UNIVERSITY OF NAIROBI

CHEMOSYSTEMATICS, ANTIMICROBIAL AND ANTIPROLIFERATIVE ACTIVITIES OF THE KENYAN *ZANTHOXYLUM* SPECIES AND *DODONAEA VISCOSA* POPULATIONS

BY

KAIGONGI MUTHONI MAGRATE

BSc (1st Class Honors), MSc (University of Nairobi)

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DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination or award of a degree. Where other people's work has been used, this has been properly acknowledged and referenced in according with the University of Nairobi's requirements.

Signature : ..... **Date:** 2/12/2020

Kaigongi Muthoni Magrate
(Reg. No. I80/52651/2018)
School of Biological Sciences
University of Nairobi

This PhD thesis is submitted for examination with our approval as research supervisors

Signature: ..... **Date** 2/12/2020

Dr. Catherine Lukhoba
School of Biological Sciences
University of Nairobi
P.O. Box 30197
NAIROBI KENYA
Email: clukhoba@uonbi.ac.ke

Signature: ..... **Date** 02/12/202

Prof. Abiy Yenesew
Department of Chemistry
University of Nairobi
P.O. Box 30197
NAIROBI KENYA
Email: ayenesew@uonbi.ac.ke

DEDICATION

This thesis is dedicated to my dear husband Dr. Fredrick Musila and children Ethan and Noreen in gratitude for their patience, sacrifices and support they accorded to me during this study. Their sacrifice gave me the much needed moral support to pursue this study.

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LIST OF ACRONYMS

ANOVA:	Analysis of Variance
CC:	Column Chromatography
CMR:	Centre for Microbiology Research
COSY:	Correlation spectroscopy
CTMDR:	Centre for Traditional Medicine and Drug Research
DCM:	Dichloromethane
DMSO:	Dimetyl sulfoxide
ESIMS:	ElectroSpray Ionization Mass Spectrometry
EtOAC:	Ethylacetate
HMBC:	Heteronuclear Multiple Bond Correlation
HPLC:	High Pressure Liquid Chromatography
HSQC:	Heteronuclear Single Quantum Coherence
IR:	Infra Red
KEFRI:	Keny Forestry research Institute
KEMRI:	Kenya Medical Research Institute
LC-MS:	Liquid Chromatography-Mass Spectrometry
MDR:	Multi drug resistance
MeOH:	Methanol
MIC:	Minimal Inhibition Concentration
MRSA:	Methicilin Resistant Staphylococcus aureus
NMR:	Nuclear Magnetic Resonance
PTLC:	Preparative Thin Layer Chromatography
SBS:	School of Biological Sciences
SD:	Standard Deviation
TLC:	Thin Layer Chromatography
UoN:	University of Nairobi
UV:	Ultra Violet
WHO:	World Health Organization
ZCB:	*Zanthoxylum chalybeum* stem bark
ZCL:	*Zanthoxylum chalybeum* leaves
ZCR:	*Zanthoxylum chalybeum* root bark
ZGB:	*Zanthoxylum gilletii* stem bark
ZGL:	*Zanthoxylum gilletii* leaves
ZGR:	*Zanthoxylum gilletii* root bark
ZHB:	*Zanthoxylum holtzianum* stem bark
ZHL:	*Zanthoxylum holtzianum* leaves
ZHR:	*Zanthoxylum holtzianum* root bark
ZPB:	*Zanthoxylum paracanthum* stem bark
ZPL:	*Zanthoxylum paracanthum* leaves
ZPR:	*Zanthoxylum paracanthum* root bark
ZUB:	*Zanthoxylum usambarense* stem bark
ZUL:	*Zanthoxylum usambarense* leaves
ZUR:	*Zanthoxylum usambarense* root bark

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ABSTRACT

The aim of this study was to establish the relationship in *Zanthoxylum* species, and *Dodonaea viscosa* populations based on the metabolite profiles. The study also included the evaluation of the crude extracts and pure compounds obtained from *Zanthoxylum* and *Dodonaea* species for antimicrobial activity and cytotoxicity.

The chemosystematics study was carried out using metabolomics approach for *Zanthoxylum* species and *D. viscosa* populations in Kenya. Through phytochemical investigations some compounds were isolated and characterized with eight compounds from *Z. paracanthum* (myristic acid (**1**), stigmasterol (**2**), sesamin (**3**), 8-acetonyldihydrochelerythrine (**4**), arnottianamide (**5**), 10-methoxycanthin-6-one (**6**), canthin-6-one (**7**) and 8-oxochelerythrine (**8**)), from *D. viscosa* subsp *viscosa* three compounds (dodonic acid (**9**), 5,7,4',5'-tetrahydroxy-3,6,2'-trimethoxyflavone (**10**) and hautriwaic acid lactone (**11**)) and from *D. viscosa* subsp *angustifolia* one compound (catechin (**12**)) were isolated by the use of a combination of chromatographic techinques. These compounds were evaluated for antimicrobial activity and cytotoxicity along with crude extracts.

Agar well and broth dilution were used to test antimicrobial activity of root, stem and leaf extracts of five *Zanthoxylum* species (*Z. chalybeum, Z. gilletii, Z. holtzianum, Z. paracanthum* and *Z. usambarense*) as well as leaf extracts of ten populations of *D. viscosa* (Coastal, Makueni, Machakos, Karura, Nanyuki, Maimahiu, Narok, Loita Forest, Marigat and Homa bay) were tested against six microbes (*Bacillus cereus* Methicilin Resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Aspergillus flavus*). Eight compounds isolated from *Z. paracanthum* as well as the four compounds isolated from *D. viscosa* were evaluated for antimicrobial activity against methicilin resistant *Staphylococcus aureus* (MRSA), typed *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans.* The positive controls were omacilin 1000µg/ml for bacteria and fluconazole 1000µg/ml for fungi. The antiproliferative activity of the eight compounds isolated from *Z. paracanthum*, and three compounds from *D. viscosa* as well as the crude extracts (*Z. paracanthum* roots, *D. viscosa* subsp *viscosa* and *D. viscosa* subsp *angustifolia* leaves) was determined using MTT assay against kidney normal cell line from African green monkey (E6 vero), human breast cancer cell line (HCC 1395) and human prostate cancer cell line (DU 145).

Chemometric analysis of relatedness of *Zanthoxylum* group of plants conformed to the current classification of *Zanthoxylum* species in Kenya established on morphology. Based on phytochemical production, the five *Zanthoxylum* species segregated into two clusters; cluster one was composed of *Z. gilletii Z. usambarense* and *Z. paracanthum* while *Z. chalybeum* and *Z. holtzianum* constituted cluster two. On the other hand, analysis of *D. viscosa* populations showed that Nanyuki, Machakos and coastal collections were found to be closely related while Narok and Nairobi populations were found to be related. This is in contrast with the existing morphological classification of *D. viscosa* where the coastal population is believed to be composed of *D. viscosa* subsp *viscosa* and the Kenyan inland populations are composed of *D. viscosa* subsp *angustifolia*.

The antimicrobial activity results of *Zanthoxylum* species showed that the root bark extracts were most active followed by leaf and stem bark extracts respectively. The extracts from the root and stem bark of *Z. paracanthum* were the most active compared to root and stem bark extracts of other species while leaf extract of *Z. usambarense* was the most active compared to leaf extracts from other *Zanthoxylum* species. On the other hand, Nanyuki population recorded the highest antimicrobial activity overall followed by Coastal population while Homabay, Makueni and

Machakos populations followed in that order. Leaf extract from Karura population was the least in terms of antimicrobial activity. The eight compounds isolated from *Z. paracanthum* roots, canthin-6-one (**6**) was the most active followed by 10-methoxycanthin-6-one (**7**) while myristic acid (**1**) and arnottianamide (**5**) did not show any activity against the tested microbes. Hautriwaic acid lactone (**11**) was the most active compound from *D. viscosa* Subsp *viscosa* while 5,7,4',5'-tetrahydroxy-3,6,2'-trimethoxyflavone (**10**) did not show any activity against the tested microbes. Catechin (**12**) on the other hand exhibited good activity against the tested microbes.

In the order listed below, stigmasterol, Sesamin, 8-oxochelerythrine, canthin-6-one, 8-acetonyldihydrochelerythrine, hautriwaic acid lactone, root bark extract of *Z. paracanthum* and 10-methoxycanthin-6-one showed antiproliferative potential against HCC 1395. While antiproliferative potential against DU 145 was highest in 10-methoxycanthin-6-one followed by canthin-6-one, 8-oxochelerythrine and leaf extract of *D. viscosa* subsp *angustifolia.*

Chemosystematics of *Zanthoxylum* spp conformed to the morphological classification while that of *D. viscosa* disagreed. This study reports isolation of myristic acid, stigmasterol, 8-acetonyldihydrochelerythrine, arnottianamide, 10-methoxycanthin-6-one and 8-oxochelerythrine from *Z. paracanthum* for the first time while 5,7,4',5'-tetrahydroxy-3,6,2'-trimethoxyflavone is reported for the first time in *D. viscosa*. Canthin-6-one, 10-methoxycanthin-6-one and 8-oxochelerythrine were all broad spectrum in terms of antimicrobial and antiproliferative activities. This study provides scientific evidence for the popularity of *Zanthoxylum* spp and *D. viscosa* as traditional medicine plants.

This study recommends invivo studies of the most active compounds (canthin-6-one, 10-methoxycanthin-6-one, hautriwaic acid lactone and catechin) isolated in this study as well as increased scope to cover other microbes of economic importance and other cancer cell lines. Additionally, revision of the current classification of *D. viscosa* using modern taxonomy tools such as molecular phylogenetics and chemosystematics is recommended.

Key words; Chemosystematics; metabolomics; phytochemicals; antimicrobial; antiproliferative; *Zanthoxylum*; *Dodonaea*

CHAPTER ONE

GENERAL INTRODUCTION

1.1. Background information

Exploitation of plants for food and medicine since antique times and is passed in communities from generation to generation (Raven, 2004). Traditional medicine is refered to by World Health Organization (WHO) to be constituted of knowledge, approaches, health practices as well as beliefs incorporating plants among other therapies in treatment, diagnosis and prevention of illnesses or maintenance of wellbeing (WHO, 2002). In low income countries, 80% of the populace rely on traditional remedies for healthcare needs (Ekor, 2014).

Africa is endowed with a great flora biodiversity with 25% of higher plants globally being found in sub-Saharan Africa (Linder, 2014). This unique resource is barely exploited for the pharmaceutical and agrochemicals industries despite Africans strong religio-cultural usage of herbal medicines in handling common infectious illnesses and other sicknesses (Fabricant & Farnsworth, 2001). African communities need to be endowned with scientific knowledge on the utilization and protection of medicinal which can improve the socio-economic circumstances, such as the high unemployment rate, especially in low-income rural areas (Okigbo & Ogbogu, 2008).

In Kenya, medicinal plants are utilized by all cadres of persons to treat different ailments including cancer, diabetes and HIV/AIDS (Mwangi *et al*., 2005; Mwangi & Gitonga, 2015). Herbal clinics that are known to provide treatment in Kenya include; Murugu, the famous Babu Loliondo, Makini (Nyandiba, 2013). Similar to most countries in Africa, Kenya is composed of multiple ethnic groups and each group has distinct beliefs and traditional knowledge including

utilization of medicinal plants (Kigen *et al*., 2013). Despite the extensive usage of herbal medicines in Kenya, little effort has been applied to document and scientifically evaluate the potency of these plants against the diseases they are known to treat (Kigen *et al.*, 2013). Like other low income countries, dosage and safety remains to be a key challenge associated to herbal remedies in Kenya (Rukangira, 2001).

Zanthoxylum and *Dodonaea* species are widely distributed in Kenya with wide usage in herbal medicine in Kenya, as in other parts of the globe. *Zanthoxylum* species have various ethnomedicinal uses reported such as management of gastrointestinal disorders, malaria, lung diseases, gonorrhea, tumours, rheumatism, dental pains, tumours, convulsion, diabetes, skin diseases, helmintic infections in humans and animals. Members of this genus are also used as aphrodisiac, analgesic, febrifuge, stimulant, tonic, and homeastatic agents (Abbasi *et al*., 2013; Aiyeloja & Bello, 2012; Ibrar & Hussain, 2009; Kaigongi & Musila, 2015; Kala, 2007). Additionally, some species used as pesticides, textile dyes and building materials (Okogun, 2002). Similarly, *Dodonaea* species have a rich ethnobotanical uses as they have been used by many cultures across the world as a medicinal species, and and are used for the treatment of sicknesses like arthritis, snake bites, haemorrhoids, fractures and also inflammation (Venkatesh *et al*., 2008).

There is currently significant taxonomic confusion in *D. viscosa* and *Zanthoxylum* species due to the classification criteria shifting from a purely morphological to the more popular molecular phylogenetic and chemosystematics utilised to delineate taxa (Brizicky, 1962; Mahar *et al*., 2017; Waterman, 1975). The significance of proper identification of medicinal plants is always emphasized because it is key in connecting the ethnobotanical uses with the existing chemical and biological knowledge (Joharchi & Amiri, 2012). Thus, to be sure of the efficacy, safety and therapeutic potency of herbal remedies resulting from *Dodonaea* and *Zanthoxylum* species, right

identification of plants has to be practiced using current classification. This study aims at solving the challenges encountered in the classification of *D. viscosa* and *Zanthoxylum* species in Kenya using metabolomics as a tool for chemistematics.

There is need to conduct studies aimed at identifying and characterizing plant substances against multidrug resistance and cancer infections (Levy & Marshall, 2004). In a previous work, (Kaigongi *et al*., 2014), the organic crude extract of *Z. chalybeum* stem bark showed high inhibition against *Pseudomonas aeruginosa, Bacillus cereus,* MRSA and *Candida albicans* with toxicity against Brine shrimp larvae. Further investigations need to be carried out based on previous reports on *Zanthoxylum gilletii* (Gaya, 2011), *Z. usamabarense* (Were *et al*., 2010) and *Z. holtzianum* (Buyinza, 2012), *Z. paracanthum* (Omosa *et al*., 2019) and *D. viscosa* (Omosa *et al*., 2014). The results of detailed chemosystematics, antimicrobial activity and toxicity studies of *Dodonaea viscosa* and *Zanthoxylum* species in Kenya is reported here.

1.2 Problem statement

Zanthoxylum species and *Dodonaea viscosa* are used in traditional medicine to manage ailments ranging from bacterial, viral and fungal diseases to conditions such as cancer and malaria (Patiño *et al*., 2012; Sankaranarayanan *et al*., 2010). However, the classification of the Kenyan *Zanthoxylum* species and the different subspecies of *Dodonaea viscosa* is based on their morphological characters and is reported to be confusing due to lack of clear –cut morphological synapormophies (Cao *et al*., 2014). Similarly, the occurence of synonymy within subspecies of *D. viscosa* and *Zanthoxylum* species has also made it difficult to place some taxa to the right positions and assemble together their uses (Appelhans *et al*., 2018). Use of chemical characters has been

applied successfully as additional taxonomic characters or to support the classification based on morphological characters, though none have been used in these two genera (Kim *et al*., 2016).

There has been a global development of conventional medication to manage illnesses such as cancer and microbial infections. Nevertheless, treating cancer in Kenya ranges from Ksh 6,000 (US$ 60) to Ksh 600,000 (US$600) per treatment course, making it a financial drain for most households (Atieno *et al.* 2018). In addition the chemotherapy drugs are rendered ineffective by the tendancy of cancer–inducing microbials as well as the resistance developed by the cancerous cells, which is causing a major challenge (Prakash *et al*., 2015). It has been recorded that most victims have recurrence of microbial infections and cancer even after a prior successful treatment using chemotherapy (Saraswathy & Gong, 2013). Natural products have thus been suggested as leads to overcome MDR in cancer cells and microbial infections (Kuete *et al*., 2017).

The current study therefore aimed at conducting a chemosystematics of species of *Zanthoxylum* and populations of *D. viscosa* in Kenya, determining their chemical profiles as well as evaluation of their antimicrobial and antiproliferative potencies.

1.3. Justification

Zanthoxylum is a genus known to produce various metabolites like amides, flavonoids alkaloids, terpenes, sterols as well as lignans which can be applied in the classification of this genus (Negi *et al*., 2011). *Dodonaea* genus is also known to produce an array of metabolites such as saponins, terpenes as well as complex mixtures of phenolics (Al-Snafi, 2017). Many of these metabolites may be the reason why *Zanthoxylum* and *Dodonaea* species are effective in curing common ailments like diarrhoea, vomiting, malaria, abdominal pains, rheumatism, colds, snake

bites, inflammation, tumors and dental problems (Patiño *et al*., 2012; Sankaranarayanan *et al*., 2010).

The synonymy in the two genera is an indication of the high level of usage in traditional medicine as seen in another genus - *Plectranthus* (Lukhoba *et al*., 2006). In order to properly utilise *Zanthoxylum* and *Dodonaea* species medicinally, the taxonomic confusion in these taxa should be resolved through generation of different types of data: morphological, anatomical, molecular and biochemical data. Identification of the most effective biochemicals from these plants and subsequent evaluation of their potential as antimicrobial and anticancer agents, shall contribute in solving the increasing cases of multiple drug resistant microbial infections and cancer

1.4. Hypotheses

HO: There are no differences in the types and concentrations of secondary metabolites produced within *Zanthoxylum* species and *Dodonaea viscosa* populations in Kenya. The extracts and chemicals isolated from *Zanthoxylum* species and *Dodonaea viscosa* varieties have no antimicrobial and antiproliferative activities.

HA: There are differences in the types and concentrations of secondary metabolites produced within *Zanthoxylum* species and *Dodonaea viscosa* populations in Kenya. The extracts and compounds isolated from *Zanthoxylum* species and *Dodonaea viscosa* varieties have antimicrobial and antiproliferative activities.

1.5. Objective

1.5.1. General Objectives

To determine chemosystematics values, *in vitro* antimicrobial activity and cytotoxicity of the extracts and compounds of the Kenyan medicinal plants *Dodonaea viscosa* and *Zanthoxylum* species.

1.5.2. Specific objectives

1. To establish the chemosystematics values of the constituents of *Zanthoxylum* species and *Dodonaea viscosa* populations through metabolomics.
2. To evaluate *in vitro* antimicrobial activity of the extracts and compounds from *Zanthoxylum* and *Dodonaea* species.
3. To isolate compounds with antimicrobial activity from *Zanthoxylum* and *Dodonaea* species.
4. To determine cytotoxicity of crude extracts and isolated compounds against normal and multi-drug resistant breast and prostate cancer cell lines.

1.6. Relevance of the study

This work has characterized chemicals that exhibit activity against microbes of economic importance. Some of the chemical compounds isolated showed significanct potency against cancer cell lines, with higher selectivity index compared to doxorubicin (a cancer chemotherapy drug). The study has also generated chemical profiles (diversity, variation and abundance) of compounds occuring in the Kenyan species of *Zanthoxylum* and *D. viscosa* populations that may provide an alternative criterion to help increase the taxonomic discrimition leading to better inter- and intra-specific resolution in the two groups of plants. Additionally, the study has also established a scientific basis for the utilization of members of these genera in traditional medicine by linking the identified active compounds therein to their related pharmacological activities. There is a high possibility therefore that this may lead to possible antimicrobial and anticancer drug discoveries.

1.6. Thesis structure

The work in this thesis is distributed in eight chapters. Chapter one provides the general introduction of the study and chapter two addresses the general literature review. The next four chapters are standalones on the basis of the specific objectives; chapter three addresses the general material and methods, chapter four chemosystematic studies of *Dodonaea viscosa* populations and *Zanthoxylum* species in Kenya using a metabolomics approach, chapter five covers the phytochemical evaluation of extracts from root bark and leaves of *Zanthoxylum paracanthum* and *Dodonaea viscosa*, respectively, chapter six addresses the antimicrobial activity of extracts of five *Zanthoxylum* species and leaves of different *Dodonaea viscosa* populations and the compounds isolated in chapter five while chapter seven covers the antiproliferative activity of the isolated compounds in chapter five and the crude extracts from which these compounds were isolated. Chapter eight provides the general discussions of the entire study together with conclusions and major recommendations. Havard referencing style has been used throughout this document using Mendley.

CHAPTER TWO

LITERATURE REVIEW

2.1. Use of medicinal plants of the world

The use of plants as source of food and medicine is recorded to be since old ages (Cotton & Wilkie, 1996). It is estimated that there are between 250,000 and 500,000 plant species globally with approximately 1-10% of these being used as food and medicine (Houghton, 1995). Much of information from the antique period, is also determined to be concurrent with the utilization of traditional medicine in many countries (Farnsworth, 1994). In the developed nations, traditional medicine is increasing with approximately 40% of the populace recording herbal medicine usage (Demma *et al*., 2009).

Herbal healers have offered treatment which has sustained primary health care in Africa since the antique times (Evans, 2002; Houghton, 1995). Herbal medicine is excercised by people of different categories in Kenya due to its accessibility and affordability (Kigen *et al*., 2013) either to treat an illness or to boost ones immunity as a preventive measure to different kinds of ailments (Mothupi, 2014). Scientific evaluation of plants for the ailments they are claimed to treat in herbal medicine is required to provide a scientific based evidenc of plants uses in Kenya as this remains one of the challenges facing herbal medicine in Kenya (Kigen *et al*., 2013).

2.2. Chemotaxonomy as a tool for plants classification

Chemotaxonomy is the classification of plants based on comparisons of biochemical composition of the plants in question (Singh, 2016). Plants produce secondary metabolites to help them defend themselves against natural enemies such as herbivores and diseases as well as to act

as signals in attracting pollinators and seed dispersing animals (Schorr *et al*., 2006). Taxonomy of plants has evolved over the years leading to many approaches such as anatomical, morphological, chemotaxonomic and molecular classification. Anatomy and morphology are recorded to be utilized in traditional classifications whereas chemosystematics and molecular phylogenetics are utilized in the modern classification of plants (Schorr *et al*., 2006). The secondary metabolites structures and their production pathways are mainly restricted and specific to taxonomically interrelated plants and are therefore useful in classification (Singh, 2016).

The alkaloids, phenolics, terpenes and non-protein amino acids, are the major significant classes of compounds applied in chemotaxonomic classification. These compounds show a range of differences in chemical distribution, diversity and function (Larsson, 2007). The chemotaxonomic classification of plants is used as a prerequisite to successful natural products research as it improves the existing system of plants differentiation and develops the present day knowledge of natural relationship of plants (Elias *et al*., 2018). There is therefore a need to carry out chemosystematics studies of taxonomically confusing groups of plants such as *Zanthoxylum* and *Dodonaea* genera as well as profile metabolites present in such plants.

2.3. Bioactive phytochemicals

This is defined as compounds with a potential of interacting with constituent(s) of a living tissue to cause an effect (Guaadaoui *et al*., 2014). Depending on characteristics and structures, phytochemicals are grouped into six key classes; alkaloids, phenolics, terpenoids, lipids, carbohydrate, and nitrogen-containing chemicals. Biosynthesis pathways gives further divisions in each class (Scalbert *et al*., 2011).

Recently, the word ‘phytochemical’ has been applied to denote plant compounds different from essential nutrients. Various phytochemicals are known to induce effects in biological systems, including humans; thus the term ‘bioactive phytochemicals’. Leitzmann (2016) defined phytochemicals as non nutrient chemicals in plants connected to minimizing the threat of key non communicable long-lasting ailments.

2.4. Antibiotic resistance

Microbial infections are the leading cause of mortalities globally with approximately 50,000 deaths daily (Somsundaram, 2018). Antibiotic resistance by most microbes causing infections is on the rise globally due to unselective usage of antibiotics (Levy, 2002). This has further made it difficut in treating communicable diseases especially in persons with low immunity like in cancer and HIV/AIDS patients (Miceli *et al*., 2011). The rise in multiple drug resistance by microbes of medical importance against antibiotics such as cephalosporins, penicillins and chloromphenical is due to reduced permeability or increased efflux of antibiotic material from the cells as well as enzymatic inactivation (Blair *et al*., 2015).

Exploration of novel drugs from plants is on the rise each day to counter the development of new infections and disturbing side-effects from conventional medicine. These novel drugs are likely to be sourced from various plants (Kumar *et al*., 2000).

2.5. Cancer causes and development

The main causes of cancer include; genetics, age, exposure to carcinogens and weight (Macharia *et al*., 2019). Tobacco smoke, chemicals, automobile exhaust pollutants, occupational exposure to mutants and carcinogens, bacterial or viral infections and radiation such as ultraviolent rays from the sun are some examples of cancer-causing environmental exposures

(Lemkebthomas *et al*., 2008). Cancer develops once the orderly processes that regulate the growth and life span of normal cells go amiss or when cells loose responsiveness to growth regulators. The abnormal cell multiplies and gives rise to a clone of mutated daughter cells (Gogvadze *et al.,* 2008). Under normal circumstances, cells multiply and split to make fresh ones as necessitated by the body and once they grow old are spoilt, they decease and new cells take their position (Stille, 2006). In cancer development, this well-ordered process ceases and the cells continually become abnormal due to existence of old or damaged cells and formation of new cells when not needed leading to formation of tumors (Weinberg, 1996).

Cancer development is a multistep process that causes damage to the cell's DNA leading to an irreversible change in the cell division process (Farber, 1984). When the cells have been mutated by an initiator, they become susceptible to the effects of promoters. As a result, these compounds lead to cell proliferation hence raising a large number of daughter cells containing the mutation created. The mutated cells then acquire additional mutations that allow them to proliferate even more rapidly expanding the size of the tumor. This tumor progression process concludes when cells metastasize to the surrounding tissues and other areas throughout the body (Cooper *et al.,* 2000).

2.5.1. The burden of cancer disease to human health

Cancer kills more people globally than tuberculosis, HIV/AIDS and malaria combined (Ferlay *et al*., 2010). All cancer types have similar features or genotypes which include lack of sensitivity to signals that prevent cell development causing their replication to be unlimited. Apoptosis is avoided and angiogenesis is continued in the tumour tissue which allows the existence of cancer cells (Iqbal *et al*., 2017).

It is projected that by the year 2030, there will be annual 17 million mortalities with 26 million new cancer cases globally with prostate and breast cancers leading in males and females correspondingly (Siegel *et al*., 2019). Africa alone is projected to record 1,400,000 new episodes and 1,000,000 mortalities per year by the aforementioned year. Limited funding and inadequate access to cancer care are the main causes of Africa's high prevalence of cancer deaths. The late diagnosis of cancer cases in Africa contributes to poorer outcomes due to reduced chances of survival (Siegel *et al*., 2019). Cancer burden in Africa is mainly felt by women in contrast to the United States as cancer prevalence in African women is almost twin-fold that of males (Dent *et al*., 2017).

A prevalence study done in Kenya using the data from Nairobi cancer registry found breast, cervical, esophageal, stomach and ovarian cancers to be the most incident in women while in men, prostate, esophageal, large bowel, stomach, oral and liver cancers were the most incident (Korir *et al*., 2015). Another study carried out in 2019 in the Kenyatta National Hospital and Moi Teaching and Referral Hospital indicated that female cancer cases were more compared to the male with 60% and 56.4% respectively (Macharia *et al*., 2019).

2.5.2. Treatement methods of cancer and their downsides

The main cancer treatment methods include: surgery, which may damage nearby tissues resulting to complications and change of patients quality of life, radiation which damages nearby tissues and cannot kill microscopic tumor cells, chemotherapy using drugs which may be toxic to normal cells and tumor cells are resistant to drugs (Wheeler *et al*., 2013).

Chemotherapy is mostly used method to treat different forms of malignancies due to its effectiveness. Generally, chemotherapy destroys proliferation of cancer cells by hindering DNA

production and constrain cellular mechanism leading to cell cycle process resulting to programmed cell death (Cheung-Ong *et al*., 2013). Despite their usefulness, chemotherapeutics lack specificity and damages the normal cells leading to side effects like tiredness, vomiting, hair damage, nausea, numbness, memory impairment and sometimes death (Aslam *et al*., 2014). The effectiveness of these drugs is established on the ability to attack malignant cells by genotoxicity means, which is to some extent is affected by the synthesis of reactive oxygen species. This causes them to lack selectivity required in cancer treatment (Serment-Guerrero *et al*., 2011). Amongst cancer medications accepted by Food and Drug Administration (FDA), 56 out of 132 drugs are shown to lead to oxidative stress (Chen, *et al*., 2007). Most of these treatments have a narrow therapeutic index and present unspecific biodistribution once administered to patients (Davis *et al*., 2010). This disadvantage of current chemodrugs has necessitated novel approaches to provide solutions to counter these problems, by looking for alternatives that exhibit high selectivity index by targeting only the cancerous cells. Certain complementary and alternative medicines are being turned to as they have been occasionally claimed to have anticancer potentials with no or negligible side effects (Lee *et al*., 2012; Onyancha *et al*., 2019). Plant compounds have exhibited ability to hinder production and multiplication of cancer cells (Greenwell & Rahman, 2015).

2.6. Plants used medicinally in Kenya

Over a hundred plants species of different families have been recorded as being used in Kenyan traditional medicine (Kokwaro, 2009; Ochwang'i *et al*., 2014). Some of medicinal plants are unique to different communities as the distribution of the plants in Kenya is different based on ecological zones (Korir *et al*., 2015). Most medicinal plants are reportedly used in mixture with others for them to achieve a synergistic results required to treat a given ailment. The most common preparation methods carried out in Kenyan traditional medicine include boiling, maceration and

poultices where they are administered orally or applied topically based on the ailment treated (Kigen *et al*., 2013; Kokwaro, 2009).

2.7 Order Sapindales

This order is comprised of woody plants (trees, shrubs, and lianas). The leaves of most members of Sapindales are pinnately compound and are rarely palmately compound or simple with raceme or single inflorescence in the axils which are mainly pollinated by insects. The fruits are capsules but modified in some genera such as follicles in *Zanthoxylum*. The evolutionary relationships of families in this order are todate elucidated by molecular analysis, morphological similarities, and phytochemistry. The order has four groups. Group one comprises of Rutaceae, Meliaceae and Simaroubaceae. Group number two is made of Burseraceae, Kirkiaceae and Anacardiaceae. Thes third group includes Sapindaceae (Muellner-Riehl *et al*., 2016).

2.7.1 The family Rutaceae Juss.

This family is famously known as citrus family with 150 genera and 900 species (Kubitzki *et al*., 2010). Majority of species are trees or shrubs with a rare herbceous (*Boenninghausenia* and *Dictamnus*) (Auld, 2001). The leaves are generally compound and opposite lacking stipules with pellucid glands (cavities containing oil with aromatic smell) which are key synapomorphic features of members of this famly (Appelhans *et al*., 2018). Flowers are solitary or in cyme mainly hermaphroditic and pollinated by insects. The fruits range from drupes, berries, hesperidiums, capsules, samaras, and follicles with varied seed numbers (Souza *et al*., 2004).

This family is important for a large number of food plants and ornamentals. For instance, many varieties of *Citrus* are extensively cultivated as fruits including oranges, lemons, grapefruit, mandarins, tangarines and limes (Rahmatullah *et al*., 2010) with the fruit of *Citrus australis* and

C. australasica being used for making jam. Several species are grown as ornamentals, particularly those of *Boronia, Crowea, Eriostemon, Phebalium* and *Correa* (Fajinmi *et al*., 2014). Timber is obtained from majority of the rainforest species like *Halfordia* and *Flindersia* (Kubitzki *et al*., 2010). Several genera including *Zanthoxylum* are medicinal sources (Mabberley, 2004; Patiño *et al*., 2012).

The most prominent classes of compounds in this family are sulfur containing amides, quinolines and quinolones, coumarins, flavanones, carbazoles, acridones, and quinazolones (Ivanova *et al*., 2004). Islation of new sulfur containing amides has been reported from different Glycosmis species (Yang *et al*., 2015). These amides are derived from methylthiopropenoic or methylsulfonylpropenoic acid in combination with phenethylamine derived amino component (Hofer *et al*., 2000).

