AAMPS Association for African Medicinal Plants Standards
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BOOK OF EXTENDED ABSTRACTS

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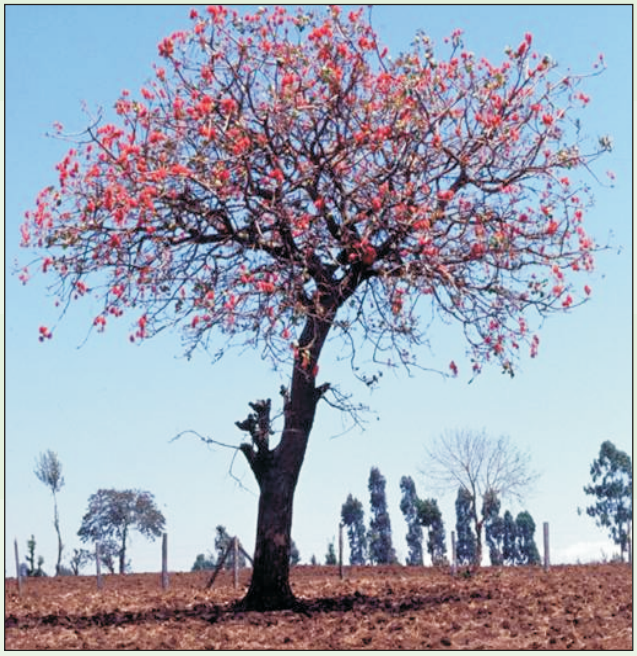

Erythrina Abyssinica

The 14th NAPRECA Symposium and AAMPS Ethnoveterinary Medicine Symposium

8th - 12th August 2011

International Center for Insect Physiology & Ecology (ICIPE) Kasarani, Nairobi, Kenya

Theme:
Natural products from African biodiversity

A SYMPOSIUM IN CELEBRATION OF THE INTERNATIONAL YEAR OF CHEMISTRY (IYC) 2011

International Science Programme, ISP

DAAD Deutscher Akademischer Austausch Dienst
German Academic Exchange Service

Pan Africa Chemistry Network

NOVUS INTERNATIONAL, INC.

RSC Advancing the Chemical Sciences

Pan-African Natural Products Library

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SUB-THEMES

Structure Elucidation
Chemotaxonomy
Pharmacology and Bioassay on Natural Products
Modern Chromatographic Techniques
Herbal Remedies
Role of Ethnobotany in Drug Discovery
Bioassay screening
Medicinal Chemistry
Synthesis of Natural Products
Drug Development
Ethics and IPR of Drug Development
Green Chemistry in Natural Product Research

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Dr. Stanley Mukanganyama (Zimbabwe)

FOREWORD

The Natural Products Research Network for East and Central Africa (NAPRECA) was established in 1984 and was subsequently affiliated to UNESCO as a network program in 1988. Since then it has held regular biennial Symposia which have continuously attracted top class research professionals to its Symposia. The current Symposium is the 14th edition and is attended by researchers from 22 countries spread in four continents- Asia, America, Europe and Africa. It is being held in conjunction with the ethno-veterinary symposium organized by the Association for African Medicinal Plants Standards (AAMPS). This is the first time we are collaborating with AAMPS and I believe this makes the meeting even more exciting.

The Symposium is going to have a total of 111 presentations- one keynote address, 25 plenary, 38 short lectures, 26 young scientist presentations and 22 posters which is quite a heavy serving. The abstracts indicate that there will be a lot of substance in the presentations and we hope that delegates leave satisfied. The presentations cover the whole range of Natural Products fields with reports of quality results from East and Central African Institutions. There are papers discussing bio-prospecting project strategies in Africa and elsewhere; due to the high throughput bioassay techniques that are now well established these presentations will be of particular interest to NAPRECA members because they offer opportunity for wide collaboration across many institutions. For this reasons, NAPRECA members are particularly encouraged to attend the Pan African Natural Products Library meeting so as to determine how they would participate it in the future. The AAMPS Symposium also offers a unique opportunity for researchers to interact with Industry and therefore reflect on how they could develop product prototypes from their research results and search for commercial outlets. Traditional medicine practitioners will also be participating in this ethno-veterinary function; we are lucky as NAPRECA members for this opportunity to interact with these people who are primary sources of information.

Due to problems we have had with publication of books of proceedings for recent Symposia we have decided to produce only one publication for the symposium in the form of this extended book of abstracts; we believe that you will find it useful as a reference material. Enjoy the booklet and the symposium.

Finally we wish you a nice stay in our beautiful, 'city in the sun' as Nairobi is fondly referred to. You should take some time and enjoy tourist attraction spots in and around the city after the Symposium.

Jacob O Midiwo, PhD
Executive Secretary, NAPRECA.

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Programme for the

The 14th NAPRECA Symposium and AAMPS Ethnoveterinary Medicine Symposium

AAMPS

MONDAY, AUGUST 8TH 2011

08:00-09:00 Registration

SESSION I: OPENING SESSION

Chairperson: Prof. Ahmed Hassanali

09:00 – 09:05	SPEECH 1	**NAPRECA-K**
09:05 – 09:10	SPEECH 2	**Prof. J.O. Midiwo**
09:10 – 09:15	SPEECH 3	**ICIPE**
09:15 – 09:20	SPEECH 4	**DAAD**
09:20 – 09:25	SPEECH 5	**ISP**
09:25 – 09:30	SPEECH 6	**NCST**
09:30 - 10:15	**KEYNOTE ADDRESS** Chemical Sciences in Africa – Historical Insights and Major Milestones	**Prof. Berhanu Abegaz** Executive Director African Academy of Sciences
10:15 – 10:30	**Reaction to the Keynote Address**	

10:30 – 11:00 HEALTH BREAK

SESSION II - PLENARY LECTURES I

Chairperson: Prof. J. O. Midiwo

PL 1	11:00 – 11:40	**Natural Products: Evolution of Structural and Functional Diversity and Implication for R&D Targeting Useful Candidates**	**Prof. Ahmed Hassanali**
PL 2	11:40 – 12:20	Shifting the paradigm to the ethnobotany-based drug discovery	**Prof. P. Rasoanaivo**
PL 3	12:20 – 13:00	Why has there been hardly any success in developing antimicrobial products from medicinal plants?	**Prof. Kobus Eloff**

13:00 – 14:00 LUNCH BREAK

SESSION III - PLENARY LECTURES II

Chairperson: Prof. Ermias Dagne

PL 4	14:00 – 14:40	Phytochemical Investigations of leaves and bark of *Croton gratissimus* (Euphorbiaceae)	**Prof. D. Mullholland**
PL 5	14:40 – 15:20	Challenges and opportunities of traditional/herbal medicine	**Prof. Ivan Addae-Mensah**
PL 6	15:20 – 16:00	Approaches to the synthesis of tricarbonyl metabolites: An Isoxazole Strategy	**Prof. Ray Jones**

16:00 – 16:30 Health break

SESSION IV - PARALLEL SESSIONS – SHORT LECTURES I

LECTURE ROOM A		**LECTURE ROOM B**	
Chairperson: Prof. I.O. Jondiko		**Chairperson: Dr. Cathrine Lukhoba**	
16:30 – 16:50	**[SL 1A] Sakina Yagi** Phytochemical and biological activity studies on *Aloe sinkatana*	16:30 – 16:50	**[SL-1B] David R. Katerere** Application of *in vitro* drug metabolism and disposition studies to assess risk of drug interactions with *Sutherlandia frutescens* extracts
16:50 -17:10	**[SL 2A] Moses Langat** Chemical constituents of East European Species	16:50 -17:10	[SL 2B] Alice Njue In vitro inhibition of tomato Fusarium wilt causative agent by zearalenone from a soil inhabiting fungus
17:10 – 17:30	**[SL 3A] Robert Byamukama** Anthocyanins from selected plant species in Uganda	17:10 – 17:30	**[SL 3B] Hayder Abdelgader** Side Effects of some Botanicals on the egg parasitoid *Trichogramma spp*.
17:30 – 17:50	**[SL-4A] V. Jayakumar** Bioactive constituents from *Hyptis suaveolens*	17:30 – 17:50	**[SL-4B] M.M.O. Omolo** Blends of Chemicals in Smelly Feet Switch Malaria Mosquitoes on and off.
17:50 – 18:10	**[SL 5A] A. Mohammed** Extractives from the genus *Heteropyxis*	17:50 – 18:10	**[SL-5B] Theoneste Muhizi** Essential oil from some Rwandese plants and their antibacterial activity
18:10 – 18:30	**[SL-6A] Nour A. Osman** Chemical constituents of the essential oil of *Cymbopogon proximus* and their potential for the treatment of otomycosis	18:10 – 18:30	**[SL 6B] Machocho, A.K.** Antimicrobial Activity and Phytochemical Studies of Some Selected Medicinal Kenyan Plants

19:00 Mixer Cocktail

TUESDAY, AUGUST 9TH 2011

SESSION V -PLENARY LECTURES II

CHAIRPERSON: Prof. Philippe Rasoanaivo

PL 7	08:00 – 08:40	Absolute Stereostructures by LC-CD Coupling in Combination with Quantum-Chemical CD Calculations	**Prof. G. Bringmann**
PL 8	08:40 – 09:20	**Technology Platforms to Facilitate Natural Product-Based Drug Discovery from African Biodiversity**	**Prof. Kelly Chibale**
PL 9	09:20 – 10:00	From past traditions to a Herbal Pharmacopoeia – Africa's Green Gold	**Prof. Ameenah G-Fakim**
PL 21	10:00 – 10:20	The pan-African Natural Product Library (p-ANPL): Giving steam a direction	**Andrae-Marobela K.**

10:20 – 10:40 Health break

SESSION VI - PARALLEL SESSIONS – SHORT LECTURES II

IV A		**IVB**	
LECTURE ROOM A		**LECTURE ROOM B**	
Chairperson: Prof. Joseph Keriko		**Chairperson: Prof. Kobus Eloff**	
10:40 – 11:00	**[SL-7A] Abiy Yenesew** Antiplasmodial and Radical Scavenging Activities of Flavonoids from Kenyan *Erythrina* species	10:40 – 11:00	**[SL 20A] Margart Ng'ang'a** Antiplasmodial Compounds from the leaves of *Drypetes gerrardi*
11:00 – 11:20	**[SL-8A] Claudia Steiner** Isolation and structure elucidation of bioactive compounds of the tropical liana *Ancistrocladus congolensis*	11:00 – 11:20	**[SL-8B] Inshirah. A. Elfahal** The efficacy of extracts of the plant *Argemone mexicana* on mosquito species, *Anopheles arabiensis*
11:20 - 11:40	**[SL-9A] Bonaventure T. Ngadjui** Arylbenzofurans, Prenylated Flavonoids and Diels Ader Adducts with biological activities from *Morus mesozygia*	11:20 - 11:40	**[SL-9B] Danielle A. D. Rakoto** *In vitro* effects of extracts from five Malagasy endemic species of *Albizia* (Fabaceae) on vegetable seeds germination
11:40 – 12:00	**[SL-10A] Stephen S. Nyandoro** Aristolactams, indolidinoids and other metabolites from *Toussaintia orientalis* - An endangered Annonaceae species endemic to Tanzania.	11:40 – 12:00	**[SL-10B] Ester Innocent** From laboratory to field application of phyto-larvicides: An outreach community based experience in Bagamoyo District, Tanzania.
12:00 – 12:20	**[SL-11A] Josiah O. Joo Odalo** Bioactive furanoditerpenoids, a dibenzopyranone, nor-isoprenoid and	12:00 – 12:20	**[SL-11B] I.O. Jondiko** *Toddalia Asiatica.* Lin: A Potential Source and Model of Materials and Services for Control of Diseases and Implications for Herbal Medical

	biflavonoids from medicinal *Stuhlmania moavi verdc*.		Practice in Kenya.
12:20 – 12:40	**[SL-12A] Danstone L. Baraza** Fungitoxic C-18 hydroxy unsaturated fatty acids from fruiting bodies of *Cantharellus* species	12:20 – 12:40	**[SL- 12B] Alvaro Viljoen** Application of vibrational spectroscopy and planar chromatography in the quality control of South African medicinal and aromatic plants
12:40 – 13:00	**[SL-13A] Bernard F. Juma** Synthesis of 2,6-Dioxo-1,2,3,4,5,6-hexahydroindoles and their Transformation into 5,8,9,10-Tetrahydro-6*H*-indolo[2,1-*a*]isoquinolin-9-ones	12:40 – 13:00	**[SL-13B] Najma Dharani** Medicinal Plants of East Africa: *Importance, Uses in Traditional Medicine, Challenges and Conservation Status*

13:00 – 14:00 Lunch

SESSION VII - PLENARY LECTURES III

CHAIRPERSON: Prof. Dulcie Mulholland

PL 10	14:00 – 14:40	NMR Analysis of the Molecular Structure of Flexible Molecules in Solution	**Prof. Máté Erdélyi**
PL 11	14:40 – 15:20	Bioassay of natural products for cosmetics	**Dr. Alain Meybeck**
PL 12	15:00 – 15:40	Multiple anti-infective properties of selected *Combretum* species from Zimbabwe	**Dr. S. Mukanganyama**
PL 13	15:40 – 16:20	The Center for World Health & Medicine at Saint Louis University: A New Translational Research Model to Develop Novel Therapies for Neglected Diseases and Other Unmet Medical Needs	**Prof. Peter G. Ruminski**

16:20 – 17:30 Health break/Poster Session

SESSION VIII – PLENARY LECTURES V

PL 14	17:30 – 18:10	Towards Gaining Recognition as an African Centre of Excellence in Applied Nanomedicine Research and Training for Poverty Related Diseases – Focus on the DST/CSIR Nanomedicine Platform.	**Dr. Hulda Swai**
PL 15	18:10 – 18:50	Application of UV-Vis Spectroscopy to Evaluate Quality of Medicinal and Edible Oils	**Prof. Ermias Dagne**

WEDNESDAY, AUGUST 10TH 2011

AAMPS **ETHNOVETERINARY MEDICINE SYMPOSIUM**

SESSION A

Chairperson: Prof. Berhanu Abegaz

08.45 08:55	Welcome	**Thad Simons** **Prof. Ameenah Gurib Fakim** **Prof. Jacob Midiwo**	President & CEO Novus International Chairman of AAMPS Executive Secretary of NAPRECA
PL I	9:00 – 9:40	From ethnoveterinary medicines to phytomedicines	**Dr. David Katerere** Medical Research Council, Cape Town, Editor of Ethno-veterinary Botanical Medicine: Herbal medicines for Animal Health
PL II	9:40 – 10:00	The role of botanical gardens in ethno-veterinary research	**Dr. Wendy Applequist** Assistant Curator, Missouri Botanical Garden, USA
SL I	10:00 - 10:15	Novel control strategies for the Southern Cattle Tick	Representative from ICIPE
10:15 -10:45		Question and Answer	

10.45 – 11:15 HEALTH BREAK

SESSION B

Chairperson: Prof. Jacob Midiwo

PL III	11:15 – 11:50	***Research on the use of plant extracts to enhance animal productivity in Southern Africa***	**Prof JN Eloff** Phytomedicine Programme, University of Pretoria
SL II	11:50 – 12:10	Validation of some plants used by Eastern Cape farmers in the control of internal and external parasites of livestock	**Prof. Patrick Masika** University of Fort Hare Zimbabwe
SL III	12:10 – 12:30	***Evaluation of plants used traditionally to protect animals against myasis***	**Ms. Lilian Mukandiwe** University of Harare, Zimbabwe
SL IV	12:30 – 12:50	Funding opportunities for research and the potential value of an African Herbal Pharmacopoeia for animal health and productivity	**Prof. Ameenah Gurib-Fakim** University of Mauritius
12:50 – 13:00		Question and Answer	

1300 – 1400 Lunch

SESSION C

Chairperson: Prof Ameenah Gurib-Fakim

PL IV	14:00 – 14:30	***Highlights of East African ethnoveterinary medicine research***	Dr. John Githiori
SL V	14:30 – 15:00	**Animal and human Trypanosomiasis: Challenges to medicinal plant research in Africa.**	Prof. Clement Adewunmi, Obafemi Awolowa African Journal of Traditional, Complementary& Alternative Medicine
SL VI	15:00 – 15:20	**A brief review of the use of medicinal plants in veterinary medicine in Mali.**	Prof. Drissa Diallo Dept. of Traditional Medicine Bamako, Mali
	15:20 – 16:00	***General panel discussion and concluding remarks***	Chairmen & speakers

16:00 – 16:30 Health Break

16:30 Annual General Meeting of Association for African Medicinal Plant

SESSION IX – PARALLEL LECTURES- SHORT LECTURES III

IV A		**IVB**	
LECTURE ROOM A		**LECTURE ROOM B**	
Chairperson:		**Chairperson:**	
16:30 – 16:50	**[SL 14A] Okoth, M.O.** Crystallization for Long Range Molecular Order Structure Elucidation	16:30 – 16:50	**[SL 16A] Tanayen, J.K.** Assessment of *Azadirachta Indica* and *Cassia Spectabilis* for Some Immunomodulatory Properties
16:50 – 17:10	**[SL 15A] Elwaleed E. Hassan** Antileishmanial Activity of Petroleum ether, *n*-hexane Crude Extract and (2E)-methyl 3-((1E, 4E)-7-methyl-4-(2-oxopropylidene) cyclohept-1-enyl) acrylate from *Xanthium brasilicum* Vell. leaves.	16:50 – 17:10	**[SL 17A] Maud K.-Mugisha** Evaluation of the Biosafety of Selected Botanical Pesticide Plants Used by Subsistence Farmers Around the Lake Victoria Basin
17:10 – 17:30	**[SL 18A] Claude Kirimuhuzya** The *in vitro* antimycobacterial activity of medicinal plants used by traditional medicine practitioners (TMPs) to treat tuberculosis in the Lake Victoria basin in Uganda	17:10 – 17:30	**[SL 19A] Faiza E. E. Salah** Effects of Aqueous Extracts of Basil, *Ocimum basilicum* L., Sodom's apple, *Calotropis procera* Ait and Coriander *Coriandrum sativum* L. on leaf miner, *Liriomyza Spp.*, on okra Crop.

19.30 Novus International Sponsored Dinner

Thursday 11TH August 2011

SESSION X - PLENARY LECTURES VI

Chairperson: Prof. D. Mullholland

PL 16	08:00 – 08:40	Natural Products from Plant Diversity and their Potential in Management of Neglected Diseases.	**Prof. J. O. Midiwo**
PL 17	08:40 – 09:20	Changes in Plants Metabolites with Location of Growth and Agronomic Practices: Some Lessions from Black Tea Quality Studies	**Prof. P. Okinda Owuor**
PL 18	09:20 – 10:00	Challenges of Isolation, Characterization and Profiling of African Medicinal Plants: Analytical Prospective of Standardization and Quality Control Methods	**Prof. Mathew M Nindi**

10:00 – 10:30 Tea break

SESSION XI –PARALLEL SESSION- YOUNG SCIENTIST COMPETITION I/p-ANPL Meeting

IV A		**IVB**		**IV C**
LECTURE ROOM A		**LECTURE ROOM B**		**LECTURE ROOM C**
Chairperson: Prof. Gerhard Bringmann		**Chairperson: Prof. Kelly Chibale**		**Chairperson: Prof. B. Abegaz**
10:30 – 10:50	**[YS-1] Ndinteh D.T Derek Tantoh** The Genus *Erythrina* a source of many useful phytochemicals.	10:30 – 10:50	**[YS-2] Elizabeth V.M. Kigondu** Antimalarial and Antileishmanial Activity and Cytotoxicity of Selected Medicinal Plants from Kenya	**pan-African Natural Product Library (p-ANPL) General Meeting.**
10:50 – 11:10	**[YS-3] Lois Mwikali Mutisya** Terpurinflavone: Antiplasmodial Flavones from the Stem of *Tephrosia purpurea*	10:50 – 11:10	**[YS-4] Beatrice. N. Irungu** *In vitro* antiplasmodial and cytotoxicity activities of selected medicinal plants from Kenya	
11:10 – 11:30	**[YS-5] Milkyas Endale Annisa** Antiplasmodial quinones from selected *Pentas* species	11:10 – 11:30	**[YS-6] Justin N. Kabera** Therapeutic (Verucidal) effect study of juice of Tetradenia (Iboza) riparia leaves on the warts.	
11:30 – 11:50	**[YS-7] Ivan Gumula** New Prenylated Isoflavanones From the Stem Bark of *Platycelphium Voense*	11:30 – 11:50	**[YS-8] Chris J.D. Obbo** Antitrypanosomal, Antileishmanial and Antiplasmodicidal Activities of *Khaya*	

			anthotheca, a Plant used by Chimpanzees for Self Medication.	
11:50 – 12:10	**[YS-9] Francis Machumi** Antiplasmodial and antileishmanial studies on carvotacetone derivatives from *Sphaeranthus bullatus*	11:50 – 12:10	**[YS-10] Rechab S. Odhiambo** *In vitro anthelmintic effect of Prosopis juliflora (Sw.) DC on Haemonchus contortus, an abomasal nematode of sheep*	
12:10 – 12:30	**[YS-11] Fredrick Munga Ng'ang'a** Application of Solid Phase Extraction Gas Chromatography Mass Spectrometry in Geographical Profiling and Characterization of Volatile Organic Compounds in Kenyan Honey	12:10 – 12:20	**[YS-12] Gladys Nyamoita Mokua** Evaluation of larvicidal and phytoextract induced morphological activities of V*itex schiliebenii* extracts against A*nopheles gambiae* larvae	
12:30 – 12:50	**[YS-13] Gomotsang Bojase** The Relative Stabilities and Reactivities of the First Six Members of the Dendralene Family		**[YS-14] Philip K. Bett** Fumigant and Contact Toxicity of *Cupressus lusitanica* and *Eucalyptus saligna* Essential Oils Against Insect Pests of Stored Cereals and Legumes	
12:50 – 13:10	**[YS-15] Mihigo, S.O** Rhuschalcone VI: Synthesis, Re-Isolation and Bioactivities in its Analogues		**[YS-16] Robert Opiro** Acaricidal Effects of Four Plant Species on *Rhipicephalus appendiculatus* Neumann (*Acarina ixodidae*) Ticks	

13:10 –14:00 Lunch

SESSION XII – PLENARY LECTURES VII

Chairperson:Prof. Philip Owuor

PL 19	14:00 – 14:40	Exploiting the chemistry of African biodiversity in pest management: from extraction of the chemicals to expression in GMOs	**Prof. John Pickett**
PL 20	14:40 – 15:20	Development of Medicines from African Medicinal Plants: Experiences in West Africa	**Prof. Drissa Diallo**

SESSION XII - YOUNG SCIENTIST COMPETITION III/SHORT LECTURES III

IV A		**IVB**	
LECTURE ROOM A		**LECTURE ROOM B**	
Chairperson:		**Chairperson:**	
15:20 – 15:40	**[YS-17] Ruth A. Omole** Anti-Malarial Activity and Phytochemical Studies of *Cissampelos Mucronata* And *Stephania Abyssinica*	12:20 – 12:40	**[YS-18] Anastasia Nandwa** Effects of *Sida Cuneifolia* (A.Gray) herbal extracts on the reproductive system functioning in male and female laboratory rats
15:40 – 16:00	**[YS-19] Jenipher Odak** Phytochemical Evaluation of *Elaeodendron buchananii* Stem Bark for Microbial Activities	12:40 – 13:00	**[YS 20] Joward Baluku** Risk-factors and the indigenous knowledge in the management of Newcastle Disease. A case study of Kasese District, Western Uganda.

16:00 – 16:20 HEALTH BREAK

SESSION X - YOUNG SCIENTIST COMPETITION III

16:20 – 16:40	**[YS-17] Ruth A. Omole** Anti-Malarial Activity and Phytochemical Studies of *Cissampelos Mucronata* And *Stephania Abyssinica*	16:20 – 16:40	**[YS-18] Anastasia Nandwa** Effects of *Sida Cuneifolia* (A.Gray) herbal extracts on the reproductive system functioning in male and female laboratory rats
16:40 – 17:00	**[YS-19] Jenipher Odak** Phytochemical Evaluation of *Elaeodendron buchananii* Stem Bark for Microbial Activities	16:40 – 17:00	**[YS 20] Joward Baluku** Risk-factors and the indigenous knowledge in the management of Newcastle Disease. A case study of Kasese District, Western Uganda.
17:00 – 17:20	**[YS–21] Sylvia A. Opiyo** Further Phytochemical and Antimicrobial Activity Studies of *Warburgia Ugandensis* Against Sweet Potato Pathogens	17:00 – 17:20	**[YS-22] Nalumansi Patricia** Medicinal Plants used in Disease Management Among Children in Namungalwe Sub County.
17:20 – 17:40	**[YS-23] Charles Ochieng** Antiplasmodial and Antinociceptive constituents from *Caesalpinia volkensii* Harms (Caesalpiniaceae) Root Bark	17:20 – 17:40	**[YS-24] Kosgey Janet Cheruiyot** Documentation of Medicinal Plants Found in Keiyo County Cherebes and Endo Village
18:00 – 18:20	**[YS -25] Fotso Ghislain Wabo** Antimicrobial Dihydroisocoumarins from *Crassocephalum Biafrae*	18:00 – 18:20	**[YS – 26] Chrian Marciale** Antimycobacterial and Cytotoxicity Activity of Extracts from Zanthoxylum rhalybeum and Hallea rubrostipulata

18:20 – 19:00 CLOSING CEREMONY

KEYNOTE ADDRESS

Chemical Sciences in Africa – Historical Insights and Major Milestones

Berhanu Abegaz

African Academy of Sciences, Nairobi, Kenya

Key words: *Chemistry in Africa; historical perspectives; natural products*

The idea to address this topic was prompted by the declaration of 2011 as the International Year of Chemistry. The intention is to outline the major contributions of those scientists and research groups that laid the foundation of chemistry in general and natural product sciences in particular in Africa. Historically the applications of natural products such as incense, opium, castor oil, etc can be traced through ancient Egyptian heliographic documents like the Ebers papyrus writings to 1500 BC. Despite these ancient beginnings, there is little documented on indigenous chemical science emerging during the post Lavoisier era up to the end of the 19th century. Information on Africans who may have trained in Europe or the US during this period is also very scanty, although one finds that a Ghanian by the name of Anthony William Amo was most probably the first African to study in Europe and to even become professor in two German Universities (Halle and Jena) around 1730 (Abraham, 1996). Amo eventually returned to Ghana, but there is no record of him having started a modern school of learning and research. The development of African universities like Ibadan, Makerere and Khartoum during colonial times was linked to the University of London. The research and teaching of chemistry in these pioneering institutions dates back to nearly seventy years, and most of the research was focused on natural products (Abegaz and Davies-Coleman, 2009). The development of chemistry in South Africa dating back to nearly one hundred years is relatively well documented (Mulholland and Drewes, 2004). Key names that feature prominently in the early development of South African chemistry include: Theiler, du Toit, Marais, Warren, Enslin, Roux, etc. with others, like Steyn, Bull, Drewes, Ferreiera, Nyokong, Michael, etc., forming some of the notable contemporary chemists. Some of the outstanding research from their efforts led to the discovery of compounds like geigerin from *Geigera aspera*, monofloroacetic acid from *Dichapetalum cymosum, caespitin* from *Helichrysum caespititium,* rooperol from *Hypoxis hemerocallidea,* ocholbullenone from *Ocotea bullata.* Pioneering Ghanian chemists include Torto and Quartey who returned from training in the UK and initiated research in Ghana. Torto is known for starting research on anti-sickeling agents in West Africa (Torto et al., 1973), while Quartey contributed on several fronts including the development of the Birch reduction, and became a life-long friend of AJ Birch (Birch et al., 1952). The development of chemistry in Nigeria is a shared contribution of Nigerian as well as European chemists with the most prominent ones being: Bevan, Taylor, Akinsanya, Ekong, Ogan, Powell, Nwaji, Arene, Eshiet, Adesogan, Olagbemi, Okogun, etc. who began active research on natural products and discovered a whole range of natural compounds including the now well known substance Gedunin (Akinsanya et

al., 1960). Looking for anti-sickling compounds has been a major trust for West African chemists, and this eventually led to the FDA approved drug (called Nicosan) based on a traditional phytomedicine preparation (Wambebe, 2008). The development of chemistry in Ethiopia was related to efforts to find applications to some widely used plants like Kosso (which engaged well known chemists like Birch and Todd and companies like Merck) (Abegaz et al., 1999) and the soap berry plant, *Phytolacca dodecandra* and the discovery made by Lemma of the properties of this bush plant in killing bilharzia-causing snails (Lemma, 1990). In Kenya, the establishment of the international Center for insect physiology (*icipe*) has made direct contributions to the study of natural products that have insecticidal properties as well as the study of semiochemicals from insects. The lecture will also describe the roles of pioneer chemists in Cameroon, Malawi, Kenya, Tanzania, etc. Chemistry has developed relatively well during the last decades with several research groups actively discovering natural compounds, while others are undertaking synthesis and analysis. Green chemistry is also taking a foothold in many countries through the collaborative efforts of European and Ethiopian scientists. The celebration of the international year of chemistry should therefore allow reflections on the role of chemistry for development especially for Africa. Many countries in Africa will probably not meet the MDGs in 2015, and hence they will unquestionably remain as key drivers for policy and actions in subsequent years. Chemists should have significant roles in this regard since chemistry can make definite contributions to at least the six of the eight Millennium Development Goals.

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PLENARY LECTURES

[PL 1] Natural Products: Evolution of Structural and Functional Diversity and Implication for R & D Targeting Useful Candidates

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Key Words: Natural products & ecology, structural & functional diversity, blend effects, bio-rational prospecting, opportunities & challenges

Introduction

Despite their dominant role in human culture, in the past, natural products ("secondary metabolites") were often dismissed as "evolutionary relics" or "waste products" of primary metabolism without any specific biological function. Earlier scientific interest in secondary metabolites was driven partly by search for bioactive agents from plants, as targets or as models for synthetic analogues useful in pharmacognosy, chemotherapy and pesticidal science. However, it was the fascination of chemists for structural diversity, synthetic challenges and biogenetic origin that saw rapid expansion of our knowledge of natural products. By 1070s, there was growing recognition that natural products play very important ecological functions in ecosystems in which they have evolved. An evolutionary view accounts for the origin, diversity, range of bioactivities, and long-term performance of natural products.

Important consequences resulting from co-evolutionary interactions within and between organisms mediated by secondary metabolites include (i) structural and analogue diversity of compounds often acting as blends to provide effective protection against specific predators or invaders (Cates, 1996) and to mitigate against speedy resistance development (Feng and Isman, 1995; Isman et al.,1996); (ii) functional diversity of individual constituents or blends to respond to diverse groups of predators/invaders (Berenbaum and Zangerl, 1996); and (iii) subtlety in the actions of secondary metabolites, often relying on repelling (or deterring) specific functions of predators/invaders, and/or inhibiting their normal physiology and development, and rarely on their acute toxicity (Romeo el., 1996). These have important implications on our approach to bioprospecting.

Bio-prospecting: examples, highlights of results and challenges

The presentation seeks to highlight the importance of a 'bio-rational' approach to discovering useful natural products that build on chemo-ecological insights, and the different challenges they present in practical exploitations. These will be illustrated by the following on-going research activities.

- Two types of blend effects will be illustrated: (a) anti-*Plasmodium* effects of artemisinin and crude blends of *Artemisia annua* leaves constituents obtained by aqueous or solvent extraction, including performance of optimally dried whole-leaf *A. annua* tablets in a clinical trial; and (b) rate of resistance development in repeated cycles of exposures of malaria parasite cultures to

pure artemisinin and a comprehensive blend of the dried *A. annua* leaves. *A. annua* constituents (Elford *et al*., 1987; 2005; Kangethe *et al*., in preparation) as well as potential resistance-mitigating effect of the full *A. annua* phytochemical blend relative to pure artemisinin (Kangethe *et al*., in preparation).

- Approaches to identifying constituents that contribute to a bioactive blend illustrated by research on herbal plants used traditionally in post-harvest crop protection (e.g. *Ocimum kilimandcharicum*) and in repelling mosquitoes and other disease vectors (e.g. *Conyza newii*); challenges encountered in downstream exploitation of the essential oils, particularly the problem of chemotypic differences in the composition and efficacy of the essential oils of plants collected from different agro-ecological sites (Bekele and Hassanali, 2001; Omolo *et al*., 2004, 2005; Aswalam et al., 2008; Mayeku et al., submitted).
- Potential integration of chemo-ecological studies relating to semiochemical-mediated location of preferred feeding site by the vector (the Brown Ear Tick, *Rhipicephalus appendiculatus*) of the cattle disease East Coast Fever (and a related species, *Rh. evertsi*), research findings on ethno-veterinary practices based on the use of anti-tick products (Wanzala *et al*., 2004; Wanzala *et al*., submitted; Wanzala et al., in preparation), toward the development of on-host 'push-pull' strategy to control the Brown Ear Tick.

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[PL 2] Shifting the Paradigm to the Ethnobotany-based Drug Discovery

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Key words: Ethnobotany; Drug discovery; Reverse pharmacology; Malaria; Brain-related disorders; Commercialisation.

Introduction

There are approximately 60,000 plant species in African Union countries, which represent roughly a quarter of the world plants. Unfortunately, despite the wealth and endemicity of the African plant biodiversity and associated cultures, Africa has only contributed **83** of the world's **1100** leading commercial medicinal plants.

Investigation of medicinal plants for drug discovery has mainly followed the Western approach of single active constituents using standard pharmacological methods. Unfortunately, there are several examples of herbal medicines with reputedly excellent therapeutic effects, which subsequently produce disappointing results when evaluated in the laboratory with the standard biological screenings. Conversely, there are several bioactive compounds isolated from plants with excellent biological activity in the laboratory, which are ineffective or too toxic to use in human patients.

The advent of today-modern techniques, namely combinatorial chemistry, high throughput screening (HTS), bioinformatics, 'omics' methods, etc., has revolutionised drug discovery. Unfortunately, biopharmaceutical companies attempting to increase productivity through these novel technologies have fallen short of achieving the desired results (1). Following the trend of these novel technologies, there has been a paradigm shift in the investigation of plants, focusing more on chemical diversity than traditional uses. The exploration of biodiversity for new sources of natural products termed bioprospecting is based on massive random collecting, systematic extraction and medium to high throughput biological screening. Disappointingly, the success in terms of new medicines reaching the market has not also increased with the application of the modern technologies (2).

The World Health Organisation estimates that up to 80% of populations in Africa depend on traditional medicine for their primary health care requirements. This is attributed to cultural acceptability, efficacy against certain types of diseases and ailments, physical accessibility and economic affordability as compared to modern medicine. African scientists should therefore look at other possibilities, get "outside the box", think otherwise on how to better harness traditional knowledge to drug discovery and formulation, taking into account the evolving equilibrium of living organisms including humans in ecosystems. We report here our recent results in malaria and brain-

related disorders, using the reverse pharmacology approach. Challenges in translating discovery into commercialisation will be also discussed.

Material and methods

Field work

For the past six years, field works were conducted in two very different ecosystems: the rainforest of the Eastern region of Madagascar, and the dry, xerophytic forests in the South and South-west. The rural communities were involved in a complete contributively and participative manner. Before commencing the research, the field team used an informed consent protocol that he had developed. Communities were asked if they were willing to allow photographs or films to be taken. Focus was on learning from/with local people, listening to them and considering their ideas (using ears more than mouth). Emphasis was also put on understanding local health concepts and disease classifications in connection with the environment.

Selection, collection and processing of plants

Medicinal plants were selected and then collected on the basis of ethnobotanical outcomes and target diseases. Botanical identification was made the by the Department of Botany, *Parc Botanique et Zoologique de Tsimbazaza*. Voucher specimens were kept at the *Institut Malgache de Recherches Appliquées* (IMRA). Extraction was carried out according to the methods used by the local populations.

Antimalarials tests

In vivo antimalarials tests were based on the 4-day suppressive test. Parasitemia, number of mice survival, and appearance/proliferation of lymphocytes were respectively recorded from D-4 until mice death. *In vitro* antiplasmodial tests were based on the inhibition of tritiated hypoxanthine uptake by *Plasmodium falciparum* cultured in human blood.

Brain-related tests

Anticonvulsant activities were assessed chemically by the pentylenetetrazole- and picrotoxin-induced seizure tests, and electrically by the maximal electroshock-induced seizure test. The Morris water maze test was used to evaluate cognitive behaviour.

Isolation of bioactive constituents and structure elucidation

Silica gel column chromatography was used to isolate bioactive constituents by bioassay-guided fractionation. Structure elucidation was mainly based on concerted interpretation of NMR data.

Results and Discussion

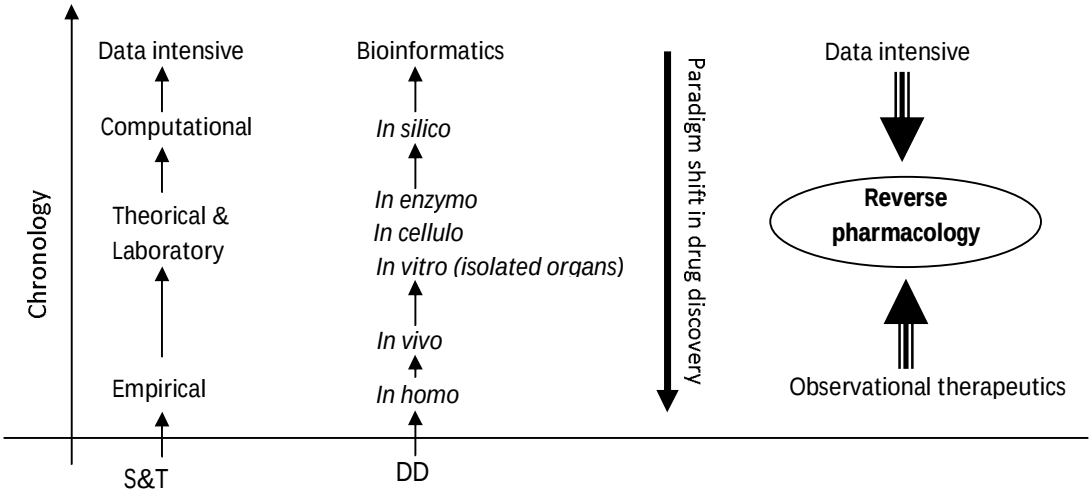
From a medicinal plant reputedly used to treat malaria, we found that the aqueous extract, in combination with sub-therapeutic doses of conventional drugs, exhibited strong immuno-stimulating effects. From a plant used to treat convulsions and related diseases, we showed that an extract obtained by specific plant processing had both anticonvulsant activities in different animal

models and positive effects on cognitive behaviour. Regarding the biological tests, we went beyond the boundary of classical test protocols, and devised modified protocols to demonstrate biological activities.

Research on antimalarial drugs has been mainly focused on killing the parasites but rarely consider other mechanisms. Many anti-malarial herbal remedies may exert their anti-infective effects not only by directly affecting the parasite, but also by stimulating natural and adaptive defence mechanisms of the host. The immune system is the first line host defence, and it is always associated with a complex inflammatory process which is partly responsible for the disease symptoms. Unfortunately, little work has been devoted to the investigation of the immuno-stimulating and anti-inflammatory effects of these herbal remedies. Curcumin was reported to possess both immune-stimulating and anti-inflammatory effects, but its poor bioavailability has limited its clinical uses (3). Our preliminary results showed a promising approach for malaria treatment and protection.

Epilepsy is the most common neurological disorder in young humans. Ion channels, neurotransmitters and second messenger systems constitute molecular targets of antiepileptic drugs (AEDs). The same targets regulate brain processes essential both for propagation of seizures and for learning, memory and emotional behaviour. Thus, AEDs which are used to treat seizures in infants, children and pregnant women can cause cognitive impairment, microcephaly and birth defects (4). All currently available AEDs are synthetic in nature. Our preliminary results showed that a clearly defined extract from a traditional antiepileptic plant had both anticonvulsant and behaviour-improving activities.

As outlined in the figure below, drug discovery (DD) process has evolved following the evolution of science and technology (S&T).



Reverse pharmacology is defined as the combination of traditional knowledge and the application of modern technology and processes to provide better and safer drugs. Its aim is to reverse the routine 'laboratory-to-clinic' pathway to 'clinics-to-laboratories' (5).

The today-dominating paradigm of drug discovery in biopharmaceutical companies is to find new single entity drugs acting selectively on individual drug targets. Natural product drug discovery based on traditional knowledge may also be considered as attractive strategic options. Nevertheless, it is important to bear in mind that most of pure natural products do not comply with the Lipinski 'Rule of Five'. During evolution and natural selection, mammals, including humans, have developed biological systems for effluxing them, to prevent xenobiotics being absorbed (example of curcumin). It may be therefore necessary to shift the paradigm from the notion that a single molecular abnormality is the cause of complex diseases, and focus on developing standardized extracts with multiple mechanisms of action. At this point, complex, pleiotropic diseases may require multi-component, multi-functional therapies, and complex molecular interactions produce effects that may not be achieved by single components. Treating malaria may involve killing the parasites, boosting the immune system and managing the inflammatory process. Treating epilepsy may involve the necessity of maintaining a clinical equilibrium between seizure control and potential behavioural and cognitive expressions that may implicate social and vocational life aspects. Standardized plant extracts may bring answers to these innovative treatments. And surprisingly, the next paradigm of drug discovery is reported to be network pharmacology, considering multi-target strategies over single-target approaches (6).

Regarding the commercial aspect of discovery, there is a skill gap and a funding gap to translate research finding in academia into marketable products. *"It is now time to break the walls between science and commerce and to start building bridges instead"* (H.A.M. Dzinotyiweyi, Minister of Science and Technology Development, Zimbawe).

In conclusion, our results reinforce the therapeutic potential of the two species and point out to the biological and cultural value of studying traditional folk medicine as a source of innovative therapeutic treatment. Where there are no modern drugs, … there could be a safe and effective local treatment.

Acknowledgements

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[PL 3] Why Has There Been Hardly any Success in Developing Antimicrobial Products from Medicinal Plants?

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Key words: Extractant, serial dilution assay, synergism, tetrazolium violet

Introduction

It is widely accepted that resistance of microorganisms to currently used antibiotics is growing at an alarming rate. Some authors have even stated that we are entering the post antibiotic era. Before the discovery of antibiotics infections of even simple wounds have led to the death of many people. Investigation of plants has led to the development of many pharmaceutical products in the therapy of many diseases. In the order of 25% of prescription drugs in the United States were based on products isolated from plants (Farnsworth, 1990). There have been thousands of publications of authors investigating plants for antimicrobial activity, yet no commercially useful antibiotic has yet been developed from plants. Several aspects that could be responsible for this situation will be discussed.

Material and Methods

Many aspects that could have an influence on development of commercially useful antibiotics from plants were investigated to try to get an answer to the question. These include: selection of plant material, extraction of the plant material, determination of antimicrobial activity, isolation of antimicrobial compounds, test organisms used, safety of compounds from plants or extracts, synergism of activity between different compounds.

Results and Discussion

Only a small proportion of the c. 250 000 plant species have been investigated in some detail to date. In selecting plant species to investigate most scientists examined plants that have been used traditionally to treat infections. Other possibilities are random screening or screening plants on a taxonomic basis (Eloff, 1998a). Many parameters play a role in selecting the best extractants for plants and the best part of the plant to examine. We decided to focus on leaves and specifically tree leaves based on sustainable use considerations and the high diversity of compounds present in leaves. We have repeatedly found that acetone is the extractant of choice based on its ability to extract compounds with a wide range of polarity, ease of removal due to volatility and low toxicity to test organisms (Eloff, 1998b).

In examining methods to determine the antimicrobial activity of plant extracts we found that agar diffusion was not trustworthy because the polarity of the compounds in an extract had a major

effect on the diffusion within an agar matrix. We also found that serial dilution and measuring turbidity with a microplate reader gave variable results due to precipitation of compounds in more concentrated extracts. We developed a method based on measuring growth after serial dilution by the reduction of tetrazolium violet to a purple formazan due to respiratory enzymes (Eloff, 1988c). This method provides reproducible results and has since then been widely used. We also decided to use four ATCC strains of the most important nosocomial bacterial pathogens as a standard to ensure that variation on strain susceptibility would not invalidate comparing data found in different laboratories.

Traditional healers mainly have water as extractant available. We have repeatedly found that water extracts of plant leaves have a very low antimicrobial activity compared to other extracts (Kotze and Eloff, 2002). By focusing on plant species used traditionally scientists may have missed plants with high antimicrobial activity. In comparing different plant species it also became clear that not only the MIC of the extract but also the quantity extracted from a plant plays a role. The concept of total activity was developed by dividing the mass in mg extracted from 1 g of plant material by the MIC in mg/ml. The result in ml/g provides an indication of the volume to which the antimicrobial compounds present in 1 g of the plant can be diluted and still inhibit the growth of the microorganism (Eloff, 2000). This approach is also useful in bioassay guided isolation of bioactive compounds. If there is no loss of activity through deactivation of the bioactive compound there should not be a difference in the total activity of the crude and the sum of the total activities of the fractions (Eloff, 2004).

We determined the antimicrobial activity of acetone extracts of leaves of more than 600 tree species against eight important bacterial and fungal pathogens to establish if taxonomic relationships can be useful in predicting which taxa would be useful to investigate in depth. We discovered that many plant extracts had excellent activities. For example about 5% of extracts of species examined had a minimum inhibitory concentration of 40 µg/ml or lower and 2% of 20 µg/ml or lower against the non pathogenic *Mycobacterium smegmatis* closely related to the species causing tuberculosis (Figure 1).

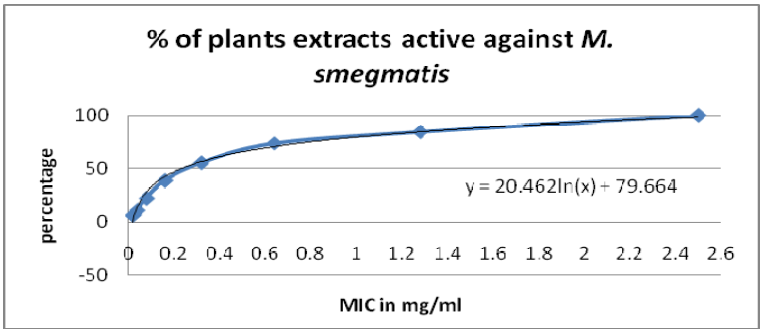

Figure 1 Cumulative percentage of tree leaf extracts active against Mycobacterium smegmatis at different MIC values

By examining extracts of species with high activity many compounds were isolated. It became clear that even though bioautography indicated practically only one antimicrobial compound in some cases, if that compound was isolated the activity would be orders of magnitude lower than what would have been expected after removing the inactive compounds. This shows that in a crude extract there is a synergism not necessarily between different antimicrobial compounds, but between the antimicrobial compound and other compounds that may affect one or more of the absorption, distribution, metabolism or excretion of the antimicrobial compound in the microorganism.

We could show that a simple manipulation of the crude extract could increase the activity per mass unit and this have led to several patents and in one case to a product in the market. We also have evidence that the development of resistance was much slower with a crude extract than with a single product antibiotic. Some examples will be discussed.

Conclusion

We conclude that the main reason why there have been no development of new antibiotics from plants are not the different methodological problems discussed above, but that plants appear to use a mixture of compounds to address microbial infection. The focus in developing effective antimicrobial products from plants should therefore move from a pure compound basis to an extract based product. In this way we may be able to use the biodiversity resources of Africa to address primary health needs of its people.

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[PL 4] Phytochemical Investigations of Leaves and Bark of *Croton gratissimus* (Euphorbiaceae)

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Key words: Euphorbiaceae, *Croton gratissimus,* diterpenoids, cembranolides.

Introduction

Of the approximately 750 species of *Croton* L. (Euphorbiaceae) distributed throughout the tropics, some 50 are found in Africa (Mabberley 2008), and only 10 species are native to the *Flora of southern Africa* region. *Croton gratissimus* Burch. (syn. *C. zambesicus* Müll. Arg.; *C. microbotryus* Pax.) is a semi-deciduous tree species, widespread in sub-Saharan Africa, occurring on stony or rocky hillsides throughout much of the warmer and drier regions, from South Africa northeastwards to the horn of Africa. The leaves produce a pleasant lavender-like scent when crushed, and are used dried and powdered for their perfume (Palmer and Pitman 1972). Across its range it is an important ethnomedicinal species: the Zulu use milk infusions of the bark as purgatives for stomach and intestinal disorders, despite its toxic reputation (Bryant 1966). Elsewhere several other crotons including the Asian *C. tiglium* L., and *C. flavens* L. from the Caribbean have been so employed, although diterpenes from both have been implicated in indirect carcinogenesis (oesophageal cancer) through activation of the Epstein-Barr virus (Hecker 1981; Bruneton 1995). The Zulu further treat unspecified uterine disorders with powdered bark blown into the womb. They also remedy pleurisy or pleurodynia by rubbing the powdered bark into chest skin incisions to act as a counter-irritant, given its cutaneous eruptive irritant and stimulant properties (Bryant 1966). As a cure for insomnia and restlessness, the leaves are ground with goat fat and those of two other *Croton* species, the paste heated on coals and the fumes inhaled (Palmer and Pitman 1972). Gerstner (1941) further recorded the purgative properties of the roots, and documented their application in treating fevers. Zimbabweans treat coughs with smoke from leaves, and take root infusions for abdominal pains and as an aphrodisiac (Gelfand *et al*. 1985). In Botswana a decoction prepared with leaves is taken for coughs (Hedberg and Staugård 1989). Watt and Breyer-Brandwijk (1962) documented the use of *C. gratissimus* bark in treating painful respiratory conditions (including intercostal neuralgia), unspecified plant parts as a remedy for fevers, charred, powdered bark for bleeding gums, and leaves to treat both eye disorders and rheumatism. In Venda, leaves are dried and smoked for influenza, colds and fevers (Mabogo 1990). Doubts about the toxicity of this species have been raised due to the esteem with which leaves have been held as a stock food in Namibia (Watt and Breyer-Brandwijk 1962). In Namibia roots and leaves of this taxon have found application as a treatment for colds and coughs, bark for ear

problems, and roots for chest ailments and fever (Von Koenen 2001). From the above usage profiles of *C. gratissimus* the following diverse bioactivities are indicated: analgesia, febrifugal, aphrodisiac, purgative, emetic, soporific, antibiotic and antiviral. The febrifugal activity of *C. gratissimus* (in the context of malaria) has earlier been demonstrated: Clarkson *et al*. (2004) found DCM extracts of the leaves to show a high antiplasmodial activity *in vitro* of 3.5 μg/ml, thus compounds **3** and the acetyl derivative of **12** were screened for antiplasmodial activity against the *P. falciparum* (CQS) D10 strain. In the current study, congnisance has been taken of the use of this species for abdominal pains (Gelfand *et al*. 1985), an indication of potential antineoplastic applications (Charlson 1980), especially as the Zulu treat unspecified uterine disorders with powdered bark preparations (Bryant 1966). Accordingly, isolates from the bark were screened against PEO1 and PEO1TaxR ovarian cancer cell lines.

Previous investigations of the genus *Croton* have yielded pimarane (Block *et al.* 2004), kaurane (Kuo *et al*. 2007), labdane (Garcia *et al*. 2006), clerodane (Garcia *et al*. 2006) and cembrane (Pudhom *et al*. 2007) diterpenoids, isoquinoline alkaloids (New World species only) (Charris *et al*. 2000) and triterpenoids (Block *et al*. 2004).

Materials and Methods

Leaves and bark of *Croton gratissimus* Burch. var. *gratissimus* were collected from a mature tree cultivated on the campus of the University of KwaZulu-Natal, Durban, South Africa, and a voucher retained for verification purposes (*Crouch 1051*, NH).

The ground stem bark of *C. gratissimus* was extracted using a Soxhlet apparatus for 24 h successively using hexane, methylene chloride, ethyl acetate and methanol. The hexane and methylene chloride extracts were separated using column chromatography over silica gel using a hexane/methylene chloride/methanol step gradient to yield the novel cembranolides, **1-4,** lupeol, 4(15)-eudesmene-1β,6α-diol and α-glutinol.

The ground leaves of *C. gratissimus* were extracted and compounds purified similarly to give the following compounds: cembranolides **3** and **5-14**, α–glutinol, 24-ethylcholesta-4, 22-dien-3-one, lupeol and eudesm-4(15)-ene-1β, 6α–diol.

Results and Discussion

Fourteen novel cembranolides were isolated from the stem bark and leaves of *C. gratissimus* and are shown in **Figure 1.** Structures were determined using extensive 2D NMR spectroscopy and mass spectrometry, and the structure of **1** was confirmed by single crystal X ray analysis and using the LSD program (Nuzillard 2003). NMR data for these compounds are reported elsewhere. (Mulholland, Langat et al. 2010)

Compounds **1** and **3** were screened against the PEO1 and PEO1TaxR ovarian cancer cell lines and were found to have IC_{50} values of 132 and 125nMolar (cf. paclitaxel 2.3) against PEO1 and 200 and

135 respectively against PEO1TaxR (cf. paclitaxel 30.5). The acetate derivative of **12** was prepared in an attempt to produce a crystalline product for single crystal X-ray analysis, or a more stable compound for screening. Unfortunately, the derivative was not crystalline but was stable enough to undergo *in vitro* anti-plasmodial screening against *P. falciparum* (CQS) D10 strain. Both compounds **3** and **12** acetate showed moderate activity (IC_{50} values of 20.80 and 13.52 µg/ml respectively), compared to chloroquine (IC_{50} 27.04 ng/ml), validating the traditional usage of this plant.

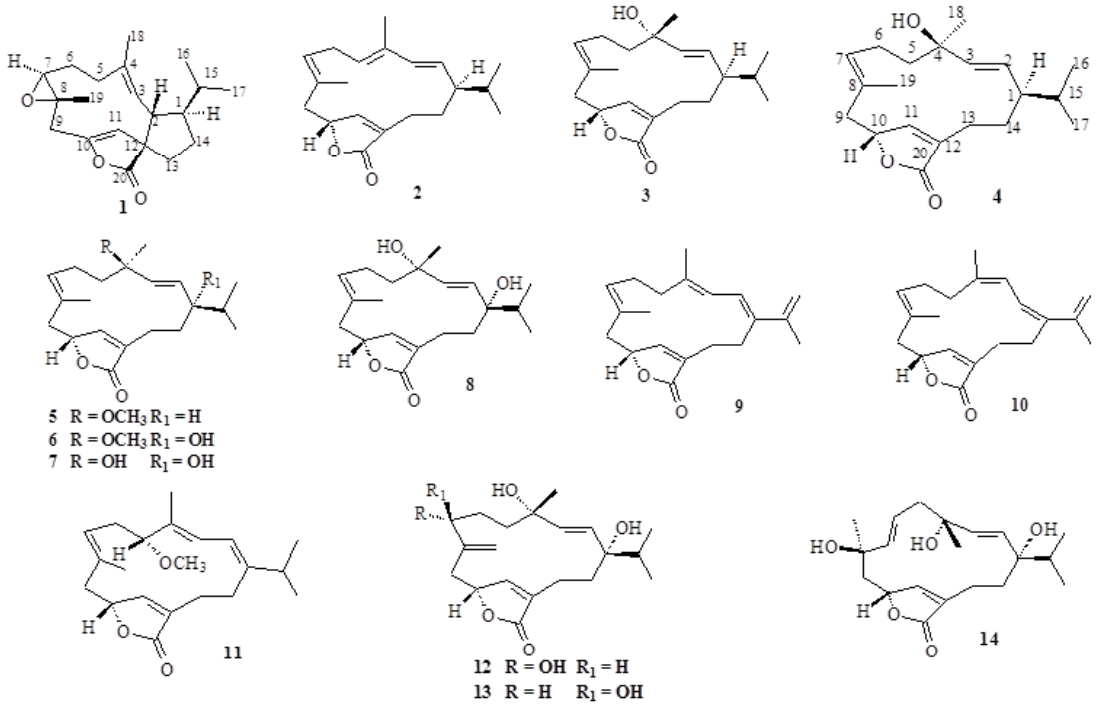

Figure 1. Cembranolides from the leaves and stem bark of *C.gratissimus*

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[PL 5] Challenges and Opportunities of Traditional/Herbal Medicine

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Key Words: Traditional Medicine; Herbal Medicine; WHO strategic plan; Research paradigm; Neglected tropical diseases; Biodiversity; Intellectual Property rights; Indigenous knowledge.

Herbal medicine has become a multi-billion dollar enterprise worldwide. The world market for herbal medicines based on traditional knowledge is estimated at over US$60 billion, which is about 30 percent of total world pharmaceutical sales.Africa accounts for only 2.1 billion dollars (1.2%) of the world's total pharmaceutical sales. The developed world, which constitutes only 20 percent of the world's population, consumes 80 percent of the total pharmaceutical output. So how do Africa's almost one billion people take care of themselves if they consume only 1.2% of the total output of pharmaceutical products? The answer lies in traditional/complementary alternative medicine (TM/CAM).

A recent survey on treatment of fevers in children has shown that the cost of herbal treatment is only about 6 percent of the cost of clinical or hospital treatment (WHO 2002 p13). In a situation where it may be the only available treatment within a radius of more than 30 kilometres, it will be far better than nothing in circumstances where no treatment at all will mean certain death.The WHO's 3-year Strategic Plan for traditional medicine envisages that member countries should integrate Traditional Medicine (TM) with national health care systems, promote the safety, efficacy and quality of TM by expanding the knowledge base, increase the availability and affordability of TM/CAM with emphasis on access for poor populations and promote therapeutically sound use of appropriate TM/CAM by providers and consumers (WHO 2000; WHO 2002).

The official national norm for primary health care in Ghana is that no citizen should travel more than 8 kilometres to the nearest health facility, no matter the kind of facility. But currently in all the regions, whereas people in over 90 percent of all localities can reach a traditional healer within a radius of 5 kilometres, conventional hospitals are available in only 12 percent or less of localities. Over 60% of patients have to travel up to 30km or more to get to the nearest hospital. The national average allopathic doctor to patient ratio is about 1 to 11,000 with some districts having a ratio of about 1 doctor to 256,000 population, whereas that of traditional healer to patient is about 1 to 900. (Ghana Statistical Services 2005) The situation cannot be any different in other African countries.

This situation makes research and development of traditional medicine a priority in the quest for adequate primary health. But research is extremely expensive. It can cost over $600 million to develop a major drug, but about $400 million of this goes into finding the best candidate, and

discarding all other possibilities on the way. Rejection rate is very high. Hardly any African country can afford that sort of research, whether for the development of synthetic or plant-based natural medicines. There is therefore the need to adopt a completely different approach to research and development of traditional medicine.

The continents of South America and Asia have both made major contributions from their biodiversity to the treatment of malaria, one of the most important tropical diseases. The discovery of quinine and quinidine from the South American cinchona bark, and compounds that emerged from this discovery were the mainstay of malaria chemotherapy for decades. The artemisinin-based anti-malarials which are now the mainstay of first-line malaria treatment are also of a Chinese plant origin. What has Africa got to offer from its vast biodiversity resource? This is the challenge we face as African Researchers.

This Plenary Lecture will focus on a selection of neglected tropical diseases such as malaria, helminthic diseases such as trypanosomiasis and leishmaniasis as well as cancer and HIV Aids and examine what research is being, or has been done in this area in the search for potential lead compounds for possible development into usable drug products for these diseases. The chemical constituents and biological activities of a selection of medicinal plants from various families including the Rutaceae, Chailetaceae, Meliaceae and Periplocaceae, will be discussed as illustrative examples. The challenges posed to African researchers in this quest will be discussed. The need for a major paradigm shift in traditional medicine research will be discussed. It will be suggested that a thorough re-examination of the constituents of plant species previously considered to be already thoroughly investigated, may reveal these as potential sources of compounds that could serve as new scaffolds for developing drugs for neglected tropical diseases.

The issue of intellectual property rights and the protection of indigenous knowledge will also be critically examined. The need for regulatory measures for conservation and protection of biodiversity as well as a rational and sustainable exploitation of this biodiversity will also be addressed. The pivotal role of African Governments in issues of scientific research and S&T governance structures will be dealt with. Our governments should realise that our science will never make any meaningful global impact if spending on S&T research continues to depend primarily on foreign donor funded programmes. Governments must take the lead in research funding, and devote a substantial portion of GDP to research.

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[PL 6] Approaches to the Synthesis of Tricarbonyl Metabolites: An Isoxazole Strategy

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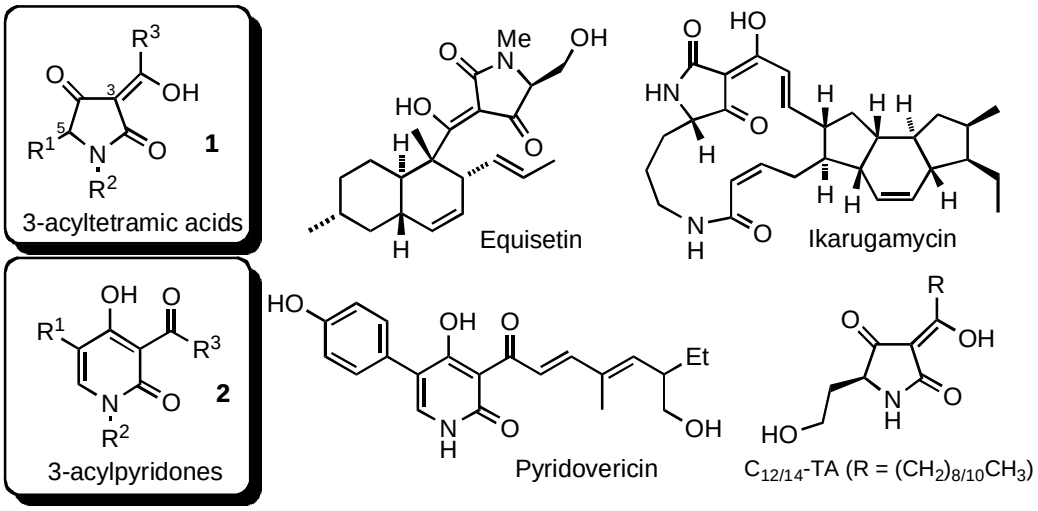
Keywords: Acyltetramic acid; acylpyridone; nitrile oxide; dipolar cycloaddition; pyrroloisoxazole; isoxazolopyridine

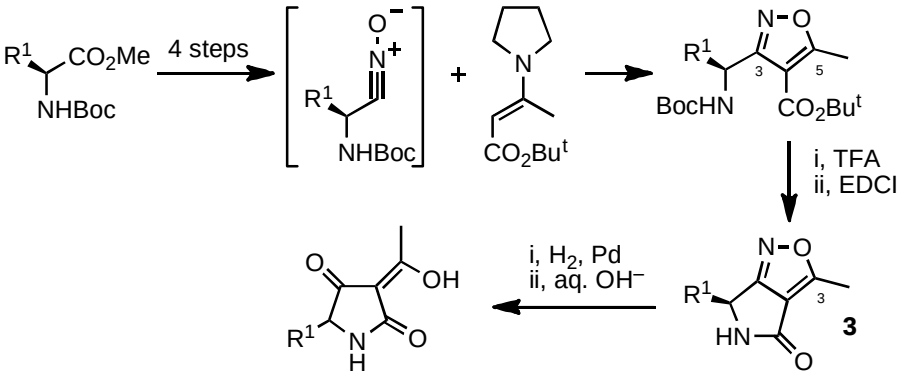
Introduction

Natural products displaying the common acyltetramic acid motif **1** show a wealth of structural features, and significantly, a diversity of biological activity including antibiotic, antiviral, antitumour, antiulcerative, fungicidal, cytotoxic and mycotoxic properties.[1] Examples are equisetin, a selective HIV integrase inhibitor, and the mycotoxin ikarugamycin. Acyltetramic acids are also implicated in a number of interesting biosynthetic processes: the 3-decalinoyl derivatives are believed to arise from intramolecular cycloaddition of a polyketide-derived side chain; acyltetramic acid ring expansion is believed to lead to bioactive 3-acyl-4-hydroxypyridone metabolites **2**, e.g the protein tyrosine kinase inhibitor pyridovericin; and $C_{12/14}$-TA formed from acylated homoserine lactones, are involved in bacterial quorum sensing. Recent renewed interest in these metabolites with reports of new natural products and synthetic work, confirms the relevance of our own approach.



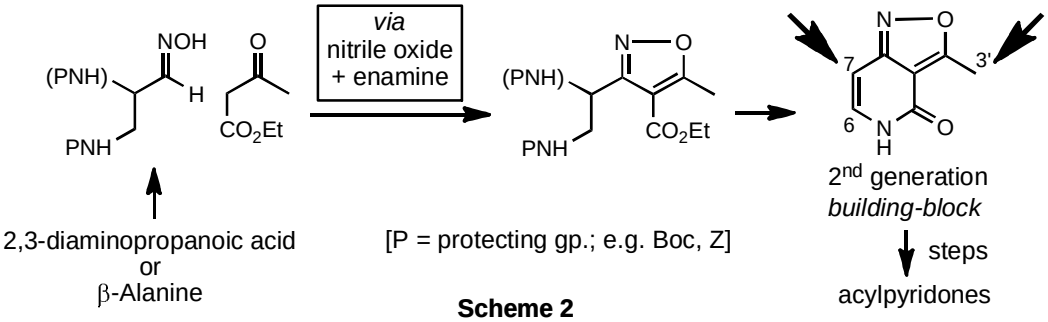
Results and Discussion

We have developed a flexible synthetic strategy for this moiety, using dipolar cycloaddition of alpha-amino acid-derived nitrile oxides with beta-ketoester enamines, via pyrroloisoxazole building blocks **3**[2]. This is illustrated in Scheme 1 for the acyltetramic acids.



Scheme 1

A key principle of our strategy is to mask the highly polar, potentially reactive beta, beta'-tricarbonyl (heterocyclic trione) moiety of the acyltetramic acids and acylpyridones as an isoxazole until required, allowing elaborations around the non-polar 'core' pyrroloisoxazole structure **3**. We will report elaboration at the C-3(methyl) of **3** using aldol-type chemistry, and subsequent unmasking of the tetramic acid moiety.[3] In a related approach to the acylpyridones (Scheme 2) we will report on the elaboration of isoxazolopyridones at C-3(methyl), again by aldol-type reaction, and at C-7 by palladium-mediated cross-couplings, followed by unmasking of the acylpyridone moiety.[4]



Scheme 2

Acknowledgements:

We acknowledge Loughborough University for studentships, Novartis & Syngenta for financial support, EPSRC Mass Spectrometry Service Centre for some high resolution MS data, & EPSRC National Crystallography Service, Prof V McKee, Dr M R J Elsegood for X-ray crystal data.

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[PL 7] Absolute Stereostructures by LC-CD Coupling in Combination with Quantum-Chemical CD Calculations

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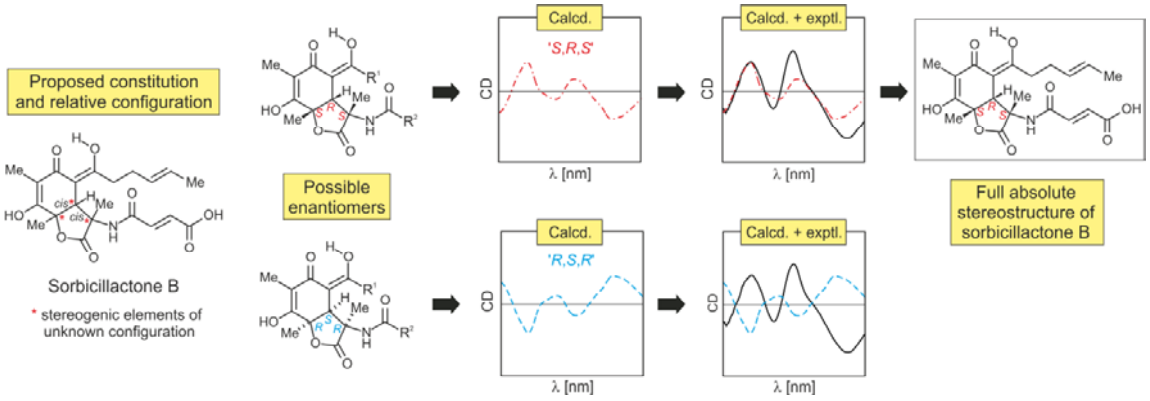
The search for novel bioactive compounds from nature is a rewarding, but also demanding task. It requires a set of modern methods to trace up these compounds, even from complex mixtures, to recognize their novelty and originality, and to assign their full absolute stereostructures reliably.

By our analytical triad HPLC-MS/MS-NMR-CD (CD = circular dichroism), we can rapidly identify novel-type compounds and establish their full absolute stereostuctures online, right from the peak in the chromatogram. Of particular importance is the LC-CD option, which we have introduced into natural products chemistry (Bringmann *et al.* 1999). The interpretation of the CD spectra (whether measured online or offline!) can be done empirically, by comparison with those of known compounds (but how related do they have to be?) or by applying empirical rules (but do they really apply in the present case?). But, much more reliably, the assignment can be achieved by quantum-chemical CD calculations (Bringmann *et al.* 2008b, Bringmann *et al.* 2009, Bringmann *et al.* 2011).

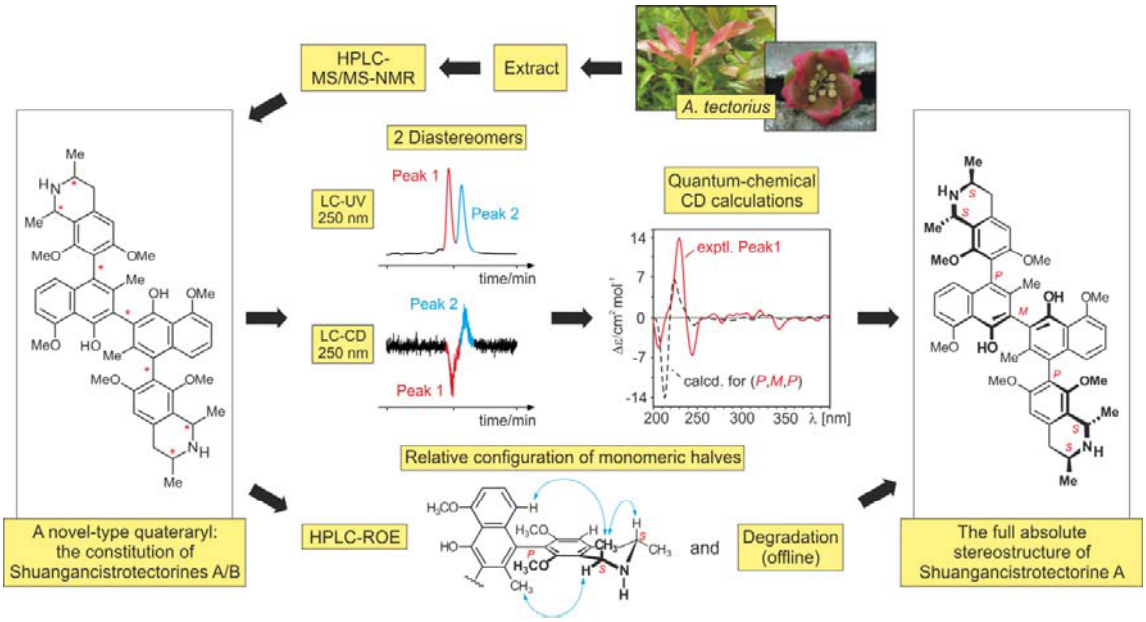
Scheme 1 outlines the strategy: For a new compound whose absolute configuration we want to assign, we simulate the CD spectrum for each of its possible enantiomers. The comparison of these two predicted CD spectra with the actual experimental CD curve will, if successful, give a good agreement for one enantiomer (top), and a mirror-like opposite situation for the other (bottom), which, thus, will permit a clear assignment of the absolute configuration.



Scheme 1. General strategy for the assignment of absolute configurations by quantum-chemical CD calculations, here for the marine natural product sorbicillatone B (Bringmann *et al.* 2005).

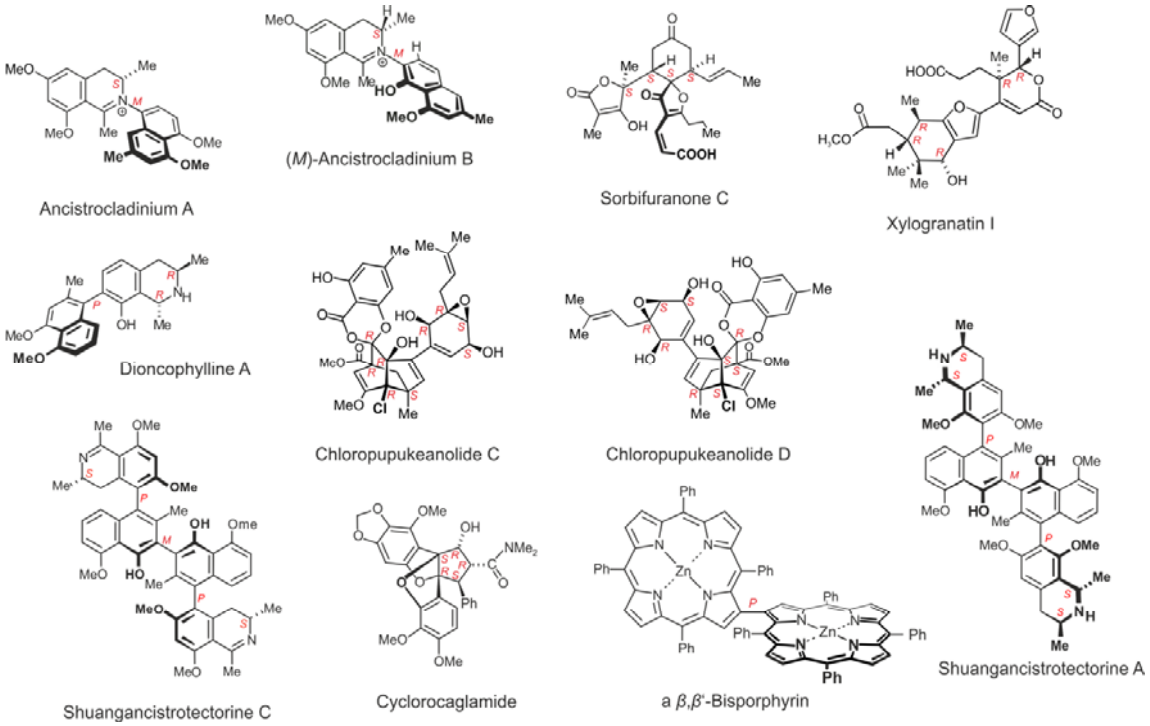
For the calculation of CD spectra one has to take into account that the CD of a molecule strongly depends on its conformational behavior - like for no other spectroscopic method! This requires a thorough conformational analysis beforehand, and that will be useful also for the interpretation of NMR data.

As an example, Scheme 2 below shows the stereochemical assignment of shuangancistrotectorine A (Xu *et al.* 2010), a demanding molecule with seven stereogenic elements: four stereocenters and three consecutive chiral axes, discovered in a tropical *Ancistrocladus* plant. Its constitution was established by NMR, like also the relative configurations at the centers and the outer axes. The absolute configuration at the central axis can be assigned by LC-CD in combination with quantum-chemical CD calculations; only the elucidation of the absolute configurations of the centers has to be done offline, in this case by chemical degradation.



Scheme 2. Structural elucidation of the novel quateraryl alkaloid shuangancistrotectorine A.

The lecture presents the strategy and illustrates examples out of most different classes of structures configurationally assigned by the method, among them ancistrocladinium A and B, shuangancistrotectorine A and C, dioncophylline A, chloropupukeanolides C and D, cyclorocaglamide, xylogranatin I, sorbifuranone C, and β,β'-bisporphyrins (Bringmann *et al.* 2006, Xu *et al.* 2010, Bringmann *et al.* 2001, Liu *et al.* 2011, Bringmann *et al.* 2003, Wu *et al.* 2008, Bringmann *et al.* 2010b, Bringmann *et al.* 2008a) (see Scheme 3). Some of the structures have already been confirmed by total synthesis (Bringmann *et al.* 2010a).



Scheme 3. Some selected examples of chiral structures whose absolute configurations have been determined by the combination of experimental and calculated CD investigations.

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[PL 8] Technology Platforms to Facilitate Natural Product-Based Drug Discovery from African Biodiversity

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In order for diseases that primarily affect the African population to receive worldwide scientific attention, African researchers must take a more active role in modern drug discovery approaches while making the most of the indigenous biodiversity available on the continent.[1] While African scientists do not have the luxury of access to large synthetic chemical libraries and other sophisticated technological platforms due, amongst other things, to limited financial and infrastructure resources, they have a powerful resource in uniquely endemic natural products and/or general biodiversity, which are yet to be exploited for health and economic benefits.

In order for natural product-based drug discovery research in Africa to translate into tangible modern pharmaceuticals, or at the very least contribute more positively to the global drug discovery value chain, it is necessary for drug discovery in Africa to adopt a more integrated and multidisciplinary approach and take advantage of modern, available drug discovery technologies. Such a paradigm shift is likely to contribute immensely to the development of research infrastructure on the continent, providing not just the potential for exciting new discoveries, but also the opportunity to expose students and scientists to multi-disciplinary research even as a new generation of African scientists are trained in modern drug discovery approaches. We have recently[2] proposed an approach to the integration of African natural products in modern drug discovery, which is summarized in **Figure 1**.

African scientists working in the area of drug discovery, to a large extent, must adopt pharmaceutical industry approaches to drug discovery through lead identification and optimization in typical hit to lead (H2L) and Lead Optimization (LO) campaigns. Increasingly integration of *in silico*, *in vitro* and *in vivo* drug absorption, distribution, metabolism and excretion (ADME) studies in the discovery and development of new chemical entities (NCE) has become a feature of medicinal chemistry programmes in the pharmaceutical industry and some academic and related institutions.[3]

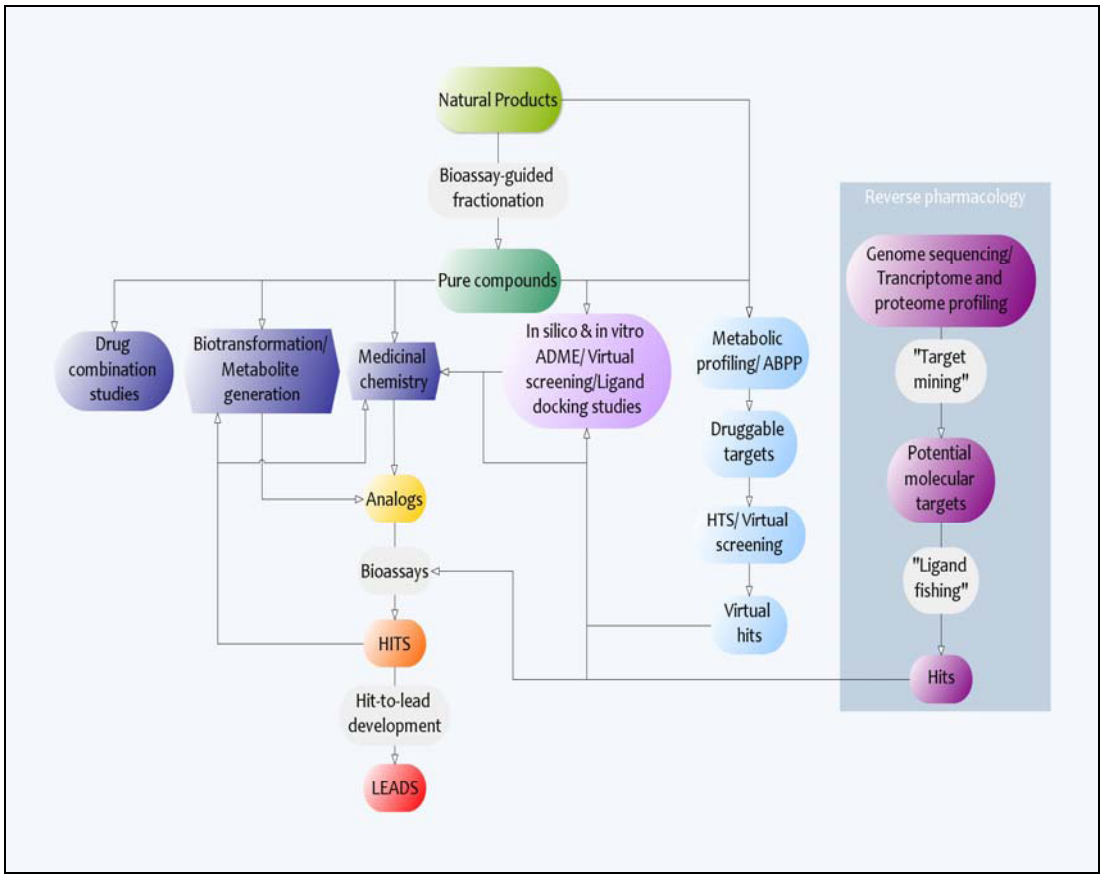

Figure 1: Development of leads from natural products.[2]

This lecture will highlight some technology platforms that have been set up in our laboratories at the University of Cape Town (UCT) to facilitate the integration of African natural products into modern drug discovery paradigms.

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[PL 9] From Past Traditions to a Herbal Pharmacopoeia – Africa's Green Gold

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Keywords: Medicinal plants, herbal medicine, validation, African Herbal Pharmacopoeia

Plants have formed the basis of sophisticated traditional medicines that have existed for thousands of years and continue to provide Mankind with remedies. According to the WHO, over 80% of the world's population still depend on traditional medicine for their primary health care (WHO 1999). The interest in Nature continues not only as a potential source of herbal medicines, which is finding increasing acceptance in the developed world, but also as chemotherapeutic agents. It is a fact that natural products and their derivatives represent more than 50% of all drugs in clinical use in the world (Farnsworth *et al*, 1985).

Whilst modern allopathic medicine usually aims to develop a patentable single compound or a 'magic bullet' to treat specific condition, traditional medicine often aims to restore balance by using chemically complex plants, or by mixing together several different plants in order to maximise a synergistic effect or to improve the likelihood with a relevant molecular target. In most societies and increasingly in western societies, allopathic and traditional systems of medicine occur side by side in a complementary way. The former treats serious acute conditions while the latter is used for chronic illnesses, to reduce symptoms and improve the quality of life in a cost-effective way.

The African continent is blessed with a unique biodiversity accounting for almost 25% of the global pool of genetic resources. Paradoxically, this continent is experiencing the highest rate of destruction. The conservation of plant genetic resources, the documentation and validation of the traditional knowledge are key issues that will need to be addressed (Neuwinger 2000). The industrial potential of these plants is to be demonstrated especially as medicinal plants have not only the potential of addressing the Millennium Development Goals (MdGs) but also provide to Mankind cheap and efficacious remedies.

African herbal medicine relies more on wild harvested plants than any continent on earth yet the sustainability of this indigenous resource is increasingly endangered with an average annual loss of 1% as opposed to 0.6% at the global level (Iwu, 1993). Fortunately the rate of this loss is reported to be slowing down. Loss of plants also means loss of accompanying traditional knowledge. The value of countless generations of observations of the application of certain plants on humans and animal disorders is impossible to value, especially in relation to present day global bioprospecting activities. The African Herbal Pharmacopoeia is one way of showcasing the important plants of Africa (Brendler *et al*, 2010).

This presentation will show case not only the potential of important African medicinal plants but also the work being carried out on the validation of traditional herbal recipes against non communicable diseases (diabetes for example) and infectious diseases in general.

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[PL 10] NMR Analysis of the Molecular Structure of Flexible Molecules in Solution

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Keywords: NMR, dynamics, ensemble, NAMFIS, residual dipolar coupling, pseudocontact shift

Introduction

Living organisms biosynthesize a broad variety of substances from macromolecules to small molecular natural products of an astonishing chemical diversity. The identification and structural studies of both type of compounds is generally performed by NMR spectroscopy, which analysis provides different challenges for the two groups: the structure elucidation of biopolymers is often hampered by extensive signal overlaps, whereas that of small natural products may become difficult originating from their dynamic nature. In this presentation the focus will be on small flexible compounds that are commonly present in solution as rapidly equilibrating mixtures of low-energy conformations and which cannot be accurately represented in form of a single structure as conventionally derived by experimentally-restrained structure calculations for biopolymers. (Nevins *et al*). The limitations of X-ray crystallography for reflecting highly dynamic solution structures are well-known, whereas computational efforts without experimental parameters can only provide predictions, but not description of the structure or dynamics of a molecule. Solution NMR is to date the method of choice for gaining an improved understanding of the behavior of flexible molecules, however, should be applied with care as for example the simplified derivation of an average structure from time averaged data, such as NOEs and scalar couplings, results in a non existing, erroneous structure Methods for the deconvolution of time-averaged NMR variables into structural families well-representing the solution ensemble and satisfactorily fulfilling the structural restraints are available, although still scarcely utilized.

In this presentation solution NMR methods for the proper structural description of dynamic small molecules will be discussed on the examples of natural products and their synthetic analogues.

Results and Discussion

The method NAMFIS-NMR Analysis of Molecular Flexibility in Solution (Cicero *et al*)-was applied for determination of the relative configuration of Angiotensin IV analogues (Andersson *et al*) and for the elucidation of the conformational properties of natural as well as C3-modified epothilones (Erdelyi *et al.*2008 and 2010) and of chroman-4-one tetrahydropyrimidines (Fridén-Saxin *et al*.). For the above examples the experimental data (*J* couplings and NOEs) were fit to a priori computed set of theoretical structures generated by Monte Carlo conformational search.

Determination of relative configuration was performed by identification of a conformationally well-defined, comparably rigid core of the studied 13- and 14-membered analogues followed by comparison of the quality of fit of the computed ensembles of all possible diastereomers to the experimental data. This procedure permitted the identification of the point of racemisation of three sets of synthetic Angiotensin IV (Ang IV) analogues and gave insight in the tremendous importance of chirality on the insulin-regulated aminopeptidase (IRAP) inhibitory activity (50-500 fold) of these substances (Figure 1.; Andersson *et al*).

Figure 1. Overlaid backbones of the low energy conformations of Ang IV analogues possessing IRAP inhibitory activity, as identified by NAMFIS analysis.

For conformational studies, the population distribution of the solution ensembles was evaluated by selection of feasible structures from a theoretically possible conformation pool and by estimation of their molar fractions. This procedure allowed the evaluation of the conformational directing role of the C3 substituents of the microtubule-stabilizing agent natural product epothilone A and provided a proof for its previously determined NMR-derived tubulin-bound conformation (Carlomagno *et al*) over the one proposed by electron crystallography (Nettles *et al*). These findings were further confirmed by investigation of the binding mode of epothilone-tubulin complexes based on transferred NOE experiments (Erdelyi *et al*, 2010). The enormous potency of the NAMFIS-based procedure is further shown by the elucidation of the conformational properties of novel synthetic chroman-4-one tetrahydropyrimidines that revealed their potential applicability as type VIII β-turn peptidomimetics (Fridén-Saxin *et al*).

A second methodology applying deconvolution of residual dipolar couplings (RDCs) and pseudocontact shifts (PCSs), as observed by NMR, was used to describe the dynamic properties of oligosaccharides. A large series of distance and orientation dependent RDCs and PCSs was collected for two different oligosaccahrides, lactose (Erdelyi *et al*, 2011) and *N,N'*-diacetylchitobiose (Yamamoto *et al*), by a paramagnetic tagging-based technology (Figure 2). The technology is shown to successfully distinguish dynamic linkages from rigid ones and estimate the probabilities of conformations present in solution, even for compounds for which the NOE- and scalar coupling-based methods often are inadequate.

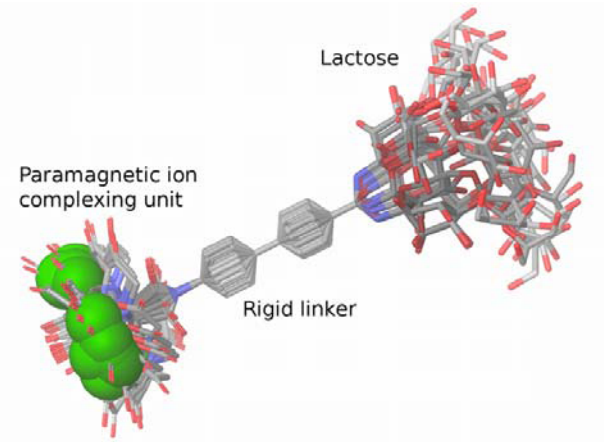

Figure 2. Overlaid conformations of lactose connected to a paramagnetic centre through a rigid biphenyl-EDTA-type paramagnetic tag that allows rotation only around a single axis and keeps the paramagnetic centre (green) at a well-defined distance from the saccharide to control paramagnetic relaxation-induced line broadening. Such utilization of the anisotropic magnetic susceptibility tensor of paramagnetic lanthanides offers long distance information on conformation and dynamics by induction of residual dipolar couplings and pseudocontact shifts.

Simultaneous fitting of the probabilities of computed conformations and the orientation of the magnetic susceptibility tensor of a series of lanthanide complexes of lactose shows that its glycosidic bond samples syn/syn, anti/syn and syn/anti ϕ/ψ–regions of the conformational space in water. The obtained results demonstrate that the applied paramagnetic tagging-based technique allows for the description of the motion of the glycosidic linkage in saccharides. It permits the collection of several complementary series of RDC and PCS data of the same ensemble by simple variation of the complexed paramagnetic ion. The increased number of available experimental parameters provides greatly improved reliability for the investigation of dynamic processes. In a related study, we demonstrated on the example of *N*,*N*-diacetylchitobiose (Yamamoto *et al*) that the applied paramagnetic tagging technique is capable of identifying rigid glycosidic linkages of sugars and thereby have proven that the approach is capable of differentiating between rigid and dynamic structures.

The high importance of the proper description and the thorough understanding of the dynamic properties of small molecules with respect to their biological activity is demonstrated and the exceptional applicability of solution NMR techniques for the ensemble analysis of flexible, complex structures, such as natural products, is shown.

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[PL 11] Bioassay of Natural Products for Cosmetics

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Cosmetic products are preparations intended to be placed in contact with the external parts of the human body with a view to clean them, perfume them, change their appearance and/or protect them or keep them in good conditions.

Anti-aging activity of 20-hydroxyecdysone (20-E). 20-E or beta-Ecdysone or Ecdysterone, is the most common member of the ecdysteroid family. It can be found in insects, in plants such as *Cyanotis arachnoidea (C.a.)*, and even in edible plants like spinach. It has many types of activities in mammals [Lafont et al, 2003] such as improvement of skin healing [Meybeck et al, 1990], and stimulation of skin keratinocyte differentiation [Detmar et al, 1994]. It is known that many proliferative cell types like lung or skin human diploid fibroblasts (HDFs), exposed to subcytotoxic stress (UV, H_2O_2, etc.), undergo stress-induced premature senescence or SIPS which is closely related to replicative senescence. SIPS can be defined as the long term effect of subcytotoxic stress on proliferative cell types, including irreversible growth arrest of a majority of the cell population. The proportion of HDFs positive for senescence-associated ß-galactosidase (SA ß-gal) activity increases in SIPS [Toussaint et al, 2002].