2.7.2. The genus *Zanthoxylum* L.

Zanthoxylum is a genus in the Rutaceae family with over two hundred species of aromatic prickly trees or shrubs with global distribution (Dharani, 2011). It is sometimes referred to as 'pricky ashes' because of the half-inch (1.2-cm) spines that cover its whitish bark **(figure 1)**, is represented by about 40 species in Africa, and seven (7) in Kenya (Adesina, 2005; Beentje *et al*., 1994).

Figure 1; *Zanthoxylum chalybeum* tree

Z. chalybeum tree with pale grey bark, a rounded crown and compound leaves. **Source:** Photograph taken by Magrate Kaigongi at Ruungu, Tharaka Nithi County, April 2018.

The major ethnobotanical uses associated with this genus include, treatment of malaria, relief of dental problems, management of digestive illnesses and lung ailments, (Kala *et al*., 2005). Members of this genus are also used as aphrodisiac, analgesic, febrifuge, stimulant, tonic, and homeastatic agents (Iwu, 2014; Williams, 2012). Additionally, some species used as pesticides, textile dyes and building materials (Koomson, 2003; Nussinovitch, 2009).

Alkaloids,lignans, amides and coumarin are commonly reported phytochemicals recognized to have a chemotaxonomic function in this genus (Patiño *et al.*, 2008). Also other metabolites found include terpenes, sterols and flavonoids (Adesina, 2005).

2.7. 3. Taxonomic treatment of *Zanthoxylum* and some challenges encountered

Zanthoxylum genus occupies the most primitive position in the family Rutaceae due to its unspecialized floral morphology (Beurton, 1994). Generally, the classification of this genus has been unsettled. Linnaeus in 1759 divided the genus into *Zanthoxylum* (one perianth) and *Fagara* (two perianth) based on the sufficiently heterogenous periath (Cao *et al*., 2014). Saunders (1934), systematically monographed the genus *Zanthoxylum*, analyzed the taxa in a comprehensive view, and split it into two subcategories, *Zanthoxylum* and *Fagara*. Majority of taxonomists acknowledged the Saunder's circumscription (Brizicky, 1962; Hartley, 1966) and proposed the one perianth of *Zanthoxylum* species was probably a minor situation resulting from *Fagara* due to abortion of sepals, and suggested a combination of the two taxa under *Zanthoxylum* and treated *Fagara* as a subgenus under *Zanthoxylum* (Waterman, 1975). Cao et al. (2014) in pursuit of understanding the connection between *Zanthoxylum* and *Fagara* compared the pollen character in detail and found the type of pollen in the two taxa was similar suggesting a close association between them. The difficulties encountered in classifying members of this genus have brought about confusion in the herbal utilization thus hindering safety, therapeutic potency, and efficacy of herbal medicines derived from the species of *Zanthoxylum* (Joharchi & Amiri, 2012).

More studies are needed to investigate whether *Zanthoxylum*, as currently circumscripted, is monophyletic (Waterman, 1975). Determination of chemical profiles have successfully been used elsewhere (Patiño *et al*., 2012) and in this case may be suitable to support a comprehensive chemotaxonomy of the genus. One suggestion is to conduct metabolomics studies of the different species of *Zanthoxylum* occurring in Kenya to determine whether they may illuminate possible inter- and intraspecific relationships.

2.7.4. Description of *Zanthoxylum* species found in Kenya

a. *Zanthoxylum chalybeum* Engl.

Z. chalybeum is a tree growing to 12m high with a pale grey bark, dark scales or prickles and a rounded crown. It has compound that when crushed produces a strong citrus aroma. The flowers are inconspicuous, yellow-greenish and strongly scented. The fruits are spherical. It grows in low altitude areas, dry woodlands and savannah grasslands (Beentje *et al.,* 1994).

Leaves are used as fodder and can be brewed to make tea while the bark is a source of durable timber and charcoal (Bamford & Henderson, 2003). Leaf decoction is rubbed on swelling for the treatment of oedema in kwashiorkor and snake bites, root decoction is drunk to manage chest pain, pneumonia and bark decoction drunk for the treatment of cold, malaria, dizziness and also chewed for toothache (Kokwaro, 2009) and diabetes (Keter & Mutiso, 2012). Seed extracts of *Z. chalybeum* has antiviral activity against measles virus (Olila & Opuda-Asibo, 2001).

Z. chalybeum has shown *in vitro* antiplasmodial activity (Nguta *et al*., 2010; Rukunga *et al*., 2009), antifungal activity against *Saccharomyces cerevisiae* and *Penicillium crustosum* (Hamza *et al*., 2006), antimicrobial activity and cytotoxic against Brine-shrimp larvae (Kaigongi *et al*., 2014; Musila *et al*., 2013; Nguta *et a*l., 2011) *Z. chalybeum* has quinoline (Kato *et al*., 1996), alkamide and fagaramide alkaloids (Omosa *et al*., 2019) and organic extract has flavonoids, alkaloids, saponins and sesquiterpene lactones (Musila *et al*., 2013; Kaigongi *et al*., 2014). Reseach to compare antimicrobial potential of different parts of *Z. chalybeum* is lacking.

b. *Zanthoxylum gilletii* (De Wild.) Waterman

This is a deciduous tropical rainforest species growing 10 to 35 m tall. In Kenya it is found in the forests of Mount Kenya, Kakamega and Mau block (Peltorinne, 2004). It is a monoecious plant with alternate leaves and terminal clusters with sparse prickles in the midrib, the flowers are

clustered with Inflorescence of terminal and axillary panicles, fruits are reddish, subglobose, sessile or with a short stipe and a persistent calyx, the seeds have a shiny black testa (Beentje *et al*., 1994). The stem wood is bright yellow with sweet scent and termite resistant (Wanga, 2018).

The bark is utilized in treating stomachache (Kokwaro, 2009). A decoction from the stem bark is used to manage back pain and applied superficially to treat urinogenital complaints. Decoctions from the fruit pulp and the root bark are used as analgesics and young leaves treat coughs, bilharzia and gonorrhoea (Nyunja *et al*., 2009).

Several phytochemical studies have reported various compounds such as alkaloids, saponins, phenolics, xanthophylls, and coumarins with medical potential (Islam & Ahsan, 1997). Phytochemical investigations of Nigerian *Z. gilletii* indicated the occurrence of various kinds of alkaloids like skimmianine, furoquinoline, fagaramide, chelerythrine, benzophenanthridine, alkaloids (Adesina & Reisch, 1988). Other compounds reported include, vanilic acid, volatile oils, and hydroxyl benzoic acid (Adesina, 2005). Studies have shown that the plant has agents that can be used as anticancer (Patiño *et al*., 2012), antimicrobial (Gaya *et al*., 2013) and antimalarial (Masinde, 2014).

c. *Zanthoxylum holtzianum* (Engl.) Waterman.

It can be a shrub or small tree with 2-15 m height with a grey bark containing conical corky bosses in the trunk and recurved prickles in the branches. It grows well in dry or moist woodlands, coastal forest, bushland and occasionally on coral (Beentje *et al*., 1994).

Little information has been recorded about this species as it has not been widely exploited scientifically. Currently, there are no records available about traditional uses of this species. Antimalarial and antimicrobial activities have been reported by (Buyinza, 2012) and (Akampurira,

2013) respectively. Seven compounds were isolated (Buyinza, 2012; Akampurira, 2013) of these, three were benzophenanthridine alkaloids (dihydrochelerythrine, 8-acetonyldihydrochelerythrine and 8-oxochelerythrine, canthin-6-one alkaloid [N-methylflindersine), a flavanone (hesperidine), a fatty acid (hexadecanoic acid), and an amide (2E, 4E)-N-isobutyltetradeca-2, 4-dienamide.

d. *Zanthoxylum paracanthum* Kokwaro

It is a shrub or a small tree growing to 10 m high in evergreen forests, bush land and sometimes in riparian areas 100–1500 m. The branchlets are glabrous, dull red with brown lenticels. The prickles are short and up curved 3–11 mm long. The leaves are 15–37 cm long with 0.5-2mm long petiolule or rarely to 13 mm on the terminal leaflet. The leaflets are 5–11 pairs, subopposite below, progressively more opposite above, oblong or elliptic-oblong to obovate-oblong with caudate or acuminate apex, cuneate to oblique base and a crenate margin. The midrib is prominent and occasionally armed beneath (Beentje *et al*., 1994). The inflorescence are composed of whitish green flowers. The fruit is a paired rhomboid follicles with a common stipe but individually sessile. The seeds are shiny black 4–5 mm in diameter (Samita, 2003).

Three new larvicidal alkaloids (Zanthoxoaporphines A–C) were obtained from purification of stem bark extract of this plant (Samita *et al*., 2013). Elsewhere, purification of stem bark yielded dihydrochelerythrine, lupeol, sesamin and canthin-6-one where canthin-6-one was active against leukemia cancer cell lines (Omosa *et al*., 2019).

e. *Zanthoxylum usambarense* (Engl.) Kokwaro

According to Beentje et al. (1994), *Z. usambarense* is a tree that grows to 15m tall. It does well in wooded savannah, montane dry forest and forest remnants, rocky slopes, evergreen bush land and marshy woodland at 1200-2600m altitude. The leaves are imparipinnate compound and opposite with 2–8 pairs of leaflets which are10–24 cm long. Flowers are unisexual with ovate to

elliptical sepals, elliptical to elliptical-oblong petals with white to cream or greenish yellow colour. The Fruit is commonly a duo of nearly globose, mucronate and reddish green in colour with ovoid dark blue-black and shiny seed.

In East Africa, *Z. usambarense* is a vital medicinal plant. Young twigs are chewed to manage oral infections. The Maasai people use stem and root bark decoction to treat backache, cough, malaria, pneumonia, rheumatism, painful joints and as purgative and emetic agent (Kariuki, 2018; Maundu *et al*., 2001). Leaf poultices are swallowed to cure oral infections, diarrhoea, nausea, cough and stomach-ache (Kokwaro, 2009). A mixture of fruit infusion and milk is used to manage sore throat, fever, chest pains and tonsillitis. A seed decoction is taken orally to manage respitatory infections and malignant catarrhal fever (Schmelzer & Gurib-Fakim, 2013). In Kenya the plant is also used for dying cloth; the root and stem bark produces a yellow and beige dye respectively. The wood is used in house construction and to make furniture and bows; it is reported as hard and durable, it is considered good firewood beacause of its easy burning. *Z. usambarense* is additionally utilized as a life fence (Dharani *et al*., 2010).

A diversity of compounds such as magnoflorin, aporphine, berberine, usambarine, usambanoline, benzophenanthridine, and chelerythrine were noted in both the stem and roots (Kato *et al*., 1995, 1996). Additional chemicals reported from the root and stem bark sesamine, pellitorin and aliphatic amide (He *et al*., 2002). Coumarins isolated from the roots include; phellopterin, toddaculin, pimpinellin and toddalolactone (Kariuki, 2017).

Stem bark exhibited important antimalarial activities against *Plasmodium falciparum* strains (Kirira *et al*., 2006). A stem bark extract exhibited good antimalarial potency against *Plasmodium bergei* and *Plasmodium knowlesi* (Were *et al*., 2010). Extracts of *Zanthoxylum usambarense* have also been shown to have antimalarial effects in an animal model *(Nanyingi et*

al., 2008). Both stem and the root bark extracts were found to possess significant antibacterial activities (Matu & Van Staden, 2003) and inhibited the growth of cancer cell lines MDA-MB-231 and MCF-7 (Özkan *et al*., 2013). Antimicrobial studies on extracts from different parts of *Z. usambarense* are lacking which requires to be addressed.

2.8. The family Sapindaceae Juss.

It is generally refered as Soapberry with 138 genera and 1858 species occurring from temperate to tropical regions (Buerki *et al*., 2011). Members of this family can either be woody or herbaceous with compound leaves which are alternate and sometimes opposite with extipulate petiole and swollen base (Beentje *et al*., 1994). The inflorescence are cymes grouped in panicles with small unisexual flowers though plants may be monoecious or dioecious. (Umadevi & Daniel, 1991). Many species from this family are exploited as source of useful oils (Kalayasiri *et al*., 1996), ornamental as well as medicinal value (Acevedo-Rodríguez *et al*., 2010). Members of Sapindaceae have been reported to have saponins terpenes and flavonoids in their tissues (Olaoluwa & Aiyelaagbe, 2015; Umadevi & Daniel, 1991).

2.9. Taxonomic treatment of the genus *Dodonaea* L.

The genus *Dodonaea* commonly referred to as hop bushes was named after Rembertus Dodoens and beongs to the family Sapindaceae. It has nearly 70 species distributed widely ranging from tropics to warm temperate areas found in Africa, Australia, Asia and America with the high variety occurring in Australia (Harrington & Gadek, 2009).

The genus is composed of dioecious trees or shrubs **(figure 2)**. The leaves are simple and the inflorescence is a raceme-like or paniculate thyrsi with actinomorphic, unisexual or (rarely) bisexual flowers. The sepals are 3-7, sometimes slightly connate with petals if present. The

Stamens are 5-8 and ovary has 2-4 loculi. The fruit is winged capsule with 2-6-loculi. The seed lacks an arillode (Beentje *et al*., 1994).

Figure 2; *Dodonaea viscosa* bush

Dodonaea viscosa bush showing leaves and bark**. Source:** Photograph taken by Magrate Kaigongi at Nanyuki, Laikipia County in April, 2018

The genus is exploited in traditional medicine to manage toothache, cuts and stingray stings (Al-Snafi, 2017). Early European Australians utilized members of this genus in making beer hence the name hop bush (Rani *et al*., 2009). Flavonoids, terpenes and saponins have been isolated from these plants (Harrington & Gadek, 2009).

2.9.1. Morphology, medicinal uses and phytochemistry of *Dodonaea viscosa* (L.) Jacq.

It is a shrub or a small tree commonly 2- 8 m with a slender stem with a peeling, grey bark and a light crown. Branches are red and sticky with thin, narrow leaves which are stiflly erect to 10 cm, tapering to a stalk, young leaves light green, shiny and sticky. The flowers are small and dioecious with distinctive papery winged fruits bearing small seeds inside (Beentje *et al*., 1994).

There are seven varieties recognized in Australia (var. *angustifolia*, *angustissima*, *burmanniana*, *cuneata*, *mucronata*, *spatulata* and *viscosa*). Each variety has a different environment with ability to cope in varying levels of temperature. Major differences that exist

among these sub-species is related to growth form and morphological characteristics, and distribution (Christmas *et al*., 2015).

Traditionally, this plant is utilized in management of fever, sore throat, burns, wouds, haemorrhoids, gout, indigestion, ulcers, diarrhoea, constipation, trachoma, fractures, snake bites malaria and rheumatism (Venkatesh *et al*., 2008). Other uses reported are; firewood, poles, tool handles, apiculture, ornamental, land reclamation (marshes, poor or sandy soils), dune fixation, live fence and toothbrushes (Rani *et al*., 2009). *D. viscosa* was shown to possess antimicrobial, antiinflammatory, muscle relaxer, antioxidants effects among others (Getie *et al*., 2003; Khalil *et al*., 2006; Rojas *et al*., 1996).

Overall, chemicals reported from *D. viscosa* are; terpenes, flavonoids and saponins (Van Heerden *et al*., 2000). The healing potential of the plant is connected to polyvalent phytochemical characteristic from synergistic effect of several compounds (Al-Snafi, 2017).

2.9.2. Challenges of the existing taxonomy of *D. viscosa* in Kenya

In Kenya, two species commonly recognised are *D. viscosa* in the coast of Kenya and *D. angustifolia* in the inland (Beentje *et al*., 1994). The two were later merged to form namely *D. viscosa* with two varieties (var. *angustifolia* and *viscosa)* (Harrington, 2008). *D. viscosa* subsp *viscosa* a thick bush about 3-4 m. The fruit capsule usually has only 2 wings which occurs naturally along the coast of Kenya and referred locally as Mkaa pwani (Swahili) while *D. viscosa* var. *angustifolia* (L.f.) Benth. is widely distributed in Kenyan inland (Beentje *et al*., 1994) and known in local languages as Hidesa (Boran), Kithongoi/ Muthongoi (Kamba), Murema muthua (Kikuyu), Muendu (Luhya), Oking' (Luo), Olgeturai/Oltuyesi (Maasai), Tobolokwo (Pokot), Tombolokwa (Sabaot), Msidu (Taita), Tabilikuet (Tugen) *(*Beentje *et al*., 1994; Names Description, 2009). The wide distribution in Kenya has led distinctive populations being classified as separate species

(Harrington, 2008), a practice that has resulted in much taxonomic confusion among the Kenyans especially those practicing herbal medicine as this compromises safety and efficacy of herbal remedies associated with *D. viscosa*.

This study aimed to address the challenges encountered in the taxonomy of both *Zanthoxylum* species and *Dodonaea viscosa* in Kenya using metabolomics. Identification of the most effective biochemicals from these groups of plants as well as evaluation of the potential of biochemicals produced within these genera to counter the multiple drug resistant microbial infections and cancer types are reported.

CHAPTER THREE: GENERAL METHODOLOGY

3.1. Collection of plant materials

All plant materials were collected from different localities in Kenya in April 2018. Specimens were correctly identified using morphological characters as described by Beentje et al. (1994) and voucher samples were prepared (Alexiades, 1996) and kept at University of Nairobi Herbarium (NAI) **(table 1** and **figure 3)**.

Table 1: Specific geographical collection sites for plants studied

Showing details of where different plant samples were collected such as county, specific location, specific site characterization, GPS coordinates and the voucher specimen numbers

Species Name	**County of Collection**	**Specific location**	**Site characterization**	**GPS (S)**	**GPS (E)**	**Voucher No**
D. viscosa .	Machakos	Kwa Mbuu	Farmland	1º 29' 55.8" S	37º 21' 58.2" E	MK2/2018
	Laikipia	Kahurura	Forest	0º 02' 06.0" S	37º 07' 49.4" E	MK4/2018
	Kwale	Gazi	Forest	4º 25' 29.1" S	39º 30' 22.5" E	MK5/2018
	Nairobi	Karura Forest	Forest	1º 14' 43.6" S	36º 50' 17.4" E	MK8/2018
	Narok	Maasai Mara Researve	Game reserve	1º 28' 36.5" S	35º 05' 33.3" E	MK9/2018
Z. chalybeum	Tharaka Nithi	Ruungu	Farmland	0º 07' 16.8" S	37º 51'36.1" E	MK13/2018
Z. gilletii	Kwale	Muguga	Forest	1º 11' 49.9" S	36º 37' 00.2" E	MK14/2018
Z. holtzianum	Kwale	Mrima Hills	Forest	4º 29' 01.6" S	39º 15' 30.8" E	MK13/2018
Z. paracanthum	Kwale	Mrima Hills	Forest	4º 29' 01.6" S	39º 15' 30.8" E	MK14/2018
Z. usambarense	Kakamega	Kakamega Forest	Forest	0º 16' 48.4" N	34º 51' 45.1" E	MK15/2018

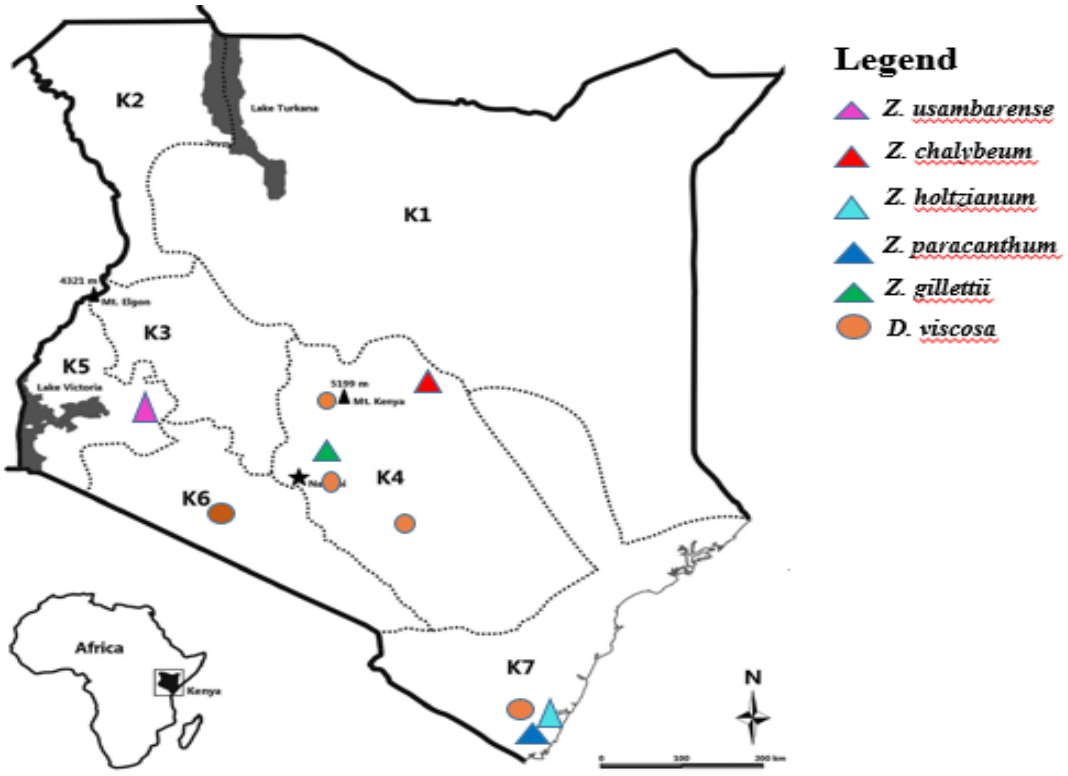


Figure 3: Kenyan Floral of Tropical of East African map showing the collection sites of plants samples used in this study

3.2. Preparation of plant materials

The collected plant materials were washed and dried in an open place under shade at room temperature (25°C) for one month. This was followed by grinding each sample with electric grinder into fine powder separately and in a very clean manner to avoid contamination at the Kenya forestry research institute (KEFRI) medicinal plants laboratory (Soni & Sosa, 2013). All the plant samples collected were analysed for their metabolomics relationship in chapter four and evaluated for antimicrobial activity against six microbes of economic importance in chapter five. Based on the antimicrobial activity results, the most active extracts were subjected to phytochemical isolation in chapter six and antiproliferative activity in chapter seven.

3.3. Analytic methods used in the study

Liquid chromatography-Mass spectrometry analysis was done on a Waters Synapt G2 quadrupole time-of-flight mass spectrometer (Milford, MA, USA). Standards used in LC-MS were obtained from Sigma-Aldrich Column chromatography was done on silica gel 60 (70 230 mesh); TLC on silica gel 60 F254, Merck); The NMR spectra were documented using Bruker Avance 500 MHz spectrometers. COSY, HSQC and HMBC spectra were acquired by use of standard Bruker software. Chemical changes were recorded in ppm in δ values relative to standard tetramethyl silane (TMS). Omacilin and fluconazole were acquired from Sigma-Aldrich (USA). The cell lines used in the cytotoxicity tests were obtained from Manassas, VA, USA. Doxorubicin was obtained from Johnson & Johnson, USA. Bacteria and fungi were gotten from the Centre for Microbiology Research (CMR), Kenya Medical Research Institute (KEMRI).

CHAPTER FOUR: CHEMOSYSTEMATICS

CHEMOSYSTEMATICS OF *ZANTHOXYLUM* AND *DODONAEA VISCOSA* IN KENYA

4.1. INTRODUCTION

Systematics work involving plants has transformed substantively in a few decades caused by innovative approaches in phylogenetics where phytochemical data has enabled comprehension of plant phylogenetic relations (Dos Santos *et al*., 2017). Metabolomics is reported to be an efficient tool in chemotaxonomy (Martucci *et al*., 2014) comprehending organisms evolution (Dos Santos *et al*., 2017) as well as biogeographic developments in plant change (Padilla-González *et al*., 2017). Additionally, metabolomics has great uses in natural products investigations like regulation of medicinal plants value, detection of bioactive compounds and resolving phylogenetic difficulties (Chagas-Paula *et al*., 2015).

Compound structure of metabolites as well as their production pathways are majorly specific and limited to taxonomically linked plants which is essential in classification (Singh, 2016). Geographical difference influences the type of metabolites produced by plants of the same species (Sampaio *et al*., 2016). Additionally, disparity in metabolites production amongst plants of a given species is influenced by age or season of plants collection. Production of phytochemicals in plants is influenced by climatic and seasonal differences (Ullah *et al*., 2012). This disparity nevertheless does not account for genetic difference. Chemical variety is determined by biology and geography of plants (Cragg & Newman, 2013). Plants materials are composed of myriad of active phytochemicals compared to all compound libraries constituted by man. Furthermore, biochemical constituents of plants collected at varied periods and sites can differ significantly

(Raskin, 2003). The aim of this chapter was set to establish quantity and distribution of biochemicals in *Zanthoxylum* species and *D. viscosa* populations and their utilization in the chemosystematics of the taxa.

4.1.1. Objective

To apply metabolomics as a tool in chemosystematics of *Zanthoxylum* and *Dodonaea*.

4.2. MATERIALS AND METHODS

4.2.1. *Zanthoxylum* species and *D. viscosa* samples preparation for metabolite profiling

Each sample was weighed separately and 30 g extracted with 50 ml methanol for 20 h at room temperature. This was followed by removal of debris by filtering through a filter paper and removal of solvent through rotavaporation. Dilution of the obtained extracts was done to constitute a concentration of 30mg/ml on the samples in preparation for LC–MS analysis. Three biological and technical replicates were analyzed. The standards; quercetin and catechin in calibration series of 10, 20, 50, 100, 200 ppm were used. This study was carried out Central Analytical Facilities (CAF) in Stellenbosch University, South Africa.

4.2.2. Liquid chromatography- mass spectrometry (LC–MS) analysis

The LC-MS was done using both positive and negative modes. The desolvation gas utilized in both modes was nitrogen at 650 l h−1 at 275 °C. *Dodonaea* samples were analysed in the negative mode while *Zanthoxylum* samples were analysed using both positive and negative modes.

Alkaloids were in the positive mode analysed using a Waters UPLC BEH C18 column where 3 µl was added into the machine for every analysis. Formic acid was used as solvent A while acetonitrile was used as solvent B. The gradient was initiated at 100% by use of formic acid for half a minute after which 22% acetonitrile was added for 2.5 minutes, this was followed by

44% acetonitrile for 4 minutes and lastly 100% of acetonitrile for 8 minutes totalling to a 15 minutes run. The speed of flow was set as 0.4 ml/ min. Phenolics were analysed in the negative mode performed as described in the positive mode except that a longer column was utilized with total run time being 22 minutes. The gradient was initiated at 80% formic acid and speed of run was 0.35 ml/ ml.

4.2.3. Analysis of data

Data was acquired from the liquid chromatography- mass spectrometry machine and processed using Masslynx version 4.1 (Waters) and MZmine 2 version.40.1 correspondingly. Targetlynx within Masslynx software was utilized in quantifying metabolites while MetaboAnalyst, a free online software was used to perform the chemometric and hierarchical analysis. The Principal Component Analysis (PCA) with pareto scaling was applied as unsupervised multivariate cluster technique. Pareto scaling enhances the influential contribution of less concentrated chemicals without amplifying the noise and artefacts connected to metabolomics data (Goodacre et al., 2007). Consequently, this makes the understanding of loading score plots easy (Heyman & Meyer, 2012). Inter- relations were shown through Principal Component Analysis and Hierarchical Cluster Analysis (HCA) established on three factors (mass data, metabolite concentration and distribution) of each sample. Where, members of different groups (*D. viscosa* populations) or species (*Zanthoxylum* spp) appeared to cluster together, Orthogonal partial least squares discriminant analysis (OPLS-DA) test was done using paretal scaling to further separate them. The discriminants (chemical markers) were identified using MetFrag, a free online software.

4.3. RESULTS

4.3.1. Grouping of *Zanthoxylum* spp.

Twelve discriminants (chemical markers) were identified as the cause of the separation of the five *Zanthoxylum* species (*Z. chalybeum, Z. gilletii, Z. holtzianum, Z. paracanthum* and *Z. usambarense*) and are shown in the **Figure 4** below. The discriminant chemicals are as listed in **Table 3**. *Z. usambarense* and *Z. gilletii* appeared to cluster together based on the two PC scores leading to the need for OPLS-DA test to separate them.

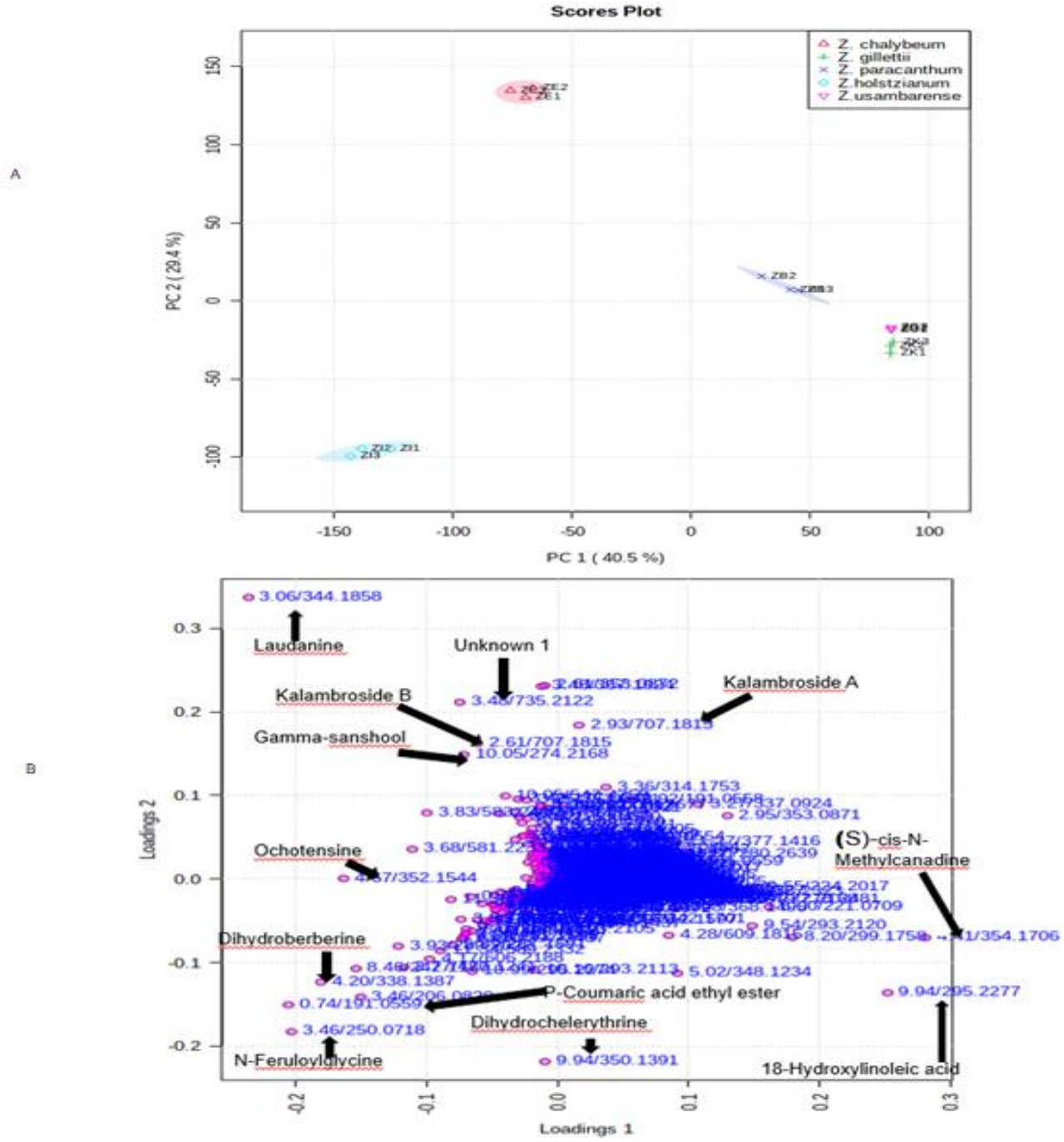


Figure 4: Principal component analysis and loadings based on liquid chromatography-Mass spectrometry spectra of *Zanthoxylum* spp

Figure A) PCA score plot showing variation of five *Zanthoxylum* species in Kenya *Z. usambarense* (purple colour), *Z. gilletii* (green colour), *Z. chalybeum* (red colour), *Z. holtzianum* (cyan colour) and *Z. paracanthum* (blue colour). **B)** PCA loadings plot of compounds that influenced the separation of species into separate clusters.