A study was undertaken in order to determine the potential anti-photoaging effects of 20-E extracted from *C.a.* in a model of dermal aging *in vitro.*

The results show that 20-Hydroxyecdysone provides human BJ foreskin fibroblasts with some kind of protection against premature cellular senescence induced by repeated UV insults (UV-SIPS) as showed by the dramatic decrease by 20-E of the proportion of cells expressing SA β-gal. Moreover it seems that this "protection" is due to a transient stimulation of p53, often called the guardian of the genome, which probably prepares cells to face UVB injuries and induces an efficient repair process of the damages caused by UVB radiations. These findings reinforce the notion that UVB-induced premature senescence of HDFs can be used to screen potential anti-photoageing compounds, and allow the development of 20-E containing cosmetic formulations which will better protect the cells against chronic sunlight damage leading to premature ageing of exposed skin [Meybeck et al, 2006], characterized in particular by senescent fibroblasts expressing markers associated with the processes of inflammation and destruction of the dermal matrix [Funk et al, 2000].

Further studies have shown that the mechanism of action of 20E might pass through its binding to the alternative active site of the Vitamin D receptor responsible for the rapid effects.

Anti-melanogenesis activity of 5-hydroxy-tryptophan (5-HTP). There is a great demand worldwide but particularly in Asia for products aimed at controlling skin pigmentation. This is why *Griffonia simplicifolia* (*G.s.*) seed extracts were screened for an eventual inhibitory activity on melanogenesis in skin cells. (*G.s.)* is a tree growing in West African countries like Ivory Coast and Ghana, the seeds of which are the main industrial source of 5-HTP widely used as a food supplement to help reduce depression, migraines, insomnia, appetite…[Lemaire et al, 2002] .

An evaluation of the activity of *G.s.* extracts and 5-HTP on melanin pigment synthesis was carried out on normal human epidermal melanocytes (NHEM) and murine B16 melanoma cells. In order to have a better appreciation of 5-HTP activity, a test on B16 melanoma cells previously stimulated by the α-MSH analog NP-MSH, has been carried out in comparison with Kojic acid and Arbutin. This protocol disconnects melanogenesis from the basic metabolism of melanocytes and allows to measure significant inhibitions at lower concentrations of active compounds.

In this assay, 5-HTP induced 31 % melanogenesis inhibition already at 8 µg/ml, 70 % at 40 µg/ml, and 90 % or nearly complete inhibition at 100 µg/ml. This performance was better than that of Kojic acid (only 70 % inhibition at 200 µg/ml), and similar to that of Arbutin (97 % inhibition at 200 µg/ml).

This new protocol , together with more classical tests , has allowed to determine that *G.s.* seed extracts and 5-HTP inhibit melanin synthesis , thus confirming the results of Kim K.T. et al [2006] who found also that 5-HTP stimulates the ERK pathway therefore downregulating MITF and tyrosinase biosynthesis.

The results obtained show that *G.s.* seed extracts and their active molecule 5-HTP might be efficient "whitening" or "brightening" ingredients in cosmetic formulations [Meybeck 2006].

Protection by *Notoginseng* root saponins (NRS) against UV-induced immuno suppression. The root of *Panax Notoginseng* contains up to 10% saponins (which are thought to be the major active components) , flavonoids , polysaccharides , polyacetylenes , aminoacids …The *Panax Notoginseng* Root Saponin Fraction (NRS) contains five major compounds (content > 4%) : ginsenosides Rg1 , Rb1 , Rd and Re , and notoginsenoside R1 .

In a recent study [Sene et al, 2007] , it was found that NRS have the effect in human skin fibroblasts , of up-modulating the m-RNA coding for Heme Oxygenase-1 (HO-1) , a very important protecting enzyme since it leads to the formation of biliverdin which is a very powerful natural antioxidant , and of carbon monoxide which has been shown to protect Langerhans cells from photo-immunosuppression (Langerhans Cells located in the epidermis , are responsible for the immune protection of skin against aggressions . But when skin is exposed to UV light, they disappear from the epidermis, and as a result the skin becomes more vulnerable) .

In a first experiment on cultivated normal human dermal fibroblasts (pool of NHDF at 8th passage), it was found through the use of a c-DNA array , that the expression of the m-RNA of HO-1 had been significantly enhanced (189%) by a 24 h treatment with Notoginseng Root Saponins at 0.2 mg/ml , although it was not significantly affected by Ginseng Root Saponins. Then , in a second experiment on another culture (NHDF at 8th passage) , a quantitative determination was achieved , after PCR amplification , and it was found that the expression of HO-1 m-RNA in the cells after 4h of contact with NRS at 0.2 mg/ml was increased to 313 % of the base level (277% at 8h ; 115% at 24h) .

In a third experiment carried out on human skin ex-vivo, it has been put in evidence that NRS are able to protect the skin against Langerhans cells depletion by UV exposure . The observed protection was up to 63 % (at 1mg/ml of NRS), and almost as much as that of a UV filtering commercial formulation of SPF 20 .

Finally, it was shown that NRS could provide a 40% protection (at 0.3mg/ml) against the appearance of SA-β-Gal positive senescent fibroblasts induced by an H_2O_2 stress (senescent cells are unable to undergo mitosis and are characterized by the expression of SA-β-Galactosidase) . This work has therefore established that *Panax Notoginseng* root saponins can protect skin cells against certain oxidative stresses and that this protection most probably results from stimulating the expression of the enzyme HO-1. And it is possible that HO-1 induction might as well be responsible for some known effects of Notoginseng on wound healing, in the treatment of cardio-vascular diseases, on memory improvement …

Anti-Wrinkle Effect of Extracts of Boswellia serrata

Expression lines are produced by the mechanical stress exerted on the skin by facial muscles. So relaxing skin can help prevent there formation.Three extracts of Boswellia serrata were tested on a nerve-muscle coculture model which makes it possible to recreate a motor arc by innervations of human striated muscle cells with explants of spinal cord and of spinal ganglia from rat embryos [Meybeck et al, 2004].

This test predicts an anti-wrinkle effect, as was demonstrated in the case of diazepam, which inhibited muscle fibre contractions in this model, and showed an anti-wrinkle activity in vivo. Human muscle cells derived from human muscle samples from a healthy donor are seeded in 15 mm-diameter wells (24-well culture dishes). After culturing for 10 days, these cells form a monolayer and fuse. At this stage, spinal cord explants from 13-day-old rat embryos, containing the spinal ganglion, are deposited onto the culture. The first muscle fibre contractions are observed after coculturing for one week. After coculturing for 3 weeks, the muscle fibres are striated and possess mature differentiated neuromuscular junctions.

A muscle fibre having regular contractions (at least 60 contractions per minute) is then selected in three different culture wells and the number of contractions is counted over 30 seconds using image analysis software. The extracts tested, diluted in ethanol, are then incubated for 60 seconds

in these wells, at the concentrations C1 and C2 of 0.005% and 0.01%. At the end of the incubation, the number of contractions is again counted over 30 seconds.

The percentage of inhibited contractions is then determined. The three Boswellia extracts tested induced inhibitions. The most active extract induced 72% of contraction inhibition at the concentration of 0.005%. The four pentacyclic triterpene acids found in extracts of Boswellia serrata, namely beta-boswellic acid, 3-*O*-acetylboswellic acid, 11-ketoboswellic acid and 3-*O*-acetyl-11-ketoboswellic acid, were tested in a model of calcium flux in order to evaluate their capacity for inhibiting calcium channels and therefore their ability to relax muscle fibers. The relaxing effect of 3-*O*-acetyl-11-ketoboswellic acid was found significantly greater than that obtained for the other three acids tested. This effect was confirmed with the muscle-nerve coculture test which showed a contraction-inhibiting effect of 74.7% at 5 microM and of 87% at 10 microM for this compound. Anti-wrinkle cosmetic products can therefore be formulated with Boswellia extracts.

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[PL 12] Multiple anti-infective properties of selected Combretum species from Zimbabwe

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Key words: Antifungal, anti-inflammatory, antibacterial, Drug efflux, *Mycobacterium aurum*, *Candida albicans*, Rhodamine 6G, antimycobacterial, metabolism

Introduction

Medicinal plants are therapeutic resources used by traditional population specifically for health care and which may serve as precursors for the synthesis of useful drugs (Okigbo *et al*., 2009). Plants have served as a source of new pharmaceutical products and inexpensive starting materials for the synthesis of many known drugs. Natural products and their derivatives represent more than 50% of drugs in clinical use in the world (Cowan, 1999). A number of interesting outcomes have been found with the use of a mixture of natural products to treat diseases, most notably the synergistic effects and polypharmacological application of plant extracts (Ncube *et al*., 2008). The search for antimicrobial agents has mainly been concentrated on lower plants, fungi and bacteria as sources. Much less research has been conducted on antimicrobials from higher plants. Since the advent of antibiotics, in the 1950s, the use of plant derivatives as antimicrobials has been virtually nonexistent. The interest in using plant extracts for treatment of microbial infections has increased in the late 1990s, as conventional antibiotics become ineffective (Cowan, 1999). Of interest in this study is the use of *Combretum* species in the treatment of diseases. At least twenty four species of *Combretum* are well known in African traditional medicine and are used for the treatment of a variety of ailments and diseases, ranging from scorpion and snake bites, mental problems, heart and worm remedies to fever and microbial infections. The fruits and seeds are, although, in general considered poisonous by traditional healers in various African countries and have been reported to give toxic effects on human (Fyhrquist, 2007). Many species of Combretum have been found to possess powerful antibacterial and antifungal effects. Among antimicrobial active compounds isolated from *Combretum* species are combretastatins (bibenzyle compounds), acidic tetracyclic and pentacyclic triterpenes/triterpenoids, ellagitannins, phenanthrenes, flavonoids, saponins and cycloartane glycosides (Eloff *et al*., 2005). The leaves, roots and stem bark of *C. zeyheri* are used medicinally. The leaves are used frequently and have a variety of uses in African traditional medicine. The smoke of burnt leaves is inhaled for treatment of coughs. The leaves of *C. imberbe* are used in the treatment of diarrhea and cough, symptoms that can be related to bacterial and fungal infection. The wide spread use of Combretum species in treatment of many ailments makes them potential sources of anti-infective target. Phytoconstituents from these plant species could prove to be more effective than the current available drugs. The study was undertaken to test the antimycobacterial activity of the selected Combretum species plant species against *Candida albicans, Mycobacterium aurum, E. coli, B .subtilis* and human recombinant cyclooxygenase.

Materials and Methods

All the chemicals used were obtained were obtained from Sigma Aldrich (Darmstadt, Germany). Other chemicals used were of the highest grade available and were obtained from different sources. *Mycobacterium aurum A+* were obtained were obtained from Prof. Smith of the Department of Pharmacology, University of Cape Town and and *Mycobacterium smegmatis*, was obtained Prof. Steenkamp, of the Department of Clinical Laboratory studies, University of Cape Town. *Candida albicans* strain ATCC 10231, *B. subtilis and E.coli* were obtained from Department of Biological Sciences, University of Botswana. The fungi were maintained on nutrient agar slants. The isolate was sub cultured regularly and stored at 4ºC as well as at -30ºC by making their suspension in 30% glycerol. The plants used in this work : *Combretum imberbe, Combretum molle, Combretum apiculatum, Combretum elaegnoides*, and *Combretum krussaii*, were collected from the National Botanical Gardens, Harare Province, Harare, Zimbabwe. *Combretum platypetalum* and *Combretum zeyheri* were obtained from Norton in Mashonaland West Provinces of Zimbabwe. The plants were authenticated by Mr Christopher Chapano, a taxonomist at the National Botanical Gardens. The voucher specimens of the plants investigated were kept in the Department of Biochemistry, University of Zimbabwe.

Plant leave material was dried in an oven at 50 ^{o}C and ground in a two speed blender (Cole Parmer Instrument Company, Connecticut, USA) to a fine powder. A volume of 10 ml of absolute ethanol (or methanol) was added to 2 g of powder and shaken for 5 minutes on a vortex and left to sit for 24 hours. A syringe was prepared by inserting a piece of fine sieve. The plant suspension was then transferred into syringe and filtered into a small glass vial. The sterile suspension was filtered again using 0.45 µM Millipore® sterile filter (Sigma-Aldrich, Taufkirchen, Germany) into a labeled small glass vial. Ethanol was left to evaporate overnight in fume hood with air stream to quantify extraction. A constant dry weight of each extract was obtained and the residues were stored at 4^{o}C until when required. To determine the activity of the plant extracts as antimicrobial, initial screening was carried out using the agar disc diffusion method. Once identified as being potent, growth inhibition parameters were determined using the broth microdilution method in a 96 wells microtitre plates. The S9 metabolites from *C. zeyheri* extract were prepared using fractions from rat livers of male Sprague-Dawley rats in which drug metabolizing enzyme induction had been done *in vivo* using phenobarbitone. The inhibitory effects of Combretum species on cycloxygenase I and 2 were also investigated using the Cox assay kit (Cayman, Estonia).

2. Results and Discussion

Table 3.1 shows the effects of selected Combretum species on the growth of *Candida albicans*. *Combretum imberbe* had the highest zone of inhibition followed by *Combretum zeyheri*. All the *Combretum* species used had some antifungal activity on *C. albicans*.

Table 3.1:

Plant name	Zone of inhibition (mm)	MIC (*mg/ml*)	MFC (*mg/ml*)
Combretum imberbe	20	0.31	0.52
Combretum elaeagnoides	16	0.31	>10
Combretum apiculatum	13	0.31	0.31
Combretum zeyheri	19	0.31	>10
Combretum molle	10		
Combretum kraussii	9		
Miconazole (+ve control)	20	1.25	1.25
Dimethylsufoxide (DMSO)	6		
Ethanol(-ve control)	6		

Antifungal activity of plant extracts as determined by the agar disc diffusion method (diameter in mm). These were the results obtained following 24 hours incubation at 37ºC on nutrient agar.

It was also observed that 3 Combretum species, *C. imberbe, C. apiculatum and C. zeyheri* had efflux inhibitory activity on *C. albicans* when ciprofloxacin was used as the probe drug (Figure 3.1). This means that the antimicrobial action of these plants could also be due to efflux pump inhibition.

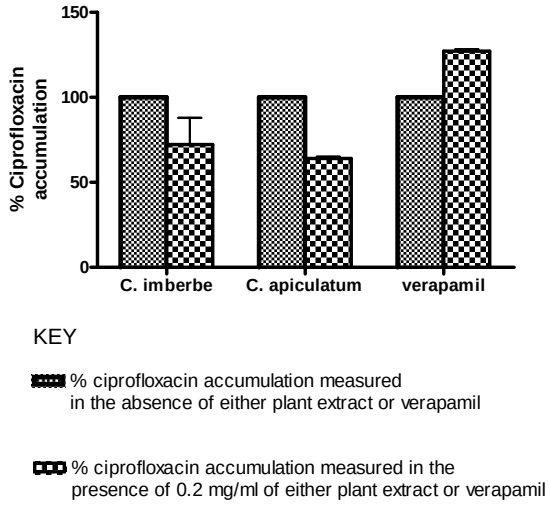

Figure 3.1: Accumulation of Ciprofloxacin in *Candida albicans* fungal cells at the final concentration of 0.2 mg/ml *Combretum apiculatum* and *Combretum imberbe* plant extracts.

The effects of the Combretum species were also investigated in Mycobacterial species and it was found that only *C. imberbe* had inhibitory activity on the bacteria (Table 1.3). It was also observed that *C. imberbe* and *C. hereroense* had drug efflux inhibitory activity using ciprofloxacin as the probe drug.

Table 3.2: Antimycobacterial activity of plant extracts as determined by the agar disc diffusion method (diameter in mm).

	Zone of inhibition (mm)		*MIC*	*MBC*
Plant name	*M. smegmatis*	*M. aurum*	µg/disc	
C. elaeagnoides	*na*	*na*		
C. hereroense	*na*	*na*		
C. imberbe	*9.5*	*na*	*125*	*>1000*
C. zeyheri	*na*	*na*		
Rifampicin 50 µg/ml	*33.0*	*21.75*	*0.2*	*50*
DMSO	*na*	*na*		

na - no activity.

Metabolic studies using the liver S9 fractions from male Sprague-Dawley rats showed that the metabolites rather than the parent extracts were responsible for the antifungal effects observed in *C. albicans* (Figure 3.2) and that administration of the aqueous extract to the animals increased the activity of glutathione transferases (Figure 3.3)

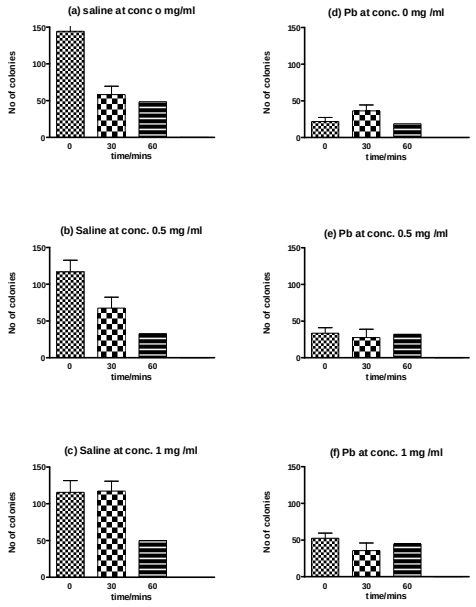

Figure 3.2

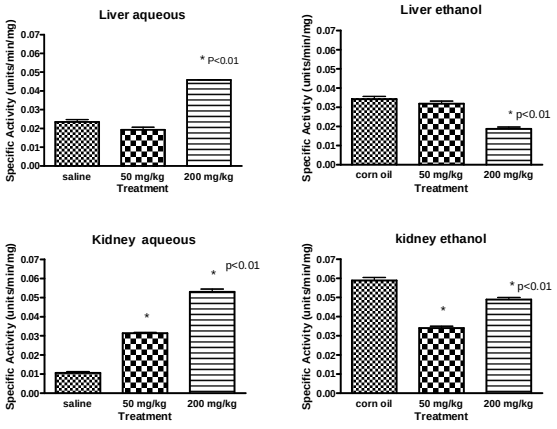

Figure 3.3

It was also noted that *C. Zeyheri* had antimicrobial activity against *B. subtilis* and *E. coli* and also showed drug efflux inhibition using Rhodamine 6G as the probe substrate (Figure 3.4).

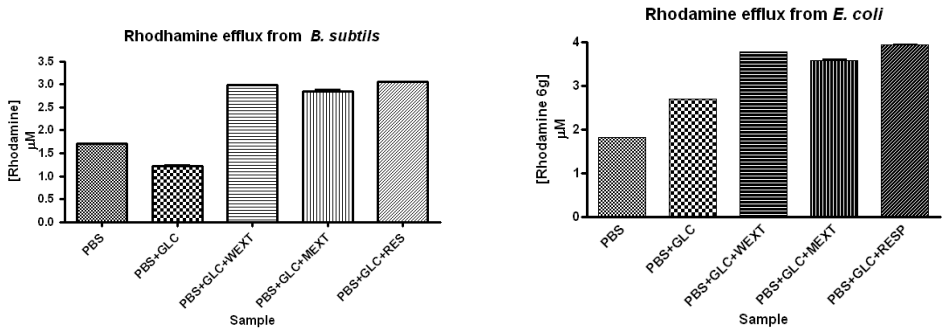

Figure 3.4 Effects of *C.zeyheri* exyracts on Rhodamine 6G effelux in bacteria.

Table 3.3 shows the activties of Combretum species on Cox activity. *C.platypetalum* showed potent inhibition of COX2 and had an IC_{50} of 571 µg/ml compared to 414 for indomethacin**.**

Table 3.3: Summary of results obtained from COX Activity assay showing the prostaglandin concentrations for the various test extracts

Enzyme	Extract (2500 µg/ml)	[PG] mg/ml	% Inhibition	Selectivity (concentration)	IC_{50} (µg/ml)
COX-1	*Combretum platypetalum*	6.1240	41.5	0.5	
	Combretum zeyheri	0.362	97.4	2.3	
	Combretum molle	0.733	93.3	1.4	
	Combretum molle (1250 µg/ml)	2.723	76.4	3.5	
	Indomethacin	0.396		1.4	
COX-2	*Combretum platypetalum*	0.733	84.9		571
	Combretum zeyheri	2.76	42.2		
	Combretum molle	0.509	67.6		
	Combretum molle (1250 µg/ml)	1.556	22.0		
	Indomethacin	0.280			414

The aqueous leaf extract and the methanolic leaf extract of *C. zeyheri* showed antimicrobial activity against *E. coli* and *B subtilis*. The plant extracts had an inhibition effect on drug efflux pumps with *E. coli* being inhibited the most. The water extract had a higher antibacterial and efflux pump inhibition effect as compared to the methanolic extract. The conclusion that can be drawn from this study is that S9 metabolites of an aqueous extract of *Combretum zeyheri* inhibit growth of *Candida albicans*.

Extracts from all the six plants investigated possessed inhibitory activity against *Candida albicans*. The *Combretum imberbe* plant extract showed almost comparable antifungal activity with miconazole, which was used as a positive control and reference antifungal. *Combretum imberbe* and *Combretum apiculatum* possessed fungicidal properties while *Combretum elaeagnoides* did not show such activities at the highest concentration tested. *Combretum imberbe* and *Combretum apiculatum* ethanolic plant extracts were found not to have an effect on the *Candida albicans* efflux pumps. *Combretum platypetalum* is likely source of compounds with COX 2 inhibitory activity and thus may serve as a source of anti-inflammatory novel compounds. This study has identified Combretum species plants with

potential antifungal, antibacterial, antimycobacterial and anti-inflammatory activities that could be used as sources for the isolation of active compounds that may serve as lead compounds in development of phytomedicines. Further studies are being carried out to determine the cytoxicity of these compounds using cancer cell lines.

Acknowledgments

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[PL 13] The Center for World Health & Medicine at Saint Louis University: A New Translational Research Model to Develop Novel Therapies for Neglected Diseases and Other Unmet Medical Needs

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Key words: CWHM, ICCL, *echistatin*, RGDX, integrin

The Center for World Health & Medicine (CWHM) (www.cwhm.org) at Saint Louis University is a not-for-profit institution dedicated to the discovery and development of safe, effective and affordable therapies for neglected diseases of poverty in the developing world (especially infants and children), as well as rare and orphan diseases and other unmet medical needs. It evaluates promising drug candidates to find therapeutic solutions to debilitating and life threatening global health problems that pharmaceutical companies typically don't explore. Located in the Doisy Research Center at the Saint Louis University School of Medicine, the CWHM consists of a full team of former Pfizer pharmaceutical research and development scientists with specialized skills and over 200 years of combined experience in successful drug development. The CWHM team possesses the entire spectrum of the critical skill sets needed to translate basic science discoveries into clinically useful drug candidates. These skill sets range from medicinal chemistry and structure based drug design, in vitro assay development, in vivo pharmacology, development of animal models of human disease, PK/PD, and biomarker development and analysis. CWHM scientists are redirecting their specialized talents toward these global unmet medical needs. CWHM has established an efficient operating model that marries basic science with clinical opportunity and by forming international collaborations and partnerships with disease experts and institutions. A brief overview of the CWHM model, some of our disease targets, and how we are applying our expertise in advancing promising candidates to the clinic will be presented. It will also highlight our proposed partnerships in Africa, which includes a significant partnership opportunity as a member of the International Clinical Compound Library (ICCL) Consortium.

The International Clinical Compound Library (ICCL), which is being coordinated by the CWHM, has the goal of accumulating compounds that have been discontinued (shelved) at clinical stages by pharmaceutical companies over the years to a central repository. There are more than 11,000 of such compounds that never made it to market. This pool is comprised of late-stage pre-clinical through phase III clinical trial compounds. These compounds were discontinued for lack of adequate efficacy for the original indication, lack of perceived commercial potential, company reorganization, companies exiting the therapeutic area, etc. Information about such discontinued compounds is often hard to find due to lack of public disclosures. And yet, these are high quality compounds with drug-like characteristics that would have tremendous value to the international research community. They represent a wide variety of structural diversity and mechanisms of

action. A lead generated from such a library would already be highly advanced, have passed safety assessments, and have either been in man or deemed appropriate for clinical trials. Such discontinued, or abandoned clinical compounds represent untapped potential.

The ICCL would be made available for re-purposing or screening for new indications to academic and institutional investigators worldwide, researching a wide variety of diseases, including neglected diseases of the developing world, rare diseases and many additional diseases in need of therapies or more effective therapies. Integration of researchers into collaborative repositioning efforts would substantially increase the knowledge base and the pool of methodologies available for proof-of-concept studies. These matches will undoubtedly increase the number of approved drugs for new indications and considerable public benefit. Obtaining, managing and making available such a library to international research investigators has the potential to accelerate the advancement of new therapies for many unmet medical needs on a global scale.

Adding global approved (marketed) drugs, active metabolites of approved drugs, veterinary drugs, and other appropriate high quality drug substances and diverse bioactive natural products to the ICCL would collectively constitute this world-class "one stop shop" screening and re-purposing library for the international research community.

High quality bioactive natural products isolated from Africa's rich biodiversity would be a valuable component to the ICCL. Although not technically "clinical" compounds, the fact that a large majority of drugs used in clinical practice today are natural products or have their origins from a natural product lead, would allow for the screening of these novel compounds against a variety of diseases on a global basis. The potential benefits of this will be discussed as well as a brief overview of the ICCL project, and Africa's proposed partnership in it.

Pharmaceutical Companies abandoned natural products as a screening resource a number of years ago in favor of high throughput combinatorial chemistry, fragment based screening, *in silico* computational tools, etc. These were thought to be a more cost effective and imparted an attractive "design tailored" approach to new drug discovery as compared to the perceived costly and empirical approaches of natural product screening. In many regards this was ill-conceived, given the prior wealth of drugs that resulted from natural products. The view that combinatorial chemistry would provide millions of compounds with diverse structures to quickly and more cheaply (as opposed to natural products) provide leads for a new generation of drugs has not turned out to be the panacea envisioned. A shift back to a more balanced approach to new molecule drug discovery should be encouraged. Natural products can still provide a wealth of structural diversity with which to provide novel substrates, ripe for optimization into novel drug candidates for a wide range of disease targets. The fact that there are still numerous untapped and untested natural products in existence (especially marine natural products) opens the door to those that want to bring such a balance back to new drug scaffold identification. With current analytical and separations technologies and advanced high throughput biological assays, the past perceived

barriers to natural product screening should now be minimized. Controversy over ownership rights related to natural products identified in a native country is a separate topic, but one that should not preclude development of innovative new therapies for diseases in need. It can also present an opportunity for these countries to benefit from their natural riches.

Several prime examples of extremely profitable and efficacious drugs that directly resulted from natural product leads (other than antibiotics and cancer agents) will be highlighted. A detailed case example of clinical compounds that were developed in our labs from a natural product lead will then be presented, as described below:

The venom from the viper snake *Echis carinatus* produces dangerous systemic symptoms, with hemorrhage and coagulation defects being the most striking. These observations ultimately led to the isolation of the venom protein *echistatin*, and its subsequent clinical use as an anticoagulant. Isolated proteins from similar snake venoms, also with anti-coagulant activity, revealed a consensus RGD-X peptide sequence common to all of these venom proteins. It was further determined that this RGD-X sequence interacts with the integrin $a_{IIb}b3$ (IIb/IIIa), a cell surface protein up-regulated on activated platelets, and thus interfering with platelet aggregation and clotting. Additional work demonstrated that the RGD-X peptide sequence itself was an antagonists of the IIb/IIIa receptor.

Medicinal chemistry and *in vitro* and *in vivo* pharmacology efforts to transform this natural product lead into long acting, orally available, small molecule anti-platelet clinical compounds will be discussed. Additionally, and capitalizing on the work leading to these clinical anti-coagulant compounds, detailed drug development research leading to the discovery of potent, orally available clinical compounds as antagonists of the avb3 integrin receptor in my lab will also be shown. This will include the strategies employed that led to these avb3 antagonists which were designed to be selective against IIb/IIIa. These avb3 antagonists have potential utility in several therapeutic areas, such as oncology, osteoporosis, virology and angiogenesis.

[PL 14] Application of UV-VIS Spectroscopy to Evaluate Quality of Medicinal and Edible Oils

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Keywords: *Nigella sativa, Brassica carinata, Sesamum indicum*

Introduction

We report here simplified methods for the isolation and quantitative determination of thymoquinone (TQ), the main bioactive substance of the medicinal oil obtained from *Nigella sativa*, commonly known as black cumin or black seed. We also report a practical method of determining the extent of adulteration of the edible oil of *Sesame indica* by the cheaper and less desirable edible oil *Brassica carinata*.

In folk medicine of many countries black cumin seeds and its expressed oil are used as medicines. Biological activities of black cumin corroborated by scientific studies include: analgesic and ant-inflammatory (Hajhashemi*, et al*., 2004), antifungal (Khan, *et al*., 2003), antiasthmatic (Boskabady, *et al*., 2010), antiallergic (Kalus, *et al*., 2003) etc.

Sesame (*Sesamum indicum L*.) is an ancient oilseed crop comprising of 50–60% oil (Arslan*, et al*., 2007). It is thought to have originated in Africa, although today India and China are the leading producers of sesame. In Ethiopia, three varieties are known: Humera, Gonder and Wellega. The oil extracted from sesame seeds is used mainly for cooking and in the production of margarine. Despite sesame oil's high proportion of polyunsaturated fatty acids, it is least prone, among cooking oils to turn rancid. This is believed to be due to the presence of endogenous antioxidants such as sesamol, sesamolin, sesamin etc.

Brassica carinata, although now widely cultivated in different parts of the world, its origin is from the Ethiopian highlands, where its cultivation goes back to 4000 years B.C. In Amharic its leaves and seeds are known as Yabesha Gomen and Yegomen Zer respectively. The leaves are eaten cooked as vegetable whereas the seeds are used to oil the pottery baking pan (Mitad) of Injera.

Materials and Methods

NSPO (*Nigella sativa* pressed oil) was obtained by pressing black seeds or by purchasing oil from local commercial producers. Thymoquinone was isolated from powdered seeds of black cumin (10 g) by first soaking in 80% aq. MeOH (70 ml), on a shaker for 4 h and filtering. Water was added to the filtrate to make the solution 50% aq. MeOH. This was then extracted twice using 50 ml $CHCl_3$. The lower organic layer was separated, dried with anhyd Na_2SO_4, and concd to give dark brown gummy extract, that was subjected to CC on silica gel. Elution was carried out using hexane/CH_2Cl_2

(7:3), monitoring the separation of TQ by TLC (1:1, hexane/ CH_2Cl_2), which yielded pure TQ, confirmed by its ^1H, C-13, DEPT-135 NMR and UV spectra. TQ reference solutions were prepared (10 mg/10 ml).

Relative comparison of TQ levels in different NSPO: Each sample of NSPO (10 mg) was dissolved in Solvent 1 [10 ml hexane: ethanol (1:9)]. For UV-Vis measuremnet 0.1 ml aliquotes from each sample was taken and diluted to 10 ml to give 0.01 mg/ml.

Monitoring transformation of TQ in sunlight by UV-Vis: NSPO (200 mg) was placed in sunlight and about 10 mg was withdrawn after every 15 min, which was then dissolved in Solvent 1 and UV-Vis measured.

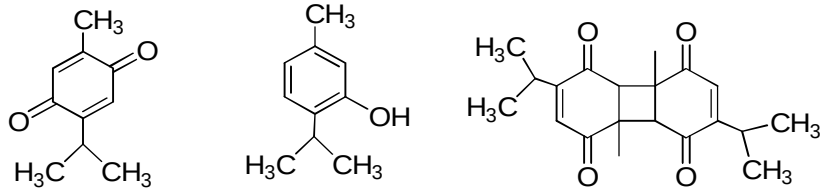
Qualitative detection *B. carinata* oil: 5 mg and 20 mg of pure sesame oil were separately dissolved in 1 ml of Solvent 1 and their UV-Vis measured. Same procdure was repeated for *B. carinata* oil.

Quantitative determination of *B. carinata* oil: Stock solutions of sesame (A) and *B. carinata* (B) oils were prepared by dissolving each time 100 mg of oil in 20 ml of Solvent 1. The volume ratio of A:B in each 5 mg was as follows: 5.0:0, 4.5:0.5, 4:1, 3.5:1.5, 3:2, 2.5:2.5, 2:3, 1.5:3.5, 1:4, 0.5:4.5, and 0:5. In each case absorbance was measured at 287 nm.

Instruments: ^1H and ^{13}C NMR spectral measurements were done on Bruker ACQ 400 AVANCE Spectrometer operating at 400 MHZ; UV-Vis on T 60 U spectrophotometer (PG instruments, UK) equipped with deuterium and tungsten lamps. The running parameters are controlled by UV-win software.

Results and Discussion

It is well known that the substance which is most responsible for the diverse biological activity of black cumin seeds and its pressed oil is thymoquinone (TQ).



Thymoquinone (TQ) Thymol Dithymoquinone (DTQ)

The potential of using UV-Vis measurements for the detection and relative comparison of TQ levels in different oils is evident. In line with this, the UV-Vis spectra of different concentrations of reference TQ (0.002, 0.004, 0.006 & 0.008 mg/ml), generated in ethanol (λ_{max}: 253 nm), are shown in Fig. 1. A similar linear curve was obtained when the spectra were generated using 0.1, 0.2, 0.3 and 0.4 mg/ml of freshly prepared TQ dissolved in hexane/EtOH (1:9)/Solvent 1 (Fig. 2). Moreover,

the UV-Vis spectrum of NSPO after exposure to direct sunlight for 0 (C1), 30 (C2) and 45 (C3) minutes was obtained (Fig. 3). The result indicated that there is a gradual decline of the starting TQ (C1) and appearance of D1 and D2 peaks most likely due to its conversion products DTQ and thymol.

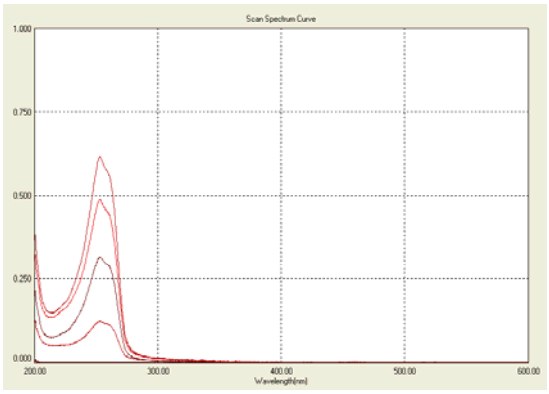


Fig. 1: UV-Vis spectrum of different concentrations of TQ samples

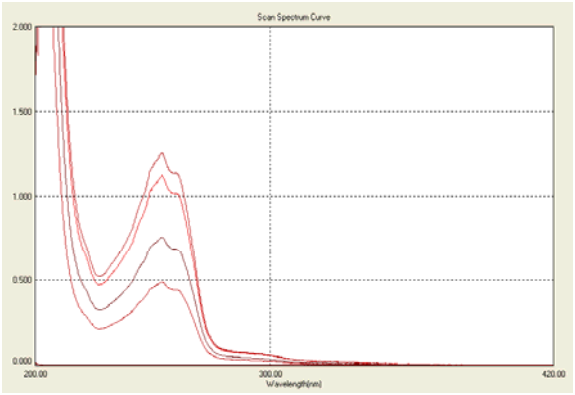


Fig. 2: UV-Vis spectrum of different NSPO

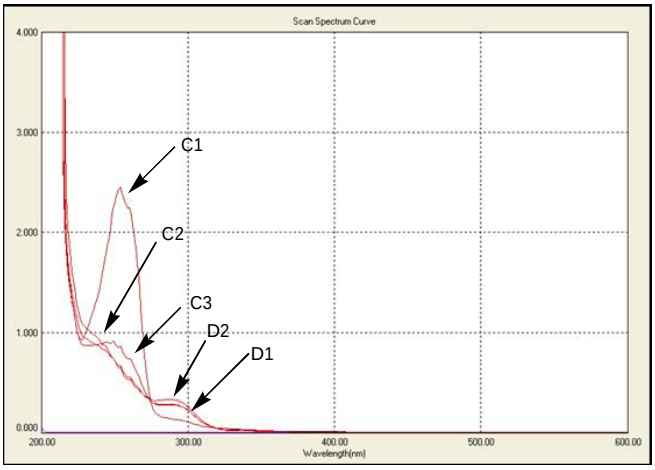


Fig. 3: UV-Vis spectrum of TQ solutions after exposure to sunlight for 0 (**C1**), 30 (**C2**) and 45 (**C3**) minutes. Peaks **D1** and **D2** may be due to formation of DTQ and thymol.

Sesame oil is widely believed to be adulterated by *Brassica carinata* oil. We attempt here to offer a simple and quick method to detect this adulteration. The UV-Vis spectra of the two oil samples were obtained at 5 mg/ml (Fig. 4) and 20 mg/ml (Fig. 5) concentrations of the two pure oil samples. As shown in Figs. 4 and 5 their UV spectra are clearly different enough to enable one to distinguish the two oils. The spectrum of *B. carinata* (A) revealed three bands in the region between 400 - 500 nm, insignificant in the spectrum of sesame oil (Fig. 5). On the other hand sesame oil displayed a typical band (B) with max absorption at 287 nm (Fig. 4). Therefore, these bands were used to

qualitatively distinguish between the two oil samples since addition of small amount of *B. carinata* to sesame oil changed the form of the spectrum of sesame oil.

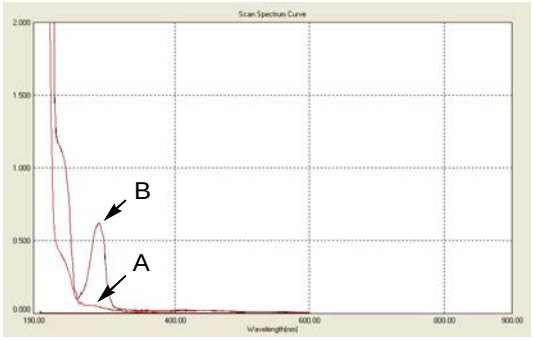


Fig. 4: Overlayed UV-Vis spectrum of oils of *B. carinata* (A) and sesame (B), each 5 mg/ml

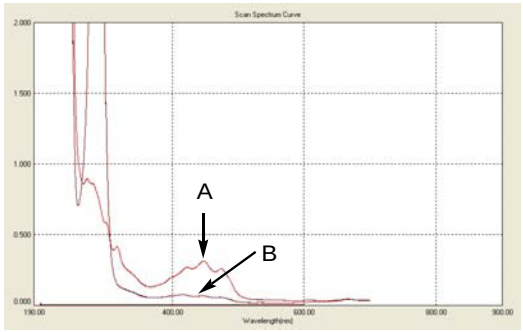


Fig. 5: Overlayed UV-Vis spectrum of oils of *B. carinata* (A) and sesame (B), each 20 mg/ml

In order to estimate sesame oil adulteration with *B. carinata*, different samples were prepared by mixing reference sesame oil with different volume ratios (10, 20, 30, 40, 50, 60, 80, and 90 %) of *B. carinata* oil. The absorbance for all samples including the pure and the blended oils were measured in triplicates. As observed from the spectrum, when the amount of *B. carinata* oil added to sesame oil increased, the absorbance of the blend at 287 nm decreased linearly while on the other hand the absorbance in the region 400 - 500 nm increased (Fig. 6).

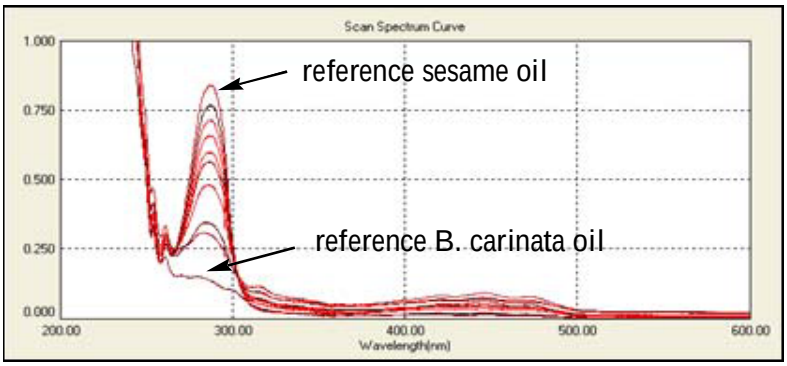


Fig. 6: UV-Vis spectrum of oils of *B. carinata* and sesame in different concentrations

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[PL 15] Towards Gaining Recognition as an African Centre of Excellence in Applied Nanomedicine Research and Training for Poverty Related Diseases – Focus on the DST/CSIR Nanomedicine Platform

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Keywords: Nanomedicine, nanoparticles, Tuberculosis, HIV/Aids, malaria, drug delivery, bioavailability, centre of excellence

Introduction

Sub-Saharan Africa bears the brunt of Poverty Related Diseases (PRDs) such as Tuberculosis and malaria. Current therapies against PRDs are inadequate and warrant a quantum leap in drug development approaches. Nanomedicine is a rapidly advancing area of biomedical research with great potential to radically improve health, by enhancing shortfalls such as poor drug bioavailability, safety, efficacy, stability, and resistance, of new and existing therapeutic agents, used against diseases of poverty like TB and malaria.

Technical Progress

The DST/CSIR nanomedicine platform has made significant advances in TB drug delivery, and embarked on nanomedicine for HIV/Aids and malaria. We have successfully nanoencapsulated all four first line anti-TB drugs, in polymeric nanoparticles. *In vitro* release assays showed the drugs were released sustainably for up to 6 days. Intracellular drug delivery studies in two human cell lines demonstrated that the particles are taken up by the cells and delivered from the phagosomes into the cytoplasm. We also illustrated that the bacterial growth index in THP-1 cells treated with encapsulated rifampicin was reduced significantly, compared to cells treated with free rifampicin. Extracellular bacteria were also killed by the encapsulated drug over a period of time. Drug release was observed in vivo over a period of seven days. Further characterisation of the particles revealed that the particles did not elicit any inflammatory response when orally administered to both TB challenged and unchallenged mice. Preclinical studies on TB infected mice demonstrated that the encapsulated drugs, administered once weekly, over a period of 6 weeks, showed comparative efficacy against the TB bacterium, when compared to the free drugs that were administered once daily. Furthermore, we actively targeted TB infected macrophages with nanoparticles that are functionalised with aptamers against the target protein, and noted that intracellular delivery and slow release of the drugs is feasible.

Focus on the Centre of Excellence

The DST/CSIR nanomedicine platform is in a unique position in Africa; having built a substantial knowledge base in human capital, equipment/facilities and infrastructure, and is pioneering nanomedicine-based drug delivery research for PRDs. Given this advantage, the platform sees an urgent responsibility to advance nanomedicine research in Africa, while simultaneously generating highly qualified human capacity, in order to impact meaningfully at not only continental but also at a global level. In line with this goal, the platform is growing towards recognition as an "African Centre of Excellence (CoE) in Applied Nanomedicine Research and Training". The proposed CoE will seek to deliver nanomedicine as an alternative therapy against PRDs through sharing of resources, know-how and technologies, which will avoid wasteful duplication of efforts and allow the most efficient use of pre-existing structures.

Also geared at building and transforming human capital in Africa, the proposed CoE will concentrate existing capacity and resources to facilitate collaboration across disciplines and across organisations on long term programmes and projects of direct relevance to PRD drug development needs and aspirations. The proposed CoE will offer researchers a stimulating and dynamic research environment by providing: Guidance/Support – through mentoring, providing expertise, standards, methods, tools and knowledge repositories; Shared learning – through training such as sensitisation seminars, workshops, summer schools, lab/researcher exchange programs, sabbaticals; Monitoring and Evaluation – through conferences, publications, patents, technology transfer; Governance – through coordinating of activities to enable valuable delivery.

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- All collaborators

[PL 16] Natural Products from Plant Diversity and their Potential in Management of Neglected Diseases

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Introduction

Conventionally, neglected diseases are considered as a group of 13 infectious diseases that are endemic in the low income populations in the tropical developing world. They can be classified as those caused by trypanosomal parasites, helminthes, bacteria and viruses. They cause death to an estimated 0.5- 1m people annually. Trypanosomal diseases are represented by Kala-azar or visceral leshmaniasis, African sleeping sickness (African trypanosomiasis) and Chaga's disease (American trypanosomiasis); the current drugs for these diseases are relatively toxic even though the disease is not that lethal. Helminth infections include schistosomiasis treated with the inexpensive praziquantel but which cannot stop re-infection; onchocerciasis (river blindness), on which anthelmintic treatment is being tried; dracunculiasis (guinea worm), which should have been eradicated in 2009; lymphatic filiriasis (elephantiasis), managed by anthelmintic treatments. The others are soil transmitted worms such as ascariasis (round worms), trichuriasis (whipworms) and hookworms which are really best controlled by good hygienic practices. Leprosy, trachoma, Buruli ulcer and cholera represent the prevalent bacterial problems. Viral infections are yellow and dengue fevers caused by flavivirus transmitted by *Aedes aegyptii* and Japanese encephilitis caused by a flavivirus transmitted by *Culex tritaeniorhynchus*; the viral infections can be controlled through vaccination (WHO, 2008).

However the WHO Innovative and Intensified Disease management (WHO-IDM) group considers NTDs to be only: Buruli ulcer, Chaga's diseases, cholera, sleeping sickness and leshmaniasis. This is because they get less funding than the "big three" – HIV/AIDS, malaria and tuberculosis. However other groups led by Drugs for Neglected Diseases Initiative (DNDi) organization do not agree and consider African trypanosomiasis, leshmaniasis, Chaga's disease, and malaria as NTDs. This list leaves out Buruli ulcer but includes malaria with the argument that the disease does not receive adequate funding relative to its perilous effects on society. Their argument is that "neglected diseases" should be conditions that do not get enough funding relative to their impact on society (DNDi, 2003); in the presentation, this definition is considered to be the correct one.

Clearly there is African folklore (mostly, medicinal herbal concoctions) about management of these neglected diseases even though such information is mostly scattered and may have run out of vogue at the advent of "civilization". It would be pertinent to explore the potential of certain local herbs for the development of phytomedicines for local populations for the diseases. Such practices have been recorded in monographs such as Medicinal Plants of East Africa (MPEA) (Kokwaro 2009) and Kenya Trees and Shrubs (Beentje 1994).

Helminthiasis

Helminthiasis as a disease group covers many of the neglected diseases. More than five hundred East African plants are listed by Kokwaro in MPEA as emetics/ purgatives, intestinal worm infections alleviators for hookworm, roundworm, tapeworm, threadworm, general anthelmintic, bilharzia and filarial infections. A few of these plants have been studied in our laboratory. They are the Myrsinaceae which are mentioned in several categories of anthelminthiasis and *Rumex* (Midiwo,2002) species listed as laxatives which can also be used to expel helminths from bowels. We have looked at the phytochemistry of the Kenyan Myrsinaceae reasonably and we find that it corresponds with that already reported in the literature. They are harbingers of long alkyl side chain 2, 5-dihydroxybenzoquinones. We managed to chemotaxonomically group them into two sub-families, the Myrsinodae and the Maesodae using these markers which coincides with their morphological delimitation. The Myrsinodae**,** which includes the species *Myrsine africana, Rapanea melanphloes, Embelia schimperi* and *E. keniensis* are chemically typified by the existence of embelin/ rapanone while the Maesodae represented monotypically by *Maesa lanceolata* in Kenya is typified by the existence of maesaquinone. We have isolated several other benzoquinones from Kenyan Myrsinaceae and reported them in the literature (Midiwo, 2002). *Rumex* species are widely used locally for control of intestinal helminthic conditions. There are five Rumex species in Kenya - *Rumex abyssinicus*, *R. usambarense*, *R. bequaertii, R. ruwenzoriensis* and *R. crispus*, according to their grouping in the "Key to Species". The compounds that are found in high concentration are the common anthraquinones, emodin, physcion and chrysophanol along with the polyketide naphthalenic compound, nepodin, whose distribution is in accordance with the Key (Midiwo, 2002). The anthraquinones have been reported to have purgative effect; no doubt this is the mechanism by which they exert their traditional anthelminthic activity.

Considering the high concentration of bioactive principles in these Myrsinaceae, *Rumex* species and similar anthelmintic plants, it is suggested that they could be pursued for formulation of cheap phytomedicines for use by the local populations. Some of the herbs, like the berries of the Myrsinaceae, are usually sold in market places to be added food as medicinal spices for the desired effects for intestinal worm expulsion; they can be developed as food supplements to control intestinal worm proliferation.

Malaria

Malaria is a serious disease whose progression may lead to death. Its symptoms of high fever, chills, weakened joints, and flu-like illness are however well recognized by people in malaria endemic areas such as Kenya. Illness and death from malaria are largely preventable. Malaria is caused by *Plasmodium* parasites which are transmitted by mosquito vector. The most common parasite in Kenya which is the most virulent is *Plasmodium falciparum*; the others are *P. vivax, P. ovale* and *P. malariae.* The main mosquito vector in Africa and which, unfortunately, is also the most efficient is *Anopheles gambiae.* There are 300-500 million malaria infections world wide every year. About 80% of these are in Africa leading to 1.75- 2 m deaths annually, mostly children under five years old. Worldwide 3000 children die everyday from malaria. Approximately 90% of malaria deaths are in Africa. Malaria constitutes 10% of Africa's total disease burden; 40% health expenditure and 30-50% of in-patient cases (WHO 2001). Total African cost estimate is in the range of US$12b annually.

In Kenya, 22 million people are at risk, 70% of them are in rural areas. About 34,000 Kenyan children die every year from malaria compared to a total estimate of 42,000 people dead (DMS Kenya 2006).

Malaria can be controlled using three approaches- drug therapy, vector eradication and use of vaccines (in the future). Drug therapy has been beset by development of resistance in virtually all the drugs developed by synthetic method or obtained from nature; the latter ones tend to take a longer period to experience resistance by the microorganisms. Malaria has been usefully been controlled using herbs such as *Artemisia annua* (Francois, 1993) and Cinchona which gave rise to quinine. These natural compounds have been noticed not to succumb to resistance as fast as the synthetic ones such as chloroquin, mefloquin and sulfadoxin/pyrimethamin. So it is attractive to look at new plant sources for these drugs. We have looked at several examples of these in our laboratory including Erythrina *abysinica* (Abiy, 2004) and Polygonum *senegalense* (Midiwo 2007) among others (Abiy 2003, Vlodomir Samolyenko, 2009).

Conclusion

Medicinal plants are useful sources of anti-plasmodial compounds that can be packaged as new anti-malarial drugs. It is worth pursuing the concept that mixtures of such compounds from plants can be formulated together for synergistic activity in which case the effective concentrations can be significantly reduced. It is the belief that this "shift in paradigm" from single or small number compound therapies may in the future lead to development of more efficient and cheaper drugs which also circumvent resistance development.

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[PL 17] Changes in Plants Metabolites with Location of Growth and Agronomic Practices: Some Lessions from Black Tea Quality Studies

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Key words: Tea, *Camellia sinensis,* geographical location, yield, quality parameters

Introduction

Tea (*Camellia sinensis* (L.) is an intensively cultivated perennial crop in many regions varying from 49ºN, outer Carpathians to 30ºS, Natal, South Africa[1] and from altitudes ranging from sea level in Japan and Sri Lanka to above 2700m above mean sea level (amsl) in Olenguruone, Kenya and Gisovu, Rwanda[2]. Despite the large variations in geographical locations of production, the recommended processing technologies and agronomic inputs are largely the same. For example, agronomic recommendations from Kenya[3] are used throughout tea growing areas in East and Central Africa. Tea growers also have a desire to for high yielding and good quality panting materials in the hope that whatever identified good material in a location would replicate the good attributes in new areas of production. This paper examines the role of geographical location of production on black tea yields, processing, and overall quality.

Material and Methods

In one trial[2], clones TRFK 6/8 and TRFCA SFS 150 grown in at the Tea Research Foundation of Central Africa (TRFCA), Mulanje, Malawi, (altitude 650 m above mean sea level (amsl), latitude 16^o 05' S, longitude 35^o 37' E) and Tea Research Foundation of Kenya (TRFK), Kericho, Kenya, (altitude 2180 m amsl, latitude 0^o 22' S, longitude 35^o 21' E) for changes in quality parameters with fermentation time. The plants were grown under recommended agronomic practices. The cultivars were plucked both at the TRFCA and TRFK and processed by miniature CTC method under similar conditions at the respective institutions. Fermentation was varied at 30, 50, 70, 90 and 110 minutes at 28-30^{o}C before firing. The processing was done in replicate. The unsorted black teas were subjected to chemical analysis. The total theaflavins were analysed by the Flavognost method[4] while thearubigins, brightness and total colour were determined by the method of Robert and Smith[5] while the individual theaflavin ratios were determined using HPLC[6,7] as explained previously[8]. The astringency of the black teas was estimated using the modified theaflavins digallate equivalent factor[9]. Simultaneous steam distillation-extraction[10] was used to extract the volatile flavour compounds (VFC) with cumene as an internal standard. The dried (anhydrous Na_2SO_4), ether-VFC mixture was analysed using chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) [11].

In a second trial[12] 20 widely cultivated (commercial) genotypes of tea were planted in Kangaita Tea Farm (latitude 0^o 30'S, longitude 37^{o}16'E, altitude 2100 m amsl), TRFK, and Kipkebe Estate, Sotik (latitude 0^o 41'S longitude 35^o 5'E, altitude 1800 m amsl) were evaluated. At each site the plots were arranged in a randomised complete block design with three replicates[12]. Nitrogenous fertiliser

(NPKS 25:5:5:5) was applied at a single dose of 300 kg N ha^{-1} $year^{-1}$. Plucking was done at 10–14 day intervals, depending on leaf availability. The plants were under uniform management and agronomic practices. One kilogram of leaf was plucked from each plot and processed by the miniature CTC method[2, 8]. The unsorted black teas were subjected to plain tea quality parameters chemical analysis[4, 5] and sensory evaluations.

Another set of trials were laid out in five main tea growing regions of Kenya at Karirana (altitude 2260 m amsl, latitude 1^0 6'S, longitude 36^0 39'E), TRFK, Changoi (altitude 1860 m amsl, latitude 0^0 29'S, longitude 35^0 14'E), Sotik Highlands (altitude 1800 m amsl, latitude 0^0 35'S, longitude 35^0 5'E) and Kipkebe. Clone BBK 35 p uniformly managed and with known past cultivation history, were selected from each site. Fertilizer was applied at 0, 75, 150, 225 and 300 kg N $ha^{-1}year^{-1}$ all in November, while plucking was done at 7, 14 and 21 days. The trials were laid out at each site as 5 by 3 factorial design in randomized complete block arrangement. The data were analyzed as Randomised Complete Block in 5 by 3 factorial design at each site split for the five locations. Samples for miniature manufacture and fatty acids analyses were obtained when all plucking intervals coincided. Fatty acids[13] and plain black tea quality parameters[4, 5] were analysed according to published methods.

Results and Discussion

The optimal fermentation duration was reached much faster in Malawi tea leaves than tea leaves from Kenya[2]. Indeed, the quality parameters were lower in Malawi tea leaves than Kenyan tea leaves demonstrating that the plant metabolites responsible for making tea quality (polyphenols and volatile flavour compounds) and enzymes responsible for their transformation were not equal in the same clone produced in different regions. The individual theaflavins ratios in the same clone produced in different regions were not the same suggesting that the clones were not producing the flavan-3-ols in the same amounts and ratios. While black tea produced from Kenyan leaf was more aromatic, the same black tea from Malawi had high amounts of C_6 alcohols and aldehydes which tend to lower black tea aroma quality. Thus production of the metabolites responsible for tea quality is influenced by the growing environment.

The plain black tea quality parameters of the 20 clones varied with geographical area of production[14]. The order of the variations and preferences of the clones also changed with location of production. However, some cultivars showed remarkable stability, with very little variations with geographical area of production. The results demonstrate need for assessing cultivars in new areas of intended cultivation before they are released to farmers for wide spread production, as it is not possible to predict the production rates and levels of the metabolites even in a single genotype when grown in different environment.

Although the same clone was subjected to different rates of nitrogenous fertiliser and plucking intervals, the yields[15], black tea quality parameters[15, 16] and fatty acids[17] levels changed with area of production. These results demonstrate that same level of production or black tea quality cannot be obtained from the same cultivar when it is grown in different geographical regions even when

agronomic inputs are the same. The magnitudes and rates of changes in the individual parameters due to rates of nitrogenous fertiliser and plucking intervals changed with regions causing significant ($P \leq 0.05$) interactions effects. Thus the magnitude and rates of the changes in any parameter at one site cannot be used to predict responses at different locations.

These results explain the general inability of tea growers to obtain same yields and/or quality of black tea from same clone grown in different locations. There is therefore the need for development of region specific agronomic recommendations including cultivar selection to obtain high yields and quality. These results have further implications to phytochemical investigations. Levels of the metabolites detected in a plant from a given area may not be replicated in another area. Indeed in some cases, some metabolites detected in one area maybe absent altogether in another area. For, example nerolidol found in Kenya[2] and Longjing[18] tea has not been determined in Assam teas[11].

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[PL 18] Challenges of Isolation, Characterization and Profiling of African Medicinal Plants: Analytical Prospective of Standardization and Quality Control Methods

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Keywords: *Harpagophytum procumbens*, isolation, standards, profiling, quality control.

Profiling African medicinal plants is a challenge due to the lack of standards required for quantification. In this study a HPLC-DAD method is developed for quality control of Devil's Claw (*Harpagophytum procumbens)* products. Reference standards/compounds were isolated and purified using chromatographic methods. The standard compounds were used for profiling and quantification of Devil's Claw (*Harpagophytum procumbens)* species. Compounds such as harpagoside, acteoside, isoacteaoside, bioside and procumbide were isolated at high purity using chromatographic techniques. The purity and identification of the isolated compounds was determined using TLC, ^{1}H-NMR and HPLC.

Introduction

Harpagophytum procumbens is a plant found in dry and sandy regions of Namibia, Botswana, South Africa, Zambia and Zimbabwe [1]. *Harpagophytum procumbens* is commonly known as Devils' Claw and has several vernacular names such as Sengaprile in Botswana. The root extracts of *Harpagophytum procumbens* have been reported to be pharmacologically active. Clinical studies have shown that root extracts are effective in the treatment of degenerative rheumatoid arthritis, osteoarthritis and tendonitis, kidney inflammation and heart disease [2]. The tuber of this plants is commercially available and is sold on large quantities to European countries especially Germany [3]. Currently there are no known quality assurance and standardization methods developed for Devil's Claw in supplying countries despite the fact that the importing countries require that the percentage of harpagoside should be at least 1 %. This work is an effort to develop suitable analytical procedures for quality control, standardization or profiling of *Harpagophytum procumbens* samples.

Quantification and quality control studies require the availability of high purity standards. The major challenge in this work is that the reference standards for the analytical work for African traditional plants are either not available, limited or are very expensive in the market. For Devil's claw only one reference standard (harpagoside) is currently available in the market. Such a limitation necessitates the isolation of high purity standards (> 90 – 98 %.) from the plant. Thus the aim of this work is to isolate known compounds from the plant using chromatographic methods and

subsequently use the isolated compounds as reference standards for profiling Devil's Claw products using HPLC.

Material and Methods

The compounds were isolated from the Kalahari Devil's Claw tea packaged by Thusano Lefatsheng (Gaborone, Botswana). Pharma Tech Model 1000 High Speed Counter Chromatography (HSCCC), (Baltimore, Maryland USA) was used for chromatographic isolation of the tea. The isolated components were further cleaned-up by Sephadex LH-20 and Silica gel 60 (0.040-0.063) mm gravity column. In addition Dionex Ultimate 3000 HPLC (Germering, Germany) was used for semi-preparative HPLC for the isolation and clean up of reference standards. Prep Xterra® MS C_{18} (3.5 µm × 7.8 mm × 100 mm) column was used for semi-preparative HPLC work. Chloroform, ethyl acetate, n-butanol, methanol, dichloromethane and ethanol were of GPR grade and were used for the preparation of crude sample, gravity column and HSCCC separation. All the GPR grade solvents were distilled prior to use. In addition ultra high purity (UHP) water from a Milli-Q system (Millipore, Bedford, MA, USA) was used as part of the mobile phase. Silica GF_{254} Thin Layer Chromatography (TLC) plates from Merck (Darmstadt, Germany) were used for the isolated fractions. In addition Bruker Advance 300 MHz spectrometer (Karlsruhe, Germany) was used for ^1H proton structural elucidation. The identification was achieved by comparing of the obtained spectra with published spectra [4]. Devil's Claw (*Harpagophytum procumbens*) secondary roots (3.0 kg) were extracted using CH_2Cl_2/ MeOH (1:1 v/v) for 24 hrs followed by MeOH for 1 hr. The volume of the extracts was reduced by evaporation before application to HSCCC and gravity column. The HSCCC solvent systems used in this study were prepared by mixing ethyl acetate–*n*-butanol–ethanol–water (4:0.6:0.6:5, v/v). The upper organic phase was used as the mobile phase and the lower aqueous phase as the stationary phase. In gravity column, separations were achieved by using $CHCl_3$/MeOH gradients saturated with H_2O as the eluting solvent system. Further clean up was achieved on a column packed with Sephadex LH-20 using $CHCl_3$/MeOH, 2:1 v/v as the eluting solvent.

Result and Discussion

A number of compounds are known to exist in *Harpagophytum procumbens* in appreciable quantities and these are 6-o-acetyl acteoside **1**, iso-acteoside **2**, bioside **3**, acteoside **4**, Harpagoside **6**, procumbens **9** and cinnamic acid **10** (Table 1**)**. In this study 4 iridoid glycosides **(5-6, 8-9)**, 3 phenylethanoid 2-4 glycosides **(2-4)** and cinnamic acid **10** were isolated by means of HSCCC as well as column chromatography on Sephadex LH-20 and silica gel. The presence of compounds **1** and **7** was detected in trace amounts on ^1H NMR spectra of other compounds.

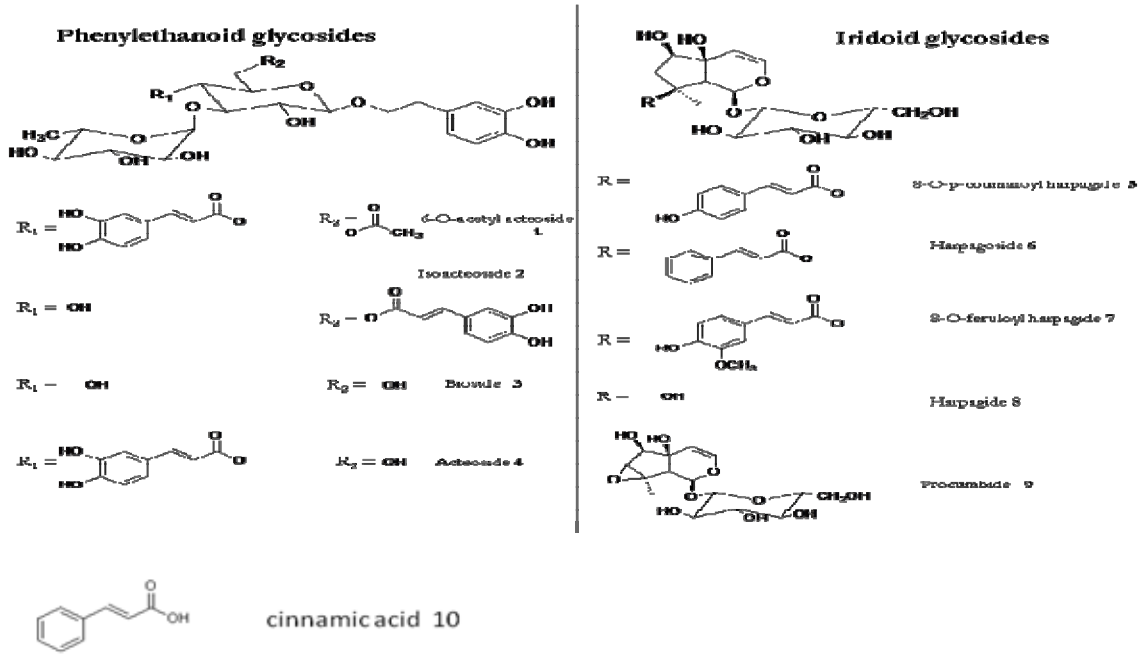

Fig 1 Structures of Compounds isolated from *Harpagophytum procumbens*

Table 1 Compounds isolated from *Harpagophytum procumbens*

Reference compound Isolated	Quantity (mg)
Harpagoside	359
Harpagide	67
Procumbide	98
8-0-p-coumaroylharpagide	50
Acteoside	179
Iso acteoside	194
Bioside	4
Cinnamic acid	10

Preparative HPLC showed promising results for the isolation of known compounds at higher purity. Fig 2 shows a separation of the crude extract of *Harpagophytum procumbens.* From the chromatogram 16 peaks have been separated. Isolation of these peaks could result in an increased number of reference compounds needed for the profiling of the plant.

Conclusions

This study has shown that it is possible, although challenging, to isolate and purify compounds which can be used as reference standards. A HPLC-DAD method for the quality control of Devil's

Claw *Harpagophytum procumbens* products has been developed using the isolated reference standards.

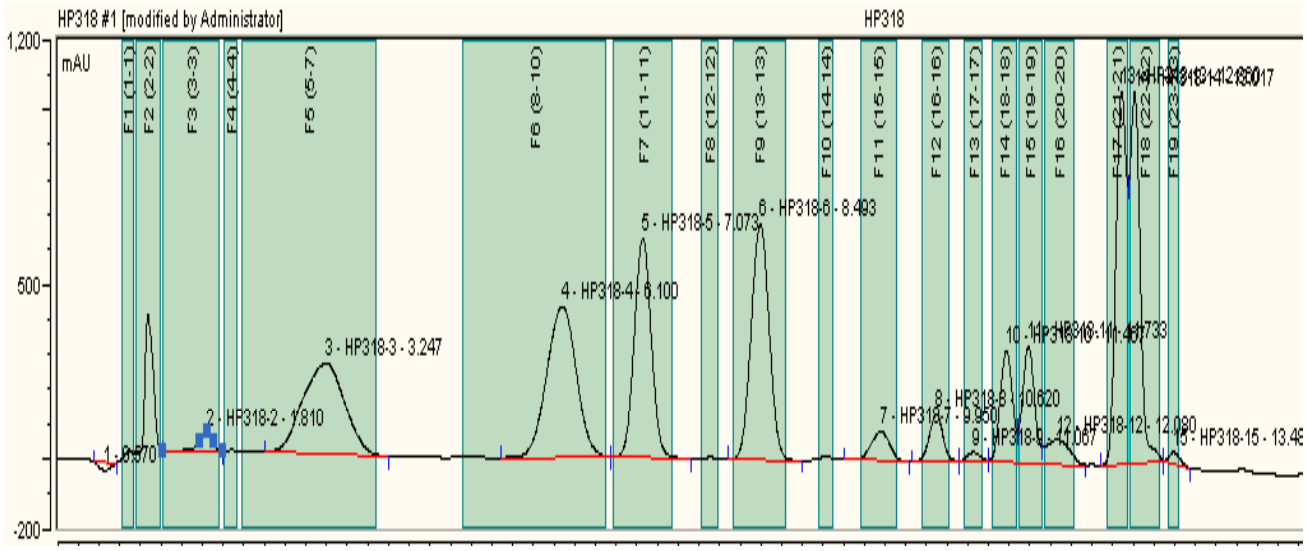

Fig 2. HPLC-DAD analysis of crude extract Prep Xterra® MS C_{18} 3.5 µm × 7.8mm × 100 mm, CH_3CN+0.1% HCOOH: 0.1% HCOOH, 20 min gradient, room temp

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[PL 19] Exploiting the Chemistry of African Biodiversity in Pest Management: from Extraction of Plant chemicals to Expression in GMOs

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Key words: Africa, biodiversity, pest control, natural products, pheromones, semiochemicals, push-pull

Introduction

Natural products have long been viewed as providing leads for pest management, and particularly as insecticides. Indeed, many recently developed insecticides have followed natural product leads, such as the neonicotinoids, and some are indeed natural products themselves, for example spinosad. Perhaps the most well known are the synthetic pyrethroids, such as permethrin, cypermethrin and deltamethrin invented by Michael Elliott and his colleagues at Rothamsted from lead compounds, specifically pyrethrin 1, in the pyrethrum daisy, *Chrysanthemum (= tanacetum) cinerariifolium* (Asteraceae), currently grown commercially in Kenya for these natural insecticidal compounds.

Here not only do we describe providing lead compounds and products directly for the international pest control market, but, by exploiting African biodiversity, compounds can be identified for exploitation locally in pest management. This can either be as plant products or their extracts, developed locally for feedstocks for pest control agents, or as pest management agents released from plants as wild-type cultivars or after genetic modification (GM). Although natural products do not represent intrinsically more benign agents than synthetic, by choosing those natural products that control pests, diseases and weeds, without the involvement of direct toxic modes of action then such intrinsically benign properties can be incorporated.

Discussion

Repellents against insect vectors of pathogens

In global agricultural use, the neonicotinoids have now overtaken the pyrethroids, but for intervention against vectors of human pathogens, the pyrethroids still lead. In protecting against malaria, pyrethroids such as permethrin in long-lasting bednets act principally as toxicants, but there is evidence of repellency. The Sumitomo Chemical Company Ltd has introduced metofluthrin which acts aerially as a consequence of its very high vapour pressure, determined by a high level of fluorine substitution, and which appears to show marked repellency. For natural products with non toxic repellency, there are many potential essential oil components, for example eucamalol from *Eucalyptus camaldulensis* (Myrtaceae), and a number of African herbs, e.g. *Ocimum spp* (Lamiaceae), yield essential oils with similarly useful properties. Although many, for various reasons, are not as useful repellents as the commercial gold standard DEET, the fact that they can

be produced locally could make these materials more useful. Also, African ethnobotany is able directly to offer indicators of this type of biological activity and recently we showed that gum haggar from *Commiphora holtziana* (Burseraceae), growing in the arid regions of Kenya and to the East, contains volatile sesquiterpenes e.g. (*R*)-germacrene D, highly active against ticks and mites attacking cattle and camels, acting as repellents and which, although highly unstable, were protected by the gum matrix (Birkett *et al*. 2008). Recently, we and others have shown that DEET acts on the insect olfactory system in a similar way to the plant-derived volatiles (Pickett *et al*., 2008; Stanczyk *et al.* 2010) and this paves the way for more rapid electrophysiological screening of putative repellents.

Although many haematophagous insects feed on floral nectar, when foraging for blood meals haematophagous arthropods are mostly repelled by plant-derived volatile compounds. However, these can be overcome by the insect detecting host chemicals in spite of the presence of the plant-derived signals. Exploiting mechanisms by which hosts are selected from within the host family, or even species via variation within the species, is likely to provide more durable and powerful repellents. Thus, although within the host family Bovidae, the water buck, *Kobus defassa*, releases volatile repellents against tsetse flies, which can mask the attractancy of hosts such as domestic cattle (Gikonyo *et al*. 2003). Even from cattle, and human subjects, repellents can also be identified from unattractive representatives of these animals, and can have potential commercial value as repellents in animal husbandry and against biting insects including the malaria mosquito *Anopheles gambiae s.s.* (Birkett *et al.* 2004, Logan *et al.* 2008, Logan *et al*. 2010).

Antifeedants against crop pests

Repellents have been largely unsuccessful as pest control agents in crop protection, but plant derived antifeedants, which interfere by non-toxic modes of action with normal feeding by herbivorous pests, have shown much greater promise. For these, East Africa has a range of plants yielding potentially valuable antifeedants, such as *Ajuga* spp. (Lamiaceae), particularly *A. remota*, yielding ajugarin 1, and the tree *Warburgia ugandensis (Canellaaceae)*, yielding ugandensidial (Pickett *et al*. 1987). However, although these could be used locally and also form the basis of exports to the north, such products have not yet been exploited.

Direct production of crop protection agents by companion crops

For generation directly by plants, it is possible to exploit the technique of companion cropping, referred to in kiSwahili as "kilimo cha mchanganyiko", and the famous push-pull system, or "vuta sukuma" (pull-push), pioneered with the International Centre of Insect Physiology and Ecology (*icipe*) in Kenya www.push-pull.net, is an excellent example. Thus, by searching East African plant biodiversity for candidate repellent and attractant plants, a system has been developed for pushing away pests, and at the same time attracting beneficial enemies of these into the crop, so that the pests can be trapped in a peripherally grown trap crop (Khan *et al*. 1997; Hassanali *et al*. 2008). Each companion crop is used also as forage for cattle or dairy goats. Although knowledge intensive, once the technology has been acquired, this approach is extremely popular with farmers (Khan *et*

al. 2011). Of the intercrops one, which comprises forage legumes in the genus *Desmodium* (Fabaceae), also dramatically controls the African parasitic witchweeds in the genus *Striga* (Orobanchaceae), particularly *S. hermonthica* (Hassanali *et al*. 2008).

Release from GM plants

By identifying the chemistry of the companion crops that is responsible for repelling pests and attracting beneficial insects we have also created new targets for genetic modification, for example, increasing production of 4,8,12-trimethyl-(*E,E*)-trideca-1,3,7,11-tetraene which both repels pests and attracts parasitic wasps (Bruce *et al*. 2008; Matthes et al 2011). Insect-derived elicitors will provide a valuable tool by which to "switch on" defence based on this type of chemistry through breeding and also by GM approaches. Currently we are working on elicitors from the eggs of maize stem borers to identify elicitors that can have dramatic effects on the defence chemistry of African grasses (Bruce *et al.* 2010). The compounds from *D. uncinatum* that interfere with the development of the parasitic weeds *Striga* spp. comprise *C*-glycosylated flavonones and we have recently elucidated the mechanism by which these compounds are biosynthesised (Hamilton *et al.* 2009). By heterologously expressing the *C*-glycosyltransferase enzymes involved into edible beans, we would create companion intercrop plants useful as human food but embodying the novel trait for controlling *Striga* spp. (Pickett *et al*. 2010; Khan *et al*. 2010).

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[PL 20] Development of Medicines from African Medicinal Plants: Experiences in West Africa

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KEY WORDS: African pharmacopeia, Improved Traditional Medicines, Gastrosedal, Antianeamia, Dysenteral, Antitussif

Introduction

African flora contains many medicinal plants. Less than 10% of these plants have been investigated so far. Plants are sources of new molecules which can be developed as news medicines. From plants to medicines there are different steps. How Africans can improve the health state of their population by using natural products research?

Material and Methods

In West Africa many research institute are developing medicines from plants. Researches should use standardized methods and assure efficacy, safety and quality. Studies have been performed on different plants: *Vernonia kostchyana* (roots), *Guiera senegalensis* (leaves), *Combretum micranthum* (leaves), *Euphorbia hirt*a (aerial part), *Zanthoxylum zanthoxyloïdes* (root bark), *Crossopteryx febrifuga* (fruits) *Dissotis rotundifolia, Sclerocarya birrea, Cochlospermum tinctorium*, and *Cassia italica*.

Result and Discussion

Vernonia kostchyana root powder has shown effect on gastric ulcer in Mali (Diallo et al., 1990). Coumarins, flavonoids, tannins, sugars, mucilage, sterols and triterpens were identified during the chemical screening (Diawara, 1989), more investigation by biological tests showed the activity on gastric ulcer of rats (Sanogo et al 1996); Saponins and polysaccharides were identified as responsible of the activity (Sanogo et al 1998; Sogn et al 2005). The Root powder of *Vernonia kostchyana* is being sold in Mali as Gastrosedal a phytomedicine used against gastric ulcer. "Sirop Elooko"is a phytomedicine prepared from the leaves of *Guiera senegalensis* and used against cough in Senegal . *Dissotis rotundifolia* is also used against cough in Guinea Conakry, a cough syrup has been prepared from this plant. After different researches, *Euphorbia hirta* was show to be efficacious against dysenteria, phytomedicines have then been prepare from it Dysenteral in Mali and sirup Amibex in Burkina Faso. The fruits of *Crossopteryx febrifuga* contain saponins, Balembo syrup has been made from their ethanol extract and being use in Mali against cough. *Zanthoxylum zanthoxyloïdes* is used in west Africa against sickle cell anemia in, Benin, Burkina Faso, Mali Nigeria and Togo. Chemical investigations showed that alkaloids, benzoic acid derivatives (p-hydroxybenzoic acid, 2-hydroxymethyl benzoic acid and vanillic acid); essential oil, tannins; flavonoïds and saponins are present in the roots bark (Sofowora.E.A. Lloydia *et al*, 1971, Lamba S. *et*

al., 1990, Emerson *et al*., 2006, Pousset, 2007). *Cassia italica* is used against constipation, the phytomedicine Laxa cassia has been prepare from its leaves in Mali. Further studies are ongoing for plant against diabetes, hypertension and AIDS. The main aim of all these researches is to valorize traditional medicine and its products by making available to low income population quality medicines that are efficient safe and affordable.

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Traditional Healers

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[PL 21] The pan-African Natural Product Library (p-ANPL): Giving Steam a Direction

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African scientists have in various ways shown immense creativity in efforts to take advantage of natural product diversity on the African continent to identify lead compounds to use against locally relevant, but globally neglected diseases, including many parasitic infections.

However, mainly due to a lack of resources many efforts remain uncoordinated and involve little cooperation between African natural product researchers. The exploitation of the African biodiversity for drug discovery has been largely confined to a model in which natural resources from Africa are extracted and evaluated in industrialized countries with minimal participation or direction from African collaborators. But to develop a more accurate global health perspective African scientists need to be in the forefront of drug research involving their unique resources in order to combat major health challenges effectively.

To this end the pan-African Natural Product Library (p-ANPL) consortium was established in 2009, signed by representatives from seven African research institutions and a common declaration outlined the major goal of the consortium as bringing together biologically diverse natural compounds and extracts from the African continent and compounds originating from Africa that are held in other countries. This library will - in a first step - be subjected to screening for non-peptide nematode G-protein coupled receptor agonists which are likely to act on specifically on multiple receptors preventing the development of anthelmintic resistance. However, other antibiotic or additional drug discovery programs can be included in the future.

In this presentation we will discuss the current status of efforts to build p-ANPL, introduce our screening platforms and above all we would like to invite African natural product researchers to actively participate in the p-ANPL initiative.

SHORT LECTURES

[SL 1A] Phytochemical and Biological Studies on *Aloe sinkatana* Berger

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Kew wards: *Aloe sinkatana*, anthraquinones, antiglycation activity, T-cell proliferation, cytotoxicity.

Introduction:

Aloe species (family Liliaceae) have enjoyed a wide folkloric usage and are also used in modern medicine and cosmetic products in many parts of the world. Carter (1994) and Lavranos (1995) reported the presence of 14 *Aloe* species in Sudan with 2 endemics namely; *Aloe sinkatana* Berger and *A. macleayi* Reynold. *A. sinkatana* is a shrub native to the Red Sea Hills in the Sudan. In folk medicine, the leaves and leaf exudates are used for the treatment of constipation, fever, skin disease and inflamed colon (MAPRI, 1997).

Material and methods:

The plant, *A. sinkatana* was collected from Arkawit, Sudan, in February 2009. Dry leaves (200g) were extracted with hexane, ethyl acetate and methanol respectively. Pure compounds were isolated from the ethyl acetate and methanol extracts using different chromatographic techniques. Their structures were identified on the basis of IR, UV, and 1D and 2D NMR and mass spectroscopic analysis. Evaluation of protein glycation was determined by α-gluconolactone assay (Rahbar and Nadler, 1999). Antiglycation property of isolated compounds was evaluated by the method described by McPherson *et al.* (1988). Cell proliferation was evaluated by standard thymidine incorporation assay following a method reported by Nielsen *et al*. (1998). Cytotoxicity was performed according to method reported by Dariusz *et al*. (1993) with some modifications.

Results and discussion

From the leaves of *A. sinkatana* one new anthraquinone (2, 8 -dihydroxy -6-(hydroxymethyl)-1-methoxyanthracene-9,10-dione) (**1**), aloe-emodin (**2**), aloin A & B, (**3** & **4**) chrysophanol (**5**), feralolide (**6**) microdontin (**7**), homoaloin (**8**) and β-sitosterol (glycoside) (**9**) were isolated. Antiglycation activity of extracts and compounds **1** and **2** was carried out. The results obtained showed that the MeOH and EtOAc extracts as well as compound **1** showed inhibitory effect of early stage of protein glycation. Compound **1** also showed significant inhibitory effects against glucose-induced advanced glycation end-products formation. The immunomodulatory and cytotoxic properties of some of the isolated compounds were also evaluated. Two of these compounds (**2** and **6**) were found to exert significant suppressive effects on T-cell proliferation with an IC_{50} of 9.2

and 7.4 μg/mL respectively and a suppressive IL-2 production activity with an IC_{50} of 1.1 and 1.9 μg/mL for **6** and **2** respectively. The cytotoxicity of these two compounds was evaluated using two cell lines and *in vitro* MTT assay, where none of them showed significant effect on the viability of either of the two cell lines.

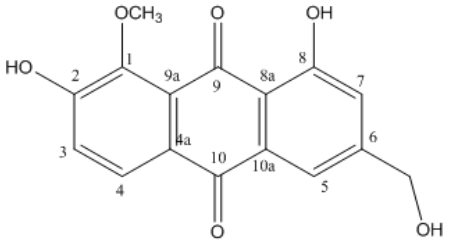

Fig. 1: Structure of compounds 1 isolated from *A. sinkatana*.

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[SL 1B] Application of *in vitro* Drug Metabolism and Disposition Studies to Assess Risk of Drug interactions with *Sutherlandia frutescens* Extracts

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Key Words: Drug interactions, Sutherlandia frutescens, apparent permeability, CYP inhibition

Introduction

The interactions of drugs with herbal drugs is an emerging area of interest in drug discovery studies and clinical use. Interactions may occur at luminal transporter level (e.g. pg-p), or by inhibition or induction of drug metabolizing enzymes (DME) impacting on the bioavailability of the victim drug(s). Most drug-herb interactions studies to date have used phytomedicines popular in industrialized countries but little attention has been paid to herbs which are used in Africa and Asia where co-medication with herbal medicine is rife (WHO, 2002; Okigbo et al, 2008). We investigated the potential for drug-herb interactions of extracts of ground leaves of *Sutherlandia frutescens* (so-called cancer-bush) which is widely marketed and used in Southern Africa for stress disorders, cancer, diabetes and more recently as an "immunebooster" in HIV / AIDS.

Material and Methods

The assay for Pg-p inhibition utilized the MDCK-MR1 cell line culture as a monolayer on Transwells® (Corning) and dosed with sutherlandia extracts (SU) (6.8 mg / ml to 1.7 μg /ml). Amprenavir was used as the permeability marker and GF120918 the standard Pg-p inhibitor. After pre-inculabtion the Transepithial Electrical Resistance (TEER) was measured, and then the concentration of amprenavir analyzed by LC-MS in positive ion mode (API 4000 QTRAP, Applied Biosystems) post-incubation.

The effect of SU on cytochrome P450 was determined by the P450-Glo™ assay kit (Promega, Madison, WI, USA). Three controls were used viz. one with control membranes, one with vehicle (DMSO < 3.2%) and the third with standard inhibitors specific to the target enzyme i.e. α-naphthoflavone (for 1A2), sulfaphenazole (2C9), troglitazone (2C19), sertraline (2B6) and ketoconazole (3A4). SU extract (25mM stock solution) was used to give final serial dilutions from 100 to 1 μM. Luminescent activity was read using the Wallac® luminometer with Envision® software (Perkin Elmer, Mass, USA). The data was processed by MS Excel and then transformed by GraphPad to give IC50 values.

Result and Discussion

Doses of SU >850 μg /ml reduced TEER readings implying possible damage to tight junctions. Amprenavir permeability increased in a dose-dependent manner in cells exposed to SU >53 μg /ml.

This suggests that the extract is inhibiting Pg-p and reducing amprenavir efflux. In the CYP inhibition assays, the IC50 values for sutherlandia extracts were above 10 µg / ml for all enzyme isoforms which implies a diminished potential for drug interactions at the tested concentrations.

In conclusion this study suggests that persons self-medicating with sutherlandia may be prone to intoxication if they are also co-medicating with drugs which are pg-p substrates e.g. HIV protease inhibitors, digoxin, cyclosporine, macrolide antibiotics, anti-neoplastics and verapamil (Lee et al, 1998; Huisman et al, 2002; Mills et al, 2005; Bauer et al, 2005). On the other hand sutherlandia does not cause clinically significant CYP enzyme inhibition.

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[SL 2A] Chemical Constituents of East European Forest Species

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Key words: *Pinus*, *Larix*, *Picea,* Pinaceae, Salicaeae, diterpenoids, lignans and stilbenoids

Introduction

FORESTSPECS is an EC 7th framework programme (FP7-KBBE-2008-2B-227239) consortium consisting of researchers from the UK (University of Surrey), Germany (Trifolio-M), Finland (Granula, VTT and University of Helsinki), Switzerland (FiBL) and Russia (FEFRI, NRIF and SPSMA). The overall aim of FORESTSPECS project is to utilize different types of wood residues from wood processing industry including *Pinus sylvestris, Pinus pumila, Picea abies, P. Ajanensis Larix gmelinii, L. sibirica, L. sukaczewii, L. decidua*, *Abies nephrolepis* (Pinaceae) and *Populus tremula* (Salicaceae) as raw materials to produce bioactive compounds and environmentally benign industrial chemicals and polymers as well as remediation chemicals.

The Pinaceae (Pine) is an ancient and important family of trees which occur in the cooler parts of the northern hemisphere, and mountains further south [Polunin, 1976]. The family consists of about two hundred and sixty species worldwide, with about eighteen native to Europe [Polunin, 1976]. The chemical composition of the wood varies from genus to genus and also between the species of the same genus. The compounds isolated from this family include, flavonoids, alkaloids, phenols, terpenoids, glucosides, phytosterols and lignans [Challen and Kucera, 1967; Slimestad, 2003]. The Salicaceae or Willow family is widely distributed in the northern hemisphere and is a typical temperate family. This family is dispersed across the whole of Europe [Ding, 1995] and consists of over six hundred and fifty species worldwide with three genera, *Chosenia Nakai, Populus L.* and *Salix L.* [Ding, 1995]. Previous chemical investigations of the plants from the Salicaceae family have resulted in the isolation of a wide range of compounds including flavonoids, terpenoids, aromatic alcohols, glycerides and steroidal compounds [Hartonen, 2007].
Chemical constituents of *Larix gmelinii*, *L. sukaczewii*, *L. sibirica*, *Pinus sylvestris*, *P. Pumila* and *Picea abies* will be presented.

Materials and Methods

The barks of ten east European forest species were collected from Arkhangelsk Territory, Northern Russia and Finland. The barks were air-dried and ground using a Glen Creston cross beater mill and extracted using a CEM Corporation, microwave assisted extraction system (MARS). Tests were performed to compare the performance of the MARS microwave extraction system against the two conventional extraction techniques, Soxhlet extraction and room temperature solvent extraction by shaking. Compounds were separated using silica gel (Merck Art. 9385)/sephadex (LH20100) packed column using different diameter-sized columns ranging from 2-6 cm depending on the amount of sample available and thin layer chromatographic techniques (Merck Art. 9385). Final purification

was carried out using 1 cm diameter column, packed with silica gel (Merck Art. 9385) in dichloromethane, dichloromethane/ ethyl acetate or dichloromethane/ methanol systems. Structural determinations were done using 1D and 2D NMR, IR and CD spectroscopy and mass spectrometry.

Results and discussion

A comparative analysis on the performance of the MARS microwave extraction instrument against traditional extraction methods of Soxhlet extraction and room temperature solvent extraction using a shaker showed that the MARS microwave can be used instead of the conventional extraction techniques while retaining the yield and composition of the extract using considerably less solvent. Several compounds have been isolated and identified from *Larix gmelinii*, *L. sukaczewii*, *L. sibirica*, *Pinus sylvestris*, *P. Pumila* and *Picea abies* including the novel labdane diterpenoid, 6β,13-dihydroxy-14-oxo-8(17)-labdene (**1**) from *L. gmelinii*, the novel 4-acetyl-2,5-dihydroxy-3-methoxy-1-methylbenzene (**2**) from *L. sibirica*, an unusual serratane triterpenoid, 3β-methoxyserrat-14-en-21-one (**3**) and bornyl ferulate (**4**) (Figure 1) from *P. pumila*. Isolated pure compounds are being tested for the following activities: insect antifeedant activity (Helsinki University), herbicidal (Trifolio-M, Germany), fungicidal (FiBL, Switzerland), antiviral and antibacterial (Helsinki University) and anti-inflammatory and antidiabetic (St. Petersburg State Medical Academy).

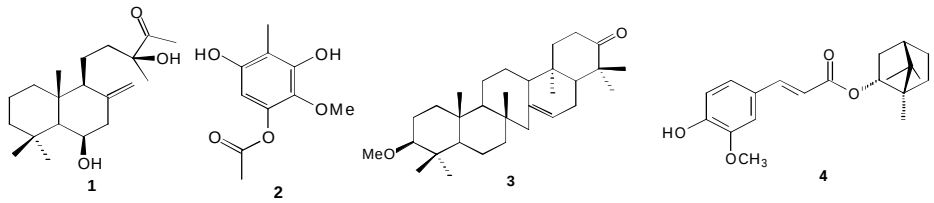

Figure 1. Compounds from *L. gmelinii*, *L. sibirica* and *P. pumila*

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[SL 2B] *In vitro* Inhibitions of Tomato Fusarium Wilt by Zearalenone from a Soil Fungus

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KEYWORDS: *Fusarium oxysporium* f. sp. *Lycosperci*, Tomato, submerged cultures, column chromatography, zearalenone

Introduction

In the course of continuous search for bioactive compounds from tropical forest which are a rich diversity of fungal genetic resources (Berdy, 2003), fungal strains were screened against *Fusarium oxysporium* f. sp. *Lycosperci* a disease of tomato in the farming fields. Synthetic chemical fungicides have been used for decades to control fungal diseases (Allen, 2004). However, the effectiveness of fungicides is threatened by development of resistance by the pathogen and in some instances there are cases of efficacy concerns. In the last 2-3 decades efforts have been reported that involve control of *Fusarium* wilt using antagonistic fungi (Nel et al., 2006; Sabuquillo,et al., 2009). These anatagonistic interactions with other fungi typically have been classified as based on antibiosis, mycoparasitism and competition for nutrients (Hjeljord, and Tronsmo, 1998; Chet and Inbar, 1994). However, the spectrum of activity of microorganisms when they are used as biological control agents is usually narrower than that of synthetic pesticides (Janisiewicz, 1996; Copping, and Menn, 2000)

Methodology

Aerial parts of infected tomato plants were collected from a greenhouse in Crops, Soils and Horticulture Department, Egerton University. The tomato fusarial causative agent was identified as *F. oxysporum* f. sp. *Lycopersici* from the cultures isolated from the infected plant and used as the test organism in the antifungal tests. The fungal strain was cultured in 35 replicates of liquid media which was prepared by dissolving 10.0g of molasses, 4.0g glucose, and 4.0g of yeast extract in 1.0L of tap water. The cultures were incubated at 25^{o}C and aerated by agitation for 21 days. The filtered fermentation broth gave 950 g of mycelium (Mex) and 30 liters of culture filtrate (Kex). Crude extract from culture filtrate were prepared using liquid-adsorption technique (Mitsubishi HP21 DIAION) packed in a glass column. The column was eluted with acetone, followed in succession by methanol.