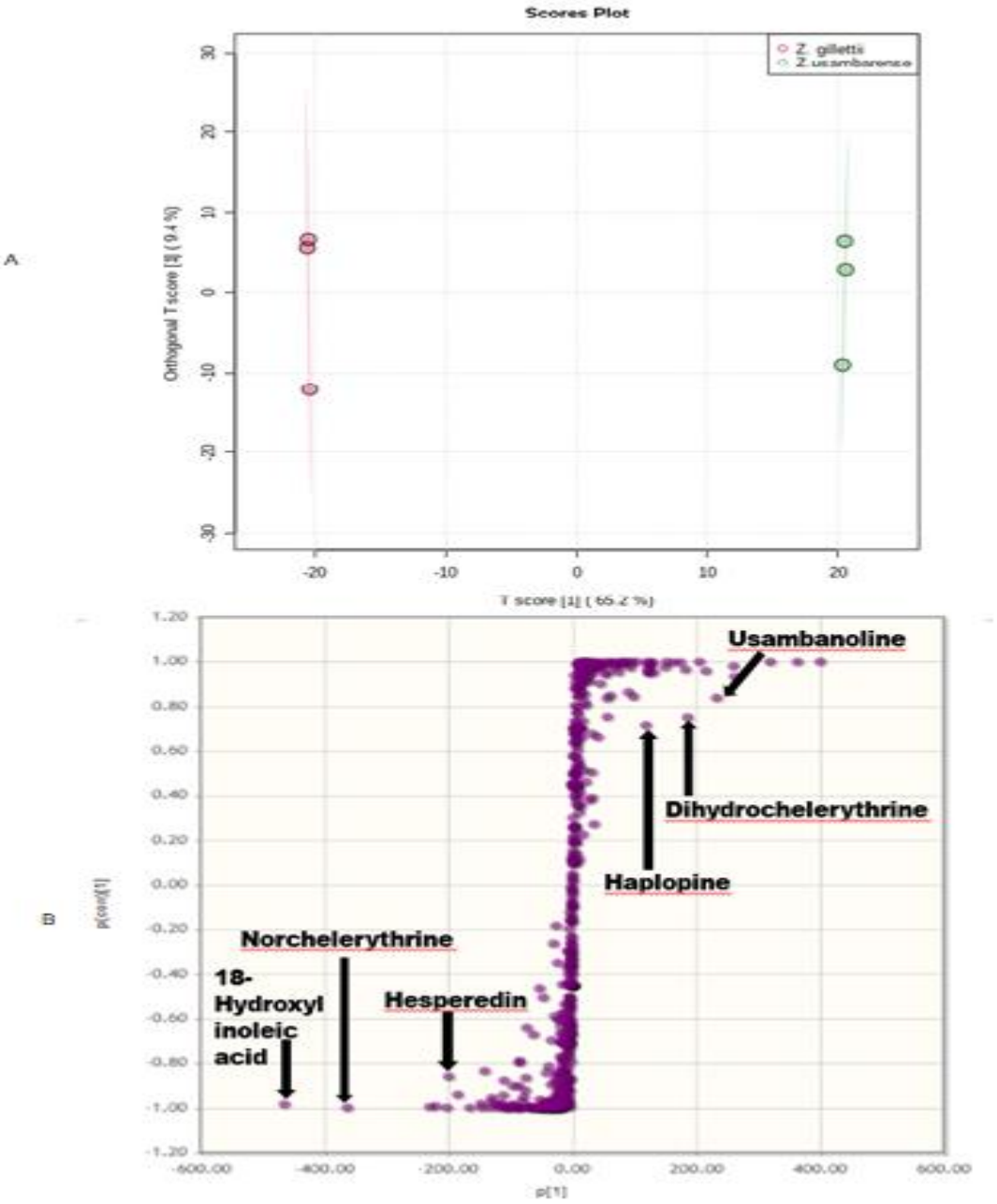


Figure 5: Orthogonal partial least squares discriminant analysis (OPLS-DA) of *Z. gilletii* and *Z. usambarense*

Figure 5 A) Orthogonal partial least squares discriminant analysis (OPLS-DA) for *Z. gilletii* and *Z. usambarense*. The OPLS-DA test separation of *Z. gilletii* and *Z. usambarense* on T score 1 which was 65.1% separating *Z. gilletii* on the left and *Z. usambarense* on the right **B)** Loadings of OPLS-DA spectra for *Z. gilletii* and *Z. usambarense* showing the annotated discriminants.

From the OPLS-DA test loadings of *Z. usambarense* and *gilletii* it was clearly shown that the two species have minor differences based on the few metabolites seen to pull *Z. usambarense* on right side and *Z. gilletii* on the left side.

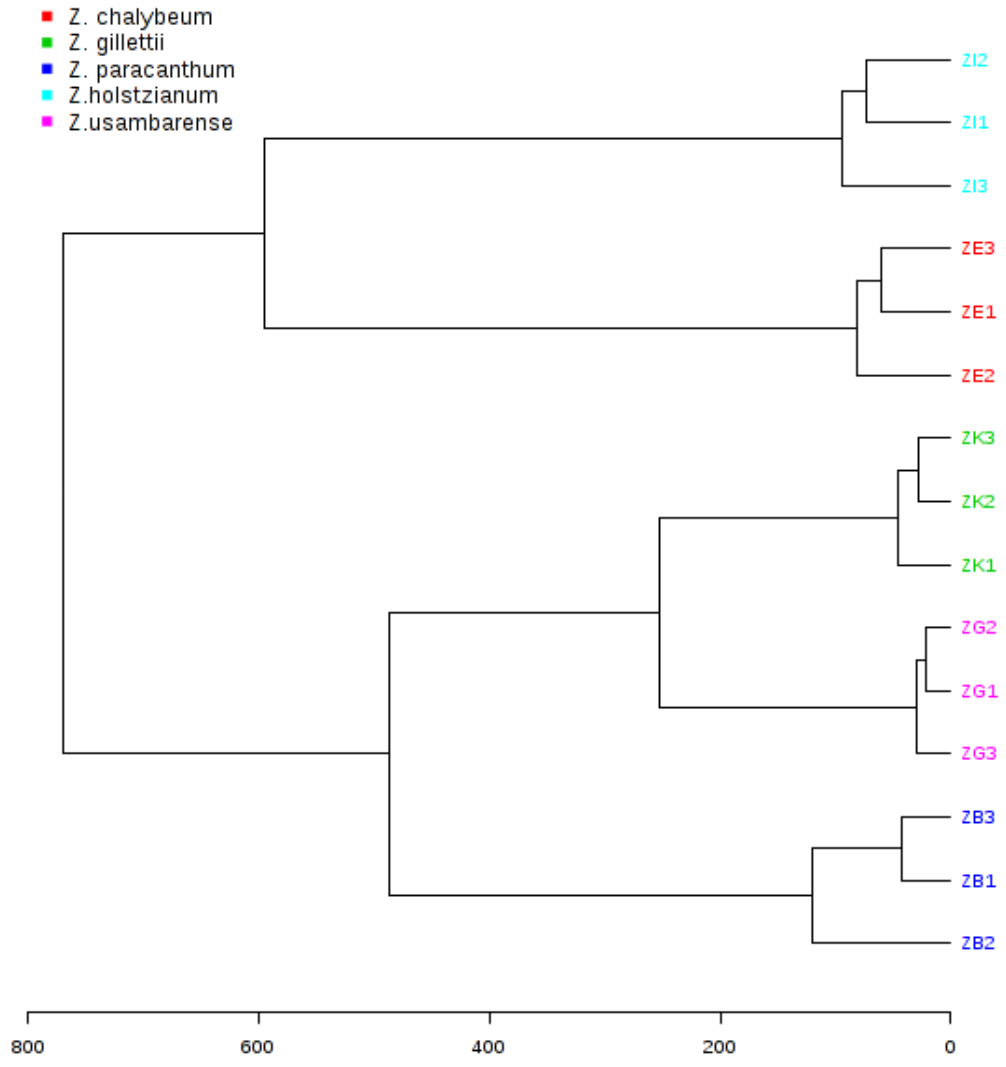


Figure 6: Dendogram/ relationship tree of five Kenyan *Zanthoxylum* spp

Dendogram/Relation tree of five Kenyan *Zanthoxylum* spp based on different phytochemicals. *Z. usambarense* (purple colour), *Z. gilletii* (green colour), *Z. chalybeum* (red colour), *Z. holtzianum* (cyan colour) and *Z. paracanthum* (blue colour).

The Hierarchical cluster analysis as shown in the tree above indicates that *Z. usambarense* and *Z. gilletii* are sister species with a common ancestor. *Z. paracanthum* is closely related to the two sister species. *Z. chalybeum* and *Z. holtzianum* were attached on the same branch from the ancestor showing that they are closely related but the length of their branches from each other indicates that they are not sister species. The two species (*Z. chalybeum* and *Z. holtzianum*) seem to cxarise from a different lineage from that of *Z. usambarense*, *Z. gilletii* and *Z. paracanthum*. The discriminants identified in *Zanthoxylum* species are as listed in **table 2.** The relative abundance of discriminants in different *Zanthoxyum* species was analysed using targetlynx (**figure 7)**.

Table 2: Discriminants compounds identified for *Zanthoxylum* species

Retention time (min)	**ESI negative $(M-H)^{-}$ (m/z)**	**ESI positive $(M+H)^{+}$ (m/z)**	**Name**
3.06		344.1858	Laudanine
3.48	735.2122		Unknown 1
2.93	707.1815		Kalambroside A
2.61	707.1815		Kalambroside B
10.05		274.2168	Gamma-sanshool
4.67		352.1544	Ochotensine
4.41		354.1706	Cis-N-Methylcanadine
9.94	295.2277		18-Hydroxylinoleic acid
9.94		350.1391	Dihydrochelerythrine
3.46	250.0718		N-Feruloylglycine
0.74	191.0559		P-Coumaric acid ethyl ester
4.20		338.1387	Dihydroberberine

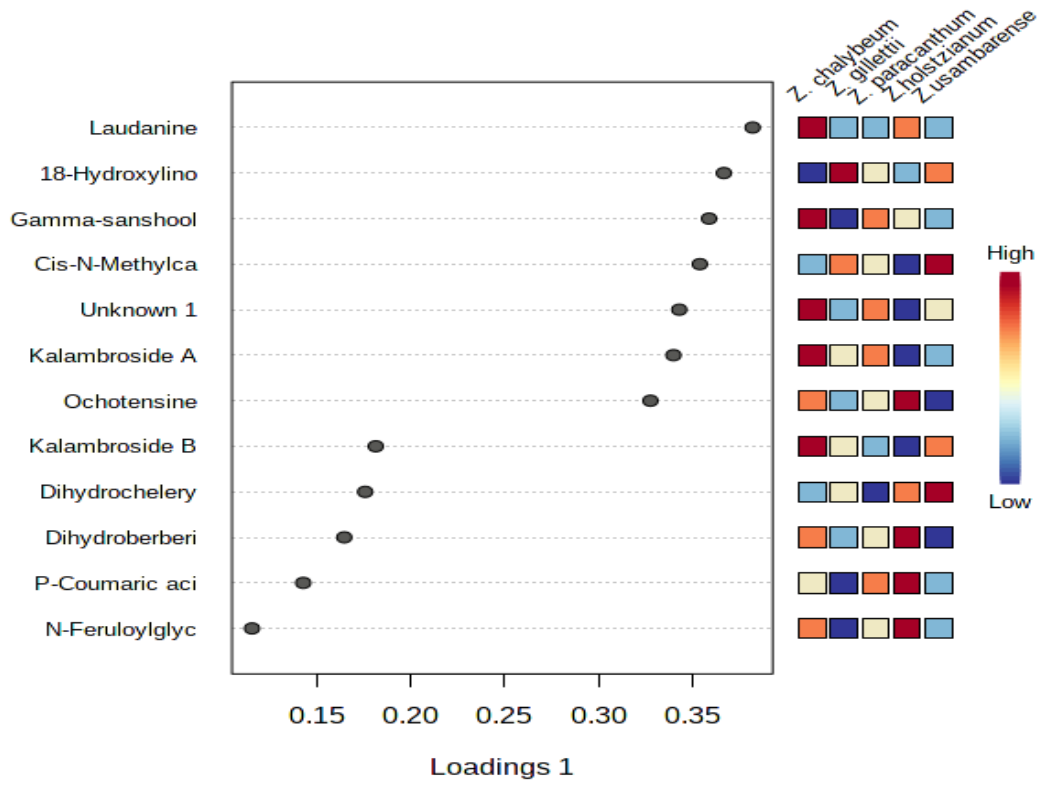


Figure 7: Concentrations of different discriminants in *Zanthoxylum* species

Relative abundance of chemical discriminants in different *Zanthoxylum* spp. The dark red shows high concentration of the individual chemical in each population while the dark green colour implies low concentration.

4.3.2. Grouping of *Dodonaea viscosa* populations

The five populations of D. viscosa segregated differently **(Figure 8A).** Eleven metabolites (Chemicals which appeared far from the pool) **(Figure 8B)** were identified as the discriminants of the 5 populations of *D. viscosa*. Chemicals were identified by comparing mass and retention time of metabolites with those of standard compounds or mass to charge ratio, mass fragment patterns, and UV absorbance (Carazzone *et al*., 2013). From PCA analysis, the Nanyuki and Machakos populations fell on the same quadrat and appeared to cluster closer to each other leading to OPLS-DA test to further separate them. The OPLS-DA **(figure 9)** test separated Machakos and Nanyuki populations on the left and right sides respectively. This clearly illustrated the minor differences present in the two collections.

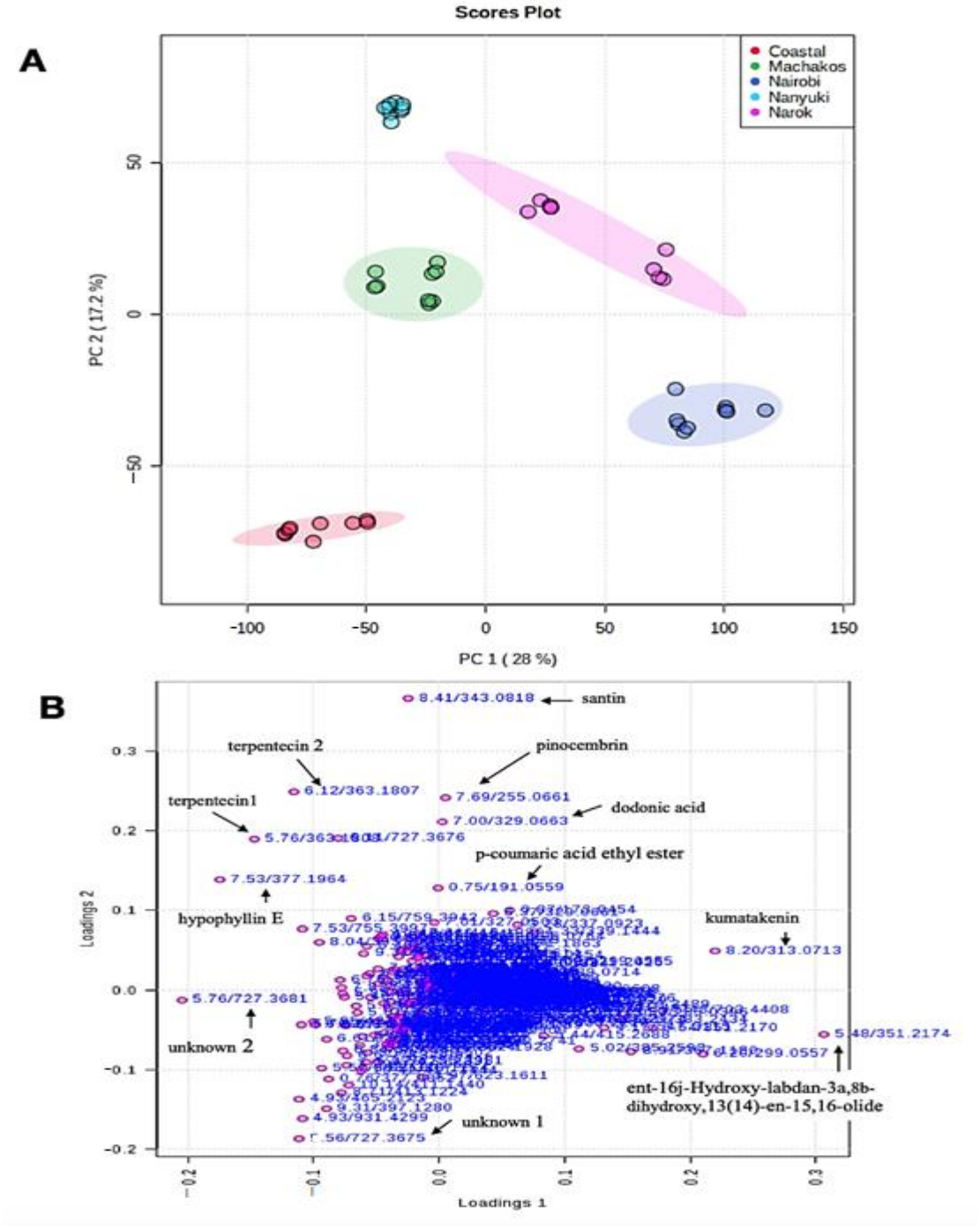


Figure 8: Principal component analysis scores and loadings of liquid chromatography-mass spectrometry spectra of *D. viscosa* populations

(A) PCA score plot showing variation of *D. viscosa* plants from different natural population five different locations in Kenya; coastal (red colour), Machakos (green colour), Nairobi (blue colour), Nanyuki (cyan colour) and Narok (purple colour). **B)** PCA loadings plot of compounds that influenced the differentiation of population into separate cluster.

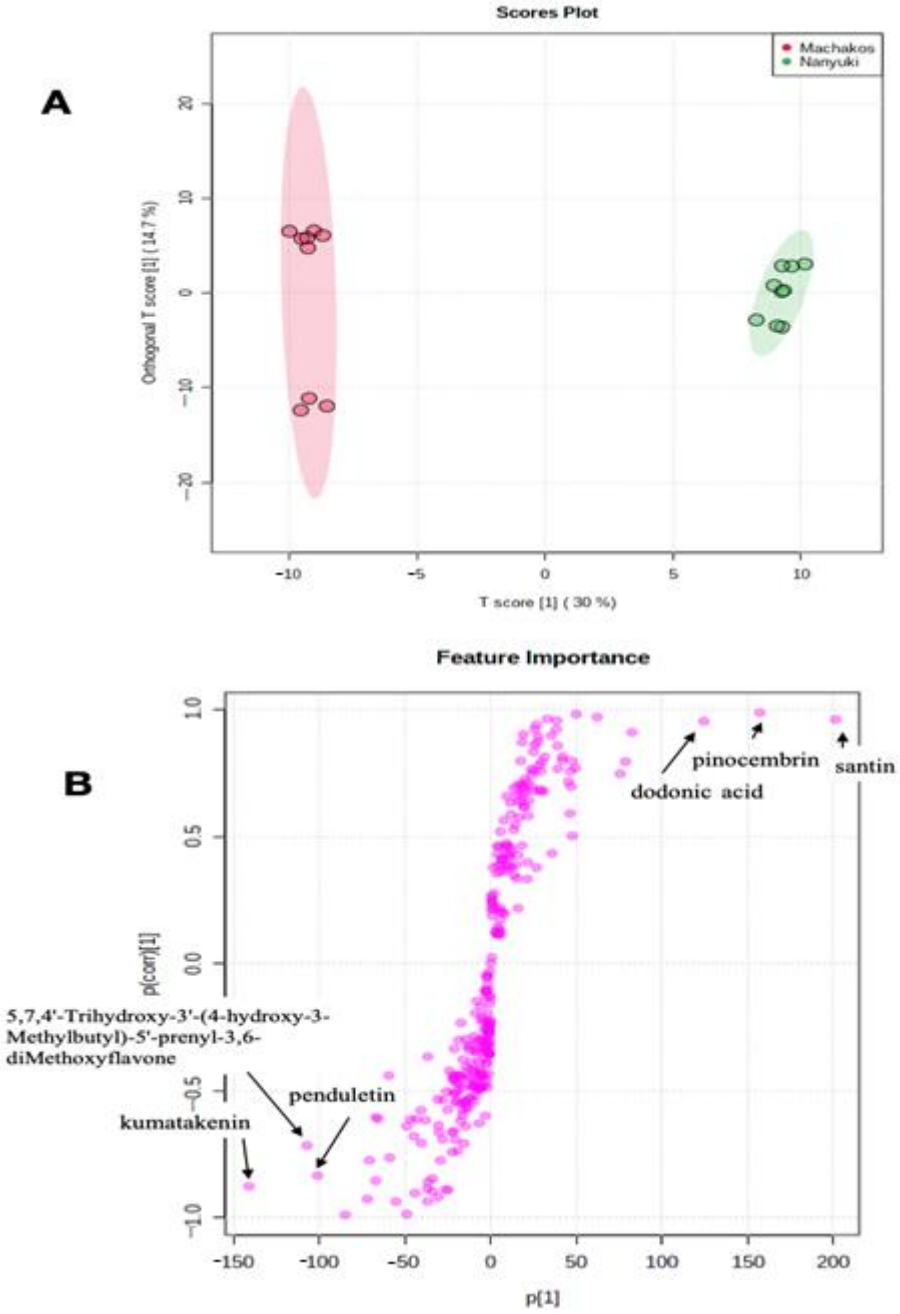


Figure 9: Orthogonal partial least square discriminants analysis (OPLS-DA) for *D. viscosa* Nanyuki and Machakos populations

Figure A) Orthogonal partial least squares discriminant analysis (OPLS-DA) for Dodonanea viscosa Nanyuki and Machakos populations. The OPLS-DA test separation of Machakos and Nanyuki populations of D. viscosa was based on T score 1 which was 30% separating Machakos population on the left and Nanyuki population on the right**. B)** Loadings of OPLS-DA of LC–MS spectra for *D. viscosa* Nanyuki and Machakos populations.

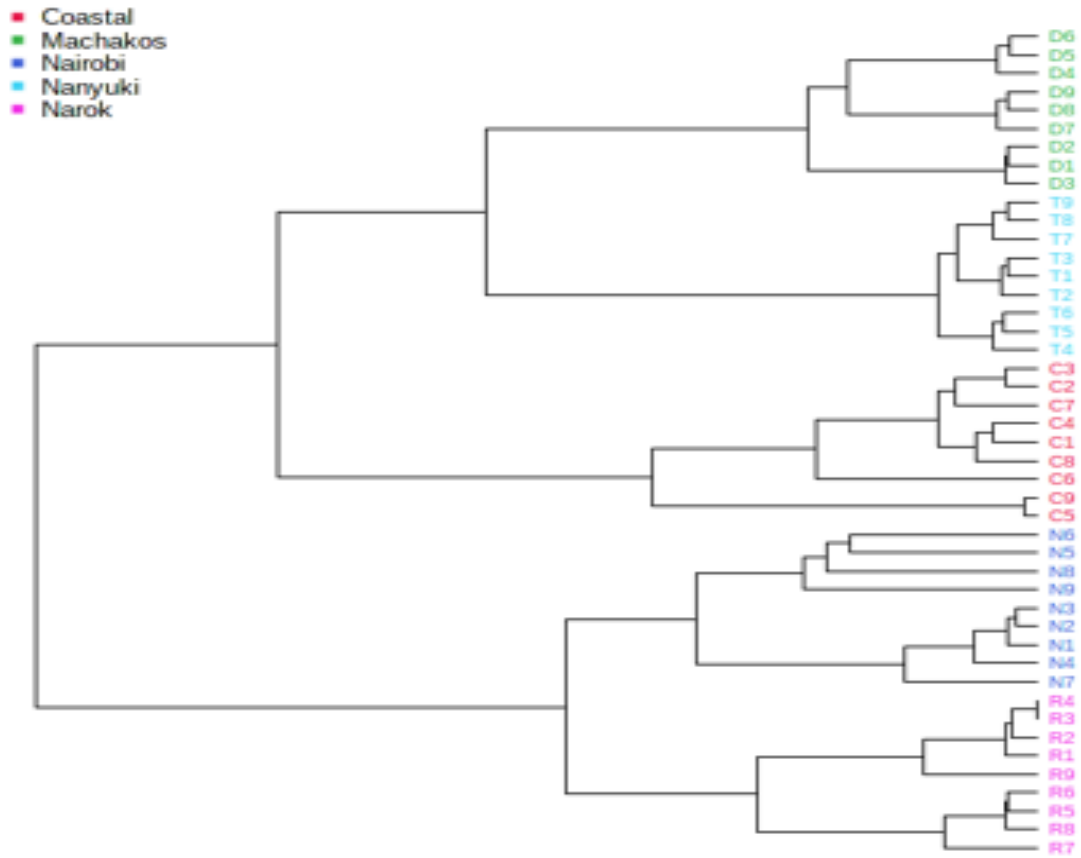


Figure 10: Dendogram of Kenyan *D. viscosa* populations

Dendogram/Relation tree of Kenyan *D. viscosa* populations from different ecological zones based on different phytochemicals. The Nanyuki population shown by (cyan colour), Machakos (green colour), coastal (red colour), Narok (purple colour) and Nairobi (blue colour).

The Hierarchical cluster analysis as shown in the dendogram above indicates that Nanyuki and Machakos populations of *D. viscosa* were closely related. The coastal population was distantly related to the two aforementioned populations but its relationship to these populations is not as strong as it is seen in the aforementioned populations. Nairobi and Narok populations are attached on the same root from the ancestor showing that they are closely related. The two populations (Nairobi and Narok populations) seem to arise from a different lineage from that of Nanyuki,

Machakos and coastal. The *D.viscosa* discriminants are as shown in **table 3** and their concentrations in different populations are shown in **figure 11**.

Table 3: Discriminants compounds identified for *D. viscosa* populations

	Retention time (min)	ESI negative $[M-H]^-$ (*m/z*)	Elemental composition	Name	Population with highest concentration
1.	0.75	191.0559	$C_{10}H_7O_4$	p-coumaric acid ethyl ester	Nanyuki
2.	5.48	351.2174	$C_{20}H_{31}O_5$	ent-16j-hydroxy-labdan-3a,8b-dihydroxy,13(14)-en-15,16-olide	Nairobi
3.	5.76	363.1808	$C_{20}H_{27}O_6$	terpentecin 1	coastal (Gazi)
4.	5.76	727.3681	$C_{40}H_{55}O_{12}$	dimer of terpentecin 1	coastal (Gazi)
5.	6.11	727.3676	$C_{40}H_{55}O_{12}$	dimer of terpentecin 2	coastal (Gazi)
6.	6.12	363.1807	$C_{20}H_{27}O_6$	terpentecin 2	Nanyuki
7.	7.00	329.0663	$C_{20}H_{25}O_4$	dodonic acid	Nanyuki
8.	7.53	377.1964	$C_{21}H_{29}O_6$	hypophyllin E	Nanyuki
9.	7.69	255.0661	$C_{15}H_{11}O_4$	pinocembrin	Nanyuki
10.	8.20	313.07	$C_{17}H_{13}O_6$	kumatakenin	Nairobi
11.	8.41	343.0818	$C_{18}H_{15}O_7$	santin	Nanyuki

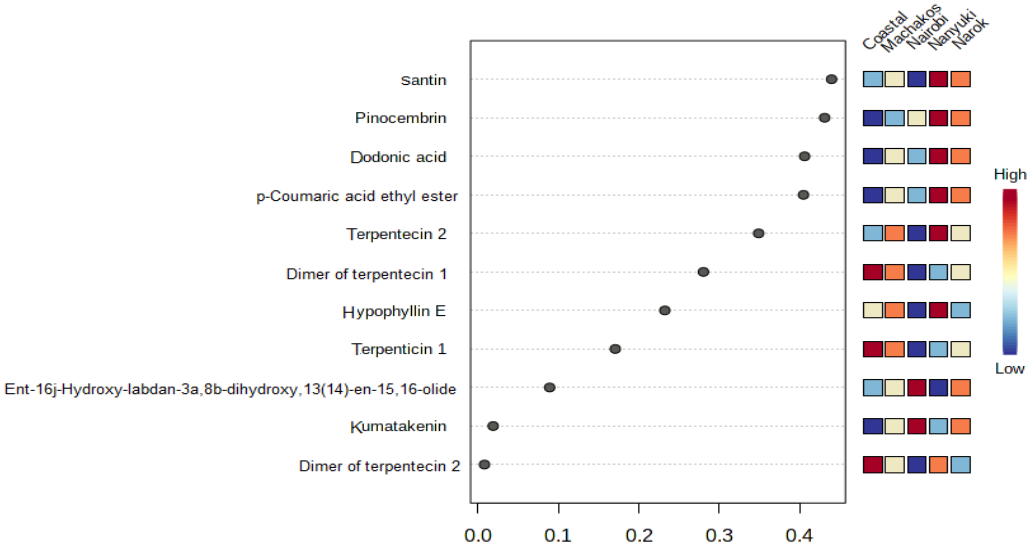


Figure 11: Concentration of different discriminants in *D. viscosa* populations

Concentration of chemical discriminants in different *D. viscosa* populations. The dark red shows high concentration of the individual chemical in each population while the dark green colour implies low concentration

4.4. DISCUSSION

Two genera of plants (*Zanthoxylum* species and *Dodonaea viscosa* populations) were analysed in chemosystematics using metabolomics.

4.4.1. Metabolomics of *Zanthoxylum* species in Kenya

Five *Zanthoxylum* species (*Z. chalybeum, Z. gilletii, Z. holtzianum, Z. paracanthum and Z. usambarense*) were analysed and 12 discriminants out of 862 chemicals were identified as the cause of the separation of the five *Zanthoxylum* species. The dendogram composed of the five species of *Zanthoxylum* resulted into two groups *Z. usambarense, Z. gilletii* and *Z. paracanthum* and that of *Z. chalybeum* and *Z. holtzianum*. *Z. usambarense* and *Z. gilletii* are closely related and *Z. paracanthum* is closely related to the two aforementioned species. *Z. chalybeum* and *Z. holtzianum* are closely related and seem to arise from a different lineage from that of *Z. usambarense, Z. gilletii* and *Z. paracanthum. Z. chalybeum* and *Z. holtzianum* can be termed as a monophyletic group as they arise from a common ancestor. Similarly, *Z. usambarense, Z. gilletii* and *Z. paracanthum* are monophyletic group because they are rooted together. The relationship between the two groups (*Z. usambarense, Z. gilletii* and *Z. paracanthum* and that of *Z. chalybeum* and *Z. holtzianum*) can be termed as paraphyletic since the two groups seem to arise from different ancestors.

The relationship of Kenyan *Zanthoxylum* species showed in this study is supported by a report by (Appelhans *et al*., 2018) who showed that *Z. gilletii* and *Z. holtzianum* were rooted to a different ancestor using molecular phylogenetics. It may therefore be argued that the relationship of Kenyan *Zanthoxylum* species in this study is not influenced by environmental conditions but evolution as species like *Z. holtzianum* and *Z. paracanthum* were collected from same locality

(Mrima Hills) and seem to appear in different groups based on the metabolites present. Plants metabolites production is determine by the genetic composition as well as environment (Cragg and Newman, 2007). Synthesis of metabolites in members of a given species could vary with age, soil browsing or season of the year (Ebuu and Sawyerr, 1994).

From morphological classification, *Z. chalybeum* is closely related to *Z. holtzianum* while *Z. gilletii, Z. usambarense* and *Z. paracanthum* are closely related (Beentje *et al*., 1994) which corroborates with this study which reports the chemosystematics of the Kenyan *Zanthoxylum* for the first time. The metabolites production of these species could be correlated to the evolution and morphology of the species (Appelhans *et al*., 2018; Beentje *et al*., 1994) and not environmental conditions and or geographical localities (Newman & Cragg, 2007) as the case of *Z. paracanthum* and *Z. holtzianum* seems to differ with the latter mentioned factors.

4.4.2. Metabolomics of *Dodonaea viscosa* populations in Kenya

Five groups of populations of *D. viscosa* (Nanyuki, Machakos, Narok, Nairobi and coastal) were analysed in this study. The chemosystematics of the five groups of populations of *D. viscosa* resulted to a dendogram composed of 3 clusters (cluster A composed of Machakos and Nanyuki, cluster B composed of the coastal and cluster C composed of Nairobi and Narok populations). The coastal collection was closely related to Machakos and Nanyuki populations. Nairobi and Narok populations are attached on the same root from the ancestor showing that they are closely related. The two populations (Nairobi and Narok populations) seem to arise from a different lineage from that of Nanyuki, Machakos and coastal. Eleven out of the total 313 recorded metabolites were identified as the discriminants (chemical markers) of the 5 groups of populations of *D. viscosa*.

Based on morphology, the coastal population is believed to be composed of *D. viscosa* subsp *viscosa* while other populations are from *D. viscosa* subsp *angustifolia* (Beentje *et al*., 1994).

The chemicals produced are thus related to the geographical and environmental conditions prevailing at the sites where each population was collected (Newman & Cragg, 2007). For example the Nanyuki population as well as Machakos population produced a group of similar chemicals irrespective of the wide distribution because of the environmental conditions in two localities were similar.

Similarly, the Narok and Nairobi populations were found to contain similar metabolites. Metabolites production in plants help plants to fight natural enemies as well as counteract with other forms of environmental stress such as pollution (Mazid *et al*., 2011). The environmental conditions prevailing in the Machakos and Nanyuki groups of *D. viscosa* populations include drought accompanied with high temperatures in the day and very low temperatures at night (Speranza, 2010). The group of chemicals produced by the two groups of *D. viscosa* populations is believed to help these plants survive such environmental conditions. Arguably, the Nairobi and Narok collections experience environmental stress such as pollution (Nairobi population) (Mazid *et al*., 2011) and browsing (Narok plants) and thus the group of chemicals produced by these plants help to counter pollution as well as browsing effects.

The coastal population on the other hand was found to have different group of discriminants arguably meant to counter high temperatures and salinity (Akula & Ravishankar, 2011; Smirnoff & Stewart, 1985). This explains why the Coastal group of populations is closely related to the Nanyuki and Machakos as compared to Nairobi and Narok populations. It can be argued that the metabolite production by the different populations of *D. viscosa* populations in Kenya is based on environmental conditions and not evolution due to the similarity that *D. viscosa* subsp *viscosa* (coastal populations) has to the Nanyuki and Machakos populations (composed of *D. viscosa* subsp *angustifolia*). On the contrary, the Narok and Nairobi populations which are

composed of *D. viscosa* subsp *angustifolia* were found to have no relatedness with Nanyuki and Machakos populations which are also composed of *D. viscosa* subsp *angustifolia*. This is the first report on the relatedness of *D. viscosa* populations in Kenya.

4.5. CONCLUSION

The chemosystematics study of the Kenyan *Zanthoxylum* species is in agreement with the current classification of the genus based on morphology where *Z. chalybeum* and *Z. holtzianum* are closely related while *Z. paracanthum*, *Z. gilletii* and *Z usambarense* clustered together. The chemosystematics of *D. viscosa* populations in Kenya is in disagreement with the current morphological classification of this species. This is because, the coastal population composed of *D. viscosa* subsp *viscosa* was closely related to Nanyuki and Machakos collections which are constituted by *D. viscosa* subsp *angustifolia*. On the other hand, Narok and Nairobi collections were closely related but differed from Nanyuki and Machakos materials irrespective of them being *D. viscosa* subsp *angustifolia*.

Laudanine, kalambroside A and B, gamma-sanshool, ochotensine, cis-N-Methylcanadine, 18-hydroxylinoleic acid, dihydrochelerythrine, N-feruloylglycine, P-coumaric acid ethyl ester, dihydroberberine and one unknown compound were recognized as the chemical markers that accounted for the differences in *Zanthoxylum* species. In *D. viscosa* populations, p-coumaric acid ethyl ester, ent-16j-hydroxy-labdan-3a,8b-dihydroxy,13(14)-en-15,16-olide, terpentecin 1, dimer of terpentecin 1, dimer of terpentecin 2, terpentecin 2, dodonic acid, hypophyllin E, pinocembrin, kumatakenin and santin compounds were responsible for the clustering of *D. viscosa* populations.

CHAPTER FIVE: PHYTOCHEMICAL ISOLATION

PHYTOCHEMICAL INVESTIGATION OF *Z. PARACANTHUM* AND *DODONAEA VISCOSA* FROM COASTAL AND NANYUKI POPULATIONS

5.1. INTRODUCTON

Phytochemicals are part of natural products obtained from plants inform of chemicals. Individual plant synthesize phytochemicals as a way of protecting itself from harsh environmental conditions brought about by both biotic and abiotic factors. Phytochemicals are viewed as plants component with non-nutritive value with the ability to prevent one from diseases (Surh, 2003).

Based on scientific evidence, phytochemicals have the potential to cause immunity stimulation, prevent inflammation, cancer and DNA damage. Phytochemicals are also known to aid in repairing of DNA, apoptosis, gene expression, activation of insulin receptors and regulation of hormone signals (Ferrari & Torres, 2003; Katiyar, 2011; Nichols & Katiyar, 2010).

Carotenoids for example, inhibit cancer growth and cardiovascular disease and boost immunity (Chew & Park, 2004; Hughes, 2001). Flavonoids fight inflammation and tumor growth while anthocyanins is associated with longevity in some animals (Kim *et al*., 2006). Proanthocyanidins and flavanols on the other hand are linked to better function of the lining of the arteries and reduced blood pressure while Sulfides and thiols help decrease "bad" LDL cholesterol (Farooqui, 2012). Isothiocyanates (sulforaphane) help protect against cancer and cardiovascular disease while quercetin help decrease inflammation and blood pressure (Rodriguez-Casado, 2016). Terpenes help slow cancer cell growth and fight viruses while lutein and zeaxanthin on the other hand are linked to eye health (Abdel-Aal *et al*., 2015).

This study targeted phytochemical isolation of the root bark of *Z. paracanthum* and leaf extracts of *D. viscosa* from coast and Nanyuki collections as they recorded high antimicrobial activity.

5.1.1. Objective

To isolate and characterize compounds from the roots of *Zanthoxylum pacanthum* and leaves of of *Dodonaea viscosa* populations in Kenya using 2D NMR and LC-MS

5.2. MATERIALS AND METHODS

5.2.1. Extraction and isolation of compounds

Each of the ground plant material was extracted separately in a thorough and sequential manner with adequate quantities dichloromethane: methanol (CH2Cl2: CH_3OH) (1:1) for 72 h. The resulting mixture was passed through filter papers to to eliminate the debris. Rotary evaporator was used to get rid of the solvent mixture from the extracts. Isolation of compounds from the extracts was done using column chromatography on Silica gel and purified using gel filtration on Sephadex as well as preparative thin layer chromatography (PTLC).

5.2.1.1. Extraction and isolation of compounds from the roots of *Zanthoxylum paracanthum*

The ground material (960g) of *Z. paracanthum* was thoroughly extracted for 72 h consecutively with adequate quantities CH2Cl2: CH_3OH (1:1) (1:1). After removal of debris and the solvent, the resulting extract was 128g. This was followed by partitioning of 77g of the extract between CH_2Cl_2 and water followed by EtOAc and water. Removal of the organic solvents yielded 45 g of CH_2Cl_2 and 3 g of EtOAc extracts. The CH_2Cl_2 extract (45g) was adsorbed (80 g) of silica gel and isolated in column chromatography packed with silica gel (700g) in 100% *n*-hexane. Elution was progressively done with *n*-hexane 100% and

systematically increasing amounts of EtOAc (from 1%, 2%, 5%, 10%, 20%, 30% … 100% EtOAc) followed by 5% CH_3OH in EtOAs increased with a factor of 10% up to 100% CH_3OH. The main column yielded 220 fractions each 400 ml eluent. The fractions with alike TLC profiles were pooled into one. Eluents made at 1- 4% EtOAc in *n*-hexane yielded nothing but oily substances and which were not investigated further. Elutions obtained at 4% EtOAc in *n*-hexane were pooled together and re-purified using Sephadex LH-20 (eluent: CH_2Cl_2/CH_3OH, (1:1) to yield compound (**1)** (50 mg) as a white powder. Fractions eluted at 6-10% of EtOAc in *n*-hexane were pooled together and separated by column chromatography over silica gel eluting with *n*-hexane: EtOAc (4:1) to yield compounds (**2**), (100mg), (**3**), (320mg) which were white amorphous solids and (**4**), (101) mg, as armophous powder. Fractions obtained at 12% EtOAc in *n*-hexane were combined, crystallized and washed with (*n*-hexane/ EtOAc; 3.5:1.5) yielded cream powder of compound (**5**), (42mg). The fractions obtained at 15-70% of EtOAc in *n*-hexane were all pooled and cleaned with Sephadex (eluent: CH_2Cl_2/CH_3OH, 1:1) to afford yellow powder of compound (**6**), 68mg, orange powder of compound (**7**), 1g and white amorphous solid of compound (**8**), 54mg. The EtOAc extract (3g) was not followed further.

5.2.1.2. Extraction and isolation of compounds from the leaves of *Dodonaea viscosa* coastal population

The ground material (800 g) of *Dodonaea viscosa* collected from Gazi (coastal) was extracted as described above. After removal of debris and the solvent, the resulting extract was 220g. Part of this extract (50g) was adsorbed in (100g) silica gel and isolated in column chromatography using silica gel (700 g) packed under 100% *n*-hexane. Elution was done using 100% *n*-hexane containing increasing amounts of EtOAc (1% to 100% EtOAc) followed

byEtOAc containing increasing amounts of CH_3OH (1% to 100% CH_3OH). Elutions made at 1-15% of EtOAc in *n*-hexane did not yield any compound but resulted to oily substances which were not followed further. Fractions eluted between 15% and 30% of EtOAc in *n*-hexane were pooled together and cleaned with n-hexane: EtOAc (3:2) to give a white amorphous solid of compound (**9**), (100mg). Elutions obtained at 40% of EtOAc in *n*-hexane were combined and washed with *n*-hexane: EtOAc (3:2) yielding a yellow powder of compound (**10**), (11mg). Fractions obtained between 40% and 100% EtOAc *n*-hexane were further combined to yield 10g and further separated by column chromatography over silica gel using *n*-hexane containing increasing amounts of EtOAc and yielded white amorphous compound (**11**)**, (**29mg) at 30% of EtOAc in *n*-hexane elution.

5.2.1.3. Extraction and isolation of compounds from the leaves of *Dodonaea viscosa* Nanyuki poplation

The ground powder (910g) of *Dodonaea viscosa* leaves collected from Nanyuki was extracted as described above. After removal of debris and the solvent, the resulting extract was 250g. This was followed by partitioning (120 g) of the extract between CH_2Cl_2 and H_2O followed by EtOAc and H_2O. Removal of the organic solvents yielded 5g of CH_2Cl_2 and 37g of EtOAc extracts. The EtOAc extract (37 g) was adsorbed on (70g) of silica gel and isolated in a column with silica gel (500 g) packed under 100% *n*-hexane. Fractions obtained at 1-10% was not followed further. Fractions obtained at 40-80% of EtOAc in *n*-hexane were all pooled (6 g) and packed in a small column and isolated using *n*-hexane containg increasing amounts of EtOAc which yielded brown crystals of compound (**12) (**73 mg) at 80% of EtOAc in *n*-hexane elution

5.2.2. Structure elucidation of of isolated compounds

Fractions were vied using TLC under (UV254 nm) and iodine vapour. ^{1}H and ^{13}C NMR, COSY, HMBC, NOESY and HSQC were used to determine the isolated compounds structures and comparison with the literature.

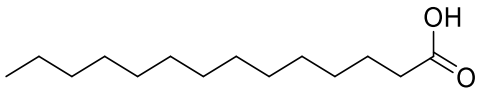
5.3. RESULTS AND DISCUSSION

5.3.1. Secondary Metabolites isolated from *Zanthoxylum paracanthum*

The CH_2Cl_2/ CH_3OH root bark extract of *Z. paracanthum* yielded 8 compounds which were characterized as described below.

Myristic acid (1)

Compound **1** was white crystals and UV inactive. The molecular formula was $C_{14}H_{28}O_2$ based on ESIMS ($[M-H]^-$ *m/z* 227). Its ^{13}C NMR spectrum showed 13 sp^3 hybridized carbons and one carbonyl carbon in the range of a carboxylic acid class. This suggested the presence of a saturated fatty acid. These were assigned as follows **(table 4)**; δ_C 179.4 (C-1), δ_C 33.9 (C-2), δ_C 24.9 (C-3), δ_C 12C.9-29.6 (C-4 to C-11), δ_C 31.9 (C-12), δ_C 22.7 (C-13) and δ_C 14.1 (C-14). HMBC was used to further confirm the structure where H-2 (δ_H 2.35, t) exhibited connections with C-1 and C-3, H-3 (δ_H 1.62, q) showed correlations with C-2 and C-5, and H-13 (δ_H 1.26, m) showed correlation with C-4 and C-11. The compound was thus identifed as the fatty acid myristic acid (Yaouba, 2018).



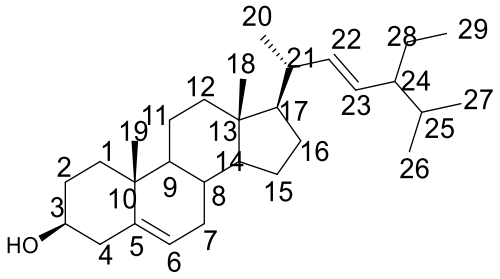
Myristic acid **(1)**

Table 4: NMR data for myristic acid (1)

Position	^{1}H NMR (δ_H)	^{13}C NMR (δ_C)	HMBC
1		179.4	
2	2.35 (t)	33.9	C-1, C-3
3	1.62 (q)	24.7	C-2, C-5
4			
5			
6	1.26 (m)	129.1-29.6	
7			
9			
10			
11			
12	12.5 (m)	31.9	
13	1.26 (m)	22.7	C-4, C-11
14	0.88 (t)	14.1	C-13, C-12

Stigmasterol (2)

Compound **2** was isolated as white armophous solid. The molecular formular was found to be $C_{29}H_{48}O$ from the ESIMS ([M-H]$^-$ *m/z* 411). The ^{13}C NMR spectra showed signals corresponding to exactly 29 carbon atoms, among which four are olefinic and one oxygenated sp^3 carbon which were assigned as follows δ_C 140.7 (C-5), δ_C 129.6 (C-6) δ_C 138.2 (C-22) δ_C 129.3 (C-23) and δ_C 71.7 (C-3) respectively. The rest of the carbons were all sp^3 carbons and were assigned in comparison with literature as shown in the table below. The presence of the olefin functional groups was further confirmed by protons resonating at δ_H 5.35(H-6, qd), δ_H 5.14 (H-22, d) and δ_H 5.02 (H-23, dd). Six methyl groups were likewise seen based on the NMR signals at δ_H 0.97 (CH_3- 19), δ_H 0.9(CH_3-24), δ_H0.75 (CH_3-26), δ_H1.31 (CH_3-27), δ_H 1.06(CH_3-28), δ_H 00.89 (CH_3-29). The assignment was further confirmed using HMBC and COSY as presented in **Table 5**. The compound was thus recognized as stigmasterol (Ali *et al*., 2015).



Stigmasterol **(2)**

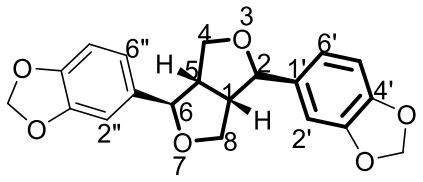
Table 5: NMR data for stigmasterol (2)

Position	**^{13}C**	**^{1}H-NMR**	**HMBC**
1	37.3	1.06(m)	C-3, C-19, C-10
2	31.7	1.88(ddt)	C-10, C-4
3	71.7	3.52(m)	C-4
4	45.8		
5	140.7		
6	129.7	5.35(qd)	C-4, C-7, C-10
7	31.9	1.83(ddq)	C-5, C-9, C-14
8	31.9	2.03 (m)	C-13
9	50.1	0.97 (d)	C-1, C-12
10	36.5		
11	21.2	1.51 (m)	C-8, C-13
12	39.8	2.27 (m);1.99 (m)	C-9, C-14
13	42.3		
14	56.8	1.06(m)	C-16
15	24.3	1.51 (m)	C-7, C-8
16	28.2		
17	56.1	1.21	
18	12.0	0.85 (d)	C-12, C-17, C-14
19	18.8	0.97 (d)	C-5
20	40.5	1.99 (m)	C-13, C-23
21	21.1		
22	138.2	5.14 (d)	C-23, C-24, C-20, C-21
23	129.3	5.02 (dd)	C-22
24	51.2		
25	26.1	1.21 (m)	C-23
26	11.9	0.75	
27	29.2	1.31 (m)	
28	20.5	1.06 (m)	
29	19.1	0.89(m)	

Sesamin (3)

Compound **3** was a white amorphous solid which was UV active (254 nm). The molecular formular was found to be $C_{20}H_{18}O_6$ from the ESIMS ($[M-H]^-$ *m/z* 354). ^{13}C NMR spectrum revealed three peaks with low intensity which revelaed the presence of the aromatic quaternary carbon. The chemical shifts at δ_C 147.9 and δ_C 147.0 were apportioned to oxygenated aromatic carbon 3′, 3″ and 4′, 4″ correspondingly. The chemical shift at 135.6 was allocated to quartenary carbon C-1′ and C-1″. Three tertiary aromatic carbon with high peak intensity were observed at δ_C 106.4 (C2′, 2″), δ_C 107.9 (C5′, 5″) δ_C 119.2 assigned to C6′, 6″. δ_C 101.2 confirmed the presence of methylenedioxy. Two oxymethyne peaks were observed one tertiary and another secondary δ_C 85.7 was assigned to C-2 and C-6. δ_C 71.7 assigned to C-4 and C-8. δ_C 54.5 was allocated the methyne carbons (C-1 and C-5). Additionally, two pairs of doublets were recognized on the ^1H NMR at δ_H 3.88 (*dd, J* = 9.2 Hz, 2H) and δ_H 4.24 (*ddd, J* = 9.1 Hz, 2H) attributed to the methylene protons at C-4 (axial) and C-4 (equatorial). A twin signal at δ_H 4.72 (*J* = 4.3 Hz) was observed and ascribed to H-2 and H-6. The downfield signal located at δ_H 5.98 (*s*, 2H) was a feature of methylenedioxy protons. The ^1H NMR spectrum additionally exhibited signals linked to four magnetically and chemically corresponding to aromatic protons at δ_H 6.82 which was allocated to H-5′, H-6′ and H-5″, H-6″ whereas the signal at δH 6.88 was ascribed to H- 2′ and H-2″.

The ESIMS data together with all NMR spectra values as recorded in the table shown **(Table 6)** were considered in the above structural elucidation. Based on the above spectroscopic data and comparison literature, compound **3** was identified as sesamin (Pelter, 1976) which was earlier isolated from the *Z. budrunga* (Mukhlesur *et al* 2003), *Z. holtzianum* (Buyinza, 2012; Akampurira, 2013) and *Z. paracanthum* (Omosa *et al*., 2019; Samita *et al*., 2013).



Sesamin **(3)**

Table 6: NMR Data for sesamin (3)

Position	**^{1}H NMR (δ_H)**	**^{13}C NMR (δ_C)**	**HMBC**
1	3.06	54.5	C-2, C-1"
2	4.72	85.7	C-1, C-4, C-2", C-6" C-1"
3		O	
4	4.24 ; 3.88	71.7	C-1, C-2
5		54.5	
6	4.72	85.7	
7		O	
8		71.7	
1'		135.6	
2'	6.88	106.4	
3'		147.0	
4'		147.9	
5'	6.82	107.9	
6'	6.84	119.2	
1"		135.6	
2"	6.88	106.4	
3"		147.0	
4"		147.9	
5"	6.82	107.9	
6"	6.84	119.2	
-O-CH_2-O-	5.98	101.2	

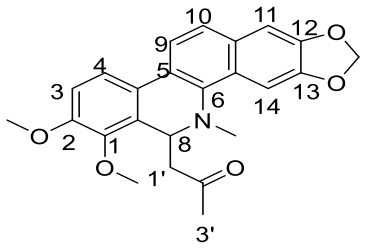
8- Acetonyldihydrochelerythrine (4)

Compound **4** was in form of colourless crystals. It showed flouresence under UV (254-366 nm) on TLC plates. The molecular formular was determined to be $C_{24}H_{23}NO_5$ based on ESIMS ($[M+H]^+$ *m/z* 406). Out of twenty-four carbon peaks in the ^{13}C NMR spectrum, sixteen were sp^2-

hybridised carbons (one of which is typical of methylenedioxy group), two methoxy and a *N*-methyl peaks. The ^{13}C NMR spectrum showed four oxygenated carbons on benzophenanthridine skeleton consisting of rings A, B,. From biogenetic consideration and chemical shift values, the four oxygen atoms were placed and assigned C-1 (δ_C 145.6), , C-2 (δ_C 152.2), C-12 (δC 147.6) and (C-13 (δ_C 148.2). The methoxy protons (δ_H 3.92, δ_C 55.8) showed a correlation with C-2 and C-3 and the other methoxy group (δ_H 3.96, δ_C 60.9) showed correlation with C-1. Therefore, methylenedioxy group is attached on ring D at C-12/C-13.

The aromatic region from ^{1}H exhibited 6 protons; two sets of *ortho*-coupled protons and two one-proton singlets. A set of one-proton doublets (δ_H 6.96 and δ_H 7.55), showed ^{1}H-^{1}H COSY correlation with each other and HMBC correlation with oxygenated carbons at C-1 and C-2. The two protons (δ_H 6.96 and δ_H 7.55) were thus assigned H-3 and H-4 respectively. The second pair of one-proton doublets (δ_H 7.71 and 7.51) was assigned to H-9 and H-10 respectively using HMBC and HSQC spectra. Futhermore, the two singlet proton peaks showed HMBC correlation with the two oxygenated carbons (C-12/C-13) of ring D and the methylenedioxy protons showed correlation with the same carbons and were hence placed at C-11 and C-14. From HMBC, the three proton singlet δ_H 2.64 (N-CH_3) showed a cross peak with the methine carbon (δ_C 55.0, δH 5.05) which confirmed fixing of the benzylic proton at C-8. The methyl singlet at δ_H 2.06 and the AMX system with J = 3.6, 7.2 and 11 Hz at δ_H 2.26, δ_H 2.64 and δ_H 5.05 due to H-8, indicated the presence of the acetonyl group (-CH_2COCH_3) with signals at δ_C 207.2 (C=O), δ_C 31.0 (-CO$\underline{C}H_3$) and δ_C 46.9 (-CO$\underline{C}H_2$) **table 7**. The attachement of the acetonyl group at C-8 was further comfirmed sing HMBC, ^{1}H-^{1}H-COSYand HSQC. Compound **4** was identified as 8-acetonyldihydrochelerythrine; a known alkaloid reported in *Zanthoxylum* species (Neg *et al*.,

2011).



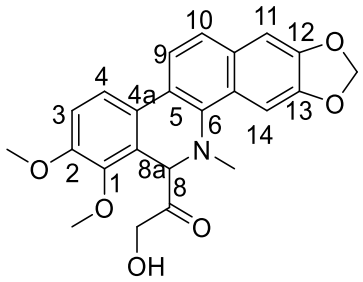
8-Acetonyldihydrochelerythrine **(4)**

Table 7: NMR Data for 8-acetonyldihydrochelerythrine (4)

Position	**^{13}C NMR (δc)**	**^{1}H NMR (δH)**	**HMBC**
1	145.6		
2	152.2		
3	111.7	6.96 (d)	C-2, C-1, C-4a
4	118.8	7.55 (d)	
4a	127.7		
5	131.1		
6	139.1		
7 N-CH_3	42.8	2.64(s)	C-8, C-6
8	55.0	5.05(dd)	C-7, C-1', C-1, C-6
8a	128.1		
9	119.7	7.71d)	C-6, C-5, C-4a, C-8a
10	124.7	7.51(d)	
10a	123.9		
11	104.3	7.1(s)	C-14, C-10a, C-13, C-12,
12	147.6		
13	148.2		
14	100.6	7.51 (s)	C-13, C-12
14a	127.7		
1'	46.9	2.26(dd),2.64	C-2', C-8, C-8a, C-2
2'	207.2		
3'	31.0	2.06 (s)	C-1', C-2'
O-CH_2-O	101.0	6.04 (d)	C-12, C-13
1-OCH_3	60.9	3.96 (s)	C-1
2-OCH_3	55.8	3.92(s)	C-3, C-2

Arnottianamide (5)

Compound 5 was isolated as cream crystals and showed fluoresence under (254-366 nm). Its molecular formular was determined to be $C_{23}H_{21}NO_6$ from the ESIMS ($[M+H]^+$ m/z 408). The 13C NMR spectrum showed 17 sp2 carbons, of which 4 of which are oxygenated, 3 are quartenary (as evident from their low peak intensities and lack of HSQC correlaions), 1 carbonyl carbon and 6 tertiary aromatic carbons. The 13C NMR spectrum also showed the presence of 6 sp3 carbons and a methylenedioxy group. The skeleton was established to be that of benzophenanthridine constituting of four rings on the bases of NMR **(Table 8)** and biosynthetic considerations (Nissanka et al., 2001). Based on the 13C NMR cgemical shift values and biosynthetic considerations, the four oxygenated sp2 crbons are assigend to C-1 (δC 146.9), C2 (δC 152.4), C-12 (δC 147.6) and C-13 (δC 147.9). The methoxy proton (δH 3.88, δC 55.8) showed a correlation with C-2 and C-3 and the other methoxy proton (δH 3.96, δC 60.9.8) showed correlation with C-1. Therefore, methylenedioxy group was attached on ring D at C-12/C-13 confirming the identification of this compound to be arnottianamide, a chemical reported from Z. holtzianum (Akampurira, 2013).



Arnottianamide **(5)**

Table 8: NMR data for arnottianamide (5)

POSITION	^{13}C	^{1}H-NMR	HMBC
1	146.9		
2	152.4		
3	113.1	6.98(d)	C-2, C-1
4	119.3	7.60 (d)	C-5 , C-8a
4a	123.9		
5	131.0		
6	140.5		
7	43.44	2.9	C-6, C-8
8	66.1	7.32	
8a	126.0		C-2', C-5, C-6, C-1
9	117.9	7.52(d)	
10	122.9	7.33(d)	C-11, C-5, C-10a
10a	122.2		
11	104.2	7.00 (s)	C-12, C-13, C-10a, C-14a
12	147.6		
13	147.9		
14	101.0	7.55	C-11, C-5, C-6,C-12, C-13
14a	125.9		
1'	181.9		
2'	42.8	2.32	
1-OCH_3	60.8	3.81	C-1
2-OCH_3	56.1	3.88	C-2, C-1, C-3
OCH_2O	101.0	6.02	C-12 ,C-13

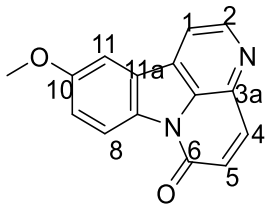
10-Methoxycanthn-6- one (6)

Compound **6** was an orange powder and showed fluoresence under (254-366 nm). Its molecular formular was $C_{15}H_{10}N_2O_2$ from the ESIMS ($[M+H]^+$ *m/z* 251). From the ^{13}C NMR spectrum a total of 15 carbons were identified, 7 of which were protonated aromatic carbons (-CH), 1 amidic carbon, 5 quaternary sp^2 aromatic carbons and 1 methoxy carbon. The δ_C 162.5 was assigned to the oxygenated sp^2 aromatic carbon (C-10) while the peak at δ_C 159.5 was asigned to

amidic carbon (C-6). The five quaternary carbons were assigned as follows: δ_C 135.5 (C-3a), δ_C 117.2 (C-11a), δ_C 130.2 (C-11b), δ_C 132.3 (C-11c) and δ_C 141.2 (C-7a). The seven protonated aromatic carbons were also assigned the following chemical shift values δ_C 139.6 (C-4) , δ_C 128.4 (C-5), δ_C 115.5 (C-8), δ_C 123.4 (C-9), δ_C 101.2 (C-11), δ_C 145.7 (C-2), and δ_C 113.6 (C-1).

The ^{1}H NMR showed a pair of mutually-coupled doublets assigned as follows **(table 9)**; δ_H 6.92 (*d, J*=9.76 Hz, H-5) and δ_H 8.02 (*d, J*= 9.76 Hz, H-4) and other signal resonating at δ_H 7.86 (*d, J*= 5.03 , H-8), δ_H 7.98(*dd, J*= 4.9, H-9), δ_H 7.09 (dd, J= 2.41 Hz, H-1) and δ_H 8.74 (d, J= 5.04 Hz, H-2). The coupling patern of the protons was further confirmed by H,H-COSY.

The structure was further confirmed using HMBC where the methoxy proton signal δ_H 4.00 (δ_C 55.9) showed HMBC corellation with C-10 confirming its placement at C-10; H-4 showed corellations with C-6, C-11a, C-11b; H-5 showed corellation with C-6, C-3a, C-7a; H-9 showed corellation with C-11, C-8, C-11b and C-10; and H-11 showed correlation with C-1, C-11a, and C-10. This compound was thus identified as the known 10-methoxycanthin-6-one (Arisawa *et al*., 1983). This appears to be the first reports on the isolation of this compound from the genus *Zanthoxylum*.



10-Methoxycanthin-6-one **(6)**

Table 9: NMR data of 10-Methoxycanthin-6-one

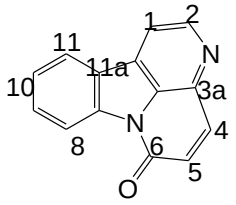
Position	$\delta_{(H)}$	^{13}C NMR (δc)	HMBC
1	7.09 (dd)	113.6	
2	8.74	145.7	
3		N	
4	8.02 (d)	139.6	C-6, C-11a, C-11b
5	6.92 (d)	128.4	C-6, C-3a, C-7a
6		159.5	
7		N	
8	7.86 (d)	115.5	C-11a, C-11c
9	7.98 (dd)	123.4	C-11, C-8, C-11b, C-10, C-6
10		162.5	
11	8.16 (d)	101.2	C-1, C-11a, C-10
11a		117.2	
11b		130.2	
11c		132.3	
7a		141.2	
3a		135.5	
-Ome	4.00 (s)	55.9	C-10

Canthin-6-one (7)

Compound **7** was an orange powder that fluoresenced under (254-366 nm). The molecular formular was determined to be $C_{14}H_8N_2O$ from the ESIMS ($[M+H]^+$ *m/z* 221). From the ^{13}C NMR spectrum, a total of fourteen carbons were identified, eight were protonated aromatic carbons (-CH), one amide carbon and five quaternary sp^2 aromatic carbons. The carbon chemical shift value δ_C 159.3 was asigned to amidic carbon (C-6). The five quaternary carbons were assigned as follows: δ_C 139.4 (C-3a), δ_C 130.3 (C-11a), δ_C135.9 (C-11b), δ_C 124.3 (C-11c) and δ_C 131.9 (C-7a). The eight protonated aromatic carbons were also assigned the following chemical shift values δ_C 139.3 (C-4), δ_C 129.0 (C-5), δ_C 117.2 (C-8), δ_C 125.6 (C-9), δ_C 130.9 (C-10), δ_C 122.6 (C-11), δ_C 145.5 (C-2), and δ_C 116.3 (C-1).