Results and Discussion

About 2.4 g of crude extract was obtained from acetone eluent while the methanol eluent afforded 5.5 g of crude extract. However, only the acetone-eluted crude extract showed significant activity of 220.2±9.0 ppm as compared to the methanol-eluted crude extract, which had weak activity of 455±15.0 ppm, hence the latter, was not investigated. From the mycelium, 2.1 g of crude extract (Mex) was prepared using acetone, with an activity of 430±15.0 ppm, which was less active than

acetone-eluted crude. The crude extracts were subjected to silica gel chromatography and the purified compound established using two-dimensional experiments, COSY, HSQC and HMBC and zearalenone was found to be the main compound. The MIC for zearalenone was found to be 550±10.5 ppm, which was less than the antifungal activity observed for the crude extracts. The diminution may be attributed concentration of zearalenone in the crude extract and partly to synergistic effects of other compounds present. The method of processing may be responsible for the reduction as well given that from the structure of zearalenone, stability factors are evident. The antifungal activity found for zearalenone is significant and can be of scientific value in the control *Fusarium* wilt in tomato farming.

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[SL 3A] Anthocyanins from Selected Plant Species in Uganda

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Introduction

Anthocyanins comprise a diverse group of intensely coloured pigments responsible for the appealing and often spectacular orange, red purple and blue colours of many fruits, vegetables, cereal grains, flowers, leaves, roots and other plant storage organs. The most common food colorants that have been used worldwide are synthetic ones some of which are deemed to be carcinogenic. Because of this, the safety of synthetic colorants has been questioned in the past years, and this has significantly increased the interest in natural colorants as food colour additives such as anthocyanins. Today, interest in anthocyanin pigments has also intensified because of their possible health benefits related to their antioxidant properties.

Methods

The anthocyanins were isolated from the plant materials by a combination of chromatographic techniques. Their structures were elucidated by online diode array detection chromatography and homo- and hetero-nuclear Nuclear Magnetic Resonance (NMR) spectroscopy and Mass spectrometry (LC-MS) techniques.

Results and Discussion:

This presentation will give the results of anthocyanin analysis (isolation and structure elucidation) from a number of plants plant species in Uganda including the novel compounds from *Ricinus communis* (caster plant) (Byamukama et al., 2008) *Synadenium grantii* (Andersen et al., 2010) and *Plumbago auriculata* (Jordheim et al., 2010), whose structures have been elucidated recently.

In the sky-blue corollas of *Plumbago auriculata*, six new anthocyanins were isolated. All the six pigments in *P. auriculata* are based on three anthocyanidins, which for the first time in natural state are reported to have methylation of both of their A-ring hydroxyl groups. Four new together with two known anthocyanins pigments were isolated from *S. grantii.* The four were the first reported anthocyanins containing the monosaccharide apiose and the disaccharide 2''-(β-apiosyl)-β-xyloside.

Cyanidin 3-xyloside-5-glucoside and cyanidin 3-*O*-β-xylopyranoside-5-*O*-(6'''-*O*-methylmalonate-β-glucopyranoside) were new anthocyanins isolated from *Ricinus communis,* and are relatively rare anthocyanins with monosaccharide xylose linked directly to the anthocyanidin 3-position.

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[SL 3B] Effects of Botanical Insecticides on the Egg Parasitoid *Trichogramma cacoeciae* Marchal (Hym. Trichogrammatidae).

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Key words: Side effects; Trichogramm; Botanical Insecticides; Neemazal; Quassin.

Introduction

Parasitoids of the genus *Trichogramma* occure naturaly worldwide and play an important role as natural enemies of lepidopterous pests on a wide range of agricultural crops. Results of augmentative releases of *Trichogramma* can be affected by the use of broad-spectrum insecticides in or near release plots (Stinners *et al.* 1974, Ables *et al.* 1979, King *et al.* 1984). The search for selective insecticides to be used with Trichogramma releases is of great importance. The recent laboratory studies were carried out to investigate the side effects on *Trichogramma cacoeciae* of two formulated products of each of two botanical insecticides: Azadirachtine (Neemazal T/S Blank and Celaflor®) and Quassin (alcoholic or water extracts) to study there possible use with Trichogramma releases, since these insecticides are coming from plant origin they are believed also to have the advantage of having the lease impact on the environment.

Materials and Methods

Two formulations of the botanical active ingredient, azadrichtine (Neemazal T/s Blank and Celaflor) as well as two extracts of Quassin (Alcoholic and Water extracts) were included in the tests. The field recommended concentrations were prepared for the tests. The study included Exposing adults (susceptible life statgae) of Trichogramma to sprayed glass plates using the method described by Hassan et al. (2000). In other experiments adults *of Trichogramma were exposed to sprayed host eggs.* The treated host eggs were either offered directly after drying of the spray or the eggs were hold at 15 °C and offered to adults after 6 days. Less susceptible life stage (parasites within their hosts) were also exposed to tested treatments. Thhe method described by Hassan and Abdelgader (2001) was followed. The study included spraying of parasitised host eggs at different interval after parasitisation ranging from 1 – 8 days. The percentage of adult emergence and the reduction in emergence relative to the control were then determind and the pesticides were categorized accordingly.

Results and Discussion

Effects on adults

Results of tested Botanicals on adults are presented in Table (1). The results showed that by exposing adults *T. cacoeciae* to residues of Neemazal formulations on glass plates (standard test method, Hassan et al. 2000), the preparations were either harmful (Neemazal-Blank) or moderately harmful (Celaflor). The two Quassin formulations tested were harmless. In another set of experiments, where treated host eggs were offered to adults *T. cacoeciae,* all tested chemicals

were almost harmless. By exposing adults to treated host eggs both Quassin formulations were harmless. Celaflor was slightly toxic for adults, both when freshly or 6-day old sprayed host eggs were offered to adults of T. cacoeciae. Neemazal-Blank formulation was only slightly toxic when 6 day old sprayed host eggs were offered to the adults.

Table 1. Effects of exposing adult *Trichogramma cacoeciae* to various treatments

Treatment	Parasitism rate eggs/female (glass plate test)	Class	Fresh residue host eggs spraying eggs/ female	Class	6 day residue host eggs spraying eggs/ female	Class
Control	18.9 abc		28.8 bc		36.0 b	
Quassin-Alcohol	21.2 bc	1	23.1 ab	1	31.6 ab	1
Quassin-Water	22.0 c	1	33.0 c	1	33.9 b	1
Neemazal-Blank	0.0 a	4	24.0 ab	1	24.0 a	2
Celaflor	1.0 ab	3	20.3 a	1-2	23.2 a	2

** = Figures followed by the same letter are not significantly different (Multiple Range Test , 5%); SE = Standard Error; % RC = Percentage Reduction relative to the control; Class = IOBC classification

Effects on immature stages

Spraying parasitized host eggs one day after parasitism resulted in a significantly lower number of black eggs (i.e. lower pupation). All tested insecticides significantly reduced pupation, when host eggs where sprayed two days after parasitism, indicating that *Trichogramma* was very sensitive during this stage. This might have coincided with the hatching of the vulnerable neonate larvae of *Trichogramma* from laid eggs. The pupation rate was not reduced as a result of treatment, when host eggs were sprayed on the third and subsequent days after parasitism (Table 2). This trend can also be seen clearly when the percentage reduction relative to the control and the categorisation according to the IOBC classification was determined (Table 3).

Table 2. Developing Black eggs after treating parasitsed eggs at various days after parasitism

Treatment	1 day	2 days	3 days	5 days	7 days	8 days
Control	427.3 c	329.0 a	388.3 ab	465.2 ab	440.2 b	355.5 ab
Quassin-Alcohol	400.8 c	189.8 b	441.7 bc	464.2 a	420.7 b	388.5 bc
Quassin-Water	401.7 c	247.8 b	448.8 c	506.3 b	412.0 b	421.3 c
Neemazal-Blank	219.0 a	219.8 b	357.5 a	437.5 a	340.3 b	325.3 a
Celaflor	334.3 b	197.0 b	466.5 c	430.2 a	420.0 b	323.2 a
SE	17.5	20.9	19.3	26.0	19.5	20.8

** = Figures followed by the same letter are not significantly different (Multiple Range Test , 5%); SE = Standard Error

Table 3. Developing Black eggs after treating parasitsed eggs at various days after parasitism (IOBC – Classification)

Treatment	1 day		2 days		3 days		5 days		7 days		8 days	
	% RC	Class	% RC	Class	% RC	Class	% RC	Class	% RC	Class	% RC	Class
Quassin-Alcohol	6.2	1	42.3	2	-13.7	1	0.2	1	4.4	1	-9.3	1
Quassin-Water	6.0	1	24.8	1	-15.6	1	-8.9	1	6.4	1	-18.5	1
Neemazal-Blank	48.8	2	33.2	2	7.9	1	6.0	1	22.7	1	8.5	1
Celaflor	21.8	1	40.1	2	-20.1	1	7.5	1	4.6	1	9.1	1

% RC = Percentage Reduction relative to the control; Class = IOBC classification

Conclusion

The results showed, in general, that both Azadirachtine and Quassin were relatively safe to the tested parasitoid and could therefore be used in combination with *Trichogramma* releases.

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[SL 4A] Bioactive Constituents from *Hyptis suaveolens*

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Key words: *Hyptis suaveolens*, (2E)-1-(2-hydroxy phenyl) pent-2-en-1-one, 1-[(3-hydroxy-5, 5-dimethyl cyclohex-3-en-1yl) oxy] hexan-3-one, Antifeedant activity, Ovicidal activity.

Introduction

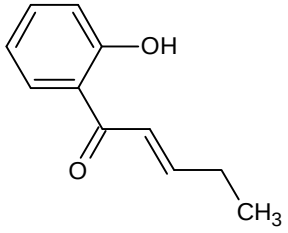
Hyptis suaveolens (L.) poit, a rigid sweetly aromatic herb belongs to the family Lamiaceae is a native of tropical America. The plant is used as green manure in India[1], the edible shoot tips are used for flavoring the dishes. In Philippines, the leaves are used for antispasmodic, antirheumatic and antisoporific baths. A decoction of the roots is used as appetizer and the root is chewed with betel nuts as a stomachic[2, 3]. The leaves are used to treat cancer ailments[4] and anti-fertility causes[5]. This plant is used for ethano botanical applications in rural communities in African countries and shows the promising results to control the *Sesamia calamistis* on Maize[6].

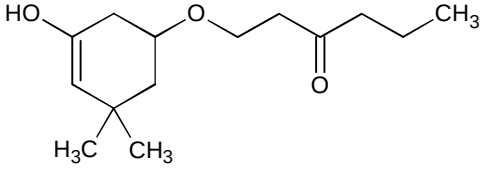
Previously isolated constituents

The presence of ethereal oil, Monoterpenes, Diterpenes, Suaveolic acid, Suaveolol, Triterpenoid, Campesterol, Fucosterol, Sesquiterpene alcohols and essential oils have been reported to be present in this plant[7].

Experimental Procedure

Shade dried, powdered leaf material (2 Kg) was subjected to sequential solvent extraction and the respective crude extract was then subjected to bio-activity studies. The crude extracts which shows promising activity alone further taken for column chromatographic isolation. Ethyl acetate crude extract (50g) showed promising activity was fractionated through flash column chromatography, using silica gel (230-400 mesh AR), column size (15cm X 100cm) using the gradient of solvent Hexane / Ethyl acetate (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 60:40, 50:50 and 100).Totally 20 fractions were obtained, each fraction was tested for its bioactivity at various concentration. Promising fractions were further studied for their bioactivity at 100, 250, 500, 1000 and 2000 ppm. Purified promising fractions were subjected to FTIR, H NMR and C NMR for identification of bioactive compounds.





(2*E*)-1-(2-hydroxyphenyl) pent-2-en-1-one (I) **1-[(3-hydroxy-5,5-dimethylcyclohex-3-en-1-yl)oxy]hexan3-one(II)**

Table1. Bioactivity of ethyl acetate extract of Hyptis suaveolens at 1000 ppm concentration

	Tested insects	
Bioactivity	*Spodoptera litura*	*Helicoverpa armigera*
Antifeedent (%)	65.3 ± 3.37	71.0 ± 1.90
Oviposition detergent	39.0 ± 3.48	24.0 ± 4.21
Ovicidal (%)	69.4 ± 2.99	65.7 ± 2.7
Insecticidal (%)	19.4 ± 2.55	11.5 ± 2.28

Values are expressed as percentage mean ± SD (n = 5).

Maximum antifeedant and ovicidal activity were recorded in ethyl acetate extract of H. suaveolens and the results are presented in Table. 1. No antifeedent and ovicidal activity was recorded in positive and negative control. Among the 11 fractions tested, fraction II and IV showed maximum antifeedent and ovicidal activity. Statistically significant antifeedant and ovicidal activity were recorded at 1000 ppm concentrations. The bioactivity of fraction II seems to be due to the presence of long aliphatic chain group containing α, β-unsaturated keto-moiety, attached to phenolic nucleus. The presence of α, β-unsaturated ketone group seems to impart synergistic activity of phenolic compound. Also, the presence of methyl residue seems to enhance the hydrophobic nature of the molecule, thereby indirectly enriching the bioactivity of the patent phenolic compound. Earlier bioactivity of polyphenolic rich fractions form the stem bark of *Streblus asper* against *Dysdercus cingulatus* has been reported[8] and several polyphenolic compounds have been reported to have insecticidal activity[9-11].

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[SL 4B] Blends of Chemicals in Smelly Feet Switch Malaria Mosquitoes on and off

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Key words: Human, foot, odours, mosquitoes, attractants, repellants

Introduction

Mosquitoes are important vectors of several tropical diseases, including malaria, filariasis, and a series of viral diseases such as dengue, Japanese encephalitis, West Nile virus, and yellow fever. Of these, malaria-transmitting species are most important. Globally, an estimated 200-300 million people are affected by malaria, of which 1.5-2.7 million die each year.

Host location in nocturnal anthropophilic mosquitoes is mediated largely by volatile human odours associated with body and breath (Mukabana et al., 2004; Okumu et al., 2010, Verhulst et al., 2010). Some studies suggest that, some humans are more attractive than others to host seeking mosquitoes (Lindsay et al., 1993; Knols et al., 1995). Human foot odour is more attractive to *An. gambiae* Giles *s.s.* than cow leg odour (Pates et al., 2001). Washing of human feet substantially reduced their attraction to the mosquito (Knols et al., 1995). Moreover, placing recently worn socks next to a blood-feeding membrane device enhanced feeding, and in turn fecundity, in *An. gambiae* and *An. stephensi* (Andreasen, 2004). These observations suggest a two-step process in the orientation behaviour of anthropophilc mosquitoes: location of hosts from some distance mediated by gross odour emanating from the body and breath and closer range avoidance of breath but attraction to preferred feeding sites that is mediated by site-specific volatiles (Suer, 2011).

In this study, we compared the attractiveness of foot odours collected on socks from 16 individuals and chemical compositions of the most and least attractive odours. Herein we report the results of our study.

Materials and methods

Masking effect of the repellents

The average catches of a counter flow geometry (CFG) trap (American Biophysics) baited with one of the following blends were compared with unbaited CFG trap under semi-field conditions in a screenhouse (11.5 x 7.1 x 3.0 m) between 20 hr in the night and 6 hr the following morning:

(i) 11-component blend of EAG-active (electrophysiologically-active) components (of isobutyric acid, isovaleric acid, 2-methylphenol, 4-ethylacetophenone, 4-ethoxyacetophenone, *n*-octanal, *n*-nonanal, *n*-decanal, *n*-undecanal *n*-dodecanal and *n*-tridecanal) in relative proportion present in typical human foot odour attractive to *An. gambiae* s.s.;

(ii) (i) minus 4-ethylacetophenone

(iii) (i) minus 4-ethoxyacetophenone

(iv) (i) minus undecanal

(v) 4-ethylacetophenone and 4-ethoxyacetophenone; and

(vi) 4-ethylacetophenone, 4-ethoxyacetophenone and undecanal.

The two traps were arranged diagonally in the screenhouse (Figure 1). The positions of the test and the control CFG traps were interchanged every following night. 200 starved female *An. gambiae* s.s. were released from a cup placed at the center between the baited trap and unbaited control trap (Figure 1) and the number of mosquitoes caught in each trap counted. Each test was replicated 8 times on different nights. The relative catch size associated with each blend was computed and analysed statistically.

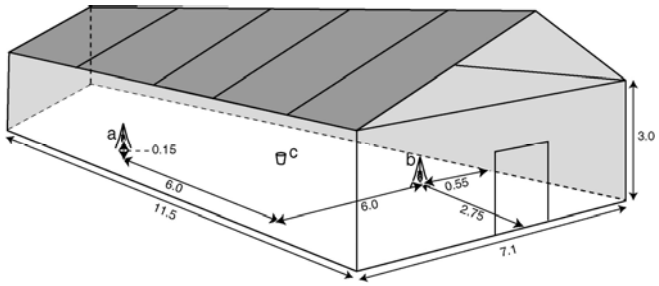

Figure 1: Sketch showing the arrangement of baited and control CFG traps in a screenhouse (a and b represent test and control CFG traps at the 2 different locations with the cup c at equidistant from the traps. All the dimensions are in meters).

Comparison of mosquito catches in traps baited with a synthetic blend and a human volunteer

The average catches of a counter flow geometry (CFG) trap (American Biophysics Corporation) baited with a synthetic blend of eight constituents that make up the attractive mixture of human foot odours (i.e. isobutyric acid, isovaleric acid, octanal, nonanal, decanal, dodecanal, tridecanal, and 2-methylphenol) was compared with those of a bed-net trap (Mathenge et al. (2002). Each comparison was performed repeatedly between 20 hr in the night and 6 hr the following morning under semi-field conditions in a screenhouse (11.5 x 7.1 x 3.0 m) also at Mbita Point on the shores

of Lake Victoria. The two types of traps in each test were located at the corners of the screenhouse and were interchanged before each replicate. 200 starved laboratory-reared female *An. gambiae* s.s. were released from a cup placed at the center between the two traps being compared and the number of mosquitoes caught in each trap counted. The relative catch sizes of each pair of traps were computed and analysed statistically.

Results and Discussion

The results of the experiment on masking effect of the repellants (Table 1) show that the presence of 4-ethylacetophenone, 4-ethoxyacetophenone and undecanal in the EAG-active 11-component foot odour blend in relative amounts found in the natural odour masks the attractiveness of the blend to the mosquito. This is reflected in higher catches in CFG traps baited with blends with one or more of these constituents missing.

Table 1. Transformed mean mosquito catches of CFG traps baited with different blends of EAG-active human foot odour blends.

Blend	Transformed mean ± SE
Blend (i)	15.86 ± 2.26^{d}
Blend (ii)	51.25 ± 7.02^{b}
Blend (iii)	27.13 ± 5.85^{c}
Blend (iv)	48.10 ± 6.01^{b}
Blend (v)	73.50 ± 2.91^{a}
Blend (vi)	69.50 ± 2.99^{a}

The CFG trap baited with the synthetic odour blend caught significantly more ($P < 0.01$; t-Test) mosquitoes with an average (± SE) of 105.3 ± 14.5 compared with 45.5 ± 5.6 in the bed-net trap.

This study has demonstrated that there is differential attractiveness of *An. gambiae s.s.* to the human feet odours. The difference in attractiveness of the human feet odours is due to the variation in the composition of the skin microflora and fauna, which lead to variation in the chemical composition of the odours emanating from the feet. The repellents and attractants identified in the human foot odour can be exploited in reducing mosquito numbers in their natural habitats in a 'push-pull' strategy wherein traps or targets attractive blends are used as baits in the 'pull' mode and repellents or repellent blends are used in personal protection or space fumigation in the 'push' mode.

Acknowledegments

This work was supported by funds from UNDP/World Bank/WHO/TDR (Grant No.) and NIH (Grant No. U19A14511-01). We thank Jactone Arija (ICIPE) for the supply of the insects, Moses Kimote (ICIPE) for his help during the bio-assays and all the sixteen human subjects for their voluntary participation in the experiments.

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[SL 5A] Non-Volatile Isolates from Two Members of the African Genus *Heteropyxis* (Myrtaceae)

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Keywords: *Heteropyxis natalensis; Heteropyxis canescens;* Psiloxyloideae; Myrtaceae

Introduction

Heteropyxis Harvey, one of two genera in the small subfamily Psiloxyloideae of the Myrtaceae (the other being *Psiloxylon* Thouars ex Tul.), comprises only three species: *H. canescens* Oliver, *H. natalensis* Harvey and *H. dehniae* Susseng (Heywood et al. 2007, Mohammed et al. 2009).

A literature survey of *Heteropyxis* revealed that only the essential oil composition of *H. natalensis* has been studied, although it is used widely in Zulu traditional medicine as a tea, a drench for stock animals, an aphrodisiac, and to treat impotence (Hutchings et al. 1996, Sibanda et al. 2004). The Vhavenda of South Africa employ the plant in the treatment of bleeding gums and noses, and for menorrhagia (Hutchings et al. 1996). No ethnobotanical usage has been documented for *H. canescens*, nor has it previously been investigated phytochemically.

The present study has considered the chemistry of two of the four subfamily members (tribe Heteropyxideae Harv.) in relation to the rest of the Myrtaceae (represented by the Myrtoideae) in view of the oftimes contentious placement of *Heteropyxis* and *Psiloxylon* in the Myrtaceae (Conti et al. 1997; refs within). *Heteropyxis* has, at various times, also been included in the Lythraceae, Rutaceae, and the monogeneric Heteropyxidaceae (Dahlgren and Van Wyk 1988), whilst the monotypic *Psiloxylon* has been placed in Bixaceae *sensu lato*, Flacourtiaceae, Myrtaceae, Guttiferae and the monogeneric Psiloxyloaceae (Schmid 1980). Darnley Gibbs (1974) earlier contemplated the familial position of *Heteropyxis,* remarking (p. 1495) that "if we knew something of the chemistry of the genus we might well be able to place it with confidence". Phytochemical data to assist in defining historical family relationships were lamented by Schmid (1980) to be meagre, and have remained so, thus providing the motivation for undertaking research on non-volatile elements in *Heteropyxis*.

Materials and Methods

Plant materials:

Heteropyxis natalensis Harvey (*N. Crouch 1057*, NH) was collected in Kloof, KwaZulu-Natal in October 2002 and *Heteropyxis canescens* Oliver (*N. Crouch & J. Burrows 1007*, NH) was collected in January 2004 at Buffelskloof Private Nature Reserve in Mpumalanga Province, South Africa.

Extraction, separation and isolation of compounds

The air-dried, milled plant material was extracted separately and successively for 24 h each, in a Soxhlet apparatus with *n*-hexane, DCM, EtOAc and MeOH. Each crude plant extract was initially fractionated by either vacuum or gravity column chromatography, generating some 250-300 fractions of 100 mL each. Fractions were pooled on the basis of their TLC profiles and further purified when necessary by further column chromatography. Separation of crude extracts was generally carried on a column using silica gel (Merck 9385). Both column and thin layer chromatographic techniques made use of varying ratios of *n*-hexane/dichloromethane, *n*-hexane/EtOAc, dichloromethane/EtOAc or dichloromethane/methanol as eluting solvents. Thin layer chromatography was carried out on 0.2 mm silica gel, aluminium-backed plates (Merck 5554). The plates were first analysed under ultraviolet light (254 and 366 nm) and then sprayed with anisaldehyde: conc. H_2SO_4: methanol (1: 2: 97) spray reagent and heated.

Characterization and identification of compounds

Compounds were characterized and identified using NMR, IR, and UV spectroscopy and LRMS or HRMS and data were compared with literature data.

Results and Discussion

The chemical investigation of the leaves and stems of *Heteropyxis canescens* and the twigs and roots of *H. natalensis* afforded a total of twenty five known compounds. Eleven known compounds, comprising two cinnamic acid esters, eicosyl *trans*-7-hydroxycinnamateand eicosyl *trans*-7-hydroxy-6-methoxycinnamate, four lupane triterpenoids, lupeol, lupenone, betulinic acid, and 3β-hydroxylup-20(29)-en-28-al, and two phytosterols, sitost-4-en-3-one and sitosterol, together with the rarely encountered 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone and 5′-methoxy-3′,4′-di-*O*-methyl-3,4-methylenedioxyellagic acid and gallic acid were isolated from the twigs and roots of this species. Fourteen known compounds were isolated from the leaves, stems and roots of *Heteropyxis canescens*: two flavanoids, strobopinin and desmethoxymatteucinol, two flavones, quercetin and apigenin, four lupane triterpenoids, lupeol, lupenone, betulinic acid, and 3β-hydroxylup-20(29)-en-28-al, an oleanane triterpenoid, arjunolic acid, three phytosterols, sitosterol-3-*O*-β-*D*-glucoside, stigmasterol and sitosterol, an ellagic acid derivative, 3,3′,4′-tri-*O*-methylellagic acid, and the phenolic acid, gallic acid (Table 1).

Table 1. Non-volatile isolates of *Heteropyxis natalensis* and *H. canescens*

Compound class	***Heteropyxis natalensis***	***Heteropyxis canescens***
Lupane triterpenoids	Lupeol lupenone betulinic acid 3β-hydroxylup-20(29)-en-28-al	lupeol lupenone betulinic acid 3β-hydroxylup-20(29)-en-28-al
Phytosterols	sitosterol sitost-4-en-3-one	sitosterol sitosterol-3-*O*-β-D-glucoside stigmsterol
Oleanane triterpenoids		arjunolic acid
C-methylated flavanoids and chalcones	,4-dihydroxy-6-methoxy- ,5-dimethylchalcone	strobopinin desmethoxymatteucinol
Ellagic acid derivatives	5-methoxy-3,4-di-*O*-methyl-3,4-methylenedioxyellagic acid gallic acid	3,3',4'-tri-*O*-methylellagic acid gallic acid
Flavonols	-	apigenin quercetin
Cinnamic acid esters	eicosyl *trans*-7-hydroxycinnamate eicosyl *trans*-7-hydroxy-6-methoxycinnamate	-

The current phytochemical investigation of the Psiloxyloideae has not revealed a significant variance from the chemical profile of the Myrtoideae (Myrtaceae). However, of the four hundred and seventy-one lupane triterpenoids reported to date from all natural sources, the Myrtoideae have yielded only four, from four species out of some one hundred and forty that have been investigated (DNP 2009). By comparison, two members of the Psiloxyloideae have currently yielded four lupane triterpenoids **(**Table 1). The phytochemical investigation of the Psiloxyloideae has not revealed a significant variance from the chemical profile of the Myrtoideae, revealing that at least on the basis of non-volatile constituents, alternative family placements of *Heteropyxis* are not presently supported (Mohammed *et al*. 2009).

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[SL 5B] Essential Oils Extracted from *Cymbopogon citratus* Leaves, *Citrus limon* and *Citrus sinensis* Peels as an Alternative to Find New Friendly Environmental Biocides

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Key words: Essential oils, *Cymbopogon citratus*, *Citrus lemon, Citrus sinensis,* antibacterial activity, *Salmonella thyphimurium, Listeria innocua, Staphylococcus aureus, Escherchia coli*

Introduction

Biocides are substances or preparations containing one or several active substances which should kill or inhibit the action from pathogen microbes (CE, 1998). The use of biocides has been undertaken over many decades and by numerous people from over the world. For example sulphur and arsenic have been used as biocides in 1000 year before Christ (Muhizi, 2008). Furthermore, during the First World War, the bioactive properties of *Pyrethrum* were accidentally discovered while those from *Tobacco*, *Derris* and *Lonchocarpus* were reported in the end of 16th century. On the beginning of 19th century, treatment of fungi was done using principally heavy metal salts containing copper, mercury and lead. Development of chemistry enabled to discover other type of biocides which are principally organic and their uses have been more intensified. According to Knight et al (2002), the world market of biocides cover approximately four milliards of US dollars and increases for 4% each year. Among these substances figure disinfectants, anti-interferences, wood protection substances, conservators substances and so on.

However, in this decade, the resistance of microorganisms towards conventional biocides is increasing (Corrégé, 2001; White et al, 2001; Prazak, 2004, Korsac, 2004,) and constitutes public health problems. For example studies have shown that among 1001 isolates of *Listeria* genus from retail foods, about 10.9% was resistant to one or more antibiotics (Walsh et al, 2001). Furthermore, Prazak *et al*. (2002) showed that about 95% of *L. monocytogenes* isolated from cabbage, water and environment samples resisted to two or more antibiotics. At the present time these different microbial resistances remarked must be highly combated to protect humanity against various diseases.

Not only the world is struggling to find solutions to resistant microorganisms but also those from the toxicity of many of the biocides used. In fact, depending on their higher toxicity some of the biocides have been removed from the market (Kamrin ,1998, GTIF, 2003, FAO/UNEP, 1996, Hanson, et al., 2007, Cooper, et al., 2008, Focant, 2002, INERIS, 2005 and 2007, CE. 2001, Gouv. F. 2004, Hughes, W. W. 1996,) and this resolution constitute a big challenge, principally to biocides users but

also to researchers. Nowadays, researchers should work hardly to discover new effective and friendly environmental biocides to replace the banned ones.

Natural organic compounds isolated from plants should be a good candidate for achieving this goal. These natural products have shown various utilities in different domains which include food, cosmetic, perfume, pharmaceutical and pesticides industries and more others (Ibrahim et al, 2001, Cimanga et al, 2002). Some authors even described some of these natural compounds as safe and strong bactericidal (Takahashi, 2002). Essential oils extracted from plants are among these natural compounds and constitute inexhaustible resources in Rwanda and in over the world.

In this study, the essential oils from *C. citratus* leaves and those obtained from the peels from *C. lemon* and *C. sinensis* were extracted, chemically characterized and evaluated on different microorganisms such us *Salmonella thyphimirium*, *Escherchia coli*, *Staphylococcus aureus* and *Listeria innocua*. We choose to work with *C. citratus* from Rwanda because of its uses as food additive and its reported biological activity (Onawunmi et al., 1984, Negrelle et al, 2007) while peels of *C. lemon* and *C. sinensis* were chosen because of their important amount found in Rwanda and their useless consideration. This study intended to valorise them by recuperating the essential oils they contain and verify their possible economic application. The biological activity study of essential oils was done for their possible application as biocides, especially in food chemistry. Note that the microbes used are generally contaminating foods and thus further studies on the obtained active essential oils should be done to verify if they can be used as food preservative compounds.

Material and methods

Plant materials

Fresh leaves of *Cymbopogon citratus* were collected from the Garden of CURPHAMETRA (Centre Rwandaise de la Recherche sur la Pharmacopée et la Médécine Tradionnelle) in the Southern Province of Rwanda while peels of *Citrus lemon* and *Citrus sinsensis* were obtained from fruits collected in Tumba sector at Huye District, Southern Province of Rwanda. All these collections were done in the beginning of dry season, precisely in the month of July.

Extraction process

In this study the hydro-distillation of fresh peels or leaves was conducted using Clevenger-type apparatus. The essential oils obtained were dried on anhydrous sodium sulphate and kept into opaque vials at 0°C for further studies.

Microbial strain and media

Staphylococcus aureus (Institut Pasteur 25923), *Escherichia coli* (Institut Pasteur 25922), *Salmonella typhimurium* (Institut Pasteur 5858) *and Listeria innocua* (ISTAB, Université Bordeaux 1) were maintained at - 4°C in 20% of glycerol. Overnight pre-cultures were performed as follows: *Staphylococcus aureus* and *Listeria innocua* were grown in Tryptose broth (Difco 262200) while

Escherichia coli and *Salmonella typhimurium* were grown in nutrient broth (Difco 234000) at 37°C for 18 h.

Antibacterial Activity Assessment

The antibacterial assessment of pure essential oils was conducted using an agar plate method performed by a diffusion method. To do this, 20 mL of culture medium prepared by mixing tryptose broth (Difco 262200) or nutrient broth (Difco 234000) with 15%(w/w) agar (Difco 215530) for *L. innocua* or *S. aureus* and *S. typhimurium* or *E. coli*, respectively, was poured into each Petri dish. Then, one-hundred microliters of 10^{-3} diluted inoculums from the microbial culture was gently spread on the surface of agar medium. Six-millimeter-diameter cellulosic disks were deposited on the agar medium surface and 10 µL of essential oil were gently deposited on the disk. Control disks without any essential oil were concurrently tested. Thereafter plates were incubated at 37°C for 24 h prior to determination of the diameters of inhibition zones surrounding the disks. Each test was performed three times and means of diameters of inhibition zones were calculated.

Gas chromatography

The essential oils were dissolved in ether prior to have a dilution of 100 times. The obtained samples were then analysed on Thermo Finnigan Gas Chromatography fitted with BP 21 SGE=FFAP column (25 m x 0.22 mm). The oven temperature was programmed from 50°C to 200°C at 4.5°C min^{-1}, the detector and injector temperatures were set at 240°C and 180°C respectively while Helium was used as carrier gas. Different internal references were used to identify essential oil components. Results obtained from GC were completed by those obtained from coupled Gas chromatography/mass spectrometry Ultra DSQ Thermo.

Results and discussion

From fresh leaves and peels, the essential oils were obtained with yields of 1.3, 0.19 and 0.16 % for *C. citratus* leaves, *C. lemon* and *C. sinensis* peels respectively. The yield from *C. citratus* was more than that found in previous reports (Onawunmi et al. (1984), 0.80-0.98% and Cimanga et al. (2002), 0.3%), but is still being in the range reported by Negrelle et al., 2007. Essential oils from *C. sinensis* and *C. limon* have been used in different studies, but no yields of these oils have been reported (Hili et al; 1996; Caccioni et al., 1998; Steuer et al, 2001). All essential oils were then analysed on GC and GC/MS to determine their chemical composition. *C. citratus* essential oil is highly composed by geranial and neral with percentages of 33.0 and 49.7% respectively (Table 1 and figure 1). These two chemical components are mainly known as trans- and cis- citral respectively. The total amount of citral found in the Rwandese *C. citratus* essential oil, 82.7%, is quite similar on what reported (Negrelle et al, 2007 (70-80%)) but higher than that reported by Cimanga et al., 2002 (32.7%). Furthermore, this oil presented also β-Myrcene and α-phellandrene with percentages of 3.8 and 2.5 respectively. The main component found from the essential oils of *C. lemon* and *C. sinensis* was limonene in the quantities of 77.5 and 83.3 respectively (Table 1 and Figures 2 and 3). These essential oils also contain β-phellandrene with percentages of 8.1 and 10.8 respectively for *C. lemon*

and *C. sinensis*. In addition, the *C. lemon* oil indicates a remarkable percentage of (Z)-hex-3-enyl acetate (6.0%).

Table 1: Chemical composition of essential oil of *C. citratus*, *C. lemon* and *C. sinsensis*

Components	*C. citratus*	*C. lemon*	*C. sinensis*
α-pinene	tr	0.8	0.5
Bicyoclo (3.1.0)hex-2-ene,4-methylene-1-(1-methylethyl)-	tr	tr	tr
Camphene		tr	
β-Phellandrene		8.1	10.8
β-Pinene	0.4	0.4	0.1
(Z)-hex-3-enyl acetate		6.0	1.3
β-Myrcene	3.4	1.5	1.3
α-Phellandrene	2.5	0.2	0.1
p-Cymene		0.5	0.1
Limonene	0.5	77.5	83.3
Eucalyptol	0.3		
3-Carene (chercher nom)		0.2	
γ-Terpinene		0.7	
Terpinolene		0.2	tr
Citronellal	1.4		
Longifolene	1.2		
Caryophyllene	0,8	1.3	
Humulene		0.3	
Citronellyl acetate	0.5		0.8
(cis)-p-(2-menthen)-1-ol			1.5
Methyl geranate	0.5		
Cis-Verbenol	0.72		
Cis-Carveol	1.19		
Trans-Citral (=Geranial)	33.0		
Cis- Citral (=Neral)	49.7		
α- Terpenyl acetate	2.4		
Total peak area	***98.51***	***97.7***	***99.8***

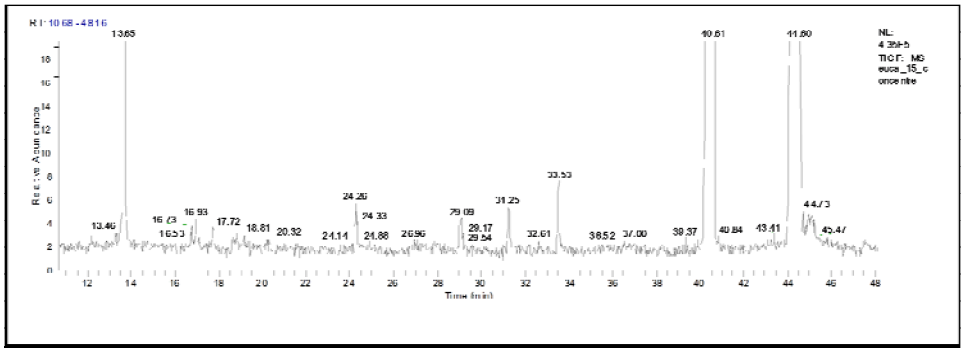

Figure 1: Chromatogram of essential oil from *C. citratus* leaves

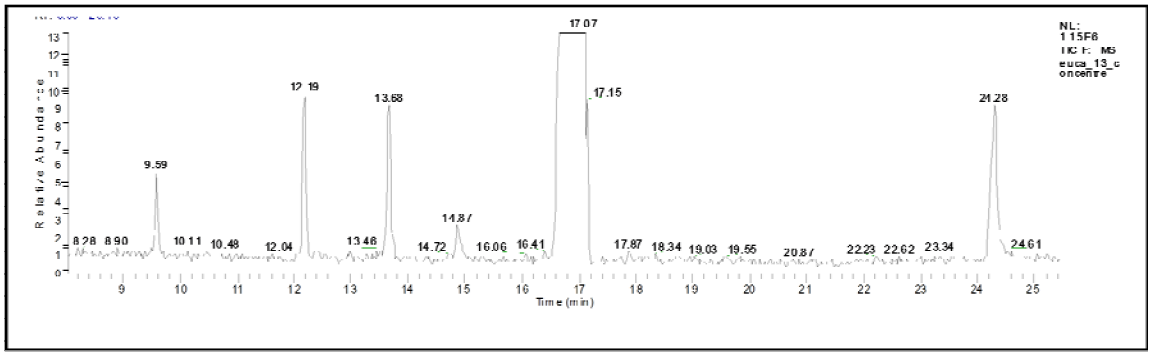

Figure 2: Chromatogram of essential oil from *C. lemon* peels

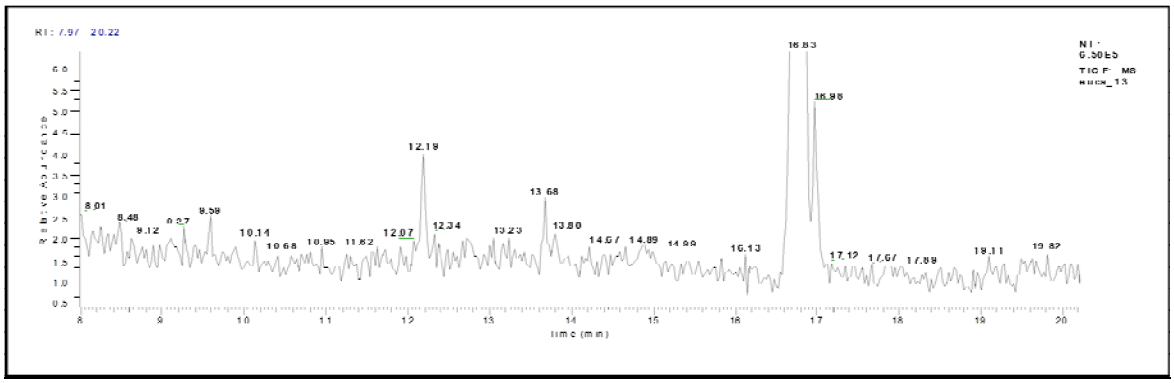

Figure 3: Chromatogram of essential oil from *C. sinensis* peels

Biological activity of these three essential oils was evaluated on four food contaminating bacteria, *E. coli*, *S. typhimurium*, *L. innocua* and *S. aureus*. Disk-diffusion method was used to assess this activity. All essential oils tested showed the antibacterial activity but at different means. The oil from *C. citratus* was the more effectiveness compared to the remaining two others. This oil inhibited the bacteria with an inhibition diameter varying from 16 to 85 mm respectively for a Gram negative bacteria, *S. typhimurium* and a Gram positive bacteria, *L. innocua* (Table 2). The antibacterial activity of the oil extracted from *C. lemon* was observed with the inhibition diameters varying from 15 mm to 18 mm for *E. coli*, a gram positive bacteria and *L. innocua*. The less active essential oil is that obtained from *C. sinensis* with an inhibition diameter of 9 mm for *L. innocua*. According to Johnson and Case (1995), the activity of biocides can be considered as effective against bacteria when the inhibition diameter is over 16 mm. In this study, the essential oil from *C.*

citratus and Citrus lemon led to an inhibition diameter close or more than 16 mm and thus they might be considered as effective against all bacteria tested. This study showed that the Gram positive bacteria were less resistant to the effective essential oils than Gram negative ones. The mechanism of action of these essential oils is not known but different sensitivity between these bacteria can be explained by the difference noted in the chemical compositions of their cell walls (Muhizi et al., 2009). Differences remarked in the antibacterial activity of essential oils are closely in relationship with their chemical composition. The more effective essential oil from *C. citratus* is mainly composed by citral (trans and cis form). The antibacterial activity of these compounds was reported in previous works (Negrelle et al, 2007). According to these authors, it is not surprising that this oil become more active than those from *C. lemon* and *C. sinensis* which don't possess any trace of citral. The essential oil from C. lemon is more active than that from *C. sinensis*. In this study we didn't be able to determine the reason of this difference since the main component, Limonene, of these two oils is found in the quite same concentration (table 1). The more potent activity of *C. lemon* may be due to the other components found in small quantity.

Table 2: Inhibition of *E. coli*, *S. typhimurium*, *L. innocua* and *S. aureus* by different essential oils

Essential oils	*E. coli*	*S. typhimurium*	*L. innocua*	*S. aureus*
C. citratus	1.9 ± 0.1	1.6 ± 0.2	8.5 ± 0.0	6.8 ± 0.3
C. lemon	1.5 ± 0.1	1.6 ± 0.1	1.8 ± 0.2	1.7 ± 0.2
C. sinensis	0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.7 ± 0.1

In conclusion, essential oils from *Cymbopogon citratus* and *Citrus lemon* showed more pronounced antibacterial activity against bacteria contaminating food and can be further studied for their possible application as food preservative.

Acknowledgements

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[SL 6A] Chemical constituents of the essential oil of *Cymbopogon proximus* and their potential for the treatment of Otomycosis

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Keywords: *Cymbopogon proximus,* automycosis, essential oil, estragole PTLC-plate

Introduction

The indigenous *Cymbopogon proximus* of the family poaeceae (Graminae) growth as wild aromatic plant in Sudan, and used locally as carminatives, stimulatives, antiseptics, and for treatment of rheumatism, cholera (Heiba, 1983). Fungal species associated with automycosis disease in Sudanese patients were *Aspergillus niger*, *Aspergillus Flavus,* and *Aspergillus aculeatus* (Elmustafa & Elmahi, 1999)

Objective

The main study objective was to test essential oil of *C. proximus* against main organisms responsible for otomycosis in Sudanese patients. Elucidating the nature of the active chemical ingredient was another target.

Material and Methods

The essential oil was prepared from the leaves of *C. proximus* by steam distillation method (Wagner, 1984). Fungal growth inhibition was obtained according to the agar plate-hole diffusion method and disc-diffusion method. Chemical analyses were carried out by FTIR, GLC, TLC, GC-MS and MS spectra.

Result and Discussion

The major fungal casual agent for automycosis in Sudan is : *A. niger* (60%), followed by *A. flavus* (30%). The extracted essential oil inhibited both of the test microorganisms in a dose-dependant manner. A dose of 20 µl/ml essential gave 100% kill for both *A. niger* and *A. flavus*. The MIC was between 0.2 and 0.6 µl/ml for *A. niger* and between 2 and 4 µl/ml for *A. flavus*. The LC_{50} was 0.6 µl/ml for *A. niger* and 8.0 µl/ml for *A. flavus* respectively. Only two out of ten components separated on PTLC-plate from leaves, of *C. proximus* essential oil, were found active against both tested microorganisms. The FTIR and MS spectra for the most active compound pointed to the presence of alcoholic compound with M.W. 198 and chemical formula : $\mathbf{C_{12}H_{18}O_1}$. According to GC-MS chromatographic analysis, the major compound of indigenous *C. proximus* essential oil is estragole (43%).

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[SL 6B] Antimicrobial Activity and Phytochemical Studies of Some Selected Medicinal Kenyan Plants

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Crude extracts of some plants species reported by herbalists to treat various bacterial and fungal complications were subjected to screening for the activity by use of various strains of microbials in vitro. The plants are *Tabernaemontana stapfiana*, *Echinops hispidus*, *Grewia similis*, *Ochna holtzii*, *Teclea nobilis* among others. The extracts that showed positive or promising activity were subjected to fractionation using separation techniques including various chromatographic methods. Structures of the obtained compounds were elucidated by use of spectroscopic techniques which included IR, MS, proton and carbon-13 NMR. 2-D NMR (COSY, NOESY, HMQC and HMBC) was employed for complete elucidation. Some of the isolated compounds were equally tested antimicrobial activity some interesting results were reported. For example, methanolic extract of *T. Stapfiana* gave an average inhibition zone of 20 mm to various bacteria strains. Compounds responsible are bis-indole alkaloids like conodurine. Crude extract of *E. hispidus* showed strong antifungal activity and compounds responsible were polyacetylene thiophenes which gave an average inhibition of 23 mm. The results call for in vivo bioassay and toxicity evaluations.

[SL 7A] Antiplasmodial and Radical Scavenging Activities of Flavonoids from Kenyan *Erythrina* species

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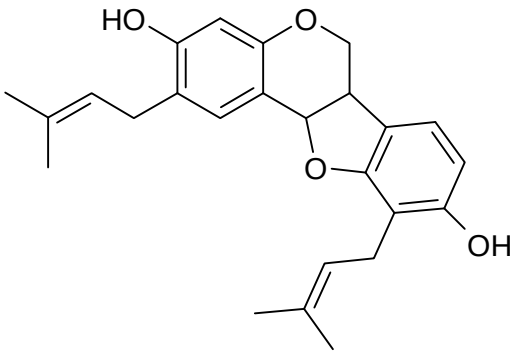
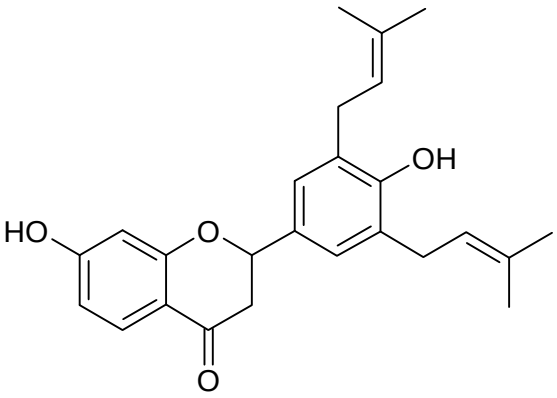
Keywords: *Erythrina*, antiplasmodial, radical scavenger, pterocarpan, flavanone, isoflav-3-ene, arylbenzofuran.

The success of quinine and artemisinin as potent natural antimalarial drugs demonstrates the importance of plants, especially those used in traditional medicine, as potential source of antimalarial agents. *Erythrina abyssinica* (Leguminosae) is one of the most widely used plants to treat malaria in East Africa. The root bark of this plant showed antiplasmodial activity against the chloroquine sensitive (D6) and chloroquine resistant (W2) strains of *Plasmodium falciparum*, with IC_{50} values of 0.64 and 0.49 μg/ml, respectively (Yenesew et al., 2003). Several compounds isolated from this plant (Kamat et al., 1981; Yenesew et al., 2003) were also tested (Yenesew et al., 2003; 2004). Activity was observed among pterocarpans (e.g. erythrabyssin-II, IC_{50} 8.1 and 6.5 μM against the D6 and W2 strains, respectively), and flavanones (e.g. abyssinone-IV, IC_{50} 9.0 and 7.7 μM against D6 and W2 strains, respectively). However the activities of these compounds individually are much lower than that of the crude extract, indicating that these flavonoids and isoflavonoids may be more effective as mixtures.



Erythrabyssin II

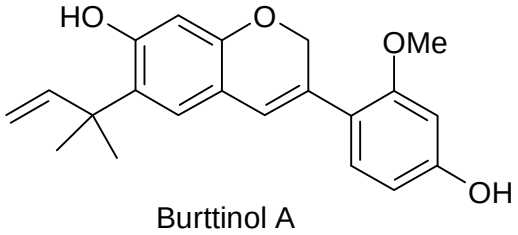


Abyssinone-IV

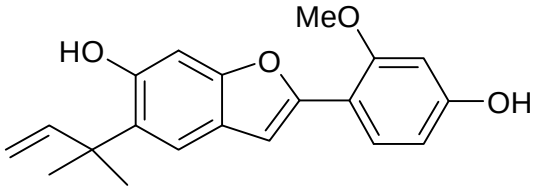
Four additional *Erythrina* species of Kenya, namely *E. burttii, E. melanacantha* and *E. sacleuxii*, have been tested for antiplasmodial activities. Among these the root bark of *E. burttii* showed good antiplasmodial activity with IC_{50} value of 0.97 and 2.0 μg/ml against the D6 and W2 strains of

Plasmodium falciparum, respectively. Flavonoids and isoflavonoids including isoflav-3-enes (eg burttinol A) and an aryl benzofuran burttinol D have been identified as the active principles. The root bark of *E. sacleuxii* was also active, with the most active compound being the isoflavone erusubin F (IC_{50} 9.0±2.1 and 7.7±1.6 μM against D6 and W2 strains respectively (Yenesew et al., 2006). In an *in vivo* assay, the extracts of the roots *E. abyssinica, E. burttii* and *E. sacleuxii* showed significant antimalarial activities against *Plasmodium berghei.*

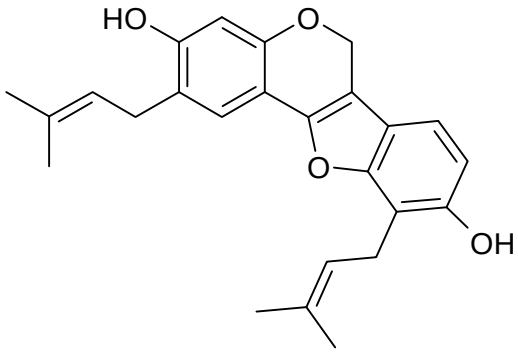
Oxidative stress normally follows malaria infection. This is due to elevated production of reactive oxygen species (Bahorun et al., 1996). It is therefore important that cells are protected from oxidative burden through the use of effective antioxidants. In a Radical Scavenging Activity (RSA) assay against 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, using spectrophotometric method, the crude acetone extract of the root bark of *E. abyssinica* at 10 μg/ml showed RSA of 82.2 %. In activity guided fractionation of the crude acetone extract, the pterocarpene erycristagallin, was identified as the most active principle with EC_{50} value of 8.2 μM (Yenesew et al., 2009). The root bark of *Erythrina burttii* also showed high RSA activity (EC_{50} values of 10.8 μg/ml) and the isoflav-3-enes burttinol A and the aryl benzofuran burttinol D have been identified as the most active principles.



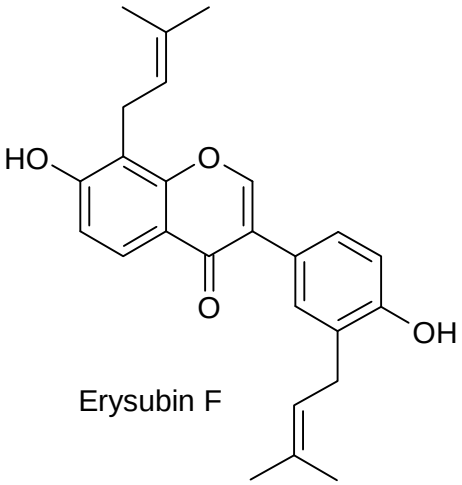
Burttinol A



Burttinol D



Erycristagallin



Erysubin F

In conclusion, the wide traditional use of *Erythrina abyssinica* for treatment of malaria in East Africa has been justified. Antiplasmodial and radical scavenging activities have also been observed in other *Erythrina* species, *E. burttii* showing the highest activity. The activities of these plants are

associated with flavonoids and isoflavonoids which appear to be more effective as mixtures rather than as pure compounds.

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[SL 7B] Inventory and Pharmacological Screening of Selected Indigenous Plant Species of Namibia for Development of New Natural Products.

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Keywords: Natural product development, Namibia, indigenous plant use, pharmacological screening, quality assurance

We present the outline and methodology of a project conducted in collaboration with Rutgers University, GIBEX, and AAMPS, which is funded by the Namibian Millennium Challenge account. This is a work in progress, having started in March 2011 and to be completed within approximately one year. We have surveyed and inventoried the utilized indigenous Namibian flora for the purpose of identifying potentially new ingredients for indigenous Namibian natural products in foods, flavors, health, nutrition, and cosmetic products in a context of local sustainable economic development. The survey and inventory has drawn from all available sources including early explorer's and other historical accounts in order to include traditional plant use which may have been lost over time (von Koenen 2008). From this inventory some 100 species are being selected for investigation based on a variety of criteria, with focus on local stakeholder's interests. Samples and herbarium specimen are being collected and uses will be verified in the field. Plant material is subjected to Screens-to-Nature technology (GIBEX 2011), where we introduce 11 portable, field-deployable pharmacological screens, and facilitate into Namibia the training associated with their use. This approach does not remove any natural resources from its country of origin for the purpose of analysis. Based on the results of the screening process, select species will be subjected to further investigation including chemical profiling, development of quality assurance standards, regional and international market assessments, sustainability (wild-crafting vs. cultivation) and feasibility of creating a product development value chain benefitting Namibian PPOs. This work will lead to a shortlist of around 10 candidate species for product development employing science-based marketing strategies and establishing public-private sector partnerships.

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[SL 8A] Isolation and Structure Elucidation of Bioactive Compounds from the Tropical Liana *Ancistrocladus congolensis*

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Keywords: Naphthylisoquinoline alkaloids · *Ancistrocladus congolensis* · michellamine · 2D NMR experiments · CD spectroscopie · oxidative degradation

Introduction

The small plant family of the Ancistrocladaceae comprises approximately 18 species in the palaeotropic regions and, up to now, one single genus named *Ancistrocladus* [Taylor *et al*. 2005]. These lianas feature hooked branches as climbing implements, and are closely related to the Dioncophyllaceae, tropical lianas with hooked leaves. The two plant families are used in traditional African and Asian medicine against dysentery, malaria, African sleeping sickness and leishmaniasis [Franois *et al.* 1997]. The bioactivity results from naphthylisoquinoline alkaloids, an extraordinary class of biaryls, only found in Ancistrocladaceae and Dioncophyllaceae species. The naphthylisoquinolines consist of a naphthalene and an isoquinoline moiety, coupled *via* a biaryl axis. The axis joins the two molecule halves at various positions and usually is rotationally hindered. Several naphthylisoquinolines, *C*,*C*-coupled ones, like dioncophylline A and C and ancistrolikokine B, and *N*,*C*-coupled ones, like ancistrocladinium B, have shown *in vitro* and *in vivo* activities against pathogens of tropical diseases.

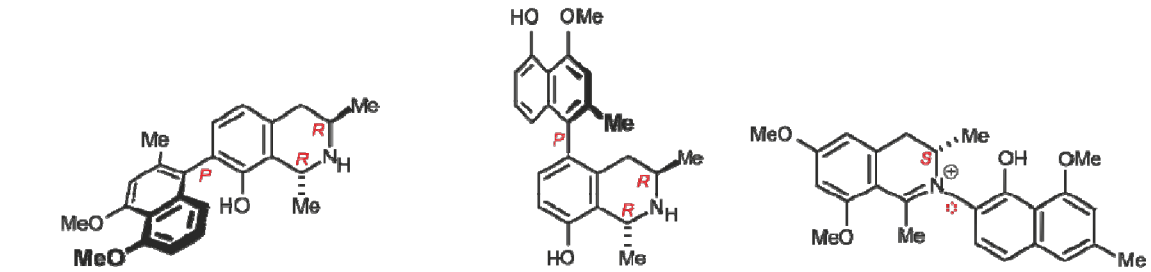

Figure 1: selected naphtylisoquinoline alkaloids with excellent bioactivities against pathogens of the African sleeping sickness, malaria, and leishmaniasis.

Previous work in our research group on *Ancistrocladus congolensis* showed the presence of numerous interesting naphthylisoquinolines and also naphthylisoquinoline dimers, which are known for their anti-HIV activity [Boyd *et al.* 1994].

Materials and Methods

Ground root bark of *Ancistrocladus congolensis* (collected in the Democratic Republic of the Congo in 2002) was extracted with methanol/dichloromethane (1:1, v/v) for several days. The solvents were evaporated in the vacuum, the resin was ground and mazerated in H_2O (dest.), supported by ultrasonic sound. The water-resin-suspension was filtered, and the water phase was extracted with dichloromethane. The organic phase was evaporated to dryness, the water phase was frozen und lyophilized. The resin from the water phase was applied to the preparative HPLC. After several isolation circles, 3 pure fractions containing monomeric naphthylisoquinolines and 7 pure fractions containing dimeric naphthylisoquinolines were gained.

Results and Discussion

The 3 monomeric naphthylisoquinolines were identified with the aid of exact mass, 2D NMR experiments, CD spectroscopie, and oxidative degradation as korupensamine A, 5'-*O*-demethyl-hamatine and an ancistrocongoline derivative (figure 2).

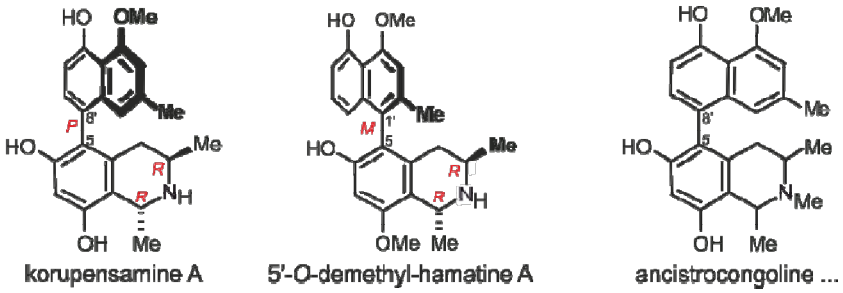

Figure 2: Korupensamine A, 5'-*O*-demethyl-hamatine, and ancistrocongoline derivative.

The first dimeric napthylisoquinoline alkaloid was identified as michellamine A (figure 3), the corresponding dimer of korupensamine A. Three of the further dimers were found to have the same mass as michellamine A, meaning to be isomers, like michellamine B and C (figure 3).

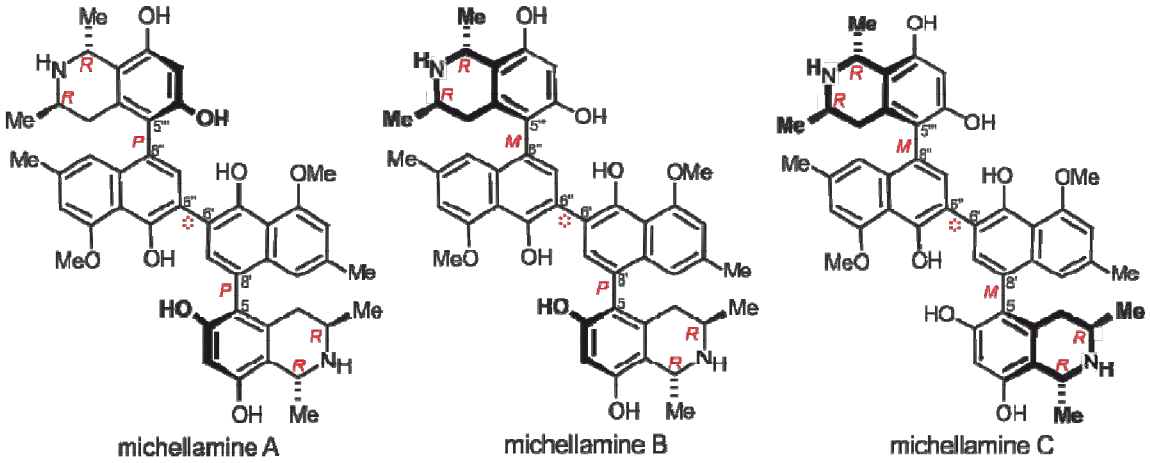

Figure 3: michellamines A, B, and C

The last two dimers corresponded to the mass m/z = 770, supposably being identical with or isomeric to michellamine D or F (figure 4).

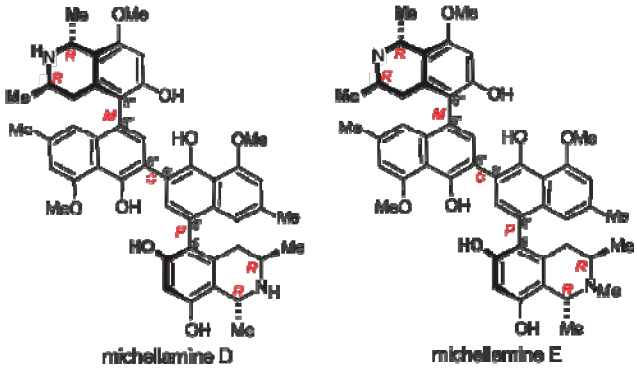

Figure 4: michellamines D and E

Acknowledgements

I thank Prof. Virima Mudogo from the University of Kinshasa (DR Congo) for the collection of *Ancistrocladus congolensis* plant material. From the University of Wuerzburg I thank Dr. Gruene and Dr. Buechner for the 2D NMR as well as the exact mass measurements. I thank B. Amslinger for CD acquisition, and M. Michel for the oxidative degradation.

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[SL 8B] The Efficacy of Extracts of the Plant Argemone Mexicana on Mosquito Species, *Anopheles arabiensis*.

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Key words: Malaria vector; insecticide resistance; Botanical extract

Introduction

The development of malaria control in irrigated Schemes of Central Sudan has gone through several phases. As a result of agricultural and irrigation practices in the Gezira, falciparum malaria transmission became perennial instead of seasonal and the mosquito vector developed resistance to several insecticides (Brown, 1986, Lee et.al., 2001, Wattal et.al.,1981). Subsequent failure to maintain control led to serious epidemics. A new control strategy of the vector is essential to help in insecticide resistance management.

Objective

In this study extracts of the plant Argemone mexicana were selected to investigate their larvicidal potential against mosquito.

Methodology

A known weight of leaf powder (range from 6 gm to 0.015 gm) was transferred to small permeable cloth cotton bags, knotted with cotton thread. The bags containing different weighs of leaf powder were placed in a fixed volume of distilled water to give different concentrations. Laboratory reared mosquito larvae species, Anopheline arabiensis were used for bioassay. Rearing was conducted under the standard conditions described by Busvine (1971) and WHO (1981). The numbers of dead larvae were assessed after 24 hours. The powder bags floated or remained close to the water surface throughout the bioassay period. The effect of pre incubation of Argemone leaf powder bags on mosquito larvicidal activity was assayed for 1, 2, 3, h etc up to 18.5 hours. The oil was extracted in n-hexane, hexane was evaporated completely and the oil was saponified according to Fadelmolla (2003) to give emulsion concentrate formulation(EC).Saponification was done by spotting 2N potassium hydroxide into freshly prepared Argemone oil.

The results showed that the LC50 and LC90 of the leaf extract were 0.16% and 0.39%, respectively. The Slop of the Ld-p line was relatively steep (5.44) indicating a homogenous response. The LC50 (0.006%) and LC90 (0.061%) of seed extract (EC formulated) were found to be lower than in leaf extract and the slope of the Ld-p line was 1.27. The mortality observed after exposing larvae of mosquito to the concentration of 0.25% of seed extract were found to be 0, 25% and 100 % after ½

h, 1.5 h and 6.5 hours of exposure. In case of leaf extract the mortality of tested larvae were 0%, 25% and 95% after 1 h, 6.5 h and 18.5 h of exposure.

Table (1a) shows the effect of pre incubation of Argemone leaf powder bags (ALPB) on mosquito larvicidal activity as assayed for 1, 2, 3, h etc up to 18.5 hours. Significant mortality was observed between 5.5 and 6.5 hours from the time of incubation of the bags. This can be taken as the time required for release of larvicidal activity from the bag. By 16.5 h from bag introduction larvicidal activity was as high as (95%). These results were largely confirmed by the data of Table (1b) where the ALPB is more incubated for 0, 24, and 48 h before testing the larvicidal activity (at interval of ½, 2, 4, 8, and 13 hours). In this separate experiment release of larvicidal active component was observed between 8 and 13 hours.

Table (1a): Effect of exposure time on response of mosquito larvae to leaf powder. Tested larvae 20. Bags containing 0.25 g each, of Argemone leaf powder/100 volume of water.

ALPB incubation h.	%Mortality
1	0
2	5
3	10
3.5	10
4.5	10
5.5	10
6.5	25
7.5	60
16.5	95
18.5	95

Table (1b): Effect of incubation time of Argemone leaf powder bags in bioassay aqueous medium

Powder bag incubation period	Bioassay observation time				
	.5h	2h	4h	8h	13h
48 hr	14.5 b	16.5 b	17.0 b	18.5 b	19.0 c
24 hr	15 b	16 b	16 b	17 b	19.0 c
0hr	0.0 a	0.0 a	0.0 a	0.0 a	13.0 b
control	0.0 a	0.0 a	2.0 a	3.0 a	3.0 a
SE + CV%	2.76 79	3.17 79	3.29 79	3.55 79	1.31 18

Bags containing 0.75g/300 ml of Argemone leaf powder were introduced onto the aqueous bioassay medium & allowed to stand for 0, 24 & 48 h.20 larvae were introduced in each jar & larval mortality was observed at interval shown (1/2, 2, 4h etc)

By 24 hours most of the ingredient was released to the aqueous medium, the 48 h incubation having little effect on the release. As for the exposure time necessary to kill larvae, it seems that the process is fast and can occurs in half an hour time (Table 2c, 24 and 48h pre incubation).

Conclusion

Larval mortality increased in a dose dependent manner. A100% mortality was reached by (0.25%) concentration .The minimum inhibitory dose was below (0.005%). Significant mortality was observed between 5.5 and 6.5 hours from the time of incubation of the bags. This can be taken as the time required for release of larvicidal activity from the bag. The fact that 48 h pre incubated medium was larvicidaly active for a further 13 hours implies good stability for more than 2 days application. Thus it seems that formulating Argemone leaf powder simply in permeable cloth bag release the larvicidaly active constituent in about 8 hours or just less. The activity remained highly effective for nearly 60 hours from introduction of bags. As for the exposure time necessary to kill larvae, it seems that the process is fast and can occur in.5 hours time. The Argemone plant produces a numerous number of seeds that separate easily during drying of shoots from the fruits. These seeds produce a fixed oil (30-40%) that was shown to have larvicidal activity when used as EC formulation.

Acknowledgment: Thanks to the Agricultural Research Corporation for providing the financial support. The help of the staff of the Blue Nile Research and Training Institute in the bioassay is highly appreciated.

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[SL 9A] Arylbenzofurans, Prenylated Flavonoids and Diels Ader Adducts with Biological Activities from *Morus mesozygia*

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Key words: *Morus mesozygia*, Moraceae, Arylbenzofurans, Diels Alder adducts, mesozygin

Introduction

Morus mesozygia Stapf. (Moraceae) is a small to medium-sized tree found in the tropical forests of Africa. The leaves and fruit provide food to the Mantled Guereza, a colobus monkey native to tropical Africa, and chimpanzee in West and Central Africa (Fashing, 2001). Traditionally, *M. mesozygia* is used to cure diabetes, arthritis, rheumatism, malnutrition, debility, stomach disorders, veneral diseases, and pain (Burkill, 1997). Recently, five new antioxidant and antimicrobial arylbenzofuran derivatives from the stem bark of this plant have been reported by us (Kapche et *al*. 2009; Kuete et *al*. 2009). Successive investigation on the bioactive constituents of *M. mesozygia* resulted in the isolation and identification of four new compounds from the leaves.

Material and Methods

General Experimental Procedures

Optical rotations were measured on a JASCO P-2000 using a Glan-Taylor Prism as Polarizer. Melting points were determined on a Buchi 535 apparatus. IR and UV spectra were recorded on SHIMADZU 8900 and Thermo-Evolution 300 spectrophotometers, respectively. EI-MS (ionization voltage 70 eV) and FAB-MS were measured on JEOL MS Route and JEOL HX 110 mass spectrometers, respectively. NMR spectra were run on Bruker AV- 400 and AV-500 MHz NMR spectrometers.

Plant Material

The leaves and trunk bark of *Morus mesozygia* Stapf. were collected in Yaoundé, Cameroon in January 2010 and identified by Mr. Nana, a botanist at the National Herbarium, Yaoundé (Cameroon), where a voucher specimen (No. 4228/SRFK) is deposited.

Extraction and Isolation

The air-dried leaves and trunk bark were ground into powder and extracted with MeOH at room temperature. Evaporation of the solvent under reduced pressure provided a MeOH extract. The EtOAc soluble part of the MeOH extract was subjected to consecutive column chromatography over silica gel and Sephadex LH-20 to give twenty compounds including six new stilbenoids: moracins O, Q- U(**1–6**) and three new Diels Alder adducts : mesozygins A- C. (**7-9**).

Phosphodiesterase I Inhibition Assay. Activity against snake venom was assayed by taking 33 mM Tris–HC1 buffer pH 8.8, 30 mM Mg–acetate with 0.000742 U/well final concentration of the enzyme using a micro titer plate assay and 0.33 mM bis-(*p*-nitropheny1) phosphate (Sigma N-3002) as a substrate. EDTA was used as a positive control ($1C_{50}$ = 274 μM $\pm$ 0.007).

Results and discussion

The MeOH extract of the trunk bark and leaves of *M. mesozygia* was subjected to consecutive column chromatography over silica gel and Sephadex LH-20 to give twenty compounds including six new stilbenoids: moracins O, Q- U(**1–6**) and three new Diels Alder adducts : mesozygins A- C. (**7-9**). Compounds 1-6 were characterized as 2-arylbenzofuran derivatives and were noted to have the following common features; their UV spectra displayed the characteristic two absorption bands in the regions 204- 237 and 290-320 nm (Nomura and Fukai, 1981). Compound **1-9** gave the expected colours upon reaction with methanolic ferric chloride confirming the presence of free phenolic hydroxyl substituent. Their IR spectra disclosed absorptions at ν_{max} ca. 3400 (OH stretch) and typical aryl absorptions and overtones from 1610-1450 cm^{-1}. The ^{1}H-NMR spectra of **1-6** showed a sharp singlet at δ 6.88- 7.19 ppm characteristic of H-3 of the 2-arylbenzofuran derivatives and resonances for aryl A_2X spin system [δ 6.88- 7.05 (2H, d, J = 2.0 Hz, H-2'/6') and 6.39- 6.49 (1H, t, J = 2.0 Hz, H-4')]. and 2,5,6-trisubstituted benzofuran moiety [δ 6.88 (1H, s, H-3), 7.26 (1H, s, H-4), and 6.91 (1H, s, H-7)] for compounds **1**,**2**,**4** and **6** (Kapche et *al*. 2009). Compounds **7**-**9** derived from Diels Alder reaction between the chalcone **10** and the flavone **11**.

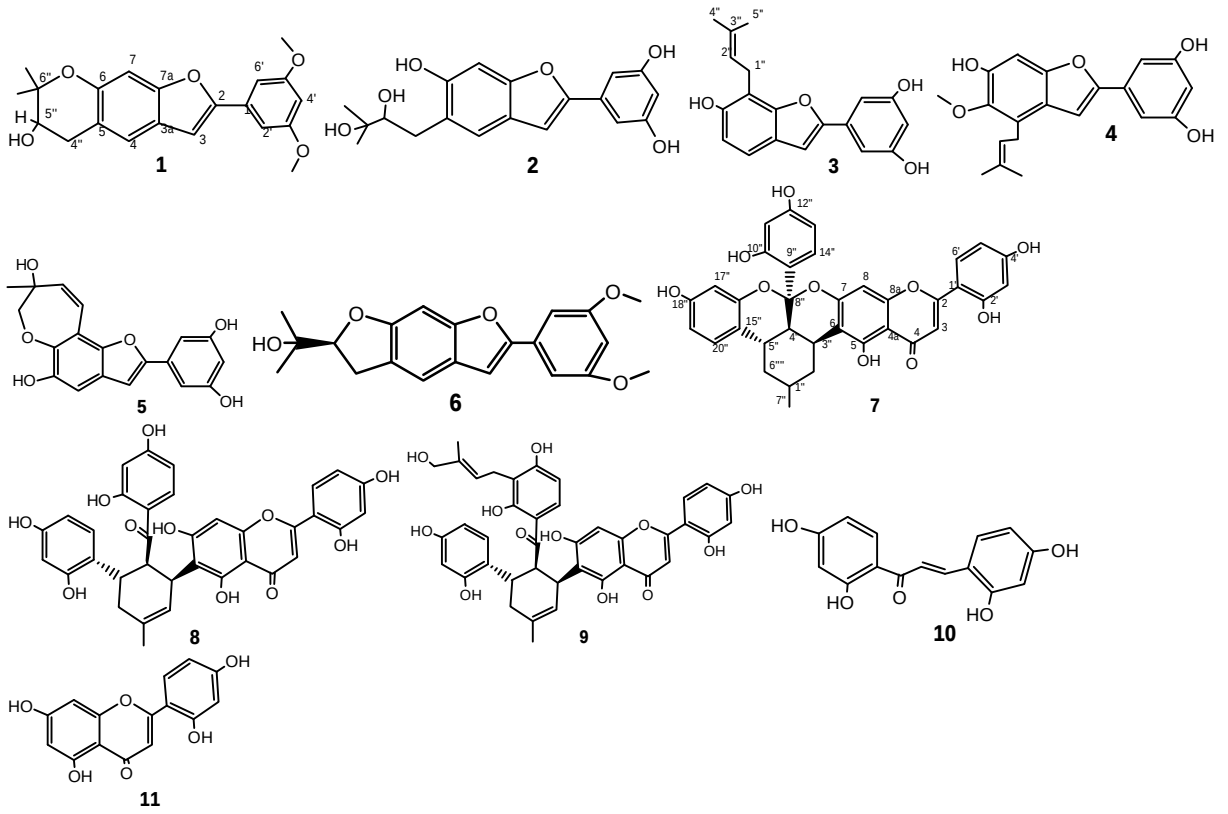
Mesozygin A (**7**), $[\alpha]_D^{28}$ + 343.2 (*c* 0.29, MeOH), displayed a quasi-molecular ion $[M+H]^+$ at *m/z* 607.1640 in the HR-FAB-MS, indicating the molecular formula of $C_{35}H_{26}O_{10}$ (calcd for $C_{35}H_{27}O_{10}$, 607.1604). The broadband-decoupled ^{13}C NMR spectrum of **7** displayed 35 resonances, which were differentiated via a DEPT spectrum as one methyl, one methylene, 15 methine, and 18 quaternary carbons. The ^{1}H and ^{13}C NMR spectra showed resonances assignable to a 6-*C*-substituted-5,7,2,4-tetrahydroxyflavone moiety [δ_H/δc 7.23 (1H, s)/107.8 (CH-3), 6.67 (1H, s)/95.3 (CH-8), 6.61 (1H, d, J = 2.4 Hz)/104.5 (CH-3'), 6.51 (1H, dd, J = 8.7, 2.4 Hz)/108.7 (CH-5'), and 7.83 (1H, d, J = 8.7 Hz)/130.7 (CH-6'); δc 163.3 (C-2), 183.7 (C-4), 106.0 (C-4a), 161.6 (C-5), 108.9 (C-6), 159.2 (C-7), 156.7 (C-8a), 110.2 (C-1'), 160.9 (C-2'), and 163.1 (C-4'); and δ_H 13.97 (1H, s, 5-OH)]. The resonances observed in the ^{1}H and ^{13}C NMR spectra at δ_H/δc 6.43 (1H, br. s)/122.2 (CH-2''), 3.42 (1H)/36.8 (CH-4''), 3.41 (1H)/34.3 (CH-3''), 2.91 (1H)/28.3 (CH-5''), 2.70, 2.03 (1H each)/36.3 (CH_2-6''), and 1.77 (3H, s)/23.8 (CH_3-7'') and δc 134.1 (C-1'') indicated the presence of a methylcyclohexene ring (Zhang et *al*, 2007). Consistent with the resonances of methylcyclohexene ring, a quaternary carbon in the ^{13}C NMR spectrum at δc 103.3 (C-8'') revealed a ketal function. Additionally, two sets of ABX system observed in the ^{1}H NMR spectrum were ascribed to two 2,4-dihydroxyphenyl rings [δ_H 7.11 (1H, d, J = 8.4 Hz, H-14''), 6.21 (1H, dd, J = 8.4, 2.1 Hz, H-13''), and 6.43 (1H, d, J = 2.1 Hz, H-11'') and δ_H 7.11 (1H, d, J = 8.7 Hz, H-20''), 6.49 (1H, dd, J = 8.7, 2.1 Hz, H-19''), and 6.37 (1H, d, J = 2.1 Hz, H-17'')]. The HMBC correlations between H-20''/C-5'' and H-2'', H-4''/C-6 revealed that the 2,4-dihydroxyphenyl and the flavones moieties were attached to the methylcyclohexene ring through $C_{3''}$-C_6 and $C_{5''}$-$C_{15''}$ bonds. Also correlations of H-14'' and H-4'' with C-8'' showed that the remaining

2,4-dihydroxyphenyl ring and the methylcyclohexene ring were connected to the ketal moiety. It is well established that structurally similar compounds possess absolute configuration of the chiral centers in the methylcyclohexene ring as *R* (C-3''), *R* (C-4''), and *S* (C-5'') for trans adducts, *S* (C-3''), *R* (C-4''), and *S* (C-5'') for cis-trans adducts, and *S* (C-3''), *R* (C-4''), S (C-5''), and *R* (C-8'') for ketal adducts; all trans adducts exhibit negative optical rotation values while the other two types show positive ones (Hano et al., 1988). Since (**7)** displayed a positive optical rotation, characteristic for cis-trans ketal adducts, the absolute configuration of the four chiral carbons were determined to be *S* (C-3''), *R* (C-4''), *S* (C-5''), and *R* (C-8''). The structure of (**7)** was established as mesozygin A.

Mesozygin B (**8**), $[\alpha]_D^{28}$ + 139.6 (*c* 0.16, MeOH), displayed a quasi-molecular ion at *m/z* 625 $[M+H]^+$ in the FAB-MS and its molecular formula, $C_{35}H_{28}O_{11}$, was established by HR-FAB-MS (*m/z* 625.1688, calcd for $C_{35}H_{29}O_{11}$, 625.1710). The ^1H and ^{13}C NMR spectral data of **8** were close to those of **7** except that a carbonyl carbon resonance (δ_C 209.0, C-8'') in **8** replaced a ketal quaternary carbon (δ_C 103.3) in **7**. As a result, the resonance of an additional hydrogen-bonded hydroxyl proton was observed at δ_H 12.49 (1H, s, OH-10''). The HMBC correlations between H-2'', H-4''/C-6 and H-20''/C-5'' suggested that the flavones and 2,4-dihydroxyphenyl moieties were connected to the methylcyclohexene ring at C-3'' and C-5'', respectively. In addition, the correlations of H-4'' and H-14'' with a carbonyl carbon at δ_C 209.0 (C-8'') supported the existence of an oxo group between the 2,4-dihydroxyphenyl and methylcyclohexene rings. The positive optical rotation observed for **8** is in favour for a cis-trans adduct as explained in discussion of **7** and supported the *S*, *R*, and *S* configuration at C-3'', C-4'', and C-5'', respectively. Thus, the structure of compound **8** was established as mesozygin B.

The HR-FAB-MS of **9** provided a quasi-molecular ion $[M+H]^+$ at *m/z* 709.2315 corresponding to the molecular formula $C_{40}H_{36}O_{12}$ (calcd for $C_{40}H_{37}O_{12}$, *m/z* 709.2285). Compound **9** was also found to be an optically active Diels-Alder adduct ($[\alpha]_D^{28}$ + 85.7 (*c* 0.4, MeOH)); its ^1H and ^{13}C NMR spectra were very similar to those of **8**, except for significant change in one of the 2,4-dihydroxyphenyl rings. This is reflected by the presence of an AB doublet [δ_H/δ_C 6.43 (1H, d, J = 9.0 Hz)/108.3 (C-13'') and 8.31 (1H, d, J = 9.0 Hz)/132.0 (C-14'')] in the ^1H NMR of **9** instead of one of the ABX spin systems in **8**. In addition, the resonances for an hydroxyisoprenyl moiety were observed in **9** The HMBC correlations of H_2-21'' with C-10'' and C-12'', and H-22'' with C-11'' (Figure 1) revealed the position of hydroxyisoprenyl at C-11'' in a 1,2,3,4-tetrasubstitutedphenyl ring. Based on the facts explained in **8**, the configuration at C-3'', C-4'', and C-5'' was determined to be *S*, *R*, and *S*, respectively, due to a positive optical rotation observed for **9**. Finally, the structure of **9** was elucidated as mesozygin C.

Compounds **7**-**9** were tested for phosphodiesterase I inhibitory activity against snake venom using EDTA as a positive control and found to be potent inhibitors compared to standard (Table 3). Compounds **8** showed the most potent phosphodiesterase I inhibitory activities [IC_{50} 8.9 μM ± 1.26 (IC_{50} for EDTA 274 μM ± 0.007)].



Acknowledgement

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[SL 9B] *In vitro* Effects of Extracts from five Malagasy Endemic Species of *Albizia* (Fabaceae) on Vegetable Seeds Germination

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Key-words: Albizia, crude extracts, purified extracts, saponins, alkaloids, inhibition, germination.

Introduction

Trees belonging to the genus *Albizia* (Fabaceae) grow in tropical areas such as Africa, Asia and South-America where they are widely used in traditional medicine (Kang et al., 2000; Zou et al., 2006; Rukunga et al., 2007). Chemical and pharmacological investigations on number of them have led to the isolation of novel structures with various properties, indicating the efficacy of the healers herb preparations.

Thus, ethanolic extract from *A. lebbeck* exhibited anticonvulsive activity (Kasture et al., 2000). The structure of cytotoxic triterpenoidal saponins from *Albizia julibrissin* was established (Zou et al., 2006). Sedative activity of flavonol glycosides from this species was reported (Kang et al., 2000). Antiplasmodial spermin alkaloids were isolated from *Albizia gummifera* (Rukunga et al., 2007). Antimicrobial activity of several species such as *A. ferruginea*, *A. gummifera*, *A. lebbeck*, was reported (Agyare et al., 2005; Geyid et al., 2005; Sudharameshwari et al., 2007). It is also of importance to note that some *Albizia* species are toxic (Gummow et al., 1992; Agyare et al., 2005)

However, reports on the effects of these species on other plants are rare. Now, high application of herbicides leads to resistance of many weeds.

In Madagascar, *Albizia* is represented by 25 endemic species. No previous report on both the chemical constituents and the pharmacological activities of these plants could be found in the literature.

The purpose of this study was to carry out assessment of the effects of extracts from five Malagasy species of *Albizia*: A_2, A_4, A_5, A_6 and A_7 on Monocotyledons and Dicotyledons. Vegetables were used in germination assays since calibrated seeds were available.

Materials and Methods

Plant materials

Collection and processing of plants

Five species of *Albizia* encoded A_2, A_4, A_5, A_6 and A_7 were used in this study. Plant parts were collected in western and southern regions of Madagascar. The organs used for each plant are shown in Table 1.

Table 1: Organs used for the 5 species

Species	**A_2**	**A_4**	**A_5**	**A_6**	**A_7**
Organs	Teguments	Empty pods	Seeds	Leaves	Seeds

Seeds (for A_2, A_5, A_7) were washed and all plant materials (seeds, pods and leaves) were sun-dried. Dried seeds (for A_5, A_7), empty pods (for A_4) and leaves (for A_6) were ground into a fine powder, using a microgrinder Culatti. For A_2, teguments were separated from almond by several cycles of grinding/sieving. Thereafter, teguments were washed to remove almond residues, sun-dried and ground into a fine powder.

Tests-seeds

Calibrated vegetable seeds used for germination tests came from the collection of *Foibe Fikarohana momba ny Fambolena* (Fofifa, Antananarivo) seed bank. For each test, experiments were carried out with one representative of Monocotyledons and one representative of Dicotyledons.

Extracts preparation

Crude extracts

Cold extraction (A_2, A_4, A_7)

Powdered dried teguments (A_2), pods (A_4) and seeds (A_7) were extracted with 75% ethanol, distilled water and 50% ethanol, respectively. Prior to extraction, A_7 powdered dried seeds were defatted by Soxhlet extraction with hexan at 45°C for 18 h.

Hot extraction (A_5, A_6)

Powdered dried seeds (A_5) were defatted by extraction with petroleum ether (60-80°C) in a Soxhlet's extractor. Using the same procedure, powdered dried leaves (A_6) were depigmented with acetone. Both defatted powdered seeds and depigmented powdered leaves were extracted with absolute ethanol, using a reflux heating system.

Purified extracts

A_2, A_5, A_6 and A_7 crude extracts were purified using methods based on solubility, molecular weight or electric charge properties of active principles.

Phytochemical screening

Extracts were subjected to preliminary phytochemical testing for the major chemical groups (Fransworth, 1966; Marini-Bettolo et al., 1981).

Assays on plants

Assays on seedlings growth

The effects of the crude (A_2, A_4, A_7) or purified (A_5, A_6) extracts were studied on epicotyl and hypocotyl growth. Seven batches of 10 seeds were soaked for 48 h at 30°C in darkness, then transfered onto Petri dishes layered with cotton. Six batches among the seven ones were germinated and regularly watered with different concentration levels of the tested extract. One

batch was watered with distilled water as a control. Epicotyl and hypocotyl lengths were measured at 2 days intervals during 14 days.

Assays on axillary bud growth

Assays were carried out on 15-day-old pea seedlings previously sectionned above the second axillary bud. Effects of extracts were compared with those of the plant growth regulators giberellin and auxin. Tested solutions (1µl) were mixed with lanolin and deposited on the top of the sectionned part. Five groups of 5 plants each were studied: group 1received 50 µg giberellin; group 2: 50 µg of auxin; groups 3 and 4: 50µg of extracts and group 5: 1µl distilled water. Axillary bud growth was measured at 2-days intervals during 10 days.

Statistical analysis

One-way analysis of variance (ANOVA) followed by Newman Keuls comparison test with Statitcf® software were used for statistical analysis. Statistical estimates were made at confidence interval of 95%.

Results and discussion

The extracts used, corresponding to the different species of *Albizia,* and the vegetable seeds tested in germination assays are shown in Table 2.

Table 2: Extracts from for the 5 species and vegetable seeds used

Plants	**A_2**	**A_4**	**A_5**	**A_6**	**A_7**
Extracts	CE = E_{23} PE = E_{24}	CE = E_{41}	PE = A_5	PE = E_6	CE = E_{71} PE = E_{72}
Seeds	Rice/Bean	Rice/Bean	Rice/Bean	Maize/Pea	Rice/White tissam

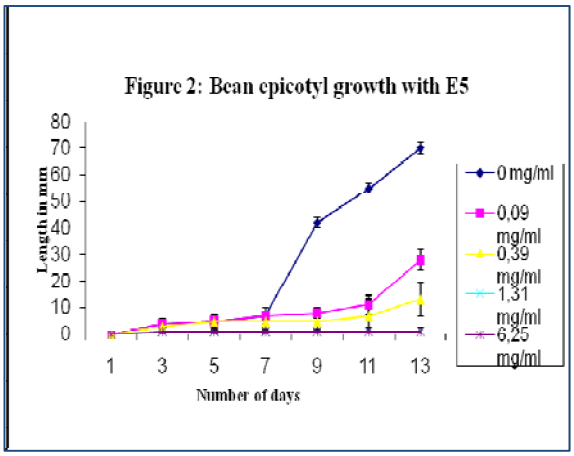
CE = Crude extract; PE = Purified extract

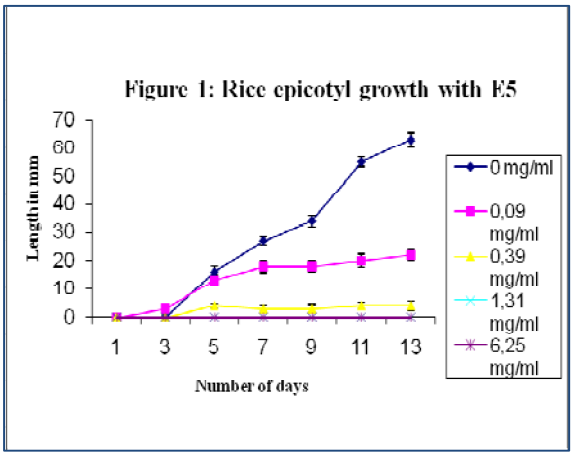
Vegetable seeds (from Monocotyledons and Dicotyledons) were chosen among those which did not germinate during preliminary tests with extracts at 1 mg/ml.

All extracts inhibited the epicotyl and hypocotyl growth of seedlings tested at the used concentration levels. However, a slight stimulation effect was exhibited by some extracts at low concentrations, such as 0.23 mg/ml and 0.46 mg/ml for E_{23} or 0.035 mg/ml for E_4. Above these concentrations, dose-effect was observed ($p<0.05$). In some cases (A_5 and A_6), epicotyl appeared to be more susceptible than hypocotyl.

E5 at 1.31 mg/ml was the most active extract as demonstrated by the total inhibition of growth noted for epicotyl of rice and beans seedlings (Figures 1 and 2).

Crude extract E_2 slightly inhibited axillary bud growth. This effect was lower than auxin at the same level. On the contrary, purified extract E_{24} exhibited no effect. Active principles were probably removed by purification.





The inhibition activity of the extracts from the various parts of the investigated plants appears to be due to saponins (A_4, A_5, A_7) or alkaloids (A_2, A_6) identified by phytochemical screening (Table 3).

In conclusion, these natural products were demonstrated to be toxic on seeds at the levels used in this study. On the other hand, these substances could be involved in plant-plant interactions, warranting studies of relationships between plants and their environment. Further investigations are necessary to determine their action mechanism, before undertaking research for the purpose of their probable use as alternatives for herbicides.

Table 3 : Phytochemical screening of extracts from species A_2, A_4, A_5, A_7, A_6

Phytochemical compounds	**Extracts**						
	E_{23}	E_{24}	E_{41}	E_5	E_6	E_{71}	E_{72}
Alkaloids	+	+	–	–	+	–	–
Flavonoids	–	–	–	–	+	–	–
Anthocyanins	+	–	–	–	–	–	–
Phenols	–	–	–	–	–	–	–
Quinons	–	–	–	–	–	–	–
Unsaturated sterols	+	+	–	+	–	+	+
Triterpenes	+	+	+	+	–	+	+
Deoxysugars	–	–	+	+	–	+	+
Saponins	–	–	–	+	–	+	+

Legend for extracts : see Table 2

Acknowledgements

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[SL 10A] Aristolactams, Indolidinoids and other Metabolites from *Toussaintia orientalis* - An Endangered Annonaceae Species Endemic to Tanzania

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Keywords: *Toussaintia orientalis*; Annonaceae; Aristolactams; Indolidinoids; Antimicrobial; Anti-inflammatory; Cytotoxicity.

Introduction

During our on-going investigations of Tanzanian Annonaceae species over the past two and half decades we have continued to focus particular interests on those species considered vulnerable to extinction (Nkunya, 2005). From our investigations as well as others reported elsewhere, it has been established that Annonaceae species are rich sources of structurally diverse natural products, some of which are also reported to exhibit wide spectra of biological activities (Leboeuf et *al.*, 1982; Nkunya, 2005). In continuation with our studies it was noted that *Toussaintia orientalis* Verdc that has almost certainly gone extinct in Kenya and can still be found only in fragmented patches of coastal forests in Tanzania is under severe threat of extinction. For example the plant can no longer be found in Pugu Forest, and probably around Ifakara in the Kilombero river valley, localities which used to be its habitats (Verdcourt, 1971; Lovett and Clarke, 1998; IUCN, 2010). Therefore, this prompted us to include *T. orientalis* (Odalo *et al*., 2010; Samwel *et al*., 2011) in our on-going investigations for chemical constituents of rarely occurring Tanzanian Annonaceae species (Nkunya, 2005). We hereby review the results from those investigations.

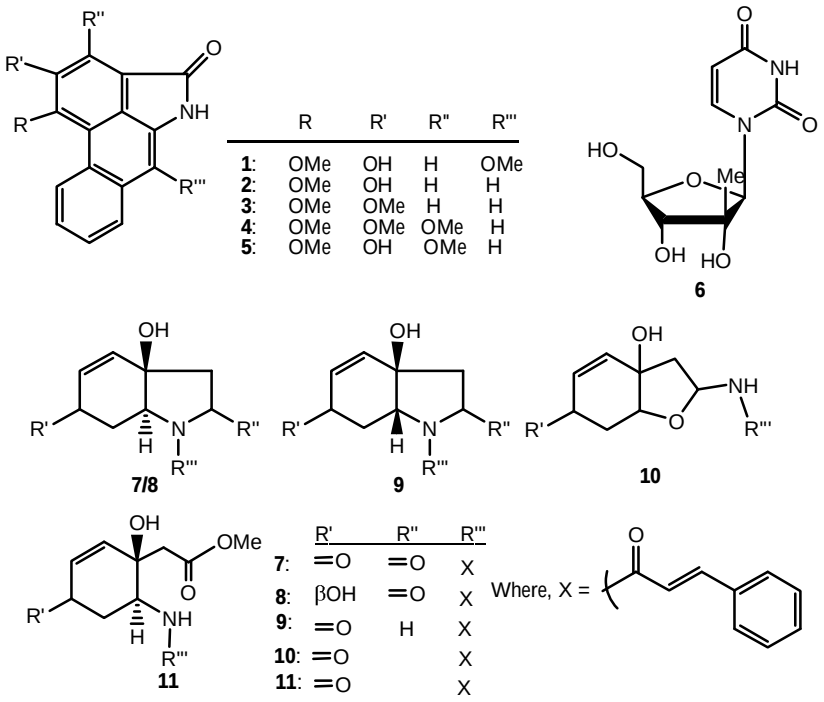
Material and Methods

The stem and root barks were collected in October 2004 while the leaves were collected in August 2007. Both collections were made from Zaraninge Forest Reserve at the edge of Saadani National Park, Bagamoyo District in Tanzania. The identity of the plant species was confirmed at the Herbarium of the Department of Botany, University of Dar es Salaam, where a voucher specimen is preserved (specimen No. FMM 3330). Extraction, chromatographic and spectroscopic methods and instrumentations are reported in Odalo *et al.*, 2010 and Samwel *et al.*, 2011. Antimicrobial assays were done as described by Perez, *et al*., 1990 and Elloff, 1998. Cytotoxicity, anti-inflammatory and antiproliferative effects were assessed as described by Meyer *et al*., 1982, Penning, 1985 and Dahse *et al*., 2001, respectively.

Results and Discussion

The cytotoxic stem and root bark extracts upon extraction and repeated chromatography (Odalo *et al.*, 2010 and Samwel *et al.*, 2011) yielded the hitherto new aristolactam alkaloid toussalactam (**1**), as well as the known ones, namely aristolactam AII (**2**), aristolactam BII (**3**), piperolactam C (**4**) and aristolactam FII (**5**); and 1-(2-C-methyl-β-D-ribofuranosyl)-uracil (**6**), 3,4,5-trimethoxyphenyl-β-D-glucopyranoside, 3,4,5-trimethoxyphenyl-β-D-glucopyranoside, 2-Hydroxy-3,4,6-trimethoxychalcone**,** 2-Hydroxy-3,4,6-trimethoxydihydrochalcone**,** (+)-Dependensin and Quercitin. The structures of the isolated metabolites were established based on extensive analysis of spectroscopic data, aprticularly 2-D NMR (HSQC, HMBC and NOESY interactions, Odalo *et al.*, 2010). The aristolactams exhibited antimicrobial, cytotoxic and antiinflammatory activities, aristolactam FII **(5)** showing almost the same level of activity as the standard anti-inflammatory agent Indomethacin. The compounds also exhibited either mild or no antiproliferative and cytotoxic activities, except aristolactam FII that showed the same level of cytotoxicity as the standard drug Camptothecin (Odalo *et al*., 2010). This was the first time for the isolation of the *pseudo*-nucleoside **6** from a plant source.

The unprecedented isolation of **6** that was previously known only as a synthetic product (Beigelman *et al*., 1987), prompted us to search for this type of compounds also from the leaves of *T. orientalis*, considered as renewable sources of that pharmacologically potent metabolite. However, the leaves yielded neither **6** nor its related compounds, nor those we obtained from the stem and root barks. Instead, the leaves yielded a series of variously cyclized aminocinnamoyl-tetraketide derivatives **7-11** (Samwel *et al*., 2011). These displayed similar spectroscopic features suggesting their structural similarities, consisting of a cinnamoyl moiety and indolidinoid or hydrobenzofuranoid, or cyclohexenoyl systems (1- and 2-D NMR, MS and IR). The compounds exhibited varying inhibitory potency and selectivity against the tested bacterial and fungal strains at 40 µg/mL concentration. The antibacterial compounds showed minimum inhibitory concentration (MIC) values that ranged between 5 and 20 µg/mL, the cynnamoylhydrobenzofuranoid **10** being the most potent (MIC = 5 µg/mL), but showing less potency compared to the standard antibiotic Ampicillin (MIC = 2.5 µg/mL).



These results once gain demonstrate the significance of the less common Annonaceae species to be important sources of bioactive compounds having unprecedented chemical structures (Leboeuf et al., 1982; Nkunya, 2005), thus justifying the need for extended efforts for their continued conservation.

Acknowledgement

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[SL 10B] From Laboratory to Field Application of Phyto-larvicides: An Outreach Community Based Experience in Bagamoyo District, Tanzania

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Keywords: Phyto-larvicide, *Annona squamosa* L., Traditional medicine, Mosquito-borne diseases, Ethno-knowledge, operational research, Livelihood improvement.

Introduction

The burden of mosquito-borne diseases such as malaria, filariasis and yellow fever continue to lead in terms of morbidity and mortality. In the absence of promising vaccine for these diseases, high cost and detrimental effects caused by synthetic chemical insecticides, plants continue to play a very important role in traditional medicine and in protection against mosquito vectors especially in African communities. Since time immemorial, the use of plants in management of mosquitoes and other insects is well recognised in traditional (through ethno-knowledge passage) and academic (through publications) circles however, not given the deserving attention. Likewise, communities represent the greatest resource available for mosquito control, but least exploited. Although, communities have knowledge on the potential of plants growing in their environment, they lack proper processing technology. Therefore, simple and cheap-technological methods of harvesting and using bioactive agents that can be adopted by individuals and communities in order to improve their livelihood are encouraged.

Objective

To create awareness to the communities in Bagamoyo District of Tanzania on the potential of *Annona squamosa* L. as accessible and affordable phyto-larvicide for mosquito control.

Material and Methods

Semi-structured questionnaires to assess knowledge, magnitude and attitude of using plants as an alternative to mosquito control was administered to individuals in the communities followed by participatory rural appraisal (PRA). Also, an outreach inspection of mosquito habitat and presence of *Annona squamosa* in selected villages was done by researchers in collaboration with village representatives.

Results and Discussion

Initial results show that, most of the individuals in the rural communities do use plants in managing mosquito population. However, very few individuals were aware of the habitants of immature mosquitoes and *Annona squamosa* as an insecticide of which detailed results will be presented.

Conclusion

Annona squamosa is used as food, medicine and insecticide. The potential of this plant species continue to be extensively studied with an average of 12-20 publications been released annually since 1990's hence its inclusion in operational research is justified.

Acknowledgement

This study was funded by DelPHE-British council. We are thankful to the support of the communities, The Office of District Medical Officer (DMO) and the District Community Development Officer (DCDO) in Bagamoyo District.

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[SL 11A] Bioactive Furanoditerpenoids, a Dibenzopyranone, Nor-isoprenoid and Biflavonoids from Medicinal *Stuhlmania moavi* Verdc.

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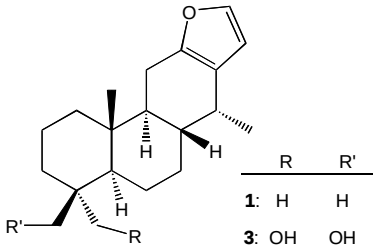
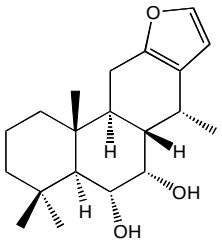
Keywords: *Stuhlmania moavi* Verdc., Ceasalpiniaceae, biflavonoids, phenolic lactone, glucopyranose, antimicrobial, anti-proliferative, cytotoxic.

INTRODUCTION

Recent investigations have revealed bioactive and novel constituents from some Tanzanian *Caesalpiniaceae* species (Freiburghaus et al., 1998; Chin et al., 2006; Kihampa, 2008). The recent investigations on the roots and stem bark extracts of the Caesalpiniaceae species *Stuhlmania moavi* that grows in Tanzania and is used for the management of skin infections revealed the presence of cytotoxic, antiproliferative and antimicrobial furanoditerpenoids voucapane (**1**), voucapane-6α,7α-diol (**2**), voucapane-18,19-diol (**3**) and 18-hydroxyvoucapan-19-al (**4**) (Odalo et al, 2009).



	R	R'
1:	H	H
3:	OH	OH
4:	OH	CH=O



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This prompted the investigation of the most redeemable part (leaves) of the plant for bioactive constituents. We thus report the isolation, structural determination, and antiproliferative, cytotoxic and antimicrobial activities of the 3-hydroxy-2,8-dimethoxydibenzo[b,d]pyran-6-one (**5**), (*E*)-4-hydroxy-4-(3-hydroxybut-1-enyl)-3,5,5-trimethylcyclohex-2-enone (**6**), 4',4''',5,5'',7,7''-hexahydroxy-3',8''-biflavone (**7**), 4',4''',5,5'',7,7''-hexahydroxy-3'',6''-biflavone (**8**) and 1,2,3,4,6-penta-*O*-galloyl-*6-D*-glucopyranose (**9**).

MATERIALS AND METHODS

Plant materials

Leaves of S. moavi were collected from Kwedijela forest in Handeni District and Wami valley below Wami Bridge on the road to Segera-Tanga in Tanzania, respectively. The plant species was

authenticated at the Herbarium, Department of Botany, University of Dar es Salaam where a voucher specimen (No. FMM 3326) is preserved.

Extraction and isolation

The air-dried, powdered plant materials were extracted sequentially at room temperature with pet ether, CH_2Cl_2 and MeOH (each 2 x 72 h). The dichloromethane extract of the leaves of *S. moavi* was subjected to repeated pet ether/EtOAc gradient elution chromatography over silica gel coupled with Sephadex® LH-20 chromatography ($CHCl_3$/MeOH, 2:3 v/v) resulting into isolation of compounds **5** and **6**. The methanol extract of the leaves of *S. moavi* when fractionated by vacuum liquid chromatography (VLC, pet ether/EtOAc gradient elution) and then separation by repeated CC yielded compound **7**. Further purification of the polar fractions of the CC by reversed phase (RP_{18}) HPLC chromatography (H_2O/MeOH) yielded compounds **8** and **9**.

Brine Shrimp Test

The crude extracts were assayed in the brine shrimp test (BST) in artificial seawater and DMSO according to standard procedures (Meyer et al., 1982) and Cyclophosphamide was used as the standard toxic agent. LC_{50} values (the concentration required to kill 50% of the shrimp larvae) were determined using Probit analysis (Finney, 1971).

Antiproliferative and cytotoxicity assays

The antiproliferative assay was carried out as described in the literature (Dahse et al., 2001) using the cell lines K-562 (human chronic myeloid leukaemia) and L-929 (mouse fibroblast) and the activity was expressed as GI_{50} values (concentration which inhibited cell growth by 50%). Cytotoxicity assay was carried out against HeLa cells and the activity expressed as GC_{50} values (concentration at which cells are destroyed by 50%; used partially in referring to the lysis of cells). For both the assays Taxol®, Colchicine and Camptothecin were used as the standard anticancer agents.

Antibacterial and antifungal assay

The agar diffusion method (Jorgensen et al., 1999) was used in the assays against microorganisms obtained from the Hans Knöll Institute for Natural Product Research and Infection Biology (HKI), Jena in Germany, Ciprofloxacin and Amphotericin being used as the standard antibacterial and antifungal agents, respectively. Antibacterial and antifungal activity was expressed as the average diameter of inhibition zones.

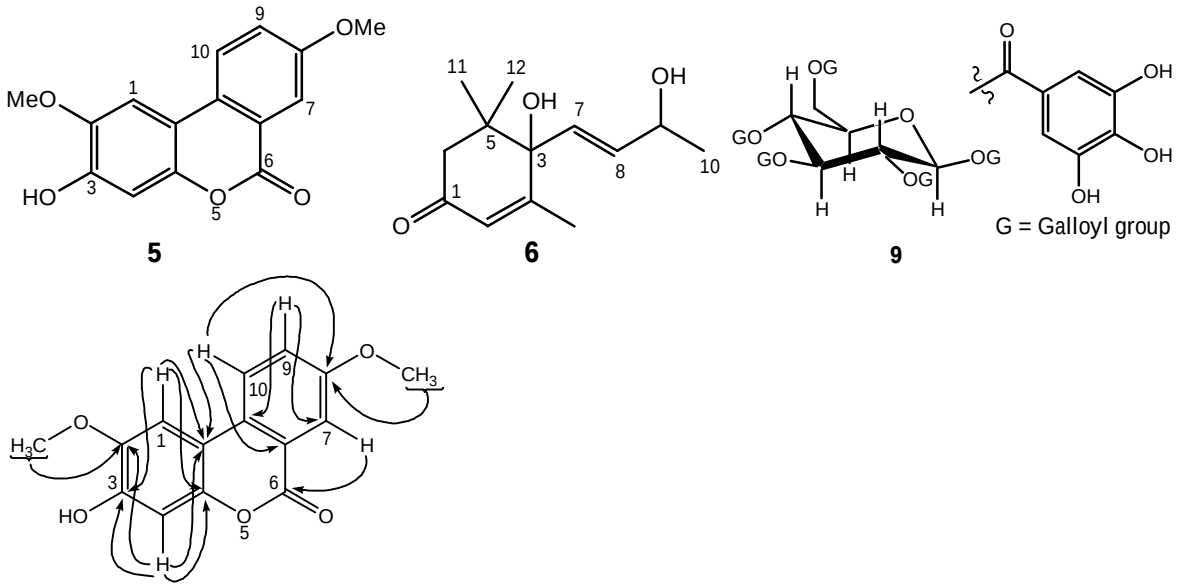
The minimum inhibitory concentration (MIC) was determined for compound **9** in a serial microplate dilution assay against each test bacterial species (Elloff, 1998), with two-fold serial dilution of the compound dissolved in DMSO, beyond the level where no inhibition of growth of the bacterial strains *Bacillus subtilis* [ATTC 6633 (IMET NA)], *Staphylococcus aureus* [SG511 (IMET 10760)], *Mycobacterium smegmatis* (SG987), *M. aurum* (SB66), *M. vaccae* (IMET 10670) and *M. fortuitum* was observed. Ciprofloxacin was used as the reference antibiotic.

RESULTS AND DISCUSSION

Repeated chromatography by silica gel and reverse phase chromatography (HPLC) of the dichloromethane and methanol extracts from the leaves of *S. moavi* resulted into isolation of the metabolites **5**-**9**.

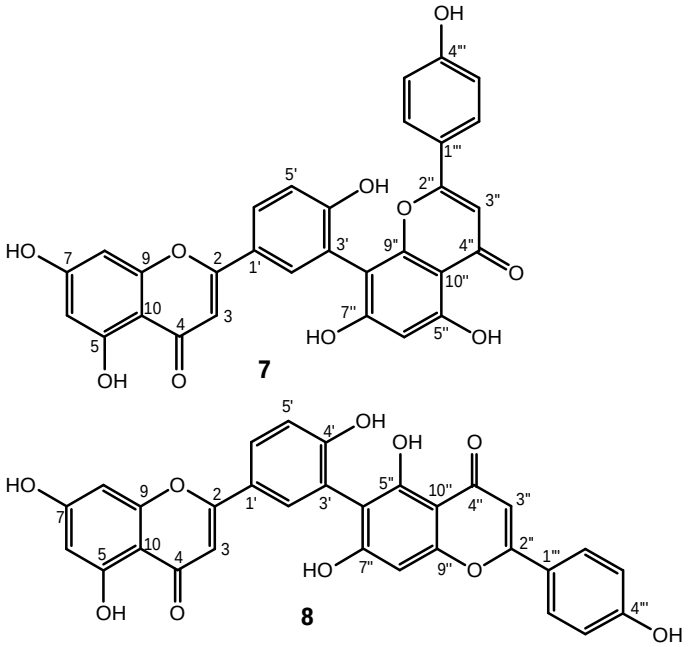
The TLC profile of the dichloromethane extract from the leaves of *S. moavi* indicated presence of a blue fluorescing compound **5**, which was isolated upon repeated chromatography. The HR-ESIMS established a molecular formula $C_{15}H_{12}O_5$ (MW 272.0685, calcd. for $C_{15}H_{12}O_5$ 272.2528) for the compound, indicating presence of 10 degrees of unsaturation. The IR spectrum showed strong absorptions at 3391, 1711, 1608, 1571 and 1491 cm^{-1}, this being consistent with the presence of phenolic hydroxyl, carbonyl and benzenoid C=C groups, respectively (Kemp, 1991). The presence of a conjugated carbonyl group was evident from the appearance of the corresponding ^{13}C NMR resonance at a particularly high field (δ 161.7). The ^{1}H NMR spectrum exhibited signals due to two methoxyl groups at δ 4.00 and 3.91 (each *s*), two aromatic proton singlets at δ 6.93 and 7.24 and signals due to three other aromatic protons forming and ABX coupling pattern (δ 7.35, *dd*, *J* = 8.8, 2.7 Hz; 7.77, *d*, *J* = 2.7 Hz and 7.86, *d*, *J* = 8.8 Hz; Table 1). The aromatic proton coupling patterns suggested the presence of a tri- and a tetra-substituted benzene moiety in the compound. The presence of the methoxyl groups at C-2 and C-8, and a hydroxyl group at C-3 was indicated by the H/C HMBC correlations observed from H-1 and H-4 to C-2, C-3 and H-10 to C-8 as well as other H/C interactions (Fig. 1). Thus, based on these spectral data the structure of the isolated compound was established to correspond to 3-hydroxy-2,8-dimethoxy-dibenzo[b,d]pyran-6-one. The MS fragmentation pattern for **5** was typical of dibenzopyranone compounds (Concannon et al., 2000) and it consisted of fragment ion peaks at *m/z* 197, 213, 214 and 229 that were attributed to cleavage of CO_2 and CO units from the parent skeleton.

The spectroscopic data for the compounds **6**-**9** agrees with those already reported in the literature (Gonzalez et al., 1994; Markham et al., 1987; Chen and Hagerman, 2004).



5 **6** **9**

Fig. 1. Important H/C HMBC correlations for 3-hydroxy, 2,8-dimethoxy-dibenzo[b,d]pyran-6-one (**5**)



7 **8**

Table 1. ^{1}H and ^{13}C-NMR spectral data for 3-hydroxy-2,8-dimethoxy-dibenzo[b,d]pyran-6-one **1.**

H/C	δ_H	*J* (Hz)	δ_C	H/C	δ_H	*J* (Hz)	δ_C
1	7.24	*s*	102.9	8	---	---	159.2
2	---	---	144.3	9	7.35	*dd*, 8.8, 2.7	124.4
3	---	---	145.7	10	7.86	*d*,8.8	122.6
4	6.9	*s*	103.8	10a	---	---	128.7
4a	---	---	147.3	10b	---	---	110.3
6	---	---	161.7	2-OMe	4.00	*s*	56.4
6a	---	---	121.3	8-OMe	3.91	*s*	55.8
7	7.77	*d*, 2.7	111.1				

The brine shrimp assay of the crude leaves extracts indicated potent levels of activity (Dichloromethane extract; LC_{50} 13.01 μg/ml), being comparable with the efficacy shown by the standard cytotoxic agent Cyclophosphamide (LC_{50} 16.33 μg/ml). Additionally, the isolated compounds from the crude extracts when assayed for *in vitro* antiproliferative and cytotoxic activity against L-929, K-562, and HeLa cell lines, respectively showed different levels of mild activity, compared with the standard anticancer agents Taxol®, Colchicine and Camptothecin. The pyranolide **5**, which is reported for the first time, demonstrated better activity as a cytotoxic agent as compared to antiproliferative activity (CC_{50} 7.9 μg/ml against HeLa cell line and GI_{50} 16.4 and 18.3 μg/ml against L-929 and K-562, respectively).

The compounds were evaluated for antibacterial and antifungal activities so as to establish possible activities that would corroborate the ethnomedicine application of the crude extracts of *S. moavi* in the treatment of skin infections. The compounds **5**-**9** were assayed for these activities *in vitro* against bacteria *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Mycobacterium vaccae*, and against the fungal species *Sporobolomyces salmonicolor*, *Candida albicans* and *Penicillium notatum*. The galloylglucoside, **9** exhibited both antibacterial and antifungal activity. The compound was active against *S. aureus* (B3), *E. coli* (B4), *M. vaccae* (M4) and *P. notatum* (P1), displaying inhibition zones of 13, 18, 19 and 14 mm, respectively.

The minimum (bacterial) inhibitory concentration (MIC) was determined for the more active compound **9** against *S. aureus*, *M. smegmatis*, *M. aurum*, *M. vaccae*, *M. fortuitum* and was found to be post potent against *M. vaccae* (MIC 12.5 μg/ml).

The demonstrated antibacterial and antifungal activities of the galloylglucoside **9** tend to further corroborate the traditional use of the crude extracts of *S. moavi* for the treatment of skin infections. It would be interesting to carry out further bioassays of combination formulations of the reported antimicrobial furanoditerpenoids from *S. moavi* and the galloylglucoside, so as to establish possible synergistic activities of the active plant metabolites.

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Financial support from Germany Academic Exchange Services (DAAD) through NAPRECA and that Sida/SAREC is highly acknowledged. We thank Mr. F. Mbago of the Herbarium, Department of Botany at the University of Dar es Salaam for identification of the investigated plant species.

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[SL 11B] *Toddalia asiatica.* Lin: A Potential Source and Model of Materials and Services for Control of Diseases and Implications for Herbal Medical Practice in Kenya.

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The systematic diseases caused by physiological problems and infectious ones caused by helminthes, protozoans , viruses, bacteria and fungi and remain terrible killer diseases in tropical Africa due to development of drug resistance to disease germs and vectors and expensive insecticides which may be used for therapeutic and insect vector management respectively. The published ethno-botanical uses, phytochemical, pharmacological and biological evaluations of *Toddalia asiatica* attracted us to investigate the potentials of this plant for the management of these diseases (Orwa et al 2008).The ethno-botanical (Kokwaro 1976, Beentje,1994) ,phytochemical (Buckingham J. 1994 , Rashid et al 1995, Ishii et al 1991) and pharmacological (Okech-Rabbah H.A. et al 2000,Gakunju et al 1995, Ishi et 1991, Heather et al 2002, Hao et al 2004, Lu et al 2005, Iwasaki et al 2006 , Guo et al 1998, Kavimani et al 1996) and larvicidal (Korir ,2002) including repellency properties rejuvenated our interest in *T. asiatica* .

This paper reports the approach that can be used as a model for the aim of validation of the traditional medicinal practices in Kenya. Thus the study guided by both ethno-botanical, pharmacological, agronomic, biological activity studies and chromatographic isolation of active principles led to identification and structural studies of many compounds which can be used as markers for efficacy, safety and quality in traditional medicinal practice by other researchers. In our own laboratory two compounds with invitro antiplasmodial activity against *Plasmodium falciparum* and essential oils with repellent activity against adults of mosquitoes as well as several essential oils with larvicidal activity against mosquito larvae of *Anopheles gambiae* were isolated and structurally elucidated. Further the extracts and isolated pure compounds of this plant exhibited interesting antibacterial and antifungal properties. This paper further discusses the implication of information being developed on *T. asiatica* that is required for purposes of implementation of the recently completed draft policy on traditional herbal medicinal practice in Kenya.

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[SL 12A] Fungitoxic *C*-18 hydroxy Unsaturated Fatty Acids from Fruiting Bodies of *Cantharellus* Species

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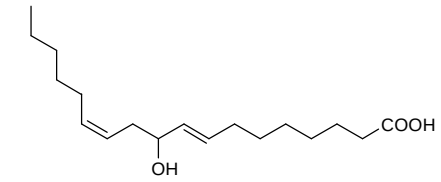
Key words: Cantharellus, fungitoxicity, unsaturated fatty acids

Introduction

The mushroom genus *Cantharellus* belongs to the fungi class Chanterellales that has been reported to consist of a number of species, some of which were only very recently described, growing wildly in the Miombo woodlands during the rainy season (HARKONEN et al. 2003, BUYCK et al. 2000). Although *Cantharellus* species are among the most popular edible mushrooms in East Africa and Northern Europe (HARKONEN et al. 2003, BUYCK et al. 2000), so far very few studies have been carried out to evaluate the constituents of *Cantharellus* and other mushroom species wildly occurring in East Africa. Recently, it was observed that there could be many mushroom species in East Africa that have not yet been scientifically documented (KARHULA et al. 1998, BUYCK et al. 2000). These studies and the fact that previous chemical investigations of mushrooms had yielded compounds with interesting pharmacological properties (KAWAGISHI et al. 1994, SIMON et al. 1995, KAWAGISHI et al. 1997, MEKKAWY et al. 1998) prompted us to analyze extracts of the *Cantharellus* species *C. isabellinus*, *C. cibarius*, *C. platyphyllus*, and *C. tubaeformis* with respect to their constituents, as part of our on-going investigations for nutritional and biologically active chemical substances from wildly occurring edible mushroom species, part of whose results on the analysis of amino acid constitution were recently published (MDACHI et al. 2004). We now report the isolation and structural determination of fungitoxic C_{18} unsaturated fatty acids from *Cantharellus isabellinus* and *C. platyphyllus.* 10-Hydroxy-8*E*,12*Z*-octadecadienoic acid (**1**), (9*Z*,14*Z*)-octadecadien-12-ynoic acid (**2**), and 9-hydroxy-10*E*,14*Z*-octadecadien-12-ynoic acid (**3**), which was previously reported as an oxidation product of **2** (PANG & STERNER 1991, PANG et al. 1992), were obtained upon HPLC separation of the extracts of the dried fruiting bodies of *C. isabellinus*, *C. cibarius*, *C. platyphyllus*, and *C. tubaeformis*. Cibaric acid (**4**) also isolated from *C. cibarius* (PANG & STERNER 1991, PANG et al. 1992) could not be observed.



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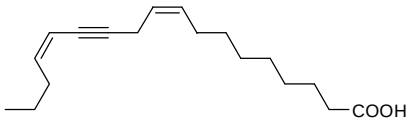

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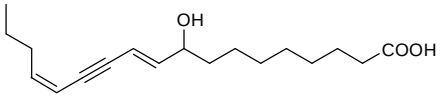

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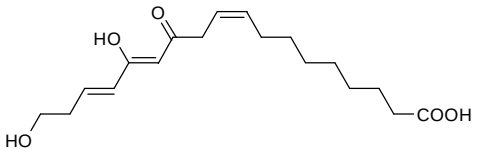

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Materials and methods

1D-NMR spectra were recorded on a Varian 300 spectrometer (300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C NMR); 2D-NMR on a Varian Inova 500 instrument at 500 MHz. Analytical HPLC was carried out on Lichrospher 100-RP 18 (5 μm) column (4 x 125 mm) using a Merck-Hitachi D-7000 system. Preparative HPLC was carried out on Lichrospher 100-RP 18 (10μm) column (10 x 250 mm) with a Merck-Hitachi L-6250 low-pressure gradient pump with L-4250 UV/Vis detector, and thin layer chromatography (TLC) on plastic or aluminium SiO_2 plates (solvent system: *n*-hexane : EtOAc 10 : 3), visualization by anisaldehyde/sulphuric acid after spraying and heating at 120ºC (blue colour). Column chromatography was performed using silica gel of particle size 230 – 400 mesh ASTM (Merck), eluting with mixtures of *n*-hexane and ethyl acetate. High-resolution ESI mass spectra (HRMS) were obtained from a Bruker Apex III Fourier Transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker Daltonics, Billerica, USA). IR spectra were measured on a Bruker IFS 28 spectrophotometer; specific rotations were measured on a JASCO DIP-1000 polarimeter. The fungitoxicity assays for crude extracts and the isolated fatty acids **1–3** were carried out according to Gottstein et al. (1982). An aliquot of a $CHCl_3$ solution of test sample (20, 50, 100 and 200 μg) was spotted on a thin layer silica gel plate (0.5 mm layer thickness) and then the plate was sprayed with an aqueous nutritive suspension of the phytopathogen *Cladosporium cucumerinum* Ell. & Arth.

Results and Discussion

Compound **1** ($C_{18}H_{32}O_3$ by HRMS) exhibited IR ((ν_{CO} = 1710, $\nu_{C=C}$ = 3025 and 1610 cm^{-1}) and NMR absorptions due to carboxylic carbonyl and olefinic functionalities, the latter showing the presence of two double bonds whose positions, as well as that of the secondary alcohol function (δ_H = 4.08, and δ_C = 72.5 ppm), were established from C/H correlations in the HMBC plot of **1**, and from the ESI-CIDMS with key ions at *m/z* 183 (base peak), 155 (CO) and 139, characterizing the position of the double bonds (MURPHY et al., 2001). The 8*E* and 12*Z* geometry of the double bond in **1** was deduced based on the magnitude of the $J_{8,9}$ and $J_{12,13}$ values (15.9 and 10.9 Hz respectively) as indicted in the ^{1}H-NMR spectrum. The rest of the ^{1}H- and ^{13}C-NMR spectral features were in agreement with those previously reported for unsaturated fatty acids similar to **1** (KOSHINO et al. 1987). The structure of (9*Z*,14*Z*)-octadecadien-12-ynoic acid (**2**, $C_{18}H_{28}O_2$, by HRMS) was established

upon comparison of its spectral features with those previously reported for similar compounds (HADACEK et al. 1987, PATIL et al. 1989). ^{1}H- and ^{13}C-NMR parameters, and the MS fragmentation pattern established the positions of the triple and double bonds, while the *Z* geometry at C-9 and C-14 was deduced from the magnitude of the corresponding ^{1}H-NMR coupling constants. 9-Hydroxy-10*E*,14*Z*-octadecadien-12-ynoic acid (**3**) displayed spectral features that indicated structural similarities of this compound with **1** and **2**, whereby the ^{1}H- and ^{13}C-NMR, IR and UV spectra of **3** exhibited absorptions due to a triple bond that is conjugated to two double bonds, one of which has a Z geometry and is vicinal to a CH_2 group while the other double bond has an *E* configuration and is adjacent to a secondary alcohol function. The ESI-CIDMS of **3** exhibited characteristic fragment ion peaks at *m/z* 119 and 171. These spectral features, as well as the long range CH correlations observed in the HMBC spectrum, established the positions of the triple and double bonds, the secondary alcohol function, and hence structure **3**. HPLC analysis of *Cantharellus isabellinus*, *C. platyphyllus C. tubaeformis,* and *C. cibarius* extracts indicated the presence of compounds **1** – **3,** and ergosterol. The fatty acids **1** - **3** exhibited mild antifungal activity against *C. cucumerinum*.

The results reported herein have shown that *Cantharellus* species are a source of fungitoxic, unsaturated C_{18}-fatty acids, which is in agreement with previous investigations that indicated *Cantharellus* mushrooms as producers of polyunsaturated free fatty acids as well as triglycerides, steroids and carotenoids (PANG & STERNER 1991, PANG et al. 1992).

Acknowledgement

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[SL 12B] Application of Vibrational Spectroscopy and Planar Chromatography in the Quality Control of South African Medicinal and Aromatic Plants

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It is reported the approximately 80% of the population of African countries rely on medicinal plants for their primary health care while 70-80% of the population of developed countries use herbs as a form of alternative or complementary medicine. Quality control procedures are vital in plant-based medicines to guarantee the authenticity and quality of products. A major challenge in quality assurance of botanicals is their complex phytochemistry. It is now widely accepted that many molecules may work in an addictive / synergistic manner and that is it short-sighted to standardize plant extracts on a couple of molecules. Profiling a greater part of the plant metabolome is hence more desirable and vibrational spectroscopy is a useful tool in this regard. Furthermore, cost and efficiency remains a determining factor in the analysis of plant extracts. The equipment used in planar chromatography has greatly advanced in the past decade and remains an indispensible chromatographic technique in the quality assessment of botanicals. Various examples ("Buchu"*, Pelargonium, Sutherlandia, Hoodia,* etc) will be discussed to show the powerful application of these two techniques to assess the quality of both the raw material and commercial products derived from indigenous South African plant.

[SL 13A] Synthesis of 2,6-Dioxo-1,2,3,4,5,6-hexahydroindoles and their Transformation into 5,8,9,10-Tetrahydro-6*H*-indolo[2,1-*a*]isoquinolin-9-ones

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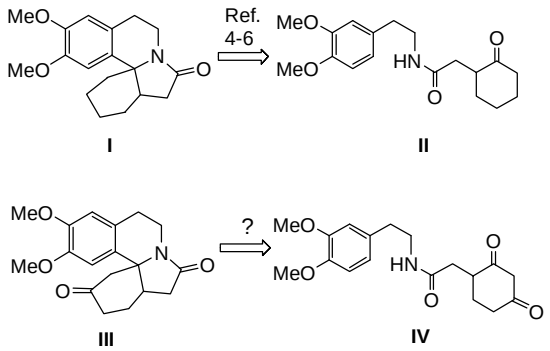
Keywords: Alkaloids; amides; C-C bond formation; cyclization; nitrogen heterocycles

Introduction

Erythrina alkaloids have been prepared through different ways but an important strategy relies on the acid-mediated domino reaction of (2-oxocyclohex-1-yl)acetic amides (Stanislawski, P. C. *et. al*. 2006, Tietze, L. F. *et. al*. 2008, Padwa, A. *et. al*. 2006).For example, spirocycle **I** has been directly prepared from the amide **II** under various conditions (Scheme 1). However, the preparative scope of this reaction is very narrow and its success strongly depends on the structure of the substrate.

To address this problem, we planned to prepare the unknown *erythrina* derivative **III**, which contains an additional carbonyl group, from the corresponding amide **IV** (Scheme 1). The carbonyl group of **III** was expected to be a useful tool for the synthesis of *erythrina*-type natural products and their non-natural analogues.



Scheme 1. Strategy for the synthesis of the novel *erythrina*-type spiro-compound **III** containing an additional carbonyl group

Experimental Section

General

Chemical shifts of the ^{1}H and ^{13}C NMR are reported in parts per million using the solvent internal standard (chloroform, 7.26 and 77.0 ppm, respectively). Infrared spectra were recorded on a FTIR spectrometer. Mass spectrometric data (MS) were obtained by electron ionization (EI, 70 eV),

chemical ionization (CI, isobutane) or electrospray ionization (ESI). Column chromatography was performed using 60 A silica gel (60 – 200 mesh). Cyclization reactions were carried out in Schlenk tubes under an argon atmosphere. Crystallographic data were collected on a Bruker X8Apex with $Mo_{K\alpha}$ radiation (λ = 0.71073 Å).

2-(1,4,8,11-Tetraoxadispiro[4.1.4.3]tetradec-12-yl)acetic acid (7a)

To a stirred water solution (250 ml) of $NaIO_4$ (15.00 g, 196.0 mmol) and $KMnO_4$ (0.63 g, 3.9 mmol) was added an acetone solution (39 ml) of **6a** (2.30 g, 11.7 mmol). The solution was stirred at room temperature until a colour change from violet to red was observed. The solution was then extracted with EtOAc (3 x 100 ml) and the combined organic layers were dried ($MgSO_4$). The solution was filtered and the filtrate was concentrated *in vacuo* to give **7a** (1.61 g, 65%) as a light brown gummy substance which required no further purification.

Typical procedure for the synthesis of amides 10

To a CH_2Cl_2 solution (20 mL) of **7a** (200 mg, 0.8 mmol) was added *N*-hydroxysuccinimide (88 mg, 0.78 mmol) and dicyclohexylcarbodiimide (162 mg, 0.8 mmol) at 0 °C and the mixture was stirred for 1 h at the same temperature. After stirring for 12 h, the mixture was filtered, 1-amino-2-phenylethane (0.01 mL, 0.85 mmol) was added to the filtrate and the mixture was stirred for 2 h. The mixture was filtered and washed for several times with water (50 mL for each washing). The organic layer was dried ($NaSO_4$), filtered and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (silica gel, heptanes/EtOAc) to give **10k** (180 mg, 64%) as a colourless solid.

General procedure for the synthesis of 11a-z

An acetone solution of amide **10** and of a catalytic amount of *p*-toluenesulfonic acid (PTSA) was heated under reflux for 6 h. The solution was cooled to 20 °C and concentrated in vacuo to give a solid residue which was purified by column chromatography (silica gel, heptanes/EtOAc).

2,3-Dimethoxy-5,8,10,11-tetrahydroindolo[2,1-*a*]isoquinolin-9(6*H*)-one (14a)

A CH_2Cl_2 solution (16 ml) of **11o** (150 mg, 0.5 mmol) and of TfOH (0.7 mL) was heated under reflux for 4 h, cooled to room temperature, and quenched with water. The aqueous layer was extracted with $CHCl_3$ (3 x 80 ml) and the combined organic layers were dried ($MgSO_4$). The solution was filtered and the solvent of the filtrate was removed under reduced pressure. The residue was purified by flash chromatography (silica gel, heptanes/EtOAc) to give **14a** (120 mg, 84%) as white crystals which proved to be unstable at room temperature.

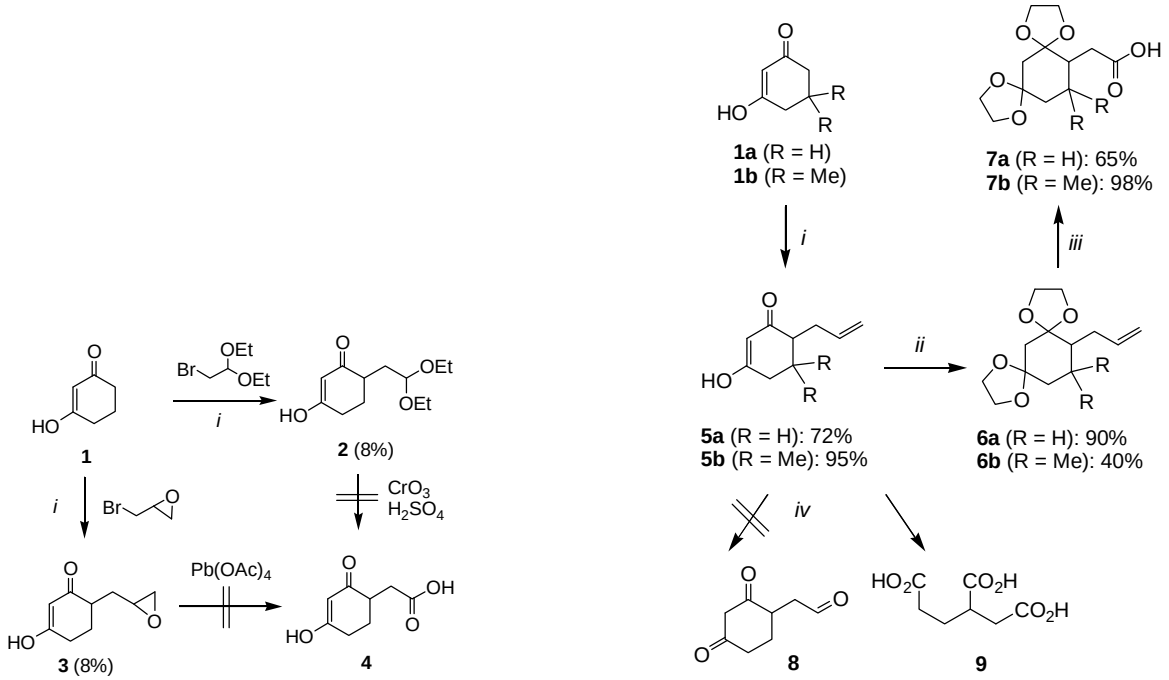
1,4,5,10,11,12,13,13a-Octahydro-7,8-dimethoxy-2*H*-indolo[7a,1-*a*]isoquinolin-2-one (12).

The synthesis was carried out following the procedure as given for the synthesis of **14a**. Starting with **10x** (380 mg, 1.2 mmol), PTSA (5 mg, 0.02 mmol) and dry acetone (70 mL), **12** (309 mg, 86%) was isolated as a colourless viscous oil.

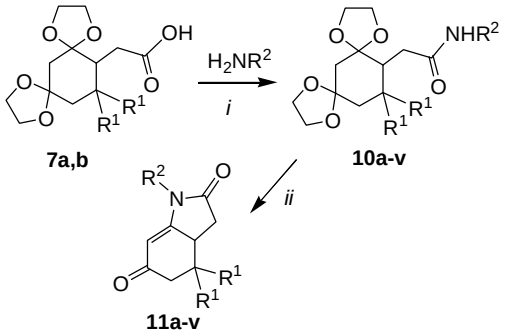
Results and Discussion

The synthesis of (2,4-dioxocyclohex-1-yl)acetic acid (**4**), despite its structural simplicity was done for the first time by our group. Accessing it through reactions of cyclohexane-1,3-dione (**1**) with 1-bromo-2,2-diethoxyethane and epibromohydrin and similar bromides failed.

Ozonolysis of 4-allylcyclohexane-1,3-dione (**5a**), following previous reports(Guay, B. et al. 2003), prepared by reaction of the dianion of **1a** with allylbromide, (Zhang, W. *et al* 2003, **59**) afforded the triacid **9** rather than the desired aldehyde **8**. The formation of **9** can be explained by oxidative cleavage of the enolic double bond. The problem was solved by protection of the carbonyl groups of **5a** to give the bis(acetal) **6a**. The oxidation of **6a** by $KMnO_4/NaIO_4$ (in acetone) afforded the acid **7a**. Likewise, derivative **7b** was prepared in three steps from **1b**. The bis(acetal) **7a** can be deprotected to give the desired (2,4-dioxocyclohex-1-yl)acetic acid (**4**) which, however, proved to be unstable. Therefore, bis(acetals) **7a,b** were directly used for all further transformations.



The DCC-mediated reaction of **7a** with various amines afforded the **10a**-like amides. Reflux of an acetone solution of **10a** in the presence of *para*-toluenesulfonic acid (PTSA) afforded the 2,6-dioxo-1,2,3,3a,4,5-tetrahydroindole **11a**. The formation of an *erythrina*-type spiro-compound, such as **III** (see Scheme 1), was not observed.



The structures of all products were established by spectroscopic methods. Some structures e.g. **10o**, **11o**, and **11e** were independently confirmed by X-ray crystal structure analyses (Figures 1-2).[6]

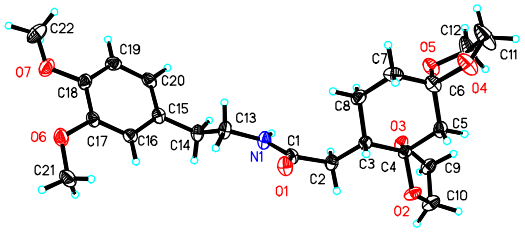


Figure 1. Ortep plot of **10o (**50% probability level)

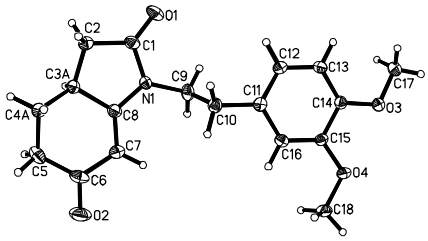
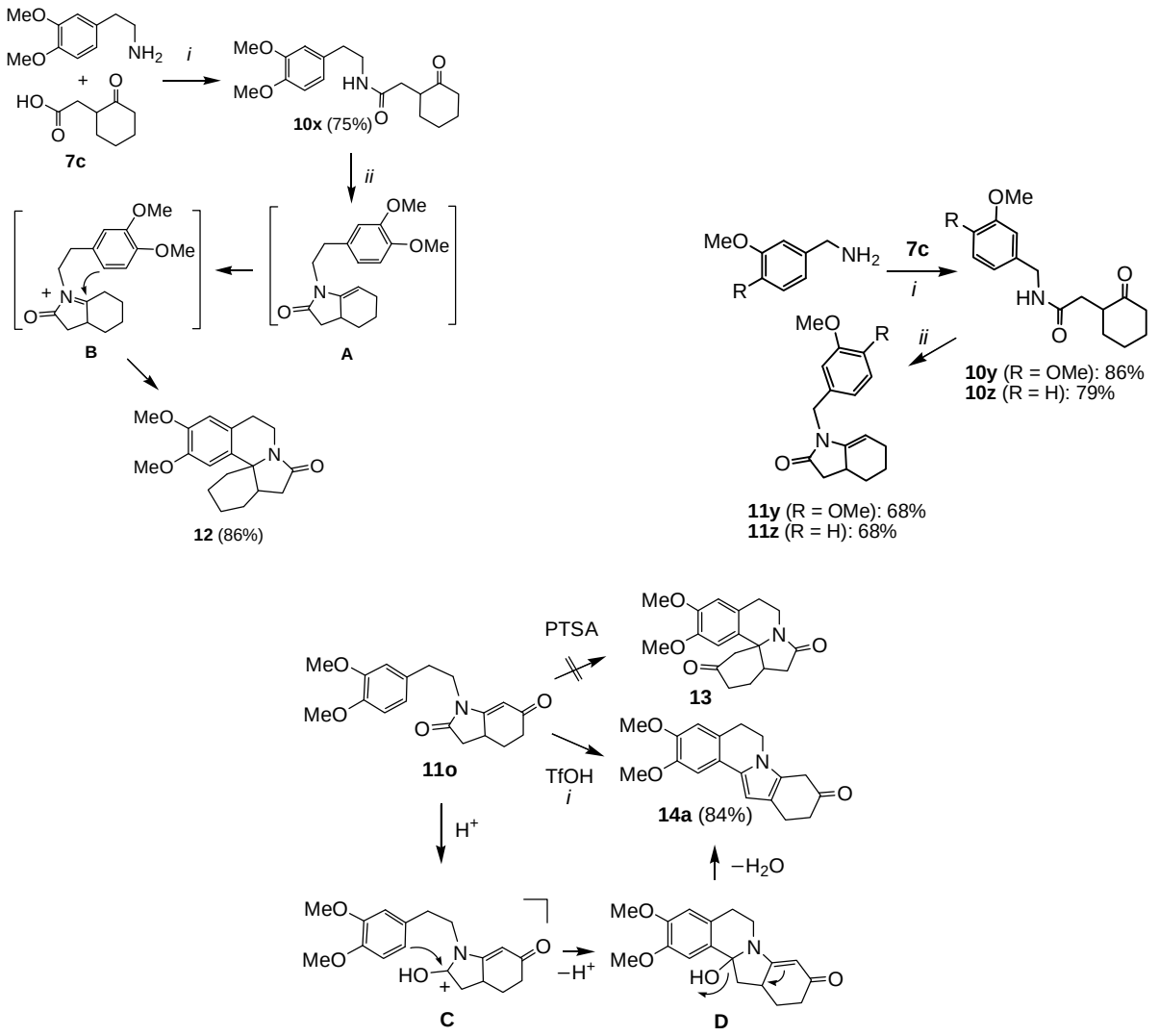


Figure 2. Ortep plot of **11o** (50% probability level)

For comparison, we studied the reaction of PTSA with amide **10x** which contains one free carbonyl group. The reaction of **10x** with PTSA afforded the *erythrina*-type spiro-compound **12** in excellent yield. The formation of **12** can be explained by acid-mediated reaction of the keto group with the electron-rich phenyl group to give intermediate **A**, protonation of the enamine moiety to give iminium salt **B**, and subsequent Pictet-Spengler reaction. It is important to be noted that this reaction is not general: The reaction of PTSA with amides **10y,z**, again prepared from **7c** in good yields, afforded the 2-oxo-1,2,3,4,5,6-hexahydroindoles **11y,z** rather than the expected spirocyclic products. This can be explained by the higher strain of a 5,5,6- compared to a 5,6,6-spirocyclic system.

Our next plan was to study the transformation of 2,6-dioxo-1,2,3,4,5,6-hexahydroindoles **11** into *erythrina*-type spirocycles, such as **III**, under more forcing conditions. Heating of 2,6-dioxo-1,2,3,4,5,6-hexahydroindole **11o** in the presence of PTSA for an extended period of time (48 h) did not result in any conversion. The reaction of **11o** with triflic acid (TfOH) afforded the 5,8,9,10-tetrahydro-6*H*-indolo[2,1-*a*]isoquinolin-9-one **14a** (84% yield) rather than the *erythrina*-type spirocycle **13**. The formation of **14a** can be explained by protonation of the amide oxygen atom to give the cationic intermediate **C**, cyclization via the electron-rich aryl group (intermediate **D**), and subsequent extrusion of water and double bond migration.



In conclusion, we have reported the synthesis of the first (2,4-dioxocyclohex-1-yl)acetic amides. Their reaction with PTSA provides a general method for the synthesis of 2,6-dioxo-1,2,3,4,5,6-hexahydroindoles. The reaction of the latter with triflic acid afforded 5,8,9,10-tetrahydro-6*H*-indolo[2,1-*a*]isoquinolin-9-ones rather than *erythrina*-type spirocycles.

Acknowledgements:

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6. CCDC-xxx contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[SL 13B] Medicinal Plants of East Africa- *Importance, Uses in Traditional Medicine, Challenges and Conservation Status*

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Medicinal plants are important part of "Traditional Health Systems or Primary healthcare systems" in most of the East African Region. Where access to clinics dispensing modern medicine is either, limited or prohibitively costly; affordable traditional herbal remedies are still the primary means of meeting the health and medical needs of most rural communities. According to World Health Organization, of the 700 million population of sub Saharan Africa, fewer than 30% have access to modern health care and pharmaceuticals. Remaining 70% people still depend entirely on traditional herbal remedies. WHO has listed 10,000 medicinal plant species that people use regularly on the African continent.

Common ailments such as stomach problems, diarrhoea, skin infections, infected wounds and sores, fevers, colds, coughs; parasitic diseases such as malaria, bilharzia and trypanosomiasis ; treatment of sexually transmitted diseases (STD's), tuberculosis, pneumonia and devastating afflictions associated with HIV/AIDS all can be prevent, treated and cured by using Medicinal Plants.Natural products and their derivatives represent more than 50% of all drugs in clinical use in the world. Medicinal Plants contribute no less than 25% to the total.

Malaria, a deadliest - life threatening disease especially during pregnancy contributes to maternal anemia, premature delivery and low birth rate leading to increased child mortality. Due to high cost, limited availability of malaria drugs and remoteness of the area, medicinal plants have clearly played an important role in malarial treatment for centuries.

Traditional herbal medicine in East Africa been prominently in existence for centuries remains almost wholly unregulated. Cut off from the mainstream economy, traditional herbal medicine receives little or no development support and importance. Catastrophic habitat loss is threatening the survival of many important Medicinal plants. Steps must be taken to ensure use of local plants is sustainable and does not threaten biodiversity.

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[SL 14A] Crystallization for Long Range Molecular Order Structure Elucidation

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Key words: Crystallization, Structure elucidation, X-ray crystallography, NMR

Introduction

Natural products are very important in the pharmaceutical industry. Within pharmaceutical analysis, there exist a hierarchy of activities related to molecular structure. Typically, structure characterization is a collection of primary spectroscopic data obtained from IR, UV, NMR, MS and 'clues' from chromatography among others.

However, more often than not, such structure characterization are neither conclusive nor involve interpretation of the data in a structural context. As such, structural confirmation methods i.e. the use of these data to confirm the proposed molecular structure of the chemical substance are still necessary. In effect the process of structure determination can be long, costly and without as much details.

X-ray crystallography can be an invaluable tool in structure elucidation with which molecular structure can be worked out without preconception. Using this technique it is possible to solve molecular structures that are either related to known chemical structures (e.g. degradants and metabolites) or those from unknown origins (e.g. impurities) more cheaply, faster and more completely. However, the real bottleneck in this is the preparation of suitable crystals. For years crystallization has been a standard technique for purification of materials but in recent years it is a lost art that needs attention at all levels.

Through rediscovery of the lost art of crystallization and utilization of recent advances in X-ray diffraction methods, elaborate structure elucidation has never been made easier for natural product chemistry

Materials and Methods

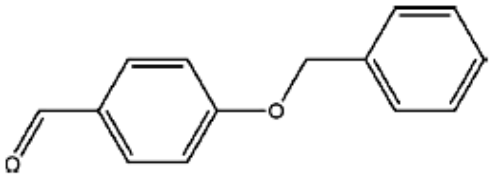
In the course of typical organic reactions, several intermediates were monitored using TLC and some isolated and characterized using NMR, IR etc. All synthetic reactions were performed under an atmosphere of nitrogen. Some of the intermediates were liquids but were crystallized using standard crystallization techniques. The resulting crystals were taken through a recrystallization process in appropriate solvents for purification and then subjected to the single crystal X-ray diffraction studies. Data collection was using a CrysAlis CCD and Bruker–Nonius APEXII CCD diffractometers. Various softwares were used; Data collection: CrysAlis CCD (Oxford Diffraction, 2007) and COLLECT (Hooft, 1998); cell refinement: CrysAlis CCD and DENZO (Otwinowski & Minor,

1997) and COLLECT; data reduction: CrysAlis RED (Oxford Diffraction, 2007) and DENZO and COLLECT; program(s) used to solve structure: SHELXS97 (Sheldrick, 2008); program(s) used to refine structure: SHELXL97 (Sheldrick, 2008); molecular graphics: ORTEP-3 (Farrugia, 1997); software used to prepare material for publications: SHELXL97.

Results and Discussion

Although some results were obtained using IR, NMR, MS and 'clues' from chromatography, they were not as conclusive as those from X-ray crystallography. The X-ray diffraction revealed the structure of two compounds in more details including Hydrogen bonds.

4-(Benzyloxy)benzaldehyde



Crystal data

$C_{14}H_{12}O_2$
$M_r = 212.24$
Orthorhombic, $Pna2_1$
$a = 11.4772\ (11)$ Å
$b = 12.9996\ (12)$ Å
$c = 7.2032\ (6)$ Å
$V = 1074.71\ (17)$ Å^3
$Z = 4$
Mo $K\alpha$ radiation
$\mu = 0.09$ mm^{-1}
$T = 123$ K
$0.42 \times 0.20 \times 0.14$ mm

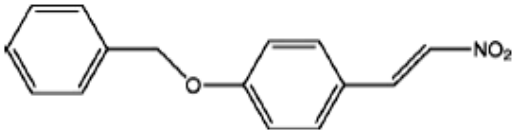
Hydrogen-bond geometry (Å, °).

D—H···A	D—H	H···A	D···A	D—H···A
C1—H1A···O2^{i}	0.99	2.50	3.324 (2)	141
C1—H1B···O2^{ii}	0.99	2.53	3.478 (2)	160

Symmetry codes: (i) $-x+\frac{3}{2}, y+\frac{1}{2}, z+\frac{1}{2}$; (ii) $x-\frac{1}{2}, -y-\frac{1}{2}, z$.

The compound, $C_{14}H_{12}O_2$, has an essentially planar conformation with the two aromatic rings forming a dihedral angle of 5.23 (9)° and the aldehyde group lying in the plane of its aromatic group [maximum deviation = 0.204 (3) Å]. Weak intermolecular C—H...O contacts are found to be shortest between the aldehyde O-atom acceptor and the H atoms of the methylene group.

1-Benzyloxy-4-(2-nitroethenyl)benzene



Crystal data

$C_{15}H_{13}NO_3$
$M_r = 255.26$
Triclinic, $P\overline{1}$
$a = 9.9522\ (8)$ Å
$b = 14.0456\ (13)$ Å
$c = 14.2506\ (10)$ Å
$\alpha = 74.416\ (5)°$
$\beta = 84.188\ (5)°$
$\gamma = 80.888\ (4)°$
$V = 1891.0\ (3)$ Å^3
$Z = 6$
Mo $K\alpha$ radiation
$\mu = 0.09$ mm^{-1}
$T = 120$ K
$0.12 \times 0.10 \times 0.05$ mm

Hydrogen-bond geometry (Å, °).

D—H···A	D—H	H···A	D···A	D—H···A
C3—H3···O8^{i}	0.95	2.56	3.503 (5)	175
C8—H8···O8^{i}	0.95	2.38	3.322 (5)	175
C5—H5···O9^{ii}	0.95	2.58	3.493 (4)	162
C12—H12···O4^{iii}	0.95	2.46	3.284 (5)	145
C20—H20···O5^{iv}	0.95	2.49	3.373 (4)	155
C22—H22···O5^{iv}	0.95	2.59	3.448 (4)	150
C18—H18···O6^{v}	0.95	2.40	3.326 (4)	166
C33—H33···O3^{ii}	0.95	2.40	3.325 (4)	163
C45—H45···O1^{vi}	0.95	2.58	3.413 (5)	147

Symmetry codes: (i) $-x+2, -y, -z+2$; (ii) $-x+1, -y, -z+2$; (iii) $x, y, z-1$; (iv) $-x+2, -y+2, -z+1$; (v) $-x+1, -y+2, -z+1$; (vi) $-x+1, -y+1, -z+1$.

The compound, $C_{15}H_{13}NO_3$, crystallizes with three independent molecules per asymmetric unit ($Z' = 3$). One of these molecules is found to have a configuration with a greater twist between its two aromatic rings than the other two [compare 70.26 (13) and 72.31 (12)° with 84.22 (12)°]. There are also differences in the number and nature of the weak intermolecular C—H….O contacts formed by each of the three molecules.

The art of crystallization should be strengthened to enhance structure elucidation.

Acknowledgements

We are grateful to the National Crystallography Service, University of Southampton, for data collection. Dr Okoth also thanks the Commonwealth Scholarship Commission and the British Council for funding, Dr Alan Kennedy and the University of Strathclyde for hosting him as a visiting scholar and Moi University for sabbatical leave to do this work.

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[SL 15A] Antileishmanial Activity of Petroleum ether, *n*-hexane Crude Extract and (2E)-methyl 3-((1E, 4E)-7-methyl-4-(2-oxopropylidene) cyclohept-1-enyl) acrylate from *Xanthium brasilicum* Vell. leaves.

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Introduction

Leishmaniases are a group of parasitic diseases of multifaceted clinical manifestations, world-wide spread with epidemiological diversity. It is caused by at least 20 species of protozoan parasites of the genus *Leishmania.* The parasites are transmitted by the bite of sandflies of the genus *Phlebotomous* in the Old World and *Lutzomia* in the New World. About 30 species of the sandflies are proven vectors (Desjeux, 1996). Most leishmaniases are zoonosis and the reservoir hosts are mammals other than man. Man is secondarily infected, being an incidental host (Ashford, 1996). Leishmaniasis is endemic in 88 countries. The disease ranges from self-healing disease of the skin (cutaneous leishmaniasis; CL), disfiguring disease of the mucous membranes (mucosal leishmaniasis; ML) to a fatal disease if not treated (visceral leishmaniasis; VL). More than 90% of VL is reported from Bangladish, Brazil, India and Sudan, and more than 90% of CL from Afaganistan, Iran, Saudi Arabia, Syria, Brazil and Peru (Desjeux, 1996; WHO, 2002; Guerin *et al*., 2002). WHO estimates the worldwide prevalence to be approximately 12 million cases, with annual mortality of about 60 000. The size of the population at risk is about 350 million (WHO, 2010). Every year 1-1.5 million new cases of CL, and 0.5 million of VL are reported, and the incidence is substantial when subclinical infections are included; asymptomatic infections outnumber symptomatic infections by a ratio ranging from 10:1 to 100:1 (Murray, 2002). Leishmaniasis is associated with about 2.4 million disability adjusted life years (DALY). (Murray *et al*., 2005).

Folk medicine is very often a good source for researchers looking for bioactive substances potentially useful against many diseases. Plants were used for leishmaniasis treatment by the people who live far from modern medicine; these plants offered many lead substances for new antileishmanial drugs discovery (Carvalho and Ferreira, 2001). Natural products provided highly successful new drugs such as artemisinine. Further more screening natural products found in all environments such as the deep sea, rain forests and hot springs, and produced by all sorts of organisms ranging from bacteria, fungi and plants to protozoa, sponges and invertebrates (Kayser *et al*., 2003). There are many problems associated with it's treatment, like development of resistance to current treatment in many areas of the world where the disease is endemic, their high

costs, there are some serious side effects associated with many of them, and need for hospitalization (Croft *et al*., 2006).

Methods

Extraction

X. brasilicum was collected from Gezira in August- 2008 and identified by Dr. Wail Elsadig and Hayder Abd el-Gadir M. Ahmed at Medicinal and Aromatic Plants Research Institute, National Center for Research, Khartoum. Extraction of *X. brasilicum* was done first using (50 gm) successively with six solvents in order of increasing polarity: petroleum ether, *n*-hexane, chloroform, ethylacetate, ethanol and methanol for at least 12 hrs. For isolation and characterization of the active compounds 1 kg of the powdered plant was used for extraction. Extracts were dried under reduced pressure using rotary evaporator. Stock solutions with a concentration of 10 mg/ml were made in DMSO and kept at 4˚C.

Antileishmanial activity (promastigotes)

Antileishmanial activity screening was carried using Atta-ur-Rahman *et al*. (2005) method. In a 96 well microtiter plate; 90 µl of the parasite culture with count of $2x10^6$ parasites/ml were taken into each well. 10 µl of each concentration was taken and mixed well. Amphotericin B was set as the positive control with the concentrations up to 1µg/ml. The plate was incubated in the dark at 25^oC. Counting of the living parasites was done after 72 hours.

Five concentrations 5, 10, 100, 500 and 1000 µg/ml were prepared of each extract of *X. brasilicum*. *N*-hexane extract was further assayed with concentrations of 5, 6.5, 8, 9.5 and 11µg/ml. The activity of the 14 chromatographic fractions were assayed using 5 and 10 µg/ml. Fraction 10 and 11 were further investigated using concentrations of 2.5, 5, 7.5 and 10 µg/ml. The test was performed using the above mentioned method.

Anti-amastigotes

Culture and preparation of human monocytes (THP-1)

THP-1 cell lines were used for infection with the promastigotes. Cells were cultured in RPMI-1640 complete medium at 37˚C, 5% CO_2/ 59% air mixture. THP-1 cells were transferred to 50 ml centrifuge tube and centrifuged at 4˚C, 2000 rpm for 10 minutes. The supernatant was discarded and the pellet gently resuspended in a small volume of fresh culture medium, and then counted using Neubauer hemocytometer. Phorbol 12-myristate 13-acetate (PMA) 20 ng/ml was added to allow cells differentiation and adherence. The cells were seeded in 16-well tissue-culture slides at a density of 40,000 cells/well i.e. $4x10^5$/ml, 100µl/well and maintained at 37ºC, 5% CO_2/ 95% air mixture for 48 hours. After that the cells were washed by replacing the overlay with 100 µl fresh culture medium without PMA and incubated for further 24 hours.

Infection of the macrophages with Leishmania promastigotes

Adherent macrophages were infected with late-stage promastigotes and incubated at 37˚C, 5% CO_2/ 95% air mixture, for 24 hours. The infected cells were washed with cold (4˚C) culture medium.

The overlay was removed with sterile Pasteure pipette, and with a multi-channel pipette, 100 µl of the cold medium gently dispensed and withdrawn 2-3 times. This dislodges the majority of any extracellular promastigotes. Finally, 100 µl of the complete medium was added prior administration of the drug. Sample was applied when the infection average reached 80

Drug application

Stock solution of the drug with concentration of 20 mg/ml was prepared; from this solution 2x dilution of 60µg/ml was made by taking 3µl of the stock solution + 997µl complete medium. After that serial dilutions were made in the wells as follows: the first well contained 75µl and the remaining wells each had 100µl fresh complete medium. 75µl of the 2x dilution of 60µg/ml was placed into the first well and mixed. Using 4 channels of a multichannel pipette, 50µl is removed, transferred and mixed (Conc 2) and so on, to produce a 3-fold dilution series and leaving 100µl in each well.

Isolation of compound A & B

After cooling the petroleum ether and *n*-hexane extracts at room temprature a pale yellow crystalline precipitate was formed on the bottom and the wall of the flask. The precipitate was collected by decantation of the extract, then subjected to further decolorization by dissolving it in chloroform and passing the chloroform solution through a small column (30 x 1.5 cm) containing activated charcoal loaded on a piece of cotton wool and sand. The filtrate was collected in small vials and the solvent was evaporated to dryness to obtain a pale yellow crystals. Further more the compound was recrystallized.

Recrystallization of compound A & B

Compounds A & B were dissolved in a little amount of chloroform, and then petroleum ether was added gradually with stirring till complete solubility, and then allowed for sometime for recrystallization of the compound. The solvent was removed by decantation, and the crystals were dried on a filter paper. This process was repeated three times. Colourless crystals were obtained.

Results and discussion

General Antileishmanial screening

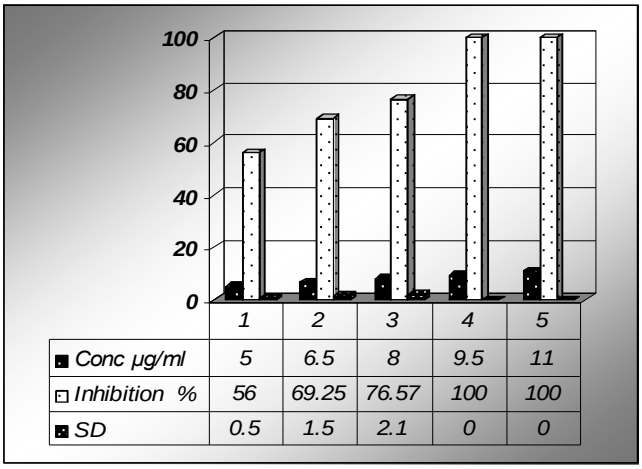
Table 1.

No	Plant	Inhibition %		
		Petroleum ether	Chloroform	Methanol
1	*Cassia obtusifolia*	+	+++	-
2	*Indigofera oblongifolia*	+++	++	+++
3	*Xanthium brasilicum*	+++	+++	++
4	*Tinospora bakis*	+++	-	-
5	*Striga hermonthica*	+++	+++	-
6	*Anogeissus leiocarpus*	+	+++	+++
7	*Annona spp.*	-	+++	+++
8	*Croton zambesicus*	+++	+++	+++
9	*Pulicaria crispa*	+++	+++	-
10	*Lwasonia innermis*	+	+++	++
11	*Argemone mexicana*	ND	+++	-

(-): No or very weak activity, (+) Inhibition % < 50%, (++): Inhibition % <75%, (+++): Inhibition % 75-100.
ND: Not determined.

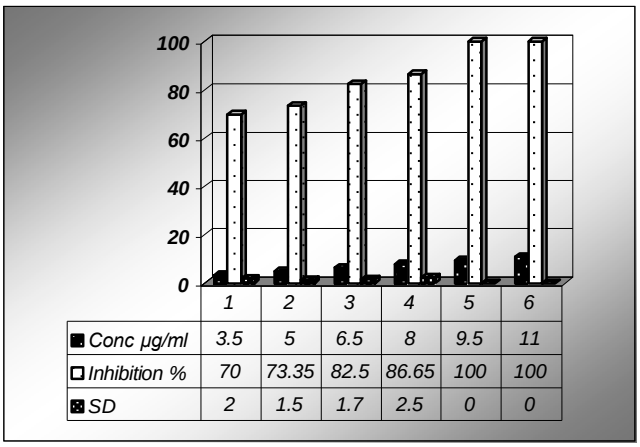
Table 2. Antileishmanial activity of *X. brasilicum* crude extracts, with petroleum ether, *n*-hexane, chloroform, ethylacetate, methanol and ethanol crude extracts obtained by successive extraction using six concentrations as shown in the table following incubation period of 72 hours. Petroleum ether, *n*-hexane and chloroform extracts showed good activity. *N*-hexane extract showed the highest activity among the six extracts, while the polar extracts showed weak or no activity.

Conc µg/ml	Inhibition%					
	Pet. ether	Hexane	Chloroform	EtOEt	MeOH	EtOH
5	6.7 ± 2.6	51.25 ± 2.1	0	86-	33-	48-
10	59.25 ± 4.0	100	50 ± 2.0	46-	49-	46-
50	100	100	80± 2.6	17-	25-	41-
100	100	100	100	100	25-	20-
500	100	100	100	100	41-	25-
1000	100	100	100	100	1-	85



	1	2	3	4	5
Conc µg/ml	5	6.5	8	9.5	11
Inhibition %	56	69.25	76.57	100	100
SD	0.5	1.5	2.1	0	0

Fig. 1. Inhibition % for *n*-hexane crude extract obtained successively, after 72 hours incubation. Inhibition was dose dependent. The parasites morphology and motility were completely changes even at small doses.



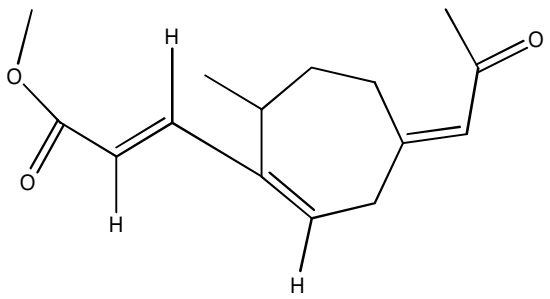
	1	2	3	4	5	6
Conc µg/ml	3.5	5	6.5	8	9.5	11
Inhibition %	70	73.35	82.5	86.65	100	100
SD	2	1.5	1.7	2.5	0	0

Fig. 2. Inhibition % for compound B, isolated from *n*-hexane successive extract, after 72 hours incubation period. The compound was more active than the original crude extract, reaching100% inhibition at a dose of 9.5µg/ml and 70 at 3.5µg/ml marked changes in the morphology of the remaining parasites. Inhibition occurred in a dose dependant manner.

Table 3. Inhibition % for compound B against the intracellular amastigotes. The compound was toxic to both macrophages and the parasites at the concentration of 3.3µg/ml indicating its low selectivity towards the parasites.

Conc µg/ml	Inhibition %
3.3	T
1.1	29.8
0.37	7.4

T: toxic to both macrophage and the parasite



Chemical name: (2*E*)-methyl 3-((1*E*,4*E*)-7-methyl-4-(2oxopropylidene)cyclohept-1-enyl)acrylat

Molecular formula: $C_{15}H_{20}O_3$
Molecular weight: 248.32
Melting point: 94°C

Fig. 5. The proposed structure of compound (B). This structure was based on the above spectroscopic data. However it is not the final suggestion, further analysis like two dimensional NMR is needed to confirm this suggestion.

Conclusion

This study showed the importance of plants and plant-derived compounds as source for new molecules and important leads for drug discovery and development. *X. brasilicum* possesses very good antileishamial activity, in its nonpolar extracts mostly *n*-hexane extract. Bioactivity guided fractionation had led to isolation of an active compound which was identified with the means of spectroscopic method as: (2E)-methyl 3-((1E,4E)-7-methyl-4-(2-oxopropylidene)cyclohept-1-enyl)acrylate, beside this compound we have other active compounds. Intracellular amastigotes studies showed that the compound exhibited some toxicity against human macrophages.

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[SL 16A] Assessment of *Azadirachta Indica* and *Cassia Spectabilis* for Some Immunomodulatory Properties

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Key words: *Azadirachta indica, Cassia spectabilis*, immunomodulatory properties, medicinal plants, traditional medicine.

Introduction

It has become a common practice to search for contemporary drugs among herbs used in traditional medicines partially because the plants contain bioactive substances. Also there is the general belief that the herbal medicines are safer. In developing countries herbal medicines are more available and affordable. It is thus necessary to explore this area to provide a scientific rationale for the use of herbal medicines on which 80% of our rural populations rely for primary healthcare (Kamatenesi-Mugisha et al, 2000).

Methods

Healthy male swiss albino mice were orally administered 2.0 and 4.0mg/kg bodyweight and, 100 and 200mg/kg bodyweight of *Azadirachta indica* and *Cassia spectabilis* respectively, for 7days while the control group was given distilled water. Each group contained five animals (n=5). The animals were then sacrificed humanely and blood collected by cardiac puncture. After centrifugation, the blood was analysed for adenosine deaminase (ADA) activity (Matinek 1963), total protein, serum albumin and serum globulin.

Results

There was a statistically significant ($p<0.05$) decrease in ADA activity in mice treated with *A. indica* while those treated with *C. spectabilis* showed a statistically significant increase in ADA activity. For serum globulin there was a significant decrease in values for mice treated with *A. indica* and *C. spectabilis* at 4.0mg/kg and 100mg/kg bodyweight respectively. No significant change was observed in total protein and albumin.

Table: Adenosine deaminase activity and total protein levels of mice treated with *A. indica* and *C. spectabilis* at various dose levels. (Mean ±SD).

GROUPS	EXTRACT/DOSAGE	ADA activity (IU/l)	TOTAL PROTEIN (g/dl)	SERUM ALBUMIN (g/dl)	SERUM GLOBULIN (g/dl)
A	*A. indica* 2mg/kg	1.34±0.3	7.03± 0.9	4.31± 0.5	2.72 ±1.3
B	*A. indica* 4mg/kg	6.65± 2.5*	5.76± 0.8	4.66± 2.5	1.10± 1.4
C	*C. spectabilis* 100mg/kg	5.15± 2.3	5.65± 0.6	4.59 ±2.3	1.06± 0.7
D	*C. spectabilis* 200mg/kg	19.34 ±3.2*	7.60± 0.9	4.75 ±3.2	2.85 ±1.9
E	Control	6.85 ±2.6	7.12± 1.1	4.39 ±0.7	2.73± 0.4

* Significant at $p \leq 0.05$ and n=5

Conclusion

A. indica directly affects the pathogens while *C. spectabilis* modulates the immune system response.

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[SL 17A] Evaluation of the Biosafety of Selected Botanical Pesticide Plants Used by Subsistance Farmers Around the Lake Victoria Basin

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Key words: botanical pesticides, biosafety, oral acute toxicity, Lake Victoria Basin

Introduction

There is a very long history of use of botanical extracts for human and veterinary medicines as well as for the protection of field and stored crops (Berger 1994). In the recent decades however, due to the introduction of synthetic pesticides, the adoption of these traditional approaches of crop and post harvest protection have not been improved (Berger 1994). Today, the use of plant extracts for controlling pests has been limited to small holder farmers, who in most cases have been supported by various Non Government Organizations (NGOs) and women groups (Mugisha-Kamatenesi *et al*. 2008). The use of synthetic pesticides has undoubtedly increased crop production**.** This has been possible through reduced losses caused by crop pests but many of these chemicals are hazardous to both humans and the environment at large. According to Blackman *et al.* (1999), it has been estimated that hardly 0.1% of the agrochemicals used for crop protection reach the target pest leaving the remaining 99.9% to enter the environment and cause hazards to non-target organisms including humans.

Materials and methods

Collection of plant materials

Leaves of the selected pesticide plants were collected from Mukono district, Uganda.

Preparation of plant materials

Identification of the collected plants

The plants were identified by taxonomists in the Herbarium Department of Botany, Makerere University. This was done to ensure that the plants are rightly identified to avoid any confusion.

Drying of plant materials

The collected plant materials were separately exposed under shade until they become dry.

Extraction and concentration of Plant extracts

Ethanol and water extracts of plant materials

Leaves of *Cupressus lusitanica, Ocimum suave, Tithonia diversifolia* and *Eucalyptus globulus* were pounded. Known weights of the pounded material were separately soaked in ethanol (95 %) for

three days and then filtered. The solutions were filtered by use of filter papers and the extracts were thereafter concentrated to thick residues on a rotary evaporator and water bath maintained at 70°C. The concentrated ethanol extracts were put in well labeled, dry, clean bottles. In order to obtain the water extract, known amounts of the pounded materials were separately soaked in distilled water for two days to obtain the aqueous extract. The solutions were then filtered through a cheese cloth before further filtration using a Whitman No.A-1 filter paper. The filtrates were concentrated in a hot air oven maintained at 50°C for tow days and were subsequently air-dried to thicker residues.

Extraction of Essential oils

For *Cupressus lusitanica, Ocimum suave* and *Eucalyptus globulus*, essential oils were extracted by steam distillation from fresh leaves. The leaves will be cut into small pieces, put into a distillation flask. Steam was allowed to pass through each batch of leaves for two hours. Essential oils were trapped in collecting tubes and put in clean, dry and well labeled bottles, which were then kept in a fridge maintained at low temperatures to freeze the water which had been trapped together with the oil.

Preparation of the test animals

Test animals (wistar mice and rats) were purchased at 4 weeks of age from the faculty of veterinary medicine, Makerere University. They were randomly group housed in stainless wire cages living enough space for clear observation of each animal. The animals were kept on a 12 hour artificial light and dark cycle at 22 + 30°C. Conventional laboratory diets were used to feed the animals with unlimited supply of drinking water. This was done for 5 days prior to dosing so as to get them acclimatized to laboratory conditions. During this period, the mice were observed to asses their health conditions basing on their external appearance, nutritional conditions and general behavior.

Determination of the acute and chronic toxicity of the plant extracts using mice

Acute toxicity studies

In order to determine the preliminary acute toxicity of the different plant extracts, 4 dose levels were prepared for each plant extract. Each dose level was assigned 2 test animals (mice). The administration of the test substances was done by use of intragastric plastic tubes to the different groups of test animals. The rough LD 50 was then used to determine the accurate LD_{50}. For the accurate LD 50, five dose levels were set within the range of the rough LD_{50} for each extract. Each group was assigned 6 members (mice).

Sub chronic toxicity studies using rats

Sub chronic studies were done for two essential oils (*Eucalyputus globulus* and *Cuppresus lusitanica*). Test animals were divided into three groups of 10 members for each essential oil. A group of 10 members was kept as a control group and received 1 ml of the carrier substance (oil/emulsifier/water respectively) in the ratio 4:2:1 .Tween 80 2 % was used as the emulsifying agent.

Haematology

Blood samples for bioassays were obtained from the tails of rats under anesthesia every after two weeks of administration. Heamatological parameters including haemoglobin (Hb), total leukocyte count (TLC), differential count including total lymphocyte, poly-morphonuclear leucocytes, and eosinnophil counts, platelet count, protrombin time and packed cell volume (PCV) were analyzed using standard techniques.

Table of results showing the LD 50s of the different plant extracts

NO.	Plant name	Extract	LD 50 Value
1.	*Ocimum suave*	Essential oil	4,677 mg/kg
2.	*Ocimum suave*	Ethanol extract	13,182 mg/kg
3.	*Ocimum suave*	Aqueous extract	
4.	*Cupressus lusitanica*	Essential oil	2,951.2 mg/kg
5.	*Cupressus lusitanica*	Ethanol extract	14,791 mg/kg
6.	*Cupressus lusitanica*	Aqeous extract	
7.	*Eucalyptus globulus*	Essential oil	2,290 mg/kg
8.	*Eucalyptus globulus*	Ethanol extract	12,589.3 mg/kg
9.	*Eucalyptus globulus*	Aqeous extract	
10.	*Tithonia diversifolia*	Ethanol extract	11,748 mg/kg
11.	*Tithonia diversifolia*	Aqeous extract	11,885 mg/kg
12.	*Tithonia diversifolia*		

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[SL 18A] The *in vitro* Antimycobacterial Activity of Medicinal Plants Used by Traditional Medicine Practitioners (TMPs) to Treat Tuberculosis in the Lake Victoria Basin in Uganda

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Key words: *In vitro* activity; Anti-mycobacterial; Medicinal plant; *Mycobacterium tuberculosis; Mycobacterium avium;* rifampicin; isoniazid

Introduction

Tuberculosis (TB) is one of the dreadful infectious diseases and the leading cause of mortality worldwide, with approximately 9 million people developing the disease and 2 million people dying annually (WHO, 2007; Sanjay 2004; Navin *et al.*, 2002). Globally, more than one-third of the world's population (more than 2 billion) is infected with MTB (CDC Report, 2005; Navin *et al.*, 2002). Outbreaks of multi-drug resistant (MDR) and extensively drug-resistant (XDR) tuberculosis have also compounded the problem. The emergence of XDR TB is cause for concern because it is widely distributed geographically (now in over 50 countries on all inhabited continents), and renders patients virtually untreatable with available drugs. New drugs have to be developed to deal with MDR and XDR TB strains, which have already become a problem especially where there is co-infection with HIV/AIDS. There is an urgent need to search for and develop new, comprehensive, safer and more effective quick acting and affordable anti-TB agents, and this also includes searching for leads from natural products of plant origin. This presentation is a report of results of a study that was carried out to identify the medicinal plants used by Traditional Medicine Practitioners in the Lake Victoria Region of Uganda, and their subsequent screening against rifampicin-resistant mycobacterium tuberculosis. The objectives of the study included documenting indigenous knowledge and the existing practices used by traditional medicine practitioners (TMPs) in the treatment of TB and to scientifically validate the TMPs' claims.

Materials and Methods

The study area included three districts of Mayuge, Mbarara and Mukono as part of a wider East African regional survey in the Lake Victoria basin. Data was mainly gathered using key informant interviews, guided questionnaire interviews and direct observation techniques. A total of 31 TMPs were interviewed as well as 16 patients who had received TM treatment for TB about their health seeking behaviour and attitudes towards use of TM. After the survey, a list of the most frequently mentioned plant species was prepared and parts of 11selected plants were collected from various areas, their crude petroleum ether, chloroform and methanol extracts prepared and tested in a

bioassay on three strains of *Mycobacterium*. The antimycobacterial tests were done according to Parish and Stroker (1998). Susceptibility tests were carried out using the disc diffusion method on Middlebrook 7H10, while MIC and MBC tests for the active extracts were carried out using the Microtitre plate method where Middle brook 7H9 broth was used. Phytochemical screening (Edeoga *et al.,* 2005) and acute toxicity tests (Gosh 1984) were also done for the most active extracts. *Mycobacterium* strains used were obtained from Joint Clinical Research Centre (JCRC) Mengo in Kampala, Uganda, where the mycobacteriology work was carried out, and included a rifampicin-resistant strain (TMC -331strain) to serve as an indicator of MDR, a fully susceptible strain (H37Rv) as a control, and *Mycobacterium avium* (MA) a wild strain from a Ugandan patient to represent the Mycobacterium other than tuberculosis (MOTT) group. Acute toxicity tests were done on the most active extracts according to Ghosh (1984).

Results and Discussion

Over 50 plant species were mentioned by the TMPs. Herbal drugs were prepared as mixtures of four or more plants. The most frequently used plant parts were leaves, root wood, stem bark and fruit. Of the screened plants, four were found active against TB, two of which were active on all the three strains of *Mycobacterium* used, including the rifampicin-resistant. All the active extracts were bactericidal although their activity was lower compared with isoniazid and rifampicin. However, they had an advantage over rifampicin, one of the first-line anti TB drugs, by being active against rifampicin – resistant TB. With regard to susceptibility tests the highest activity was registered with *Erythrina abyssinica* (VT8), *Cryptolepis sanguinolenta* (VT10), *Warburgia ugandensis* (VT2) (Wube *et al*., 2005), *Mangifera indica* (VT6) with zones of inhibition ranging between 10.7 and 23mm (including diameter of the disc which was 6mm). The concentrations of the extracts were at 50 mg/ml (25 mg/ml for *C.sanguinolenta*). Rifampicin was not active on *Mycobacterium avium* complex and a rifampicin resistant strain TMC-331 but it showed a zone of inhibition of 26 mm for H37Rv (a pan sensitive strain) at a concentration of 0.1 mg. Isoniazid cleared the quadrant for two strains at a concentration of 0.05mg but it was also not effective on *M.avium*. The MICs of the active crude extracts ranged between 1.17 and 6.25 mg/ml while for rifampicin and isoniazid they were between 0.25 and 9.38µg/ml. The MBCs for the active crude extracts were between 0.20 and 6.25 mg/ml while for rifampicin and isoniazid they were between 0.25 and 1.0 µg/ml (but rifampicin was inactive on TMC-331). Alkaloids were found mainly in *C. sanguinolenta* (Gibbons *et al.,* 2003) and flavones mainly in the extracts of *E. abyssinica.* Acute toxicity tests on *E. abyssinica* and *C. sanguinolenta* gave LD_{50} between 700 and 800mg/kg body weight which were in the relatively safe range. Phase III of the project involving isolation, characterization and identification of the compounds that are active on *M. tuberculosis* is under way.

Conclusion

The bioassays conducted on the selected plant species further vindicated some of the claims by the TMPs by showing activity against *M. tuberculosis* although more research is required especially in the area of standardization. However, isolation and screening active compounds and more *in vitro*

and *in vivo* studies on the toxicity of the plants are needed before declaring them completely safe for use in humans.

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[SL 19A] Effects of Aqueous Extracts of Basil, *Ocimum basilicum* L., Sodom's apple, *Calotropis procera* Ait and Coriander *Coriandrum sativum* L. on leaf miner, *Liriomyza Spp.*, on okra Crop.

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Introduction

Okra, *Ablemoschus* (*Hibiscus*) *esculentus* (L.) (Moench), is ranking third of the major vegetable crops, and is one of the most popular and main Sudanese dishes. The most famous cultivars in the Sudan are Khartoumia, Momtaza, Karrari, Kassala, Dwarf long green and Clemson spineless (Bugstaler *et al.,* 1984). Okra is grown under irrigation all year around, but partienlarly in summer. It is attacked by a number of insect pests one of which is the vegetable leafminers, *Liriomyza spp*. These are one of the largest groups or genera with over 300 species through out the world. Only about 15 different species are known to feed on cultivated plants and thus have some actual or potential economic significance. In the Sudan two species of *liriomyza* were reported: *L. trifolii* (Burgess) (Sharaf EL-Din *et al.,* 1997) and *L. sativae* (Blanchard) recorded by Martinez and Brdat (1996).

Materials and Methods

An experiment was conducted at the U. Of Gezira Experimental farm to evaluate the effects of 10% aqueous leaf and fruit extracts of Basil, (*Ocimum basilicum* L.), leaf extracts of Sodom's apple, (*Calotropis procera* Ait) and fruit extract of Coriander , (*Coriandrum sativum* L.) on the vegetable leaf miner, *Liriomyza Spp* .on okra. The experimental design comprised 16 plots assigned to 4 treatments (replicated 4 times), arranged in a completely, randomized block design. Un dressed okra seeds were sown on 7/7/2008. Three natural products were used in the study Sodom's Apple (Usher), *Calotropis procera* Ait, Basil (Rehan), (*Ocimum basilicum* L.) and coriander (Kasbra), *Coriandrum sativum* L. The treatments consisted of spraying okra plants with either Basil, Sodom's apple and Coriander 10% aqueous extract or distilled water (control). The efficacy of the extracts was assessed in terms of active mines in okra leaves i.e., where leaf miner larvae were alive and feeding. Fresh leaves of Usher plant, leaves and fruits of Rehan plant and fruits of Kasbara plant, were dried in the laboratory at room temperature of 30°C. Dried plant materials were first crushed by hand then ground by an electric blender mixer, the powder was then stored in tightly covered glass jars and kept at room temperature in the laboratory ready for extraction.

Results

The results (Table 1) indicated that the three aqueous extracts significantly ($P<0.05$) reduced the infestation level by the leaf miner on okra leaves. Sodom's apple extract resulted in the lowest infestation level by the leaf miner (mean number of active mines =106), followed by coriander (124) and Basil (132) while the control treatment scored 489 active mines. In addition, the between

treatment mean values for Usher, Kasbara and Rehan were significantly different from each other and the mean value recorded for the untreated okra.

Table (1): Mean number of active mines of the leafminers *Liriomyza spp.* **on okra leaves during the experimental period.**

Treatment	Means*1							Mean*2
	count 1	count2	count3	count4	count5	count6	Count7	of all counts
Control	405 a	457 a	492 a	505 a	513 a	526 a	537 a	489 a
Usher	348 b	117 b	113 b	69 c	33 b	33 b	32 b	106 d
Rehnan	399 a	218 c	155 c	113 d	17 c	12 c	12 c	132 b
Coriander	396 a	236 d	215 d	23 d	0 d	0 d	0 d	124 c
S.E. ±	2.624	2.217	2.107	1.493	1.987	2.072	2.098	0.49
CV%	14.22	14.43	14.54	10.00	22.40	24.01	24.19	16.30

Means followed by the same letter (s) were not significantly different (P <0.05) at Duncan MRT.

*1 Means of 4 replicates

*2 Means of 7 counts.