The ^{1}H NMR showed eight clearly resolved peaks in the aromatic zone which included;two pairs of mutually-coupled doublets assigned as follows; δ_H 6.94(*d, J*=9.7 Hz, H-5) and δ_H 7.98 (*d, J*= 9.7 Hz, H-4); δ_H 7.90 (*d, J*= 4.9 Hz, H-1) and δ_H 8.78 (*d, J*= 4.9 Hz, H-2). The presence of multiplet signals indicated the presence of unsubstituted aromatic ring.

The NMR assignments were equally reasoned from NOESY correlations which resembles COSY. Cross peaks were observed on close protons. This was further confirmed by the correlations adduced from ^{1}H-H COSY which confirmed that H-1 and H-2 were coupling partners. Also found to be coupling partners were; H-4 and H-5, Using Heteronuclear Multiple Bond Connectivity, H-1 correlated with C-11a and C-11c, H-2 correlated with C-11b, H-4 correlated with C-6 and C- 11c while H-5 correlated with C-3a, C-6,C-7a. Other observed correlations were; H-8 with C-10, C-11a and C-11c, H- 9 with C-7a and C-11, C-8, C-11b, C-10 and finally H-11 with C-1, C-11a, C-10 and C-11b **(table 10)**. The compound was thus identified as canthin-6-one, previously isolated from stem bark of *Z. paracanthum* (Omosa *et al*., 2019).



Canthin-6-one (**7**)

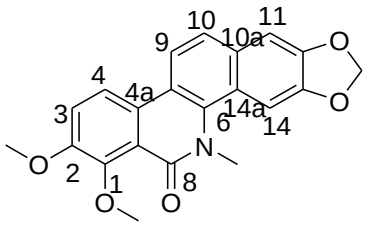
Table 10: NMR Data for canthin-6-one (7)

Position	$\delta_{(H)}$	^{13}C NMR (δc)	HMBC
1	7.90 (d)	116.3	C-11c, C-7a, C-11b, C-2
2	8.78 (d)	145.5	C-1, C-11c, C-11a, C-11b
3		N	
4	7.98 (d)	139.3	C-6, C-7a
5	6.94 (d)	129.0	C-11b, C-6, C-11c
6		159.3	
7		N	
8	8.6 (dt)	117.2	C-11c, C-9, C-10, C-11
9	7.49 (td)	125.6	C-8, C-11c, C-10
10	7.66 (dd)	130.9	
11	8.03 (m)	122.6	C-8, C-11c,C-10
11a		130.3	
11b		135.9	
11c		124.3	
7a		131.95	
3a		139.4	

8-Oxochelerythrine (8)

The molecular formular of compound **8** was determined to be $C_{21}H_{17}NO_5$ from the ESIMS ($[M+H]^+$ *m/z* 364). The ^{13}C NMR spectral data showed 16 sp^2 hybridised carbons, constituting a four ring system typical of benzophenanthridine alkaloids, with 1 amidic carbonyl carbon δ_C 163.8 (C-8), 4 oxygenated carbons δ_C 136.5 (C-1), δ_C 152.8 (C-2), δ_C 148.8 (C-12) and δ_c 149.2 (C-13); six quaternary carbons δ_C 120.0 (4a), δ_C 147.9 (C-8a), δ_C 134.5 (C-5), δ_C 135.6 (C-6), δ_C 130.9 (C-10a) and δ_C 128.3 (C-14a) and tertiary sp^2 carbons assigned as follows; δ_C 103.5 (C-3), δ_c 125.1 (C-4), δ_C 127.8 (C-9) and δ_C 127.4 (C-10) **(table 11)**.

In the HMBC spectrum, the methoxy protons at δ_H 3.65 correlated with C-1, the second methoxy protons at δ_H 3.79 showed correlation with C-2 confirming their placement at C-1 and C-2, respectively. This implied that the methylene proton is placed at C-12 and C-13 as in the other related alkaloids of this plant, and was confirmed by HMBC correlation where the methylene dioxy proton δ_H 6.15 showed correlation with C-12 and C-13. The ^{1}H NMR spectrum showed a pair of ortho-coupled protons. The first pair resonated at δ_H 6.55 (H-3) and δ_H 6.74 (H-4) and the second pair resonated at δ_H 7.21 (H-9) and δ_H 7.81 (H-10). These were also confirmed using COSY. The two protons δ_H 7.43 (H-11) and δ_H 6.98 (H-14) appeared as singlets in agreement with the proposed structure. This compound was identifed as 8-oxochelerythrine, a compound previously isolated from *Z. holtzianum* (Buyinza, 2012)**.**



8-Oxochelerythrine **(8)**

Table 11; NMR data for 8-oxochelerythrine

Position	**^{13}C NMR (δc)**	**^{1}H NMR (δH)**	**HMBC**
1	136.5		
2	`152.8		
3	103.5	6.55	C-4a, C-1
4	125.1	6.74	C-8a, C-5, C-2
4a	120.1		
5	134.5		
6	135.6		
7	33.1	2.87	C-8, C-6
8	163.8		
8a	147.96		
9	127.8	7.21	C-10a, C-6, C-4a
10	127.4	7.81	C-14a, C-11, C-5
10a	130.9		
11	104.5	7.45 (S)	C-14a, C-13, C-10

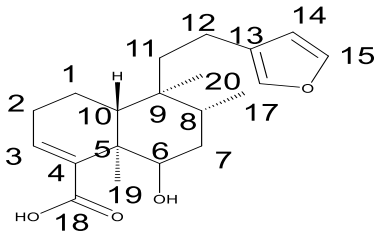
Position	^{13}C NMR (δc)	^{1}H NMR (δH)	HMBC
12	148.0		
13	149.2		
14	98.9	6.98	C-10a, C-12, C-6
14a	128.9		
C-1 OMe	60.6	3.65	C-1
C-2-OMe	55.9	3.79	C-2
O-CH_2-O	101.9	6.15	C-13, C-12

5.3.2. Secondary Metabolites isolated from *Dodonaea viscosa* coastal population

D. viscosa subsp *viscosa* yielded 3 compounds identified as hautriwaic acid lactone, 5, 7, 4', 5' -Tetrahydroxy-3, 6, 2' –trimethoxyflavone and hautriwaic acid as described below.

Dodonic acid (9)

In the ^{13}C-NMR spectrum six quaternary carbon signals, five methylene carbon signals, three methyl carbon signals and five methine signals were observed. The methyl carbon chemical shift for methyl were assigned δ_C15.3 (C-17), δ_C16.3 (C-19) and δ_C17.8 (C-20).The carbon chemical shift at δ_C71.9 was assigned to oxygenated carbon, C -6. This was justified by HMBC associations where C-5 and C-7 correlated to C-6) **(table 12)**. From the HMBC data, the attachment of the alkyl chain ta C-9 was determined by the relations between C-9 and C-12, C-20, C-17 and C- 10. From the 1H-NMR spectrum, there was evidence of deshielded proton chemical shifts at δ_H 6.32 (H-14), δ_H7.38 (H-15) and δ_H7.26 (H-16) suggested the presence of furan ring and thus the compound was identified as dodonic acid. This compound was formerly reported in *D. viscosa* (Muhammad *et al*., 2015; Omosa *et al*., 2014).



Dodonic acid (**9**)

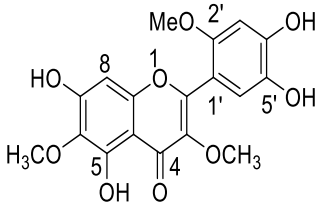
Table 12; NMR data of dodonic acid acid (9)

Position	δc	δ_H (m, J in Hz)	HMBC
1	17.2	1.74 (m)	
2	27.5	2.35 (m)	
3	143.9	7.25 (m)	
4	140.2		
5	44.7		
6	74.6	3.77 (dd, 4.9, 10.8)	
7	35.8	1.67 (m)	
8	33.6	1.77 (m)	C-9
9	38.7		
10	45.7	1.42 (m)	C-2, C-4, C-9
11	38.6	1.66 (m)	
12	17.2	1.77 (m)	C-9
13	125.3		
14	138.4	7.26 (br s)	C-13, C-15, C-16
15	142.6	7.38 (br s)	C-13
16	110.9	6.32 (br s)	C-13, C-14, C-15
17	15.3	0.9 (d, 6.6)	C-7, C-9
18	173.1		
19	16.3	1.26 (s)	C-3,C-6, C-10
20	17.8	0.8 (s)	C-8, C-10, C-11

5, 7, 4', 5' -Tetrahydroxy-3, 6, 2' -trimethoxyflavone (10)

Compound **10** was isolated as a yellow powder. From the ^{13}C NMR spectrum, 5- Oxygenated aromatic carbons at a chemical shifts of δ_C130.2 (C-6), δ_C155.9 (C-7), δ_C151.2(C-2'), δ_C150.2 (C-4')

and δ_C139.9(C-5'). The ^{13}C NMR chemical shift at δ_C177.8 indicated the presence of carbonyl group adjacent to a sp2 carbon and was assigned to C -4. The chemical shift at δ_C130.2 was assigned to C-6 which is ortho- para substituted with electron donating groups. The chemical shift at δ_C155.3 was assigned to C-7. Ring B is tri-substituted at C-2' (151.8), C-4' (δ_C150.2) and C-5' (δ_C 139.9). Also from ^{13}C NMR spectrum, 3-methoxy carbon chemical shift were observed and assigned as follows: δ_C 60.73 (C-6), δ_C 61.9(C-3) and δ_C 56.15 (C-2'). In the ^{1}H- NMR spectrum 7- singlets were observed, three of which were methoxy protons (δ_H 3.98, δ_H 3.88 and δ_H 4.05). The aromatic protons were assigned as follows δ_H 6.60 (s) (H-8); δ_H 7.26 (s) (H-6',); δ_H 6.67 (H-3',) while the hydroxyl groups were assigned to δ_H 7.84 (s) (OH-4', OH-5'), δ_H 6.67 (s) (OH- 7) and δ_H 12.74 (s) (OH-5). The HMBC correlation confirmed the assignment of the substituents as summarised in **table 13**. Compound **10** was identified as 5, 7, 4', 5'-tetrahydroxy-3,6,2' –trimethoxyflavone. This is the first report of this compound in the genus *Dodonaea* previously isolated in *Gymnosperma glutinosum* (Yu *et al*., 1988).



5,7, 4',5' -Tetrahydroxy-3,6,2'-trimethoxyflavone **(10)**

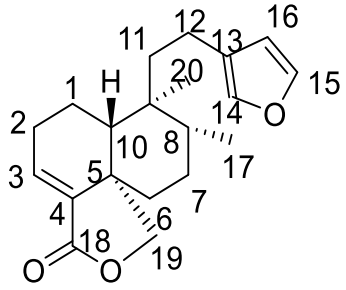
Table 13: NMR data of 5, 7, 4', 5' -tetrahydroxy-3,6,2' -trimethoxyflavone (10)

Position	δc	δ_H	HMBC
2	155.9		
3	136.4		
4	177.8		C-4
4a	106.0		
5	151.2		
6	130.2		
7	155.3		
8	93.3	6.60 (s)	C-4a, C-6, C-8a, C-7
8a	152.8		
1'	109.8		

Position	δc	δ_H	HMBC
2’	151.8		
3’	102.2	6.66	C-1’, C-6, C-5’, C-2, C-8a, C-4’, C-5
4’	150.2		
5’	139.9		
6’	113.3	7.26 (s)	C-5’
2 ’-OMe	56.15	3.98	C-2’
3-OMe	61.90	3.88	C-3
6-OMe	60.73	4.05	C-6
OH-4’(-5’)		7.84 (s)	C-3’ C-1’, C-5
OH-7		6.67 (s)	C-7, C-8
OH-5		12.74 (s)	

Hautriwaic acid lactone (11)

The ^{1}H and the ^{13}C NMR of this compound resemble that of hautriwaic acid except that in this compound the carboxylic acid group cyclised with the hydroxy group at C-6 to make a lactone, and the hydroxyl group at C-6 was lost **(table 14)**. The compound was thus determined as hautriwaic acid lactone, previously, this compound was reported in *D. viscosa* (Omosa *et al.*, 2014).



Hautriwaic acid lactone **(11)**

Table 14: NMR data for hautriwaic acid lactone (11)

Position	^{13}C NMR (δc)	^{1}H NMR (δH)	HMBC
1	17.9	1.65, 1.47	
2	27.6	2.19	
3	135.9	6.68	C-18,C-4, C-5
4	141.5		
5	42		

Position	^{13}C NMR (δc)	^{1}H NMR (δH)	HMBC
6	34.5	1.42	
7	26.6	1.68,1.38	
8	16.7		
9	19.5		
10	45.7	1.14	
11	38.6	1.48	
12	17.2	2.43	
13	125.3		
14	110.9	6.31	C-13, C-16, C-15
15	142.7	7.37	
16	138.5	7.26	
17	15.3	0.88	
18	169.6		
19	65.1	4.12, 3.72	C-18, C-4,C-6,C-10, C-5
20	17.3	0.78	C-19, C-17

5.3.3. Secondary Metabolites isolated from *Dodonaea viscosa* Nanyuki population

The dried and ground leaves of *D. viscosa* collected from Nanyuki were extracted using CH_2Cl_2/ CH_3OH (1:1) yielded one compound which was characterized as catechin.

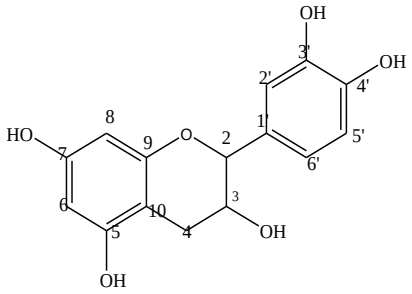
Catechin (12)

Details of mass spectroscopy of compound **12** showed a molecular formula of $C_{15}H_{14}O_6$ with $[M-H]^-$ m/z 289.0718. The ^{1}H NMR spectrum revealed that there are two aliphatic proton signals at 2.51 and 2.85 with the chemical shifts indicating they are not vicinal to oxygen atoms. Slightly further downfield are two signals at 3.98 and 4.57 which originate from protons on carbons bearing oxygen atoms or other electron withdrawing group such as an unsaturated carbon. The spectrum also shows two mutually coupled doublets at 5.86 and 5.93, suggesting vinylic or aromatic protons, while a similar set of coupled signals at 6.72, 6.76 and 6.84 suggest a tri−substituted aromatic ring.

Cosy spectrum confirmed that the protons resonating at 2.51 and 2.85 are mutually

coupled, and the latter is coupled to the proton resonating at 3.98, in a spin system isolated from the rest of the downfield protons. This spectrum also showed that the protons resonating at 5.86 and 5.93 are coupled in second isolated spin system that contain unsaturated carbons, while the aromatic protons resonating at 6.72, 6.76 and 6.84 form a third isolated spin system.

The ^{13}C spectrum shows 15 distinct carbon signals, which is in consistent with the formula suggested by high resolution mass spectrometry. Presence of seven quaternary carbons at 100.87, 132.27, 146.23, 146.26, 156.93, 157.57 and157.85 as indicated by their low intensities relative to the other signals in the spectrum. The most upfield carbon signal at 28.51 is due to sp3 hybridised carbon atom and was assigned to position 4. Signals at 68.83 and 82.88 are due to oxygenated sp3-hybridised carbons. From the HSQC experiment there is only one carbon that is connected to two protons which is the sp3 hybridised carbon atom at 28.51 (It is bonded to the protons resonating at 2.51 and 2.85. This can be confirmed by the coupling constants for these protons indicated by the 1H spectrum − a J value of 16.1 Hz for both indicates geminal coupling. HMBC spectrum was used to confirm the placements. The proton NMR signal at 5.86 showed a correlation with C-8 and C-10 while its meta coupling partner at 5.93 showed a correlation with C-6 and C-10; this confirmed the assignments on ring A. The proton NMR signal for H-4 showed HMBC correlations with C-2, C-3, C-5, C-9 and C-10 confirming its assignment at position 4. The NMR description as well as the ESIMS confirmed the compound **12** to be catechin.



Catechin (**12**)

Table 15; NMR data for catechin (12)

Position	δc	δ_H (m, *J* in Hz)	HMBC
2	82.9	4.57 (d, 7.5)	C-3, C-1′, C-2′, 6 ′
3	68.8	3.98 (td, 5.4, 7.8)	
4	28.5	2.51 (dd, 8.1, 16.1)	C-2, C-3, C-5, C-9, C-10
5	157.6		
6	96.4	4.93 (d, 2.3)	C-8, C-10
7	157.9		
8	95.6	5.86 (d, 2.3)	C-6, C-10
9	156.9		
10	100.9		
1′	132.3		
2′	115.3	6.84 (d, 2.0)	C-2, C-4′ C-6′
3′	146.2		
4′	146.3		
5′	116.1	6.76 (d, 8.1)	C-2, C-1′ C-3′
6′	120.0	6.72 (dd, 2.0, 8.2)	C-2, C-2′, C-6′

5.4. CONCLUSION

The chemical analysis of the root bark of *Z. paracanthum* led to characterization of eight known compounds, namely one fatty acid (myristic acid **1**), one sterol (stigmasterol **2**), one lignan (sesamin **3**), two β-carboline alkaloids (canthin-6-one **7** and 10-methoxy-canthin-6-one **6**) and three phenanthridine alkaloids (8-acetonyldihydrochelerythrine **4**, arnottianamide **5** and 8-oxochelerythrine **8**). The *D. viscosa* coastal population yielded one flavone (5, 7, 4', 5' -tetrahydroxy-3, 6, 2’ -trimethoxyflavone **10**) and two diterpenoids (hautriwaic acid **9** and hautriwaic acid lactone **11**) and catechin **12** from D. viscosa, Nanyuki collection. This study reports isolation of myristic acid, stigmasterol, 8 acetonyldihydrochelerythrine, arnottianamide, 10-methoxycanthin-6-one and 8-oxochelerythrine from *Z.paracanthum* for the first time while 5,7,4',5'-tetrahydroxy-3,6,2'-trimethoxyflavone is reported for the first time in *D.viscosa*

CHAPTER SIX: ANTIMICROBIAL ACTIVITY

ANTIMICROBIAL ACTIVITY OF EXTRACTS AND PURE COMPOUNDS FROM *ZANTHOXYLUM* SPECIES AND *DODONAEA VISCOSA* POPULATIONS

6.1. INTRODUCTION

Majority of plants are a wealthy source of natural antimicrobial resources. Globally, Plants are utilized to derive most effective and stong medications (Inta *et al.*, 2013). Numerous herbs are recognised to contain healing potential such as antimicrobial ability (Kalaivani *et al*., 2012). Even though several plants are investigated for antimicrobial potential, only a few have been evaluated adequately (Ríos & Recio, 2005). Based on scientific evidence, several plants are reported to prevent microbial infections (Prabuseenivasan *et al*., 2006).

Some of the isolated plants metabolites known to hinder microbial infections include; phenols and phenolic acids**.** The hydroxyl groups are associated with determining the antimicrobial potency, increament in hydroxylation causes high toxicity (Cragg & Newman, 2013). The antimicrobial mode of action of these compounds include acting like enzyme inhibitors due to oxidation resulting from compound reaction with sulfhydryl or other general interaction with microbe protein (Daciana, 2007). Quinones probably aim at the surfaces of microbes containing adhesins, polypeptides and enxymes on the cell. Quinones also act as substrate inhibitors to the microorganism (Wales, 1995). The activity of flavonoids, flavones, and flavonols can be attributed to their potential to complex the microbial proteins with their cell walls (Tanaka *et al*., 2004).

Like quinones, tannins act by inactivating adhesions of microbes, food transport and enzymes as well as complexing the polysaccharide (Akiyama *et al*., 2001). Tannins are also toxic to fungal and mbacterial microbes (Myint *et al*., 2013). Coumarins stimulate macrophages Additionally, hydroxycinnamic acids associated to coumarins have the potential to inhibit the

Gram positive bacteria (Peng *et al.*, 2016). Quaternary alkaloids act by intercalating the DNA (Wink, 2015).

The opportunities triggering research for antimicrobial drugs is on the rise as a result of occurrence of new infections coupled to the side-effects conventional medicine. These novel drugs are sourced from various known to be of use in traditional medicine. This study aimed to assess the antimicrobial ability of extracts from leaves, stem and root bark of five species of *Zanthoxylum* (*Z. paracanthum, Z. holtzianum, Z. chalybeum, Z. usambarense and Z. gilletii*) as well as leaf extracts of ten populations of *D. viscosa* were evaluated for antimicrobial potency using agar well diffusion and broth dilution methods.

6.1.1. Objective

To evaluate *in vitro* antimicrobial activity of both extracts and pure compounds from *Zanthoxylum* species and *D. viscosa* populations.

6.2. MATERIALS AND METHODS

6.2.1. Samples preparation

Three concentrations were prepared from each of the CH_2Cl_2: CH_3OH extracts of leaf, root and stem of the five *Zanthoxylum* species (*Z. chalybeum, Z. gilletii , Z. holtzianum, Z. paracanthum* and *Z. usambarense*) and leaf extracts of the ten populations of *D. viscosa* (Coastal, Makueni, Machakos, Karura, Nanyuki, Maimahiu, Narok, Loita Forest, Marigat and Homa bay) . The three concentrations were: 1000, 100 and 10 µg/ml. To prepare 1000 µg/mL, 10 mg of the extracts as well as compounds were dissolved in 1 ml of DMSO and then topped to 10 ml using distilled water. Concentrations 100 and 10 µg/ml were made through serial dilution as shown in **table 16**.

Table 16: Sample preparation for antimicrobial testing

Solution	Preparation	Total solution volume	Concentration of the solution
A	10 mg of a given crude extract/compound + 1ml DMSO + 9 ml distilled water	10 ml	1000 µg/mL
B	1 ml of A + 9 ml distilled water	10 ml	100 µg/mL
C	1 ml of B + 9 ml distilled water	10 ml	10 µg/mL

6.2.2. Reviving of stock cultures

The bacteria microbes were collected from Centre for Microbiology Research, Kenya Medical Research Institute (CMR, KEMRI) while the fungal microbes were obtained from the microbiology laboratory in the School of biological Sciences, University of Nairobi (SBS, UoN) from Microbiology laboratory in the School of Biological Sciences, University of Nairobi.The bacteria were revived in nutrient broth and incubated at 37^0C for 24h while the fungi were revived in Sabouraud agar for 72 hours at 30^0C.

Table 17: The microbes used in the study

Microbes Name	Microbe Class	Type	Source of microbes
Bacillus cereus	Gram positive bacteria	ATCC 11778	CMR, KEMRI
Methicilin Resistant *Staphylococcus aureus* (MRSA)	Gram positive bacteria	Un-typed isolate	CMR, KEMRI
Staphylococcus aureus	Gram positive bacteria	ATCC 259213	CMR, KEMRI
Escherichia coli	Gram negative bacteria	ATCC 25922	CMR, KEMRI
Candida albicans	Yeast	ATCC 10231	SBS, UoN
Aspergillus flavus	Filamentous fungus	Untyped isolate	SBS, UoN

6.2.3. Antimicrobial Screening

Agar well diffusion technique was used as described by Valgas et al. (2007). The three concentrations used were 1000 µg/ml, 100 µg/ml and 10 µg/ml. Bacterial or fungal culture adjusted to the 0.5 McFarland standard equivalents to 10 CFU/mL (1 ml) was pipetted onto the centre of a sterilised plastic petri dish (100 × 15 mm) in a laminar flow. Cooled molten nutrient

agar was dispensed into the petri dish with the inoculum. Four wells (6 mm) were prepared in each agar plate having inoculum adjusted to the 0.5 McFarland standard equivalents to 10 CFU/mL following solidification of the agar by means of a sterile cork borer in a laminar flow. Thereafter, 100 µl of individual samples was added to their own wells. The plates were left in a laminar flow for 30 minutes to set and let the samples diffuse well into the agar before being placed in an incubator at 37°C for 24 hours for bacteria and 30°C for 72 hours for fungi. Antimicrobial activity was noted by the zone of inhibition (including the wells diameter) after the incubation. Ten percent DMSO was used as a negative control while omacilin and fluconazole obtained from Sigma-Aldrich (USA) at 1000, 100 and 10 µg/ml served as positive controls for bacteria and fungi respectively. All tests were done in triplicates.

6.2.4. Minimal Inhibition Concentration (MIC)

A broth dilution where serial dilution of sample was carried out from 1000 to 0.011 µg/ml in 96 micro well plates to determine the MIC. All the dilutions were carried out in triplicate. One mL of 24 h and 72 h culture for bacteria and fungi correspondingly ($\cong 10^6$ CFU/mL) adjusted to McFarland turbidity was added to each dilution and placed in incubator at 37 ^oC for 24 h for bacteria and 30^oC for 72 h for fungi. The lowermost dilution with undetectable bacterial growth was noted as MIC this confirmed by absence of turbidity after inoculating into agar followed by incubation (Kaloyou *et al*., 2012).

6.2.5. Data Analysis

This was done by use of SPSS version 23. Using the software; descriptive statistics such as means, standard errors of the mean, variance, range, confidence interval of the mean of inhibition zones were computed and tabulated. Additionally, one way ANOVA was performed to find out the presence of significance differences in terms of activity among the samples tested. Further ANOVA (post hoc ANOVA) was also performed using Dunnett T Test (recommended when there is a positive control in the experiment) to determine presence of any

significant difference between the antimicrobial activity of the samples and the positive controls (Musila *et al.*, 2017) at probability level ≤ 0.05.

6.3. RESULTS

6.3.1. Antimicrobial activity of *Zanthoxylum*

6.3.1.1 Antimicrobial activity of leaf extracts of *Zanthoxylum* species

Leaves of different *Zanthoxylum* species (*Z. paracanthum, Z. holtzianum, Z. usambarense, Z. chalybeum* and *Z. gilletti*) were tested against *Candida albicans, Bacillus cereus,* MRSA, *Aspergillus flavus, Escherichia coli,* and *Staphylococcus aureus.*

Z. usambarense exhibited highest inhibition zone against *C. albicans* amongst all the 5 species tested at both 1000 and 100 µg/ml followed by *Z. paracanthum* while *Z. gilletii* did not have any activity in all the three concentrations. The positive control (Fluconazole) at 1000µg/ml had an inhibition zone of 21.67mm which was way more than that of *Z. usambarense* at similar concentration **figure 12**.

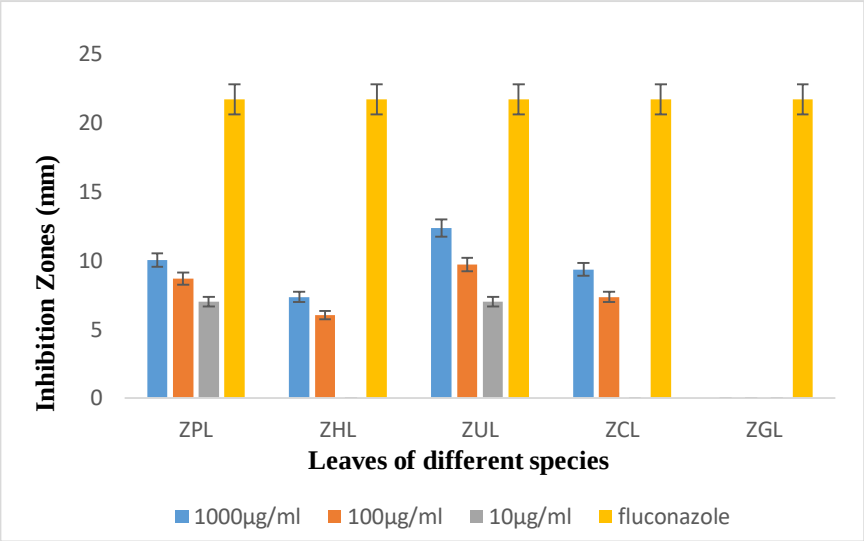


Figure 12; Size (mm) of inhibition zones of *C. albicans* by leaf extracts of different *Zanthoxylum* spp

ZPL-*Z. paracanthum* leaves, ZHL-*Z. holtzianum* leaves, ZUL-*Z. usambarense* leaves,ZCL-*Z. chalybeum* leaves,ZGL-*Z. gilletii* leaves

Z. usambarense exhibited highest inhibition zone against *E. coli* amongst all the 5 species tested at both 1000 and 100 µg/ml followed by *Z. chalybeum* while *Z. holtzianum* had the least activity at both 1000 and 100 µg/ml. All the species tested did not have any activity at 10 µg/ml. The positive control (Omacilin) at 1000µg/ml had an inhibition zone of 19.67mm which was way more than that of *Z. usambarense* at similar concentration **(figure 13)**.

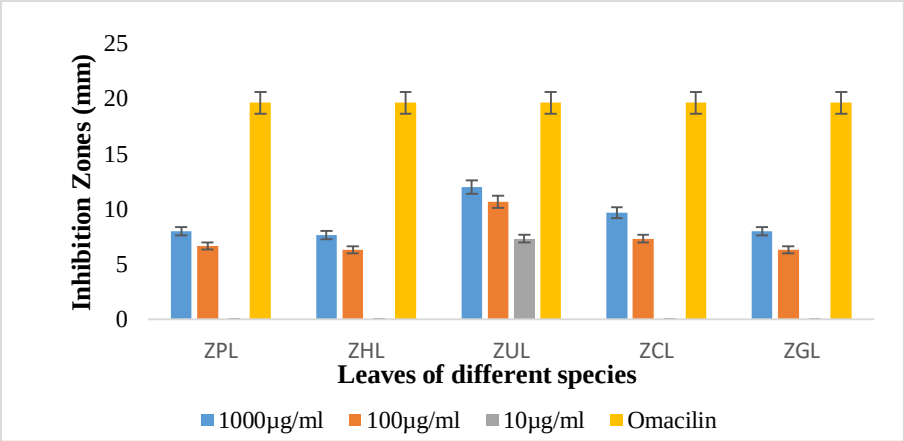


Figure 13; Growth inhibition of *E. coli* by leaf extracts of different *Zanthoxylum* spp
ZPL-*Z. paracanthum*, ZHL-*Z. holtzianum*, ZUL-*Z. usambarense*, ZCL-*Z. chalybeum*, ZGL-*Z. gilletii*

Z. holtzianum exhibited highest inhibition zone against MRSA amongst all the 5 species tested at both 1000 and 100 µg/ml followed by *Z. usambarense* while *Z. gilletii* had the least activity at 1000 µg/ml and no inhibition zone at both 100 and 10 µg/ml was recorded. Omacilin at 1000µg/ml had an inhibition of 24 mm which was way more than that of *Z. holtzianum* at similar concentration **(figure 14).**

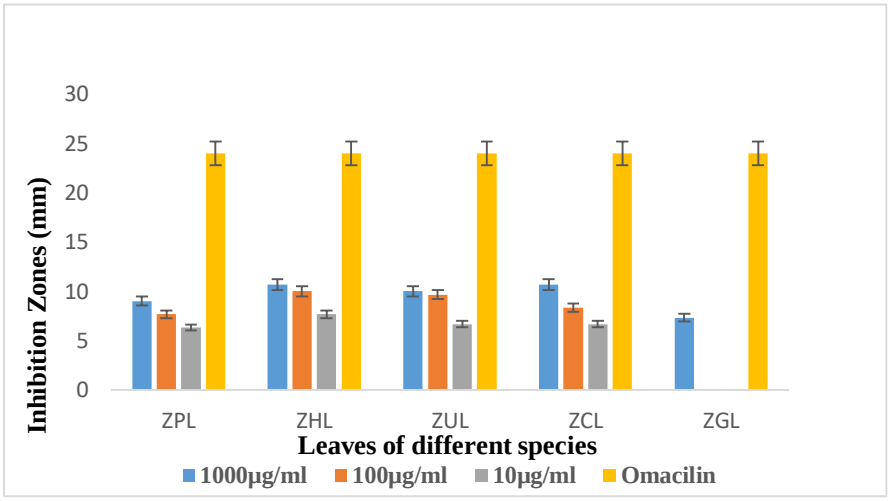


Figure 14; Growth inhibition of MRSA by leaf extracts of different *Zanthoxylum* spp

ZPL-*Z. paracanthum*, ZHL-*Z. holtzianum*, ZUL-*Z. usambarense*, ZCL-*Z. chalybeum*, ZGL-*Z. gilletii*

Z. usambarense exhibited largest inhibition zones against *A. flavus* amongst all the 5 species tested at both 1000 and 100 µg/ml followed by *Z. chalybeum* while *Z. gilletii* had the least activity at 1000 µg/ml with no activity at both 100 and 10 µg/ml. The positive control (Omacilin) at 1000µg/ml had an inhibition zone of 20.67 mm which was way more than that of *Z. usambarense* at similar concentration **(figure 15)**.

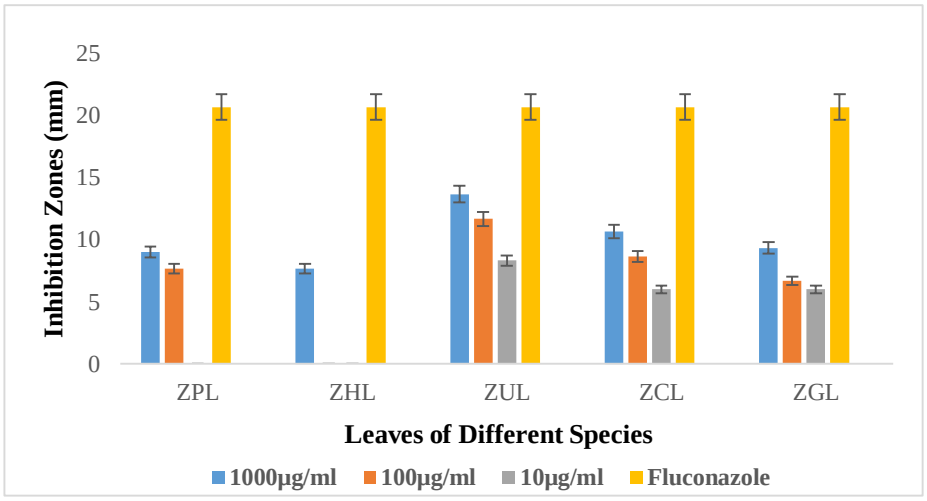


Figure 15; Growth inhibition of *A. flavus* by leaf extracts of different *Zanthoxylum* spp

ZPL-*Z. paracanthum*, ZHL-*Z. holtzianum*, ZUL-*Z. usambarense*, ZCL-*Z. chalybeum*, ZGL-*Z. gilletii*

Z. chalybeum exhibited largest inhibition zone amongst all the 5 species tested against *B. cereus* at both 1000 and 100 µg/ml followed by *Z. paracanthum* and *Z. gilletii* while *Z. usambarense* and *Z. holtzianum* had no activity in the three concentrations tested. The positive control (Omacilin) at 1000µg/ml had an inhibition zone of 20.33 mm which was way more than that of *Z. chalybeum* at similar concentration. All species tested did not show any activity at 10 µg/ml **(figure 16)**.

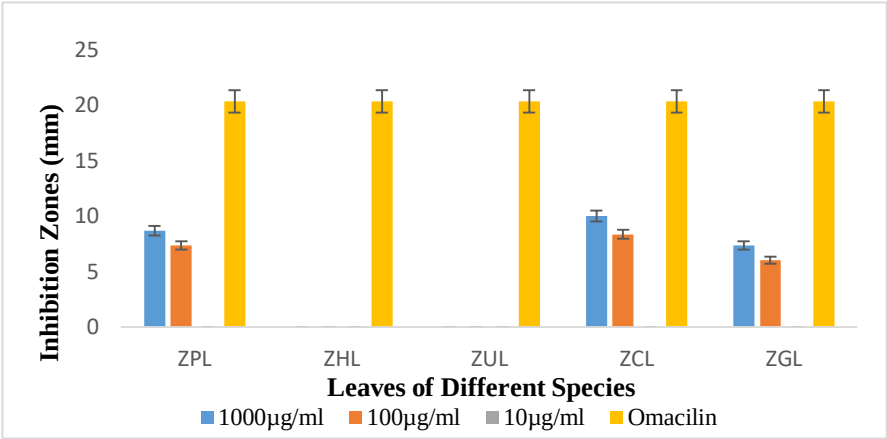


Figure 16; Growth inhibition of *B. cereus* by leaf extracts of different *Zanthoxylum* spp
ZPL-*Z. paracanthum*, ZHL-*Z. holtzianum*, ZUL-*Z. usambarense*, ZCL-*Z. chalybeum*, ZGL-*Z. gilletii*

Z. usambarense exhibited highest inhibition zone amongst all the 5 species tested at both 1000 and 100 µg/ml followed by *Z. holtzianum.* All samples tested showed activity in all the three concentrations. The positive control (Omacilin) at 1000µg/ml had an inhibition zone of 26.33 mm which was way more than that of *Z. usambarense* at similar concentration **(figure 17)**.

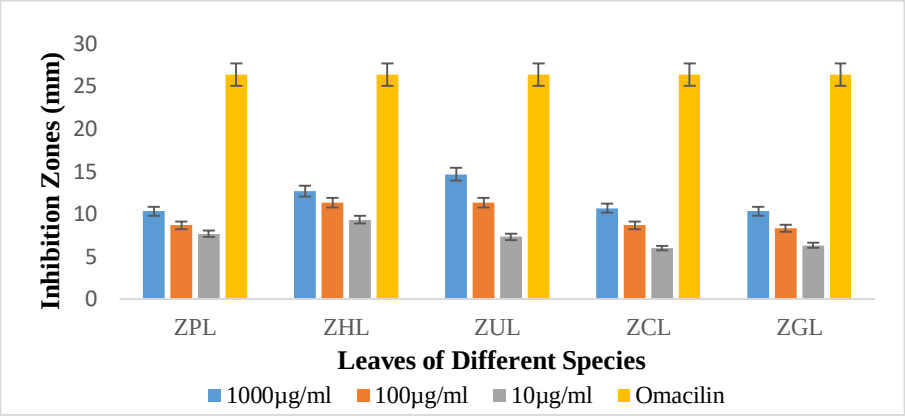


Figure 17; Growth inhibition of S. aureus by leaf extracts of different *Zanthoxylum* spp

ZPL-*Z. paracanthum*, ZHL-*Z. holtzianum*, ZUL-*Z. usambarense*, ZCL-*Z. chalybeum*, ZGL-*Z. gilletii*

6.4.1.2. Antimicrobial activity of stem bark extracts of *Zanthoxylum* species

Z. paracanthum stem bark extract exhibited highest inhibition zone against *C. albicans* amongst all the 5 species tested at both 1000 and 100 µg/ml followed by *Z. chalybeum*. *Z. paracanthum*, *Z. chalybeum* and *Z. usambarense* showed antimicrobial activity in the three concentrations while *Z. holtzianum* showed activity at 1000 and 100 µg/ml. *Z. gilletii* exhibited antimicrobial activity only at 1000 µg/ml. Fluconazole at 1000µg/ml had an inhibition zone of 21.67 mm which was way more than that of *Z. paracanthum* at similar concentration **(figure 18)**.

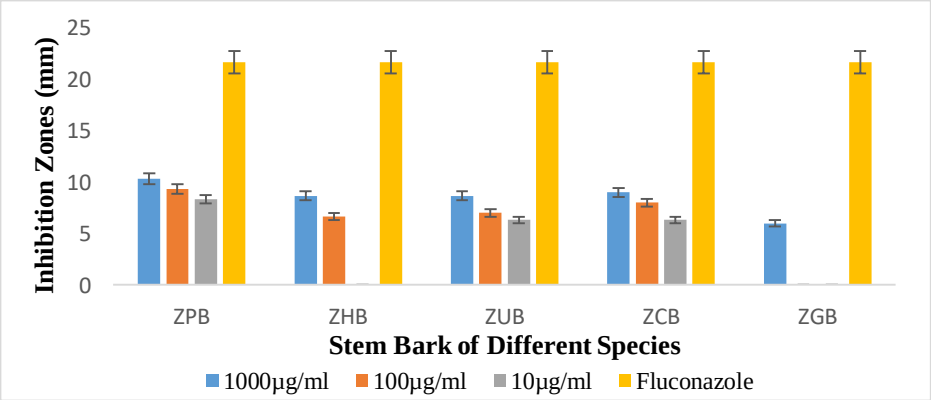


Figure 18; Growth inhibition of *C. albicans* by stem bark extracts of *Zanthoxylum* spp

ZPB-*Z. paracanthum*, ZHB-*Z. holtzianum*, ZUB-*Z. usambarense*, ZCB-*Z. chalybeum*, ZGB-*Z. gilletii*

Z. paracanthum exhibited highest inhibition zone amongst all the 5 species tested at 1000, 100 and 10 µg/ml against *A. flavus* followed by *Z. holtzianum*. *Z. usambarense*, *Z.*

chalybeum and *Z. gilletii* showed activity at 1000 µg/ml and 100 µg/ml with no inhibition zone at 10 µg/ml. Fluconazole at 1000µg/ml had an inhibition zone of 20.67 mm which was way more than that of *Z. paracanthum* at similar concentration **(figure 19)**.

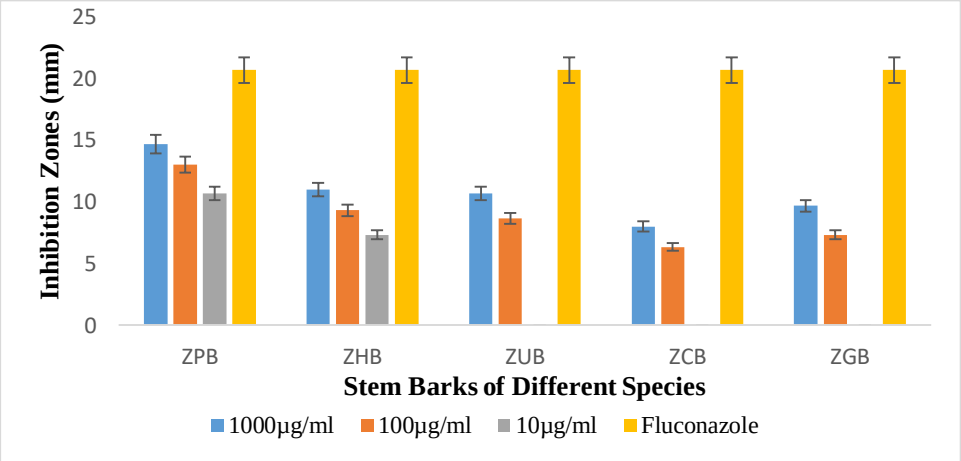


Figure 19; Growth inhibition of *A. flavus* by stem bark extracts of *Zanthoxylum*
ZPB-*Z. paracanthum*, ZHB-*Z. holtzianum*, ZUB-*Z. usambarense*, ZCB-*Z. chalybeum*, ZGB-*Z. gilletii*

Z. paracanthum exhibited the highest inhibition zone against *E.coli* amongst all the five species tested at 1000, 100 and 10 µg/ml followed by *Z. usambarense. Z. holtzianum, Z. chalybeum* and *Z. gilletii* showed activity at 1000 and 100 µg/ml. *Z. gilletii* exhibited activity only at 1000 and 100 µg/ml. Omacilin at 1000µg/ml had an inhibition zone of 19.67 mm which was way more than that of *Z. paracanthum* at similar concentration **(figure 20)**.

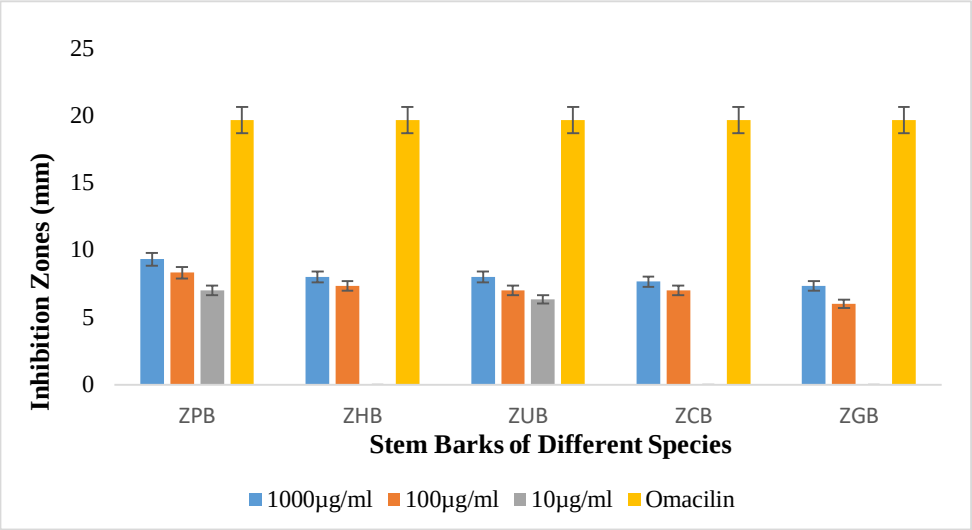


Figure 20; Growth inhibition of *E. coli* by stem bark extracts of *Zanthoxylum* spp

ZPB-*Z. paracanthum*, ZHB-*Z. holtzianum*, ZUB-*Z. usambarense*, ZCB-*Z. chalybeum*, ZGB-*Z. gilletii*

Z. paracanthum showed the highest inhibition zones amongst all the five species tested against MRSA at 1000 100 and 10 µg/ml of 10.67mm, 9.33mm and 7.33 mm respectively followed by *Z. holtzianum* and *Z. usambarense*. *Z. chalybeum*, showed activity at 1000 and 100 µg/ml while *Z. gilletii* only showed activity at 1000 µg/ml. The positive control (Omacilin) at 1000µg/ml had an inhibition zone of 24 mm which was way more than that of *Z. paracanthum* at similar concentration **(figure 21)**.

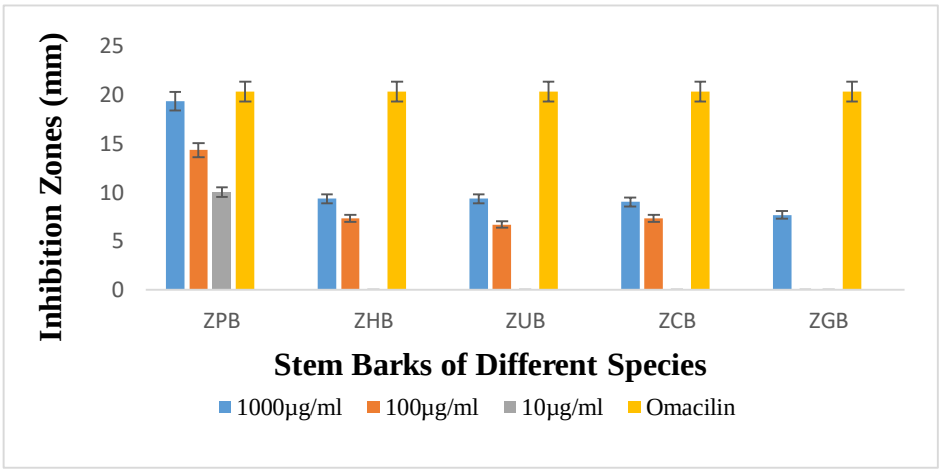


Figure 21: Growth inhibition of MRSA by stem bark extracts of *Zanthoxylum* spp

ZPB-*Z. paracanthum*, ZHB-*Z. holtzianum*, ZUB-*Z. usambarense*, ZCB-*Z. chalybeum*, ZGB-*Z. gilletii*

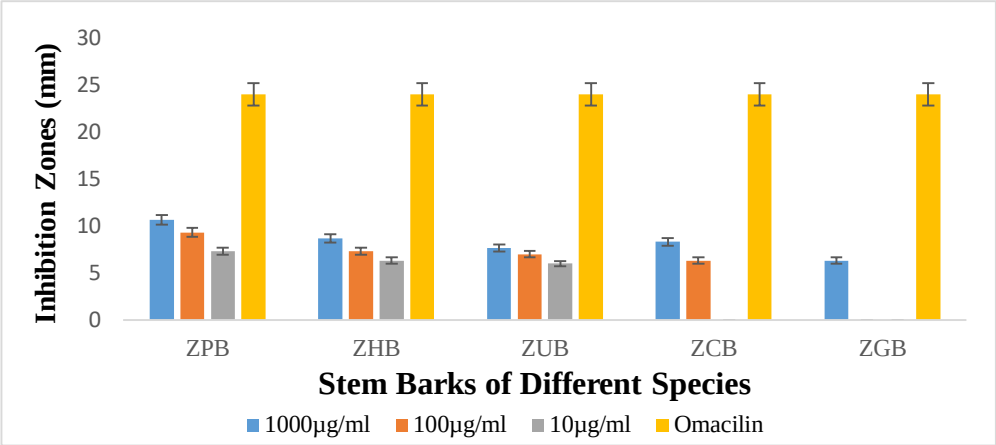


Figure 22: Growth inhibition of *B.cereus* by stem bark extracts of *Zanthoxylum* spp

ZPB-*Z. paracanthum*, ZHB-*Z. holtzianum*, ZUB-*Z. usambarense*, ZCB-*Z. chalybeum*, ZGB-*Z. gilletii*

Z. paracanthum exhibited the highest inhibition zones against *B. cereus* amongst all the five species tested at all the concentrations, 1000, 100 and 10 µg/ml. *Z. holtzianum*. *Z. usambarense*, *Z. chalybeum* showed activity at 1000 and 100 µg/ml with 10 µg/ml failing to record inhibition while *Z. gilletii* was active against *B.cereus* only at 1000 µg/ml **figure 22**. Inhibition zone against *B. cereus* by *Z. paracanthum* stem bark extract at 1000 µg/ml was insignificantly different compared to omacilin at the same concentration **(table 18)**.

Table 18: Comparison of inhibition of *B. cereus* by different stem extracts with positive control

Multiple Comparisons
Dunnett t (2-sided)[a]

Dependent Variable	(I) Crude extracts	(J) Crude extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Z. paracanthum stem against *B. cereus*	1000µg/ml	Omacilin (1000µg/ml)	-1.00000	.40825	.095	-2.1756	.1756
	100µg/ml	Omacilin (1000µg/ml)	-3.66667*	.40825	.000	-4.8423	-2.4910
	10µg/ml	Omacilin (1000µg/ml)	-5.33333*	.40825	.000	-6.5090	-4.1577
Z. holtzianum stem against *B. cereus*	1000µg/ml	Omacilin (1000µg/ml)	-11.00000*	.40825	.000	-12.1756	-9.8244
	100µg/ml	Omacilin (1000µg/ml)	-13.00000*	.40825	.000	-14.1756	-11.8244
	10µg/ml	Omacilin (1000µg/ml)	-20.33333*	.40825	.000	-21.5090	-19.1577
Z. usambarense stem against *B. cereus*	1000µg/ml	Omacilin (1000µg/ml)	-11.00000*	.40825	.000	-12.1756	-9.8244
	100µg/ml	Omacilin (1000µg/ml)	-13.66667*	.40825	.000	-14.8423	-12.4910
	10µg/ml	Omacilin (1000µg/ml)	-20.33333*	.40825	.000	-21.5090	-19.1577
Z. chalybeum stem against *B. cereus*	1000µg/ml	Omacilin (1000µg/ml)	-11.33333*	.33333	.000	-12.2932	-10.3734
	100µg/ml	Omacilin (1000µg/ml)	-13.00000*	.33333	.000	-13.9599	-12.0401
	10µg/ml	Omacilin (1000µg/ml)	-20.33333*	.33333	.000	-21.2932	-19.3734
Z. gilletii stem against *B. cereus*	1000µg/ml	Omacilin (1000µg/ml)	-12.66667*	.33333	.000	-13.6266	-11.7068
	100µg/ml	Omacilin (1000µg/ml)	-20.33333*	.33333	.000	-21.2932	-19.3734
	10µg/ml	Omacilin (1000µg/ml)	-20.33333*	.33333	.000	-21.2932	-19.3734

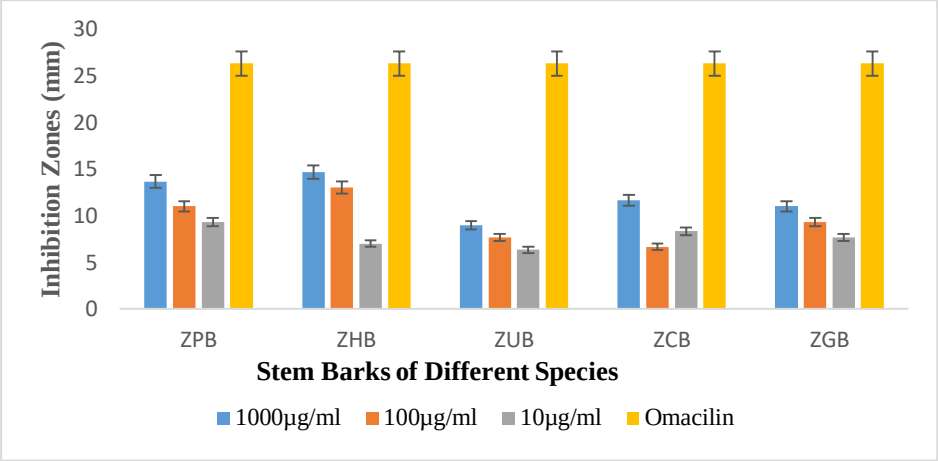


Figure 23: Growth inhibition of *S. aureus* by stem bark extracts of *Zanthoxylum* spp
ZPB-*Z. paracanthum*, ZHB-*Z. holtzianum*, ZUB-*Z. usambarense*, ZCB-*Z. chalybeum*, ZGB-*Z. gilletii*

Z. holtzianum exhibited the highest inhibition zones amongst all the five species tested at 1000 and 100 µg/ml followed by *Z. paracanthum*. All the species tested showed activity at 1000, 100 and 10 µg/ml. *Z. usambarense* showed the least activity in comparison to other species. The positive control (Omacilin) at 1000µg/ml had an inhibition zone of 26. 33 mm which was way more than that of *Z. holtzianum* at similar concentration **(figure 23)**.

6.4.1.3. Antimicrobial activity of root bark extracts of *Zanthoxylum* species

The root bark extracts of 5 *Zanthoxylum* species were tested for antimicrobial activity against 6 microbes.

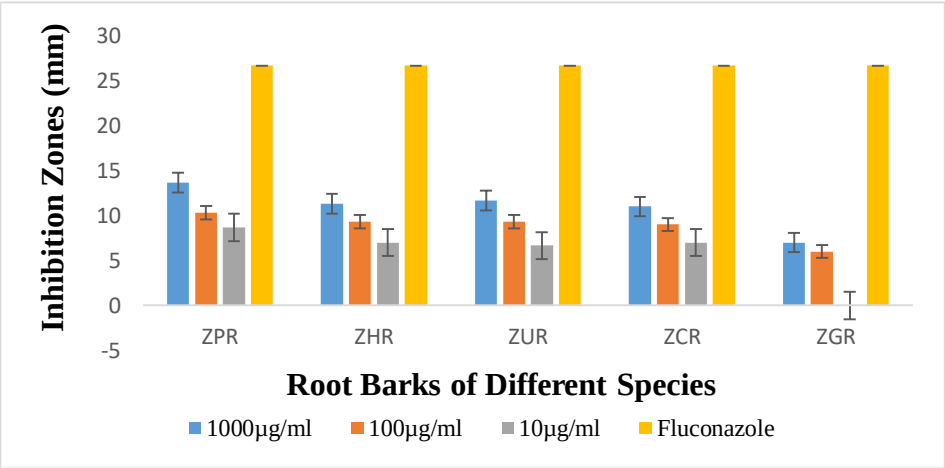


Figure 24: Activity of root bark extracts of *Zanthoxylum* spp against *C. albicans*

ZPR-*Z. paracanthum* root bark, ZHR-*Z. holtzianum* root bark, ZUR-*Z. usambarense* root bark, ZCR-*Z. chalybeum* root bark, ZGR-*Z. gilletii* root bark

The highest inhibition zone was for *Z. paracanthum* against *C. albicans* in all the three concentrations followed by *Z usambarense* while *Z. gilletii* was the least. The inhibition zones of the extracts from all the species was significantly different compared to fluconazole **(figure 24)**.

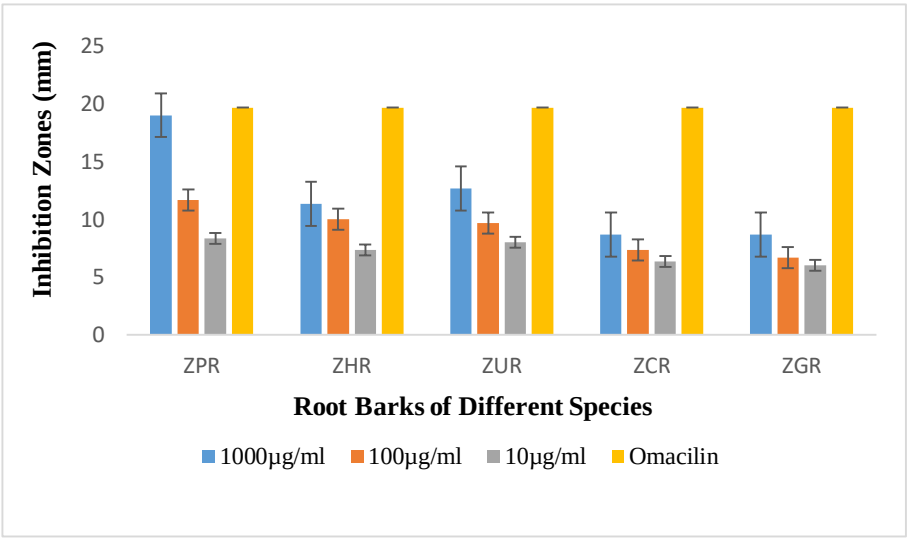


Figure 25: Activity of root bark extracts of *Zanthoxylum* spp against *E. coli*

ZPR-*Z. paracanthum* root bark, ZHR-*Z. holtzianum* root bark, ZUR-*Z. usambarense* root bark, ZCR-*Z. chalybeum* root bark, ZGR-*Z. gilletii* root bark

Z. paracanthum root bark showed highest antimicrobial activity against *E. coli* with no significant difference at 1000µg/ml compared to omacilin (P≥0.05) **(table 19)**. This was followed by Z. *usambarense* **(figure 25)**.

Table 19: Inhibition of *E. coli* comparison of different root extracts with positive control

Multiple Comparisons

Dunnett t (2-sided)[a]

Dependent Variable	(I) Crude extracts	(J) Crude extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Z.paracanthum root against *E.coli*	1000µg/ml	Omacilin (1000µg/ml)	-.66667	.57735	.548	-2.3292	.9959
	100µg/ml	Omacilin (1000µg/ml)	-8.00000*	.57735	.000	-9.6626	-6.3374
	10µg/ml	Omacilin (1000µg/ml)	-11.33333*	.57735	.000	-12.9959	-9.6708
Z. holtzianum root against *E.coli*	1000µg/ml	Omacilin (1000µg/ml)	-8.33333*	.40825	.000	-9.5090	-7.1577
	100µg/ml	Omacilin (1000µg/ml)	-9.66667*	.40825	.000	-10.8423	-8.4910
	10µg/ml	Omacilin (1000µg/ml)	-12.33333*	.40825	.000	-13.5090	-11.1577
Z. usambarense root against *E.coli*	1000µg/ml	Omacilin (1000µg/ml)	-7.00000*	.57735	.000	-8.6626	-5.3374
	100µg/ml	Omacilin (1000µg/ml)	-10.00000*	.57735	.000	-11.6626	-8.3374
	10µg/ml	Omacilin (1000µg/ml)	-11.66667*	.57735	.000	-13.3292	-10.0041
Z.chalybeum root against *E.Coli*	1000µg/ml	Omacilin (1000µg/ml)	-11.00000*	1.59861	.000	-15.6035	-6.3965
	100µg/ml	Omacilin (1000µg/ml)	-12.33333*	1.59861	.000	-16.9368	-7.7299
	10µg/ml	Omacilin (1000µg/ml)	-15.33333*	1.59861	.000	-19.9368	-10.7299
Z.gilletii root against *E.coli*	1000µg/ml	Omacilin (1000µg/ml)	-11.00000*	.40825	.000	-12.1756	-9.8244
	100µg/ml	Omacilin (1000µg/ml)	-13.00000*	.40825	.000	-14.1756	-11.8244
	10µg/ml	Omacilin (1000µg/ml)	-13.66667*	.40825	.000	-14.8423	-12.4910

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

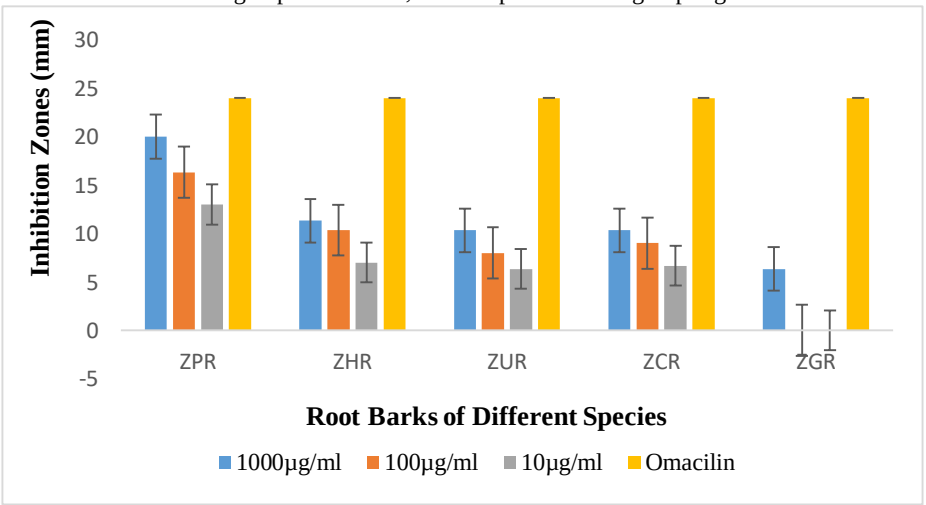


Figure 26: Activity of root bark extracts of *Zanthoxylum* spp against MRSA

ZPR-*Z. paracanthum* root bark, ZHR-*Z. holtzianum* root bark, ZUR-*Z. usambarense* root bark, ZCR-*Z. chalybeum* root bark, ZGR-*Z. gilletii* root bar

Z. paracanthum root bark exhibited highest activity against MRSA followed by *Z. holtzianum* while *Z. gilletii* was the least. The difference in the inhibition zones of the root extracts of all the species was significant compared to omacilin (P<0.05) **(figure 26)**.