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[SL 20A] Antiplasmodial Compounds from the leaves of *Drypetes gerrardi*

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Key words: *Drypetes gerrardi*, Antimalarials, Antiplasmodial activity, Cytotoxicity

Introduction

Malaria is one of the most important parasitic infections of humans due to its high morbidity and mortality, a threat to over 2 billion people living in areas of high incidence (Andrade-Neto *et al*., 2004). *Plasmodium falciparum*, the causative agent of the malignant form of malaria, has high adaptability by mutation and is resistant to various types of antimalarial drugs, a serious setback to antimalarial programs, since it precludes the use of cheap and previously effective drugs like chloroquine. New families of active compounds are needed as well as poly chemotherapy associating molecules with independent mechanism of action, in order to decrease the risk of resistance. In this paper we report the isolation of eight compounds (**1-8**) from *Drypetes gerrardii* (Euphorbiaceae) and there *in vitro* antiplasmodial activities against P. falciparum and their cyctotoxicity.

Materials and Methods

Extraction and Isolation

The leaves of *D. gerrardii* were collected in Kilifi district Coast province in Kenya, in July 2004 and authenticated by Simon Mathenge, of Nairobi University, Kenya. A voucher specimen (MM/07/04) is deposited in Nairobi University herbarium, Chiromo Campus. The dried and powdered leaves (1 kg) of *D. gerrardii* were exhaustively and sequentially extracted with petroleum ether, CH_2Cl_2, EtOAc and MeOH. The petroleum ether and the DCM crude extracts were combined based on their similarity on the TLC plate. The combined extract (32.6 g) was subjected to column chromatography on silica gel using petroleum ether, petroleum ether-EtOAc, EtOAc-MeOH and finally, pure MeOH as the mobile phase to yield 95 fractions (F_{1-95}). Fractions $_{15-35}$ were combined and further separated by silica gel column chromatography eluting with petrol ether-EtOAc (3:1) to give white cotton needles of friedelin (**1**, 50 mg) and epifriedelanol (**2**, 10 mg). Similarly, repeated column chromatography of $F_{54-67,}$ which were eluted with petroleum ether: EtOAc (3:2) furnished friedelanol methyl ether (**3**, 12 mg). Further purification of F_{70-75} [petroleum ether-EtOAc (5.5:4.5)] and F_{78-85} [petroleum ether-EtOAc (3:7)] on a Sephadex ® LH-20 column with CH_2Cl_2: MeOH (7:3) as eluant combined with repeated crystallization using acetone afforded 5β,24-cyclofriedelan-3-one (**4**, 8.6 mg).

The crude ethyl acetate extract (15 g) was similarly chromatographed on a silica gel column and eluted with a gradient of petroleum ether, CH_2Cl_2, EtOAc, and MeOH yielding 65 fractions (F_{1-65}).

Repeated column chromatography of F_{20-30} using a 5 % stepwise gradient of petroleum ether and ethyl acetate afforded 3-epimoretenol (**5**, 6.5 mg). Similarly repeated CC of fraction F_{31-35}, eluted with a mixture of petroleum ether-EtOAc (8:2) and further purification in a Sephadex column using CH_2Cl_2: MeOH (1:1) gave resinone (**6**, 4.0 mg). Fraction F_{38-45} on CC using CH_2Cl_2-EtOAc (4:6), gave β-sitosterol glucopyranoside (**7**, 50 mg). Preparative TLC of F_{50-58} eluted with ethyl acetate: MeOH (9.5:0.5) from the column, using CH_2Cl_2: MeOH (7:3) as the solvent system yielded 5 fractions. The polar fraction was further purified on a Sephadex LH-20 column using CH_2Cl_2:MeOH (1:1) and furnished amentoflavone (**8**, 6 mg) as a yellow powder.

In Vitro drug sensitivity Protocol

The semi automated micro dilution technique of Desjardins *et a*l., (1979) for assessing the *in vitro* anti malarial activity as modified by le Bras and Deloron (1983) was adopted in the drug sensitivity studies for crude extracts, pure compounds and standard drugs against *P. falciparum*. This test evaluates the ability of the crude and pure compounds to inhibit growth of *Plasmodium falciparum* by preventing the uptake of [^{3}H]-hypoxanthine *in vitro* was carried out at the Kenya Medical Research Institute (KEMRI). For each assay chloroquine (Sigma C6628) and artemisinin (Arteannuin, Qinghaosu; Sigma 36,159-3) were used as the standard drugs with the highest concentration at 200 ng/ml as positive control.

Cytotoxicity assay

In vitro cytotoxicity assay was carried out at KEMRI following a modified rapid calorimetric assay Of Mosmann, (1983) using Vero (199) cells.

Results and Discussion

The DCM and EtOAC extract of the leaves of *D. gerrardii* afforded one new flavone dimer, four friedelane-type triterpenoids namely friedelin (**1**) (Patra *et al*., 1990), epifriedelanol (**2**) (Bentacor *et al*., 1980), friedelanol methyl ether (**3**) (Samaraweera et al., 1983), and 5β,24-cyclofriedelan-3-one (**4**) (Connolly et al., 1986) together with 3-epimoretenol (**5**) (hopane-type triterpenoid) (Khastgir *et al*., 1967), resinone (**6**) (lupane-type triterpenoid) (Pyrek and Baranowska, 1977), β-sitosterol glucopyranoside (**7**) (Seo *et al*., 1978), and amentoflavone (**8**) (Goh *et al*., 1992; Lin et al., 2001) by comparison of ^{1}H and ^{13}C NMR data with reported data.

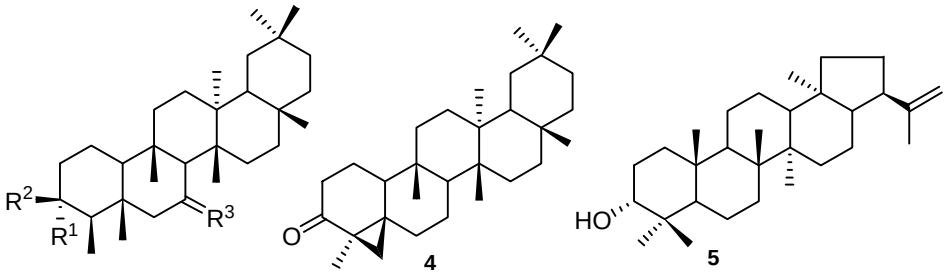
In vitro *antiplasmodial activity of pure isolated compounds from* D. gerrardii

Nine compounds isolated from *D. gerrardii*, were tested for *in vitro* anti-plasmodial activity against a K1 multidrug resistant strain of *P. falciparum.* The IC_{50} values are tabulated in Table 1 and their chemical structures are given in Figure 1. The antiplasmodial activity for the pure compounds (**1-8**) was considered high when IC_{50} < 1 µg/ml, moderate when between 1 and 5 and low when between 5 and 10 µg/ml. Compounds with IC_{50} exceeding 10 µg/ml were considered to be inactive (Likhitwitayawuid *et al*., 1993). The definition of the cytotoxicity used: CC_{50} < 1.0 µg/ml – high

toxicity; CC_{50} 1.0-10.0 µg/ml – moderate; CC_{50} 10.0-30.0 µg/ml – mild toxicity; and CC_{50} > 30 µg/ml – non toxic. Podophyllotoxin (CC_{50} 0.009+0.00003 µg/ml) was used as standard toxin

Selectivity index (SI) defined, as the ratio of IC_{50} (concentration required to cause visible alterations in 50% intact cells) of vero cells to IC_{50} *P. falciparum* was also determined. Selectivity index (SI) is used as a parameter of clinical significance of the test samples by comparing general toxins and selective inhibitory effect on *P. falciparum* (Wright and Phillipson, 1990). A pure compound is considered a hit if it is active *in vitro* against *Plasmodium* species with an IC_{50} of $\leq$ 1µg/ml and selective if it 10-fold more active against parasite than against a mammalian cell line (Likhitwitayawuid *et al*., 1993).

Resinone (**6**) exhibited high antiplasmodial activity against K1 strain of *P. falciparum* with an IC_{50} value of 0.09±0.01 µg/ml as well as satisfactory selectivity index. Amentoflavone (**8**) and 5β,24-cyclofriedelan-3-one (**4**) also exhibited moderate antiplasmodial activity of IC_{50} 2.6+0.01 µg/ml and 2.2±0.02 µg/ml respectively. Interestingly, amentoflavone (**8**) had high toxicity of CC_{50} 0.34±0.00 µg/ml as compared to 5β,24-cyclofriedelan-3-one (**4**) that displayed mild toxicity This clearly indicated that the high antiplasmodial activity observed for amentoflavone (**8**) was probably due to cytotoxicity rather than the activity against the parasites The other compounds (**1-3**,**5-7**) gave antiplasmodial activity ranging from 4.8±0.11 µg/ml to >10 µg/ml. In addition 5β,24-cyclofriedelan-3-one (**4**) that exhibited good antiplasmodial activity, did not demonstrate sufficient selectivity to kill the parasites without damaging mammalian cells. The selectivity index observed suggested that the antiplasmodia activity might be due to general toxicity. The antimalarial activity reported herein may explain the therapeutic efficacy claimed for these plants in traditional medicine and the compounds with appreciable activity may be used as scaffolds to generate leads with enhanced antiplasmodial activity, reduced cytotoxicity and improved bioavailability.



4 5

1 R^1, R^2 = O, R^3 = H

2 R^1 = H, R^2 = OH, R^3 = H

3 R^1 = OCH_3, R^2 = H, R^3 = H

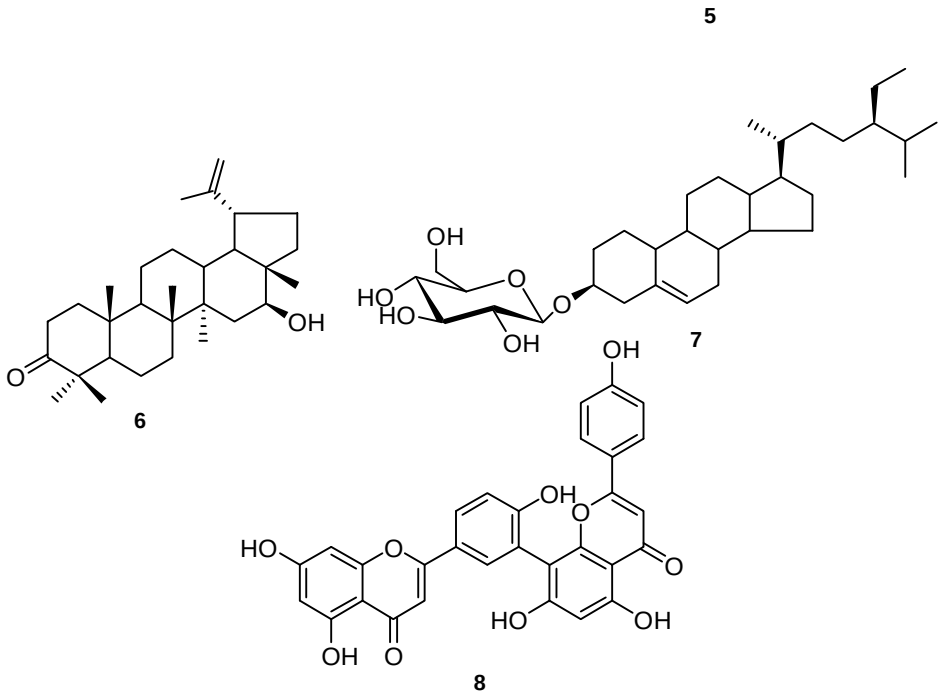


Figure 1. Compounds **1–8** isolated from *D. gerrardii*

Table 1. Antiplasmodial and cytotoxicity activity[a] of compounds **1-8** of *D. gerrardii*

Compound	IC_{50} (µg/ml)	CC_{50} ± S.E (µg/ml)	SI
1	4.8±0.11	>90	18.75
2	>10	90.0±1.30	>9.00
3	>10	32.8±0.90	3.28
4	2.2±0.02	21.2±0.01	9.64
5	>10	7.9±0.05	> 0.79
6	0.09±0.01	84.8±2.34	942.2
7	5.4±0.02	14.3±0.03	1.46
8	2.6+0.01	0.34±0.00	0.13

[a] *P. falciparum* –K1 strain, Vero 199 cells, IC_{50} – inhibitory concentration for 50% of tested parasites, CC_{50} – cytotoxic concentration for 50% of tested, cells, chloroquine IC_{50} 0.063+0.03, artemisinin IC_{50} 0.002+0.000, Pdx - podophyllotoxin CC_{50} 0.009+0.000

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YOUNG SCIENTIST PRESENTATIONS

[YS 1] Flavonoids with Anti-Diabetic Activiy from *Erythrina Abyssinica*

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Key words: *Erythrina abyssinica*, Flavonoids, PTP1B inhibitor.

Introduction

Since the beginning of humankind people have relied primarily on plants for nourishment. Through trial and error they discovered that some plants are good for food, that some are poisonous, and that some produce bodily changes such as increased perspiration, bowel movement, urination, relief of pain, hallucination, and healing. Over the millennia these observations were passed orally from generation to generation, with each generation adding to and refining the body of knowledge. Every culture the world over has in this manner developed a body of herbal knowledge as part of its tradition. Some examples which have been historically proven as example are the India Aryuveda the Chinese Pen Tsao etc.

Unlike many other genera of forage tree legumes, *Erythrina* is pan-tropical, consisting of some 112 species, 70 neo-tropical, 31 African and 12 Asian. Only one species, *Erythrina fusca,* occurs in both the New and Old Worlds. The genus is probably of South American origin but the ability of the seeds to float and retain viability after prolonged immersion in salt water and the probable riverine, coastal or estuarine environments inhabited by the ancestral species have resulted in worldwide distribution. Pollination by birds and a marked ability to hybridize have resulted in a tremendous amount of ecological and morphological diversity, both within and between species, but with rather close cytological and phytochemical relationships. They have been known to possess predominantly flavonoids and have also been shown to have a considerable amount of alkaloids. The alkaloids of *Erythrina* are distinct from those of other legumes and they all possess an unusual high activity, low affinity nitrate reductase system distinct from known nitrate reduction patterns in other angiosperms (Neill, 1988).

Erythrina abyssinica is medium-sized tree, usually 5-15 m in height, deciduous, thickset, with a well-branched, rounded, spreading crown; trunk short; bark yellow-buff when fresh, otherwise grey-brown to creamy brown, deeply grooved, thickly corky and often spiny; when damaged the tree exudes a brown, gummy sap. Leaves are compound, trifoliolate, alternate; leaflets almost as broad as long, 5.5-15 x 6-14 cm, with the terminal leaflet being largest; lateral leaflets rather smaller than this, if 3 lobed then obscurely so, densely woolly when young, losing most of these hairs by maturity; midrib and main veins on the undersurface often bear scattered prickles. Flowers spectacular, in strong, sturdy racemes on the ends of branchlets, orange-red, up to 5 cm long; calyx

joined to form a tube, split along the under surface almost to the base and separating away into long, slender, distinctive lobes at the apex; calyx and standard petal striking scarlet to brick red. Fruit a cylindrical, woody pod, 4-16 cm long, deeply constricted between the seeds, densely furry, light brown in colour, opening to set free 1-10 shiny, red seeds with a grey-black patch.

Diabetes is the body's failure to metabolize blood sugar properly. There are two widely known forms of diabetes. **TYPE 1** is a failure of the pancreas to produce insulin. Daily injection of insulin replacement is the treatment, itself a triumph of twentieth century science. **TYPE 2** is insulin resistance or impaired glucose tolerance. Insulin appears to be the key factor in developing Type 2 diabetes. Some people have impaired glucose tolerance. What happens is that there is just a moderate rise in blood sugar--enough so that it silently triggers heart disease. In others, they develop insulin resistance. The insulin is produced in adequate quantity, but the body no longer responds effectively to it.

Insulin resistance is one of the characteristic pathogenic signs of type-2 diabetes, and several drugs that increase the insulin sensitivity are currently in clinical use. However, these drugs have a number of limitations, which include adverse effects and high rates of secondary failure. Of the various potential drug targets for treatment of type-2 diabetes, protein tyrosine phosphathase-1B (PTP1B) has recently been considered as a major negative regulator in the insulin signaling pathway. It has been suggested that compounds reducing PTP1B activity or the genetic expression levels of PTP1B may be useful in the treatment of type-2 diabetes and possibly obesity as well. Although there have been a number of reports on the development of PTP1B inhibitors new types of PTP1B inhibitors having improved pharmacological properties remain to be discovered. It is with this in view that we under took the phytochemical and pharmacological study of Erythrina abysinica stem bark. (Rob Hooft van Huijsduijnen et al, 2004).

Materials and Methods

The optical rotations were obtained in MeOH using a Rudolph Autopol IV polarimeter, whereas the IR spectra (KBr) were recorded on a Bruker Equinox 55 FT-IR spectrometer. The UV spectra were taken in MeOH using a Shimadzu spectrophotometer, with CD spectra obtained in MeOH using a JASCO J-710 spectrometer. The nuclear magnetic resonance (NMR) spectra were obtained on Varian Unity Inova 500 MHz spectrometer using TMS as the internal standard. Mass spectra were performed on a Micromass QTOF2 (Micromass, Wythenshawe, UK) mass spectrometer. Silica gel (Merck, 63–200 lm particle size) and C-18 silica gel (Merck, 75 μm particle size) were used for column chromatography (CC). TLC was carried out using Merck silica gel 60 F254 and RP-18 F254 plates, whereas HPLC employed a Gilson system with a UV detector and an Optima Pak C18 column (10-250 mm, 10μm). Enzyme PTP1B (human, recombinant) was purchased from BIOMOL International LP. The solvents used for the bioassay were of analytical grade and obtained from Merck and Sigma–Aldrich.

Results and Discussion

Erythrina abyssinica stem bark was collected from Mukono, Kampala, Uganda in June 2005 and was authentified and a voucher specimen No 0001 was deposited at the Department of Botany, Makerere University, Kampala, Uganda. The stem was air dried chopped and ground. It was then extracted repeatedly in Ethyl Actetate and about 500 grams of crude extract were obtained. The extract was tested positive for flavonoids using hydrochloric acid and Magnesium chips. The extract underwent a series of various chromatographic separations and pure compounds were isolated, their structures elucidated and their Protein Tyrosine Phosphate Inhibitory activity tested. Over seven known flavanones and twenty one novel flavanones were isolated and characterized using usual characteristic spectroscopic techniques. (Long Cui et al, 2007)

The known flavanones were, Sigmoidin A, Sigmoidin B, Sigmoidin C, Sigmoidin D, Sigmoidin E, Sigmoidin F, Sigmoidin G. Abyssinin. The novel flavanones were given the trivial names Abyssinoflavanones V- XXV. Some of the structures are given below.

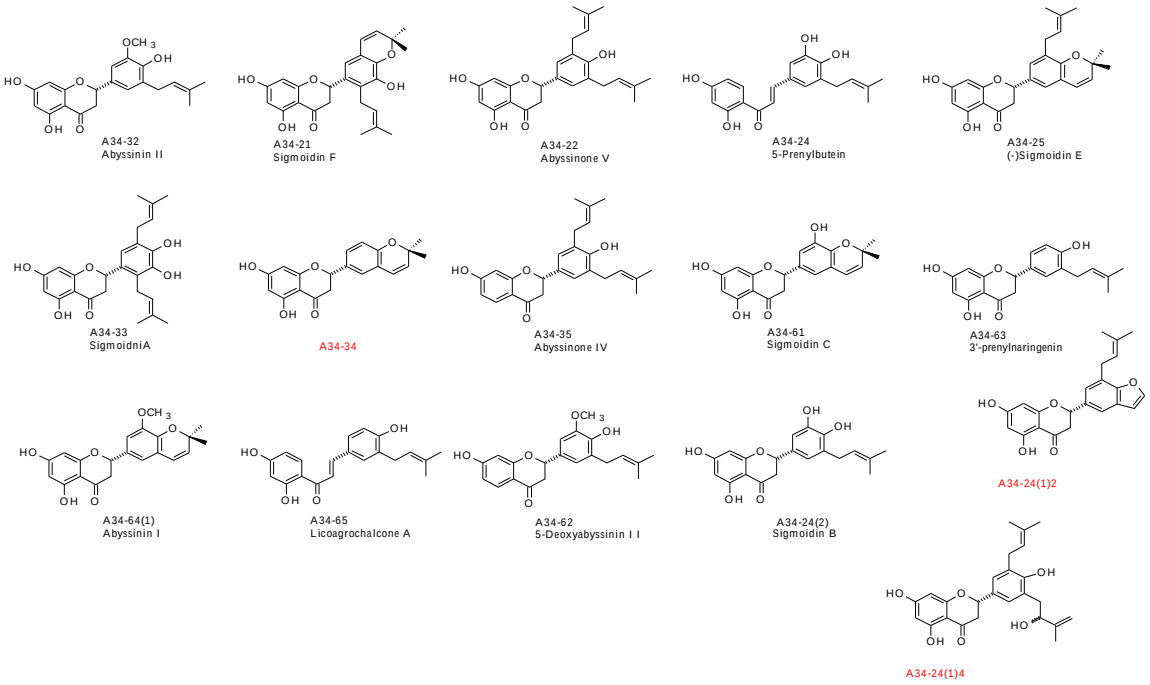

Figure 1: Some compounds isolated from *Erythrina abyssinica*.

Table 1: Inhibitory Activity of some compounds against PTP1B

compound	inhibitory activity[a]
abyssinoflavone V	>60
abyssinoflavone VI	18.9 ±1.9
abyssinoflavone VII	15.7 ±0.4
sigmoidin F	14.2± 1.7
sigmoidin B	19.4± 2.3
abyssinin II	17.3± 1.4

sigmoidin A	14.4 ± 0.8
sigmoidin C	>60
5-deoxyabyssinin II	19.2 ±1.1
3-prenylnaringenin	26.7 ± 1.2
abyssinin I	18.2 ± 1.4
abyssinone-VI	20.6 ± 2.1
licoagrochalcone A	16.9± 0.7
RK-682*b*	4.5 ± 0.5
ursolic acid[b]	3.6 ± 0.2

a Results are expressed as IC_{50} values (μM), determined by regression analyses and expressed as the mean (SD of three replicates.

b Positivecontrol. (Na M et al, 2006)

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[YS 2] Antimalarial and Antileishmanial Activity and Cytotoxicity of Selected Medicinal Plants from Kenya

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Key words: Plant extracts, Anti-plasmodial, Anti-leishmanial, Cytotoxicity, *In vitro*

Introduction

Malaria is perceived as the world's worst health problem. This burden of mortality is not equally shared, falling most heavily on sub-Saharan Africa. Global figures for deaths from malaria range from 1.5 to 2.7 million each year, most of whom are children under 5 years of age and pregnant women (WHO, 2006). In Kenya, more than half of the population is exposed to malaria transmission. Areas endemic are: Western, Coastal, parts of Rift Valley, Central and Eastern provinces (Ministry of Health, 2006). Indigenous rural communities in the tropics manage parasitic diseases, like malaria and leishmaniasis, using herbal drugs. The efficacy, dosage, safety and active principles of most of the herbal preparations are not known (Njoroge *et al.,* 2004).

Materials and methods

Extraction

The leaves, stem bark and root bark of the plants were air dried and ground using an electrical mill. Extraction of the various parts of the medicinal plants was carried out using organic solvents and water. Extracts were subjected to anti-leishmanial and anti-malarial bioassay (Harborne, 1994).

Anti-malarial *Plasmodium falciparum in vitro* assay:

Two strains of *P. falciparum* parasites (D6, chloroquine sensitive and W2 chloroquine resistant) were used. Parasite cultivation was done on *in vitro* technique described by Trager and Jensen (1976). *In vitro* assay semi-automated microdilution assay technique that measures the ability of the extracts to inhibit the incorporation of [G-^{3}H] hypoxanthine into the malaria parasite was adapted (Matile & Pink, 1990; Desjardins *et al*. 1979). Computation of the concentration of drug causing 50% inhibition of [G-^{3}H] hypoxanthine uptake (IC_{50}) was carried out by interpolation after logarithmic transformation of both concentration and cpm values using the formula,

$$IC_{50} = \text{antilog}\ (\log X_1 + [(\log Y_{50} - \log Y_1)(\log X_2 - \log X_1)]/(\log Y_2 - \log Y_1)])$$

Where; Y_{50} is the cpm value midway between parasitized and non-parasitized control cultures and X_1, Y_1, X_2, and Y_2 are the concentrations and cpm values for the data points above and below the cpm midpoints (Sixsmith *et al.,* 1984).

Anti-leishmanial *in vitro* bioassay: Anti-promastigote assay

Leishmania major promastigotes were cultured in NNN media overlaid with Schneider's *Drosophila* insect medium supplemented with 20% FBS, 100 μg/ml streptomycin and 100 U/ml penicillin-G, and 5-fluorocytosine at 25°C, in tissue culture flasks and the assay was done as described by Delornzi *et al.* (2001). Counting of the promastigotes using an improved neubauer chamber. Calculation of percentage mortality (PM).

$$PM = \frac{\text{Number of dead parasites} \times 100}{\text{Total number of parasites}}$$

Anti-amastigote (Macrophage) assay)

Harvesting of mouse peritoneal macrophages – BALB/c mice was carried out and the macrophages were cultured in RPMI-1640 medium, 37°C, 5% CO_2, in 24 microwell plates. Infection of macrophages with *Leishmania major* (Strain IDU/KE/83 = NLB-144) amastigotes followed and then introduction of plant extracts. Determination of infection rate (IR) and multiplication index (MI). The assay was carried out as described by **(**Delorenzi *et al.,* 2001).

IR = No. of infected macrophages in 100 macrophages

$$MI = \frac{\text{(No. of amastigotes in experimental culture/100 macrophages)} \times 100\ \%}{\text{No. of amastigotes in control culture/100 macrophages).}}$$

Nitric oxide determination

Nitric oxide release in supernatants of macrophage culture was measured by the Griess reaction for nitrites (Holzmuller *et al.,* 2002).

Results and Discussion

After screening extracts from the six selected plant species, for *in vitro* anti-plasmodial and anti-leishmanial activity, against 2 laboratory-adapted *Plasmodium falciparum* isolates (D6, CQ-sensitive and W2, CQ-resistant) and *Leishmania major* (IDU/KE/83 = NLB-144 strain), respectively, the methanol extract of *Suregada zanzibariensis* leaves exhibited good anti-plasmodial activity (IC_{50} 4.66±0.22 and 1.82±0.07μg/ml for D6 and W2, respectively). Similarly, the methanol extracts of *Albizia coriaria* (IC_{50} 37.83±2.11μg/ml for D6) and *Asparagus racemosus* (32.63±2.68 and 33.95±2.05μg/ml for D6 and W2, respectively) had moderate anti-plasmodial activity. *Acacia tortilis*

(IC_{50} 85.73±3.36µg/ml for W2) and *Albizia coriaria* (IC_{50} 71.17±3.58µg/ml for W2) methanol extracts and *Aloe nyeriensis* var *kedongensis* (IC_{50} 87.70±2.98 and 67.84±2.12µg/ml for D6 and W2, respectively) water extract exhibited mild anti-plasmodial activity. The rest of the extracts did not exhibit any anti-plasmodial activity.

Although the leishmanicidal activity of extracts were lower than for pentosam (80%), reasonable activity was observed for *Aloe nyeriensis* methanol (68.4±6.3%), *Albizia coriara* water (66.7±5.0%), *Maytenus putterlickoides* methanol (60.0±6.23%), *Asparagus racemosus* methanol and water (58.3±8.22 and 56.8±6.58%, respectively), *Aloe nyeriensis* water (53.3±5.1%) and *Acacia tortilis* water (52.9±6.55%) extracts at 1000µg/ml. *Leishmania major* infected macrophages treated with methanol extracts of *Suregada zanzibariensis* and *Aloe nyeriensis* var *kedongensis* and Pentostam® had infection rates of 28±2.11, 30±1.22 and 40±3.69%, respectively at 1000µg/ml, indicating better anti-leishmanial activity for the extracts. The methanol extract of *Albizia coriara* (44.0±3.69%) and aqueous extracts of *Asparagus racemosus* (42±3.84%) and *Acacia tortilis* (44±5.59%) had similar activity to pentosam®. Multiplication indices for *Leishmania major* amastigotes treated with methanol extracts of *Albizia coriaria*, *Suregada zanzibariensis* and *Aloe nyeriensis* var *kedongensis*, aqueous extract of *Acacia tortilis* and pentosam® were 28.5±1.43, 29.4±2.15, 31.1±2.22,35.9±3.49 and 44.0±3.27%, respectively, at 1000µg/ml, confirming better anti-leishmanial activity for the extracts. Aqueous extracts of *Aloe nyeriensis* (46.7±3.28%) and *Albizia coriaria* (47.5±3.21%) had similar activity level to pentosam®. The plant extracts have better inhibitory activity while pentosam® has better leishmanicidal activity. All extracts exhibited very low cytotoxicity (CC_{50} >500µg/ml) against human embryonic lung fibroblast (HELF) cells. The investigations demonstrated the efficacy and safety of some extracts of plants that are used by rural indigenous communities for the treatment of parasitic diseases.

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[YS 3] Terpurinflavone: Antiplasmodial Flavones from the Stem of *Tephrosia purpurea*

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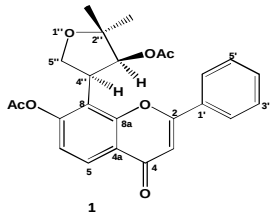
Introduction

Tephrosia Pers (Leguminosae- Papilionoideae) is a large tropical and sub-tropical genus estimated to contain about three hundred species (Waterman and Khalid, 1980; Abou-Douh et al., 2005) out of which thirty are found in Kenya (Tarus et al., 2002). The extracts of the some *Tephrosia* species have shown various biological activities including antiplasmodial (Muiva et al., 2009), antibacterial (Abou-Douh et al., 2005). Various biological activities including antibacterial (Hegazy et al., 2009), antidiabetic and cancer chemoprevetive activities (Chang et al., 2000) have been reported for extracts and pure compounds from this plant. *Tephrosia* is rich in prenylated flavonoids including flavones (Hegazy et al., 2009; Pelter et al., 1981). Flavanones (Pelter et al., 1981); chalcones (Chang et al., 2000; Pelter et al., 1981) and rotenoids (Ahmad et al., 1999). In the search for compounds with antiplasmodial activity from Kenyan plants, the stem of *T. purpurea* has been investigated. This report is on the isolation and characterization of new prenylated flavones, named terpurinflavone (**1**), with antiplasmodial activity along with three known flavonoids.



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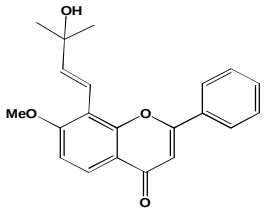

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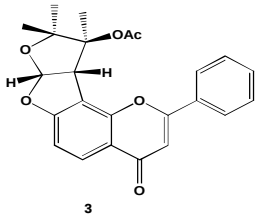

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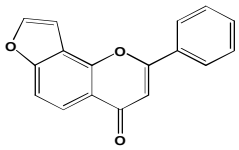

4

Results and Discussion

Compound **1** was obtained as a white amorphous powder with an R_f value of 0.45 in n-hexane/ethyl acetate (3:2). It showed $[M+H]^+$ peak at m/z 437.1593 in its positive electrospray ionization time of flight mass spectrum (ESI-TOF-MS) constituting the molecular formula $C_{25}H_{24}O_7$. The presence of a flavone skeleton was deduced from the UV (λ_{max} 295, 325 nm), ^1H (δ 6.79 *s* for H-3) and ^{13}C (163.9 for C-2, 108.5 for C-3 and 177.6 for C-4) NMR spectroscopic data (Table 1).

The presence of unsubstituted ring-B was clearly shown in ^1H (δ 7.63 *m* for H-3'/4'/5', δ 8.18 *m* for H-2'/6') and ^{13}C (δ 127.9 for C-2'/6', δ 130.7 for C-3'/5', δ 133.1 for C-4' and 133.6 for C-1') NMR

spectra. In ring-A, an AX protons which are *ortho*-coupled at δ 8.00 and 6.94 (*J* = 8.5 Hz) were assigned to H-5 and H-6, respectively, with C-7 and C-8 being substituted with an acetoxy (at C-7) and a tetrahydrofuran ring (at C-8) derived from a modified prenyl group as in tephrorin B (Chang et al., 2000). The HMBC spectrum showed correlation of H-4'' (δ 4.44) with C-5'' (79.1), C-3'' (78.7), C-2'' (84.0), C-7 (167.9), C-8 (116.0) and C-8a (155.9) confirming that the tetrahydrofuran ring is placed at C-8. The presence of a second acetate group was also evident from the NMR spectra (Table 1) and placed at C-3'' of the tetrahydrofuran group based on the HMBC spectrum which showed correlation of H-3'' (δ 5.35) with acetoxy carbon (δ 170.3) and the two methyl carbon atoms (δ_C 22.4 and 24.4) at C-2''). The ^{1}H and ^{13}C NMR chemical shift values of the tetrahydrofuran ring of **1** were quite similar to those reported for tephrorin B (Chang et al., 2000). The coupling constant (J = 8.5 Hz) between H-3'' and H-4'' indicated that the relative orientation of these two protons is *cis* as in tephrorin B. In the NOESY spectrum, NOE interaction of H-3'' with H-4'' supported the *cis* geometry. The new compound was therefore characterized as 7-acetoxy-8-[3''-acetoxy-2'',2''-dimethyltetrahydro-4''-furanylflavone (**1**) for which trivial name terpurinflavone was assigned. The isolation of this new compound once again demonstrated the unique capacity of *T. purpurea* to oxidize the C-7 methoxy group in compound **2** and cyclize it with the adjacent 2-hydroxy-2-methylbut-1-enyl group into complex C-8 substituted flavonoids (Pelter et al., 1981).

The EtOAc fraction of the CH_2Cl_2/MeOH (1:1) extract of *T. purpurea* showed moderate antiplasmodial activity against chloroquine-sensitive (D6) and chloroquine-resistant (W2), strains of *Plasmodium falciparum* with IC_{50} values of 10.47 ± 2.22 µg/ml and 12.06 ± 2.54 µg/ml, respectively, respectively. The pure compounds isolated from this plant were also tested with the new compound terpurinflavone (**1**) exhibiting the highest activity with IC_{50} values of 3.12 ± 0.28 µM and 6.26 ±2.66 µM against D6 and W2 stains of *P. falciparum*, respectively (Table 2). The activity of the crude extract could be due to these compounds, especially that of compound **1** which showed the highest activity.

Table 1: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data along with HMBC correlations for compound **1** in acetone-d_6

Position	δ_H (in Hz)	δ_C	HMBC (^{2}J, ^{3}J)
2		163.9	
3	6.79 *s*	108.5	C-1', 2, 4, 4a
4		177.6	
4a		119.9	
5	8.00 *d* (8.5)	129.3	C-4, 7, 8a
6	6.94 *d* (8.5)	110.1	C-4a, 8
7		167.9	
8		116.0	

Position	δ_H (in Hz)	δ_C	HMBC (^2J, ^3J)
8a		155.9	
1'		133.6	
2'/6'	8.18 *m*	127.9	C-1', 2
3'/5'	7.63 *m*	130.7	C-2'/6', 4
4'	7.63 *m*	133.1	
2''		84.0	
3''	5.35 *d* (8.5)	78.7	C-2'', 5'', COMe-3''
4''	4.44 *ddd* (2.0, 8.0, 8.5)	42.3	C-2'', 3'', 5'', 7, 8, 8a
5''	4.84 *dd* (8.0, 9.5)	79.1	C-3''
	5.02 *dd* (2.0, 9.5)		C-4'', 8
COMe-3''		170.3	
COMe-3''	1-61 *s*	20.9	COMe-3''
Me-2''	1.76 *s*	22.4	C-2'', 3'', Me-2''
Me-2''	1.61 *s*	24.4	C-2'', 3'', Me-2''
COMe-7		170.8	
COMe-7	2.00 *s*	23.0	COMe-7

Table 2: In vitro IC_{50} values of pure compounds isolated form *T. purpurea* against the D6 and W2 strains of *P. falciparum*.

Sample	IC_{50} (µM ± SD)	
	(D6)	(W2)
Terpurinflavone (**1**)	3.12 ± 0.28	6.26 ± 2.66
Lanceolatin A (**2**)	11.36 ± 2.97	14.97 ± 3.09
Semiglabrin (**3**)	25.77 ± 6.08	35.58 ± 5.41
Lanceolatin B (**4**)	27.02 ± 2.65	35.99 ± 4.24
Mefloquine	-	0.013 ± 0.002
Chloroquine	0.035 ± 0.003	-

Methods and Materials

T. purpurea was collected from Kilifi District, Coast province, Kenya in August, 2007. The plant was identified by Mr. Patrick C. Mutiso of the the University Herbarium, Botany Department, University of Nairobi, where a voucher specimen (Mutiso-520-August 2007) is deposited.

Extraction and Isolation

Air dried and ground stems of *T. purpurea* (2 kg) were extracted with dichloromethane/methanol (1:1) by cold percolation at room temperature (3 x 1.5 L). The extract was filtered and the solvent removed under vacuum using a rotary evaporator at 35^{o}C. This gave dark oily extract that was partitioned between water and ethyl acetate. The organic layer (36 g) was subjected to CC on silica gel (400g) eluting with n-hexane containing increasing percentages (2%, 4%, 6%, 8%, 10%, 12.5%, 15%, 17.5%, 20%, 25%, 30%, 40%, 50%, 75%, and 100%) of ethyl acetate and gave 15 fractions each

of 1.5L. The fraction eluted with 15% ethyl acetate in n-hexane was separated on Sephadex LH-20 (CH_2Cl_2-MeOH; 1:1) to give terpurinflavone (**1**, 34 mg).

In vitro antiplasmodial activity assay

Antiplasmodial activities of crude extract and pure compounds against chloroquine-sensitive Sierra Leone 1 (D6) and chloroquine-resistant Indochina 1 (W2) strain of P. falciparum was tested using a non-radioactive assay technique (Smilkstein et al., 2004) with modifications. This method use the fluorochrome called "SYBR Green 1", a non-radioactive DNA dye that accurately depicts in vitro parasite replication.

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[YS 4] *In vitro* Antiplasmodial and Cytotoxicity Activities of Some Medicinal Plants from Kenya

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Key Words: Malaria, medicinal plants, Plasmodium falciparum, cytotoxicity

Introduction

Malaria still remains a major cause of morbidity and mortality especially in sub-Saharan Africa despite intensive efforts to control it. This pervasiveness is compounded by emergence and spread of drug resistant parasites combined with the absence of a vaccine and lack of systematic vector control strategies. As a result, efforts are being directed towards discovery and development of novel and affordable malaria control tools including the search for new drugs leads from plants.

Materials and Methods

Plants materials were collected from Meru and Mombasa regions of Kenya. They were air dried and ground. Cold extraction was done at room temperature ~ 25^{o}C successively using dichloromethane and methanol. They were dried using a rotary evaporator. Water extracts were extracted for 6 hours in a waterbath at 70^{0}C and dried in a freeze drier. Continuous *in vitro* cultures of asexual erythrocytic stages of Plasmodium strains {NF54 from unknown origin- chloroquine sensitive and K1 from Thailand-CQ/pyrimethamine resistant} were maintained following a modified procedure described by Trager and Jensen (1976). Drug assay was done using a modification of the semi automated micro dilution technique of Desjardin et al. (1979) which measures the ability of the extracts to inhibit the incorporation of [^{3}H] hypoxanthine into the malaria parasite. Chloroquine was used as the reference drug.

Cytotoxicity assay was done following the method of Page et al. (1993) and Ahmed et al. (1994) where rat skeletal myoblast L6 cell line was used. Podophyllotoxin (Polysciences Inc. USA) was used as a positive reference.

Results and Discussion

26 extracts from 14 plants were tested for their antiplasmodial properties *in vitro* (**Table 1 & 2**). Water extracts showed no activity with IC50 > 50 µg/ml in both strains except for *S. heningsii*, which had moderate activity. Methanol extracts had moderate activity except for *T. robusta* that was highly active with an IC50 of 3.5 and 2.4 µg/ml against K1 and NF54 strains respectively. Dichloromethane extracts showed the highest activity with IC50's ranging from 35.2 - 1.4 µg/ml against the two strains except for *S. heningsii* which had dichloromethane extract as the least active. This phenomenon of high activity on dichloromethane extracts over water and methanol

extracts was also reported by Koch et al. (2005). A probable explanation could be due to lack of tannins, polysaccharides and other water-soluble molecules that have no antiplasmodial properties. It was also noted that sensitivity of the extracts to both CQ-resistant and CQ-sensitive strains did not differ significantly.

Table 1: In vitro antiplasmodial activities against K1 strain

	IC's 50 in µg/ml		
	Type of extract		
Plant/part	**water**	**MeOH**	**CH_2Cl_2**
Caesalpinia volkensii	68.7	51.4	25.6
Clerodendrum eriophyllum	82.7	47	2.7
Clerodendrum myricoides	64	48.2	15.8
Harrisonia abyssinica	91.1	52.3	4.4
Strychnos heningsii	29.6	14.6	35.2
Turraea robusta	91.5	3.5	
Vernonia auriculifera	84.5	53.8	32.7
Vernonia lasiopus	52.2	31.2	4.7
Warbugia ugandensis	31.8	17.8	1.4
Chloroquine	0.091	0.05	0.061

Table 2: In vitro antiplasmodial activity against NF54

	NF54 strain: IC's 50 in µg/ml			
Plant/part	**water**	**MeOH**	**CH_2Cl_2**	
Caesalpinia volkensii	100	65.1	11.9	
Clerodendrum eriophyllum	100	79	5.3	
Clerodendrum myricoides	94.3	51.5	10.9	
Harrisonia abyssinica	100	55.4	5.6	
Strychnos heningsii	33.7	17.9	33.3	
Turraea robusta	100	2.4		
Vernonia auriculifera	100	60.8	27.3	
Vernonia lasiopus	100	50.5	4.9	
Warbugia ugandensis	64	24.3	2.2	
Chloroquine	0.003	0.003	0.004	

The dichloromethane extracts and methanol extract of *T. robusta* were tested for their cytotoxicity properties *in vitro* using L6, rat skeletal myoblast cells (**Table 3**). Most extracts showed low cytotoxicity with IC50 value of > 20 µg/ml (Zirihi et al., 2005). Selectivity index was also calculated with *V. lasiopus* having the highest selectivity of malaria parasite with an SI >10. Though *W. ugandensis* possessed a high antiplasmodial activity (1.4 µg/ml against K1), it also expressed the highest cytotoxicity with an IC50 of 0.34 µg/ml and a SI of 0.24. This indicates that the high

antiplasmodial activity noticed is probably due to cytotoxicity rather than activity against the parasite themselves.

Table 3: Cytotoxicity results and SI of dichloromethane extracts

Plant/ part	L6 cells IC50's (µg/ml)	K1 IC50's (µg/ml)	SI
Caesalpinia volkensii	82.4	25.6	3.2
Clerodendrum eriophyllum	7.9	2.7	2.9
**Clerodendrum myricoides*	90	15.8	5
Harrisonia abyssinica	32.8	4.4	7.5
**Strychnos heningsii*	90	35.2	2.6
***Turraea robusta*	14.3	3.5	4.1
Vernonia auriculifera	84.8	32.7	2.6
**Vernonia lasiopus*	90	4.7	10.7
Warbugia ugandensis	0.34	1.4	0.24
Chloroquine	37	0.061	607
Podophyllotoxin	0.01		

According to our classification, dichloromethane extract of *V. lasiopus* exhibited promising antimalarial activity with relatively minimal cytotoxicity. This plant is commonly used in traditional medicine in Kenya to manage malaria (Beentje, 1994). At least from ethnomedicinal use there is some evidence that the plant may be safe in humans. It is therefore planned to evaluate the activity of *V. lasiopus in vivo* using *P. berghei* mouse model then followed by isolation of the active compound (s). It's hoped that a lead compound may be identified that could be developed further as antimalarial agent.

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[YS 5] Antiplasmodial Quinones from the Roots of two *Pentas* Species

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Keywords: *Pentas longiflora*; *Pentas lanceolata*; Rubiaceae; anthraquinone; 5,6-dihydroxydamnacanthol; malaria

Introduction

Malaria, caused by the protozoan parasites of the genus *Plasmodium,* is a major disease in the tropical and subtropical regions of the world. Out of the yearly 300 to 500 million clinical episodes, 1.5-2.7 million cases are lethal [Snow *et al.,* 2005]. The emergence of multidrug-resistant strains of the parasite *P. falciparum* and the rising resistance of the vector (*Anopheles* spp.) to insecticides on top of poverty and lack of a well-functioning healthcare system are the main causes for the relentless increase of malaria morbidity and mortality over the past decade. To date, over a thousand herbal species are employed in indigenous health care systems as a means of treating malaria and managing fever associated to the disease. However, neither the efficacy of most of such plants established nor the active components responsible for activity identified.

In Kenya, a decoction of *Pentas longiflora* mixed with milk and is taken as a cure for malaria [Kokwaro, 2010].We report here the isolation, structure elucidation, antiplasmodial and cytotoxicity studies of the secondary metabolites isolated from the roots of *Pentas longiflora* and *Pentas lanceolata*.

Results and Discussion

The roots of *P. longiflora* and *P. lanceolata* were extracted with CH_2Cl_2:MeOH (1:1) and tested for antiplasmodial activities and both extracts showed good activities (IC_{50} <3 μg/mL). From the root extract of *P. longiflora*, the naphthoquinone derivatives pentalongin (**1**), psychorubrin (**2**) and mollugin (**3**) were chromatographically isolated, identified (Fig 1) and tested for antiplasmodial activities. The major constituents **1** and **2** showed high (IC_{50} < 1 μg/mL) activity, whereas **3** has moderate inhibition potencies against the W2 chloroquine-resistant and D6 chloroquine-sensitive strains of *P. falciparum* (Table 1). Though compounds **1**-**3** were previously reported [De Kimpe *et al*., 1993, Hari *et al*., 1991] and evaluated for antibacterial [Tuyen Nguyen *et al*., 2004], antifungal

[Van Puyvelde *et al.*, 1985], and antiviral [Li-Kang *et al.,* 1994] properties, their antiplasmodial activities are reported here for the first time. Unfortunately, compounds **1** and **2** showed high cytotoxicity (LD_{50} 0.80 µg/mL for **1** and 0.89 µg/mL for **2).**

Compounds **4**-**13** were isolated from the root extract of *P. lanceolata* of which 5,6-dihydroxydamnacanthol (**11**) is a new compound; while nordamnacanthal (**7**), lucidin-ω-methyl ether (**9**), and damnacanthol (**10**) are reported here for the first time from the genus *Pentas.* Compounds **4-11** (Fig 2, Table 2), especially **9** and **11**, showed a valuable compromise between the antiplasmodial activity and cytotoxicity, revealing that by reaching better understanding of the factors governing their activities against *P. falciparum* and against mammalian cells, anthraquinones may become promising targets for further lead optimization.

The anthraquinones isolated from the roots of *Pentas lanceolata* have a hydroxyl, and/or methoxyl and carbon (CH_2, CHO, CH_3, etc) substitution at C-2 of ring A [Kusamba *et al.*, 1993; Han *et al.*, 2001], and in the case of compound **11**, additional hydroxyl groups at positions 5 and 6 of ring C are observed. These latter oxygens are introduced at a late stage of the biogenesis [Han *et al.*, 2001].

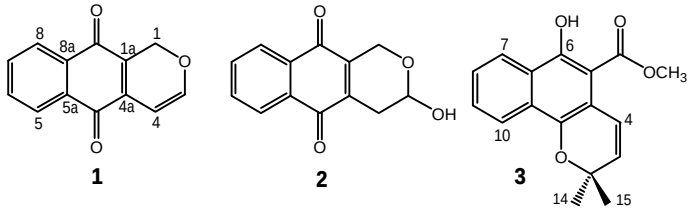

Figure 1. Compounds isolated from the roots of *Pentas longiflora*

Compound	R^1	R^2	R^3	R^4	R^5
4	H	CH_3	H	H	H
5	OH	CH_3	OH	H	H
6	OCH_3	CH_3	OH	H	H
7	OH	CH_2OCH_3	OH	H	H
8	OH	CHO	OH	H	H
9	OCH_3	CHO	OH	H	H
10	OCH_3	CH_2OH	OH	H	H
11	OCH_3	CH_2OH	OH	OH	OH
12	OH	CH_3	OPrimveroside	H	H
13	OCH_3	CH_3	OPrimveroside	H	H

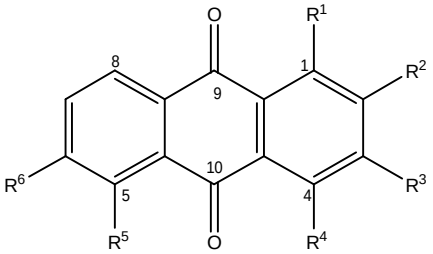

Figure 2. Compounds isolated from the roots of *Pentas lanceolata*

Table 1. *In vitro* antiplasmodial activity and cytotoxicity of crude extracts and pure compounds

Sample (purity in %)	Antiplasmodial acitivty IC_{50}* (µg/mL)		Cytotoxicity LD_{50}§ (µg/mL)	Selectivity Index	
	W2 clone (CQ-R)	D6 clone (CQ-S)		W2	D6
Pentas longiflora (root extract)	0.93 ± 0.16	0.99 ± 0.09			
Pentalongin (**1**, ≥ 98%)	0.27 ± 0.09	0.23 ± 0.08	0.80	2.96	3.48
Psychorubrin (**2**, ≥ 98%))	0.91 ± 0.15	0.82 ± 0.24	0.89	0.98	1.09
Mollugin (**3**, ≥ 95%)	10.22 ± 1.37	7.56 ±1.13	20.0	1.96	2.65
Pentas lanceolata (root extract)	2.55 ± 0.30	1.33 ± 0.15			
Tectoquinone (**4**, ≥ 98%)	10.78 ± 1.33	6.74 ± 1.73	> 10#	> 0.93	>1.48
Rubiadin (**5**, ≥ 98%)	8.36 ± 2.19	5.47 ± 0.70	53.0	6.34	9.69
Rubiadin-1-methyl ether (**6**, ≥ 98%)	18.91± 0.39	12.08 ± 2.28	64.0	3.38	5.30
Nordamnacanthal (**7**, ≥ 99%)	9.33 ± 2.98	9.29 ± 0.00	51.0	5.47	5.49
Damnacanthal (**8**, ≥ 99%)	10.88 ± 2.09	7.67 ± 0.36	73.0	6.71	9.52
Lucidin-ω-methyl ether (**9**, ≥ 98%)	13.19 ± 2.15	12.08 ± 3.69	> 100	>7.58	> 8.28
Damnacanthol (**10**, ≥ 98%)	31.42 ± 2.32	16.07 ± 1.15	> 100	> 3.18	> 6.22
5,6-Dihydroxydamnacanthol (**11**, > 99%)	19.33 ± 6.36	15.02 ± 4.28	> 100	> 5.17	> 6.66
Chloroquine	0.07 ± 0.01	0.01 ± 0.01			
Mefloquine	0.004 ± 0.38	0.06 ± 0.04			

* Data are the mean of at least 3 independent experiments. § The mean value of at least 6 independent experiments are given; 95% confidence interval and dose-response curves are presented. # Reference [Costa *et al.*, 2001].

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[YS 6] Study of Verucidal Effect of the Crashed Leaves of *Tetradenia riparia* on the Warts

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Keywords: *Tetradenia riparia leaves,* warts, Juice, Nitrogene, Magnesium and Chrome.

Introduction

Tetradenia riparia was previously classified under the genus *Iboza*, which was derived from its Zulu name and apparently this refers to the aromatic qualities of the plant. The Zulu people have many uses for the plant including the relief of chest complaints, stomach ache and malaria. Inhaling the scent of the crushed leaves apparently also relieves headaches. In Rwanda, the plant is used to treat mainly the fever, cough, you find also others medicinal uses such us respiratory problems, stomach ache, diarrhea, dropsy, *angina pectoris*, fever, malaria and dengue fever, yaws, headache, toothache and as an antibiotic. (Hakizamungu, E. et al, 1986).

The natural habitat of *Tetradenia riparia* is along river banks, forest margins, dry wooded valleys and hillsides in areas where there is little frost. The natural distribution ranges from KwaZulu-Natal, Northern Province, Mpumalanga in South Africa to Swaziland, Namibia, Angola and northwards through tropical east Africa into Ethiopia (Codd, L.E, 1985).

Figure 1: distribution map

Botanic description

Synonyms
Iboza bainesii N. E. Br.
Iboza galpinii N. E. Br.
Iboza riparia (Hochst.) N. E. Br.
Moschosma riparium Hochst.

At Macroscopical level

Soft much-branched dioecious shrub or small tree 1-3m in height, with brittle, semi-succulent stems and sticky-aromatic foliage; **leaves** petiolate, ovate-oblong to round, 35-80 × 35-70mm, sparsely to densely glandular-pubescent on both surfaces, margin coarsely crenate to dentate,

variable in size, shape and degree of hairiness; **flowers** (May-Aug) in large branched terminal panicles, the male flower-spikes longer than the female, small (corolla 3-3.5mm long), white to pale mauve (Codd, L.E, 1985)

Figure 2: line drawing (female flowers)

At Microscopical Level

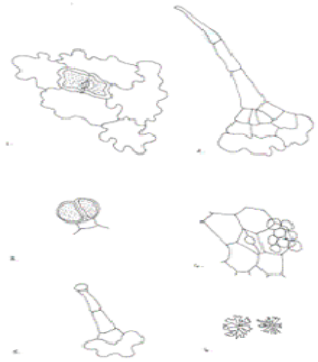

Figure 3: Microscopical features

Characteristic features are: the cells of the lower leaf epidermis with sinuous walls and numerous anomocytic stomata (1); the polygonal cells of the upper leaf epidermis with occasional stomata and underlying palisade layer (4); the numerous glandular hairs of leaf and stem, of two types: those with 2-3 celled stalk and unicellular head, up to 650μ in length, raised on papillae (5), particularly abundant on main leaf veins and those having a unicellular stalk and bicellular head up to 25μ in diameter, filled with yellow-brown contents (3); the uniseriate clothing hairs of both leaf surfaces, up to 800μ long, thin-walled, smooth, 2-3 cells long, with swollen base (2); the micro-rosettes of calcium oxalate, 10-12μ in diameter, in cells of the leaf palisade and mesophyll. (Codd, L.E, 1985); (Pyvelde, 1983), (Davies Coleman et al, 1995)

Chemical constituents

Previously, several new substances have been isolated from the leaves of this plant, including a new diterpene diol, i.e. 8(14), 15-sandaracopimaradiene-7 alpha, 18-diol. This new diterpene diol exhibits significant antimicrobial activity against several bacteria and funga. (Bodenstein, J. W. ,1977) diterpenes e.g. ibozol (Zelnik, R et al,1978), 7 α-hydroxyroyleanone, 8 (14), 15-sandaracopimaradiene-7α,18-diol (Puyvelde Van et al, 1987) α-pyrones e.g. umuravumbolide[4, 5], tetradenolide[6]

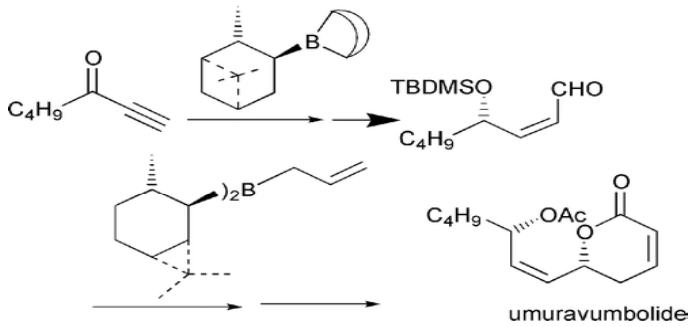

Figure 4: *Umuravumolide*

Essential oil (1.9%) of which the main components are: α-terpineol (22.6%), fenchone (13.6%), β-fenchyl alcohol (10.7%), β-caryophyllene (7.9%) and perillyl alcohol (6.0%), (Campbell, W.E, 1997). But the chemical composition of essential oil can vary depending on season and time of harvest (Gazim ZC et al, 2010).

Material and Methods

Plant material

The plant material consists of *Tetradenia riparia* leaves from which the juice has been extracted.

Figure 1. Live plant

In a primary school in Butare (Ikibondo primary school) , 64 volunteers pupils aged between 12 and 17 years old with warts have been selected for the treatment.

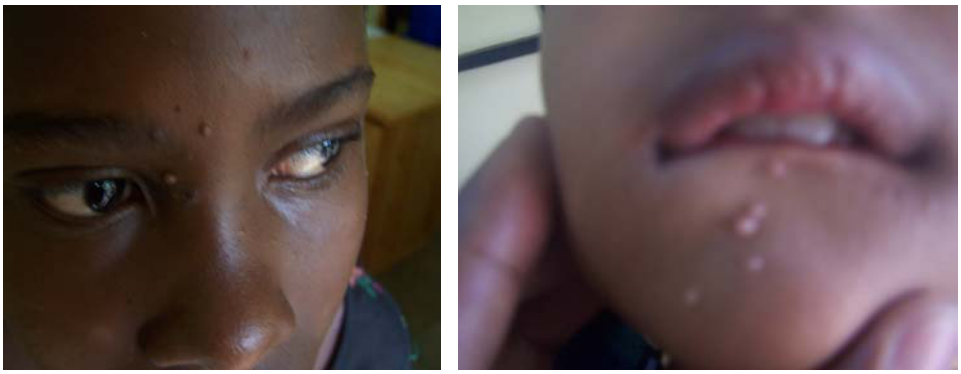

Figure 3. Photos of some Pupils with warts.

Methods

Data collection procedure

A form of structured questionnaires where convenience sampling was used. The focus was on crashed leaves of *Tetradenia riparia* used in treating the warts. Filling the form processing was done by patients or their parents. The forms were collected and analyzed after the period of treatment.

Determination of chemical constituents: Nitrogen, Magnesium and Chrome.

According the theory of DEGOS, recurrent warts are treated with magnesium salt, liquid nitrogen, and chromic acid instead of dry ice. We have previously investigated the presence of those constituents in the leaves of *Tedradenia riparia* to finally confirm or disprove the DEGOS theory, such as traditional healers had observed in treating warts. (Degos R., 1981)

Determination of Magnesium and Crome (Digestion of organic matte).

We used the method of wet digestion for the determination of magnesium (Mg) and Chromium (Cr)

or wet digestion method,(MOORS, 1967).

Determination of total nitrogen.

For determination of total nitrogen, we used the Kjeldahl digestion method, distillation and titration, (Blume, 1966).

Application of crushed leaves of Iboza riparia on warts

The crushed leaves of *Tedradenia riparia* were rubbed two to three times per day on warts .The application is distributed over a treatment period ranging between one and five weeks.

Results and Discussions

Determination of chemical constituents in the leaves of *Tedradenia riparia*

The results of the analysis and determination of chemical constituents in the leaves are presented in the table below:

Table No. 1: Concentration of chemical constituents in *Tedradnia riparia* leaves.

No	Chemical constituents	Concentration (ppm)
1	Total nitrogen (N)	4850
2	Magnesium (Mg)	6720
3	Chromium (Cr)	1072

Application of crashed leaves of *Tedradenia riparia* on the warts

The leaves were crushed and rubbed precisely on where the warts were observed in faces. Applying the juice of crushed leaves of *Tedradenia riparia* was regularly done three times a day (Morning-Noon-Night) and for a period ranging between one and five weeks. After regular application of the juice of the leaves of *Tetradenia riparia* on the warts, we observed the following results:

Table No. 2: Distribution of patients according the number of treatment day.

Number of days	Number of patients	Patients cured	Resistant cases	% healing	% Resistance
1	14	12	2	85.7	14.3
2	20	18	2	90	10
3	30	27	3	90	10
TOTAL	**64**	**57**	**7**	**89**	**11**

.

Figure 4 : Photos showing some children cured after treatment.

After carry out this study, in general 89% of cases were cured. These observations allow us to confirm the therapeutic effect of the crashed leaves of *Tedrania riparia* on warts. The plant contains essential oils, nitrogen, magnesium and chromium. All these chemical constituents have veridical activities acting on the warts. After the treatment with crashed leaves, non scars remains on the faces as you can see on the pictures above. This healing is due mainly to the presence in leaves of Nitrogen, Magnesium, and Chromium, as well as the essential oils. These results confirm the scientific work of (Degos, R., 1981), that the warts are treated by liquid nitrogen, nitric acid, chromic acid and magnesium salt.

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[YS 7] Isoflavanones and 3-Methoxyflavones from the Stem Bark of *Platycelphium voënse*

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Key Words: *Platycelphium voënse*; Stem bark; Leguminosae; Isoflavanones; 3-methoxyflavones; Glyasperin F; Sophoraisoflavanone A; 5,7-dihydroxy-4'-methoxy-[2'',3'':2',3']-furanoisoflavanone

Introduction

Platycelphium (Engl.) Wild (Leguminosae) is a monotypic genus that occurs in the drier parts of Eastern Africa (Gillett *et al.,* 1971). Prior to this report, the only phytochemical studies on *Platycelphium voënse* describe the identification of quinolizidine alkaloids through the GC-MS analysis (Asres *et al.,* 1997; Van Wyk *et al.,* 1993). As part of the ongoing study of some Leguminosae species of Kenya, we hereby discuss the isolation and characterization of eight compounds including three isoflavanones, one isoflavone, two 3-methoxyflavones and two triterpenes.

Materials and Methods

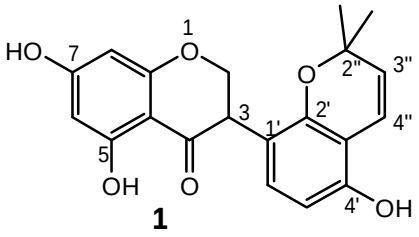
The stem of *Platycelphium voënse* was collected from Eastern Province, Kenya, in January 2009. The plant was identified at the University Herbarium, Botany Department, University of Nairobi, where a voucher specimen was deposited.

The air-dried and pulverized stem bark (1.6 Kg) of *P. voënse* was extracted with CH_2Cl_2-MeOH (1:1) at room temperature to afforded 114 g of crude extract. The extract was subjected to CC on silica gel, using increasing amounts of EtOAc in n-hexane as the mobile phase. Purification was done by repeated chromatography on silica gel, prepTLC, and gel filtration over sephadex LH-20. The structures of isolated compounds were established using a combination of spectroscopic techniques and by comparing the results with published data.

Results and Discussion

Compounds **1** and **3** were obtained as white amorphous solids with ^{1}H (Table 1) NMR spectral features characteristic of 5-hydroxyisoflavanones each with a pair of *meta*-coupled protons between δ_H 5.90 and 6.10 for H-6 and H-8 (in A-rings); and two *ortho*-coupled protons resulting in an AX spin system at *ca.* 6.40-6.70 ppm (H-5') and 6.80-7.20 ppm (H-6'). The ^{1}H, ^{13}C NMR and DEPT spectra further revealed the presence of 2,2-dimethylpyrano (δ_C 27.0, 27.5 for 2''-OCH_3, δ_C 76.4 for

C-2'', δ_C 118.4 for C-4'', δ_C 129.9 for C-3'') and isoprenyl (δ_C 23.7 for C-1'', δ_C 121.4 for C-2'', δ_C 135.6 for C-3'', δ_C 18.0 for C-4'', δ_C 25.8.8 for C-5'') moieties in **1** and **3**, respectively. The ^{13}C NMR spectrum for each of the two compounds **1** and **3** exhbited five peaks due to quaternary sp^2 hybridized enolic carbons in the δ_C 150-167 range, four of which (based on biogenesis) are attributed to C-5, C-7, C-8a and C-4'; and the fifth (based on the chemical shift range) is assignable to C-2' and not C-3'. The presence of peaks at δ_H 3.74 (3H) and δ_C 62.6 in the spectra for compound **3** was indicative of a di-*ortho* substituted methoxyl group, hence, its placement at position 2' and the isoprenyl unit at 3'. The ESI-mass spectra gave $[M+1]^+$ peaks at *m/z* 355.5 for compound **1** and 371.4 for **3**, which is in agreement with the molecular formulae $C_{20}H_{18}O_6$ and $C_{21}H_{22}O_6$, respectively. In attempts to confirm the structure of **1**, a portion of the compound was treated with dimethylsulphate and acetone in the presence of potassium carbonate resulting in compound **2** with two methoxyl carbon peaks resonating at 55.8 ppm (7-OCH_3 and 4'-OCH_3) implying that the 2'-enolic carbon (rather than the 4'-enolic carbon) was part of the pyrano ring in compound **1**. Based on these data, HMQC and HMBC experiments, compound **1** was identified as glyasperin F, previously isolated from the roots of *Glycrrhiza aspera* (Zeng, *et al.,* 1992). Compound **3** was identified as sophoraisoflavanone A, first reported from the aerial parts of *Sophora tomentosa* (Komatsu *et al*., 1978).



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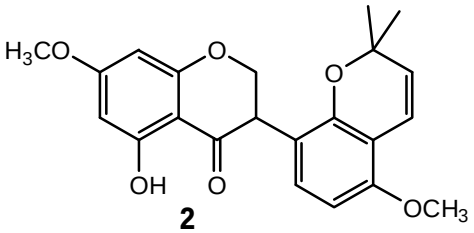

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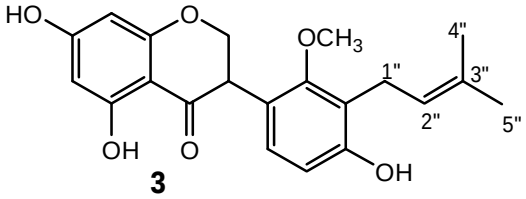

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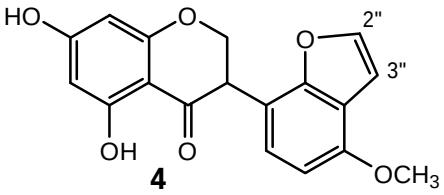

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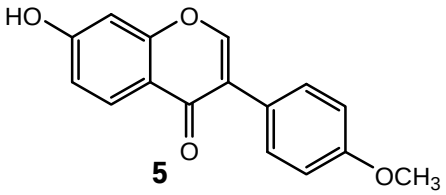

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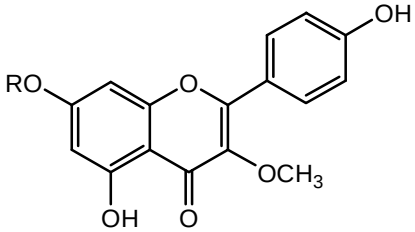

6: R = CH_3, **7:** R = H

Table 1. ^{1}H NMR Spectroscopic Data (200 MHz) for Compounds **1**, **3** & **4**

	δ_H (J in Hz)		
Position	**1** (acetone-d_6)	**3** ($CDCl_3$)	**4** ($CDCl_3$)
2 a	4.41, *dd* (11.0, 5.7)	4.46, *m*	4.75, *m*
b	4.56, *dd* (11.0, 11.0)	4.46, *m*	4.75, *m*
3	4.19, *dd* (11.0, 5.5)	4.35, *dd* (9.8, 6.4)	4.48, *m*
6	5.97, *d* (2.2)	6.00, *d* (2.0)	6.00, *d* (2.0)
8	5.94, *d* (2.2)	5.96, *d* (2.0)	5.97, *d* (2.0)
5'	6.40, *d* (8.4)	6.63, *d* (8.0)	6.64, *d* (7.8)
6'	6.87, *d* (8.4)	6.88, *d* (8.2)	7.06, *d* (8.2)
5-OH	12.40, *s*	12.21, *s*	12.13, *s*
1''	-	3.43, *d* (6.8)	-
2''	-	5.25, *t* (6.6)	7.52, *d* (2.2)
3''	5.63, *d* (10.1)	-	6.87, *d* (2.2
4''	6.67, *d* (10.1)	1.77, *s*	-
5''	-	1.65, *s*	-
2'-OCH_3	-	3.74, *s*	-
4'-OCH_3	-	-	3.93, *s*
2''-OCH_3	1.33, *s*	-	-
	1.34, *s*		

Compound **4**, obtained as a yellow gum, exhibited ^{1}H NMR spectral features similar to those of **1** and **3**. However, the presence of a pair of doublets, in the ^{1}H NMR spectrum, at δ 6.87 (J = 2.4 Hz) and δ 7.52 (J = 2.0 Hz) was suggestive of a furano moiety. The ^{1}H NMR spectrum further revealed the presence of a methoxyl group (δ 3.93, *s*, 3H) that exhibited an nOe difference interaction with a proton at δ 6.64 (*d*, J = 7.8 Hz, H-5'), hence allowing the placement of the methoxyl group at position 4' and the furano at 2' and 3'. Compound **4** was, therefore, identified as 5,7-dihydroxy-4'-methoxy-[2'',3'':2',3']-furanoisoflavanone.

The other compounds identified included formononetin (**5**), kumatakenin (**6**), isokaempferide (**7**), β-amyrin and betulin. All these compounds are reported from *Platycelphium voënse* for the first time.

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[YS 8] Antitrypanosomal, Antileishmanial and Antiplasmodicidal Activities of *Khaya anthotheca,* a Plant used by Chimpanzees for Self Medication.

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Key words: *Khaya anthotheca*, chimpanzees, self-medication, antiprotozoal, drug-resistance, *Trypanosoma, Leishmania, Plasmodium*

Introduction

Trypanosomiasis, leishmaniasis and malaria are among the major devastating protozoan diseases of the underdeveloped tropical regions of the world, the first two being neglected tropical diseases that afflict extremely poor and disadvantaged rural settings (WHO 2002, Fevre *et al.*, 2008). For each one of these diseases, there are no practical vaccines available yet to kill all parasites in infected communities and to block the transmission (Target and Greenwood 2008) Defence and protection against these diseases remains to be chemotherapy, but the number of therapeutic drugs available to each of them is very limited and unsatisfactory (Falade *et al.*, 2005). Variable and marginal efficacies, severe toxicities high costs, requirements for parenteral administration and for long course of treatment constitute some of the major drawbacks to current antitrypanosomal and antileishmanial drugs (Osorio *et al.*, 2006). Resistance to the commonly used drugs against these pathogens poses the greatest challenge to their usefulness (Croft *et al.*, 2006, Hyde 2007). The urgent need for newer, safer and more practical antiprotozoal drugs continues unabated (Bouteille *et al.*, 2003) and their searches from plant sources are among the major priorities (Wilcox *et al.*, 2004).

Khaya anthotheca (Welw.) C.D.C. belongs to the family Meliaceae. Members of this family have important roles in the ethno-pharmacological practices of tropical and subtropical communities (Phillipson and O'Neill 1986). Reports indicate that chimpanzees also ingest different parts of plants of this family in unusual feeding behaviour (Krief *et al*., 2004). Researchers have suggested medicinal benefits of such feeding behaviour for parasite control and provided chemical evidence to support the pharmacological roles of secondary metabolites in that respect (Huffman and Seifu 1989). This report presents the antiprotozoal potency of the crude petroleum ether extract and two pure compounds, grandifolione and 7-deacetykhivorin, from *K. anthotheca* seed.

K. anthotheca was selected for this study on the basis of its common use in West African traditional medicine and from documented evidence of its use by primates in Budongo forest, western Uganda. Plant materials were collected, identified at Makerere University Herbarium and a voucher specimen retained. Mature healthy seeds were collected from a pure *K. anthotheca* tree plantation at the Budongo Conservation station.

Extraction and purification Procedure

The pericarp was removed and the dried seeds ground into smaller particles. Batches of 100 grammes of the powder were packed in thimbles and successively extracted with hexane, petroleum ether, dichloromethane and methanol using a modified protocol of Rosanaivo and Ratsimamanga-Urveg (1993). The extracts were filtered and solvents removed in a *Bucchi*^{R} rotary evaporator below 50^{o}C under reduced pressure. The crude extracts were freeze-dried in a shell freezer system (Labconco^{R}/103 M BAR) at 0^{o}C. The hexane extract gave viscous colourless oil and the petroleum ether extract separated out into white crystals and a viscous oily portion. The solvent free extracts were placed in sample bottles, weighed, sealed with parafilm and stored at room temperature. The petroleum ether extract (3.22g) was purified by column chromatography eluted with dichloromethane : methanol (v/v 48: 2). Further purification was achieved with preparative thin layer chromatography. The pure compounds were identified as 7-deacetoxykhivorin, (**1**), grandifolione, (**2**), 1, 3-diacetyldeoxyhavenensin (**3**) using 2D NMR spectroscopy and by comparison against literature data (Adesogan *et al.*, 1971).

***In vitro* drug sensitivity assays**

Activity against erythrocytic stages of *P. falciparum* was determined by a modified [3H]-hypoxanthine incorporation assay (Matile and Pink, 1990) using the chloroquine- and pyrimethamine-resistant K1 strain and the standard drug chloroquine. Activity of all the extracts against *Trypanosoma brucei rhodesiense* was determined by the methods described (Räz *et al.*, 1997). *Activity against* trypomastigote forms of *T. cruzi*, Tulahuen C2C4 strain containing the β-galactosidase (Lac Z) gene, was determined by the methods of Buckner *et al.*, (1996). *Activity against* amastigotes of *L. donovani* strain MHOM/ET/67/L82 was determined according to the method described by Al-Bashir *et al.*, (1992). Cytotoxicity assays against L6-cells were performed according to the methods described by Ahmed *et al*., (1994). The IC_{50} values were calculated from the sigmoidal inhibition curves using SoftmaxPro software. The selectivity index (SI), ratio of the IC_{50} for the L-6 cells to the IC_{50} for the protozoan parasite was calculated for each compound.

Results

Table 1: Antiprotozoal activities of limonoids from *K. anthotheca* seed. IC_{50} shown are means of two independent assays. Selectivity index (SI) in brackets

Drug substance	*T. b. rhodesiense*	*T. cruzi*	*L. donovani*	*P. falciparum*	Cytotoxicity L6
	IC_{50}	IC_{50}	IC_{50}	IC_{50}	IC_{50}
Crude Ka2-b	5.72 (17)	14.51 (6)	30 (3)	0.955 (95)	90
Grandifolione	10.66 (4)	20.97 (2)	13.31 (3)	0.372 (121)	44.7
7-deacetylkhivorin	16.88 (0)	31.82 (0.5)	36.71 (0.4)	1.370 (11)	14.9
Melarsoprol	0.004	-	-	-	-
Benzinidazole	-	0.296	-	-	-
Miltefosine	-	-	0.156	-	-
Chloroquine	-	-	-	0.058	-
Podophillotoxin	-	-	-	-	0.005

Discussion

Sequential maceration of the seedcake of *K. anthotheca* with organic solvents of increasing polarities yielded crystals and gummy crude extracts with moderate antitrypanosomal, antileishmanial and antimalarial activities as compared to standard drugs. The crude petroleum ether extract demonstrated IC_{50} activities at 5.2 μg/ml, 14.51μg/ml, >30 μg/ml against *T.b. rhodesiense*, *T.cruzi* and *L. donovani*.

Purified compounds, grandifolione and 7-deacetylkhivorin, showed IC_{50} activities at 10.66, 16.88μg/ml against *T. b. rhodesiense,* 20.97, 31.82 μg/ml against *T. cruzi*, and 13.31, 36.71 μg/ml) against *L. donovani* respectively. Their selectivity indices were correspondingly low, at 4 and 0; 2 and 0.5; 3 and 0.4 respectively for the three pathogens. The pure compounds exhibited lower activities than the crude form of the drug and weak antiprotozoal activities as compared to standards.

However, *P. falciparum* (KI) was more sensitive to the crude form and pure compounds than the test trypanosomes and *Leishmania*. The crude form of the extract gave a mean antiplasmodial IC_{50} value of 0.955 μg/ml. Grandifolione gave an IC_{50} of 0.372 μg/ml and 7-deacetoxykhivorin gave an IC_{50} of 1.37 μg/ml. Selectivity indices for both compounds were 121 and 11. Grandifolione was 12x more selective than 7-deacetylkhivorin. The antiplasmodial activity of 7-deacetykhivorin was still moderate as compared to the activities of chloroquine and artemisinin. Cytotoxicity levels of the crude extracts and pure compounds were appreciably low, at 90, 44.7 and 14.9 μg/ml, making the two compounds 8940x and 2980x less toxic than podophyllotoxin.

From this study, it is evident that an ethnopharmacological use of *Khaya* seed to treat trypanosomiasis would be insufficient and perhaps that explains why there are no such anecdotal reports in ethnobotanic literature. The observed activity of grandifolione supports the claim, in

part, that chimpanzees might be using the seeds for self medication and in general, the use of *Khaya* plant material by humans in disease endemic Tropical areas to treat fevers. Their low toxicity levels and high selectivity, especially for *P. falciparum*, make them attractive as possible antiplasmodial drug candidates. The antitrypanosomal and antileishmanial properties of grandifolione and 7-deacetylkhivorin are here reported for the first time.

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[YS 9] Antiplasmodial and Antileishmanial Studies on Carvotacetone Derivatives from *Sphaeranthus bullatus*

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Key words: *Sphaeranthus bullatus*, antiplasmodial, antileishmanial, carvotacetone derivatives

Introduction

Malaria and leishmaniasis are both parasitic diseases caused by protozoan parasites and transmitted by bite of infected insects, mosquitoes and sand flies respectively. Malaria, predominant in the tropics, is caused by blood parasites of the genus *Plasmodium* and transmitted to humans by female *Anopheles* mosquito. Clinical malaria is manifested by a range of symptoms such as fever, vomiting, joint pain and convulsions [Nkuo-Akenji and Menang, 2005]. Besides contributing to over a million deaths yearly, malaria is known to be a cause of anaemia and its various complications, miscarriages, brain damage, decreased cognition and irreversible disabilities [Rugemalila *et al*., 2006]. Leishmaniasis on the other hand is transmitted by a bite of some species of sand flies which infect the blood with parasites of the genus *Leishmania* [Tonui 2006]. Two common forms of leishmaniasis are known; cutaneous leishmaniasis (CL) which causes sore at the bite site and visceral leishmaniasis (VL) which affects vital organs. Leishmaniasis is spread in tropical and subtropical regions of the world, with estimated number of new cases of CL and VL at 1.5million and 500,000 annually, respectively [Kigondu *et al*., 2009]. This study paper we report the first account of antiplasmodial and antileishmanial properties of abietane diterpenoids and carvotacetone derivatives from traditionally used medicinal plant *Sphaeranthus bullatus*.

Material and Methods

The aerial parts of *Sphaeranthus bullatus* were collected from Ngong forest Nairobi, in November 2007. They were air dried in shade and pulverized to give 2.3 Kgs which were extracted by cold percolation at room temperature using 1:1 CH_2Cl_2/MeOH (3×5 L, 24 h each), followed by 100% methanol (1×4 L, 24 h) to give 168 g of black-brown gummy extract, of which 100 g were chromatographed over silica gel giving fractions which were further purified using to give five carvotacetone derivatives. The compounds were identified using spectroscopic method (^{1}H-NMR, ^{13}C-NMR, DEPT, COSY, NOESY, HMBC, HSQC, EI-MS) and direct comparison with published spectral data and structures.

The antiplasmodial activity was measured *in vitro* by a colorimetric assay that determines the parasitic lactate dehydrogenase (pLDH) activity [Samoylenko *et al*., 2009]. The assay was performed in 96-well microplate and included two *P. falciparum* strains [Sierra Leone D6 (chloroquine-sensitive) and Indochina W2 (chloroquine-resistant)]. DMSO, artemisinin and chloroquine were included in each assay as vehicle and drug controls, respectively. Antileishmanial activity of the compounds was tested *in vitro* on a culture of *Leishmania donovani* promastigotes. In a 96 well microplate assay compounds with appropriate dilution were added to the Leishmania promastigotes culture (2×106 cells/mL). The plates were incubated at 26°C for 72 hours and growth of *Leishmania* promastigotes was determined by Alamar blue assay [Mikus and Steverding 2000]. Pentamidine and amphotericin B were used as standard antileishmanial agents. IC_{50} values for each compound were computed from the growth inhibition curve.

Results and discussion

Five known carvotacetone derivatives (Figure 1) were isolated from the aerial parts of *Sphaeranthus bullatus*. These are 3-acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (**1**), 3,7-dihydroxy-5-tigloyloxycarvotacetone (**2**), 3-acetoxy-5,7-dihydroxycarvotacetone (**3**), 3,5,7-trihydroxycarvotacetone (**4**), and 5-*O*-*β*-glucopyranosylcarvotacetone (**5**) which is reported for the first time from the genus. With excerption of **5**, the compounds showed antiplasmodial and antileishmanial activities as tabulated in Table 1. The activities seemed to be enhanced by presence of the acetyl or tiglyl substituents at 3-OH and 5-OH respectively, as witnessed by the weaker activity of **4** as compared to **1**, **2** and **3**. Compound **3** (3-acetoxy-5,7-dihydroxycarvotacetone) was the most active compound, having antiplasmodial activity of IC_{50} 0.6 and 0.7 µg/ml against chloroquine sensitive and chloroquine resistant strains of *P. falciparum* respectively, as well as antileishmanial activity of IC_{50} 0.7 against *L. donovanii*.

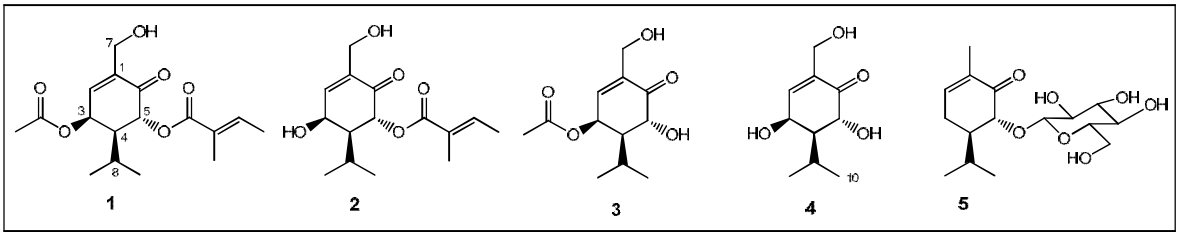

Figure 1: Carvotacetone derivatives from the aerial parts of *Sphaeranthus bullatus*

Table 1: *In-vitro* antiplasmodial activity, antileishmanial activity and cytoxicity

	Antiplasmodial activity (µg/ml)				**Antileishmanial activity**		**Cytotoxicity**
	P. falciparum D6		*P. falciparum* W2		*L. donovanii*		VERO
	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI	
Extract	9.7	NT	15.0	NT	NT	NT	NT
1	1.4	0.002	2.0	0.001	0.7	0.001	2.8
2	0.8	0.006	0.9	0.005	3.0	0.007	NC
3	0.6	0.022	0.7	0.019	0.7	0.001	0.013
4	3.4	0.001	2.8	0.002	17.0	>0.04	NC
5	NA	NT	NA	NT	17.0	0.035	NC
Chloroquine	0.01	NT	0.14	NT	NT	NT	NT
Artemisinin	0.004	NT	0.0048	NT	NT	NT	NT
Pentamidine	NT	NT	NT	NT	0.1	NT	NT

NA = Not Active (up to the maximum dose tested 47.6 µg/ml); NC = Not Cytotoxic; NT = Not Tested, IC_{50} = concentration that affords 50% inhibition of growth, SI = Selectivity index

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[YS 10] *In vitro* anthelmintic Effect of *Prosopis juliflora* (Sw.) DC (Fabaceae) on *Haemonchus contortus*, an Abomasal Nematode of Sheep

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Key Words: *Haemonchus contortus*, Tannins, saponins, anthelmintic activity, ruminants

INTRODUCTION

The economic impact of parasitic gastroenteritis caused by mixed infection with several species of stomach and intestinal round worms, as a production disease in ruminants lies in direct losses involving mortality due to the clinical form of the disease and also indirect losses due to weaknesses, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity. In Kenya, economic loss to the agricultural sector due to *Haemonchus contortus* parasite of small ruminants is estimated at over US$ 26 million per year (Githiori, 2004). Control programs based on the use of synthetic anthelmintics are no longer sustainable because of high prevalence of gastrointestinal nematode resistance, slow development of new anthelmintics, high costs to poor farmers and concerns regarding residue in food and the environment (Singh *et al.,* 2002). Alternative methods of control such as use of tanniferous plants are thus required for introduction into farm production systems (Niezen *et al.,* 2002). *Prosopis juliflora* (Sw.) DC (Fabaceae) is an evergreen tree native to South America, Central America and the Caribbean. *Prosopis* species are generally fast-growing, drought-resistant, nitrogen-fixing trees or shrubs adapted to poor and saline soils in arid and semi-arid zones. (Pasiecznik *et al.,* 2001).

MATERIALS AND METHODS

Sample collection, preparation and extraction

Leaves and root bark samples of *P. juliflora*, obtained from Endao, Marigat district, in Baringo county of Kenya were botanically identified and authenticated by field a officer from Kenya Forestry Research Institute, Marigat station and a taxonomist from Botany Department of Jomo Kenyatta University of Agriculture and Technology, where voucher specimens were also deposited. The collected materials were washed thoroughly in water, chopped; air dried for two week, pulverized in electric grinder and exhaustively extracted using ethanol. The extracts were concentrated *in vacuo*, dried and stored at 4°C until required for bioassay.

Phytochemical screening

Phytochemical screening was performed using standard procedures (Harborne, 1998) and the extracts were tested for triterpenes, sterols, flavonoids, saponins, tannins and alkaloids.

***In vitro* ovicidal activity:**

The egg hatch assay (EHA) was carried out using the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for determination of anthelmintic resistance (Coles *et al.,* 1992) with modifications that allowed the testing of the natural compounds. The number of eggs which had not hatched and number of hatched larvae were counted and percentage hatching calculated. There were three replicates for each concentration and controls.

Results and Discussion

Extraction yield

Ethanolic extraction of the roots gave a higher yield of 16.78% as compared to that of the leaves which was 6.94%, an indication that there were more polar compounds in roots as compared to the leaves.

Results for phytochemical screening

Table 1: Phytochemical profile of LEE and REE of P. juliflora

Secondary metabolite	LEE	REE
Alkaloids	+	+
Tannins	+	+
Saponins	+	++
Flavonoids	+	+
Sterols/ Triterpenes	+	+

' + ' Present, ' ++ ' Present in high concentration, **LEE**: Leaf Ethanolic extract;
REE: Root Ethanolic Extract

Phytochemical analysis showed that LEE and REE possess alkaloids, tannins, saponins, flavonoids, sterols and triterpenes. The root ethanolic extract had higher concentration of saponins as compared to the leaves ethanolic extract as exhibited by higher volume of persistent frothing.

Results for in vitro anthelmintic activity

In the search for natural anthelmintics, *in vitro* tests are used as preliminary studies of plants. In these tests, the plant extracts are directly placed in contact with the eggs larvae or adult parasites to evaluate the effect on egg hatching, larval development or motility and mortality of adult worms (Hammond *et al.,* 1997).

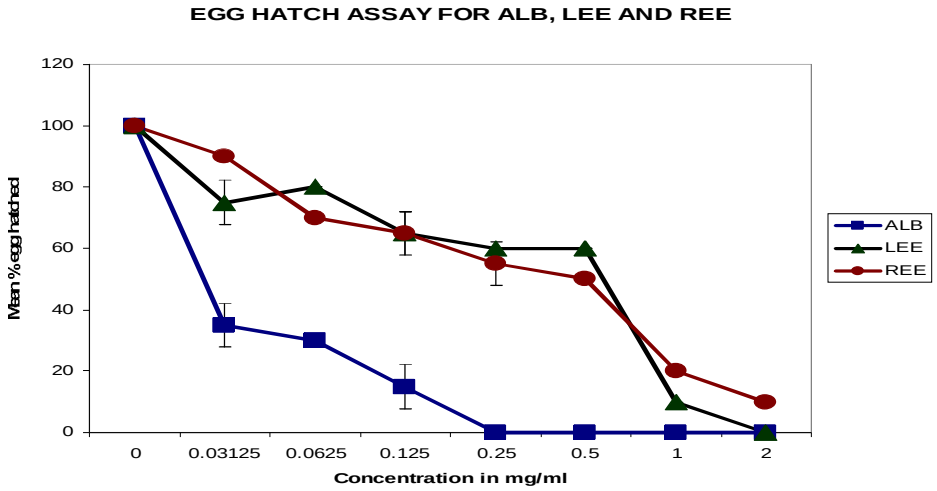

Fig1: *Graph showing mean percentage egg hatching of various concentrations of LEE, REE and ALB after 48 hours*

Both LEE and REE showed anthelmintic activity in a concentration dependent manner. However, LEE had a higher activity (LD_{50}=0.857 mg/ml) as compared to REE (LD_{50}=1.782 mg/ml). Albendazole had significantly higher activity as compared to the ethanolic extracts. (LD_{50}=0.046mg/ml). The anthelmintic activity of LEE and REE may be attributed to presence of phytochemicals such as saponins, tannins and alkaloids. Min *et al.* (2003) reported that Condensed tannins might diffuse through the external surfaces such as eggshells and bind to faecal egg proteins thus inhibiting egg hatching and larval development. Saponins destabilize membranes and increase cell permeability by combining with membrane-associated sterols (Gee and Johnson, 1988) while alkaloids may improve tonicity of the gastrointestinal tract and thus expel the worms or may have a direct effect on the nervous system of nematodes. Other phytochemicals like flavonoids and oleane type triterpenes may also have their independent or synergistic effects (Brantner *et al*., 1996). The use of botanical anthelmintics has been proposed as an alternative strategy for the control of gastrointestinal nematode infections in order to reduce the dependence on chemical anthelmintic treatments and to delay the selection and the transmission of anthelmintic resistances in worm populations (Hoste *et al.,* 2006).

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[YS 11] Application of Solid Phase Extraction Gas Chromatography Mass Spectrometry in Geographical Profiling and Characterization of Volatile Organic Compounds in Kenyan Honey

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Key Words: Honey, Solid Phase Extraction (SPE); Gas Chromatography-Mass Spectrometry (GC-MS)

Introduction

Honey as a natural product is greatly appreciated by consumers, not only for its nutritive properties, but also for its characteristic aroma and sweet taste. Aroma is caused by the presence of many different volatile compounds in it (Soria *et al*., 2003). The composition and flavor of honey varies, this mainly depends on the source of the nectar(s) from which it originates and to a lesser extent on certain external factors - climatic conditions and beekeeping practices in removing and extracting honey (White, 1975). A large number of organic compounds have been described in different types of honey. Some of the compounds have been described as characteristic of the floral source whereas other compounds like some alcohols, branched aldehydes and furan derivatives may be related to microbial purity or processing and storage of honey (Bouseta *et al*., 1992).

Because of the high price of certain honey types based on botanical and geographical origin, adulteration with low cost and nutritional value substances (Cotte *et al*., 2007) or mislabeling regarding the botanical or geographical origin (Alissandrakis *et al*., 2007a) sometimes occurs. Nonetheless, the discrimination between different types of honey is important for honeys that possess discrete aroma, taste and special nutritional properties (Lusby *et al* 2005; Ma˘rghitas *et al*., 2009) which make them preferable for consumers. Thus, the profiling and identification of Organic compounds which could be used as markers for the discrimination and classification of honey based on their geographical origin is of high importance.

Aroma compounds are present in honey at very low concentrations as complex mixtures of volatile components of different functionality and relatively low molecular weight.

Gas Chromatography – Mass Spectrometry (GC-MS) is usually the technique of choice for the determination of volatile Organic compounds in honey this is due to its high separation efficiency and sensitivity and also it provides qualitative and quantitative data for these compounds. However, GC-MS requires the previous removal of sugars and water (Soria *et al*., 2003). Several fractionation techniques have been employed for the removal of sugars and water, they include: Solvent extraction, simultaneous steam distillation extraction Method, Static headspace analysis, Dynamic headspace (Purge and Trap), Solid Phase Extraction (SPE) and Solid phase micro extraction (SPME).

In this study solid-phase extraction followed by gas chromatography-mass spectrometry (GC-MS) was used to extract and identify volatile organic compounds in honey obtained from various geographical origin in Kenya. Solid phase Extraction technique was employed as it offers the advantage of eliminating, by washing with water, some interfering substances such as sugars and acids thus making it possible to obtain the honey volatile fraction without the need of applying heat; however optimization of several parameters is necessary before applying this technique (V´azquez, *et al* 2006). Extraction conditions were optimized in order to obtain the highest yields of volatile substances.

Material and methods

Study of choice of eluting solvent

A 30 % honey solution was prepared.10ml of these solution was passed through 2 precondition Sep-Pak (waters) C18 SPEcartridges , each of the catridge was washed with 5ml of distilled water . One was eluted with Hexane while the other one with Dichloromethane. Each of the eluent was spiked with 100µl of 100ppm Internal Standard (Benzophenone). Both eluent were concentrated to 1ml and analysed by GC-MS.

Sample throughput (Volume) optimization

5,10 15and 20ml of the 30 % honey solution prepared above was passed through a precondition C18 SPE catridge at a flow rate of approximatelty 1ml/min. 5 ml of distilled water were run into each of the catridge to wash the sugars. 5ml of DCM was used to elute the volatile organic compounds from each of the cartridges at a flow rate of 1ml/min. The eluent was spiked with 100µl of 100ppm of internal standard. The DCM eluent was further preconcentrated in a vacuum sample concentrator at 30 ^{o}c to a volume of 1ml and analyzed by GC-MS.

Honey amount optimization

4 honey solutions i.e 10%, 20%, 30% and 40% honey solutions were prepared, 10 ml of each of the solution (the optimum sample volume determined previously) was passed through a preconditioned C18 SPE catridge. 5 ml of distilled water were run into each of the catridge. 5ml of DCM was used to elute the volatile organic compounds from each of the cartridges at a flow rate of 1ml/min. the eluent was spiked with 100µl of 100ppm internal standard. The DCM eluent was further preconcentrated in a vacuum sample concentrator at 30 ^{o}c to a volume of 1ml and analysed by GC-MS.

Blank preparation

10ml of water was passed through a preconditioned cartridge at a rate of 1ml/min. The cartridge was eluted with 5ml of Dichloromethane. The eluent was further preconcentrated in a vacuum sample concentrator at 30 ^{o}c to a volume of 1ml and analyzed by GC-MS.

Analysis of Volatile compounds in honey from various geographical regions in Kenya.

20 grams of honey obtained were dissolved in 100ml of distilled water. 10ml were passed through a preconditioned cartridge at a rate of 1ml/min. the volatile fraction was eluted with 5ml of Dichloromethane. The eluent was spiked with 100µl of 100ppm internal standard. The DCM eluent was further preconcentrated in a vacuum sample concentrator at 30 ^{o}c to a volume of 1ml and analysed by GC-MS.

Chromatographic conditions

GC-MS analyses were perfomed in a GC8000 Top series (CE instruments) coupled to a Voyager-Finnigan quadruople mass spectrometer detector. 1µl of each extracts were injected into the splitless mode in a DB5 (Crosslinked 5% Phenyl-95% Methyl Siloxane) capillary column (30m × 0.25mm i.d × 0.1µm film thickness) The injection temperature was maintained at 200^{0}c, while the oven temperature was kept at 60^{o}c for 3min and programmed to rise at 3oc/min to 240 where it would be held for 5min. Helium was used as the carrier gas at flow rake of 1ml/min.Mass spectra were recorded in the Electron Ionization mode at 70 ev scanning the 40-450m/z range, the ion source and transfer line temperature were maintained at 200^{o}c and 250^{o}c respectively.

Sampling

33 Fresh unprocessed honey samples were obtained from farmers in different parts of the country geographical sampling was employed.

Data analysis

Peak identifications were performed by comparison of their mass spectra with spectral data from the NIST library. Peaks which were present in the blank were not considered. While quantitation was done by comparing the peak areas of individual compounds identified with that of the internal standard used (100µl of Benzophenone.)

Results and Discussion

Optimisation studies

In the optimization study, 10 compounds identified to be present in both eluents were used so as to establish the trend. These 10 compounds identified were further used in subsequent optimization studies.

It was established that the Dichloromethane eluent was found to have higher concentrations of the Volatile Organics as compared to the Hexane eluent, identified thus it was chosen as the best solvent for elution during the sample extraction phase with the C18 SPE cartridges as illustrated in Figure 1.

In the sample optimization stage it was found that the optimum sample throughput volume was 10ml. as shown in figure 2. Lastly in the e honey amount optimization stage, a 20 % honey solution was found to give the highest concentrations of the 10 compounds analyzed in these stage. As shown in figure 3.

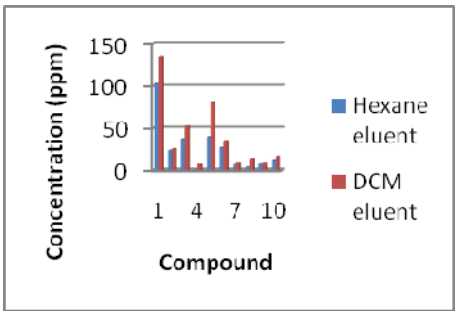

Fig 1: Comparison of the concentration of compounds eluted from SPE cartridge by Hexane and DCM

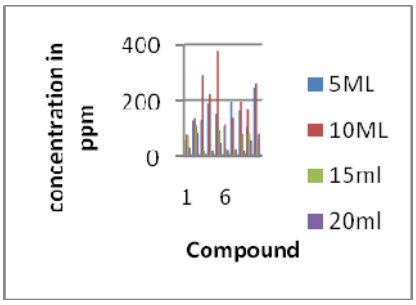

Fig 2: Comparison of concentration of compounds eluted with DCM with varying sample volume

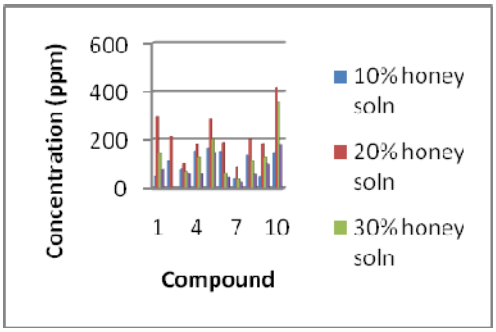

Fig 3: Comparison of concentration of compounds eluted with DCM with varying sample concentration.

Volatile compounds present in honey from different geographical regions in Kenya.

Chromatographic analysis of the extracts obtained by solid phase extraction enabled the identification of 57different compounds from samples collected in Kenya. A typical honey VOC chromatogram exhibited from 15 to 25 peaks. Differences in chromatographic profiles were observed when comparing honey samples from the different geographical origins. The volatile compounds identified were into 5 groups. Namely terpenes and derivatives, aldehydes, ketones, carboxylic acids and esters. These was in agreement with reports from researchers in other parts of the world who have been profiling volatile compounds in honey from different floral sources (Soria *et al*., 2003, Baroni *et. al* 2006, Wolski, *et al*., 2006).

Table 2 shows the profile developed form these study.

Table 2: Concentration in ppm of compounds identified from the SPE extract of honey obtained from various geographical regions in Kenya.

Compound	**Region**					
	Lower eastern Province		Upper Eastern Province	Central Riftvalley Province	South Riftvalley Province	Nairobi & Central Province
Carboxylic Acids	Mwingi	Kitui				
n-hexadecanoic acid	1970.667±351.269	176.67±55.13	35.12±9.85	230.96±11.95	46.71±2.94	556.93±114.89
2-decanynoic acid	__		__	__	53.45±9.98	__
14-methyl pentadecanoic acid	__		__	__	__	109.15±23.45
Trans-e(sup 9)-octadecenoic acid	__		__	11.78±4.35	__	29.41±6.78
16.methylheptadecanoic acid	__		__	__	__	34.47±8.78
Ketones and Aldehydes						
2-pentadecanone	__		66.2±14.56	__	__	_
E-14-Heptadecenal	35±12.34		14.94±2.43	__	61.23±25.54	32.78±8.89
3-heptadecenal	__		__	__	13.86±4.71	__
Esters						
1-Methylethyl ester tetradecanoic acid	__		27.34±7.45	__	__	__
Oleic acid,methyl ester	__		__	23.98±6.45	__	__
Palmitic acid,methyl ester	45.62±8.834		__	23.45±5.45	__	__
Stearic acid, methyl ester	__		__	17.45±4.34	__	__
Terpenes and derivatives						
2,6,10,15-tetramethylheptadecane	__		26.11±3.13	__	216.98±39.7	__
2,4-dimethyleicosane	__		44.30±7.70	__	__	__
10-MethylEicosane	__		__	__	68.03±13.61	__
n-nonadecane	38.67±6.34		15.65±5.63	__	__	__
7-n-Hexyleicosane	__		__	__	__	12.89±3.56
n-docosane	__		__	__	56.51±9.78	37.06±7.98
Others	Mwingi	Kitui				
Sandoracopimar-15-en8á-yl acetate	__	66.03±15.70	__	__	__	__
2,2'-Methylenebis(4-Methyl-6-Tert-Butylphenol)	582.46±106.98	4793.08±1205.95	__	769.45±87.98	__	__

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[YS 12] EVALUATION OF LARVICIDAL ACTIVITY AND PHYTOEXTRACT INDUCED MORHOLOGICAL DISRUPTIONS OF *VITEX SCHILIEBENII* EXTRACTS AGAINST *ANOPHELES GAMBIAE* LARVAE

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Key words: Phytochemicals, larvicidal, *Vitex schiliebenii*, Verbenaceae, *Anopheles gambiae*.

Introduction

Most synthetic larvicides act on target and non-target organisms therefore representing a danger to beneficial insects, wildlife and human beings. Consequently, environmentally safe methods must be found to enhance or minimize the use of conventional chemical insecticides. Some plants are known to contain toxic principles that are useful in the control of vectors. Such plants exhibit insecticidal and biological activities. Botanical insecticides may provide a safe and effective short-term strategy for larval and adult mosquito control. Mosquito responses to phytochemicals from different plants or parts vary and have been studied. Among the plant families studied include Meliaceae, Rutaceae, Labiatae, Piperaceae, Verbenaceae, Asteraceae, Cladophoraceae, Oocystaceae, and Annonaceae and perhaps are the most promising (Akhatar & Isman, 2004).

The genus *Vitex* in the family Verbenaceae and its species consists of shrubs or trees found mainly in tropical and sub-tropical regions although a few species may be found in temperate zones (Mokua *et al.,* 2008). In Kenya, there are 12 different species of the genera *Vitex* found naturally from the Kenyan Coast through the dry woodlands to Mt. Kenya area and across the Rift Valley to the shores of Lake Victoria. (Kimondo *et al*., 2010). *Vitex* species besides their popular use as traditional medicines in many countries have been reported to exhibit insecticidal activities against a variety of insects (Karunamoorthi *et al* 2008; Yuan *et al*., 2006; Rodríguez-Lopez *et al*., 2007; Kannathasan *et al*., 2007; Rahman& Talukder. 2006).

In this study, *Anopheles gambiae* were investigated using *Vitex schiliebenii* (Verbenaceae) a branched shrub with multiple stems and low height (4-8 m). The leaves have 3-leaflets with a smooth surface, about 10-12 cm long. In Kenya, it grows in the coastal region at Watamu which is a low and semi-arid land. The present investigation assessed the laboratory larvicidal effect of *V. schiliebenii* polar extracts against 3rd and 4th instar larvae of *An. gambiae* s.s. This is the first report on the evaluation of the acetone extracts of *Vitex schiliebenii* against *An. gambiae* through larvicidal bioassays.

Materials and methods

Plant materials

The plant species was authenticated at the field by a botanist at the National Museum of Kenya (NMK). The leaf, stem and root barks of *V. schiliebenii* were collected from North Coast at Kenya Forest Research Institute (KEFRI) near Gede along Mombasa-Malindi road 18 km from Malindi town. A voucher specimen Ref. Nos. GMN/22 was deposited at the NMK herbarium. The plant materials were dried at room temperature (29^{o}C) for three weeks, ground into powder using an electric miller, from which the extracts were prepared.

Acetone and methanol extracts of the plant materials were obtained sequentially by soaking 200 g, 400 g and 800 g of the dried root bark, leaf and stem bark respectively in 1.0 L, 2.0 L and 4.0 L acetone in separate containers with occasional stirring. The mixture was kept for 24 h then filtered under gravity and concentrated to dryness using a rotary evaporator while maintaining the water temperature at 40^{o}C in order to avoid decomposition of thermally labile compounds. This procedure was repeated three times and the filtrates were then pooled and stored at 4^{o}C. Methanol extract of the plant material was prepared in a similar manner with that of acetone.

Mosquito species

The eggs of *An. gambiae* were procured from the research insectary at the International Center of Insect Physiology and Ecology (ICIPE), Nairobi. The Kenya highland strain of *An. gambiae s.s* originated from ICIPE's Mbita Point Field Station in 2003 was used because it is the most efficient vector due to its high anthropophilic character. It is the one which transmits malaria mainly in the Sub-Saharan Africa. The eggs were obtained as rafts on a filter paper and kept in plastic tray containing distilled water at a temperature of 28±2 ^{o}C as culture medium and at laboratory conditions 30±2^{o}C. The larvae were fed on Tetramin® fish food (Terta GmbH, Germany). The water temperature was maintained at 28±2^{o}C throughout the larval development period. The larvae at third and early fourth instar stages were used for larvicidal assay.

Laboratory larvicidal assay

Bioassays were conducted following the standard World Health Organization (WHO, 1981) larval susceptibility test method at ICIPE. The extracts were dissolved in 2 ml dimethyl sulfoxide and prepared into different concentrations viz 25, 50, 100, 250 and 500 ppm with distilled water. Twenty freshly molted late 3rd and early 4th instar larvae of *Anopheles gambiae* were tested in three replicates with two controls running simultaneously. During the experiment, the larvae were fed on Tetramin® fish food (Terta GmbH, Germany) at about 1mg per beaker every 24h. The experiment room was kept at a temperature of 30^{o}C and an average humidity of 48 % and a photo period of 12 hours of light and 12 hours of darkness. The lethal concentrations LC_{50}, LC_{75} and LC_{90} were calculated using GenStat teaching edition soft ware.

Phytochemical screening

Phytochemical tests were carried out on the acetone and methanol extracts using standard

procedures to identify the constituents as described by (Egwaikhide & Gumba, 2007) with some modifications.

Results and discussion

In this study, larval mortality of *An. gambiae* 3rd and 4th instar larvae under laboratory conditions were investigated after 24h, 48h, and 72h treatment with the extracts. The highest larval mortality was observed in the acetone leaf extract (VSL-221) with LC_{50} = 14.6 ppm followed by acetone stem bark (VSSB-221) extract (LC_{50} =17.4 ppm), methanol leaf (VSL-222) extract (LC_{50} = 136.3 ppm) and acetone root bark (VSRB-221) extract (LC_{50} = 252.1 ppm). Methanol root bark (VSRB-222) and stem bark (VSSB-222) extracts exhibited moderate larvicidal activity with LC_{50} = 444 and 522.6 ppm respectively (Table 2). In the case of control, no morality was observed within 24-h and the larvae developed

Table 2: Bioefficacy of crude extracts of *Vitex schiliebenii* on 3rd and early 4th instar larvae of *Anopheles gambiae* s.s after 24h, 48h and 72h of exposure

Extract code	Time (Hr)	Lethal concentration values (ppm)					
		LC_{50}	95% CL	LC_{75}	95% CL	LC_{90}	95% CL
VSSB-221	24	16.3	13.6-19.3	22.9	19.3-27.0	31.0	26.2-36.8
	48	14.7	12.0-17.8	21.2	17.5-25.6	29.6	24.5-35.7
	72	12.4	9.8-15.5	17.7	14.1-22.1	24.5	19.6-30.6
VSSB-222	24	540.7	472.4-621.7	758.9	659.1-882.2	1029.6	885.6-1214.2
	48	104.3	92.2-118.1	150.8	133.0-171.6	210.1	184.3-241.4
	72	40.1	35.6-45.2	57.5	51.0-65.0	79.4	70.1-90.5
VSRB-221	24	251.1	222.6-283.0	352.4	312.3-399.4	478.0	421.1-547.7
	48	136.3	120.2-154.7	197.1	173.6-224.7	274.7	240.7-315.9
	72	38.7	34.3-43.6	55.4	49.1-62.6	76.5	67.6-87.2
VSRB-222	24	444.4	392.0-505.0	623.7	547.9-715.5	846.1	736.8-983.9
	48	295.9	260.8-335.7	427.8	376.7-487.6	596.1	522.2-685.4
	72	80.6	71.6-90.9	115.4	102.2-130.9	159.4	140.3-182.5
VSL-221	24	13.5	10.9-16.6	18.9	15.4-23.1	25.7	21.0-31.4
	48	12.7	10.1-15.8	18.4	14.7-22.8	25.6	20.6-31.8
	72	11.1	8.5-14.39	15.9	12.3-20.	21.9	17.0-28.2
VSL-222	24	97.1	86.3-109.4	136.3	120.8-154.8	185.0	162.7-212.6
	48	63.1	56.0-71.1	91.2	80.8-103.3	127.1	112.0-145.3
	72	40.1	35.6-45.2	57.4	51.0-64.9	79.3	70.0-90.4

VSRB-221- Acetone root bark extract of *V.schiliebenii*, VSRB-222-Methanol root bark of *V.schiliebenii* VSSB-221- Acetone stem bark extract of *V.schiliebenii*, VSSB-222- Methanol root bark extract of *V.schiliebenii*, VSL-221-Acetone leaf extract of *V.schiliebenii*, VSL-222-Methanol leaf extract of *V.schiliebenii*

The present findings are comparable with the methanol leaf extracts of *V. negundo*, *V. trifolia*, *V. peduncularis* and *V. altissima* against the early 4th instar larvae of *Culex quinquefasciatus* with LC_{50} 212.57, 41.41, 76.28 and 128.04 respectively and the petroleum ether (60-80^{o}C) extracts of the leaves of *V. negundo* against larval stages of *Culex tritaeniorhynchus* in the laboratory (Kannathasan *et al*., 2007, Karunamoorthi *et al*., 2008).

The extracts showed morphological deformation disruptions, prolongation of developmental period and highly significant reduction in adult emergence to the test larvae. The pupal-adult dead intermediates had 'question mark-like' remains and the live larvae after 24-h could not move deep into the water.

Dead deformed larva

Pupal-adult dead intermediates

Although there has been no phytochemical investigation of the polar extracts of *V. schiliebenii* reported, the plant has shown larvicidal activity against *Anopheles gambiae* larvae s.s in the current study. Phytochemical investigation of the extracts revealed the presence of flavonoids, terpenoids, steroids, alkaloids, saponins and tannins. The biological activity of the evaluated phytoextracts could therefore be attributed to the presence of these compounds which could synergistically, antagonistically or independently contribute to the activity of the crude extracts. Elango *et al.* (2010) reported the presence of these compounds in leaf extracts of four *Andrographis* species which exhibited adulticidal activity and adult emergence inhibition (EI) against a malarial vector *Anopheles subpictus* Grassi.

Semi field experiments are in progress to enable the technology to be used in small water bodies around the homesteads. In addition, isolation, purification, characterization and bioassay of the pure compounds independently and in blends are in progress.

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[YS 13] The Relative Stabilities and Reactivities of the First Six Members of the Dendralene Family

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Introduction

With oligoalkene structures, four fundamental hydrocarbon families can be defined (**Figure 1**), with each differing in the type of atom connectivity (unbranched or branched; cyclic or acyclic). [1-5]

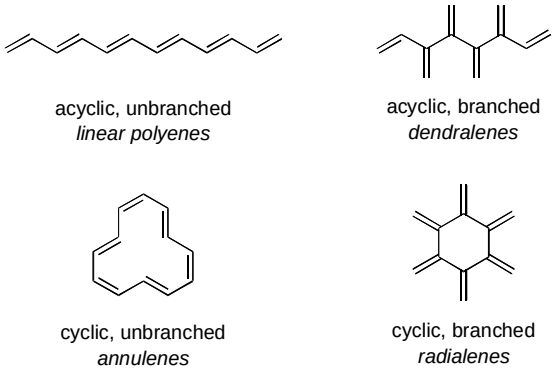


Figure 1 Fundamental classes of conjugated alkenic hydrocarbons.

The unbranched acyclic and cyclic systems, namely the *linear polyenes* and *annulenes*, respectively, have been thoroughly studied. The alternation in behaviour of the annulenes (i.e. aromaticity and antiaromaticity for odd and even numbers of conjugated alkenes) played an important role in the development of modern theories of structure and reactivity.[3] On the other hand, the acyclic and cyclic branched systems, that is, the *dendralenes* and *radialenes*, are much less well investigated.

The cross conjugated polyenes known as dendralenes are fascinating compounds with enormous untapped potential in chemical synthesis.[1-5] These hydrocarbons have remained unexplored because of limited accessibility and their reported instability. Contrary to earlier assertions that they are too prone to polymerisation to be synthetically useful, we have seen that these compounds are readily made and stored.[6-8] In this paper, it is demonstrated that these fundamental hydrocarbons exhibit alternation in their physical and chemical properties. Evidence is provided that this alternating behaviour stems from the conformational preferences of dendralenes with even- and odd numbers of alkene units.[9]

Materials and Methods

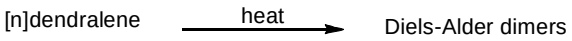
NMR spectra were recorded at 298K using a Varian Unity INOVA 500 MHz or a Varian Unity INOVA 300 MHz spectrometer. Residual chloroform (δ 7.26 ppm) was used as an internal reference for ^{1}H NMR spectra measured in this solvent. Coupling constants (J) are quoted to the nearest 0.1 Hz.

Residual chloroform (δ 77.1 ppm) was used as an internal reference for ^{13}C NMR spectra. Assignment of carbon signals was assisted by DEPT experiments. IR spectra were recorded on a Perkin-Elmer 1600 F.T.I.R, spectrometer as neat films on NaCl plates for oils. Low resolution mass spectra were recorded on a Finnigan PolarisQ ion trap mass spectrometer using electron impact (EI^+) ionisation mode at 40 or 70 eV. High resolution mass spectra were recorded on a VG Autospec mass spectrometer operating at 70 eV. Analytical TLC was performed with Merck silica gel plates, precoated with silica gel 60 F254 (0.2 mm). Flash chromatography employed Merck Kiesegel 60 (230-400 mesh) silica gel. Reactions were conducted under a positive pressure of dry argon or nitrogen in oven-dried glassware. Ether and THF were dried over sodium wire and distilled from sodium benzophenone ketyl before use. Dichloromethane was distilled from calcium hydride. Commercially available chemicals were purified by standard procedures or used as purchased.

Results and Discussion

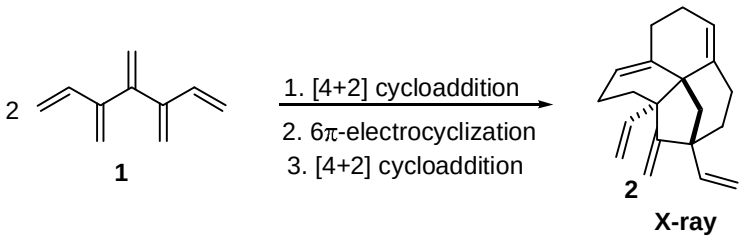
During synthesis of the first six members of the dendralene family it was observed that some members were more stable than others. This observation led to investigations into the physical and chemical properties of the first six members of the dendralene family.

It was established from the stability studies on the first six members of this class of hydrocarbons that the even numbered dendralenes are more stable than the odd numbered dendralenes. The major pathway of decomposition of these hydrocarbons is via Diels–Alder dimerisation (**Scheme 1**).



Scheme 1

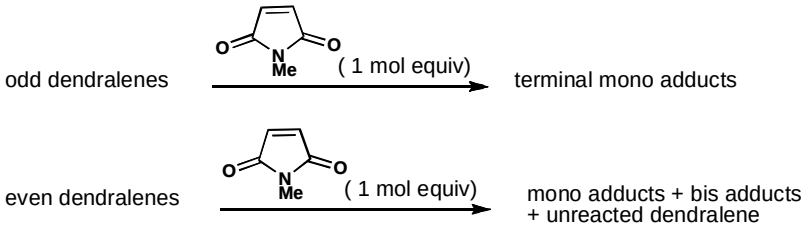
For example, [5]dendralene (**1**) underwent DA dimerisation to give dimer which underwent further 6π-electrocyclisation/[4+2] cycloaddition cascade to give a tetracyclic fenestrane (**2**) (**Scheme 2**) upon refluxing in chlorobenzene.



Scheme 2 Intermolecular [4+2]/6π-electrocyclisation/intramolecular [4+2] cascade for [5]dendralene (**7**). *Reaction conditions*: (i) 5.0M 1.7, CH_2Cl_2, 72h, 16%, (ii) PhCl, BHT, reflux, 24h, 80%.

Following work on the stabilities of these hydrocarbons the relative reactivities of the [n]dendralenes were also investigated. Thus, the Diels–Alder reactivity of the dendralenes towards the electron deficient dienophile *N*-methylmaleimide (NMM) was examined. Odd numbered dendralenes underwent a rapid and clean conversion to (predominantly) the corresponding

terminal mono adduct at ambient temperature, even in the presence of a small excess of the dienophile (**Scheme 3**). In contrast even numbered dendralenes exhibit significantly lower reactivity towards NMM, producing mixtures of starting dendralenes, mono and bis adducts. *Thus, there is clear difference in **chemical reactivity** between the "odd" and "even" dendralenes.*



Scheme 3

As is the case with the annulenes, the dendralenes exhibit alternating behaviour, with the physical and chemical properties of even members of the family being distinctly different from odd members. This alternating behaviour has been traced to conformational preferences in the dendralenes.

Acknowledgements

The authors gratefully acknowledge the Australian Research Council and The University of Botswana (scholarship for G. B.) for funding.

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[YS 14] Fumigant and Contact Toxicity of *Cupressus lusitanica* and *Eucalyptus saligna* Essential Oils Against Insect Pests of Stored Cereals and Legumes

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Key words: Botanical, extract, concentration, *Callosobruchus chinensis, Tribolium castaneum,* mortality

Introduction

Insects cause substantial quantitative and qualitative pre- and post–harvest losses varying in magnitude from 10 to 100% in tropical countries and in Kenya, 10-60 % losses of stored cereal and legume grains. These substantial losses are caused by *Sitophilus* spp., *Sitotroga cerealella* and *Prostephanus truncatus* on cereals, *Acanthoscelides* and *Callosobruchus* spp. on legumes (Mugisha-Kamatenesi *et al*., 2008). Current recommended control measures for insect pests rely on synthetic insecticides which pose health and environmental hazards. Research focus has now shifted to botanical pesticides, which are target-specific, relatively safe, affordable and readily available. The insecticidal activity of several plant essential oils, powders and other extracts have been evaluated against several insect pests of cereals and legumes and found to have contact toxic (Asawalam *et al*., 2006), repellence (Ogendo *et al*., 2008), fumigant toxicity and antifeedant (Rosman *et al*., 2007) effects. In the current study fumigant and contact toxicity of essential oils obtained from aerial parts of *C. lusitanica* and *E. saligna* were evaluated against *C. chinensis* and *T. castaneum.*

Materials and Methods

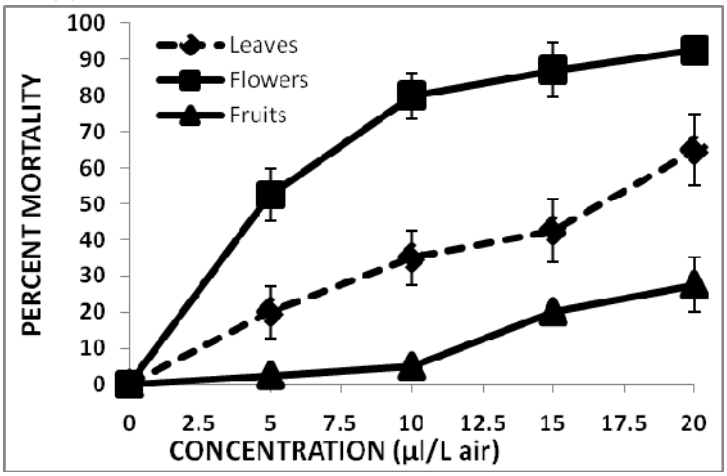
Essential oils extracted by hydro distillation using a modified clavenger apparatus. Experiments were carried out under controlled temperatures (28±2^{o}C) and relative humidity (65±5%) and laid out in completely randomized design with four replicates. The fumigant toxicity test was carried out in space fumigation chambers consisting of a 3.4L flask and suspended metallic cages carrying 20 adult insects and food. Essential oils were separately applied to provide dosages of 0, 5, 10 and 20 µl/L air on filter papers and suspended in the fumigation chamber. Insect mortality was recorded 24, 72, 120 and 168 h post fumigation. Contact toxicity was evaluated in 100ml glass jars, containing 10-20g of grain depending on size of grain. Each test oil was separately applied to provide concentrations of 0, 0.05, 0.10,0.15 and 0.20 %w/w. Number of dead insects were recorded 1, 3, 5, 7 and 10 days after treatment.

Results and Discussion

Results of fumigant toxicity bioassay reveal that *C. lusitanica,* and *E. saligna* plant parts(leaves, flowers and fruits) essential oils at the end of 168h was significantly($p<0.05$) influenced by plant species, plant part, concentration of essential oil, duration of exposure and corresponding factor

interactions. At the highest concentration (20µl/L) percent mortality for leaves, flowers and fruits was 75%, 92.5% and 32.5% for *C. lusitanica* (Fig.2a) and 60%, 20% and 22.5 % for *E. saligna* respectively (Fig.2b). Similarly analyzed results of leaf essential oils extracted from test plants and tested against *T. castaneum* were significantly (*P*<0.05) influenced by inter-plant variations, concentration applied, time after application, and corresponding factor interactions. At the highest concentration (20µl/L) *E, saligna* had a percent mortality of 53.4.9 % as compared to 81.2.4 % in *C. lusitanica*. Results of the contact toxicity test against *C. chinensis* and *T. castaneum* were significantly (P<0.05) dependent on plant species, concentration of essential oils and duration of exposure and corresponding factor interactions. Mortality of *C. chinensis* at concentration of 0.20% w/w was 61.7 % in *E. saligna* as compared to 90.9 % in *C. Lusitanica.* In *T. castaneum* mortality was 35.3% in *C. lusitanica* and 14.8% in *E. saligna* (Fig.3a&b).

(a) *C. lusitanica*



(b) *E. saligna*

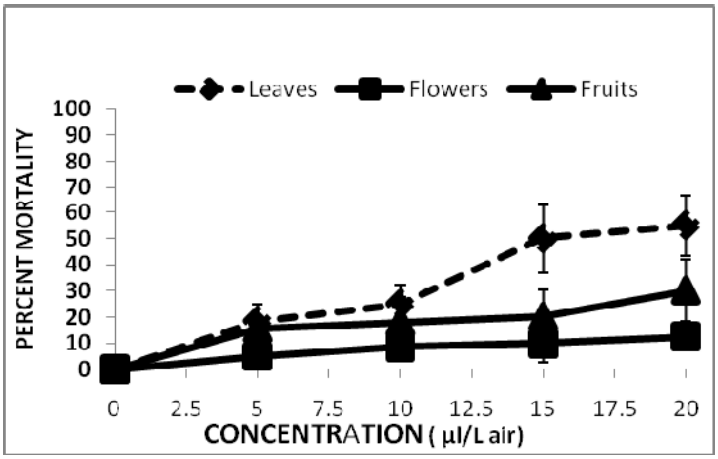


Fig.1: Percent mortality of adult *S. zeamais* after 168 h exposure to essential oils obtained from aerial parts of (a) *C. lusitanica* and (b) *E. saligna* in space fumigation chambers.

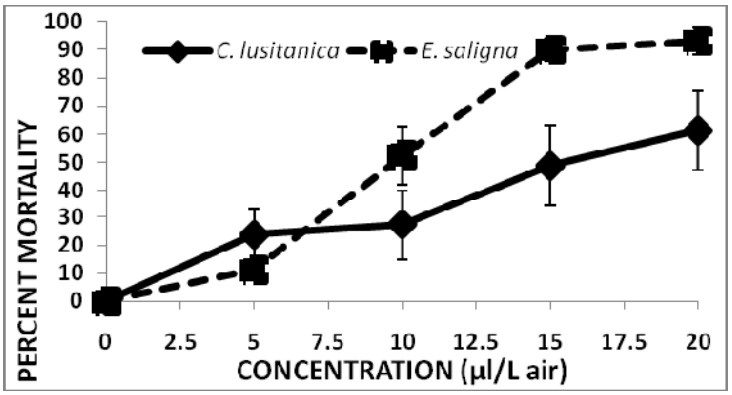
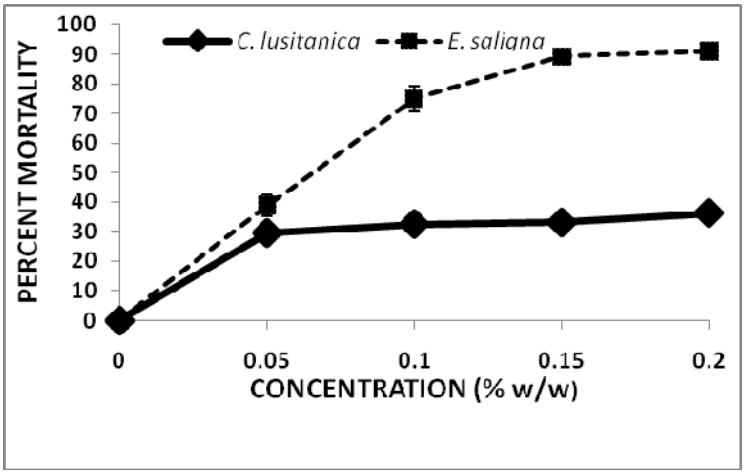


Fig.2: Percent mortality (Mean±SD; n=4) of adult *T. castaneum* after 168 h exposure to *C. lusitanica* and *E. saligna* essential oils in space fumigation chambers.

The observed differentail responses by test insect species to *C. Lusitanica* and *E saligna* essentail oils could be explained by individual/or synergistic fumigant and contact toxicity of their chemical constituents. Similarly, the toxicity differences of leaf, flower, and fruit essentail oils may be attributed to the existing intra-species variations in the quantitative chemical compositions. Results of this study point *E. saligna,* and *C. lusitanic,* essential oils as candidate substances for further bioactivity studies to determine their individual and combined effects on more insect pests as fumigants and possible integration into pest management options in subsistence agriculture.

(a) *C. chinensis*



(b) *T. castaneum*

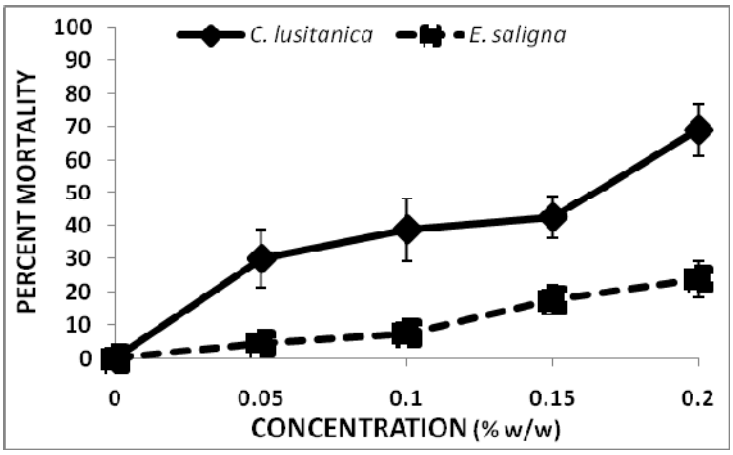


Fig.3: Percent mortality (Mean±SD, n=4) of adult (a) *C. chinensis* and (b) *T. castaneum* after 10 days contact with *C. lusitanica* and *E. saligna* essential oils

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[YS 15] Rhuschalcone VI: Synthesis, Re-Isolation and Bioactivities in its Analogues

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Key words: Suzuki-Miyaura reaction, Bichalcones, Rhuschalcones, Antiprotozoal activities, *Bodo caudatus*.

Introduction

Rhus pyroides Burch. (Anacardiaceae) is a shrub to a medium-sized tree which is widely distributed in the eastern parts of Botswana, and South Africa where it is used against epilepsy in traditional medicine. Previous reports dealing with phytochemical investigations on *R. pyroides* indicate the isolation of a number of compounds belonging to various chemical classes and of interesting biological activities, including Rhus bichalcones I-VI, but in small quantities. To date, as long as we are aware, rhuschalcone VI (**1**) is the first and unique example of a natural dimer in which two chalcones are linked by a C–C bond. Furthermore, it is remarkable that whereas (a) the total syntheses of the bi-aryl ether-type bichalcones (Rhus bichalcones and verbenachalcone) have been reported by Mdee et al. (2003) employing a novel application of the microwave assisted Ullmann synthesis, and Xing et al. (2002) employing catalytic copper-mediated oxidation coupling and the Weinreb ketone synthesis as key steps, respectively; and (b) a number of flavonoids have been employed in Suzuki reactions (Parry et al., 2002), the use of chalcones and the synthesis of any bi-aryl type rhus bichalcones have not yet been reported. In order to provide sufficient quantities of material for more complete biological studies, as well as a general route for the preparation of rhuschalcone VI and its structural analogues, we undertook and successfully achieved both the first-time total syntheses of rhuschalcone VI and analogues and the use of Suzuki-Miyaura reaction for the synthesis of AB C-C linked bichalcones.

The synthesis has been approached by constructing chalcone halides containing functionalities at the 3- or 5- positions which would be cross-coupled using transition metal (Pd) catalysis. Within this context, a total of fourteen (14) bromochalcones, of which 13 are new compounds, have been synthesized using a solvent-free methodology (Toda et al., 1990). In addition, the first total syntheses of eight rhuschalcone VI-type bichalcones were achieved, indicating that the general methodology developed by our group is of practical use in the syntheses of more congeners carrying the same carbon-framework and the creation of biologically more potent substances.

Material and Methods

a) **General:** Commercially available reagents were used without further purification. Most solvents were purified by simple distillation, apart from THF which was distilled from sodium-benzophenone under nitrogen, immediately before use.

b) Chromatographic separations: Analytical thin layer chromatography was carried out using aluminium or glass-backed plates coated with Merck Kieselgel 60 GF_{254}. Developed plates were visualized under ultra-violet light (254 nm) and/or sprayed with vanillin-sulphuric acid. Column chromatography was conducted on columns of different sizes using silica gel 60, particle size 0.040-0.063 mm, or Sephadex LH-20 (Merck). Fully characterized compounds were chromatographically homogeneous.

c) Physical and spectroscopic measurements: Melting points were determined using a Stuart® SMP3 5.0 or a Büchi mp B545 apparatus and are uncorrected. The ultraviolet and visible (UV-Vis) spectra were measured on a Shimadzu UV-2101PC UV-VIS scanning spectrometer. Infrared (IR) spectra were measured on PerkinElmer System 2000 FT-IR spectrometer as KBr pellets or on a PerkinElmer Spectrum 100 FT-IR Spectrometer. NMR spectra were recorded on Bruker Avance 300, 400 or 600 MHz spectrometers. For ^1H NMR, when complex spectra due to overlapping resonances were encountered, the range was recorded. High-resolution mass spectra were obtained on GCT Premier Instrument.

d) Chemical reactions.

1. **Solvent-free Aldol condensation:** For a typical experiment, equimolar quantities of the acetophenone and benzaldehyde derivatives and NaOH were ground in a porcelain mortar at room temperature. After a few minutes, the mixture turned to a yellow solid which was treated with water and filtered to give the desired bromochalcone.
2. **Suzuki-Miyaura cross-coupling reaction:** A mixture of equimolar quantities of the boronate ester, the bromochalcone, and $Pd(PPh_3)_4$ (5 mol%, relative to the ester) in toluene was refluxed under nitrogen atmosphere for 10 min. Afterwards, a 20% aqueous solution of tetraethylammonium hydroxide (4.2 equiv. relative to the ester) was added and the resulting mixture was refluxed following the progress of reaction by TLC. After completion of the reation, the mixture was cooled to room temperature and water was added, and the mixture extracted with diethyl ether and dried (Na_2SO_4). Then the solvent was removed and the residue was chromatographed over silica gel (with appropriate mobile solvent systems). After evaporation of solvent under reduced pressure, the resulting solid was dried overnight in a vacuum oven to afford the ketals (coupling products) as solids.

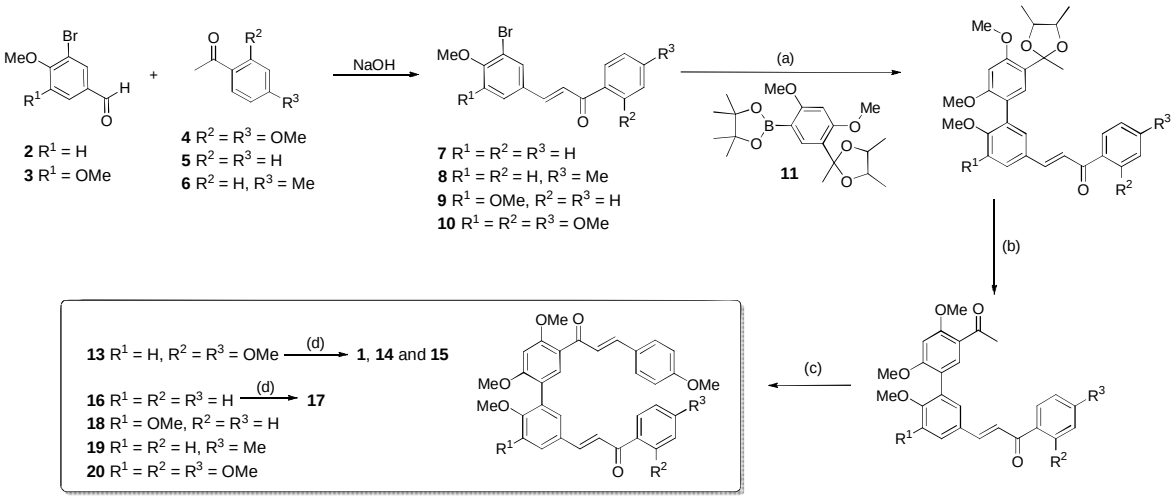
e) Biological activities: These were performed as reported by Mihigo et al. (2010).

Results and Discussion

In the last few years, special attention has been paid to the Suzuki-Miyaura reaction as one of the most popular and powerful methods for the coupling of aryl-aryl moieties. This methodology has gained prominence and found many applications (Braga et al., 2006) both in research laboratories and in large-scale industrial processes due to its compatibility with a variety of functional groups, the stability and the commercial availability of a wide range of organoboron starting materials, and the ease of working up the reaction mixtures. Another advantage of the Suzuki-Miyaura cross-coupling reaction over similar methods is its tolerance of water, which can be used as solvent or co-

solvent (Kotha et al., 2002; Miyaura and Suzuki, 1995). These desirable features made us choose the Suzuki-Miyaura coupling reaction for the synthesis of Rhuschalcone VI and analogues.

The conversion of bromochalcones to their corresponding chalconylboronate esters was envisaged via bromine-lithium exchange with *n*-BuLi followed by a reaction with 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane in THF. However, several attempts failed to yield the desired products (boronate esters); instead, *n*-BuLi (a highly reactive nucleophile) reacted with the electrophilic *α,β*-unsaturated carbonyl group (Wu and Huang, 2006) of the chalcone, and the resulting 1,2- and 1,4-addition products were isolated, and their structures confirmed from NMR data. In addition, attempts to protect the carbonyl groups of the chalcones in order to reduce the electrophilicity of the *α,β*-unsaturated carbonyl group, were not successful. At this point it was decided to form the boronate ester at an early stage of the ketal obtained from 5-bromo-2,4-dimethoxyacetophenone to give boronate ester (**11**) and then perform the Suzuki-Miyaura coupling with various bromochalcones. This would then allow the synthesis of various bichalcones by aldol condensation of the acetophenone derivatives (resulting from the deketalization of the coupling products) with variously substituted benzaldehydes. This approach was found successful and the details are presented in Scheme 1 below, where *p*-anisaldehyde (**12**) is used for the aldol condensation reaction.



Scheme 1. General synthetic strategy for Rhuschalcone VI (1) and analogues. Reagents and conditions: (a) $Pd(PPh_3)_4$, toluene, tetraethylammonium hydroxide, reflux; (b) I_2, acetone, reflux; (c) *p*-anisaldehyde (**12**), solid NaOH, rt; (d) BBr_3, dichloromethane, reflux.

The chemical structures of the newly synthesized compounds were assigned by means of extensive 1D and 2D NMR, and IR, UV, and MS analysis. For rhuschalcone VI, an authentic natural product was sought to make direct comparison possible. Such a sample was not available and hence the natural compound was re-isolated (using the synthetic material as reference) from the roots of the producing *Rhus pyroides* species. The R_f pattern, and the ^1H and ^{13}C NMR data generated for the natural product and the synthetic material were found to be in complete agreement.

The synthesized bichalcones **1**, **13**, **14**, **15**, **16**, **17**, **18**, **19**, and **20**, together with isobavachalcone **21** (a prenylated chalcone isolated from *Dorstenia kameruniana* and which has shown activity on other organisms (Mbaveng et al., 2008)), were preliminarily evaluated for their *in-vitro* anti-protozoal activities using a free-living, non-pathogenic protozoa *Bodo caudatus* (Bodonidae) as a model, and for the most active, for their cytotoxicity using the MTT viability assay (Mosmann, 1983). The results showed that compounds **1**, **15**, **17** and **21** were the most active and induced the largest reduction in viability of protozoa with an inhibition of 75-83 % compared to controls, $CuSO_4$. LC_{50} concentrations were determined for the four most active compounds. The most active compound (**21**) displayed LC_{50} concentration of 4.36 μg/mL, which was about twice less active than $CuSO_4$ (2.30 μg/mL). The other compounds (**1**, **15**, and **17**) killed protozoa with lesser efficacy at LC_{50} concentrations of 53.32, 118.20, and 20.59 μg/mL, respectively. The cytotocity test results showed that of the four most antiprotozoal active compounds, **1** and **21** induced significant cell death of BHK cells after exposure for 48 hours at a concentration of 100 μg/mL, which corresponded to CC_{50} value of 97.59 μg/mL and 86.88 μg/mL, respectively. The CC_{50} concentration of compound **21** was found to be approximately 20 times higher than its antiprotozoal concentration (LC_{50} = 4.36 μg/mL), while antiprotozoal activity of compound **1** is in the range as its cytotoxic concentration. Compounds **15** and **17** induced a small decrease in cell viability compared to non-treated cells, but did not exhibit significant cytotoxicity up to a concentration of 100 μg/mL.

Acknowledgements

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[YS 16] Acaricidal Effects of Four Plant Species on *Rhipicephalus appendiculatus* Neumann (*Acarina ixodidae*) Ticks

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Keywords: *Tick mortality, lethal concentrations, probit analysis, Interaction effects*

Introduction

There is currently a worldwide trend towards reducing the use of chemical acaricides as much as possible in several parts of the world. This has been mainly due to the development of resistance by ticks to acaricides, together with the well-documented damage these compounds cause to the environment and food chain. An alternative with proven efficacy at controlling ticks but with lower environmental impact is the use of plants with established acaricidal properties which possess numerous advantages (Liang *et al*., 2003).

So far, promising results have been obtained from some plants screened for anti-tick properties (Wilson and Surthest, 1990; Nchu *et al.,* 2005; Magano *et al.,* 2007; Kaaya and Saxena, 1998; Kaaya *et al*., 1995). However, despite the promising results, many plants are still scientifically untested for anti-tick properties (Magano *et al.*, 2007).

It is against this background that this study evaluated the acaricidal properties of four plants species. These species had been cited as useful in the control of ticks in an ethnoveterinary survey amongst cattle keepers of Gulu and Amuru districts of Northern Uganda (Opiro, 2009, unpublished data).

Materials and Methods

Tick mortality following exposure to extracts of four plants species (*Cassia didymobotrya, Kigelia africana, Euphorbia hirta* and *Cissus adenocucaulis*) at five serial dilutions (10^{-1} –10^{-5}) and three periods of exposure (24, 48 and 72 h) was assessed. Ticks were exposed to filter paper discs impregnated with the extracts for various periods of time and the number dead were determined. Plant extracts were obtained using three different solvents (methanol, dichloromethane and hexane).

Results

Data analysis using ANOVA showed significant interactions among species, time and concentration. The highest mortality was observed at 72 h and the least was at 24 h. The difference in mortality was highest among plant species at the lowest concentration of extracts, but this gap decreased as the concentration increased. Additionally, tick mortality increased with increasing concentration of

extract and period of exposure to the plant extracts; *C. didymobotrya* was the most potent species followed by *K. africana, E. hirta* and *C.adenocucaulis* in order of decreasing potency. The solvents did not show significant differences in causing tick mortality. Probit analysis showed that the LC (lethal concentration) value of the extract decreased with longer periods of tick exposure to the extracts.

Table 2: Table of means

Grand mean	54.90					
Time (h)	**24**	**48**	**72**			
	42.41	53.69	68.59			
Plant_spp	***C. didymobotrya***	***K.africana***	***E. hirta***	***C. adenocucaulis***		
	68.81	64.21	45.04	41.53		
Solvent	**Dichloromethane**	**Hexane**	**Methanol**			
	54.28	54.79	55.62			
Concn	100000	10000.0	1000.00	100.00	10.00	
	75.41	66.96	58.12	42.34	31.6	
Time	concn	100000	10000.0	1000.00	100.00	10.00
24.00		66.93	56.20	46.21	26.61	16.10
48.00		76.34	66.16	56.22	39.98	29.76
72.00		82.96	78.52	71.93	60.43	49.11
Plant_spp	concn	100000	10000.0	1000.00	100.00	10.00
C.didy		88.84	82.34	74.18	55.91	42.81
E.hirta		63.81	57.44	48.88	31.75	23.35
K.africana		82.45	72.63	64.80	56.46	44.68
S.adedacule		66.55	55.42	44.62	25.25	15.79
Solvent	concn	100000.0	10000.0	1000.00	100.00	10.00
Dichloromethane		75.25	67.36	58.03	40.73	30.04
Hexane		75.11	66.41	57.81	43.12	31.46
Methanol		75.88	67.10	58.52	43.17	33.46

Table 3: Estimated LC Values of extracts of different plants (values in mg/L=ppm)

Cassia didymobotrya

Time	methanol			dichloromethane			hexane		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
LC_{50}	5610.01	2140.81	161.641	5841.14	2493.89	172.39	5785.91	2293.64	182.27
LC_{99}	9687.52	6891.34	1398.80	9737.35	6951.12	1927.02	9928.0	7243.31	2164.25

Kigelia africana

Time	methanol			dichloromethane			hexane		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
LC_{50}	6216.39	3041.47	502.44	7286.1	3350.25	522.86	7107.59	3151.43	516.55
LC_{99}	14480.00	7714.00	1882.43	1192.65	7243.08	2242.12	11692.67	9235.12	3168.56

Euphorbia hirta

Time	methanol			dichloromethane			hexane		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
LC_{50}	64886	41841	19478	68598	33631	18425	66363	43361	20624
LC_{99}	586628	274057	68487	424096	247713	68370	570361	267415	64590

Symphostema adedacule

Time	methanol			dichloromethane			hexane		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
LC_{50}	74571	48272	21471	85050	53399	20118	75228	63182	21117
LC_{99}	451470	245831	79142	488885	251901	71476	581263	265850	68434

Discussion and Conclusions

This study indicates that all plants tested showed acaricidal activity. The analysis revealed that the interaction between plant species and concentration were significant, meaning the potencies of the former were influenced by the latter. The same applies to the effect of exposure duration. The interaction effects between plant species and concentration levels were probably caused by responses of any of the pairs of plant species reacting in the same manner i.e. mortality as described between *C. didymobotrya* and *K. africana* and also *E. hirta* and *C. adenocucaulis.*

The good correlation between concentration and mean mortality is in line with the findings of Magano *et al.,* (2007) who investigated the anti-tick properties of the root extracts of *Senna italica* subsp. *arachoides* against adults of *Hyalomma marginatum rufipes.* These authors found that the higher the concentration of the extracts, the lesser the time needed for a certain proportion of arthropod pests to die.

There were significant differences in acaricidal activities at different exposure times though this effect depended on the concentration. This probably indicates that the active compounds bound to receptor sites would be expected to increase with period of exposure to the extracts (Lullmann and Bieger, 1993). The same could be true for the significant differences in activity of the extracts with increase in concentration. This finding agrees with the observation that time is a crucial factor that increases the amount and distribution of active compounds in the body (Lullman and Bieger, 1993).

Solvent as a factor did not influence tick mortality and the observed mortality was solely due to species. This renders the discussion on effects of polarities on extraction of the plants' ingredients irrelevant, implying that any could be used to make the extracts. The observation could be attributed to the nature of the chemical compounds in the plants. It is however important to note that sharp conclusions cannot be drawn at this stage unless chromatographic, GC-MS and NMR analysis are carried out to identify precisely the components.

The LC values obtained by probit analysis decreased with an increase in the duration of exposure to the test extracts. The values obtained for the two best performing species therefore shows that they have great potential for development into commercial acaricides.

On the whole, the results indicated that by considering the % mortality as a main index, *C. didymobotrya* extracts on average performed best, closely followed by *K. africana*, and this may be attributed to their high potencies as acaricides. The traditional uses and pharmacological properties of these two plants have already been highlighted by other authors (Tuwangye and Olila, 2006; Sangita *et al.* 2008).

Conclusively, the study has demonstrated the acaricidal activity of these four plant species, as well as their potential to provide new compounds for tick control. The promising results therefore highlight the importance of these plants as a natural resource, providing a further impetus for measures to conserve them.

Acknowledgements

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[YS 17] Anti-Malarial Activity and Phytochemical Studies of *Cissampelos Mucronata* and *Stephania Abyssinica*

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Key words: *Stephania abyssinica*, *Cissampelos mucronata* hasubanan (-)-5-oxoaknadinine bisbenzylisoquinoline, (-)-curine, (-)-isocurine and (-)-pseudocurine, anti-plasmodial activity.

Introduction

Each year, there are about 500 million and 2.7 million reported malaria cases and deaths, respectively world wide (WHO 2002; Greenwood *et al*., 2005). The resurgence of malaria is partly attributed to development of drug resistance by the most common malaria parasite (*Plasmodium falciparum*). Plants are considered as important sources of lead compounds in drug development and discovery. Some of the anti-malarial in use today such as artemisinin and its derivatives were obtained directly from plants or developed using chemical structures of plants or developed using chemical structures of plant derived compounds as templates (Philipson *et al*., 1993). Previous research by Muregi *et al*. (2004) revealed that the crude extracts of *Stephania abyssinica* and *Cissampelos mucronata* exhibited strong anti-malarial activity. Isolation of the constituents that are responsible for anti-plasmodial activity has not been done. Phytochemical investigation of the crude alkaloid and dichloromethane extracts of *S. abyssinica* have resulted to characterization of two new bisbenzlyisoquinoline (BBIQ) and a new hasubanan alkaloid (-)-5-oxoaknadinine, respectively. They were largely identified by interpretation of their 2D NMR spectral data. Hexane extract of *S. abyssinica* also gave (+)-nonacosan-10-ol which was previously isolated from *Cocculus hirsutus* (Ahmad *et al*., 1987). Investigation of DCM extracts of *C. mucronata*, (-)-curine and stigmasterol were isolated. In this paper we wish to report the spectral characterization of these compounds and their biological evaluation in terms of anti-plasmodial activity.

Materials and methods:

Plant materials

Stephania abyssinica was collected from Kisii highlands and *Cissampelos mucronata* was collected from Kabondo village Rachuonyo district, Nyanza province, Kenya in August 2006. The plants were authenticated by Mr. Simon Mathenge, Department of Botany University of Nairobi and voucher specimens (RO/01/2006 and RO/02/2006) deposited at the University of Nairobi Herbarium in the Department of Botany. The plant samples were air dried under shade and ground using a laboratory mill.

Extraction

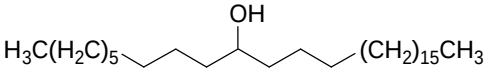
The cold organic extractions were performed by soaking 1.57kg of the *S. abyssinica* and 0.9 kg of *C. mucronata* chaff for 48h in solvents of increasing polarity. The dichloromethane of *S. abyssinica* gave the alkaloid extract after acid hydrolysis. The extracts were preserved at -20°C until used.

Instruments

^{1}H and ^{13}C NMR spectra of the compounds were recorded at 400 MHz and 100 MHz, respectively on a Bruker DRX-500 spectrometer at 292.9k with a Bruker gradient unit. Optical rotation was measured using Polax-2L polarimeter at 25°C and IR on Hyper IR. Melting points were done using Gallen Kemp apparatus and were uncorrected.

Isolation

Column chromatography was done using silica gel and Sephadex LH-20 was used as the filter gel. UV was done using CECIL 2041 UV spectrometer. Repeated column chromatography of n-Hexane extract (6 g) and DCM (2 g) extract of *S. abyssinic*a using hexane: ethylacetate followed by preparative column chromatography and recrystalization gave (-)-nonacosan-10-ol **(1)** and (-)-5-oxoaknidinine **(2)**. The alkaloid extract (266 mg) of the same plant was fractionated by Sephadex LH-20 eluting with DCM:MeOH (1:1) and then preparative thin layer chromatography (2% MeOH/DCM) with (-)-isocurine **(3)** and (-)-pseudocurine **(4)** being isolated. Repeated column chromatography of DCM extract of *C. mucronata* with n-Hexane:EtOAc, DCM:MeOH followed by preparative TLC gave stigmasterol **(5)** and (-)-curine **(6).**



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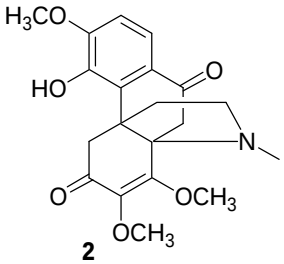

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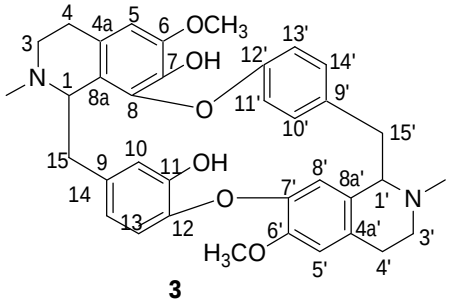

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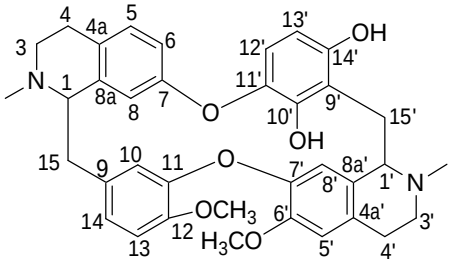

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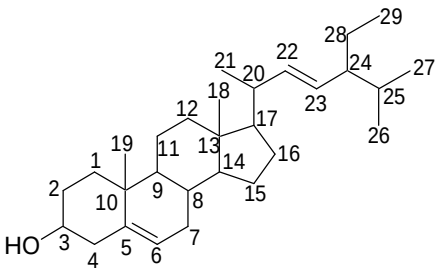

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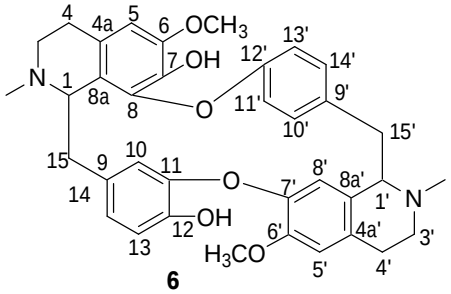

6

Results and Discussion

(+)-Nonacosan-10-ol **(1)** was isolated as white crystals. The IR spectrum showed the presence of hydroxyl group at 3337.6 cm^{-1}. Important clue for the structure was obtained from mass spectrum.

A molecular peak at m/z 424 corresponding to $C_{29}H_{60}O$.was revealed in EIMS. The peak at m/z 297 indicated loss of $CH_3(CH_2)_{18}CH(OH)$ group while the one at m/z 157 indicated loss of $CH_3(CH_2)_8CH(OH)$ group. This was consistent with the fact that –OH group was located at 10^{th} position. The structure was further established through ^1H and ^{13}C NMR which were consistent with the reported data (Dragota & Riederer, 2008). (-)-5-Oxoaknadinine **(2)** was isolated as light yellow crystals. It gave appositive test for alkaloids with Dragendoff's reagent. UV (280 nm) spectra indicated the presence of α, β-unsaturated carbonyl moiety. The structure was determined on basis of 1D (^1H and ^{13}C NMR and DEPT) and 2D (HMQC, HMBC and COSY) NMR experiments.

(-)-Isocurine **(3)** and (-)-pseudocurine **(4)** were isolated as brown amorphous solid and both gave a positive reaction for alkaloids with Dragendoff's reagents. The ^1H NMR spectrum of alkaloid **3** and **4** presented two *N*-methyl groups and two methoxy groups. The electron–impact mass spectrum presented a molecular peak ion at m/z 594 ($C_{36}H_{38}N_2O_6$) and a prominent peak at m/z at 298 for compound, indicative of a head to tail linked BBIQ (Baldas *et al*., 1972). Position of attachment of the two diaryl ether bridges and location of hydroxyl groups were established through interpretation of the HMQC spectrum of the methylated derivatives. From the ^1H NMR spectrum of alkaloid **3** it appeared related to compound **4** but the arrangement of the protons were totally different as revealed by COSY, HMQC and HMBC. Another difference between **3** and **4** one of the methoxy groups is at different position. Compound **3** the methoxy is attached to C-6 and **4** attached to C-12. The structures of the two compounds were established by careful analysis of the 1D-NMR and 2D-NMR (1H-1H correlation spectroscopy (COSY), heteronuclear multiple quantum correlation (HMQC) and heteronuclear multiple bond correlation (HMBC)

A triterpene (+)-stigmasterol **(5)** was isolated as white crystals. Its IR spectrum revealed the presence of hydroxyl group at 3422cm^{-1} and double bond at 1653cm^{-1}. A molecular formula of $C_{29}H_{48}O$ was established by EIMS at m/z 412. All the spectroscopic data confirmed the structure and were consistent with literature values (Morale *et al.,* 2003; Forgo & K□ver, 2004). (-)-curine **(6)** was isolated as white amorphous solid. The IR spectrum showed the presence of hydroxyl group at 3384 cm^{-1} and aromatic C=Cstr at 1507 cm^{-1}. The ^1H NMR spectra revealed 10 aromatic protons. Other peaks at ∂ 3.87 and 3.89 showed a presence of a methoxy group while signals at ∂ 2.28 and 2.49 indicated presence of alkyl amine thus two N-methyl groups. EIMS for (-)-curine revealed a molecular ion peak at m/z 594 corresponding to $C_{36}H_{38}N_2O_6$ The spectral data was in agreement with those reported for (-)-curine (Koike *et al*., 1981; Lengo *et al*., 2000). The anti-plasmodial activity of the isolates against *P. falciparum* D6 and W2 strains *in vitro* are reported in table 1.

Table 1: Anti-plasmodial activity for the isolated compounds

Compound	*P. falciparum* (D6) IC_{50}±SD (µg/ml)	*P. falciparum* (W2) IC_{50}±SD (µg/ml)
(+)-Nonacosan-10-ol **(1)**	13.79±1.02	4.35±2.45
(-)-5-oxoaknadinine **(2)**	10.25±1.84	3.45±2.22
(-)-Isocurine **(3)**	0.75±0.11	1.65±0.03
(-)-Pseudocurine **(4)**	0.29±0.00	0.31±0.01
(+)-Stigmasterol **(5)**	>5	>5
(-)-Curine **(6)**	0.24±0.03	0.22±0.06
Chloroquine	1.16±0.00	1.69±0.14
Artemisinin	8.34±0.14	56.87±1.27

Apart from (-)-stigmasterol **(5)**, (+)-nonacosan-10-ol **(1)** and (-)-oxoaknadinine **(2)** which were inactive, (-)-curine **(6)**, (-)-isocurine **(3)** and (-)-pseudocurine **(4)** showed a strong anti-plasmodial activity. Chloroquine and artemisinin was used as positive controls. For D6 strain the IC_{50} range for compounds was 0.24±0.03-13.79±1.02 µg/ml while that for W2 the IC_{50} range was 0.22±0.06-4.35±2.45 µg/ml. (-)-Curine exhibited the strongest anti-plasmodial activity against *P. falciparum* D6 and W2 strains as compared to other isolates.

Acknowledgement

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[YS 18] Effects of *Sida Cuneifolia* (A.Gray) Herbal Extracts on the Reproductive System Functioning in Male and Female Laboratory Rats

Anastasia N. Nandwa

Etradiol, Polymorphnuclear cells, Gonadotropin Releasing Hormone, Leutinizing hormone, Follicle Stimulating Hormone, Epithelial cells

INTRODUCTION

Sida cuneifolia whose synonym is *Billieturneria hell*eri is an ascendent to procumbent shrub. The leaves are small, being 0.5-1.5 cm long, and about as wide. It used by some communities in Kenya for contraception, however the mechanism of its action have not been documented.

Methodology

Herbs Preparation and Administration

Fifty female albino rats of the species *Rattus norvegicus*, of average weight 200g were used in the investigation. The cages were kept at room temperature i.e. 20^{o}C and exposed to twelve hours daylight and twelve hours darkness.

After a week, forty (40) rats were given root extracts from *Sida cuneifolia* at a concentration of 1g in 100ml tap water making a 1% solution. This was given as drinking water twice at four day intervals. Ten rats continued to take plain tap water. These were the controls. Ten experimental and five control rats were mated with untreated males. The toxicity level of the drug was carried out at Kenya Medical Research Institute (KEMRI) laboratories.

Results

All female rats given the *sida cuneifolia* extract and mated with normal (untreated males) failed to conceive even after staying with the males for over three months. On the other hand control rats had litter normally, that is five to six young every three weeks. Female rats that had, had litter before also failed to conceive when treated with the extract and mated with normal males. Their counterparts that were kept as controls continued to have litter normally.Treated males also failed to sire offspring while control males had offspring.

Day 1

The smears showed equal numbers of cornified and epithelial cells. Leukocytes were either minimal or lacking.

Day 2

Polymophonuclear cells esp. many neutrophilis and monocytes observed in smears from experimental rats. The slide is clean and devoid of mucus. Smears from control rats show sheets of ephilelial cells with mucous indicative of proestrus.

Sheets of ephithelial cells seen.

Day 3

Epithelial cells mostly of parabasal and intermediate type observed in smears from experimental rats. These are normally not observed during Estrus. Smears from control rats with superficial cells appearing in strings indicative of Estrus.

Day 4

High numbers of Leucocytes and total absence of superficial epithelial cells typical of metestrus and diestrus (infertile phases). Control rats showing almost equal numbers of ephithelial and cornified cells.

Day 5

A sharp rise in leukocytes and progressing decline in epithelial cells characteristic of diestrus in smears from experimental rats. The smear is devoid of mucus. The smear from control rat has increasing number of epithelial cells and declining numbers of cornified cells indicating a return to cyclicity.

Histology of sections

Uteri, Ovaries and vaginas were obtained from both experimental and control rats. Sections of Testes from experimental and control rats were also studied.

Uteri

In some areas the lining was thin while in others it was thick. There were large epithelial cells with vacuolations.

Ovaries

Whole ovaries were shrunk and sections showed degenerative granulosa cells. Few follicles were observed and these had missing or degenerate ova.

Vagina

There was peeling off of the Stratum Corneum into the lumen also few glands were observed in the Stratum Germinativum and the vagina had a narrow lumen. The vaginal walls were comparatively thinner than in control rats

Testes

Sections of testes from treated rats showed seminiferous tubules with sloughed off epithelial lining, degenerated interstitial cells with immature spermatids in the lumens. The epithelial lining was thin, two-three cell thickness and even two cell thickness in some areas.

Epididymis

Those from control rats showed complete outlines with normal epithelial lining. Their lumens were full of spermatozoa. Sections from experimental rats showed scanty broken epithelial lining and very large empty or scantly filled lumens i.e aspermia.

Acknowledgement

My heartfelt thanks go to Jehovah God who always makes a way where none exists. I thank Chepkoilel University College for facilitating the research. I am deeply indebted to Janet Kosgey who worked tirelessly with me to accomplish this project. My sincere thanks go to my supervisors; Dr. A.G. M Ng'wena and Prof. R. Ochieng for their professional guidance and superior advice during the course of the research. I also sincerely thank Dr. Ndiema of School of Medicine for always being ready to help.

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[YS 19] Phytochemical Evaluation of *Elaeodendron buchananii* Stem Bark for Microbial Activities

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Key words: *Elaeodendron buchananii* (Loes), stem bark, coumarins, sterols, triterpenoids, and antimicrobial activities.

Introduction

Despite the wide availability of clinically useful antifungal and antibacterial drugs, there is need for search for effective antimicrobials with new strategies to replace those with limited antimicrobial spectrum. Plants that are used traditionally in the management of ailments could be sources of safe and effective drugs. *Elaeodendron buchananii* (Loes) belongs to the family *Celastraceae* and is a tree of tropical Africa that grows to 20m high with dense evergreen foliage found in wooded grassland and dry evergreen forest (Burkill, 1985). Many communities in Africa use *E. buchananii* stem bark to manage fungal (Vazquez, 2000) and bacterial (Kokwaro, 1976; Bekalo *et al*., 1996; Maundu and Tengnas, 2005) infections. Previous phytochemical analyses on its fruits and root bark revealed steroids and terpenoids (Kubo and Fukuhara, 1990; Tsanuo *et al*., 1993; Tsujino *et al*., 1995). The current investigation was undertaken because no phytochemical evaluations of the stem bark had been done despite its widespread use to manage both fungal and bacterial infections.

Material and Methods

Plant material

The plant material was collected from Ngong Forest, Kenya (1°18'13.62"S; 36° 43' 07.11" E; Altitude 5808ft above mean sea level) in April 2007. A voucher specimen of the plant was deposited in the Department of Botany, University of Nairobi following identification by the Taxonomist.

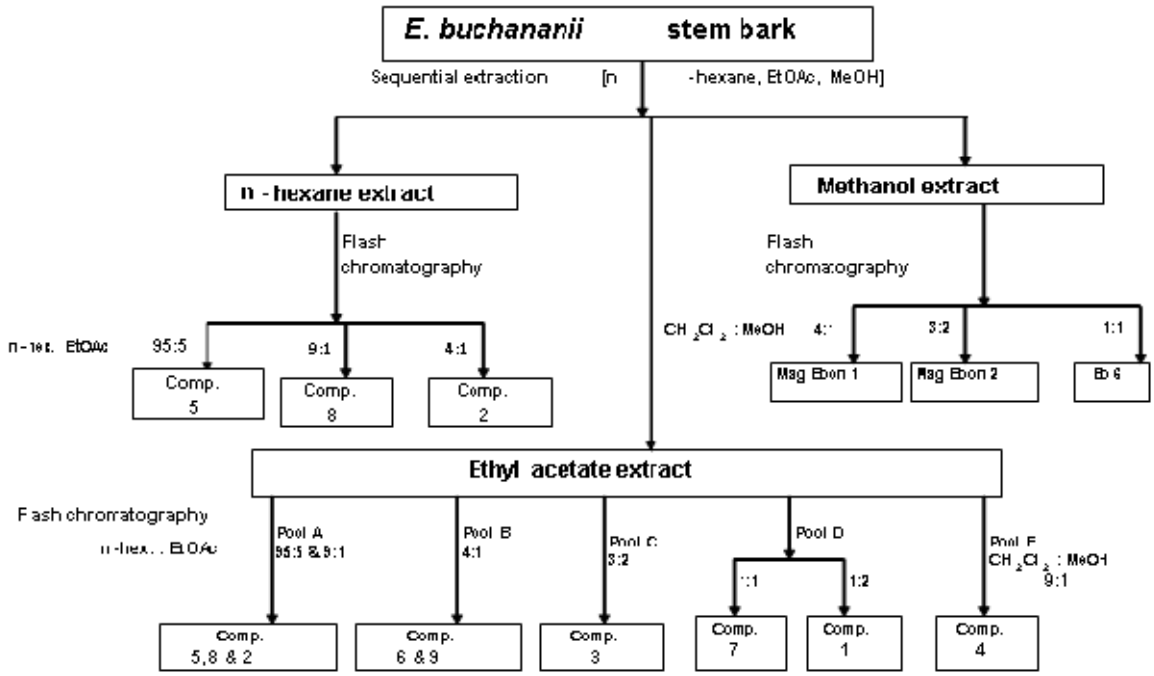
Test organisms

Gram-positive (*Staphylococcus aureus,* ATCC 25923, *Diplococcus pneumoniae* and *Staphylococcus albus*), gram-negative bacteria (*Eschirichia coli*, ATCC 25922, *Vibrio cholerae, Shigella dysenterae and Neisseria meningitidis*) and fungi (*Candida albicans,* ATCC 90028 and *Cryptococcus neoformans*) were obtained from the Microbiology Section of the New Nyanza Provincial General Hospital, Kisumu, Kenya.

Isolation of compounds and characterization

The shade-dried ground powdered stem bark (2kg) was exhaustively extracted sequentially using n-hexane, ethyl acetate and methanol at room temperature. The extracts were fractionated over silica gel packed as outlined in Figure 1. Structural elucidation was done by MS, IR and NMR.

Figure 1: Isolation of compounds from the extracts



Disc diffusion assay

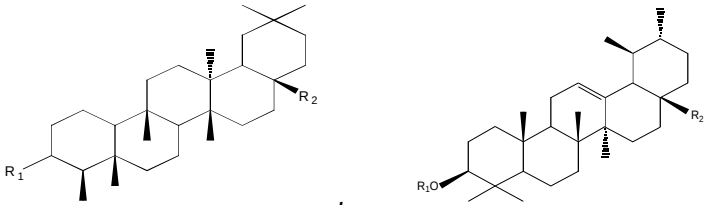
The paper disc diffusion method was employed according to method described by Murray *et al*., 1999 with some modifications. The extracts and isolates were used at concentrations 1.5 and 5mg/ml respectively. Tetracyline (30µg/disk), gentamicin (10µg/ disk), clotrimazole (3 µg/disk) and 5% DMSO ware used as control.

Minimum Inhibitory Concentration (MIC) determination

The MIC was determined by broth microdilution method according to Murray *et al*., 1999. Gentamycin and clotrimazole were used as positive control, 5% DMSO was used as negative control. The assays were done in replicate and analyzed statistically using MSTAT-C statistical package.

Results and Discussions

The n-hexane extract yielded 3α-acetylamyrin (**5**), stigmasterol (**8**) and 3-Oxofriedooleonane (**2**) while ethyl acetate extract gave coumarin (**6**)**,** β-sitosterol (**9**), 3α-hydroxyfriedooleonane (**3**), umbelliferone (**7**), carnophyllol (**1**) and ursolic acid (**4**) in addition to those obtained from n-hexane extract. Methanol extract gave negligible isolates and were not analyzed.



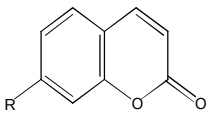
1: R_1=O, R_2=CH_2OH

2: R_1=O, R_2=CH_3

3: R_1=OH, R_2=CH_3

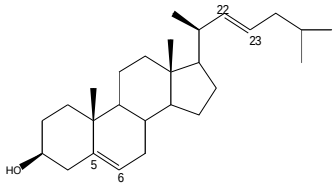
4: R_1= H R_2 =CO_2H

5: R_1=OCH_3 R_2 =CH_3



6: R = H

7: R =OH



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β-Sitosterol (**9**) differed from compound **8** by the absence of C=C at position 22. These nine compounds have been isolated from this plant for the first time.

The ethyl acetate extract exhibited strong antibacterial activities against most bacteria tested Table 1; *E. coli,* (15.2 mm), *D. pneumoniae* (16.1 mm), *S. albus* (19.2 mm), *S. aureus* (22.2mm), *N. meningititis* (24.1 mm) and the best MIC of 15.62 µg/ml against *N. meningititis*. The extract had better activity than the conventional gentamyacin used as positive control (zone and MIC of 21.13 mm and 31.3 respectively against *S. aureus*). Methanol extract displayed strong antifungal activities especially against *Candida albicans* (zone 25.13mm and MIC 31.25 µg/ml). The n-hexane extract showed mild antimicrobial activities.

Table 1: Antimicrobial activities of the extracts of *E. buchananii* stem bark

		Microorganisms								
Extract/standard		*SA*	*DP*	*SAL*	*EC*	*VC*	*SD*	*NM*	*CN*	*CA*
Sample (1.5mg/ml)	n-hexane	12.1	10.1	11.3	9.3	5.2	5.2	14.2	3.2	5.1
	Ethyl acetate	22.2	16.1	19.2	15.2	12.2	13.1	24.1	13.5	15.1
	methanol	14.2	12.2	13.2	13.1	9.1	10.2	16.2	16.1	25.1
Tetracycline (30µg /disc)		12.1	14.2	14.8	14.2	10.1	9.2	13.2	NT	NT
Gentamyacin (10 µg /disc)		21.1	17.2	18.2	17.2	15.2	14.1	16.8	NT	NT
Clotrimazole (30µg /disc)		NT	NT	NT	NT	NT	NT	NT	15.1	22.2

SA= S. aureus, DP= D. pneumoniae =SAL= S. albus, EC= E. coli, VC=V. cholerae SD=S. dysenterae, NM= N. meningititis, CN=C.neoformans,CA= C. albicans, NT=Not tested

Coumarin (**6**) and umbelliferone (**7**) showed antifungal activities (11.2mm and 13.6mm respectively against *C. albicans*) while 3-Oxofriedooleonane/friedelin (**2**) and carnophyllol (**1**) exhibited antibacterial activities. Ursolic acid (**4**) displayed antibacterial (l13.2mm against *C. albicans*) and antifungal (12.2mm against *C. albicans*) activities.

Table 2: Antimicrobial activities of the isolates from E*. buchananii* stem bark

Compound	Microorganisms								
	SA	*DP*	*SAL*	*EC*	*VC*	*SD*	*NM*	*CN*	*CA*
Canophyllol (**1**)	11.2	10.2	12.2	11.2	8.1	5.2	15.1	3.1	3.2
3β-acetyl amyrin (**5**)	10.1	8.2	11.2	3.2	3.1	7.2	10.3	0.0	0.0
Coumarin(**6**)	4.2	5.2	5.1	0.0	0.0	3.2	9.2	11.2	10.3
Umbelliferone(**7**)	9.2	5.1	7.3	4.2	3.2	3.2	5.2	10.8	13.6
Ursolic acid (**4**)	13.2	10.2	12.3	8.2	5.4	3.3	7.2	9.2	12.2
Hydroxy friedelin (**3**)	5.2	0.0	0.0	0.0	0.0	0.0	9.2	10.2	9.2
Friedelin (**2**)	14.2	13.2	10.2	12.2	10.3	9.1	14.2	3.2	7.4
β Sitosterol (**9**)	5.2	5.1	3.2	0.0	0.0	0.0	6.2	10.2	7.2
Tetracycline	11.9	14.2	14.8	14.1	10.1	9.1	13.1	NT	NT
Gentamycin	21.1	17.2	17.6	17.1	15.1	14.1	16.5	NT	NT
Clotrimazole	NT	NT	NT	NT	NT	NT	NT	15.2	21.9
CV%	1.06	1.14	1.17	1.356	1.53	1.90	0.72	0.99	0.74
LSD Comp <0.05	0.186	0.167	0.182	0.203	0.139	0.171	0.27	0.125	0.114

SA= S. aureus, DP= D. pneumoniae =SAL= S. albus, EC= E. coli, VC=V. cholerae SD=S. dysenterae, NM= N. meningititis, CN=C.neoformans,CA= C. albicans, NT=Not tested

These biological activities explain in part the use of *E. buchananii* stem bark in folk medicine for the management of bacterial and fungal related ailments. This study justifies the use of stem bark of *E. buchananii* in folk medicine for the management of bacterial and fungal ailments and recommends the conservation of the plant.

Acknowledgements

The authors are grateful to Prof. Johann Jauch, University of Saarlandes, Germany for analysing samples and availing the IR, MS and NMR spectra and Dr. Santana Odhiambo of New Nyanza Provincial General Hospital for his assistance in bioassay experiments.

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[YS 20] Evaluating Community Knowledge, Management and Economic Losses due to a Zoonotic Disease: A Case Study of Newcastle Disease in Kasese Municipal Council, Western Uganda

Baluku Joward

Key Words: Poultry, Poultry farmers, Newcastle Disease (NCD), Newcastle Disease Virus (NCDV)

Introduction

Poultry production is recognised as an important activity in all developing countries.However, over the past few decades, the focus has been on the production of commercial poultry in rural areas, while traditional village poultry systems have been largely ignored.There are many constraints to poultry production (Sonaiya *et al.* 1999) including a range of bacterial and other viral diseases, internal and external parasites (Permin and Hansen 1998), poor nutrition and predation.

Poultry farmers are disheartened by the loss of large numbers of their birds to NCD outbreaks that often occur on an annual basis.
NCDV is wide spread among several different taxonomic groups of wild birds and appears capable of infecting all species of birds (especially the domestic chickens) and other vertebrates including humans.

Collecting information on knowledge regarding folk beliefs, skills, methods and practices pertaining to the management of Newcastle Disease in poultry enables veterinarians to understand farmers' knowledge of the disease transmission process, local remedies that may be worthy of further study and the type of animal husbandry currently being practiced.

Objectives

i. To establish peoples knowledge with respect to causes and risk factors of Newcastle Disease.
ii. To evaluate the economic losses due to Newcastle Disease among households.
iii. To establish the practices for management of Newcastle Disease.

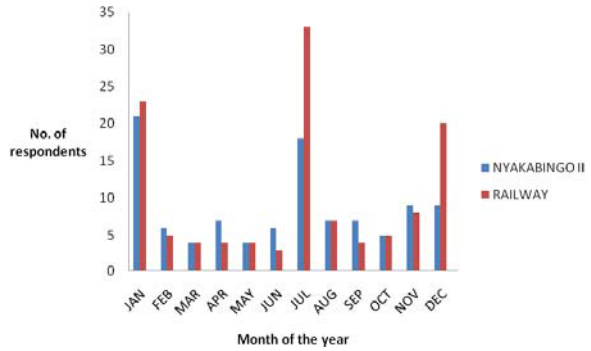
Materials and Methods

The study was conducted on poultry farmers randomly sampled from the two parishes of Nyakabingo II (far from QENP) and Railway (at the border with QENP), each with three villages in the central division of Kasese Municipality. The questionnaires were distributed randomly to 20 poultry farmers from each of the 6 villages.Data analysis was performed using the descriptive method for the qualitative data from the two parishes, to capture information with regard to household size, flock structure, signs observed, causes and management of NCD, and economic loss suffered due NCD outbreak.

Results and Discussion

Seasonality of outbreaks of NCD

Figure 1: The probability by month of an outbreak of NCD occurring according to the experience of local farmers.



Causes

Table 6: Possible causes of NCD according to the opinion of the farmers

CAUSES	Percentage	
	Nyakabingo II	*Railway*
• Too much sunshine(drought	30	31.7
• Introduction of new birds especially those from markets	18.3	
• Poor hygiene especially in the poultry houses	13.3	5
• Congestion/overcrowding in the poultry house	5	10
• Rainy weather	3.3	3.3
• Wondering of the birds mainly due to the free ranging type of production system	3.3	1.7

Risks

Table 7: Risk factors that may increase the chances of transmission and spread of NCD, according to farmers

NYAKABINGO II	RAILWAY
• Interaction with other birds • Improper disposal of the remains of birds after slaughter • Free ranging type of production system • Introduction of new birds either for re-stocking or reinforcement of an existing stock • Rodents and other wild animals especially those that predate on the chickens • Wild birds • Poor housing • Too much sunshine • Congestion in the poultry houses	• Wild birds of prey • Free ranging type of production system • Wondering • Beatings by malicious neighbours especially children, which mostly affects ducks and ducklings • Drought (too much sunshine) • The sewage dumping pit (lagoon) from which some chickens drink when the day gets hot • Introduction of new birds from anywhere • The garbage/waste plant for the Clean Development Mechanism.

Treatment of NCD

Table 8: Forms of treatments used by the respondents

Treatment	NYAKABINGO II		RAILWAY	
	Frequency	%age	Frequency	%age
Conventional only	1	1.7	1	1.7
Conventional (Name forgotten)	7	11.6	10	16.7
Traditional only	33	55	32	53.3
Both	9	15	9	15
None	10	16.7	14	23.3
Total	**60**	**100**	**60**	**100**

How the plants/items can be combined

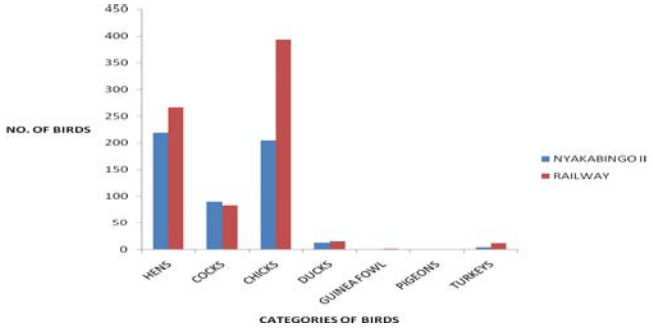
Item	Tobacco	cannabis	Red pepper	ash	pawpaw roots	Aloe	palm oil	Bitter leaf	Tall fleabane	Euphorbia	Urine	soap	Soot	Rock salt	Poke root	scales of a	garlic	Esyantonyera	moringa	Faeces of ducks	Black jack	vegetable oil	paraffin	Neem
Tobacco	0	+		+	+	+	+				+		+	+										
Cannabis	+	0	+	+	+	+	+			+	+		+		+	+	+					+	+	
Red pepper	+	+	0	+	+	+	+			+	+		+	+	+		+		+			+		
Ash	+	+	+	0	+	+	+			+	+	+	+	+	+	+	+		+			+	+	
Pawpaw roots	+		+	+	0	+	+	+		+			+	+										
Aloe	+	+	+		+	0	+	+			+		+						+					+
Palm oil	+	+	+	+	+	+	0			+														
Bitter leaf					+	+																		
Tall fleabane																					+			
Euphorbia		+	+	+			+			0														
Urine	+	+	+	+		+											+							
Soot	+	+	+	+	+	+								+	+									
Rock salt	+		+	+	+								+											
Poke root		+	+	+									+											
Scales of a snake		+		+																			+	
Garlic		+	+	+							+													
Esyantonyera																		0						
Moringa			+	+		+																		+
Faeces of ducks																				0				
Black jack									+															
vegetable oil		+	+	+																				
Paraffin		+		+																	+			
Neem																			+					
Pluchea ovalis																								

KEY

+ is combined with

0 can be used alone

Birds lost in previous encounter



4.4.2 Financial loss caused

Poultry type	Number lost		Av. Unit cost		Total cost	
	Nyakabingo II	Railway	Nyakabingo II	Railway	Nyakabingo II	Railway
Hens	219	267	10,000	10,000	2,190,000	2,670,000
Cocks	90	83	20,000	25,000	1,800,000	2,075,000
Chicks	205	393	-	-	-	-
Ducks	13	15	12,500	20,000	162,500	300,000
Guinea fowl	0	2	15,000	20,000	0	40,000
Pigeons	0	0	4,000	4,000	0	0
Turkeys	4	12	40,000	40,000	160,000	480,000
TOTAL	**531**	**772**			**4,312,500**	**5,565,000**

Conclusion

A wide range of herbal medicines were used in poultry health (NCD) management. Although these herbal drugs were used in poultry disease management, there are information gaps related to efficacy, effectiveness, lethal doses and standardized doses and active ingredients of these plants. Therefore, this study recommends that further research on information gaps be identified and investigated.

NCD causes a financial loss of more than 10million Uganda shillings annually, just in only the two parishes of Kasese Municipal council, which when extrapolated for the whole District and then country, is a lot of money. Therefore improved poultry productivity through improving disease (especially NCD) management is likely to contribute to poverty alleviation in the rural areas/villages.

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[YS 21] Further Phytochemical and Antimicrobial Activity Studies of *Warburgia Ugandensis* against Sweet Potato Pathogens

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Keywords: *Warburgia ugandensis*; Canellaceae; 7α-aceylugandensolide; antibacterial; antifungal.

Introduction

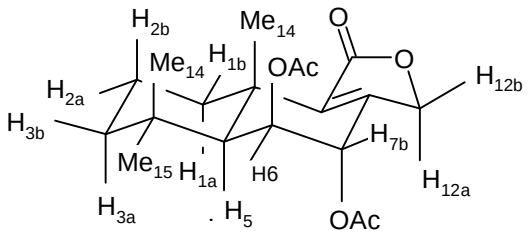
Sweet potato is important potato crop worldwide since it is drought tolerant and acts as a famine relief crop (Gibson *et al.,* 1997). However, its production is limited by viral, fungal and bacterial infections (Gibson *et al.,* 1997). Use of synthetic chemicals to manage the infections cause adverse effects to the ecosystem (Cameron and Julian, 1984) and is unaffordable by most farmers. Moreover, resistance by pathogens has rendered some chemicals ineffective. There is a need to search for affordable, readily available, sustainable and environmentally friendly means of managing the pathogens. This study evaluated the antimicrobial activity of *W. ugandensis* extracts and isolates, which are traditionally used as a remedy for fungal and bacterial infections (Kokwaro, 2009), in the management of sweet potato infections.

Materials and methods

Powdered stem bark of *W. ugandensis* was sequentially extracted with n-hexane, EtOAc and MeOH. Crude extracts were subjected to repeated column chromatography over silica gel to give pure compounds. Extracts and isolates were tested for antimicrobial activity against sweet potato pathogens: *Alternaria spp, Aspegillus niger, Fusarium oxysporum, F. solani, Rhizopus stolonifer* (fungi), *Ralstonia solanacearum* and *Steptomyces ipomoeae* (bacteria) using disc diffusion method (Barry *et al.*, 1979)**.**

Results and discussion

Phytochemical studies afforded one new sesquiterpene, 7α-aceylugandensolide (**1),** together with thirteen known ones namely bemadienolide, drimenin, polygodial, warburganal, mukaadial, ugandensidial, muzigadial, 6α-hydroxymuzigadial, 9-deoxymuzigadial, ugandensolide, deacetoxyugandensolide, cinnamolide and 3β-acetoxycinnamolide. Their structures were determined using spectroscopic methods as well as comparison with literature data.