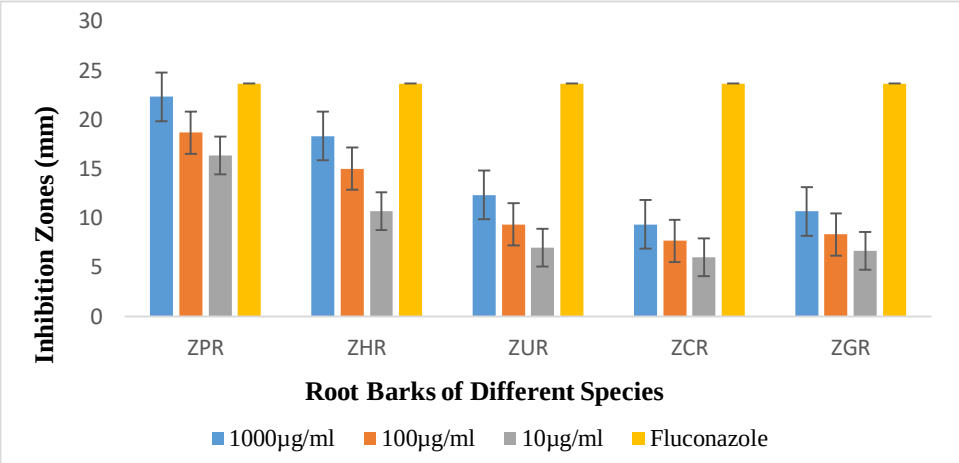


Figure 27: Activity of root bark extracts of *Zanthoxylum* spp against *A. flavus*

ZPR-*Z. paracanthum* root bark, ZHR-*Z. holtzianum* root bark, ZUR-*Z. usambarense* root bark, ZCR-*Z. chalybeum* root bark, ZGR-*Z. gilletii* root bark

Z. paracanthum root bark extract exhibited highest antimicrobial activity against *A. flavus* with no significant differerence compared to omacilin (P≥ 0.05) **(table 20)**. This was followed by *Z. holtzianum* while *Z. chalybeum* was the least **(figure 27)**.

Table 20; Inhibition of *A. flavus* comparison of different root extracts with positive control

Multiple Comparisons

Dunnett t (2-sided)[a]

Dependent Variable	(I) Crude extracts	(J) Crude extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Z.paracanthum against *A. flavus*	1000µg/ml	fluconazole (1000µg/ml)	-1.33333	.47140	.054	-2.6908	.0242
	100µg/ml	fluconazole (1000µg/ml)	-5.00000*	.47140	.000	-6.3575	-3.6425
	10µg/ml	fluconazole (1000µg/ml)	-7.33333*	.47140	.000	-8.6908	-5.9758
Z. holtzianum root against *A. flavus*	1000µg/ml	fluconazole (1000µg/ml)	-5.33333*	.57735	.000	-6.9959	-3.6708
	100µg/ml	fluconazole (1000µg/ml)	-8.66667*	.57735	.000	-10.3292	-7.0041
	10µg/ml	fluconazole (1000µg/ml)	-13.00000*	.57735	.000	-14.6626	-11.3374
Z. usambarense against *A. flavus*	1000µg/ml	fluconazole (1000µg/ml)	-11.33333*	.40825	.000	-12.5090	-10.1577
	100µg/ml	fluconazole (1000µg/ml)	-14.33333*	.40825	.000	-15.5090	-13.1577

	10µg/ml	fluconazole (1000µg/ml)	-16.66667*	.40825	.000	-17.8423	-15.4910
Z. chalybeum root against *A. flavus*	1000µg/ml	fluconazole (1000µg/ml)	-14.33333*	.40825	.000	-15.5090	-13.1577
	100µg/ml	fluconazole (1000µg/ml)	-16.00000*	.40825	.000	-17.1756	-14.8244
	10µg/ml	fluconazole (1000µg/ml)	-17.66667*	.40825	.000	-18.8423	-16.4910
Z. gilletii root against *A. flavus*	1000µg/ml	fluconazole (1000µg/ml)	-13.00000*	.47140	.000	-14.3575	-11.6425
	100µg/ml	fluconazole (1000µg/ml)	-15.33333*	.47140	.000	-16.6908	-13.9758
	10µg/ml	fluconazole (1000µg/ml)	-17.00000*	.47140	.000	-18.3575	-15.6425

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

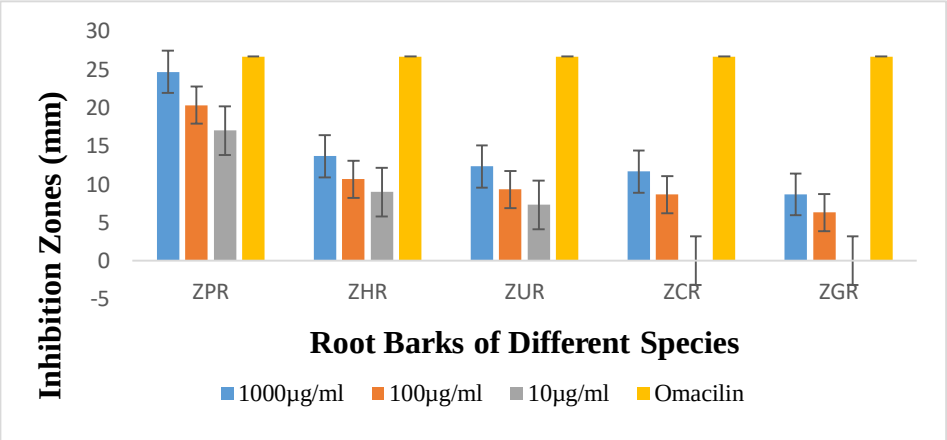


Figure 28; Activity of root bark extracts of *Zanthoxylum* spp against *B. cereus*

ZPR-*Z. paracanthum* root bark, ZHR-*Z. holtzianum* root bark, ZUR-*Z. usambarense* root bark, ZCR-*Z. chalybeum* root bark, ZGR-*Z. gilletii* root bark

Z. paracanthum exhibited highest activity compared to others followed by *Z. holtzianum* root bark extract. The inhibition of all the extracts against *B. cereus* was significantly different with omacilin (P< 0.05) **(figure 28)**.

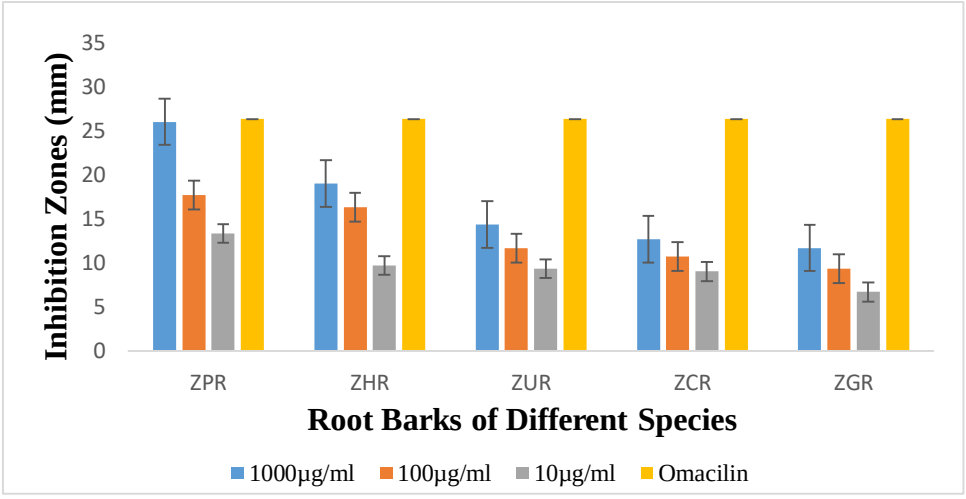


Figure 29; Activity of root bark extracts of *Zanthoxylum* spp against *S. aureus*

ZPR-*Z. paracanthum* root bark, ZHR-*Z. holtzianum* root bark, ZUR-*Z. usambarense* root bark, ZCR-*Z. chalybeum* root bark, ZGR-*Z. gilletii* root bark

The activity against *S. aureus* of *Z. paracanthum* was the highest compared to other species with no significant difference compared to omacilin. This was followed by *Z. holtzianum* while *Z. gilletii* was the least **(figure 29)**.

6.3.2. Antimicrobial activity of *Dodonaea viscosa* populations

Ten populations of *D. viscosa* (Coastal, Makueni, Machakos, Karura, Nanyuki, Maimahiu, Narok, Loita Forest, Marigat and Homa bay) were tested against six microbes *Bacillus cereus* Methicilin Resistant *Staphylococcus aureus* (MRSA), *Aspergillus flavus, Staphylococcus aureus, Candida albicans* and *Escherichia coli.*

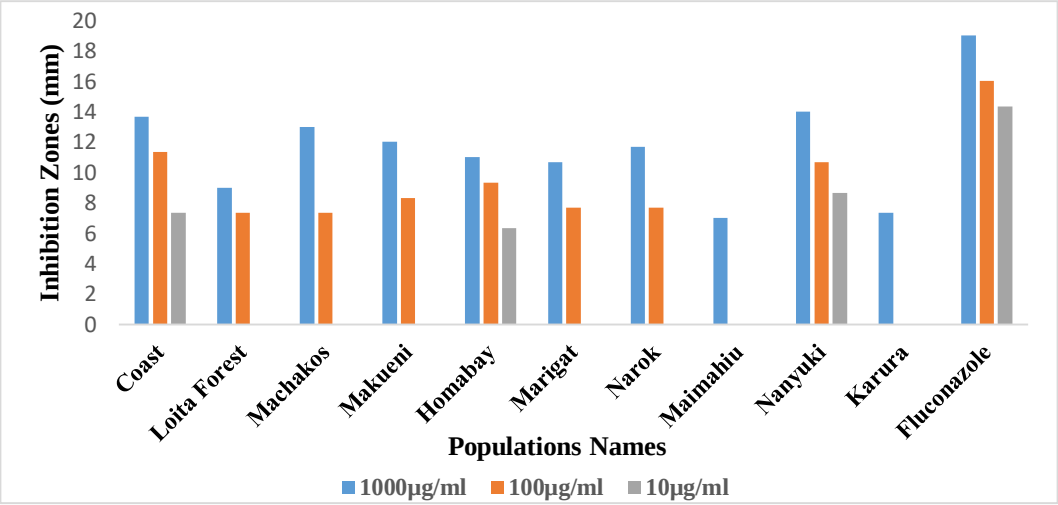


Figure 30; Growth inhibition of *C. albicans* by leaf extracts of *D. viscosa* populations

Nanyuki collection had the highest inhibition zones compared to other populations at all the tested concentrations against *C. albicans* (1000, 100 and 10µg/ml) followed by coastal. Coastal, Nanyuki and Homabay populations showed activity in all the three concentrations while Loita Forest, Machakos, Makueni, Marigat and Narok populations inhibited the growth of *C.albicans* at 1000 and 100µg/ml while Maimahiu and Karura populations showed activity at only 1000µg/ml, **(figure 30)**. All the tested concentrations of positive control (Fluconazole) exhibited more activity than the Nanyuki population.

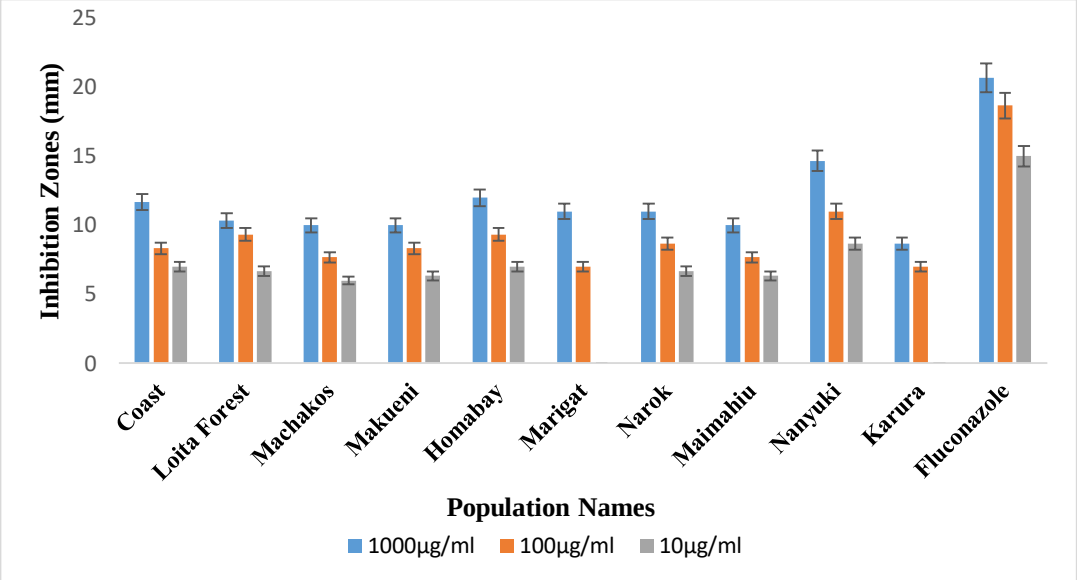


Figure 31; Growth inhibition of *A. flavus* by leaf extracts of *D. viscosa* populations

Nanyuki population had the highest inhibition zones compared to other populations at all the tested concentrations (1000, 100 and 10µg/ml) against *A. flavus* **(figure 31)** followed by Homabay. Coastal population was the third. All populations showed activity in all the three concentrations except Marigat and Karura populations. All the tested concentrations of positive control (Fluconazole) exhibited higher activity than the Nanyuki population.

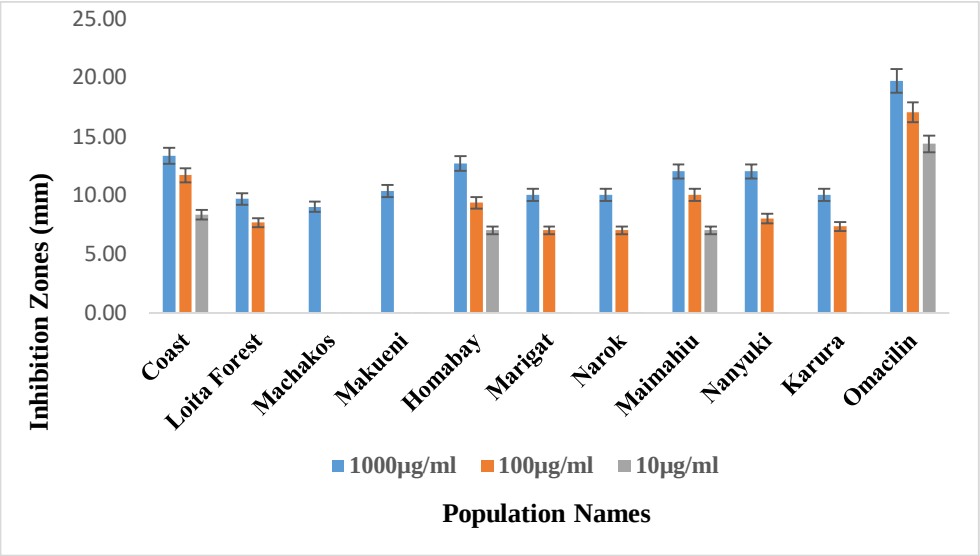


Figure 32; Growth inhibition of *E. coli* by leaf extracts of *D. viscosa* populations

The coastal collection had the highest inhibition zones compared to other populations at all the tested concentrations (1000, 100 and 10µg/ml) against *E. coli* followed by Homabay and Maimahiu **(figure 32)**. Coastal, Homabay and Maimahiu populations showed activity in all the three concentrations while Loita Forest, Marigat, Narok, Nanyuki and Karura had activities at 1000 and 100µg/ml. Machakos and Makueni showed activity at 1000µg/ml only. All the tested concentrations of positive control (omacilin) exhibited more activity than the Coastal population.

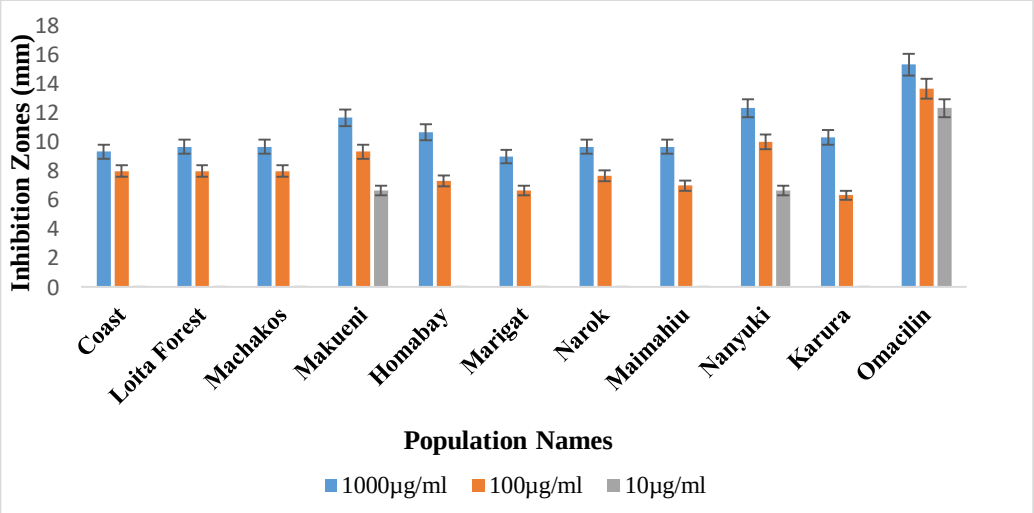


Figure 33; Growth inhibition of MRSA by leaf extracts of *D. viscosa* populations

Nanyuki collection had the highest inhibition zones against MRSA compared to other populations at all the tested concentrations (1000, 100 and 10µg/ml) followed by Makueni, **(figure 33)**. All other populations showed activity at only 1000 and 100µg/ml and was not active at 10µg/ml. All positive control (Omacilin) concentrations exhibited more activity than the Nanyuki population.

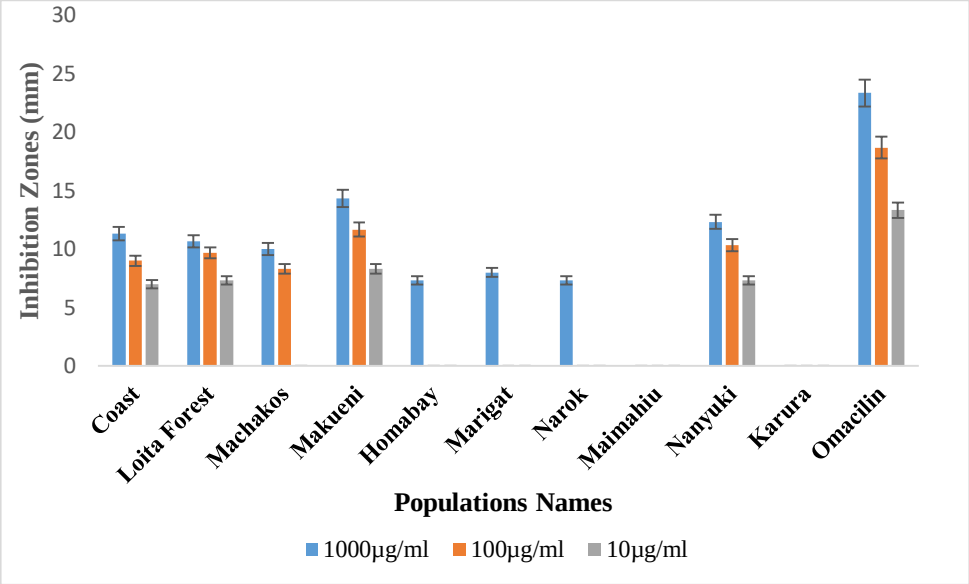


Figure 34; Growth inhibition of *B. cereus* by leaf extracts of *D. viscosa* populations

Makueni population had the highest inhibition zones against *B. cereus* compared to other populations in all the tested concentrations (1000, 100, 10µg/ml) followed by Nanyuki and coast. Coastal, Loita Forest, Makueni and Nanyuki were active at 1000, 100 and 10µg/ml while Machakos exhibited inhibition of *B.cereus* at 1000 and 100µg/ml. Homabay, Marigat and Narok had activity at only 1000µg/ml while Maimahiu and karura did not exhibit any growth at all **(figure 34)**. All the tested concentrations of positive control (Omacilin) exhibited more activity than the Nanyuki population.

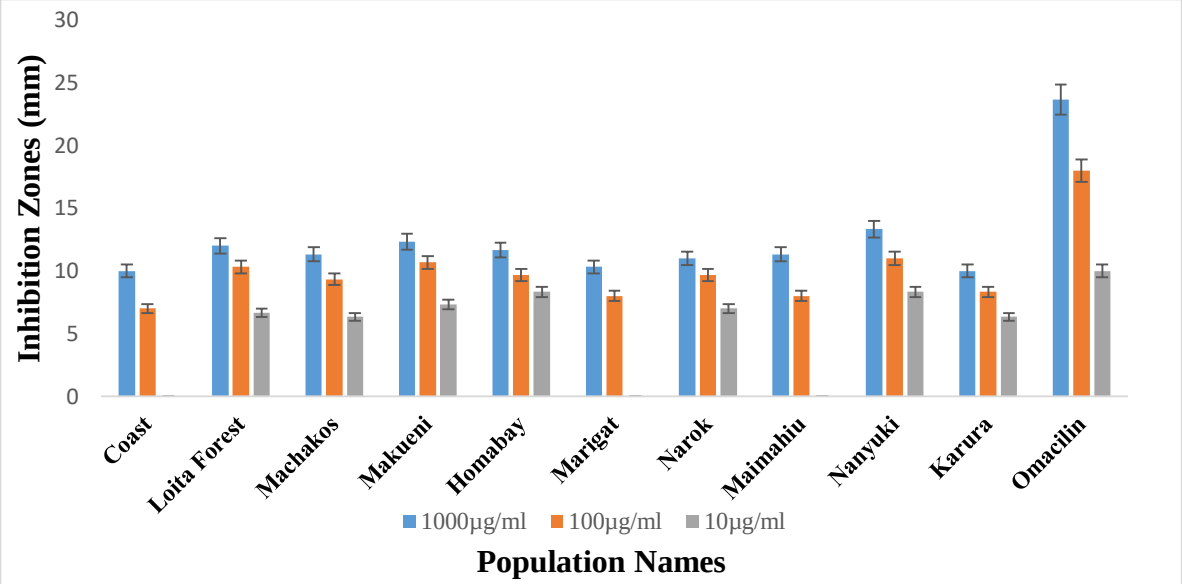


Figure 35; Growth inhibition of *S. aureus* by leaf extracts of *D. viscosa* populations

The *D. viscosa* from Nanyuki had the highest inhibition zones against *S. aureus* compared to other populations in all the tested concentrations (1000, 100, 10µg/ml) followed by population collected from Makueni population. Makueni, Homa bay, Loita Forest, Machakos, Narok, Nanyuki and Karura showed activity in all the tested concentrations (1000, 100, 10µg/ml) while Coast, Marigat and Maimahiu showed inhibition of *S.aureus* at 1000 and 100µg/ml, **(figure 35)**. All the tested concentrations of positive control (Omacilin) exhibited more activity than the Kenyan Nanyuki population.

6.3.3. Antimicrobial activity of the isolated compounds from *Z. paracanthum* root bark

All the compounds characterized from *Zanthoxylum paracanthum* (Myristic acid **1**, stigmasterol **2**, Sesamin **3**, 8-acetonyldihydrochelerythrine **4**, arnottianamide **5**, 10-methoxycanthin-6-one **6**, canthin-6-one **7** and 8-oxochelerythrine **8**) were tested against MRSA, *E. coli*, *S. aureus*, and *C. albicans*. 1000µg/ml of omacilin and fluconazole were used as positive control for bacteria and fungi correspondingly.

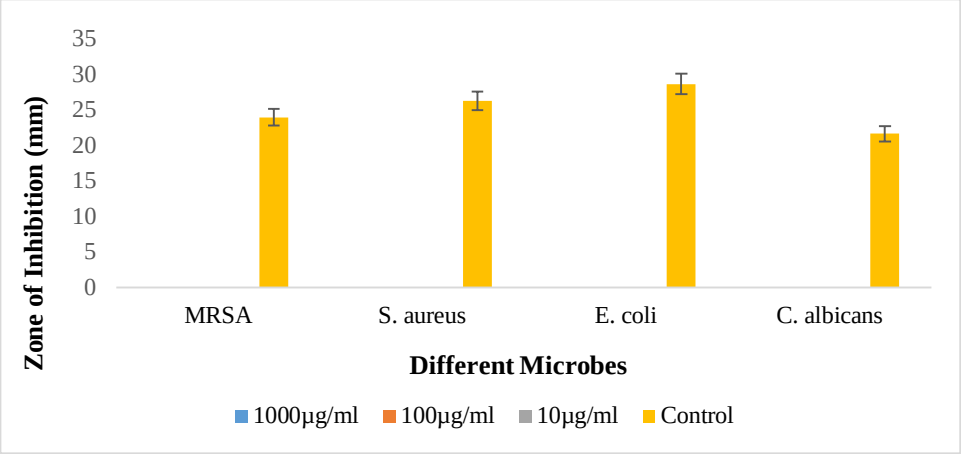


Figure 36; Antimicrobial activity of myristic acid 1

Myristic acid **1** was not active against all the tested microbes in all the concentrations **(Figure 36)**.

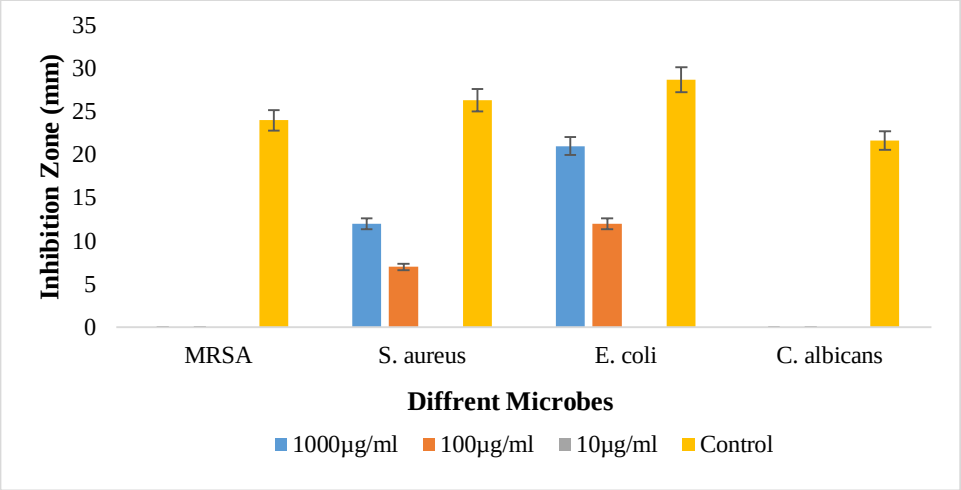


Figure 37; Antimicrobial activity of stigmasterol 2

Stigmasterol **2** recorded inhibition zones against *S. aureus* and *E. coli* both in 1000 and 100µg/ml and failed to inhibit the two microbes at 10µg/ml. There was no activity against *C. albicans* and MRSA recorded in all the three concentrations tested **(Figure 37)**.

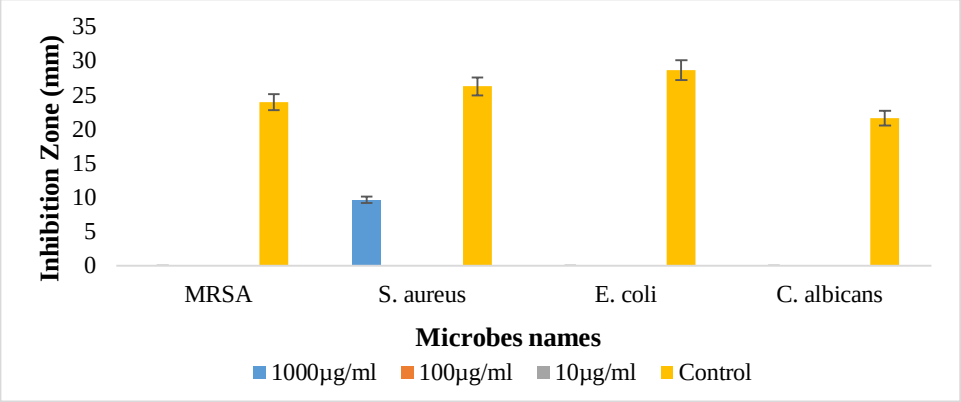


Figure 38; Antimicrobial activity of sesamin 3

Sesamin **3** recorded an inhibition zone of 9.67 mm on *S. aureus* at 1000µg/ml and failed to inhibit other microbes in all the three tested concentrations **(Figure 38)**.

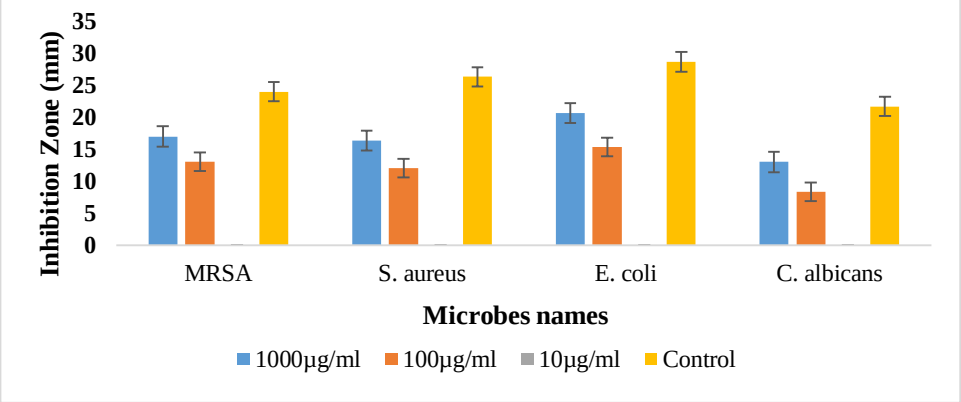


Figure 39; Antimicrobial activity of 8-acetonyldihydrochelerythrine 4

The growth of all the tested microbes were inhibited by this compound at 1000 and 100µg/ml. The inhibition zone for MRSA was 15mm at 1000µg/ml and 12mm at 100µg/ml while *S. aureus* was 16.33mm at 1000µg/ml and 12.67mm at 100µg/ml. *E. coli* was 20.67mm at 1000µg/ml and 15.33mm at 100µg/ml and *C. albicans* was inhibited by 1000 and 100µg/ml with inhibition zones of 13mm 8.33mm respectively **(Figure 39)**.

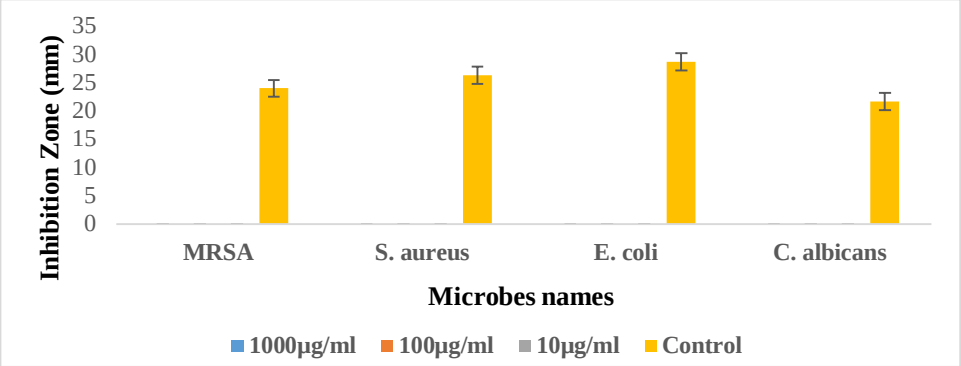


Figure 40; Antimicrobial activity of arnottianamide 5

No inhibition was recorded for arnottianamide **5** against the growth of all the four tested micro-orgarnisms in all the concentrations **(Figure 40)**.