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Antimicrobial activity

All extracts were active against the tested pathogens (Table 1). Ethyl acetate extract exhibited the highest (P≤ 0.05) inhibitory effects against the tested pathogens. *A. niger* and *R. stolonifer* were the most susceptible to the ethyl acetate extract.

Table 1: Antimicrobial activity of crude extracts

Test microorganism		*Zone of growth inhibition in mm				
		Extracts			Standard drugs	
		EtOAc	n-hexane	Methanol	Blitox	Streptocycline
Fungi	*Alternaria* spp.	22.1	19.1	9.4	27.1	ND
	A. niger	26.1	16.5	8.1	33.0	ND
	F. oxysporum	18.4	13.1	6.0	21.9	ND
	F. solani	14.4	9.0	8.5	30.1	ND
	R. stolonifer	29.5	18.7	10.6	23.3	ND
Bacteria	*R. solanacearum*	21.2	17.3	10.0	ND	23.8
	S. ipomoeae	17.3	15.2	11.2	ND	19.4
	Mean	**21.3**	**15.2**	**11.2**	**27.1**	**21.6**

*Values are means of three replicates and includes 5 mm diameter of disk; ND = Not done.

Out of the 14 isolates, polygodial and mukaadial were the most effective against *Alternaria spp* (MIC = 25 µg/ml) while warbuganal and mukaadial showed strong activity against *A. niger* (MIC = 12.5 µg/ml) (Table 2). Warbuganal and muzigadial exhibited fairly strong activity against *F. oxysporum* (MIC = 25 µg/ml) while polygodial and warburganal gave promising result with *F. solani* (MIC = 12.5 µg/ml). *R. stolonifer* was also found to be susceptible to compounds warbuganal and ugandensidial (MIC = 25 µg/ml).

Table 2: Minimum inhibitory concentration (MIC, µg/ml) of isolated compounds

Compound	MIC, µg/ml of isolated compounds	
	Test fungi	Test bacteria

	Alter spp	*A. nig*	*F. oxy*	*F. sol*	*R. sto*	*R. sola*	*S. ipo*
7α-Acetoxyugandensolide (**1**)	>200	>200	>200	>200	>200	200	>200
Bemadienolide	>200	>200	>200	>200	>200	>200	100
Drimenin	>200	>200	>200	>200	>200	>200	>200
Polygodial	25	50	50	12.5	50	25	50
Warburganal	50	12.5	25	12.5	25	50	50
Mukaadial	25	12.5	100	25	50	25	50
Ugandensidial	50	25	50	100	25	100	25
Muzigadial	50	50	25	100	50	25	50
6α-Hydroxymuzigadial	200	>200	100	>200	>200	100	>200
9-Deoxymuzigadial	>200	>200	>200	>200	>200	>200	>200
Ugandensolide	50	50	100	200	100	>200	100
Deacetoxyugandensolide	50	>200	>200	100	200	100	200
Cinnamolide	100	100	>200	200	>200	>200	>200
3β-Acetoxycinnamolide	>200	>200	>200	>200	>200	>200	>200
Blitox	50	6.25	12.5	6.25	12.5	ND	ND
Streptocycline	ND	ND	ND	ND	ND	25	12.5

ND = Not done; *Alter spp* **=** *Alternaria spp***,** *A. nig* **=** *Aspegillus niger***,** *F. oxy* **=** *Fusarium oxysporum***,** *F. sol* **=** *Fusarium solani***,** *R.. sto* **=** *Rhizopus stolonifer***,** *R.. sola* **=** *Ralstonia solanacearum***,** *S. ipo* **=** *Steptomyces ipomoeae*.

This study revealed that extracts of *W. ugandensis* have antimicrobial activity against *F. oxysporum, F. solani, Alternaria spp, R. stolonifer, A. niger R. solanacearum* and *S. ipomoeae* which are soil pathogens associated with rotting of sweet potato and other root crops (Ristaino, 1993). This suggests that the pathogens can be managed using herbal extracts as had also been observed in other studies (Okigbo and Nmeka, 2005). The herbal extracts are more environmentally safe compared to the synthetic antimicrobial drugs currently used (Masuduzzaman *et al.,* 2008; Siva *et al.,* 2008). Extracts from *W. ugandensis* are not only active against fungi and bacteria that cause disease in animals/man (Kubo and Nakanishi, 1979; Mbwambo *et al.,* 2009) but are also active against plant pathogens thus suggesting that the antimicrobial principles in the plant have broad spectrum activity.

Acknowledgement

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[YS 22] Medicinal Plants used in Disease Management among Children in Namungalwe Sub County, Iganga District

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KEY WORDS: Children, traditional medicine, Medicinal plants, diseases

Introduction

The national under-five mortality rate is 137 deaths per 1,000 live births and infant mortality rate is 75 deaths per 1,000 live births. Seventy percent of overall child mortality is due to malaria (32%), perinatal and neonatal conditions (18%), meningitis (10%), pneumonia (8%), HIV and AIDS (5.6%) and malnutrition (4.6%) (The Second NHP, 2010).

Utilisation of the health facilities in Uganda is still a challenge due to poor infrastructure, inadequate medicines and other health supplies, the shortage and low motivation of human resource (the second NHP, 2010). And poverty aggravates the prevalence of diseases such as malaria, malnutrition and diarrhea (UBOS, 2007).

Over 80% of the people World depend on medicinal plant species to meet their day today healthcare needs (WHO, 2002). The use of traditional medicine in rural Ugandan population for day-to-day health care needs is close to 90% (Kamatenesi and Oryem, 2006). The treatments of most ailments that women and children suffer especially in rural areas depend on herbs first and in case the condition deteriorates, then they seek health facilities.

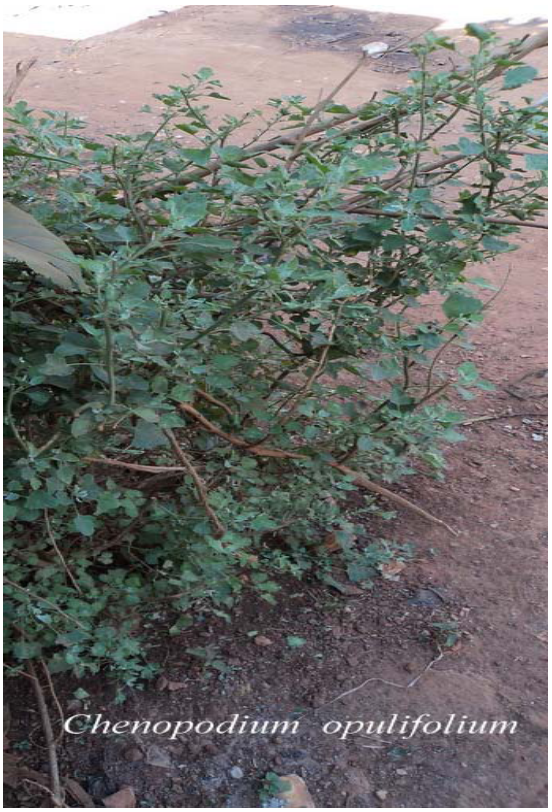
Materials and Methods

Ethno botanical data was collected by carried out household interviews, key informants traditional healers and traditional birth attendants. These techniques were complemented by direct observation, photography, collecting voucher specimens and making notes of relevant issues.

Results

A total of 67 species and one mushroom *Termitomyces microcarpus* were documented as medicinal plants used in the disease management among children. These species belonged to 38 families and 61 genera. Faboideae (6) family had the most number of plant species. Herbs (37%) were the most used plant life forms in disease management among the children. Leaves (54%) were the most used plant parts. These plant species are mainly harvested from the wild (68%). The plants were mainly boiling. *Vernonia amygdalina, Chenopodium opulifolium* and *Albizia corialia* was the most

mentioned plant species.Twenty seven percent of the recorded plant species were reported for treating malaria. The commonest ailments were digestive system disorders.



Discussion

There is a diversity of knowledge on medicinal plants known to be used in disease management among children in Namungalwe Sub County. Majority of the respondents were women (54%) and these were using the plants in the treatment of diseases among children. *Vernonia amygdalina, Chenopodium opulifolium* and *Albizia corialia* are the commonly known to be used medicinal plant species. These have been reported by different researchers as medicinal plants used by local communities country wide. A lot of knowledge has existing on the plants used in the treatment of malaria because in Uganda, it is highly prevalent and according to the (the second NHP, 2010), malaria (32%) is the leading cause of child mortality.

Conclusion

There is diversity of traditional knowledge on medicinal plants used in the management of ailments among children in Namungalwe Sub County.

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[YS 23] Antiplasmodial and Antinociceptive constituents from *Caesalpinia volkensii* Harms (Caesalpiniaceae) Root Bark

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KEY WORDS: *Caesalpinia volkensii*, voucapan-1, 5-diol, deoxycaesaldekarin D, antiplasmodial activity, antinociceptive activity

Introduction

Despite intense research, studies on more therapeutic options towards management of malaria are attractive due to the polymorphism of *Plasmodium falciparum*. *Caesalpinia volkensii* H. (Caesalpiniaceae) is used in East Africa for management of many diseases, including malaria, pain during pregnancy, aphrodisiac and retinoblastoma (Kokwaro, 2009). Medicinal values are ascribed to its root back, seed kernels and leaves (Beentje, 1994). However, pharmacogically active components responsible for the activities have not been identified. This study evaluated the antiplasmodial and antinociceptive actions of the organic extracts prepared from root bark of *C. volkensii* and characterized the active compounds.

Materials and Methods

The root bark of *Caesalpinia volkensii* was collected from Eldoret County of Kenya, identified and a voucher specimen (COO-CV- 2010-01) was deposited at the University of Nairobi Herbarium, Department of Botany. The ground-dried root bark (1.2 kg) was extracted with 95% Methanol: water, then partitioned between hexane, chloroform, ethyl acetate and *n*-butanol. The active fractions were further subjected to various chromatographic techniques (Column chromatography on silica gel and Sephadex, preparative TLC) to isolate their constituents. Antinociceptive effect of the extracts and the compounds were assessed in mice using hot plate method (Eddy & Leimbach, 1953) and acetic acid writhing tests (Koster *et al*., 1959) using Swiss albino mice (20-25 g) following the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (NIH, 1985). *In vitro* antiplasmodial activity was performed using a non radioactive assay technique described by Smilkstein *et al*., (2004). The data were analysed using one way analysis of variance (ANOVA) followed by Bonferroni posttests and Dunnett's multiple comparison tests. Values were considered significant when $P \leq 0.05$.

Result and discussion

On enteral administration of different extracts of *Caesalpinia volkensii* at doses of 100 mg/kg, chloroform and ethyl acetate extracts produced 40.6 and 40% inhibition of the writhing process in mice ($p \leq 0.05$) (Table 1) while from the hot plate test similar trend was observed as presented in

Table 1. On the other hand, the ethyl acetate extract (50 μg/ml) showed significantly strong activity against D6 and W2 strains of *Plasmodium falciparum* with IC_{50} values of 0.23 ± 0.07 and 4.39 ± 2.49 μg/ml, respectively, compared to standard drug chloroquine (P≤0.05) (Table 2). This prompted the chemical assessment of the components of active extracts by various chromatographic techniques leading to isolation and characterization seven furanoditerpene; voucapan-1, 5-diol (**1**), deoxycaesaldekarin D (**2**) voucapane (**3**), voucapan-5-ol (**4**), deoxycaesaldekarin C (**5**), caesaldekarin C (**6**), 5-hydroxy vinhaticoic acid (**7**) and three cinnamyl esters viz triacontanyl-(*E*)-ferulate (**8**), triacontanyl-(*E*)-caffaete (**9**) and 30′-hydroxytriacontanyl-(*E*)-ferulate (**10**).

Table 1: Effects of *Caesalpinia volkensii* root bark extracts on hot plate-induced pain and acetic acid-induced writhing in mice

Dose treatment (mg/kg)	Pre- treat latency	% inhibition of pain threshold (hot plate test)		Writhing response (% inhibition)
		30 min	60 min	
CV1 (100)	1.12 ± 0.37	1.25	6.92*	37.5
CV2 (100)	1.26 ± 0.15	2.09	6.05*	40.62*[a]
CV3 (100)	1.2 ± 0.45	3.13	8.13**	44.38**[a]
CV4 (100)	1.34 ± 0.19	1.19	4.40	35.63
Morphine (10ml/kg)	1.9 ± 0.1	10.07	14.70***	NT
Ibuprofen (10)	NT	NT	NT	81.88***[a]
N.saline water	1.27 ± 076	0.45	0.10	0.0

Values are mean ± SEM (n = 6). Significant activities *P<0.05, **P<0.01, ***P<0.001 vs. control (one way ANOVA followed by Bonferroni posttests). [a] P<0.05 vs control (one way ANOVA followed by Dunnet's Multiple Comparison test). NT = not tested

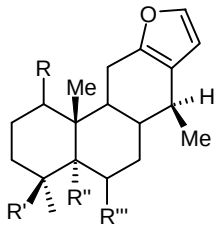
Caesaldekarin C (**6**) and its derivatives exhibited comparably stronger activity relative to the voucapane alcohols (P<0.05). Deoxycaesaldekarin C (**5**) was the most active with IC_{50} values of 13.93 ± 1.32 and 13.57 ± 1.78 μg/ml against the D6 and W2 strains of *Plasmodium falciparum*, respectively (Table 2).

Table 2: *In vitro* antiplasmodial activity (50% growth inhibition) of a fractionated MeOH extract of *C. volkensii* and some isolates against D6 and W2 strains of Plasmodium falciparum.

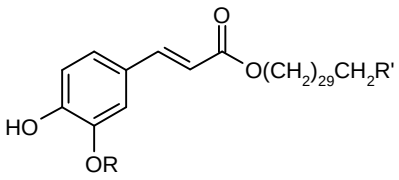
	D6 Clone (IC_{50} µg/ml)	W2 Clone (IC_{50} µg/ml)
CVR A01	**16.10±7.04[a]**	**10.84±3.85[a]**
CVR-F02	**>50**	**>50**
CVR-F03	**15.68±2.42[a]**	**13.98±4.71[a]**
CVR-F04	**0.23±0.07[b]**	**4.39±2.49[b]**
CVR-F05	**>50**	**>50**
Voucapan-1, 5-diol	**28.66±2.22**	**48.24±8.55**
Voucapan-5-ol	**>50**	**>50**
Deoxycaesaldekarin D	**18.21±5.65[a]**	**15.38±3.13[a]**
Deoxycaesaldekarin C	**13.93±1.32[a]**	**13.57±1.78[a]**
Caesaldekarin C	**19.10±1.99[a]**	**32.58±2.87**
Triacontanyl-(*E*)-ferulate	**>50**	**>50**
Triacontanyl-(*E*)-caffaete	**>50**	**>50**
Chloroquine	**0.005±0.002[b]**	**0.27±0.04[b]**

Values with same superscript in the same column are statistically similar at P<0.05 (one-way ANOVA followed by Bonferroni posttests). Samples with IC_{50} >50 did not show activity in tested range (50 µg/ml and below).

The entereal administration of **3**, **4**, **5** and **6** (100 mg/kg) caused a significant reduction in the number of writhing episodes induced by acetic acid (Fig 1) and increased pain threshold on hot plate method (Fig 2) statistically similar to the ibobrufen and morphine, respectively (p<0.01). Both antiplamosdial and antinociptive activity were thus inferred to the furanoditerpenes since the result concurred with previous reports on antiplasmodial and analgesic activities of furanoditerpenes (Linn *et al*., 2005; Duarte *et al*., 1996). The present study demonstrated, that *Caesalpinia volkensii* elaborates cassane furanoditerpenes. The antinociceptive action and antiplasmodial activities suggests that the extract and the active principles (furanoditerpenes) are potential therapeutic options for the management of malaria and pain related ailments, which validates the ethnomedicinal use of this plant.



1:R = OH, R' = Me, R'' = OH, R''' = H
2:R = OH, R' = MeOOC, R'' = H, R''' = OH
3:R = H, R' = Me, R'' = H, R''' = H
4:R = H, R' = Me, R'' = OH, R''' = H
5:R = H, R' = MeOOC, R'' = H, R''' = H
6:R = H, R' = MeOOC, R'' = OH, R''' = H
7:R = H, R' = COOH, R'' = OH, R''' = H



8: R = Me, R' = H
9: R = H, R' = H
10. R = Me, R' = OH

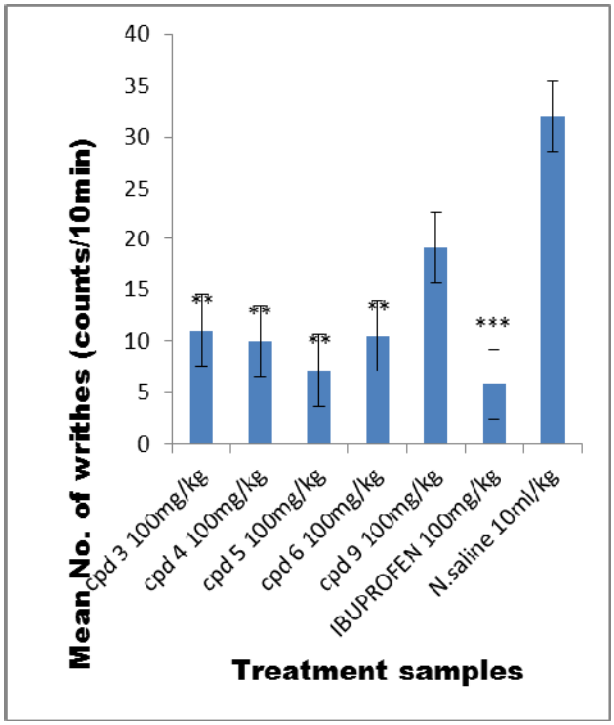


Fig 1: The antinociceptive effect of the compounds of *C.volkensii* root bark, ibobrufen, and 0.8% saline water as observed in acetic acid-induced writhing test. Values are presented as the mean ± SEM (n = 6). ***P<0.001, **P<0.01 and *P<0.05 significant difference compared to control (10 mg/kg).

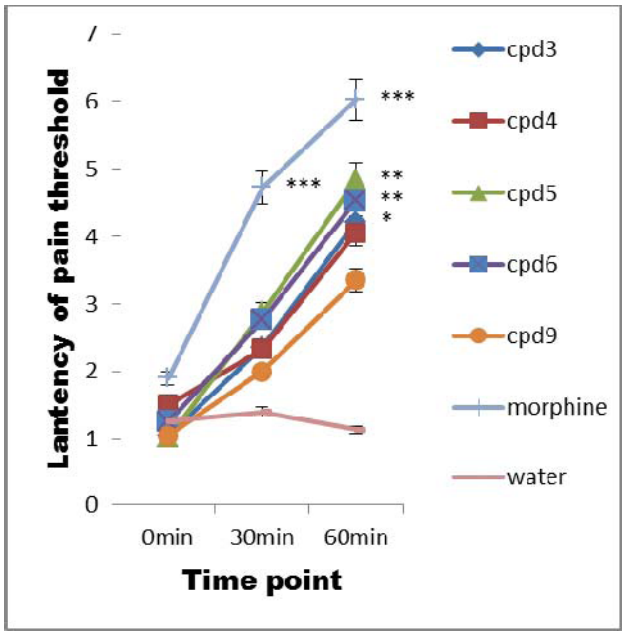


Fig. 2: The antinociceptive effect of compounds from *C. volkensii*, morphine and 0.8% saline water as observed in hot-plate test. Values were presented as the mean ± SEM (n = 6). *** P<0.001, **P<0.01 and *P<0.05, significant difference from control (10ml/kg).

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[YS 24] Documentation of Medicinal Plants Found in Keiyo County Cherebes and Endo Village

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Key words: Documentation, ethnobotany, traditional medicine, indigenous knowledge, herbalist

Introduction

Traditional herbal remedies are an important component in the provision of primary health care. They serve as an alternative to conventional medicines which are normally too expensive for most Kenyans, but the rate at which these herbal remedies are disappearing from their natural habitat is alarming. There is little or no documentation on the uses of these plants (Tildhun and Mirutse, 2007). Moreover, the indigenous knowledge on medicinal properties of these plants is secretly guarded by the herbalists and is not available to most Kenyans. The methods that were previously used to pass the information are presently not applicable. Traditional medicine has and still remains the main source for a large majority(80%) of people in Ethiopia for treating health problems and medicinal consultancy including consumption of the medicinal plants has a much lower cost than modern medicine attention (Tildhun and Mirutse, 2007). Traditional medicine is used throughout the world as it is dependent on locally available plants, which are easily accessible, and capitalizes on traditional wisdom-repository of knowledge, simple to use and affordable (Tesfaye and Sebsebe, 2009). Plants continue to be a rich source of therapeutic agents. The remarkable contribution of plants to the drug industry was possible because of the large number of phytochemical and biological studies all over the world (Kesaran *et al*, 2007). The documentation of medicinal plants in Kenya is very poor. Few of documentation have been done on plants from central Kenya as veterinary medicine, also few plants have been studied from kakamega rain forest; hence there is need to document medicinal plants found in Keiyo County. However, there is need to carry out proper identification of the medicinal plants (Tildhun and Mirutse, 2007). Equally threatened is the knowledge based on which the traditional system is based, as the ethno-botanical information is not documented and remains in the memory of the elderly practitioners (Tesfaye and Sebsebe, 2009). The purpose of documentation in ethno-botany is to try and find out how people have traditionally used plants, for whatever purpose and how is still doing so. Thus, ethno-botany tries to preserve valuable traditional knowledge for both future generations and other communities (Tesfaye and Sebsebe, 2009).

Materials and methods

An ethnobotanical study was done using a structured questionnaire to gather information on the plants used medicinally. This was administered randomly mainly targeting herbalists and adult

villagers. This information provides baseline data for collection of the medicinal plants. Plant Collection and identification was also done. Selection of the plants was based on available ethnobotanical information from traditional health practitioners consulted during the pilot study as well as available literature. The plant materials were photographed and collected in duplicates for identification, verification and storage at the herbarium in Chepkoilel University College.

Results and Discussion

A total of twenty five plants were documented. These plants are used for varied reasons ranging from stomachache, headache, dysmenorrhoea, chest infection, and increasing fertility in both males and females among others. Many plants are boiled together for better results. Some of the plants that were to be gathered were extinct as per the herbalists or are located in specific forested areas within the neighboring Kapnorok game reseave. Many other plants are endangered among them are *Zanthoxylum usambarensis, Trichillia ometca, Terminaria spinosa, Senna siamea*, *Sanseveria conspicua*, *Sanseveria suffruticosa*, *Teclea nobilis*, *Salvadora persica*, *Landolphia swynnertonii,* and *Capparis tomentosa.* From the statistics, the endangered species were ten out of twenty five collected. Some of the plants like *Terminaria spinosa* have become source of conflict among the people since in the past one would collect the plants from any region without quarrelling with the owner of the plot but presently some of the plants are jealously guarded by the plot owners.

Plant Name	Local name (Vernacular)	Traditional uses
BALANITES AEGYPTIACA	Ng'oswet	The plant roots are boiled and taken for the purpose of opening blocked or narrow reproductive tubes, for treating typhoid and for the purpose of increasing fertility in women
Balanites pedicellaris	**MUIYENG'WET**	**THE PLANT ROOTS AND THE BACK ARE BOILED AND TAKEN FOR THE PURPOSE OF RECTIFYING DYSMENORRHOEA, FOR TREATS STOMACHACHE AND FOR STOPPING DIARRHEA**
Coccinea grandis	**SOTOP-CHEPTUGE**	**THE PLANT LEAVES AND ROOTS ARE BOILED OR SOAKED AND TAKEN FOR THE PURPOSE OF SHRINKING OR TREATING FIBROIDS, DISSOLVING CLOT, TREATS PAINFUL SEXUAL INTERCOURSE AND FOR TREATING INTERNAL ORGANS, DISORDER AND INFECTION**
Acacia seyal	**LENG'NET**	**THE PLANT ROOT AND BACK ARE SOAKED OR BOILED AND TAKEN FOR THE PURPOSE OF TREATING TYPHOID KIDNEY INFECTION AND AMOEBIASIS ESPECIALLY IN CHRONIC STAGES ESPECIALLY WHEN THE PATIENT HAS BLOOD STAINED STOOL.**
Capparis tomentosa	**KUMBOLWOP KIMAGET**	**THE PLANT ROOTS AND BACK IS BOILED AND USED FOR THE PURPOSE OF TREATING GONORRHEA, INCREASING FERTILITY, FOR TREATING MASTITIS IN HUMANS AND FOR TREATING DYSMENORRHOEA**
Landolphia swynnertonii	**MOKOKWET**	**THE PLANT ROOTS ARE BOILED AND TAKEN FOR THE PURPOSE OF TREATING BACKACHE AND FOR INCREASING FERTILITY IN WOMEN**
Withania somnifera	**KUMYAP CHEPKUK**	**THE PLANT ROOTS ARE BOILED AND TAKEN FOR THE PURPOSE OF TREATING MALARIA AND YELLOW FEVER**
Salvadora persica	**CHOGOWET**	**THE PLANT BACK AND ROOTS ARE BOILED AND TAKEN FOR THE PURPOSE OF TREATING ALLERGY, COMMON COLD AND PAINFUL CHEST INFECTION**

Teclea nobilis	**KURYOT**	**THE PLANT ROOTS ARE BOILED AND TAKEN FOR THE PURPOSE OF TREATING PERSISTENT HEADACHE AND COMMON COLD**
Sanseveria suffruticosa	**MOKOLATIET**	**THE PLANT ROOTS ARE BOILED AND TAKEN FOR THE PURPOSE OF OPENING BLOCKED OR NARROWED REPRODUCTIVE TUBES AND FOR TREATING GONORRHEA**
Sanseveria conspicua	Sagaratiet	The plant roots and young leaves are either boiled or soaked and taken for the purpose of Removing placenta retained after birth in both humans and animals
BOSCIA ANGUSTIFOLIA	**LIKWOT**	**THE ROOTS ARE BOILED OR GROUND INTO POWDER AND USED FOR THE PURPOSE OF HEALING WOUNDS WHEN USED IN POWDER FORM, FOR TREATING TYPHOID AND THROAT INFECTION**
SENNA SIAMEA	**CHAKARANDAYAT**	**ITS LEAVES IS BOILED AND TAKEN FOR THE PURPOSE OF TREATING MALARIA**
CISSUS VOTINDIFOLIA	**CHEROROWET**	**ITS FLESHY ROOTS ARE BOILED AND TAKEN FOR THE PURPOSE OF TREATING AMOEBIASIS, TYPHOID, INCREASING FEMALE FERTILITY AND RECTIFYING INCONSISTENCY IN CHILDREN.**
MAERUA SUBCORDATA	**CHEPYETABEI**	**THE FLESHY ROOT IS EATEN RAW DRY OR FRESH FOR THE PURPOSES OF TREATING DIABETES, HIGH BLOOD PRESSURE, FOR IMPROVING APPETITE, PURIFIES WATER, FOR INDUCING SLEEP (INDUCES SLEEP AT HIGH DOSE)**
INDIGOFERA HOMBEI	**PARKELAT**	**ITS ROOTS ARE EATEN RAW WHEN FRESH OR BOILED WHEN DRY FOR THE PURPOSES OF TREATING ALLERGY AND FOR RELIVING TOOTHACHE.**
TERMINARIA SPINOSA	**TIKIT**	**ITS LEAVES, BACK AND ROOTS ARE BOILED FOR THE PURPOSES OF TREATING PANCREAS DISORDER, TOOTHACHE, PERSISTENT HEADACHE ACCOMPANIED BY PAINFUL TEETH AND FOR TREATING NOSE AND EYES ALLERGY. IT IS ALSO USED TO INCREASE MALE LIBIDO.**
EUPHORBIA TIRUCALLI	**KIPNAGET**	**THE LEAVES/STEM ARE BOILED AND TAKEN FOR THE PURPOSE FOR TREATING MALARIA AND STOMACHACHE.**
Trichillia ometca	**MOINET**	**THE STEM AND ROOTS ARE BOILED AND TAKEN FOR THE PURPOSE OF TREATING ALLERGY AND FOR STOPPING DIARRHEA.**
Commelina Africana	**CHESEPER**	**THE LEAVES AND POUND FRESH AND USED TO TREAT WOUNDS AND FOR REMOVING OBJECTS THAT INJURED THE BODY LIKE THORNS.**
Zanthoxylum usambarensis	**KOKCHAT**	**THE ROOTS ARE BOILED AND TAKEN FOR THE PURPOSE OF TREATING RASHES ON THE TONGUE, FOR TREATING ULCERS, COUGH AND COMMON COLD.**

The residents also do not cultivate the plants and the herbalists depend 100% from the plants from the wild. This has posed danger since they are competing with the wild and domestic animals, including among themselves. The region is also being consumed by deep gulley erosion which frequently wipes away the plants. All the plants succumb to this since the gulley erosion are so deep such that not even a single plant survives the pressure. The villagers confirmed the heredity of the knowledge and the danger of fast disappearance of the same.

Acknowledgement

I would wish to thank Mr. Wanjohi of Chepkoilel University College for naming the plants, and my supervisors Dr. Njenga, Dr. Mutai and Dr. Bii for their corrections, inspiration and guidance.

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[YS 25] Antimicrobial Dihydroisocoumarins from *Crassocephalum biafrae*

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KEY WORDS: *Crassocephalum biafrae*, Asteraceae, dihydroisocoumarins, antimicrobial activity

Introduction

Crassocephalum biafrae S. Moore (Asteraceae) and other *Crassocephalum* species are widely used as food additives and in traditional medicine in many Africa countries (Adebooye *et al*, 2004). Aqueous extracts of C. biafrae rhizomes are used in Cameroon folk medicine to treat tuberculosis, epilepsy, respiratory infections, diarrhea, wounds, and cancer (Adjanohoun *et al*, 1996). There is little phytochemical data on *C. biafrae*, although its essential oil has been characterized (Zollo *et al,* 2000).

Materials and Methods

The air-dried roots and rhizomes (2.5 kg) of C. biafrae were extracted with MeOH (1 L × 3) for 72 h at room temperature. A preliminary biological screening of this extract exhibited significant antimicrobial activity. The methanol extract (65 g) was evaporated to dryness, defatted with hexane, suspended in H2O, and partitioned between CHCl3 (400 mL × 3) and n-BuOH (400 mL× 3). The hexane (13 g), n-butanol (7 g), and chloroform (19 g) extracts were subjected to antibacterial and antifungal tests. The $CHCl_3$ extract, which showed appreciable antibacterial activity, was then concentrated to a brown, viscous mass under reduced pressure. The residue was then dissolved in a small amount of MeOH. Purification of this extract using Sephadex LH-20 column (MeOH, 3 L, 9 × 7.3 cm), and silica gel column(20mg, 40–63 μm, 5 × 48 cm) using n-hexane–AcOEt (3: 2) and n-hexane–AcOEt (1: 1) solvent systems led to the isolation of

Three new dihydroisocoumarins **(1)** (67 mg), **(2)** (53 mg) and **(3)** (89 mg) along two know triterpenoids **(4)** (43 mg) and **(5)** (23 mg) and one known ceramide **(6)** (51 mg)

In vitro antibacterial and antifungal activities were determined using the agar-well diffusion method (Atta-ur-Rahman *et al,* 2001) and the tube diffusion test (McLauglin *et al,* 1991).

Results and Discussion

As part of our ongoing research on Cameroonian medicinal plants, we report herein the isolation of three new dihydroisocoumarins: Biafraecoumarin A (**1**), Biafraecoumarin B (**2**) and Biafraecoumarin C (**3**) along with three known compounds Fernenol (**4**) (Albrecht *et al*, 1969) Sorghumol acetate (**5**)

(Jain *et al,* 2002) and (2S, 2'R, 3S, 4R, 6E)-N-(2'-hydroxytetracosanoyl) -2-aminooctadec-6-ène-1, 3, 4-triol (**6**) (Mendelsohn *et al,* 2000) from *Crassocephalum biafrae*. The structures of the new compounds wer established on the basis of the physical, chemical and spectroscopic data as shown in chart 1. Circular dichroism technique was used to determine the configuration of asymmetric carbons.

Compounds 1-6 (1 mg/mL) have been tested in vitro for their antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas picketti* and *Staphylococcus aureus* bacteria by the agar-well diffusion method [15]. DMSO was used as a control solvent, and cefixime was used as a standard drug. Minimum inhibitory concentration (MIC) values of all six isolated compounds were determined. The results showed that extract, fractions, and all compounds were active against *E. coli* and *B. subtilis.*

Compounds 1-6 (200 μg/mL) were also screened in vitro for their antifungal activity against six fungi species using the tube diffusion test [16]. Miconazole and Amphotericin (200 μg/mL) were used as standard drugs. The linear growth of the fungus was obtained by measuring the diameter of the fungal colony after seven days. The amount of growth inhibition in each case was calculated as percentage inhibition. The screening results showed that compounds **1** and **2** exhibited significant activity (> 75%) *against Candida albicans*, *Fusarium solani*, and *Trichphyton longifusus*, whereas compound **3** showed significant activity (80%) against *F. solani*. The other compounds showed no to low activity. It is worthwhile to note that biafraecoumarins A (**1**), B (**2**), and C (**3**) exhibited maximum antibacterial and antifungal activities, possibly due to the presence of the dihydroisocoumarin moiety.

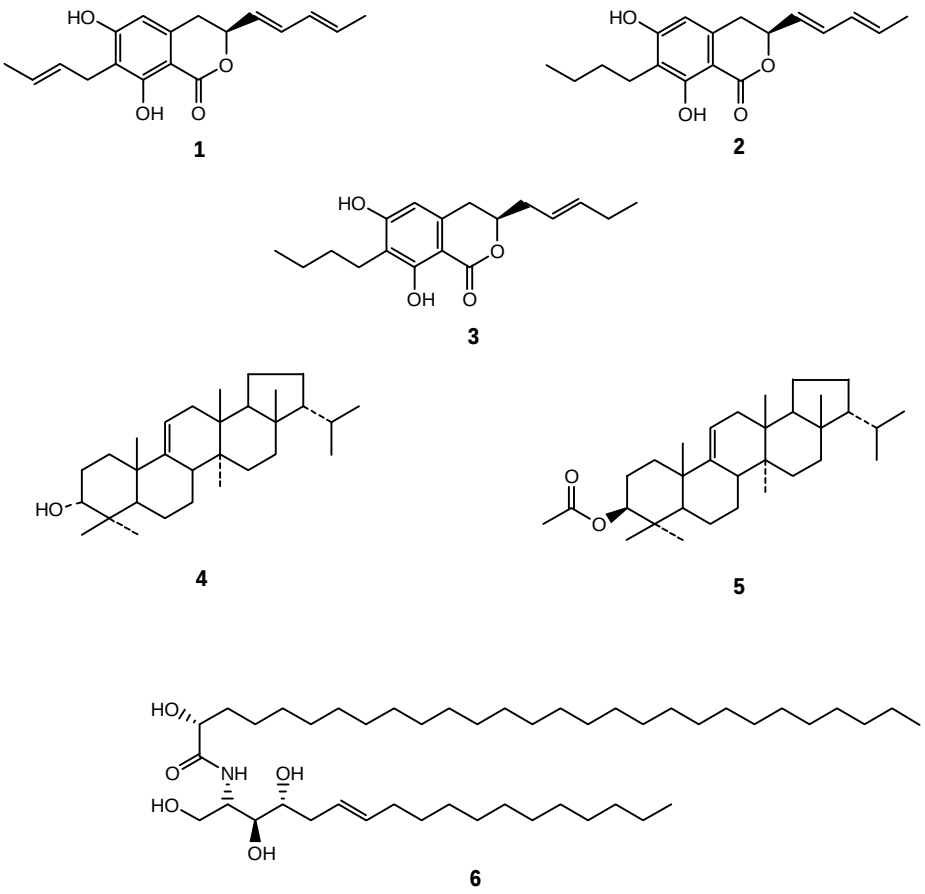

Chart 1

Acknowledgements

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[YS 26] Antimycobacterial and Cytotoxicity Activity of Extracts from Zanthoxylum rhalybeum and Hallea rubrostipulata

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Key words: *Hallea rubrostipulata, Zanthoxylum chalybeum,* Antimycobacterial activity, Minimum Inhibition Concentration, Brine Shrimp Lethality Assay**.**

Introduction

The genus *Mycobacterium* (Mycobacteriaceae) is highly diverse with 85 species known in nature (Adewole et al., 2004). The common mycobacterial diseases are pulmonary infections, leprosy, buruli ulcers and tuberculosis. Tuberculosis (TB) is one of the most pervasive diseases caused by *Mycobacterium tuberculosis* (Mtb) and *Mycobacterium africanum* (Hugo et al., 2009). The current treatment of TB is facing a challenge of emerging multidrug resistant strains that refuse to respond to the available anti-TB drugs (Gemma et al., 2006). Therefore there is a need to search for novel compounds that can be used as effective lead compounds/extracts in the development of new anti-TB drugs. Medicinal plants are among the best sources of new chemical compounds that can facilitate discovery of new anti-TB chemotherapies. Examples of such medicinal plants are *Zanthoxylum chalybeum* and *Hallea rubrostipulata.*

Zanthoxylum chalybeum (Rutaceae) and *Hallea rubrostipulata* (Rubiaceae) are medicinal plants that are used in the treatment of various diseases such as headaches, diarrhea, wound healing and respiratory tract infections. In Uganda and Kenya these plant species are used for treatment of malaria, sickle cell diseases, measles, skin infections and severe coughs (Olila et al., 2002). The plant decoction of *Hallea rubrostipulata* is used for treatment of pre-hepatic jaundice, pregnancy related illness, back ache, salpingitis and diabetes (Ssegawa et al., 2007). In this study, the crude extracts from leaves and stem barks of *Hallea rubrostipulata* and stem barks of *Zanthoxylum chalubeum* were screened against for their antimycobacterial and cytotoxicity activity.

Material and Methods

Plant materials were collected from Karagwe District in Kagera Region, Tanzania. Identification was done at the site with the aid of the taxonomist. The sample specimens were deposited in the Herbarium of Institute of Traditional Medicine at Muhimbili University of Health and Allied Sciences. Plant materials were air dried and macerated into powdery form. Thereafter, powdered plant materials were extracted separately using dichloromethane (DCM), ethyl acetate (EtOAc) and finally with methanol (MeOH). Each extraction process took 24 hours before concentrating the extracts in *vacuo* using rotary evaporator.

Antimycobacterial Activity

The crude extracts were screened against two fast growing non-pathogenic *Mycobacterium* strains namely: *Mycobacterium madagascariense* and *Mycobacterium indicus pranii* as markers for detection of potential anti-TB extracts using two folds broth microdilution method. The medium used was middlebrook 7H9 broth base containing tween 80 (0.1%) and the turbidity was adjusted to 0.5 McFarland units (approximately $1.2x10^8$CFU/ml) where the minimum inhibitory concentrations were determined according to McGaw et al. (2008). Isoniazid and ciprofloxacin were used as positive controls.

Results and Discussion

The dichloromethane extracts of the leaves of *Hallea rubrostipulata* exhibited higher activity against test organisms namely *Mycobacterium madagascariense* and *Mycobacterium indicus pranii* with the MIC values of 0.156 mg/ml and 0.625 mg/ml respectively. In the same assay conditions, the methanol extracts exhibited moderate to higher activities with the MIC values of 1.25 mg/ml and 0.3125 mg/ml respectively (Table 1). Furthermore, the dichloromethane and methanol extracts from *Z. chalybeum* showed moderate activities with MIC values of 1.25 mg/ml against *Mycobacterium madagascariense* and 2.5 mg/ml against *Mycobacterium indicus pranii*.

Table 1: Antimycobacterial activity of the extracts of *Hallea rubrostipulata* and *Zanthoxylum chalybeum*

Extracts	Minimum Inhibitory Concentration (mg/ml)	
	M. madagascariense	*M. indicus pranii*
HRSD	1.25	2.5
HRSM	5	2.5
HRSE	1.25	1.25
HRLD	0.156	0.625
HRLM	1.25	0.3125
ZCSD	1.25	2.5
ZCSM	1.25	2.5
Isoniazid	NA	NA
Ciprofloxacin	<0.05	<0.05

The most active extracts from both plant species were selected for phytochemical analysis and anti-TB studies. The preliminary phytochemical analysis showed the two extracts, dichloromethane and methanol extract of the leaves of *Hallea rubrostipulata* to be rich in alkaloids. According to Okunande et al, (2004), the naturally occurring alkaloids possess antimycobacterial activity. This may therefore be responsible for the observed higher antimycobacterial activity of their respective extracts. Further preliminary phytochemical study on the stem barks of *Zanthoxylum chalybeum* showed the extract to also contain alkaloids. Previous reports by Adesina, (2005) and Okunande et al, (2004) indicated that carbazole alkaloids are present in *Z. chalybeum* and that they possess antimycobacterial activity against *Mycobacterium tuberculosis*. Therefore, the activity exhibited by

dichloromethane and methanol extracts of *Z. chalybeum* may partly be due to alkaloids present in the extracts. Further work to isolate and characterize the alkaloids and other natural products in the bioactive extracts of the screened plant species is in progress.

The extracts from the two plant species were subjected on the brine shrimp lethality assay to test their potential cytotoxicity effect. All extracts with the exception of the dichloromethane extracts of the stem bark of *Z. chalybeum* were less or not toxic against shrimps as compared to the standard anticancer agent cyclophosphamide (Table 2).

Table 2: Cytotoxicity activity (BST) of the extracts of *Hallea rubrostipulata* and *Zanthoxylum chalybeum*

Extracts	LC_{50} (µg/ml)	95% Confidence Interval (µg/ml)
HRSD	193.5	113 – 330.5
HRSM	71.5	52.3 – 97.8
HRSE	60.3	45.7 – 79.4
HRLD	33.1	23.1 – 47.4
HRLM	67.6	51.1 – 89.2
ZCSD	5.7	3.1 – 10.5
ZCSM	87.7	71.8 – 106.9
Cyclophosphamide*	16.3	10.6 – 25.2

Abbreviations

MM	*Mycobacterium madagascariense*
MIP	*Mycobacterium indicus pranii*
HRLD	*Hallea rubrostipulata* leaves, dichloromethane
HRLE	*Hallea rubrostipulata* leaves, ethyl acetate
HRLM	*Hallea rubrostipulata* leaves, methanol
HRSD	*Hallea rubrostipulata* stem bark, dichloromethane
HRSM	*Hallea rubrostipulata* stem barks, methanol
ZCSD	*Zanthoxylum chalybeum* stem barks, dichloromethane
ZCSM	*Zanthoxylum chalybeum* stem barks, methanol

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[YS 27] Anti-Sickling Triterpenoids from *Callistemon Viminalis, Melaleuca Bracteata* Var. Revolution Gold *Syzygium Guineense* and *Syzygium Cordatum*

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***Key words*:** Anti-sickling, biological activity, maslinic, betulinic and oleanolic acids.

Introduction

All over Africa, traditional healers use medicinal plants to prepare medicines to treat a wide range of illnesses. One of these illnesses is sickle cell anaemia or drepanocytosis or sicklemia. This disease is particularly common among Sub-Saharan Africans with a clear predominance in equatorial Africa. However, it also exists in North Africa, Greece, Turkey, Saudi Arabia and India (Fleming, 1989). An estimated 50 million people are affected worldwide (Buchanan *et al*., 2004; Girot, 2003). A literature review on sickle cell anaemia revealed that a number of plants have anti-drepanocytosis activity (Neuwinger, 2000; Elujoba *et al.,* 2005, Ekeke *et al.,* 1990, Mpiana *et al*., 2007; Mpiana *et al*., 2008). *Callistemon viminalis, Melaleuca bracteata var.* Revolution Gold, *Syzygium guineense* and *Syzygium cordatum* (from Democratic Republic of Congo and South Africa) were selected for investigation on the basis of their reported medicinal uses. The main aim of this study was to isolate and characterize anti-sickling (anti-drepanocytosis) compounds from the above mentioned medicinal plants.

Materials and Methods

Materials

The leaves of *Syzygium guineense* were collected in Kinshasa, Democratic Republic of Congo (DRC) and on the campus of the University of KwaZulu Natal in South Africa, while the leaves of *Syzygium cordatum, Callistemon viminalis* and *Melaleuca bracteata* were collected in Durban, South Africa.

The blood samples used to perform the antisickling activity of the crude extracts and the pure isolated compounds in this study were collected from a known drepanocitary center named "Centre de Médecine Mixte et d`Anémie SS" located in Kinshasa area, DRC. The blood samples were first characterized by Hb electrophoresis on cellulose acetate gel, in order to confirm their *SS* nature, as previously reported by (Mpiana, 2007). They were found to be *SS* blood and were then stored in a refrigerator. These SS blood samples were so used to perform biological tests.

Methods

The selected plants were subjected to phytochemical extraction methods and crude extracts were separated using chromatographic techniques. The structures of the isolated compounds were determined by spectroscopic techniques (1D and 2D NMR, FT-IR and MS). The powdered plant materials were sequentially extracted with n-hexane, dichloromethane, ethyl acetate, methanol, and aqueous methanol 80%. Each extraction was optimized by repeating the maceration twice. Each solvent extraction was concentrated under reduced pressure and allowed to dry at room temperature and weighed to give hexane-, dichloromethane-, ethyl acetate-, methanol- and aqueous methanol-solubles, respectively. These extracts were subjected to chromatographic techniques over silica gel 60 F_{254} aluminum barking from Merck, Germany for thin layer chromatography (TLC) and over silica gel 60 (0.040 – 0.63mm [230-400 mesh]) for column chromatography. Collected fractions were monitored on TLC plate and similar fractions were combined according to their TLC pattern.

The ^{1}H, ^{13}C and all 2D NMR spectra were recorded using a 400 MHz Bruker spectrometer at the University of KwaZulu Natal, Westville Campus. All the spectra were recorded at room temperature using deuteriochloroform ($CDCl_3$) or the mixture of $CDCl_3$ and deuteriomethyl sulfoxide (DMSO). All spectra were referenced according to the central line of deuteriorated chloroform at δ_H 7.24 for ^{1}H-NMR spectra and δ_C 77.20 for ^{13}C -NMR spectra.

The FT-IR spectra were recorded using; Perkin-Elmer Spectrum 100 FT-IR spectrometer. Samples were calibrated against an air background. Crystalline samples were directly placed on the window and then analyzed. KBr was used as salt.

The mass spectrometry of compounds was recorded using an Agilent 6890 series gas chromatography system. The compound was dissolved in HPLC grade methanol (1:100) and helium (at 0.8 ml/minute), the gas carrier was maintained at 200 °C for 4 minutes and then programmed to 300 °C at 5 °C/minute.

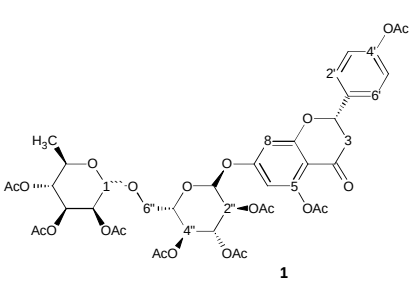
The mass spectrometry/liquid chromatography of compounds was recorded using an Agilent 1100 series LC/MSD Trap system. Compounds were dissolved in HPLC grade methanol and the following parameters were observed for the run: Injection: 6 µl; flow: 0.3 ml/min; time: 1.5 minutes; maximum pressure: 150 bar, minimum pressure: 0 bar, temperature 24.4 - 26.8 °C. Carrier solvents used were acetonitrile 95% and Millipore water 5%.

Results and Discussion

The methanol extract of the leaves of *S. guineense* of DRC gave the highest quantity of extract (25.65%), while ethyl acetate extract gave the lowest yield (0.59%) of crude extract. Dichloromethane extract of the leaves of *S. guineense* of South African origin gave the highest extract (1.05%), while ethyl acetate extract gave the lowest (0.08 %). Dichloromethane extract of the leaves of *C. viminalis* gave the highest extract (4.63%), while ethyl acetate extract gave the

lowest (2.75%) of crude extract. Dichloromethane extract of *M. bracteata* gave the highest extract (9.33%), while ethyl acetate extract gave the lowest yield (0.80%) of crude extract.

S. guineense from DRC yielded a major natural product which was a flavanone glycoside (**1**). *S. guineense* from South Africa afforded 4 compounds namely betulinic acid (**2**), sitosterol (**3**), friedelan-3-one (**4**) and a betulinic acid derivative. C*allistemon viminalis* afforded one compound, betulinic acid (**2**). *Melaleuca bracteata* afforded two compounds which were characterized as betulinic acid acetate (**5**) and ursolic acid acetate (**6**) and *Syzygium cordatum* afforded two compounds namely maslinic acid (**7**) and oleanolic acid (**8**).



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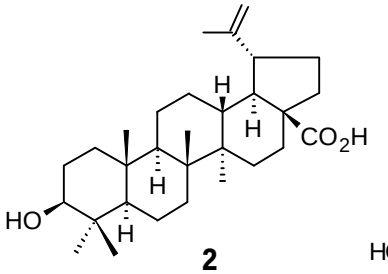

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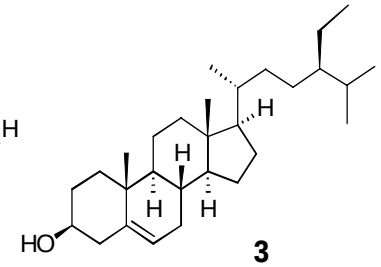

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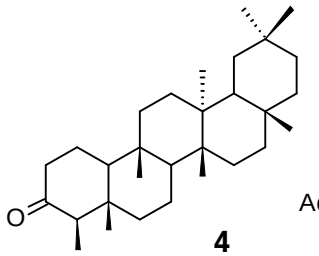

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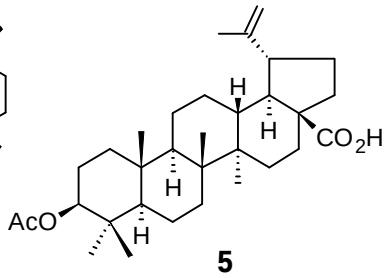

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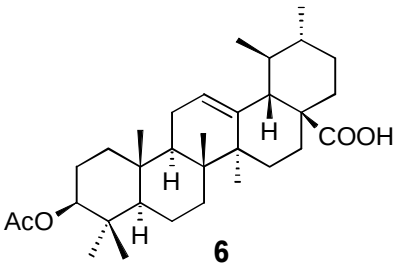

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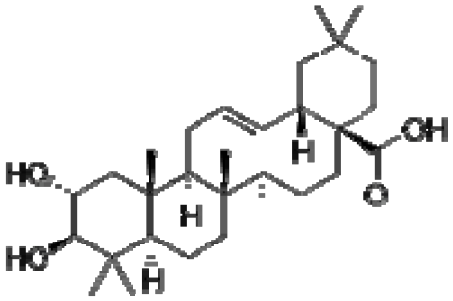

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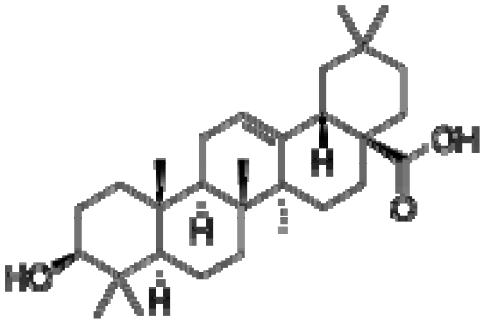

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The investigation of the anti-drepanocytosis activities of the extractives and their crude extracts showed *in vitro* effective antisickling activity. Ethyl acetate crude extracts of *Callistemon viminalis* and *Melaleuca bracteata;* hexane, dichloromethane and ethyl acetate crude extracts of *Syzygium guineense* of D R Congo, betulinic acid, betulinic acid acetate and maslinic acid showed a high antisickling activity, more than 70% of normalization. The fatty acid from *Melaleuca bracteata* was found to have an activity, between 50 and 70% of normalization and oleanolic acid showed the weakest activity, between 10 and 50 % of normalization. The maslinic acid and oleanolic acid used in this study were extracted from *Syzygium cordatum*.

However, some crude extracts and pure isolated compounds were found to have no antisickling activity. These were crude dichloromethane extract of C*allistemon viminalis*; crude dichloromethane, methanol and aqueous methanol (80%) extracts of *Melaleuca bracteata*; crude hexane, dichloromethane, ethyl acetate and methanol extracts of *Syzygium guineense* of South Africa; ursolic acid from *Melaleuca bracteata* and flavanone glycoside from *Syzygium guineense* of D R Congo.

According to these results, it can be seen that the activity of ethyl acetate extract from *Melaleuca braceta* can be attributed to the betulinic acid and its acetate. Betulinic acid and maslinic acid were found to have the highest activities. We wish to recommend further investigations of the hexane, dichloromethane, ethyl acetate, and methanol extracts of *S. guineense* from DRC, in order to identify the active principles. Furthermore, different derivatives of betulinic acid, maslinic acid and oleanolic acid should be synthesized, in order to compare their anti-sickling activities with the starting materials.

Acknowledgements

We express our deepest thanks and profound gratitude to Third World of Academy and Science (TWAS), for the 2008 Research and Advanced Training fellowship. We are also so thankful to the School of Chemistry of the Faculty of Science and Agriculture at the University of KwaZulu Natal for providing laboratory support for this study.

We are also grateful to the International Foundation for Science (IFS, Sweden) and to the Organization for the Prohibition of Chemical Weapons (OPCW) for the Research Grant F/4921-1 given to Mr Jean Paul NGBOLUA Koto-Te-Nyiwa.

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POSTER PRESENTATION

[PS 1] *In vitro* Inhibition of *Botrytis cinerea* - Causative Agent for Grey Mold by Crude Extracts of Basidiomycetes Fungi

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Key words: *Botrytis cinerea*, grey mold, basidiomycetes, submerged cultures, column chromatography.

Introduction

Botrytis cinerea the cause of grey mold is a well-known fungus with a wide host range that causes heavy economic losses of yield in more than 200 crop species including: onions, potato, strawberry, rose flowers, table grape and other ornamental plants (Guinebretiere et al., 2000). Current commercial synthetic fungicides used for its control such as 'Mancozeb' have been shown to be carcinogenic (Marta et al, 2011). In addition, there have been documented evidences on traces of these fungicide residues persisting in vegetable crops and soil (Apladasarlis et al., 1994). Resistance of *B. cinerea* isolates from vegetable crops towards the major classes commercial anti-botrytis fungicides: anilinopyrimidines, phenylpyrroles, hydroxyanilides, benzimidazoles and dicarboximides have also been recently confirmed (Myresiotis et al., 2007). It is imperative that alternative fungicides from naturally occurring compounds that are easily biodegradable and of low mammalian toxicity be explored for safe control of crop fungal pathogens since low mammalian toxicity, minimal environmental impact and novel modes of action are very important features of natural antifungal compounds.

The production of antimicrobial secondary metabolites has been reported in many fungal biocontrol agents (Turner, 2003); antibiosis mechanism best explains this phenomena, in which the antagonists produce a wide range of secondary metabolites such as antibiotics and toxins, which contribute to the antagonistic activity of fungal control agents against plant pathogens (Yazaki et al., 2008). Basidiomycetes fungi have been known to synthesize a vast array of secondary metabolites that possess beneficial biological activities, which can be exploited through research for crop protection purposes, in fact, current research on antifungal agents is based on the principle; that new generation fungicides should be practically non-toxic, except for the target organism (Komarek et al, 2009). Of relevance to this research, is the exploration of antagonistic strains belonging to the basidiomycete class of fungi, which are able to produce secondary metabolites that display antagonistic activity against *B. cinerea*. For this reason, armed with current methods in fungal biotechnology, there is high prospect of finding novel biologically active compounds that can be a potential fungicide for the control of grey mould disease.

Materials and Methods

All the glassware used in this work was standard quality and flasks as well as beakers were autoclaved before being used in the isolation of the test organism and cultivation of the submerged cultures. Crude extracts were then prepared using solvent extraction method using Acetone,

Methanol and Ethyl acetate, which were recovered by concentrating using the Rotary evaporator apparatus. Silica gel 60 (0.063 – 0.2 mm/70-230 mesh) was used as stationery phase for column chromatography. Thin layer chromatography (TLC) was done with Macherey–Nagel pre-coated silica gel 60 F254 plates (ALUGRAM® SIL G/UV254 0.25 mm, Duren, Germany). The developed TLC plate was viewed under dual fixed wavelength UV lamp (λ = 254 nm and 365 nm) and the spots visualized by spraying with freshly prepared *p*-anisaldehyde solution, then heated to 112°C. *In vitro* antifungal testing was done by impregnating filter paper disc (Rundfilter, ∅6 mm, Schleicher & Schuell) with known amounts of the crude extracts and enriched fractions. The glucose levels in the cultures was monitored using glucose testing strips (Diabur-test® 5000 (Roche). The media and flasks were initially heat sterilized using an autoclave for 15 minutes at a temperature of 115 °C and pressure of 1.5 bars. The inoculation and monitoring of growth parameters were done under a lamina flow hood backed with a hot flame produced by a Bunsen burner. Bruker ARX300 spectrometer will be used to perform NMR experiments upon successful isolation and purification.

Results and Discussion

From the initial screening crude extracts using agar diffusion assay, 22 out of 400 strains produced appreciable antifungal activities against *B. cinerea* (figure 1. below). The results significant since about 5% of the crude extracts screened showed significant activity against the *B. cinerea*, an accepted standard in microbial screening research (Rosa, 2003). The diameters of the inhibition zones were measured in millimetres and analyzed using SPSS 11.5 and all the 22 strains collectively had mean of 14.2mm, standard deviation of $\pm$1.8, the greatest inhibition zone being 17mm and the lowest being 11mm. The means were found to differ significantly at 95% confidence limit by running student t-test using the SPSS 11.5 software. The strain that gave the greatest inhibition zone was consequently selected for further cultivation.

Figure 1: Selected glass plates showing some of the bioactive basidiomycetes strains

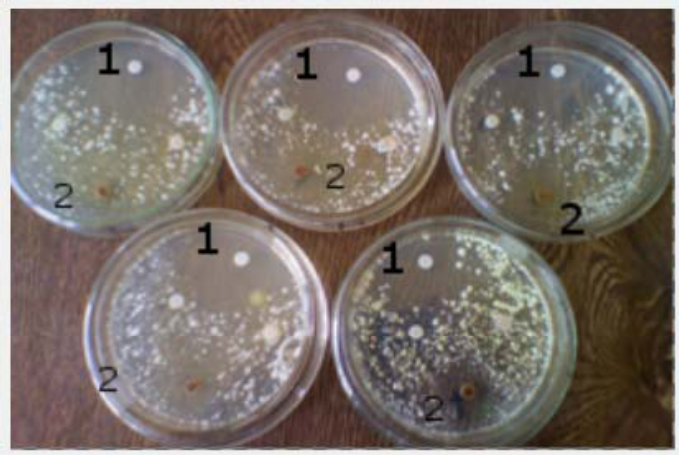

Figure 2: Comparison of "control" against selected fractions of an active crude extract

A particular fraction produced by the selected active strain (labelled 2) showed reproducible antifungal activity in 5 replicates as was tested against a 'control'- a commercial fungicide – Dithane® (labelled 1) as shown in figure 2 above. A close examination of the result above concludes that the selected strain at least produces a compound that has potent and reproducible antifungal activity that can be compared to that of the control. Column chromatography and TLC techniques are being applied in an attempt to purify the responsible compound(s) after which minimum inhibitory concentrations (MIC) tests will be carried out and followed by structure elucidation experiments.

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[PS 2] Biochemical Comparison of *Annona Squamosa* L. Leaves Growing In Different Eco-Zones In Tanzania For Mosquito Larvicidal Activity.

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Key words - *Anon squamosa* L., HPTLC analysis, HPLC analysis, mosquito larvicidal assay

Introduction

Annona squamosa L. (Annoneceae) is a medicinal plant used in treatment of different disorders such as constipation, fever, ulcer, cancer, and tumor (Saleem et al., 2009). This plant species is widely distributed in Tanzania mostly along the coastal area and Zanzibar (Nyambo et al., 2005). The leaves and root of *Annona squamosa* L have been reported to possess mosquito larvicidal activity against *Culex quinqefasciatus* (Magadula et al., 2009) and *Anopheles gambiae* (Kihampa et al., 2009) respectively. Therefore, the aim of this study was to investigate if geographical location had any effect on larvicidal activity of leaves of *A.squamosa* L.growing in different eco zones in Tanzania.

Material & Methods

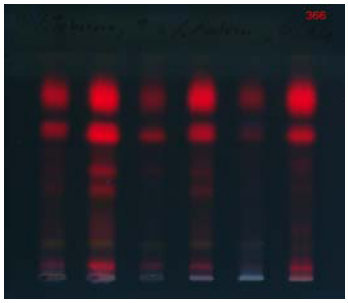
The leaves of *Annona squamosa* L. were collected from five different eco zones in Tanzania. Identification were done with the aid of the taxonomist at the site. The voucher specimens were kept in the herbarium of Institute of Traditional Medicine (ITM), Muhimbili University of Health and Allied Science (MUHAS), Tanzania. The plant materials were soaked in ethanol and the crude extracts were screened for mosquito larvicidal activity against *Culex quinqefasciatus* based on WHO protocol (1996 and 2003). Mortality was recorded after 24h of exposure. Furthermore each extract was then partitioned using DCM, EtoAc and BtOH before being subjected to HPLC for analysis.

Results

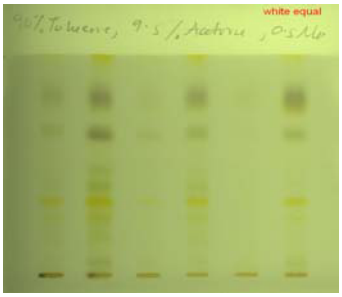
The extract from Mbeya region exhibited the highest larvicidal activity with LC_{50} value of 6.60 µg/ml and 5.20 µg/ml while the extracts from Morogoro had a lowest larvicidal activity with LC_{50} value of 416.02 µg/ml and 230.06 µg/ml after 24 and 48h of exposure. The trend of mortality increased with increase in time of exposure.

The HPTLC analysis of ethanol extracts indicated that this plant species contained compounds of different polarity but most of compounds from each extract observed to be similar with others based on their Rf values (fig. a & b).

Figure a& b: HPTLC chromatograms of crude ethanol extract of *Annona squamosa* L. from different eco zones in Tanzania viewed in (a) fluorencence (366nm) (b) at low wavelength of (254nm)



(**a**)



(**b**)

In the chromatogram (Fig.a & b) from left was extracts from Kilimanjaro, Dar es salaam, Mwanza, Morogoro(A), Morogoro (B) and Mbeya region respectively.

N.B: Mor(A)-The fresh leaves collected Morogoro region, Mor(B)-The dry leaves collected Morogoro region.

Phytochemical screening of the leaf extracts shown positive results for alkaloids from butanol fraction and terpenoids from ethylacetate fraction as indicated in (Table 1) below.

TABLE 1. Phytochemical screening of leaf extracts of *A.squamosa* L.

Class	Test used	Dichlormethane fraction	Ethylacetate fraction	Butanol fraction
Alkaloids	Dragendorff reagent	_	-	+
Flavanoids	Aluminium chloride	-	-	-
Terpenoids	Vannilin-sulphuric acid	-	+	-

+: presence **-:** absence

A comparative study of HPLC profiles showed that most of the compounds from different zones correlated in terms of peak number and retention time but differed in percentage area of the peak (fig. c)

Figure C. HPLC profile of ethylacceteate fraction.

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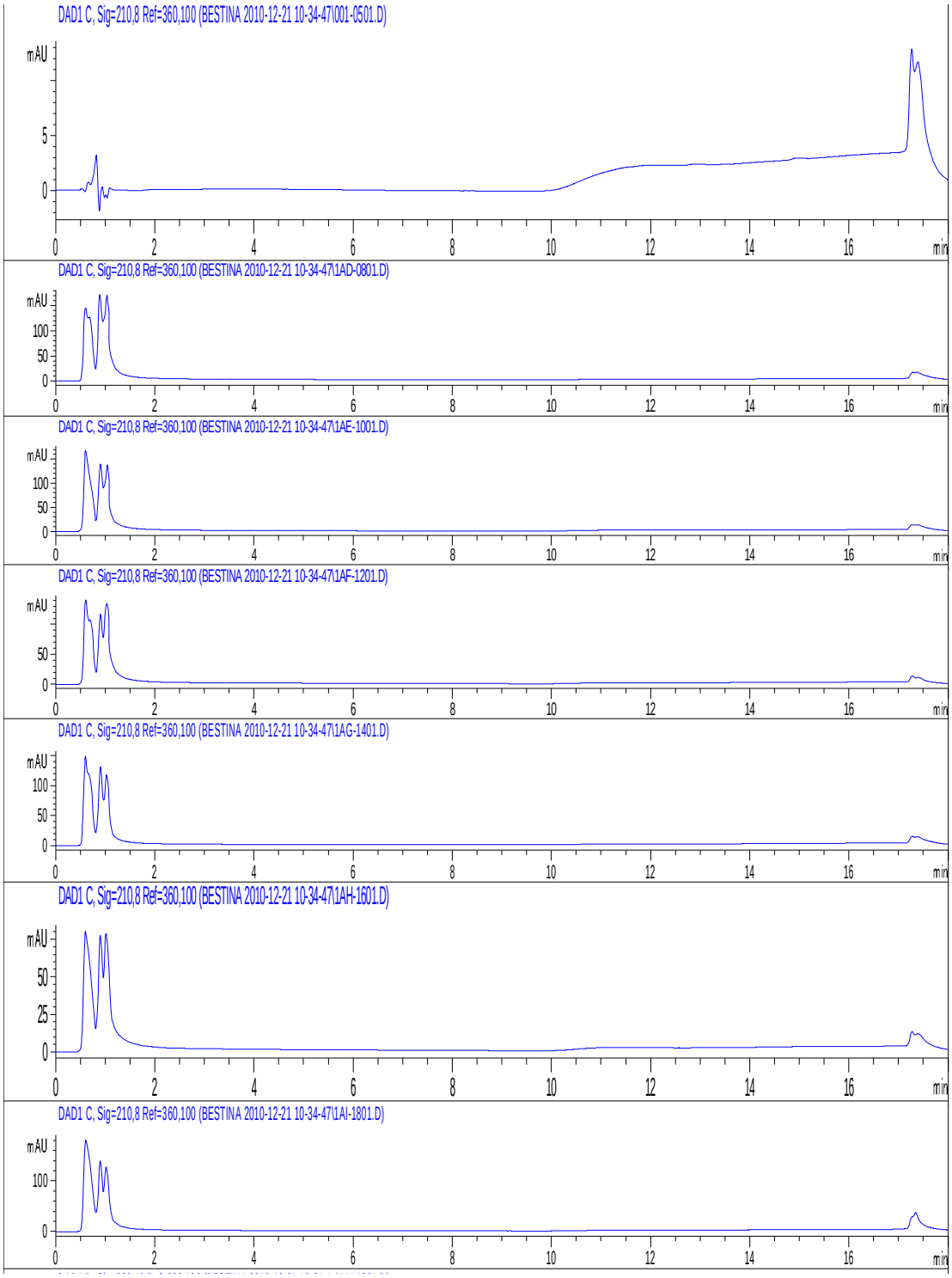

N.B;In the profiles, from top was blank, Kilimanjaro, Dar es salaam, Mwanza,Morogoro (A), Morogoro (B) and Mbeya extract.

Discussion & Conclusion

The experimental results supported that the larvicidal activity of *A.squamosa* leaves varied from one eco zone to another. Phytochemical screening indicated the presence of alkaloids and terpenoids in each extract which was in agreement with Magadula et al., (2009) and Kihampa et al., (2009). Generally, the activity showed by extracts can be further used for validation of method for development of active botanical formulation for mosquito larvae control.

Acknowledgement

I would like to acknowledge the MoHSW-Tanzania, DeIPHE –British council, NAM S &T CENTRE-India and ICCBS center- Karachi-Pakistan for their supports in funds and experimental facilities.

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[PS 3] A Toxicological Study of *Millettia usaramensis* Stem Bark Extract on *Aedes aegypti* (Mosquito), *Schistocerca gregaria* (Desert Locust) and *Mus musculus* (Mouse)

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Key words: *Millettia usaramensis*, *Aedes aegypti, Schistocerca gregaria, Mus musculus,* toxicological study.

Introduction

Some of the many insects that proliferate in tropical environments due to conduciveness of its weather conditions are crop pests both in the field and in storage. Others transmit diseases and affect the health of both man and livestock. Synthetic insecticides have been used to control them but these have shown pest resistance, they bio-accumulate and are non-biodegradable (Rembold, 1984). This has led to a search for alternative insecticides and botanical insecticides are a promising source.

The genus *Millettia* (Wight et Arn.) belongs to family Leguminosae (alternative name Fabaceae). The family has been reported to contain insecticidal rotenoids (Fukami and Nakajima, 1971). *Millettia usaramensis* stem bark extract is therefore a potential source of insecticidal rotenoids and it was interesting to investigate the activity of the extract on species of insects, and non-target organisms.

The action of several insecticides is influenced by environmental factors, temperature being one of them and is a critical factor in tropical areas (Narahashi and Chambers, 1989; Harris and Kinoshita, 1977; Kabaru, 1996; Mwangi *et al*., 1997). It was therefore worthwhile to investigate post-treatment effect of temperature on the insecticidal activity of *M. usaramensis* stem bark extract on the locust *Schistocerca gregaria*. Mice *Mus musculus* were used in toxicity testing as non-target organisms.

Materials and Methods

M. usaramensis (stem bark) used in this study was collected from Diani along the Kenyan coast in 2008. The sample was dried in the shade to constant weight and ground to fine powder in a mill, extracted using dichloromethane/ methanol at the ratio of 1: 1 (v/v) and stored desiccated at 4^{o}C. *Aedes aegypti* (L) colony, *Schistocerca gregaria* Forskal and male *Mus musculus* used in this study were obtained from the School of Biological Sciences, University of Nairobi.

A. aegypti larvae were exposed to 0-800mg/L *M. usaramensis* crude stem bark extract and larval mortality recorded after 24 h and 48 h exposure. *S. gregaria* 5th instar nymphs were exposed to 0-800mg/L *M. usaramensis* crude stem bark extract through injection (10µl/g), topical application (10µl/g) and oral administration (6ml per 200 seedlings) at 28^0C. Mortality was recorded after 24 h, 48 h, 72 h and 144 h post-exposure. The plant extract anti-feedant tests were conducted according to Butterworth and Morgan (1971) and Relative Anti-feedant Percentage (RAP) calculated. *A. aegypti* larvae were exposed to 0-100 mg/L of (+)-12a-epimilletosin, (+)-usararotenoid-A and deguelin isolated from *M. usaramensis* subspecies *usaramensis* by Yenesew (1997). After 24 h and 48 h exposure, larval mortality was recorded.

The toxicity of 10 µl/g injected *M. usaramensis* crude stem bark extract (0-1000 mg/L) to *S. gregaria* 5th instar nymphs was tested at 25^0C and 40^0C post-treatment temperatures. Mortality was observed and recorded after 24 and 48 hours. *M. musculus* were also exposed to *M. usaramensis* crude stem bark extract through intraperitoneal (50 µl of 0-1600 µg/g), oral (100 µl of 0-8000 mg/kg) and topical (200 µl of 0-2000 mg/g) administration. Observations for signs of toxicity were made after every 24 hours for 2 weeks.

Results and Discussion

Log probit analysis of the larvicidal activity of *M. usaramensis* crude stem bark extract on the 4th instar *A. aegypti* larvae showed a 48 hour activity with a median lethal dose of 50.82 mg/L. The crude extract administered to the locust *S. gregaria* elicited insecticidal activity of LD_{50} values of 445.65µg/g through injection at 48 hours, 569.77 µg/g through topical treatment at 72 hours and 504.69 µg/g through oral treatment at 144 hours post exposure. The difference in duration taken for insecticidal activity to be manifested with method of administration could be due to relative proximity to internal organs, vehicle-solvent penetration ability (Kabaru, 1996; Ware, 1982) and losses during administration. The crude extract also showed an anti-feedant activity of ED_{50} 660.71 µg/ml. The pure compounds (+)-12a- epimilletosin, (+)-usararotenoid-A and deguelin elicited LC_{50} activities of 2037 mg/L, 4.27 mg/L and 2.63 mg/L respectively at 48 hours post exposure. *M. usaramensis* crude stem bark extract is therefore larvicidal against *A. aegypti* and insecticidal as well as anti-feedant against *S. gregaria*. The moderate *A. aegypti* larvicidal activity and *S. gregaria* insecticidal activity observed in the crude stem bark extract can be attributed to (+)-usararotenoid-A, one of the major compounds in the extract.

The activity of rotenoids against insects is associated with their chemical structure. The main structural unit of all the rotenoids and associated compounds is a fused four-ring system- a chromanochromanone known as 6a, 12a-dihydrorotoxen-12 (6H)-one . The B/C ring junction in all of the active rotenoids is also cis. Compounds with modified rings and a trans- B/C ring junction are less insecticidal (Fukami and Nakajima, 1971; Joseph and Casida, 1992). (+)-12a-epimilletosin has the B/C ring junction with a trans-stereochemistry. This explains its low insecticidal activity. Despite (+)-usararotenoid-A also having a trans-B/C ring junction, its activity is relatively high. It could be an exception to the rule or its mechanism of action could be different (Yenesew, 1997). However, the

structure-activity relationship is not entirely clear as less structurally complex isoflavonoids have shown some activity against insects (Bowers, 1983).

The acute toxicity of *M. usaramensis* crude stem bark extract on *S. gregaria* has a positive temperature coefficient. Increase in temperature significantly increased toxicity of the extract in the locust *S. gregaria* by decreasing LD_{50} from 913.65 µg/g at 25^0C to 323.59 µg/g at 40^0C in a 48 hour post-treatment exposure period. This could be due to high temperature accelerating catabolic reactions or disrupting cell membrane integrity (Kabaru, 1996). This would be of great advantage as locust-prone areas are generally hot.

Neither mortality nor signs of toxicity were observed in 2 weeks in mice. This agrees with other studies (Brook and Price, 1961; Fukami and Nakajima, 1971) that rotenoids are non-toxic to mammals. This suggests that *M. usaramensis* stem bark extract is safe to mammalian non-target organisms.

Acknowledgement

We acknowledge the University of Nairobi for providing laboratory space to carry out investigations. We are grateful to Mr. Francis K. Kamau of the Animal House and Mr. Paul Ambundo of the Insectary, School of Biological Sciences, University of Nairobi, for rearing the test organisms used in this study.

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[PS 4] Bioactivity of 'Flemingin A' and other Natural Products from the Leaves of *Flemingia grahamiana*

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Key Words: *Flemingia grahamiana*; Leaves; Flemingin A; Emodin; antioxidant activity

Introduction

Flemingia grahamiana (Wight & Arn.) is an erect herb or sub-shrub up to 1.8 m tall with deep (sometimes tuberous) roots and 3-foliate alternate leaves. It is distributed in Tropical Africa and occurs in open and wooded savanna, sometimes near water in riverine vegetation, on hillside, termite mounds and along roadsides (Gillett, *et al.,* 1971; Jansen, 2005). The powder from the fruits and inflorescence of the plant is one of the principal sources of a dye and cosmetic called 'Waras (or Wurrus, or black kamala)' sold in India and Arabia (Cardillo, *et al.,* 1968; Jansen, 2005). The root decoction of the plant is used against diarrhoea and dysentery in Zimbabwe and Malawi. The plant is also used externally against skin diseases and internally as a purgative and against colds in India (Jansen, 2005).

In our search for cancer chemopreventive agents from plants, we wish to report the antioxidant properties of a known chalcone, Flemingin A (**1**) and the characterization of a new chalcone with a 3,4-disubstituted-1-methylcyclohexene moiety (**2**) from the leaves of *F. grahamiana.* Also reported, for the first time from the genus *Flemingia*, is the known anthraquinone, emodin (**3**).

Materials and Methods

The leaves of *Flemingia grahamiana* were collected from Kitale District, Western Province, Kenya, in October 2008. The plant was identified at the University Herbarium, Botany Department, University of Nairobi.

The air-dried leaves (413.2 g) of *F. grahamiana* were pulverized and extracted with CH_2Cl_2-MeOH (1:1) at room temperature to yield 29.6 g of crude extract. The extract was subjected to CC on silica gel, using gradient elution of EtOAc in n-hexane as the solvent. Further fractionation and purification was done by repeated chromatography on silica gel, PTLC, and sephadex LH-20. The structures of isolated compounds were elucidated based on a combination of spectroscopic techniques and by comparing with the data in the literature. Antioxidant property test was done as described by Ohnishi, *et al.* (1994).

Results and Discussion

Compound **1** was obtained as an orange powder with characteristic spectral features of a 2'-hydroxyl chalcone and it was identified as Flemingin A (**1**) (Cardillo, *et al.,* 1968, 1973). The compound exhibited strong *in vitro* antioxidant activity against DPPH with an EC_{50} value of 33.3 nM comparable to that of quercetin (21.5 nM, used as the standard), under the same experimental conditions.

Compound **2** was obtained as a yellow gum and exhibited spectral features typical of 2'-hydroxyl chalcone [δ_H 13.91, *s*, 1H (2'-OH); 8.14, *d*, *J*= 15.6Hz, 1H (H-β; 7.75, *d*, *J* = 15.6Hz 1H (H-α); δ_C 192.8 (C=O); 121.9 (C-α; 139.4 (C-β)] similar to those of compound **1**. Compound **2** differed from **1** by the presence of a 3,4-disubstituted-1-methylcyclohexene moiety instead of a 2-methyl-2-(4'-methylpet-3'-enyl) chromene unit. The presence, in compound **2**, of 3,4-disubstituted-1-methylcyclohexene was deduced from ^{1}H and ^{13}C NMR spectral data (Table 1): an allylic correlation observed between H-10'' and H-2'' in the ^{1}H-^{1}H COSY experiment and a long range (HMBC) interaction between H-10'' and C-4''. The closeness of R_f values, on TLC plate, for the two compounds implied that the number of hydroxyl groups is the same (three) rather than five (with extra OH groups at 4' and 7''). Therefore, it was concluded that the 3,4-disubstituted-1-methylcyclohexene, in this case, is fused with a pyrano ring as shown in structure **2** and therefore characterized as: (2*E*)-1-(6a,7,8,10a-tetrahydro-1,4-dihydroxy-6,6,9-trimethyl-6*H*-benzo[*c*]chromen-2-yl)-3-(2-hydroxyphenyl)prop-2-en-1-one. The relative stereochemistry of the pyrano-cyclohexene junction could not be established because the peaks (in ^{1}H NMR spectrum) for H-1'' and H-6'' appeared as multiplets and due to lack of NOE data. The cyclohexene moiety in **2** must have resulted, biogenetically, from the cyclisation of the geranyl group which is common in the chalcones of *Flemingia* species' (Cardillo*, et al.,* 1968, 1973). Compounds of similar moieties have been reported by Simon-Levert, *et al.* (2005), Garrido, *et al.* (2002) [meroterpenoids]; Botta, *et al.* (2003) [isoflavanones with cannabinoid-like moieties] but not from the genus *Flemingia*. Compounds **2** and **3** were not tested for antioxidant properties due to paucity of the samples.

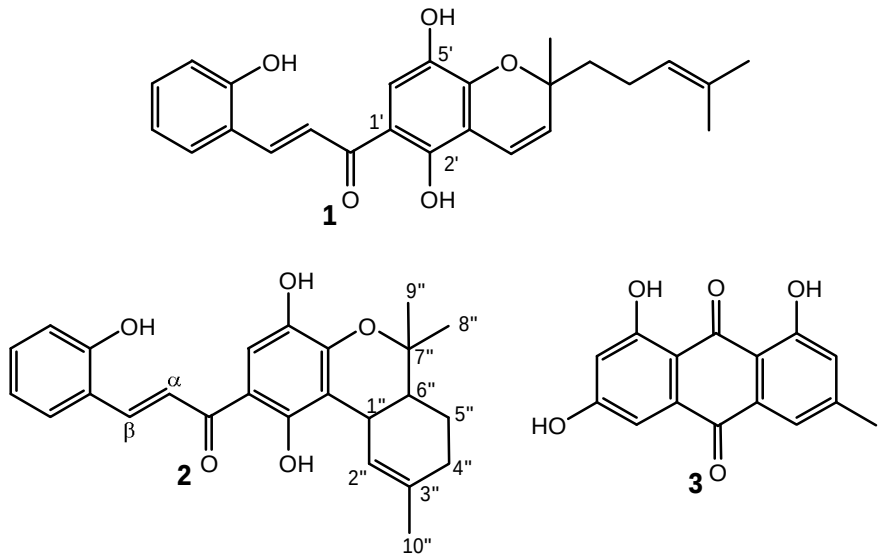

Table 1. ^{1}H (300 MHz) & ^{13}C (75 MHz) NMR Data for the 3,4-disubstituted-1-methylcyclohexene moiety in Compound **2** ($CDCl_3$)

Position	**^{1}H** (*J* = Hz)	**^{13}C**	**^{1}H-^{1}H COSY**
1"	3.65, *m*	31.5	2", 6"
2"	6.40, *d* (3.6)	121.4	6", 10"
3"	-	134.4	-
4"	2.07, *m*	29.5	-
5" ax	1.28, *m*	20.8	-
eq	2.07, *m*		8"/9"
6"	1.88 m	39.9	1", 8"/9"
7"	-	79.7	-
8"	1.51 s	25.5	6"
9"	1.37 s	25.6	6"
10"	1.72 s	23.6	2" (allylic)

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[PS 5] Seasonal Variation in the Chemical composition of the Bark *Ocotea comoriensis* Essential Oils

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Key Words: *Ocotea comoriensis*; bark; essential oil; chemical composition; seasonal variation; Comoros archipelago

Introduction

Ocotea comoriensis Kostermans (Lauraceae) is a tall beautiful and evergreen tree, 10–15 m tall, with a trunk 30-50 cm in diameter, endemic in the Comoros Islands (Kostermans, 1950). Localy, this plant is known under the names "Mnaliwa," "Ganja Mrihali" and "Mkanfure". The Ocotea comoriensis wood is particularly valued for woodworking (Said Hassani et al, 1995).

Our work reports the results of the seasonal variation (fruition period and vegetative rest) in the chemical composition of the bark *Ocotea comoriensis* essential oils.

Material and Methods

Plant Material and Extraction

Essential oils of Ocotea comoriensis were extracted by hydro distillation of the dry material of the bark collected in different periods (fruition and dormancy periods), using a Clevenger type arrangement with a 2 L flask. After 5 hours of extraction, the essential oils were recovered by decantation and dried over magnesium sulfate.

Chemical Analysis

Fruition period

GC analyses were performed on a fused silica capillary column SPB-5 (60 m x 0.32 mm, inside diameter, film thickness 0.25 m); the oven temperature was programmed from 60 °C to 200 °C at 4 °C/min and to 230°C (60 min). Nitrogen was used as the carrier gas at a flow rate of 0.7 ml/min. Injector temperature, 250°C; splitless mode; FID temperature, 300°C.

GC-MS analyses were conducted using a Hewlett-Packard chromatograph, Type 6890 and 6890N series, coupled to an HP 5972 and HP 5973N mass selective detector. The MS detector was used in the EI mode with an ionization voltage of 70 eV. Two capillary columns were used under the following conditions: (a) Supelcowax™ 10 (60 m x 0.32 mm i.d., film thickness 0.25 μm); the oven temperature programme, 50°C rising at 4°C/min to 230 °C, held for 30 min; ion source temperature, 280°C; injector temperature, 250°C; carrier gas, helium; flow rate, 0.8 ml/min. (b) SBP-5 (60 m x

0.32 mm i.d., film thickness 0.25 μm); the oven temperature programme, 60°C rising at 4°C/min to 230°C, held for 60 min; ion source temperature, 280°C; injector temperature, 250°C: carrier gas, helium; flow rate, 0.7 ml/min.

a- Dormancy period

GC analyses were performed on a fused silica capillary column (25 m x 0.32 mm, coated with OV-101); the oven temperature was programmed from 50 °C to 200 °C at 5 °C/min. GC–MS analyses were carried out on a Hewlett-Packard capillary GC-quadrupole MS system (Model 5970), operating at 70 eV and fitted with a 25 m x 0.23 μm i.d. fused-silica column with DB-5. The temperature was programmed as follows: 50 °C (3 min), 50 - 200°C at 3°C/min. Helium was used as the carrier gas at a flow rate of 0.9 ml/min.

Identification and Quantification

Retention indices of all the constituents were determined by the Kovats method; the oils were spiked on both phases with a standard mixture of n-alkanes series (C_8-C_{22}) and analyzed by GC-MS under the previous conditions. Constituents of the volatile oil were identified by comparison of their retention indices and their mass spectral fragmentation patterns with those reported in the literature (Adams, 200l and Stenhagen et al., 1974) and those stored on MS Library (NBS75K).

Results and Discussion

The essential oil yields for fruition and dormancy periods were 0.20% and 0.35%, respectively.The bark essential oil collected during fruiting contains monoterpenes (0.4%), sesquiterpenes (84.7%) and aromatic compounds (5.8%). This oil is dominated by caryophyllene oxide (11.3%), α-ylangene (8.2%), epi- α-cadinol (6.1%), γ-muurolene and α-muurolène (5.1% and 5.0%, respectively), α-amorphene (4.4%), δ-cadinene (3.3%), α-copaene (3.1%), γ-cadinene (2.8%) and β-selinene (2.4%) (Said Hassani M., 2010).

The bark essential oil collected in dormancy period exclusively consists of monoterpenes and sesquiterpenes. In fact, it includes: 67.9% monoterpenes and 27.4% sesquiterpenes. Its high content of monoterpenes is mainly owed to high percentages of camphene (18.1%), bornyl acetate (13.8%), α-pinene (13.7%), β-pinene (8.4%) and limonene (5.6%). Among the identified sesquiterpenes, there are α-cubebene (4.5%) and α-cadinol (3.0%) (Menut et al., 2002).

The essential oil yield appears somewhat more important in vegetative rest period (0.35%) than in the fruiting period (0.20%).

For the bark essential oil studied at two periods of the vegetative cycle (fruiting and vegetative rest periods) for a same specimen of *Ocotea comoriensis*, significant differences in chemical composition can be identified. Whereas in fruiting period, essential oil is largely dominated by sesquiterpenes (84.7%), in vegetative rest period, its proportion in monoterpenes becomes definitely more important, passing from 0.4% to 67.9%. The conducted study thus highlights a variation of the chemical composition of the bark essential oil according to the vegetative cycle.

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[PS 6] Proximate and Amino Acid Composition of Cowpea (*Vigna ungiculata* L.walp) Flour and Protein Isolates

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Key Words: Cowpea, Protein isolates, Amino acid Composition

Introduction

The cowpea (*Vigna ungiculata* L.walp) is a grain legumes believed to have originated in Africa and Asia (Taiwo, 1998), and is widely cultivated in the tropics (Chavan *et al.,* 1989). As a legume, cowpeas are rich and low cost sources of proteins and nutrients (Egounlety and Aworth, 2003) and they form part of staple diet in most African and Asian countries (Aykroyd and Doughty, 1964).

Material and Methods

Proteins were isolated from dehulled cowpea flour by isoelectric and micellization precipitation techniques. Cowpea protein isolate- A (CPIA) was prepared from cowpea seed flour following the method described by Fernandez-quintela *et al.,* (199**7**). Protein isolate-B (CPIB) was prepared using micella method as described by Lampart-Szczapa, (1996). Amino acids analysis was performed on (DDCF) and protein isolates (CPIA and CPIB) using amino acid analyzer according to the method described by Moore, *et al.,* (1958), using the FAO/WHO (1973) reference pattern.

Results and Discussion

Chemical composition of dehulled defatted cowpea flour (DDCF) and protein isolates (CPIA and CPIB) are presented in Table 1. The CPIB showed significantly ($P<0.05$) higher protein than the CPIA but the fat, ash and crude fibre contents of both isolates were similar. Amino acid composition of the DDCF and protein isolates CPIA and CPIB are reported as g/16g N comparing to the FAO/WHO (1973) (Table 2). The protein isolates are rich in leucine the values were 8.8 and 8.9 g/16g N for CPIA and CPIB respectively. This result was similar to that reported by Ferndez-Quaintela *et al.,* (1997) who reported 8.0 and 8.4 g/16g N for pea and faba bean proteins isolate. Methionine was the most concentrated essential amino acids in both protein isolates (CPIA and CPIB). The protein isolates showed, in general higher total essential and nonessential amino acid levels than their own original seeds (Table 3). Essential amino acids of CPIA and CPIB were 22.99 and 15.78 g/16 g N respectively, higher than FAO/WHO reference. The first limiting amino acid was cystine for DDCF and threonine for CPIA and CPIB, while lysine is the most abundant essential amino acid in DDCF (4.28 g/16g N).

Table: 1 Proximate composition of whole cowpea flour (WCF), dehulled defatted cowpea flour (DDCF) and protein isolates (CPIA) and (CPIB) % dry basis

Chemical constituents	WCF	DDCF	CPIA	CPIB	LSD
Crude protein (N x 6.25)	$22.30^{d}\pm0.20$	$26.73^{c}\pm0.06$	$75.0^{b}\pm0.06$	$76.0^{a}\pm0.12$	0.26
Crude fat	$2.10^{a}\pm0.10$	$2.30^{a}\pm0.10$	Traces	Traces	0.43
Crude fibre	$4.10^{a}\pm0.20$	$1.02^{b}\pm0.08$	Traces	Traces	0.35
Total ash	$3.77^{a}\pm0.06$	$3.87^{a}\pm0.06$	$2.63^{b}\pm0.15$	$2.3^{b}\pm0.20$	0.55
Carbohydrate (by difference)	$60.07^{a}\pm0.06$	$59.78^{a}\pm0.28$	$13.0^{b}\pm0.17$	$13.1^{b}\pm0.0$	0.43

Means in the same raw with different letters are significantly different ($P < 0.05$).

Means ± standard deviation of triplicate analysis.

LSD = Least significant differences.

Table 2: Amino acid composition of dehulled defatted cowpea flour (DDCF) and cowpea protein isolates (CPIA and CPIB)

Amino acid	DDCF	CPIA	CPIB	FAO/WHO (1973) (g/16g nitrogen)
Isolucine	**0.98**	**7.92**	**8.20**	**4.0**
Leucine	**1.58**	**8.81**	**8.85**	**7.0**
Lysine	**4.28**	**22.99**	**15.78**	**5.50**
Cystine	**0.032**	**0.06**	**-**	**3.5**
Methionine	**-**	**27.22**	**30.60**	**3.5**
Tyrosine	**3.33**	**16.31**	**19.83**	**6.0**
Phenylalanine	**2.0**	**12.37**	**11.96**	**6.0**
Threonine	**0.44**	**7.18**	**4.18**	**4.0**
Tryptophan	**ND**	**ND**	**ND**	**1.0**
Valine	**0.72**	**ND**	**6.61**	**5.0**
Histidine	**0.77**	**7.88**	**9.07**	**-**
Argnine	**2.66**	**17.09**	**19.26**	**-**
Aspartic	**ND**	**ND**	**ND**	**-**
Glutamic acid	**3.32**	**21.49**	**39.69**	**-**
Serine	**0.89**	**8.09**	**11.19**	**-**
Proline	**7.71**	**23.14**	**24.33**	**-**
Glysine	**0.68**	**3.32**	**3.97**	**-**
Alanine	**0.89**	**2.74**	**3.0**	**-**

CPIA= Cowpea protein isolate by isoelectric point precipitation

CPIB = Cowpea protein isolate by micellization precipitation

Table 3: Classification of amino acids (g/ 16 g) of dehulled defatted cowpea flour (DDCF) and protein isolates (CPIA) and (CPIB).

Amino acid description	DDCF	CPIA	CPIB
Total amino acids (TAA)	30.28	192.21	216.52
Total essential amino acids (TAA) with histidine	14.13	156.34	115.08
Total essential amino acids (TAA) with out histidine	13.36	108.46	106.01
Total non essential amino acids (TNAA)	16.92	83.75	110.51
Essential aromatic amino acid (EArAA)	5.33	28.68	31.79
Total acid amino acid (TAAA)	3.32	21.49	39.69
Total basic amino acid (TBAA)	8.79	23.98	22.05
Total sulphur amino acid (TSAA)	0.032	27.28	30.60

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[PS 7] Molecular Species Identification and the Respective Quantification of Dioscin: A Case of *Dioscorea spp*

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Key Words: *Dioscorea,* PCR, Species, dioscin, HPLC

Introduction

Dioscorea (Yams) in Kenya is a neglected crop, despite having a lot of potential as food source and as well playing a role in pharmaceutics (Mwirigi *et al,* 2009). Pharmacologically, *Dioscorea* species is known to have health promoting molecules among them the steroidal Saponin called dioscin. On acid hydrolysis of dioscin, diosgenin is derived. Diosgenin is used in the production of steroidal hormones like progesterone (Sautour et al., 2004). Apart from being used in steroidal hormones production, dioscin has other health promoting effects such as antioxidant effects and reduction of postmenopausal symptoms in women (Wang *et al*., 2002). It is therefore essential to quantify the amounts of dioscin in *Dioscorea* species in Kenya. However, for dioscin quantification to be possible it was imperative to identify the taxonomic position of these yams. The taxonomic position of cultivated yams in Kenya has been a subject of speculation over the years (Mwirigi *et al*, 2010). This project aimed at establishing the taxonomic position of Kenyan yams in relation to gene bank species and West African yams using a molecular approach and the respective identification and quantification of dioscin from the tubers.

Materials and methods

This study used three universal molecular markers (matK, rbcL, trnL_F) to investigate the species position of cultivated Kenyan yams and identify their relatedness to major African species and to species in the gene bank. The study also used reverse phase High performance Liquid Chromatography (RP-HPLC) to identify and quantify dioscin content in Kenyan yams. DNA was extracted from lyophilized tubers and leaf samples and Polymerase chain reaction (PCR) carried out on the DNA samples with direct sequencing of the PCR products. DNA sequences were assembled with sequencher® and then a multiple sequence alignment through clustalW in MEGA 5 from which phylogenetic trees were drawn. Dioscin was extracted from 5g of freeze dried tubers for consequent identification and quantification. Identification and quantification of dioscin was carried out using a Reverse Phase High Pressure Liquid Chromatography (RP-HPLC).

Results and discussion

Results indicated that there is more to the species taxonomy in Kenyan Yams than currently known. Currently yams are said to be of the species *D. minutiflora*, *D. dumetorum* and *D. bulbifera*; however from our study, the species *D. cayanensis*, *D. alata*, *D. rotundata*, *D. mangenotitana*, *D. schimperiana* and *D. bulbifera* were also shown to be growing in Kenya. HPLC analysis indicated the

presence of dioscin in Kenyan yam samples albeit at low quantities. The quantities of dioscin were variable with the least being 0.884 Parts per billion (ppb) and the highest 5.12 ppb). The dioscin quantities were not species specific and a high dioscin variability was also noted in samples identified to be of the same species. Further sampling of Kenyan *Dioscorea* samples both wild and cultivated is essential to enhance detailed dioscin identification and quantification and as well check for more species. A tool that is robust enough to identify intra – specific variability in *Dioscorea* species is also suggested for future comparative studies.

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[PS 8] Cuauthemone Sesquiterpenes from *Laggera Tomentosa* Endemic to Ethiopia

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KEY WORDS: *Laggera tomentosa*, Asteraceae, Cuauthemone sesquiterpenes, 3-*O*-(3'-acetoxy-2'-hydroxy-2'-methylbutyryl)cuauthemone

Introduction

Laggera tomentosa (Sch. Bip. ex A. Rich) Oliv & Hiern (Asteraceae) is a perennial fragment bushy herb (0.5-1.2 m high) endemic to Ethiopia. Traditionally, the juice of the crushed leaves is ingested as a treatment for stomachache, and is used against migraine. It is also used as a fumigant and for cleansing milk containers (Mesfin, 2004). Phytochemical studies on the essential oil of *L. tomentosa* have been reported before (Asfaw et al, 1999, 2003). However, there are no reports on the chemical investigation of the solvent extract of this species prior to this work.

MATERIAL AND METHODS

Plant material

Laggera tomentosa was collected from Daletti, Western Shoa of Ethiopia in November 2005. A voucher specimen (SD 6487) is deposited at the National Herbarium (ETH), Department of Biology, Addis Ababa University.

Methods

The dried and milled aerial parts of *L. tomentosa* were extracted by maceration with petroleum ether (54-93 °C) at room temperature for 24 hours, and then evaporated *in vacuo*. The residue was then soaked with ethanol twice at room temperature for up to 24 hours each and then evaporated *in vacuo*. The dried petroleum ether and ethanol extract were fractionated on column chromatography using pet. ether and ethylacetate as solvent system and the fractions monitored with TLC. The structures of the pure compounds were elucidated by spectroscopic techniques including 1D and 2D NMR techniques as well as by chemical methods.

Results and Discussion

The three cuauthemone sesquiterpenes, 3-(3'-acetoxy-2'-hydroxy-2'-methylbutyryl)- cuauthemone (**1**), 4-O-acetylcuauthemone-3-O-angelate (**2**), 4-O-acetylcuauthemone-3-O-(2'-hydroxy-2'-methyl-3'- acetoxybutyrate) (**3**) are reported for the first time from *L. tomentosa*. The compounds, 4-*O*-acetylcuauthemone-3-*O*-angelate (**2**) (Guilhon and Muller, 1996), 4-*O*-acetylcuauthemone 3-*O*-(2'-hydroxy-2'-methyl-3'-acetoxybutyrate) (**3**) (Bohlmann et ak, 1985) were identified by comparison of their spectroscopic data with reported values in the literature. The structure of compound **1** was fully characterized in this work based on 1D and 2DNMR data including DEPT-135, COSY, HSQC, and

HMBC. Dominguez *et al,* 1988 reported similar structure as **1** for a compound isolated from *Pluchea purpurescens* and the structure was elucidated from MS, IR and 1H NMR data. The MS and IR data reported are similar with those of compound **1** isolated in this study. However, the 1HNMR assignment by Dominguez *et al.* is different from some of the protons reported by us. This prompts us to do comprehensive NMR analysis of compound **1** (Table 1).

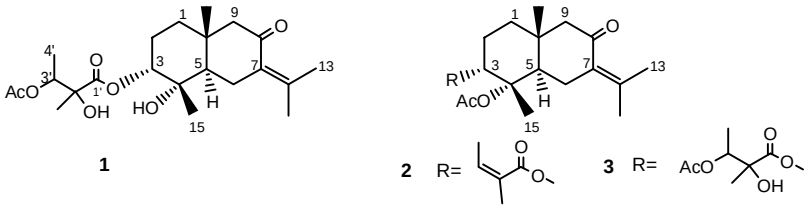

Figure 1: Cuauthemone sesquiterpenes isolated from *L. tomentosa*

Table 1. ^{1}H, ^{13}C, and HMBC (H→C) spectral data of compound **1**[a] (in $CDCl_3$).

no.	δ_C (ppm)	δ_H(ppm)	HMBC	δ_H (ppm) Dominguez et al
1	33.4	1.45 (*m*), 1.28 (*m*)	$H^{1a}\rightarrow C^{10}$, $H^{1a}\rightarrow C^{14}$ $H^{1b}\rightarrow C^{10}$, $H^{1b}\rightarrow C^{14}$	no value
2	23.9	1.79 (*m*), 1.75 (*m*)	$H^{2}\rightarrow C^{10}$	no value
3	78.9	4.89 (*br t*)	$H^{3}\rightarrow C^{1}$, C^{4}, C^{5}, C^{15}, $C^{1'}$	4.91(ht, J = 3 Hz)
4	72.2	-		-
5	46.6	1.92 (*dd*, J = 4, 8 Hz)	$H^{5}\rightarrow C^{6}$, C^{7}, C^{9}, C^{14}, C^{15}	no value
6	25.5	2.91 (*dd*, J = 4, 16 Hz), 2.17 (*dd*, J = 12, 16 Hz)	$H^{6}\rightarrow C^{5}$, C^{7}, C^{8}, C^{11}	2.94 (ddbr, J = 4, 15 Hz), 2.09 (ddbr, J = 13, 15 Hz)
7	130.5	-		-
8	202.1	-		-
9	59.7	2.22 (*s*)	$H^{9}\rightarrow C^{1}$, C^{5}, C^{7}, C^{8}, C^{14}	1.93(dd, J = 13,5)
10	35.8	-		-
11	145.9	-		-
12	23.6	2.03 (*s*)	$H^{12}\rightarrow C^{7}$, C^{8}, C^{11}, C^{13}	2.23 (brs)
13	22.9	1.82 (*s*)	$H^{13}\rightarrow C^{7}$, C^{8}, C^{12}	1.83 (brs)
14	18.7	0.94 (*s*)	$H^{14}\rightarrow C^{1}$, C^{5}, C^{9}, C^{10}	0.93 (s)
15	21.5	1.26 (*s*)	$H^{15}\rightarrow C^{5}$	1.27 (s)
1′	174.7	-		-
2′	76.4	-		-
3′	74.4	5.12 (*q*, J = 4 Hz)	$H^{3'}\rightarrow C^{1'}$, $C^{4'}$, $C^{5'}$, $\underline{C}OCH_3$	5.13(q, J = 6.5 Hz)
4′	13.3	1.28 (*d*, J = 4 Hz)		1.30(d, J = 6.5 Hz)
5′	22.4	1.40 (*s*)	$H^{5'}\rightarrow C^{1'}$, $C^{3'}$	1.42(s)
- OAc	169.8 21.0	- 1.98 (*s*)	$COC\underline{H}_3\rightarrow C^{3'}$, $\underline{C}OCH_3$	- 1.99(s)

[a] 400 and 100 MHz, respectively.

Among the 20 *Laggera* species, *L. pterodonta*, *L.alata*, *L. crispata* and *L. decurrens* have been extensively investigated and 51 eudesmanes sesquiterpenes and five flavonoids have been reported from these species (Li et al, 2007). The cuauthemone eudesmanes isolated from *L.tomentosa* have not been reported from the above mentioned species. The cuauthemone eudesmanes are rather characteristic of the genus *Pluchea* (Mukhopadhyay et al, 1983), which belong to the same tribe, *Plucheeae*, as the genus *Laggera.* This study indicated that *L. tomentosa* is chemically related to some *Pluchea* species, due to the co-occurrence of cuauthemone

sesquiterpenes, rather than to the *Laggera* species studied so far. Further studies need to be carried out to establish the chemotaxonomic relationship of the species within the genus *Laggera*, and between *L.tomentosa* and *Pluchea* species.

Acknowledgements

Financial support from NAPRECA and Addis Ababa University for the participation of 14th NAPRECA symposium is gratefully acknowledged. We thank ALNAP for the provision of NMR spectroscopic equipment used in this investigation. Prof. Wendemagegn Mamo is gratefully acknowledged for his constructive suggestions in the interpretation of the 2D-NMR spectra. We are thankful to Dr. Haregewine Taddese and School of Chemistry, University of Nottingham for recording HRMS spectrum. NA acknowledges partial support from the ChemRAWN XIV International Green Chemistry Grants Program, USA. Prof. Sebsebe Demissew is gratefully acknowledged for the collection and authentication of the plant material.

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[PS 9] Novel Limonoids and Flavonoid from the Kenyan *Vepris uguenensis* Engl. and their Antioxidant Potential

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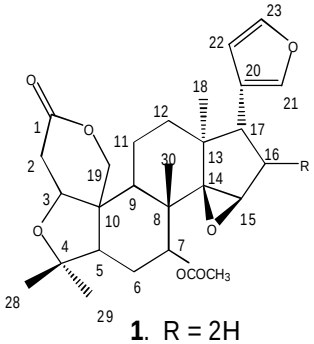
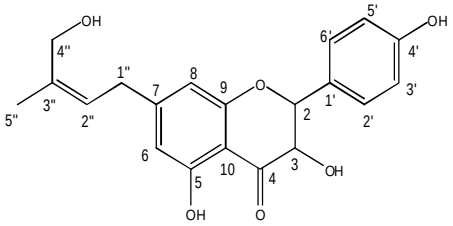
A phytochemical investigation of *Vepris uguenensis* (Rutaceae) has led to the isolation of two new A, D-*seco*-limonoids that were accorded the trivial names, uguenensene (**1**) and uguenensone (**2**) and a new C-7 prenylated flavanoid, uguenenprenol (**3**). In addition, known compounds, kihadalactone A, tricoccin S_{13} acetate, limonyl acetate, methyl uguenenoate, niloticin, chisocheton A, skimianine, flindersiamine, 8α,11-elemodiol, 7-O-methylaromadenrin, and lupeol were also isolated. The structures of the new compounds were elucidated and characterized by both 1D and 2D NMR and mass spectroscopy. The crude extracts were obtained by sequential extraction using a soxhlet apparatus and compounds were isolated and purified by repeated column chromatography. Antioxidant activity of the isolated compounds using the DPPH, Deoxyribose and Ferric Reducing Power assays showed that uguenenprenol (**3**) and 7-O-methylaromadenrin are good antioxidant agents. The isolated Limonoids displayed an interesting biogenetic relationship that might be expected for limonin biosynthesis. The current contribution adds uguenensene (**1**) and uguenensone (**2**) to the class of citrus limonoids common to Rutaceae [1].



1. R = 2H
2. R = O



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[PS 10] Need to Regulate Herbal Remedies in Kenya

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Introduction

Traditional plant based medicines play an important role in African society; supplying accessible medicines, sustaining cultures and providing income earning and enterprise development opportunities. For Kenya, the new constitution, now, recognizes herbal Practitioners and reads: "Every person has a right to health, which includes the right to Health care services, whether allopathic or complementary and alternative medicine including reproductive health care. According to WHO report estimates show that at least 80% of Kenyans have used herbal medicines at least once.

Though herbal medicines are vital in improving the health of Kenyans there is urgent need to formulate safety and governance regulations.

Herbal medicines are not regulated in Kenya. In Kenya, the only instrument available for protecting traditional knowledge is the trade secret. The Industrial Property Act Cap 509 of the Laws of Kenya, which could protect the intellectual integrity of traditional practitioners, disqualifies traditional knowledge. Medicinal plants are therefore collected and used without any regulation, opening them to indiscriminate exploitation and bio-piracy. Huge profits are made but never shared with the custodians of biological diversity.

Observations

In Kenya there is no registration system for herbal medicines and they are not included on the essential the essential drug list. These traditional medicines are sold without restriction. Due to lack of these guidelines, it has been discovered that the herbal remedies sector in Kenya is not harmony.

It was discovered that some herbalists were mixing concoctions of conventional medicines and passing them as herbal medicines. Crook herbalists targeted immunity boosting cures, sexual enhancement and contraceptives.

The ingredients currently used by most herbalists are a matter of guess since there are no machines to test the content of the herbs they recommend to patients. A draft policy formulated in 2008 lies unimplemented. Other consequences of the unregulated herbs were noted with a lot of concern by the pharmacy and poisons board of Kenya in July 2010.PPB drew guidelines on herbal and complimentary medicine but not yet in force .These guidelines are meant to harmonize the industry and also dismiss the issues as follows:

a) Misconception amongst herbalists that documentation requested for by PPB is intended to steal their indigenous knowledge and thus, there has been hesitation to submit applications.
b) Lack of documented evidence on quality, safety & efficacy of Herbal and complementary products.
c) Unethical practices that include:-
   - Adulteration of herbal and complementary products with conventional medicines.
   - Advertising of Herbal and complementary products in print media, electronic and bill boards.
   - Peddling of products with no therapeutic benefits.
   - Unsubstantiated medicinal claims by herbal practitioners.
   - Dealing with herbal products whose toxicological profile is not known.
d) Poor standards of preparation / manufacture and sale of herbal and complementary products.

This guideline will focus on the manufacture, registration and marketing of herbal and complementary medicines.

Fake herbal contraceptives being sold caused serious side effects on the users. It was noted that the women who used the contraceptives developed serious hormonal alterations that made the children develop adolescent features, including the start of menstrual cycle in three year old girls. Several women and patients and children admitted at Kenyatta National Hospital had been diagnosed with the side effects of the drug.

There were cases of cancer and HIV aids patients losing lives after using these drugs.

With the few mentioned cases of mal -practices in the herbal industry noted in the country and many more unreported, there is urgent need for Kenya to enact guidelines on the use of herbal cures.

Why regulate the industry?

It is the duty of the nation to protect the lives of its citizens. Health is a human right and in the assurance of healthy nation, the subject of safety counts a great deal.

Regulation will check the following:-

a) Safety assessment of the traditional medicines
b) Reduce the vice of counterfeit drugs
c) The standards ranging from the contents, prescription and packaging.
d) Help weed out rogue practitioners

e) Protection of intellectual property rights (patenting formulas and the ingredients used by specific herbalists)
f) Protect the indigenous knowledge /culture preservation

Recommendations

Regulations on the use of herbal medicines in Kenya ought to be issued in order to harmonize the industry. Herbalists need to undergo training, registration and the medicines having to undergo laboratory analysis.

In 2008 Kenya was singled out as one of the countries without intellectual property laws to encourage communities to share traditional knowledge. Development of laws and policies for protection of traditional knowledge like herbal medicines must be undertaken with the communities that sell them.

- There is need to draft an education capacity building initiative to Kenyans of the use of herbal medicines as an alternative source of treatment.
- There is need to intensify research on these remedies and establishment of large scale medicinal plant production.

Conclusion

- When a traditional medicine policy will be place, Kenya has the potential of improving the healthcare of its citizens. The herbal industry can and is a tool of economic empowerment. While unregulated use of traditional medicine can have negative effects, a claim that
- Herbal medicine can cure every disease brings even good practice into disrepute. With increased prevalence the questions of safety, efficacy and quality are some of the challenges that need to be overcome. More work is also needed to raise public
- awareness of appropriate use of traditional medicine

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[PS 11] Phytochemical Investigation of *Satureja abyssinica*

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Keywords: *Satureja abyssinica, Lamiaceae, Triterpene, Pulegone*, Ursolic acid, Sucrose

Introduction

The genus *Satureja* belongs to the family *Lamiaceae* (*Labiatae*). In Ethiopia, the genus is represented by eight species (1, 2). *Satureja abyssinica ssp. abyssinica* is an annual or perennial herb indigenous to Ethiopia where it is locally known as "Mutansa" (1). Previous work on the essential oil has been done and the result showed that it has a wide range of biological activities such as anti bacterial, anti fungal, anti-inflammatory and antioxidant activities (3). Here we report the preliminary phytochemical investigation on the solvent extract of *Satureja abyssinica*.

Methods

The aerial parts of *S. abyssinica* were collected in January 2011 from "Endode-mariam" near Debresina, 194 km away from Addis Ababa at an altitude of 1700 m. The air-dried leaves of *S. abyssinica* were successively extracted with hexane, chloroform and methanol. These extracts were used for further isolation on silica gel column chromatography. Isolated compounds were elucidated based on the spectral data of ^{1}H- NMR, ^{13}C-NMR, DEPT-135, 2D-NMR (H,H-COSY, HMQC, HMBC) experiments and mass spectroscopy.

Result and Discussion

Four compounds were isolated from the leaves of *S, abyssinica,* of which Pulegone and (1Z,5Z)-1,6-dimethylcycloocta-1,5-diene were isolated from hexane extract and ursolic acid and Sucrose from methanol extract. The structure of the compounds was elucidated from 1D and 2D-NMR and in comparison the data reported in the literature for the compounds. It is noteworthy that the methanol extract was found to have high content of sugar. Future work on bioassay guided analysis will be done for a better understanding of the chemical composition and bioactivities of the plant.

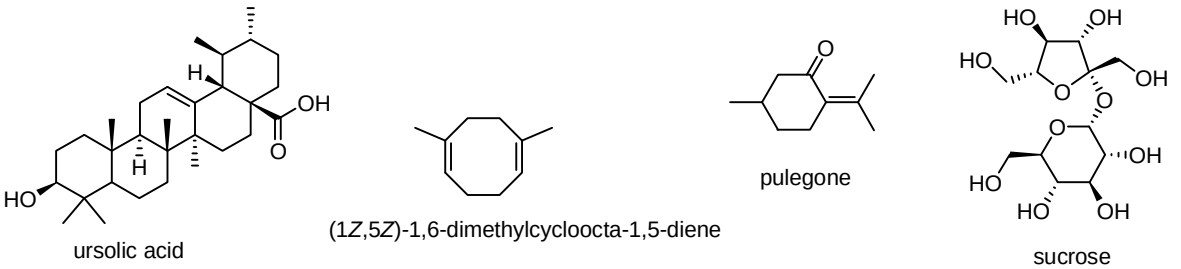

Figure 1. Isolated compounds from *Satureja abyssinica*.

Acknowledgements

Financial support from NAPRECA and Addis Ababa University for the participation on the 14th NAPRECA symposium is gratefully acknowledged. We thank ALNAP for the provision of NMR spectroscopic equipment used in this investigation. We are grateful to Dr. Mick Cooper, University of Nottingham for mass analysis of the triterpenoid. We extend our gratitude to professor Sebsebe demissew for the identification of the plant material, and to Ms. Senite dagne for her help and support in the lab work.

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[PS 12] Determination of Efficacious Praziquantel Dose in Different Mouse Strains: BALB/c and Swiss Mice for Treatment of *Schistosoma Mansoni*

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Keywords: *Schistosoma mansoni; Praziquantel*; Mice; pathology; immunological

Introduction

For 25 years Praziquantel has been the recommended treatment in mice (450mg/kg body weight) for schistosomiasis, a parasite transmitted by freshwater snails in Africa, Asia and Latin America, and infecting some 200 million people worldwide. Long experience with the WHO-recommended single dosage of 40 mg/kg has shown it to be safe and relatively efficacious. Murine models are used in *S. mansoni* studies because they could have different responses to S.mansoni infection hence vary in the effective dose of Praziquantel that can possibly eliminate the worms.

Materials and Methods

We infected BALB/c and Swiss mice with 250 *S.mansoni* cercariae.Serum were prepared and IgG ELISA carried out.Four weeks post infection mice were treated with PZQ 450, PZQ 900 and PZQ 1350.Perfusion and adult worm recovery was done 2 weeks post infection. We also carried out histopathology on livers.

Experimental Design

Mouse strains	Groups	Doses (mg/kgbw)	Week 0	Week 4	Week 6
BALB/c	Exp	450	I	T	S.(6)P(6)
	Exp	900	I	T	S.(6)P(6)
	Exp	1350	I	T	S.(6)P(6)
	IC	-	I	S(6)	S.(6)P(6)
Swiss	Exp	450	I	T	S.(6)P(6)
	Exp	900	I	T	S.(6)P(6)
	Exp	1350	I	T	S.(6)P(6)
	IC	-	I	S(6)	S.(6)P(6)

Key: Exp- Experimental**, IC** – Infected control**, I-** Infected**, T-**Treated**, S-**Sample, **P-** Perfusion mg/kgbw**- milligram/ kilogram-** body weight, **-**No activity, ( )-Number of mice per group

RESULTS

Worm maturation

Infected control BALB/c was 10% while that in Swiss mice was 14%. Swiss mice had more infecting parasites maturing into adult worms than BALB/c mice.

Schistosome Worm recovery

PZQ1350 had the highest worm reduction (69.70%) followed by PZQ900 (66.76%) and PZQ450 (53.88%) had the lowest worm reduction in BALB/c..In Swiss mice PZQ1350 had the highest worm reduction (65.92%) followed by PZQ900 (52.48%) and PZQ450 (24.34%) had the lowest worm reduction (Fig 1&2).

Ig G SPECIFIC ELISA

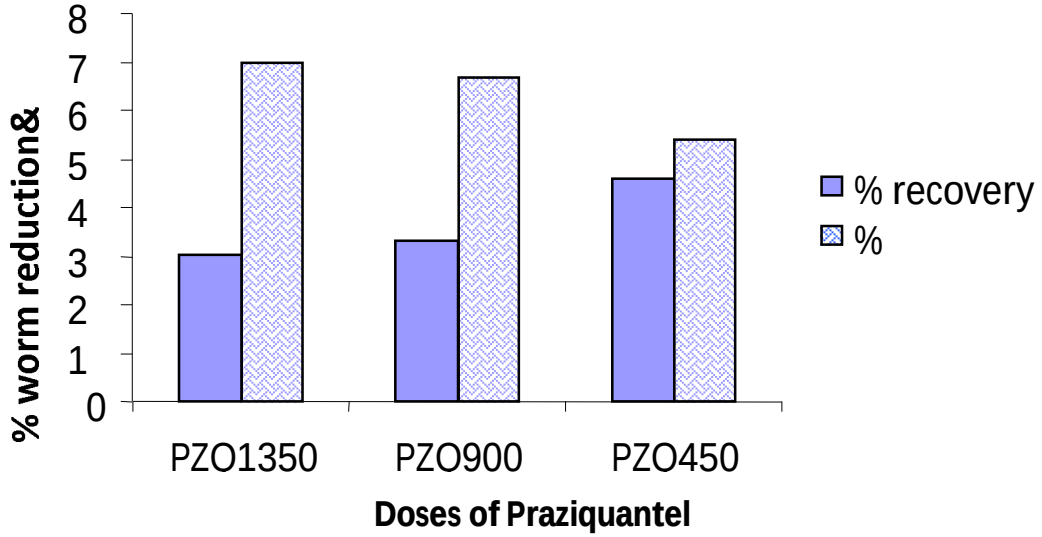

Figure 1: Percentage Schistosome worm recovery and worm reduction in BALB/c mouse strains in different treatment

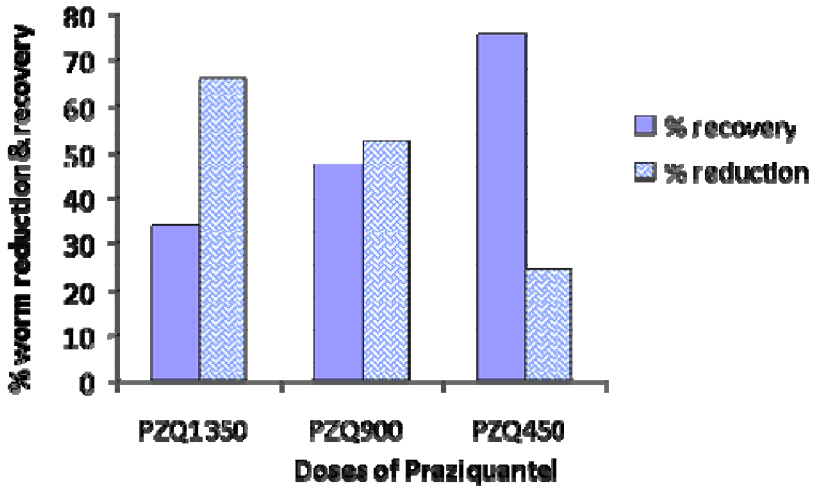

Figure 2: Percentage Schistosome worm recovery and worm reduction in Swiss mouse strain in different treatment

PZQ1350 was statistically different when compared with PZQ900 and PZQ 450 (P<0.05) in response to SSP (Fig3). PZQ1350 was statistically different when compared with PZQ900 and PZQ 450 (P<0.05) in response to SWAP (Fig.3). PZQ1350 had significance difference when compared with PZQ900 and PZQ 450 (p<0.001) in response to SSP (Fig.4). PZQ1350 dose was significantly different when compared with PZQ900 and PZQ 450 (P<0.001) in response to SWAP (Fig.4)

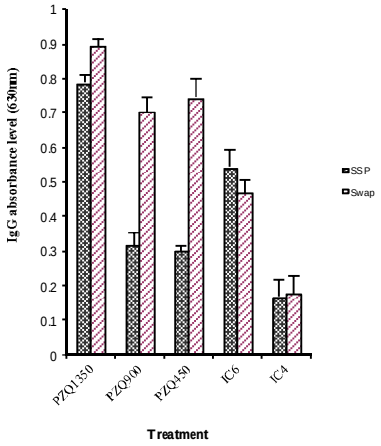

Figure 3: SSP and SWAP specific IgG antibody levels in group PZQ1350, PZQ900, PZQ450, IC6 and IC4 in BALB/c mice strain.

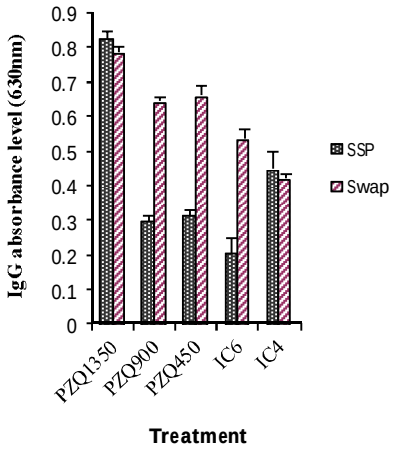

Figure 4: SSP and SWAP specific IgG antibody levels in group PZQ1350, PZQ900, PZQ450, IC6 and IC4 in Swiss mice strain.

Histopathology in BALB/c and Swiss mice strains

BALB/c and Swiss mice, normal liver tissue was encountered in PZQ 1350 and PZQ 900.We also encountered granulomas with visible eggs in PZQ 450 and cellular infiltration.

Conclusion

- The results of this work showed that PZQ 1350 had the greatest effect on worm reduction, worm recovery, pathology on liver tissue and IgG specific immunological response compared to other groups.
- The results indicated that Swiss mice were a better model for worm maturation and recovery.

Acknowledgement

The co-authors, Mount Kenya University Jomo Kenyatta University of Agriculture and Technology, Institute of primate research, family and friends for support during the entire study.

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[PS 13] Cytotoxicity of a Novel Diterpenoid from *Suregada zanzibariensis*

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Key words: Cytotoxicity, cancer cell lines, diterpenoid lactone, *Suregada zanzibariensis*, Euphorbiaceae, Bioassay-guided fractionation

Introduction

As part of the CSIR Bioprospecting platform research, 11 000 plants were collected throughout South Africa. Approximately 7 500 plant extracts were made from these plants and randomly screened for their anti-cancer properties against three cancer cell lines (melanoma UACC62, breast MCF7 and renal TK10). Based on screening results *S. zanzibariensis* (Euphorbiaceae) extract exhibited good anti-cancer activity against all the cell lines tested which prompted further research.

A novel diterpenoid-lactone was isolated through the bioassay-guided fractionation of the organic extract of *S. zanzibariensis*. The compound exhibited potent anti-cancer activity (growth inhibition GI_{50} = 20ng/ml) against the melanoma cell line and found to be more potent that the corresponding plant extract. An accelerated 96 well microtitre plate semi preparatory HPLC purification method aimed at rapidly identifying actives in complex extracts also led to the identification of the same compound as the active ingredient. 1D, 2D NMR spectroscopy and UPLC TOF MS data were used to elucidate the structure of the active compound.

Material and Methods

The stem bark of the plant species *S. zanzibariensis* was collected from Matonela Sand forest in South Africa. The plant material was authenticated at the South African National Biodiversity Institute (SANBI), where voucher specimens were deposited.

Different methods for purification of the extract was used, namely the classical method of bioassay-guided fractionation and an accelerated 96-well microtitre semi preparatory HPLC purification method.

Bioassay Guided Fractionation Method

The organic extract of *S. zanzibariensis* was purified by silica gel column chromatography resulting in the generation of thirteen semi-pure fractions which were screened against the three cancer cell lines. The active refractions were further purified using silica gel column gravimetry to isolate the active compound(s).

96 Well Microtitre Plate Semi-Preparatory HPLC Accelerated Method

The organic extract of *S. zanzibariensis* was fractionated into 96 well microtitre plates with triplicate copies using Agilent 1200 semi preparative HPLC system. The microtitre plates were separately screened against three cancer cell lines, chemically analysed by UPLC MS TOF while the third plate

is stored as a retention sample. A correlation plot was developed between biological and chemical data to identify the active compound (s).

Results and Discussion

The needle-like crystals of compound 1 as shown in **Figure 1** was isolated from the organic extract of *S. zanzibariensis* through bioassay-guided fractionation method.

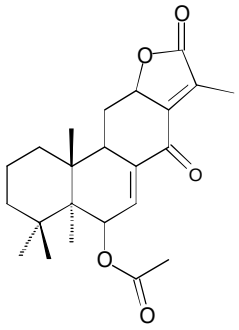

Figure 1: Compound 1

The ^{1}H NMR and ^{13}C NMR spectra and 2D experiments were used to elucidate the structure of the compound. The two double bonds, acetate group, ketone and lactone carbonyl resonances seen in the ^{13}C NMR spectrum, accounted for the five double equivalence which in turn indicated that the molecule is tetracyclic. A search on the Dictionary of Natural Product database showed no hits, indicating that the compound could be novel. Abiatanes ditepenoids lactones, were previously isolated from the related species of *Suregada*, (I. Jahan *et al*, 2002 and I.A Jahan *et al*, 2004) and cytotoxicity studies of diterpenoids from the same genus. C. L Lee *et al*, 2008.

Screening results of the 96 well microtitre plates showed that wells E3-E4 to be most active against the melanoma cells (AUCC). These active wells were analysed using UPLC MS TOF data and resulted in the confirmation that **compound 1** was present in these wells. The TOF-MS^{+} spectrum of **compound 1** showed a [M+Na+] ion peak at *mz* 395.1825, retention time (17.92 min) and UV max absorption at 263 nm. The MS spectrum indicated a mass fragmentation ion $[M-59]^{+}$ indicating loss of acetate group [-$COOCH_3$], which corresponded to a molecular formula of $C_{22}H_{24}O_5$.

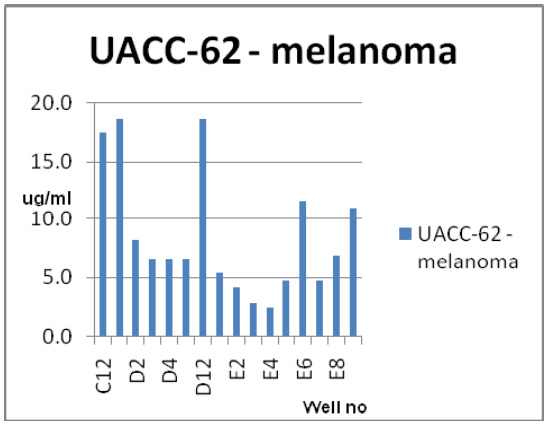

Figure 2: Histogram of UACC-62 results of most active fractions from the 96 well plate

Conclusions

Isolation of compound 1 was achieved through classical bioassay-guided fractionation approach. The 96 well plate accelerated approach confirmed the previously identified compound but also served as a validation of application the new accelerated method. The 96 well approach serve to tentatively identify compound/class responsible for activity in shorter time than that of classical bioassay guided fractionation, which took much longer. As the compound is novel, the 96 well plate approach and analysis through UPLC TOF MS analysis would not have been sufficient to elucidate the structure using only this technology.

Acknowledgements

The authors would like to thank the South African National Biodiversity Institute (SANBI) for the identification of plant specimens, NRF (YREF Young researcher Fund) and PG (Parliamentary Grant) for funding the project.

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[PS 14] Solid-state Fermentation of *Jatropha curcas* Seed Meal Using *Aspergillus niger* Eliminates Molluscicidal Activity and Changes the Phorbol Ester Composition of the Seed Meal

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Key words: *Jatropha*; solid-state fermentation; snails; Phorbol ester; *Aspergills niger*

Introduction

Jatropha curcas, commonly referred to as physic nut, is a member of Euphorbiaceae family, and grows well in the tropical region (Aderibigbe et al., 1996). The genus name for *Jatropha* is derived from the Greek *iotrós* (doctor) and *trophé* (food) which implies medicinal uses (Makkar et al., 2009). *Jatropha curcas* seeds have high potential for utilization as food or feed of high nutritional value. Although the seed contains 40-60% oil which is similar to oil used for human consumption, this oil is not used for cooking purposes because it contains some toxic substances (Rakshit et al., 2008). The seed cake obtained after extraction of oil, has a protein content of between 53 – 58% crude protein (Aregheore et al., 2003) and cannot be used as animal feed due to toxic elements such as lectins and phorbol esters. A critical obstacle in the establishment of *J. curcas* as a commercial crop could be overcome by detoxifying *Jatropha* seeds. Heat treatment followed by chemical (sodium hypochlorite (NaOCl) and sodium hydroxide (NaOH)) treatment has been used as one of the methods of detoxification (Waled and Jumat, 2009), but the method is found to be expensive and complicated. The aim of this study, therefore, was to detoxify *J. curcas* seed meal using solid state fermentation technology as a simple and an alternative method, so that seed cake can be used as animal feed or protein supplement in animals feed.

Materials and Methods

Materials

Mature sun dried seeds of *J. curcas* were provided by the Harare Polytechnical College of Zimbabwe. Snails were provided by Aquaculture Unit at University of Limpopo and *Aspergillus niger* FGSC A733 was obtained from Fungal Genetics Stock Centre (FGSC).

Methods

Seed kernels were ground to a meal using a pestle and mortar and sieved using 2 mm sieve to obtain *J. curcas* seed meal (JCSM). JCSM (10 g) was added into 500 mL Erlenmeyer flasks and the contents in the flasks were autoclaved at 120°C for 20 min and then cool to room temperature. Salt solution (0.2% K_2PO_4, 0.5% NH_4NO_3, 0.1% NaCl and 0.1% $MgSO_4$) was also autoclaved separately and left to cool to room temperature. The seed meal was moistened with 4 mL of salt solution.. The

medium was inoculated with 1 mL of *A. niger* spore suspension(5 X 10^4 CFUs/ml). The flasks were incubated at 30°C for 10 days.

After fermentation the fermented and unfermented *J. curcas* seed meal was extracted with acetone in ratio of 1:5 (w/v). The contents were stirred for 1 hr at room temperature. Acetone fraction was decanted and the extraction was repeated twice. The collected acetone fraction was filtered using Whatman No 1 filter paper. The acetone was evaporated from the extract using rotary evaporator at 40ºC. The *Jatropha* oil obtained was thoroughly mixed with 2 volumes of methanol at room temperature in a separating funnel. The methanol fraction was collected. This procedure was repeated 4 times and methanol extractions were pooled together. Methanol was evaporated using rotary evaporator at 40°C.

Snails were cultivated in an aquarium filled with fresh water, in controlled photoperiod of 12 hrs light and 12 hrs darkness. An air pump was used to supply air into the water tanks and the temperature was maintained at 24°C using heaters. The snails were fed on lettuce. After multiplication, snails were divided into separate tanks for further experiments involving exposure to *Jatropha* oil.

Tanks of 10 L capacity were filled with 10 L of fresh water and 20 snails were introduced into each tank, fractions of oil extracted from unfermented *Jatropha* seed ranging between 0.1 – 1 mL was added to each tank and mortality rate of snails was recorded every 12 hrs over a period of 48 hrs. Sunflower oil was used as control. Different trials were performed by exposing snails to 0.7 mL of *Jatropha* seed oil extracted from fermented or unfermented seed meal. Also snails were exposed to *Jatropha* oil hydrolysed using a lipase.

Results and Discussion

The lowest experimental dose where there is no measurable affect is known as the no observable adverse effects level (NOEL). Dosage range between 0.1 to 0.3 mL of *Jatropha* oil, show (NOEL) after 48 hrs of exposure. Dosage of 0.4 mL of *Jatropha* oil, 50% of snails were dead after 48 hrs of exposure. While in dosage range from 0.6 to 1 mL, all snails are died after 36 hrs of exposure. The more mortality rate increases of snails exposed to the *Jatropha* oil was thus dose dependent (Figure 1).

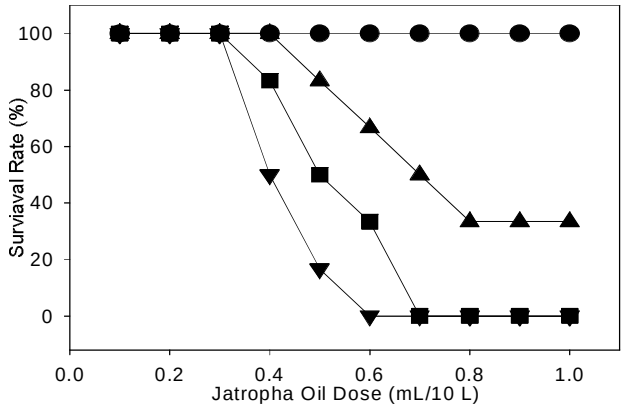

Figure: 1. Mortality of snails exposed to *Jatropha* oil at different doses of (0.1-1.0) mL per 10 L of water. 12 hrs of exposure(●),24 hrs of exposure(▲), 36 hrs of exposure(■) and 48 hrs of exposure(▼).

After 12 hrs of exposure in all treatments the snails were normal, while after 36 hrs of exposure, only 5% of snails exposed to unfermented *Jatropha* oil survived (Figure 2). In fermented oil 90% survived. Sunflower oil had no lethal effect on the snails (Figure 2). These findings reveal that solid state fermentaion detoxifies Jatropha seed oil possibly through biochemical modification of phorbol esters.

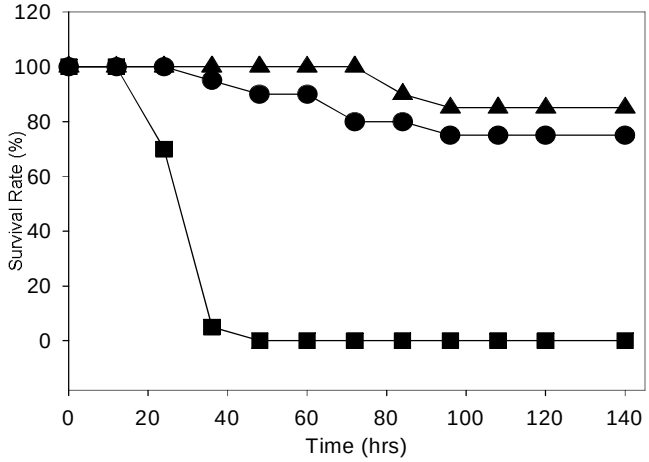

Figure 2: Snails exposed to 0.7 ml of oil over 140 hrs. Unfermented *Jatropha* oil. (■), fermented *Jatropha* oil(●) and sunflower oil(▲).

There was no difference in survival rates of snails exposed to unfermented oil (25% survival) and those exposed to unfermented oil that had been hydrolysed with lipases. Oil extracted from fermented seed meal had similar survival rates of to sunflower oil (84%) (Figure 3). Lipolysis using the current lipase therefore does not detoxify the oils as the unfermented oil that was hydrolysed using a lipase had the same toxicity to snails as untreated oil (Figure 3).

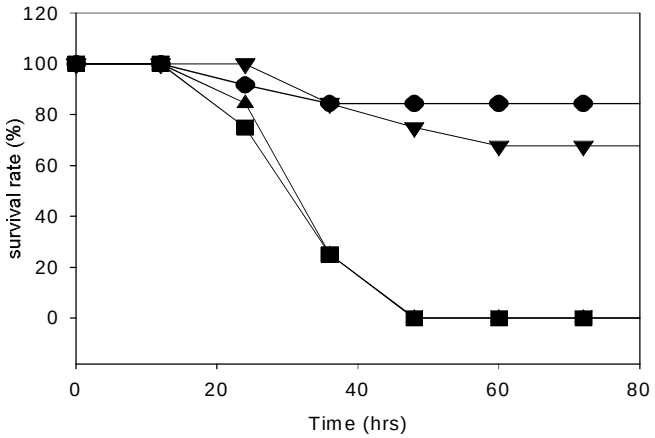

Figure: 3. Snails exposed to 0.7 mL of oil treated in different ways. Oil extracted from unfermented *Jatropha curcas* seed(■), oil extracted from fermented *Jatropha curcas* seed(▼),unfermented Jatropha curcas seed oil hydrolysed using lipase(▲) and sunflower oil hydrolysed using lipase (●).

Acknowledgements

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[PS 15] Ethnomedicinal Knowledge in the Traditional Management of Human Ailments in Lake Victoria Basin, Kenya

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Key words: Indigenous knowledge, human ailments, medicinal plants, ethnomedicine.

Introduction

Uses of plants in the indigenous cultures of developing countries are numerous and diverse. This indigenous knowledge evolved for a long time through trial and error. Though the majority of inhabitants in the Kenya rely on ethnomedicinal plant species to manage a wide range of human ailments, much the indigenous knowledge largely remains undocumented.

Materials and methods

An ethnomedicinal survey was conducted to document the plant species used medicinally in the Lake Basin. The ethnomedicinal data were based on structured interviews that sought answers to questions about the human ailments treated, local names of plant species, plant parts used, methods of preparation, and administration. In some cases, the interviews were facilitated by translators who were well conversant with the local language. This was done having first obtained verbal informed consent from each traditional healer.

Results and discussion

Traditional healers of the Lake Victoria Basin, Kenya were found to be rich in their indigenous knowledge on the use of ethnomedicinal plant species to manage various human ailments within the study area. This was evidenced by the result that a wide range of human ailments were reported to be treated using thirty four medicinal plant species distributed within twenty one botanical families.They were found to play a vital role in the primary healthcare of the local poor people as they were the main resource persons to their health problems. This may have been due to the inability by the locals to afford modern healthcare costs and the healers' capability to handle most of their health problems. The plant family reported with the highest number of medicinal plant species was|Compositae followed by Leguminosae and Labiatae. This trend is in agreement with the findings by Yineger *et al*., (2008) who reported Compositae and Labiatae as the first and third families, respectively, with the highest number of medicinal plant species, but is contrary to those of Yineger & Yewhalaw (2007) who reported the most representive families as Leguminosae, Acanthaceae and Curcubitaceae successively. The discrepancy may be due to factors such as ecological, geographical and environmental (Runyoro *et al.,* 2006) which favour the growth of some plant families and not others. There was evidence of high secrecy in the medicinal plant usage with a number of healers not ready to reveal full details of their knowledge about the medicinal plants to us. Many reported not having transferred the knowledge to the subsequent generation. The

secrecy surrounding the ethnomedicinal knowledge among the traditional healers could be attributed to the fact that traditional healers derive income from the treatments they provide. This is in agreement with findings done by Yineger *et al*., (2008) and Yineger & Yewhalaw, (2007) that apart from income, traditional healers get in-kind compensation and would therefore like the value of the indigenous knowledge maintained. Ng'etich, (2005) reported that some traditional healers regard the knowledge as personal property, Kokwaro (1993) reported that in some cases oaths are taken during passing of the information so that it is not revealed to any one else. The degree of agreement by traditional healers in dealing with ailments such as malaria which is managed using *Tithonia diversifolia* and *Schkuria pinnata, ;* sexually transmitted infections managed using *Albizia coriaria* and *Harrisonia abyssinica* and ringworms managed using *Moringa sp.* could give high validity to these species used to treat these ailments and could be due to the effective results on their usage from past experience, the species availability and existence of these ailments as the most commonly encountered ones. The use of traditional medicinal plants as mixtures by traditional healers to manage one or more human ailments was reported. In fact the majority of the ethnomedicinal plants collected were used as mixtures. This could be due to the additive effects that they could have during ailment treatment (Bussman and Sharon, 2006; Igoli *et al.,* 2002). The traditional healer may not be sure of the specific ailment the patient could be suffering from and therefore gives a mixture of several herbal medicinal preparations as a remedy to potential ailments judging from the patients' condition. The other reason could be due to the synergism action of the different preparations expected by the practitioner. Uses of traditional herbal remedies as mixtures of different herbs have also been reported in the Chinese traditional medicine by Xiao (1983). Some of the plants in the mixture could however be acting as antipyretics, immune stimulants to relieve the symptoms of the disease rather than having direct activity as reported by Philipson *et al*., (1993), and some could also be nutritive. Xiao (1983) explained that determination of the pharmacological effects and isolation of active principles from the herbal mixtures is much more difficult than in the case of single medicinal plants owing to the interaction of various constituents. In this study, it was observed that most of the medicinal plant species were used to treat more than one ailment. This could be due to the availability of the herbal plant or its effectiveness from past experience in the treatment of various human ailments. This is in agreement with reports by Lukhoba *et al.,* (2006), Boer *et al.,* (2005) Okemo *et al*., (2003) and Kokwaro, (1993). Results of this ethnomedicinal study revealed that traditional healers used additives such as the traditional ghee, Vaseline, oil during preparation. This could be attributed to the increase of potency of the medicinal plant. This result was in agreement with the findings of Otieno *et al.,* (2007) in Tanzania who reported that crude mineral *Kadosero* supplemented to other plants extracts by the herbal practitioners showed increased activity of the herbal medicine. Olembo *et al.,* (1995) also reported a similar scenario about *Dichondria repens* (Convolvulaceae) whose leaves are crushed, mixed with oil to treat dermatological ailments. The same plant showed no activity when tested against a dermatological fungus by Kariba (2000).

This study reported herbs to be the most used growth form used for remedy prepararation. The second and third being shrubs and trees respectively. High use of herbs could be attributed to the

fact that they tend to be most available in nearly all climatic conditions, have fast growth and tend to be available in conspicuous places like crop farms, disturbed areas, along the roadsides and along fences where they can easily be accessed by the practitioners. Leaves were the most cited plant parts used by the healers for the preparation of medicine followed by the roots. This finding is in line with the results of other ethnomedicinal studies such as those by Yineger *et al.,* (2008) and Yineger & Yewhalaw, (2007). Most of the ethnomedicinal plant species were reported to be processed through concoction, decoction, powdering and administered mainly through oral and dermal routes. Remedies were mostly prescribed by the traditional healers, however the dosages lacked precision as they were given in cups, water glasses or in a basin. This report was found to be in agreement with that of Erasto *et al* ., (2008) , Boer *et al*., (2005) , Kokwaro, (1993) and Yineger *et al*., (2008) who in addition mentioned that there could a rise cases of over dose which could cause serious health problems due to toxicity of some species. Our report on remedy preparation is however in contrary to the findings by Yineger & Yewhalaw, (2007) in Ethiopia who reported the principal methods of remedy preparation as crushing and squeezing. Nature of ailments treated and healers' past experience on results may have contributed to the observed difference.

Acknowledgement

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[PS 16] *In vitro* Antimicrobial Activity of Extracts from five Malagasy Endemic Species of *Albizia* (Fabaceae)

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Key-words: *Albizia*, seeds, extracts, antimicrobial, MIC, MBC.

Introduction

For centuries, most of the population in many developing countries have relied on a system of traditional medicine in which plants constitute the principal element of therapy. Plants belonging to the genus *Albizia* (Fabaceae) are trees distributed in African, Asian and South-American countries where they are widely used in indigenous pharmacopoeia (Agyare et al., 2005; Geyid et al., 2005; Murugan et al., 2007; Rukayadi, 2008). *Albizia* species have been the subject of several chemical and pharmacological studies. Thus, many structures (heterosids, alkaloids, ...) were elucidated (Zou et al., 2006; Rukunga et al., 2007) and various activities such as anthelmintic (Githiori et al., 2003), cytotoxic (Zou et al., 2006), larvicidal (Murugan et al., 2007) or antimicrobial (Agyare et al., 2005; Geyid et al., 2005; Sudharameshwari et al., 2007) were found.

In Madagascar, *Albizia* is represented by 25 endemic and 2 introduced species. No previous report on both the chemical constituents and the pharmacological activities of these plants could be found in the literature. Since infectious diseases account for the significant proportion of health problems, antimicrobial principles from five Malagasy species of *Albizia* encoded A_1, A_2, A_3, A_4 and A_5, were studied in this work. They were purified and the major secondary metabolites were identified by phytochemical screening. Extracts or pure compounds were tested *in vitro* against two Gram positive bacteria, three Gram negative bacteria and one yeast *Candida albicans*. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) were determined on susceptible germs.

Materials and methods

1- Plant materials

Seeds of plants A_1, A_2, A_3, A_4 and A_5 were used in this study. Fruits were collected in western and southern regions of Madagascar.Seeds were washed, sun-dried and ground into a fine powder, using a microgrinder Culatti.

2- Microorganisms

The pathogenic microorganisms consisted of two Gram positive bacteria: *Staphylococcus aureus*, *Bacillus subtilis*, three Gram negative bacteria: *Klebsiella pneumoniae, Escherichia coli Salmonella*

typhi and one yeast *Candida albicans*. They were isolated and identified from heterogeneous cultures available in Institut Pasteur de Madagascar.

3- Extracts preparation

3.1- *Extraction*

Powdered dried seeds were defatted by extraction with petroleum ether (60-80°C) in a Soxhlet's extractor, then extracted with distilled water, 50% ethanol or 75% ethanol.

3.2- *Purification*

Crude extracts were purified using methods based on solubility, molecular weight or electric charge properties of active principles.

4- Phytochemical screening

Extracts were subjected to preliminary phytochemical testing for the major chemical groups (Fransworth, 1966; Marini-Bettolo et al., 1981).

5- Assays on microorganisms

The antimicrobial tests were carried out by disc diffusion method in Mueller Hinton agar (Rios et al., 1988).MIC was determined by broth dilution method (Duval et Soussy, 1990; Ferron, 1994). Each medium showing no visible growth is subcultured on Mueller Hinton agar plates. After 24 hours at 37°C, MBC was the corresponding concentration required to kill 99.9% of the cells (Duval et Soussy, 1990; Ferron, 1994).

6- Statistical analysis

One-way analysis of variance (ANOVA) followed by Newman Keuls comparison test with Statitcf® software were used for statistical analysis. Statistical estimates were made at confidence interval of 95%.

Results and Discussion

1- Phytochemical screening

The major secondary metabolites identified in extracts are shown in Table 1

Table 1: Phytochemical screening of extracts from 5 Malagasy species of *Albizia* (A_1 to A_5)

Phytochemical compounds	**Extracts**					
	E_1	E_{21}	E_{22}	E_3	E_4	E_5
Alkaloids	–	–	–	–	–	–
Flavonoids	–	–	–	–	–	–
Anthocyanins	–	–	–	–	–	–
Phenols	–	–	–	–	–	–
Quinons	–	–	–	–	–	–
Unsaturated sterols	+	+	+	+	+	+
Triterpenes	–	+	+	+	+	+
Deoxysugars	+	+	+	+	+	+
Saponins	+	+	+	+	+	+

–: negative test +: positive test

E_1, E_3, E_4, E_5 : purified extracts from plants A_1, A_3, A_4 and A_5 respectively

E_1, E_{21}, E_{22} : pure compounds from plant A_1, A_2 respectively Except A_1 which didn't contain triterpenes, all extracts showed the presence of unsaturated sterols, triterpenes and deoxysugars, indicating glycosidic nature of active principles. The presence of saponins, in addition with positive foam test and hemolytic effect (not shown) mean that antimicrobial compounds may be saponins.

Saponins and other glycosides were isolated and identified from other species of *Albizia* (Pal et al., 1995; Debella et al., 2000; Zou et al., 2006).

2- Antimicrobial activity

According to these results, the extracts E_3 and E_5, respectively from **A3** and **A5**, showed activity against all the tested germs. *Bacillus subtilis* seemed to be the most susceptible bacterium (13 mm inhibition zone for E_3 and 16 mm for E_5) to these extracts. On the other hand, all the extracts inhibited the growth of *Staphylococcus aureus* and *Candida albicans* at the tested concentrations. E_{21} (pure compound) exhibited the strongest activity against the fungus (20 mm). In a general manner, Gram positive germs, including *Candida albicans,* were more susceptible than Gram negative ones.

Similar results were obtained with some other species of *Albizia* (Mbosso et al., 2010; Rukayadi, 2008; Sudharameshwari et al., 2007).

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) determined on susceptible germs are given in Table 2.

Table 2: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of extracts from 5 Malagasy species of *Albizia*

Extracts	Sensitive germs	MIC (µg/ml)	MBC (µg/ml)
E_1	*Staphylococcus aureus*	320	2500
E_{22}	*Candida albicans*	6.25	100
E_{22}	*Klebsiella pneumoniae*	50	800
E_3	*Escherichia coli*	2500	10 000
E_4	*Staphylococcus aureus*	625	10 000
E_4	*Escherichia coli*	1250	20 000
E_5	*Escherichia coli*	12 500	12 500

Pure compound E_{22} from the plant A_2, showed the lowest **MIC (6.25 µg/ml)** and **MBC (100 µg/ml)** against *Candida albicans*. With MIC values respectively corresponding to 100 µg/ml and 12.5 µg/ml, *Albizia myriophylla* and *Albizia gummifera* (Mbosso et al., 2010; Rukayadi, 2008) showed lower activity than A_2 against this germ.

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[PS 17] Phytochemical and Pharmacological Studies of Extracts of *Trichilia emetica* Used in the Treatment of Dysmenorrhoea in Mali

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Keywords: Dysmenorrhea; *Trichilia emetica*; anti-DPPH activity; Analgesic and anti-inflammatory activities.

Introduction

Dysmenorrhoea affects many women in reproductive age, and is a frequent cause of time lost from work or school as well as interfering with daily living. Treatment is usually done with NSAIDs and minor analgesics. *Maytenus senegalensis* Lam. (*Celastraceae*), *Stereospermum kunthianum* Cham. (*Bignoniaceae*) and *Trichilia emetica* Vahl. (*Meliaceae*) are traditionally used in Mali for the treatment of menstrual pains. Previous screening demonstrated the anti-inflammatory, analgesic and antispasmodic activities of these plants (Sanogo et al., 2006 and 2007). The aim of the present project is to carry out further investigations on the extracts of the leaves and roots of *T. emetica*, in order to corroborate their use in the treatment of dysmenorrhoea.

Material and Methods

A qualitative phytochemical analyse was carried out using thin layer chromatography (TLC) methods. Some chemical components were isolated and their structures were elucidated by NMR. The toxicological study was performed on mice. Pharmacological investigations were carried out on acetic acid-induced writhing (pain) and hind paw oedema in mice. The Paracetamol and Indometacin are used as reference drugs, respectively in analgesic and anti-inflammatory test. The effects of the aqueous extract were studied on the isolated uterus of rat. The Nifedipin was used as inhibition drug of the uterus muscle contraction. The active extracts were studied by bio-guided fractionation, using the antiradical (1,1-diphenyl-2-picrylhydrazyl, DPPH) on chromatograms. The phenol content of active fractions was determined using Folin-Ciocolteu method.

Results and Discussion

Phytochemical analysis of the extract revealed the presence of coumarin, flavonoids, tannins, saponin glycosides and terpenoids. The polyphenolic compounds demonstrated the anti-DPPH activity. The phenol compounds content of aqueous extract of the leaves is high than those of the roots. Lignan structures were also isolated from root extract.The aqueous extract had an oral LD (50) more than 2000 mg in mice. Results of pharmacological investigations showed that the aqueous extracts possess significant ($P<0.05$) dose-dependent anti-nociceptive and anti-inflammatory activities at the dose of 200 mg/kg *per os* in mice. The pain inhibition was 76% and

72% respectively for leaves and roots. Leaves and roots of *T. emetica*, also reduced the oedema with 67% and 76% 3h after carrageenan injection. The extract of the roots also exhibited the inhibition of spontaneous and acetylcholine-induced contractions in the uterus of rat. The best analgesic activity has been demonstrated by aqueous extracts of the leaves of *T. emetica*. In the anti-inflammatory test, the best activity was obtained with the extracts of leaves and roots bark of *T. emetica*.

These data tend to suggest that the aqueous extracts possess peripherally-mediated analgesical properties like Paracetamol. This peripheral analgesic and the anti-inflammatory effects of the decoctions may be mediated via inhibition of cyclooxygenases and/or lipoxygenases, like non-steroidal anti-inflammatory drugs, commonly employed in the treatment of inflammation induced by prostaglandins (McGaw, et al., 1997). The aqueous extracts of *T. emetica* may contribute in some way in the prevention of synthesis of prostaglandins that caused menstrual pains and uterine hyper-contractility. The results of our studies can corroborate the use of the extracts of *T. emetica* in the treatment of dysmenorrhoea.

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[PS 18] Hepatoprotective Activity of Aqueous Extracts of Leaves, Stem Bark and Roots of *Entada africana* Against Carbon Tetrachloride-Induced Hepatotoxicity in Rats.

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Keywords: *Entada africana*, carbon tetrachloride, Hepatoprotective activity, anti-DPPH activity.

Introduction

Entada africana Guillet Perr. (Mimosaceae) is used in African traditional medicine for the treatment of many diseases including hepatic syndromes, jaundice, hepatitis and other hepatic disorders (Kerharo and Adam, 1974). *E. africana,* known in Mali with the local name of "Samanéré" in the Bambara language, is one of the phyto-medicines more prescribed for liver diseases. Preclinical and clinical studies of the aqueous extract of the roots of *E. africana* demonstrated it effectiveness in hepatoprotection (Douaré, 1991, Sanogo et al., 1998). Moreover, some antiproliferative triterpene saponins were isolated from the roots of *E. africana* (Cioffi et al., 2006).

The objective of our study was to compare the hepatoprotective activities of roots with leaves and stem bark ones, in order to use them for the production of phytomedicines. The aim was to avoid the excessive exploitation of the root of *E. africana*.

Materials and Methods

Our study was designed to evaluate the hepatoprotective activity of aqueous extracts of leaves, stem bark and roots of in experimental liver injury induced by carbon tetrachloride in rats. The animals were starved for 18h with water at libitum. *E. africana* leaf, stem bark and roots aqueous extracts were administrated orally to rats respectively at the doses of 190, 110 and 100 mg/kg. The extracts were administrated once a day for seven days. On the seventh day after the extract treatment, a single dose of the carbon tetrachloride (50% in olive oil) was injected by IP at the dose of 5 ml/kg.

The levels of hepatic marker enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and biluribin were used to assess the hepatoprotective activity against carbon tetrachloride (CCl4)-induced hepatotoxicity. The hepatoprotective activity was also assessed by histopathological studies of liver tissue. The ant-DPPH activity was determined on the chromatogram of extracts of three parts of *E africana* by spraying plate with methanol solution of radical 1,1-diphényl 1-2-

picrylhydrazyle. Mains chemical constituents were analysed by colorimetric reactions, and by thin layer chromatography methods.

Results and Discussion

The highest hepatoprotective activity was observed for the aqueous extract of roots and stem bark of *E. africana,* when administrated once a day for seven days before intoxication. The protection according to the serum level was respectively for ALAT (63,84% and 61, 36%) for ASAT (63,04 and 61,22) and for biluribin (40,00% and 28,57%). For the root decoction, the hepatoprotective activity was also supported by histopathological studies of liver tissue. The high content in polyphenolic components of aqueous extracts of roots and stem bark of *E. africana* can be contributed to the hepatoprotective activity. The presence of many polyphenolic compounds and polysaccharides in the aqueous extracts of the plant can also support a good hepatoprotective activity.

The results of our researches showed the effectiveness in hepatoprotection of extract of stem bark more than leaves. Their possible use in the traditional herbal treatment in liver diseases, instead of roots extracts, should be encouraged, in order to avoid the excessive exploitation of the root of *E. africana.*

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[PS 19] The Radical Scavenging Activity of Flavonoids from *Solenostemon monostachys* (P.Beauv.) Briq (Lamiaceae).

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Key words:-Flavonoids, radical scavenging ability, Lamiaceae.

Introduction

Flavonoids are γ-benzo-pyrone derivatives, which resemble coumarin and are ubiquitous in photosynthesing cells. Their occurrence is therefore widespread in the plant kingdom and about 500 varieties of flavonoids are known (Havsteen, 1983). Flavonoids are known to have anti-inflammatory, anticancer, antimicrobial, antioxidant, antiretroviral etc activities. This made them subject of intense researches in the recent past. The different classes of flavonoid arise from the difference in the oxygenation pattern on the γ-benzo-pyrone skeleton. These compounds, which exist as aglycones and therefore lipophilic constituents, are not as universal in occurrence in higher plants as the more polar flavonoid glycosides, which occur in vacuoles in the plant cells. However, surface flavonoids are common in the Lamiaceae, especially in the subfamily *Nepetoideae*. Their presence is often correlated with the production of other lipophilic secondary products such as essential oil terpenoids (Wollenweber, 1982). *Solenostemon* monostachys (P.Beauv.) Briq (Lamiaceae) is in Ghana as a leafy vegetable while in South Western Nigeria, it is commonly employed as a recipe in herbal medicines for especially infectious and inflammatory diseases. Miyase *et al.* (1980) reported unusually rearranged diterpenoid from the plant. We now report the presence of three flavonoids from the aerial parts of the plants as well as the radical scavenging ability of the flavonoids.

Materials and Methods

General

UV spectral was recorded on Cecil UV- Spectrophotometer. ^{1}H and ^{13}C NMR spectral were acquired in deuterated chloroform using 200 MHz Varian NMR Spectrometer with TMS as internal standard. Solvents used were general reagents solvents redistilled before used.

Plant Material

The aerial part of *Solenostemon monostachys* P. Beauv. (Briq.) (Lamiaceae) was collected on Obafemi Awolowo University campus premise on October, 2006. The plant was identified by Mr. Oladele A.T. by comparison of sample with voucher specimen deposited at the herbarium of the Obafemi Awolowo University, Ile Ife.

DPPH Quantitative Spectrophotometic assay

This was carried out using the method described by Menzor *et. al.,* (2001) with a slight modification. The reaction was carried out in a 96-well microtitre plates. Stock solutions of the isolated compounds and *L*-ascorbic acid (100 µM) were separately diluted to a concentration of 100, 50, 25, 12.5, 6.25, 3.13, and 1.56 µM in methanol. Twenty microlitres of 0.25 µM DPPH solution in methanol was added to 50 µL of each concentration of sample to be tested and allowed to react at room temperature in the dark for thirty minutes. Blank solution was prepared with 50 µL of sample solution and 20 µL of methanol only while the negative control was DPPH solution (20 µL) plus 50 µL methanol. Methanol was used to blank the microplate reader and the decrease in absorbance was measured at 517 nm. Absorbance values were converted to percentage antioxidant activity (AA%) using the formula:

$$AA\% = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100]/Abs_{control}\}$$

Abs_{sample} is the absorbance of the sample, Abs_{blank} is the absorbance of the blank and $Abs_{control}$ is the absorbance of *L*-ascorbic acid. The inhibitory concentration (IC_{50}), is the concentration of the sample that brought about 50% inhibition of the DPPH free radicals. The values were obtained from the separate linear regression of plots of the mean percentage of the antioxidant activity, (AA%) against concentration of the test compounds from the replicate assays.

Extraction and Isolation

Ethyl acetate extract of the powdered dried aerial parts of *Solenostemon monostachys* (2.1 g) was adsorbed on silica gel mesh 200-400 and eluted on an open column with solvent mixtures of increasing polarity from 100% hexane through 100% ethyl acetate to 100% methanol. The thin layer chromatography (TLC) analysis of the eluates on silica gel plate using 100% ethyl acetate as mobile phase gave fractions I-IV. Fraction I (0.49 g) with DPPH active spots in the DPPH autographic assay was dissolved in 2 ml of EtOAc: MeOH (9:1) and was layered on a column of Sephadex LH-20. The column was eluted with the following solvent systems: Hex : EtOAc (7:3, 8:2, 9:1), 100% EtOAc, MeOH : EtOAc (1:9, 2:8, 4:6 and 5:5). TLC analysis of eluates on silica with *n*-hexane: EtOAc (3:7) as mobile phase led to the isolation of compound **1** (0.027 g), compound **2** (0.021g) and three other fractions. The methanol extract (6 g) was eluted on silica gel on open column with 200 ml each, of 100% ethyl acetate, ethyl acetate: methanol (9:1, 8:2, 7:3, 5: 5) and 100% methanol to give fractions I-III. Fraction III (2.3 g) that tested positive in the DPPH autographic assay (Burits and Bucar, 2000) was eluted on a column of Sephadex LH-20 pre-swollen with 100% ethyl acetate and was eluted with the following solvent mixtures 100% EtOAc MeOH: EtOAc (1:9, 2:8,3:7, 5:5), 100% MeOH and 20% water in MeOH. TLC analysis on reversed phase plate with water: methanol (6:4) led to three fractions; SMMa-SMMc. SMM3b (1.1 g extracted with 50-100% MeOH), was subjected to elution on a Lobar RP-18 column with the following solvent mixtures: MeOH: H_2O (3:7, 4:6, 5:5, 6:4) and 100% MeOH. TLC analysis of the eluates using a RP-18 plate with MeOH: H_2O (4:6) as mobile phase to give compound **3** (0.038g) and two other fractions. Compounds **1, 2** and **3** were subjected to quantitative evaluation of the antioxidant activity using the DPPH spectrophotometric assay as described above.

Table **1:** Radical scavenging activities of isolated compounds compared with quercetin.

Compl	Regression equation	R^2	IC_{50}
1	Y=1.4682x+10.849	0.9994	**26.67±0.21**
2	Y=8.4913x+4.596	0.9908	**5.35 ± 0.31**
3	Y=0.258x+2.0396	0.9789	**185.89 ± 1.02**
4	Y=11.487x+23.382	0.933	**2.32 ± 0.08**

Compound 1: ^{1}H NMR (200 MHz, DMSO). δ: 7.987 (2H, d, J=8.3 Hz, H-3′ and H-5′), 6.926 (1H, s, H-3), 6.905 (2H, d, J=8.3 Hz, H-2′ and H-6′), 6.455 (1H, d, J=0.977 Hz, H-8), 6.174 (1H, d, J=0.977 Hz, H-6). **^{13}C NMR** (50 MHz, DMSO), δ: 164.82 (C-2), 103.52(C-3), 182.43(C-4), 161.85(C-5), 99.53(C-6), 162.15(C-7), 94.65(C-8), 158.00(C-9), 104.39(C-10), 121.87(C-11), 116.64(C-12), 129.14(C-13), 164.43(C-14), 129.14(C-15), 116.64(C-16).

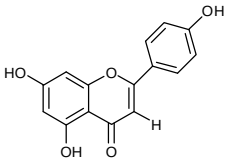
Compound 2: UV: 352, 280, ^{1}H NMR (200 MHz, DMSO). δ 7.343 (1H, d, J=8 Hz, H-5′), 6.89 (1H, dd, J=8 and 1.5 Hz, H-6′), 6.748 (1H, d, J=1.5 Hz, H-2′), 6.642 (1H, s, H-3), 6.42 (1H, d, J=1.8Hz, H-8), 6.17 (1H, d, J=1.8Hz, H-6). **^{13}C NMR,** (50 MHz, DMSO), δ: 164.3 (C-2), 103.3(C-3), 182.1(C-4), 161.9(C-5), 99.3(C-6), 164.6(C-7), 94.3(C-8), 157.7(C-9), 104.1(C-10), 122.9(C-11), 113.8(C-12), 146.2(C-13), 150.2 (C-14), 116.5 (C-15), 119.4 (C-16).

Compound 3: UV: 365, 268 ^{1}H NMR (200 MHz, DMSO), δ: 7.846 (2H, d, J=8 Hz, H-2′ and H-6′), 6.876 (2H, d, J=8 Hz, H-3′ and H-5′), 6.766 (1H, s, H-3), 6.766, (2H, d, J=1.8 Hz), 6.393 (1H, d, J= 1.8 Hz). **^{13}C NMR** (50 MHz, DMSO). δ: 164.74 (C-2), 103.17 (C-3), 182.37 (C-4), 161.39 (C-5), 100.00 (C-6), 162.23 (C-7), 95.03 (C-8), 157.36(C-9), 105.18(C-10), 120.33(C-11), 116.54(C-12), 128.84(C-13), 162.34(C-14), 128.84 (C-15), 116.54(C-16).

Discussion

Compound **1** was isolated as an amorphous powder. The UV spectrum of the methanolic solution gave two absorption bands at λ 266 and 365 characteristic of flavone UV absorption band II and band I respectively. The shift reagent studies gave a bathochromic shift of 36 nm in the NaOMe spectrum indicating a free 4′-hydroxyl group. The $AlCl_3$ spectrum indicated no ortho-dihydroxyl substitution pattern on ring B while addition of NaOAc to the methanolic solution of compound **1** gave a bathochromic shift of 36 nm indicating that 7-OH is free. The ^{1}HNMR spectrum showed 5 signals in all; an AB coupling, a signal at δ 7.90 (2H, d, J=8 Hz, H-3′,5′) was observed to be coupling with a signal at δ 6.9 (2H, d, J=8 Hz, H-2′, H-6′) suggesting a monosubstituted pattern on ring B. The singlet signal at δ 6.93 ppm integrating for 1 proton was assigned to H-3. A doublet at δ 6.17 ppm with coupling constant value of 0.977 Hz assigned to H-6 was observed to be coupling with the signal at 6.45 ppm (d, J= 0.977 Hz) assigned to H-8. The UV studies, NMR studies and comparison with literature values (Xiao *et al*, 2006) led to the characterization of compound **1** as apigenin. Compound **3** was isolated as an amorphous powder. The UV spectrum of the methanolic solution indicated a flavone with a substituted 7-OH group. The ^{1}H NMR and ^{13}CNMR spectra were similar to that of compound **1** apart from a doublet at δ 4.86 ppm, a doublet, (J=6.2 Hz) for an anomeric proton . The carbon signals showed additional four oxygenated methine carbon with one anomeric carbon and a carbonyl carbon at δ 172.52. Thus **3** was characterized as apigenin-7-*O*-β-D-

glucuronide. The UV studies of compound **2** indicated a flavone with an ortho dihydroxy group and a free 7-OH. The proton NMR spectrum showed 6 protons all in the aromatic region. In the COSY spectrum, the signal at δ 6.42 correlated with the signal at δ 6.17, a doublet with coupling constant of 1.8 Hz which is assignable to H-8 and H-6 respectively. A singlet at δ 6.64 was assigned to H-3.The signal at 7.3 ppm, a doublet, (J=8 Hz) assignable to H-5′ is coupling with a signal at δ 6.9, a doublet of doublet, (J=8 Hz and 1.5 Hz) assignable to H-6′. The signal at δ 6.7 (1H, d, J=1.5 Hz) is assignable to H-2′. In the HETCOR spectrum, there is a correlation between the proton at δ 6.17 and the carbon at δ 99.29 for H-6 and the proton at δ 6.43 correlated with the carbon at δ 94.31 for H-8. The singlet at δ 6.64 correlated with the carbon at δ 103.28. On comparison with literature values (Markham, 1982), compound **2** was therefore identified as luteolin. The radical scavenging activity of the flavonoids was evaluated as shown above. Luteolin with an ortho dihydroxy group on ring B has the highest activity compared with apigenin with no-di hydroxyl group. Glycosylation of the 4′-hydroxyl group on apigenin drastically reduced the radical scavenging ability as indicated from the IC_{50}. The presence of these compounds in *Solenostemon monostachys* may justify the ethnomedicinal uses of the plant.



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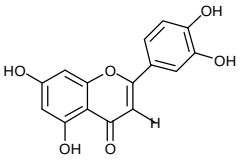

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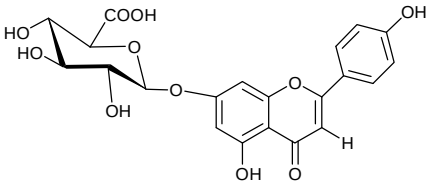

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[PS 20] Preliminary Evaluation of Anti-Diarrheal, Ulcer-Protective and Acute Toxicity of Aqueous Ethanolic Stem Bark Extract of *Ficus trichopoda* in Experimental Rodents

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Key words: Acute toxicity, anti-diarrheal, *Ficus trichopoda*, gastroprotective, loperamide, Uganda

Introduction

Genus Ficus belongs to Family Moraceae commonly referred to as fig trees. Forty-four species are known from Uganda (Berg and Hijman, 1989; Verdcourt, 1998). A number of Ficus sp. are used as food and for medicinal properties (Lansky et al., 2008). Several ficus species are traditionally used in African folk medicine in the treatment of many illnesses such as convulsions and respiratory disorders (Wakeel et al., 2004). The decoction of Ficus rhynchocarpa, Ficus sycomorus, Ficus natalensis and Ficus vasta are used to treat various stomach disorders. Several other reports have demonstrated different biological activities of Ficus plants including peptic ulcer treatment (Kokwaro, 1993; Akah et al., 1997; Mandal et al., 2000; Chiang et al., 2005; Kuetea et al., 2008; Rao et al., 2008; Singh et al.,2009). The reported medicinal uses of these plants includes: treatment of various gastrointestinal disorders, infectious diseases, fertility treatment and induction of labor (Kamatenesi-Mugisha and Oryem-Origa, 2007; Ssegawa and Kasenene, 2007). *Ficus trichopoda*, Baker, is a medicinal plant belonging to the Moraceae family used popularly as a 'multi-purpose' herb in Uganda. The aim of this study was to evaluate the anti-diarrheal, ulcer protective effects of 70% ethanolic extract of *Ficus trichopoda* stem bark (FTE) and its acute toxicity.

Materials and Methods

The laboratory animals used in these experiments were obtained from the laboratory animal facility of the department of Pharmacology and Toxicology of Kampala International University (KIU). The

stem bark and the leaves of F. trichopoda were collected in the month of June, 2009 in the morning. The plant was identified by a taxonomist at Makerere University Kampala and a voucher specimen deposited at Herbarium section of School of Pharmacy, Kampala International University. The stem bark was then ground into powder and was used for the subsequent experimentation. The powdered material was exhaustively extracted by cold aqueous ethanolic maceration for two days and the supernatant decanted. The entire process was repeated three times, and the extracts combined and filtered through Whatman No 1 filter paper. The crude extract was evaporated to dry powder at 40ºC in an oven.The anti-diarrheal effect was evaluated using castor-oil induced diarrhea model while anti-ulcer effect was evaluated using ethanol-induced ulcer model using rats. Loperamide and misoprostol were used as standard drugs for diarrhea and ulcer studies respectively. The extract was administered orally at three different doses of 125, 250 and 500mg/kg. Acute toxicity was evaluated by oral administration of the extract at 1000, 2000 and 4000 mg/kg body weight in mice. The extract exhibited a graded dose-dependent inhibition of the castor oil induced diarrhea.

Results

The onset-time and severity of diarrhea was significantly reduced ($p<0.05$). Anti-diarrhea activity exerted was significant at 250mg/kg (66.67% inhibition) and maximal at 500 mg/kg (88.89% inhibition). The inhibition at 500mg/kg compared well with the standard drug, Loperamide which produced 100% inhibition of diarrhea in rats. Also, oral administration of FTE produces a dose-dependent inhibition of ethanol-induced gastric ulcer with maximal effect at 500 mg/kg (49.05%). The oral LD50 value obtained was >4000 mg/kg in mice. Preliminary phytochemical screenings indicated the presence of reducing sugar, alkaloids, saponnins, pyrocathecolic tannins and free amino acids/amines.

Table 1: Effect of aqueous ethanolic extract of FTE on ethanol-induced ulceration (125-500mg/kg, p.o)

Treatment	**Dose**	**Ulcer index (mm)**[1]	**% Inhibition of ulceration**[2]
Ficus trichopoda	125mg/kg	80.90±17.23	1.63
Ficus trichopoda	250mg/kg	58.80±18.68	28.50
Ficus trichopoda	500mg/kg	41.90±19.23	49.05
Misoprostol	400µg/kg	16.00±6.78	80.54
Distilled water	10ml/kg	82.24±13.3	-

[1]: Values expressed as Mean±S.E.M . (n = 5); $p<0.01$ vs. control (One way ANOVA); [2]: Compared with saline control

Table 2: Effect of ethanolic extract of FTE on castor oil-induced diarrhea and loss in body weight (125-500mg/kg, p.o)

Treatment	Dose	Loss in B Wt (g)[1]	Diarrhea Score			Total Score	Percentage Inhibition[2]
			++	+	0		
Ficus trichopoda	125mg/kg	11.22±3.14	3	1	1	7	22.22
Ficus trichopoda	250mg/kg	9.98 ±3.17	0	3	2	3	66.67[3]
Ficus trichopoda	500mg/kg	7.88 ±4.13	0	1	4	1	88.89[3]
Loperamide	400µg/kg	6.84 ±4.67	0	0	5	0	100[3]
Distilled water	10ml/kg	7.04 ±1.70	4	1	0	9	-

[1]: Values expressed as Mean±S.E.M. (n = 5); [2]: Compared with saline control; [3:] Denotes statistical significance between treated groups and control.(One way ANO VA $p<0.01$); BWt: body weight

Discussion

The aim of the present study was to assess the effect of an aqueous ethanolic extract of FTE on diarrhea and ulcer using castor oil induced diarrhea and ethanol induced ulcer model respectively. Castor oil is known to cause diarrhea due mainly to the presence of ricinoleate which account for about 90% composition of the oil (Mckeon *et al*., 1999). Ricinoleate is known to cause increases in peristaltic activity of the small intestine and thus alters the permeability of Na+ and Cl- in the intestinal mucosa (Palombo, 2006). Stimulation of the release of endogenous prostaglandin has also been found to be associated with ricinoleate found in castor oil (Zavala *et al*., 1998). Acute toxicity studies showed that the plant extract is relatively safe at high doses which may not necessarily be reached in human usage. The acute toxicity study on the FTE revealed that it is relatively safe. Figs as a fruit have a very high safety profile.

Bafor and Igbinuwen (2009) demonstrated that the aqueous leaf extract of *Ficus exasperata* to be relatively safe in both 24 h and 14 days oral administration. However, research into the safety profile of ethanolic extract of *Ficus natalensis* administered orally at doses of 100 and 500 mg/kg body weight to rats for 14 days produces a significant toxicity to the liver (Kinyi and Balogun, 2009). Ficus Pretreatment of the rats in this present study with FTE exhibited significant dose-dependent antidiarrheal activity. The effect observed was similar to the standard drug, Loperamide at 3 mg/kg. This is in concordance with findings by Mandal and Kumar, 2002), Ahmadua *et al*. (2007) who demonstrated anti-diarrheal effects of *Ficus hispida* and *Ficus sycomorus*, respectively. Studies have shown that antidiarrheal properties of many plants stem from the presence of tannins, alkaloids, saponins, flavonoids, sterol and/or triterpenes and reducing sugars (Longanga *et al*., 2000; Adzu *et al*., 2003). Tannins for example are known to reduce secretion and make the intestinal mucus resistant through the formation of protein tannate (Tripathi, 1994). We may plausibly explain that

the anti-diarrheal effect observed may be due to the presence of these various phytochemicals in the extract. However, pretreatment of the rats with FTE did evoke a dose dependent inhibition of ethanol-induced gastric erosion and hemorrhage (1.63, 28.5 and 49.05% for 125, 250 and 500 mg/kg, respectively), but these observations were not statistically significant as compared to the control. It should be noted also that although the standard drug had an inhibition of 80.54% but this difference was not statistically significant either as compared to the control. The import of these findings is the need to assess the anti-ulcer activity of FTE using other *ulcer models.*

Conclusion

This study confirmed the antidiarrheal properties of this plant as it is used in traditional medicine. It has exploitable ulcer protective effects and is considerably safe on oral administration.

Acknowledgement

We are grateful to KIU-CAMRES for the financial support provided. We also declare no conflict of interest.

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[PS 21] Phytochemical and In Vitro Antimicrobial *Echinops Hispidus* Fresen

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Key words: *Echinops hispidus*, terpenoids, furofuran lignans, thiophenes, antimicrobial

The genus *Echinops* belongs to the family Asteraceae and comprises of 120 species. *E. hispidus* is widely distributed in Kenya and it is used by the Kipsigis in the treatment of bacterial infections. This work reports the isolation and anti-microbial studies of the sesquiterpenoid; cameroonan-7α-ol (**1**), the triterpenoid; acetyltaraxerol (**2**), furofuran lignans; membrin-8α-ol (**3)** and membrin-8β-ol (**4**) and polyacetylene thiophenes; 2-Octyl-5-(3,4-dihydroxybut-1-enyl)thiophene (**5**), [4-[5-(penta-1,3-dieynyl) thien-2-yl] but-3-ynyl alcohol (**6**), 2-(Penta-1,3-diynyl)-5-(3,4-dihydroxybut-1-ynyl)thiophene (**7**). Compounds **1, 2, 6** and **7** are being reported for the first time from this plant while **3, 4** and **5** have not been reported before. Compounds **5** showed moderate activity against *Staphylococcus aureus* while **6** and **7** exhibited high antibacterial activities against *S. aureus.* Compounds **5** - **7** showed no activity against *Pseudomonas aeruginosa* and *Eschelicia colli.* Compounds **1-4** exhibited no anti-microbial activities against *S. aureus, E. coli, P. eruginosa* and *Cryptococcus neoformans.* Compound **5** showed no antifungal activity but compound **6** and **7** exhibited very strong antifungal activities against *C. neoformans.* The isolated compounds **5 – 7** showed high antifungal activities against *C. neoformans*. These biologically active compounds are templates for synthesis of more potent and water soluble derivatives. These antimicrobial results support the use of *E. hispidus* for the treatment of antimicrobial related ailments by the Kipsigis in Kenya.

[PS 22] Efficacy and safety profile of some Ugandan antimalarial herbs used in Primary Health Care

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Key words; efficacy, safety, antimalarial, plants,Uganda

Introduction

Plasmodium falciparum malaria is one of the most important parasitic diseases affecting Sub-Saharan Africa. It is the most prevalent infection in Uganda, despite the availability of interventions (MoH, 2007). There is widespread resistance to the available antimalarial agents, the effective of which are too expensive for the great majority of patients, hence the need for new antimalarial drugs (MoH/MCU, 2003, 2007). In this study the efficacious and safe extracts of the commonly used medicinal plants that can be used as sources or templates in development of new antimalarial drugs were investigated.

Materials and methods

The petroleum ether, chloroform and ethanol plant crude extracts were subjected to *in vitro* screening against *Plasmodium falciparum* using the nitro-tetrazolium blue based plasmodium lactate dehydrogenase (pLDH) assay (Markler et al., 1993). Toxicity profile was done on the antiplasmodialy active extracts (Lorke D. (1983). Selected extracts were orally administered in albino mice for acute toxicity and Wister rats for subacute toxicity tests.

Results and Discussion

In the antiplasmodial activity tests a number of plants showed high sensitivity towards *P. falciparum* parasite while others were not active. The chloroform extract of *M. lanceolata* (EC_{50} 1.60 µg ml), showed the highest antiplasmodial activity followed by *R. natalensis* (EC_{50} 1.80 µg ml) as presented in Table 1 below.

Table 1*: In vitro* antiplasmodial activity (IC_{50}) of extracts of selected plants used in the treatment of malaria in western Uganda

Plant species	IC_{50} median values (µg/ml) and percent yield of the extracts					
	PE	% yield	$CHCl_3$ 02	% yield	EtoH	% yield
M. Lanceolata	>50.0	1.7	1.6	2.6	11.4	7.0
E. suaveolens	>50.0	2.8	>50.0	2.9	>50.0	4.2
Conyza Sp.	>50.0	0.8	9.1	1.5	20.5	5.8
R. natalensis	>50.0	1.2	1.8	2.8	6.6	3.7
L. trifolia	13.2	0.8	>50.0	3.4	>50.0	4.4
T. asiatica	6.6	1.0	22.4	2.5	>50.0	5.5
B. longipes	>50.0	2.4	3.7	2.7	50.0	3.9
T. bakeri	3.9	0.6	>50.0	2.1	33.2	6.6
I. emerginella	38.0	1.0	25.3	1.5	5.8	2.1
V. lasiopus	43.9	0.8	>50.0	1.3	>50.0	1.9

PE-Petroleum ether; $CHCl_3$-Chloroform; EtoH-Ethanol; EC_{50}-Effective concentration

The study showed no acute toxicity with all the plants except *B. longipes* which showed slight toxicity (LD_{50} of 4.70 g/kg) (Table below) The biochemical parameters related to liver function tests, kidney function tests and haematological analysis of the treated animals showed no significant change compared to the control group. There was no significant change observed in organ weight/body weight ratios in treated groups as compared to the control group while only slight histological changes were observed in some organs of the high dose treated animals after 28 days of oral administration.

Table 2: Summary of the effect of the different dose levels of the chloroform extract of *B. longipes* and chloroquine on the different haematological parameters of Wister rats after 28 days of treatment

GROUP	WBC	LYMP	MONO	GRAN	Hb	RBC	PLT
High dose	9.83±2.51[a]	48.0±11.41[a]	42.53±15.54[a]	9.433±4.36[a]	9.913±0.48[a]	15.80±0.32[a]	494±62.08[a]
Median dose	6.30±1.23[a]	57.10±7.30[a]	26.87±4.64[a]	15.97±3.32[a]	7.437±1.47[a]	13.87±1.13[a]	559±225[a]
Low dose	8.937±2.30[a]	54.5±10.83[a]	14.33±8.24	16.8±3.41[a]	9.49±0.20[a]	15.53±0.46[a]	633±133.9[a]
Chloroquine	13.57±1.28	67.5±6.7	19.43±7.73	13±1.13	9.053±0.53	15.07±0.62	339±76.95

[a]P > 0.05 at 95% confidence level. Normal values for the Wister Albino rats are: White Blood Cells, (6.325±0.0029); Lymphocytes, (70.95±0.3175); Granulocytes, (3.15±0.259); Haemoglobin, (8.024±0.321); Red Blood Cells, (15.88±0.266); Monocytes, (11.64±1.381).

The toxicity studies suggest that these plants maybe safe in humans if used at controlled doses. The plants can also be further investigated for identification of simple antiplasmodial active molecules that can be synthesized in the laboratory. However it should be noted that experience in malaria

control programmes has shown that *in vitro* tests of parasite susceptibility to antimalarial drugs cannot substitute for *in vivo* observations on malaria therapy, some compounds that show *in vitro* activity may not possess *in vivo* activity due to pharmacokinetic and immunological factors (Waako *et al*., 2005; WHO, 2001). Hence the need to carry out clinical trials on these active extracts.

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