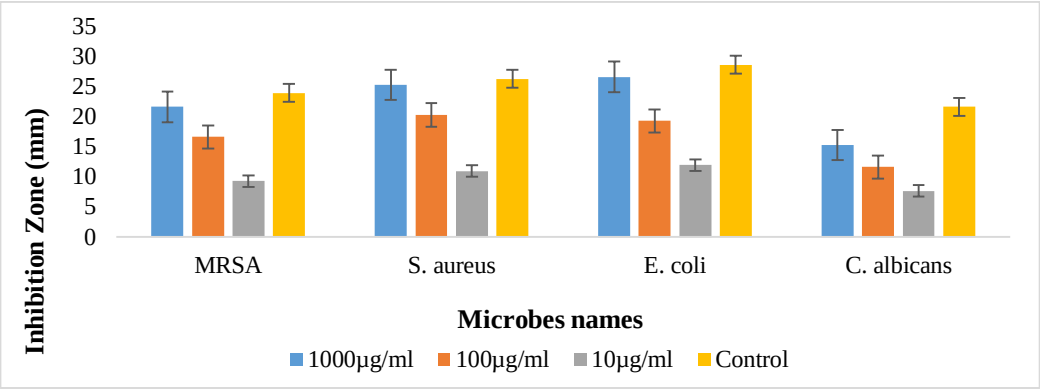


Figure 41; Antimicrobial activity of 10- methoxycanthin-6-one 6

The growth of all the microbes tested were inhibited by methoxycnthin-6-one **6** with all the microbes being inhibited by all the tested concentrations. The growth of MRSA at 1000, 100 and 10µg/ml was 21.67, 16.67 and 9.33 mm respectively while *S. aureus* was 25.33, 20.33 and 11mm respectively. The inhibition zones of *E. coli* at 1000µg/ml and 100µg/ml was 26.67, 19.33 and 12mm respectively. Additionally, the growth inhibition of *C. albicans* was 15.33, 11.67 and 7.67mm respectively **(Figure 41)**. The growth inhibition of this compound at 1000µg/ml against MRSA, *S. aureus* and *E. coli* insignificant difference **(table 21)** in comparison to omacilin at 1000µg/ml ($P \geq 0.05$) as summarized in the table below.

Table 21; Inhibition of different microbes comparison of 10-methoxycanthin-6-one 6 with positive control

Multiple Comparisons

Dunnett t (2-sided)[a]

Dependent Variable	(I) Compounds	(J) Compounds	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Activity of 10- methoxycanthin-6-one against MRSA	1000µg/ml	Omacillin:1000µg/ml	-2.33333	.91287	.081	-4.9621	.2954
	100µg/ml	Omacillin:1000µg/ml	-7.33333*	.91287	.000	-9.9621	-4.7046
	10µg/ml	Omacillin:1000µg/ml	-14.66667*	.91287	.000	-17.2954	-12.0379
Activity of 10- methoxycanthin-6-one against *S. aureus*	1000µg/ml	Omacillin:1000µg/ml	-1.00000	1.00000	.644	-3.8797	1.8797
	100µg/ml	Omacillin:1000µg/ml	-6.00000*	1.00000	.001	-8.8797	-3.1203
	10µg/ml	Omacillin:1000µg/ml	-15.33333*	1.00000	.000	-18.2130	-12.4537
Activity of 10- methoxycanthin-6-one against *E.coli*	1000µg/ml	Omacillin:1000µg/ml	-2.00000	.70711	.054	-4.0362	.0362
	100µg/ml	Omacillin:1000µg/ml	-9.33333*	.70711	.000	-11.3696	-7.2971
	10µg/ml	Omacillin:1000µg/ml	-16.66667*	.70711	.000	-18.7029	-14.6304
Activity of 10- methoxycanthin-6-one against *C.albicans*	1000µg/ml	Fluconazole:1000µg/ml	-6.33333*	.94281	.000	-9.0483	-3.6184
	100µg/ml	Fluconazole:1000µg/ml	-10.00000*	.94281	.000	-12.7150	-7.2850
	10µg/ml	Fluconazole:1000µg/ml	-14.00000*	.94281	.000	-16.7150	-11.2850

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

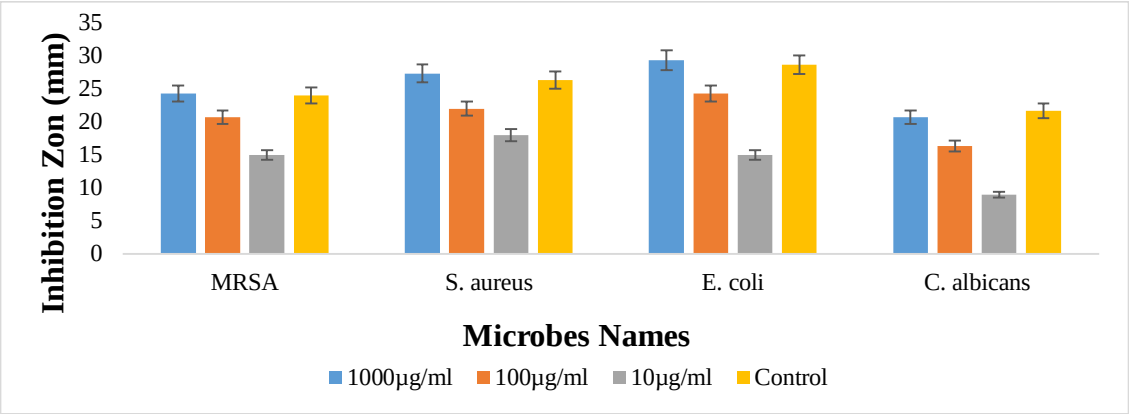


Figure 42: Antimicrobial activity of canthin-6-one 7

This compound showed high inhibition zones with insignificant variation from the positive controls at 1000µg/ml, **(table 22)**. While omacilin at 1000µg/ml against MRSA had an inhibition zone of 24mm, canthin-6-one at 1000, 100 and 10µg/ml had 24.33mm, 20.67mm and 15mm respectively. Inhibition of *S. aureus* by omacilin at 1000µg/ml was 26.33 while canthin-6-one at 1000, 100 and 10µg/ml had inhibition zones of 27.33mm, 22mm and 18mm respectively. Omacilin at 1000µg/ml inhibited *E. coli* by 28.67mm while canthin-6-one at

1000, 100 and 10µg/ml 29.33mm, 24.33mm and 15mm respectively. *C. albicans* was inhibited by fluconazole at 1000µg/ml by 21.67mm while canthin-6-one at 1000, 100 and 10µg/ml showed inhibition zones of 20.67mm, 16.33mm and 9mm respectively **(figure 42)**.

Table 22: Growth inhibition of different microbes comparison of canthin-6-one 7 with positive control

Multiple Comparisons

Dunnett t (2-sided)[a]

Dependent Variable	(I) Compounds	(J) Compounds	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Activity of canthin-6-one against MRSA	1000µg/ml	Omacillin:1000µg/ml	.33333	.88192	.963	-2.2063	2.8730
	100µg/ml	Omacillin:1000µg/ml	-3.33333*	.88192	.014	-5.8730	-.7937
	10µg/ml	Omacillin:1000µg/ml	-9.00000*	.88192	.000	-11.5396	-6.4604
Activity of canthin-6-one against *S. aureus*	1000µg/ml	Omacillin:1000µg/ml	1.00000	.88192	.561	-1.5396	3.5396
	100µg/ml	Omacillin:1000µg/ml	-4.33333*	.88192	.003	-6.8730	-1.7937
	10µg/ml	Omacillin:1000µg/ml	-8.33333*	.88192	.000	-10.8730	-5.7937
Activity of canthin-6-one against *E. coli*	1000µg/ml	Omacillin:1000µg/ml	.66667	.81650	.758	-1.6846	3.0179
	100µg/ml	Omacillin:1000µg/ml	-4.33333*	.81650	.002	-6.6846	-1.9821
	10µg/ml	Omacillin:1000µg/ml	-13.66667*	.81650	.000	-16.0179	-11.3154
Activity of canthin-6-one against *C. albicans*	1000µg/ml	Fluconazole:1000µg/ml	-1.00000	1.08012	.690	-4.1104	2.1104
	100µg/ml	Fluconazole:1000µg/ml	-5.33333*	1.08012	.003	-8.4437	-2.2229
	10µg/ml	Fluconazole:1000µg/ml	-12.66667*	1.08012	.000	-15.7771	-9.5563

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

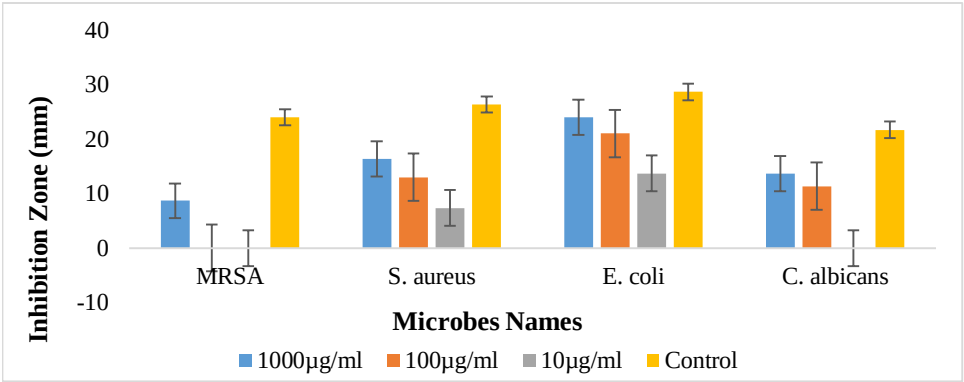


Figure 43: Antimicrobial activity of 8-oxochelerythrine 8

8-Oxochelerythrine **8** inhibited the growth of S. *aureus* and *E. coli* were inhibited at 1000, 100 and 10µg/ml with *S. aureus* inhibition zones of 16.33mm, 13mm and 7.33 mm correspondingly while *E. coli* had 24mm, 21mm and 13.67mm respectively. MRSA was

inhibited by 1000µg/ml with inhibition zone of 8.67mm. *C. albicans* was inhibited by 1000 and 100µg/ml with inhibition zones of 13.67mm and 11.33mm respectively, **(figure 43)**.

6.3.4. Antimicrobial activity of compounds isolated from *D. viscosa* coastal population

Three compounds were purified from *D. viscosa* var. *viscosa* (dodonic acid **9**, 5, 7, 4', 5' -tetrahydroxy-3, 6, 2' -trimethoxyflavone **10** and hautriwaic acid lactone **11)** were tested against four microbes, (MRSA, *S. aureus*, *E. coli* and *C. albicans*).

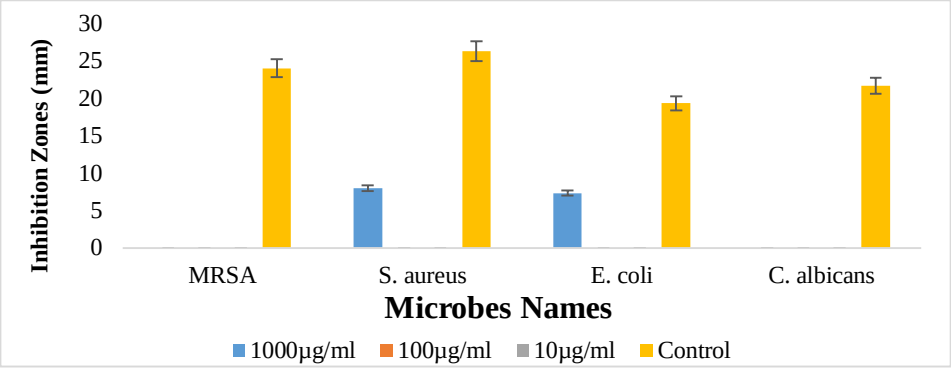


Figure 44; Antimicrobial activity of dodonic acid 9

S. aureus and *E. coli* were inhibited by 1000µg/ml as 8 and 7.33 mm respectively. No inhibition was recorded against MRSA and *C. albicans* in all the tested concentrations, **(figure 44)**.

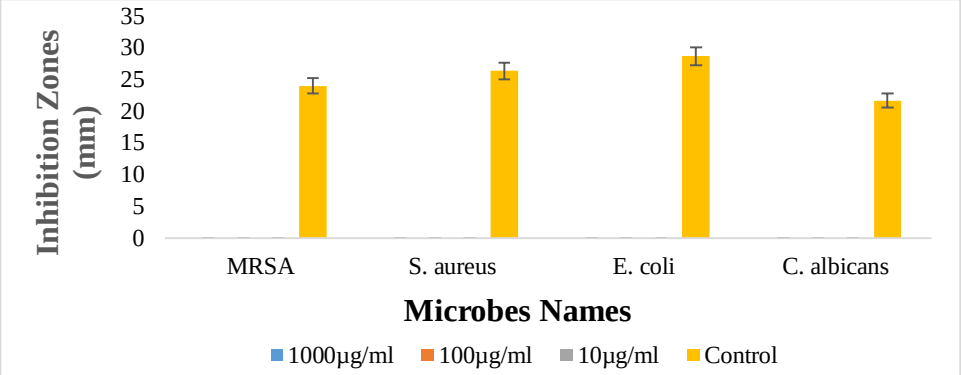


Figure 45: Antimicrobial activity of 5, 7, 4', 5' -Tetrahydroxy-3,6,2'-trimethoxyflavone (10)

No inhibition was recorded against the growth of all the tested microbes in all the tested concentrations **(figure 45).**

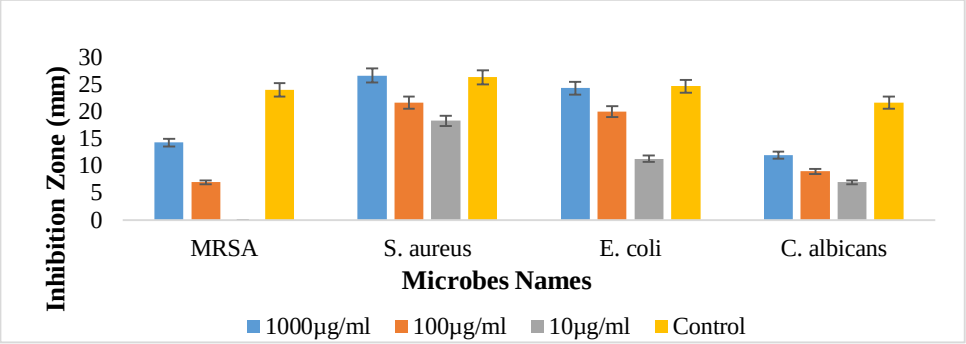


Figure 46; Antimicrobial activity of hautriwaic acid lactone 11

The growth of all the microbes except MRSA was inhibited by the three concentrations tested of hautriwaic acid lactone **11**. There was insignificant difference in the inhibition of *S. aureus* and *E coli* by hautriwaic acid lactone at 1000µg/ml compared to omacilin at the same concentration (P≥ 0.05) as summarized in **table 23**. The growth inhibition zones of MRSA at 1000 and 100µg/ml 14.33 and 7mm respectively while that of *S. aureus* at 1000, 100 and 10µg/ml was 26.67, 21.67 and 18.33mm respectively, and *E. coli* was 24.33, 20 and 11.33mm respectively while *C. albicans* was 12.9 and 7 mm respectively, **(figure 46)**.

Table 23; Inhibition of different microbes comparison of hautriwaic acid lactone 11 with positive control.

Multiple Comparisons

Dunnett t (2-sided)

Dependent Variable	(I) Compounds	(J) Compounds	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						w	Upper Bound
Activity of hautriwaic acid lactone against MRSA	1000µg/ml	Omacillin:1000µg/ml	-10.33333*	.97183	.000	-13.1319	-7.5348
	100µg/ml	Omacillin:1000µg/ml	-12.66667*	.97183	.000	-15.4652	-9.8681
	10µg/ml	Omacillin:1000µg/ml	-24.00000*	.97183	.000	-26.7985	-21.2015
Activity of hautriwaic acid lactone against *S. aureus*	1000µg/ml	Omacillin:1000µg/ml	4.00000	1.81046	.135	-1.2135	9.2135
	100µg/ml	Omacillin:1000µg/ml	-11.33333*	1.81046	.001	-16.5469	-6.1198
	10µg/ml	Omacillin:1000µg/ml	-19.33333*	1.81046	.000	-24.5469	-14.1198
Activity of hautriwaic acid lactone against *C. albicans*	1000µg/ml	Fluconazole:1000µg/ml	-21.66667*	.84984	.000	-24.1139	-19.2194
	100µg/ml	Fluconazole:1000µg/ml	-21.66667*	.84984	.000	-24.1139	-19.2194
	10µg/ml	Fluconazole:1000µg/ml	-21.66667*	.84984	.000	-24.1139	-19.2194
Activity of hautriwaic acid lactone against *E. coli*	1000µg/ml	Omacillin:1000µg/ml	-2.00000	.70711	.054	-4.0362	.0362
	100µg/ml	Omacillin:1000µg/ml	-9.00000*	.70711	.000	-11.0362	-6.9638
	10µg/ml	Omacillin:1000µg/ml	-19.33333*	.70711	.000	-21.3696	-17.2971

*. The mean difference is significant at the 0.05 level.

6.3.5. Antimicrobial activity of compounds isolated from *D. viscosa* Nanyuki population

One compound isolated from *D. viscosa* subsp *angustifolia* (catechin **12**) was tested against MRSA, *E. coli*, *S.aureus*, and *C. albicans*. 1000µg/ml of omacilin and fluconazole was used as positive control for bacteria and fungi respectively.

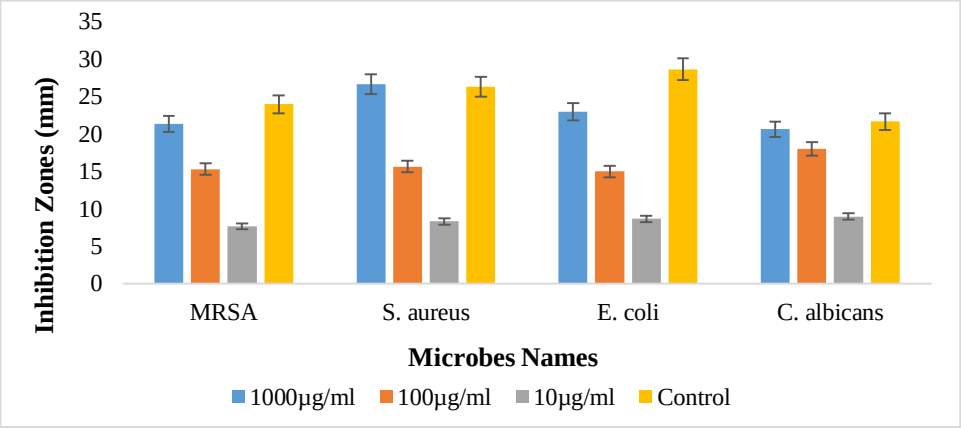


Figure 47; Antimicrobial activity of catechin 12

Catechin **12** inhbited the growth of microbes in all the three concentrations. At 1000, 100 and 10µg/ml MRSA had inhibition zones of 21.33mm, 15.33mm and 7.67mm respectively while *S. aureus* had 26.67mm, 15.67mm and 8.33mm correspondingly while *E. coli* was 23,

15 and 8.67 mm respectively and *C. albicans* had 20.67mm, 18mm and 9mm respectively, **figure 47**. The difference in the inhibition zones of all the microbes tested by catechin at 1000µg/ml was insignificant except for *E. coli* in comparison to the positive controls at the same concentrations (P≥ 0.05) as summarized in **table 24**.

Table 24; Inhibition of different microbes comparison of catechin 12 with positive control

Dunnett t (2-sided)[a]

Dependent Variable	(I) Compounds	(J) Compounds	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Activity of catechin against MRSA	1000µg/ml	Omacillin:1000µg/ml	-2.66667	1.29099	.166	-6.3843	1.0510
	100µg/ml	Omacillin:1000µg/ml	-8.66667*	1.29099	.000	-12.3843	-4.9490
	10µg/ml	Omacillin:1000µg/ml	-16.33333*	1.29099	.000	-20.0510	-12.6157
Activity of catechin against *S. aureus*	1000µg/ml	Omacillin:1000µg/ml	.33333	1.10554	.980	-2.8503	3.5169
	100µg/ml	Omacillin:1000µg/ml	-10.66667*	1.10554	.000	-13.8503	-7.4831
	10µg/ml	Omacillin:1000µg/ml	-18.00000*	1.10554	.000	-21.1836	-14.8164
Activity of catechin against *E. coli*	1000µg/ml	Omacillin:1000µg/ml	-5.66667*	1.45297	.012	-9.8507	-1.4826
	100µg/ml	Omacillin:1000µg/ml	-13.66667*	1.45297	.000	-17.8507	-9.4826
	10µg/ml	Omacillin:1000µg/ml	-20.00000*	1.45297	.000	-24.1841	-15.8159
Activity of catechin against *C. albicans*	1000µg/ml	Fluconazole:1000µg/ml	-1.00000	1.13039	.716	-4.2551	2.2551
	100µg/ml	Fluconazole:1000µg/ml	-3.66667*	1.13039	.029	-6.9218	-.4115
	10µg/ml	Fluconazole:1000µg/ml	-12.66667*	1.13039	.000	-15.9218	-9.4115

Table 25; MIC values (µg/ml) of different isolated compounds against the selected microbes

Compound Name	**Microbial Names**			
	MRSA	***S. aureus***	***E. coli***	***C. albicans***
Myristic acid **1**	> 1000	> 1000	> 1000	> 1000
Stigmasterol **2**	> 1000	62.5	15.625	> 1000
Sesamin **3**	> 1000	500	> 1000	> 1000
8- Acetonyldihydrochelerythrine **4**	31.25	15.625	15.625	62.5
Arnottianamide **5**	> 1000	> 1000	> 1000	> 1000
10-Methoxycanthin-6-one **6**	3.906	1.95	3.906	7.8125
Canthin-6-one **7**	0.98	0.49	1.95	3.906
8-Oxochelerythrine **8**	62.5	7.8125	3.906	15.625
Hautriwaic acid **9**	> 1000	500	500	> 1000
5, 7, 4', 5' -Tetrahydroxy-3,6,2'-trimethoxyflavone **10**	> 1000	> 1000	> 1000	> 1000
Hautriwaic acid lactone **11**	62.5	1.95	1.95	7.8125
Catechin **12**	7.8125	3.906	7.8125	3.906
Omacilin	**0.98**	**0.49**	**0.98**	**-**
Fluconazole				**1.95**

6.4. DISCUSSION

6.4.1. Antimicrobial activity of *Zanthoxylum* species

Three parts (leaves, stem and root barks) *Z. paracanthum, Z. holtzianum, Z. usambarense, Z. chalybeum and Z. gilletii* were tested for antimicrobial activity against six microbes (*C. albicans, B. cereus*, MRSA, *A. flavus, E. coli* and *S. aureus*). All the results for each plant parts were compared with the positive controls.

All the plant parts of *Z. paracanthum* showed inhibition against the growth of all the selected microbes. The leaves were less active compared to the stem and root barks while the root bark was the most active of them all. Comparing the activity exhibited by extracts from stem barks of other *Zanthoxylum* species, the *Z. paracanthum* recorded highest activities against all the selected microbes. The root bark extract of this species also exhibited highest inhibition on the selected microbes in relation to other *Zanthoxylum* species. This is the first report on the antimicrobial activity of *Z. paracanthum*.

The three parts of *Z. holtzianum* showed inhibition of all the selected microbes except the *B. cereus* which was not inhibited by the leaves with the root bark extract showing highest activity against all the selected microbes compared to the stem and leaf extracts of this species. The root bark extract of *Z. holtzianum* showed second highest activity compared to root bark extracts of othe *Zanthoxylum* species evaluated against MRSA, *A. flavus, B. cereus* and *S. aureus*. Moreover, the roots of *Z. holtzianum* also showed third best inhibition to *E. coli* and *C. albicans*. This is the first report of antimicrobial activity of crude extracts from *Z. holtzianum*.

Three parts of Z. *usambarense* also recorded activity against the selected microbes except *B. cereus* which was not inhibited by the leaf extract of this species. The leaf extract showed highest antimicrobial activity in all the three tested concentrations compared to leaf

extracts of other species. The root bark extract of this species showed second best activity compared to the root bark extracts of other species tested against *C. albicans* and *E. coli* and third best against *A. flavus*, *B. cereus* and *S. aureus* while MRSA was the least inhibited by this extract. This study is in agreement with findings from He et al. (2002) and Matu and Van Staden (2003) who reported the broadspectrum antimicrobial activity from this species.

The plant parts of *Z. chalybeum* had some activity against all selected microbes. This report concurs with findings from (Hamza *et al*., 2006; Kaigongi *et al*., 2014; Matu & Van Staden, 2003; Olila & Opuda-Asibo, 2001) who reported the antimicrobial activity of Z. chalybeum against different microbes of economic importance. The antimicrobial activity from the different parts of *Z. gilletii* reported in this study corroborates with studies carried out elsewhere by Gaya et al. (2013) who found this species to be active on *Saccharomyces cereveciae* among others.

Among the *Zanthoxylum* species tested, *Z. paracanthum* root bark had highest inhibition on all the selected microbes in comparison to roots of other *Zanthoxylum* species. Additionally, stem extract of *Z. paracanthum* showed highest inhibition on the selected microbes in comparison to stem barks of other *Zanthoxylum* species. Among the leaf extracts from the five tested *Zanthoxylum* species, *Z. usambarense* had highest activity on the tested microbes. This is the first report to show the best Kenyan *Zanthoxylum* species to use in treating microbial infections based on the different plant parts.

All the tested plant parts from the five *Zanthoxylum* species, root bark extracts were found to be the most active followed by leaves and lastly the stem bark. This study confirms why most communities prefer utilization of roots in the traditional medicine followed by leaves and stem as the least preferred (Kaigongi and Musila, 2015).

The difference in the activity of each plant part of different species from others lies on different biochemicals present in each plant part. The difference in biochemical distribution in

each plant part could be due to geographical distribution of each species as well as the environmental conditions as secondary metabolites in plants are produced to help the plant fight stress such as diseases, pest and natural enemies such as predators/ browsers (Bennett & Wallsgrove, 1994; Harborne, 2007; Mazid *et al*., 2011).

6.4.2. Antimicrobial activity of *Dodonaea viscosa* populations

Extracts of ten Kenyan populations of *D. viscosa* (Coastal, Makueni, Machakos, Karura, Nanyuki, Maimahiu, Narok, Loita Forest, Marigat and Homa bay), were tested against the growth of six microbes (*C. albicans, S. aureus*, MRSA, *A. flavus, B. cereus* and *E. coli*). Three concentrations, 1000, 100 and 10µg/ml of each extract were used against each microbe. All the populations showed antimicrobial activity against each selected microbe with increase in concentration but the level of activity varied in different populations as well as the microbe in question.

The Coastal population showed the second best inhibition activity against the growth of *C. albicans* compared to all the tested populations in all the three concentrations. Additionally, this population recorded third best results against *A. flavus* and *B. cereus*. Inhibition of this population on *E. coli* was the best overall and the least against MRSA and *S. aureus*. The Nanyuki population showed the largest inhibition zones copared to other populations on *C. albicans, A. flavus*, MRSA and *S. aureus* and second best against *B. cereus* while inhibition zones against *E. coli* by the Nanyuki population was the fourth compared to other popuations tested. Extract from Homa bay population recorded the third best results against *C. albicans* and MRSA and second best results against *A. flavus* and *E. coli* while activities against *S. aureus* and *B. cereus* were fourth and seventh respectively compared to other populations.

The Makueni population showed fifth best results against *C. albicans*, sixth against *A. flavus*, ninth against *E. coli*, second against MRSA and *S. aureus* and first against *B. cereus.*

Collections representing Machakos populationwas fourt against *C. albicans* and *S. aureus*, fifth against MRSA and *B. cereus*, seventh and tenth against *A. flavus* and *E. coli* respectively. Extract from Loita Forest population was eighth against *C. albicans* and *E. coli*, fifth against *A. flavus* and MRSA, fourth and third on *B. cereus* and *S. aureus* correspondingly. The Marigat population showed seventh best results against *C. albicans*, sixth best results against *E. coli* and *B. cereus*, ninth against *A. flavus*, MRSA and *S. aureus*. The Narok population fourth best results against *A. flavus*, sixth best results on *C. albicans* and *E. coli* and seventh best results against MRSA, *B. cereus* and *S. aureus*. Extract from Maimahiu population was tenth in the inhibition of *C. albicans*, third against *E. coli* and eighth against *A. flavus*, MRSA and *S. aureus* while there was no inhibition recorded against *B. cereus*. The Karura population was ninth against *C. albicans*, tenth against *A. flavus*, fifth against *E. coli*, fourth against MRSA and sixth against *S. aureus* while there was no inhibition against *B. cereus*.

It is clear from the results that the Nanyuki population recorded the highest antimicrobial activity overall followed by Coastal population while Homabay, Makueni and Machakos populations followed in that order. Extract from Karura population was the least in terms of antimicrobial activity followed by Maimahiu and Marigat in that order. This is the first report that compares the antimicrobial activity of different populations of Kenyan *D. viscosa*.

Various studies conducted elsewhere support the antimicrobial activity in *D. viscosa*. Patel and Coogan (2008) reported crude extract of *D. viscosa* leaves showed a concentration dependent inhibition of *C. albicans*. Elsewhere, it was found out that Preliminary screening of *D. viscosa* showed inhibition against *M.luteus, S.aureus, P.aeruginosa* and *E. coli* (Khurram *et al.*, 2009). Additionally, aerial parts of *D. viscosa* were active on skin disease causing pathogens; *Trichophyton rubrum, Aspergillus flavus, Microsporum gypseum, Paecilomyces*

varioti and *Aspergillus niger* (Pirzada *et al*., 2010). More over, leaf and stem were shown to act on array of microbes (Mehmood *et al*., 2013).

6.4.3. Antimicrobial activity of the isolated compounds from *Z. paracanthum* root bark

Eight isolates from roots of *Z. paracanthum*; namely myristic acid, stigmasterol, sesamin, 8-acetonyldihydrochelerythrine, arnottianamide, 10-methoxycanthin-6-one, canthin-6-one and 8-oxochelerythrine. The eight compounds were tested against the growth of four microbes, MRSA, *S.aureus, E.coli* and *C.albicans*. Three concentrations were used for each compound against each microbe 1000, 100 and 10µg/ml.

The myristic acid had no activity at all against the microbes investigated the MIC was therefore presumed to be >1000µg/ml. Elsewhere, myristic acid was reported to have moderate activity against *Fusarium* spp (Altieri *et al*., 2009) and not active on MRSA (Kitahara *et al*., 2004). Additionally, studies have shown lack of activity of myristic acid against the growth of *Bacillus subtilis, Aspergillus fumigatus* and *A. niger* (Chandrasekaran *et al*., 2011). Myristic acid is sufficiently hydrophobic to provide membrane-anchorage for proteins but not hydrophobic enough for the binding to be permanent (Stillwell, 2016) this could be the reason as to why there was no antimicrobial activity recorded because the compound could not bind to the microbes permanently to induce any visible growth inhibition.

Stigmasterol had no activity on MRSA and *C. albicans* but had moderate activity against both Gram positive and negative bacteria. Elsewhere, a study on antimicrobial activity of stigmasterol indicated limited activity against *Streptococcus* species (Laggoune *et al*., 2008; Taleb-Contini *et al*., 2003). This study also supports Jain et al. (2001) on antimicrobial activity of stigmasterol. Sesamin had no activity on MRSA, *C. albicans* and *E. coli* but exhibited inhibition on *S. aureus*. This report agrees with other antimicrobial studies of sesamin. To start with, studies on antimicrobial and antioxidant properties of sesamin revealed little antibacterial activity but antimicrobial action of sesamin against fungi was not obvious (Zhou *et al*., 2004).

Sesamin did not inhibit MRSA because it is a resistant strain of *S. aureus* (Enright *et al*., 2002). Differential sensitivity of microbes to sesamin is inferred by cell wall of bacteria. Gram negative bacteria (*E. coli*) has an outer membrane made of lipid bilayer surrounded with porins and proteins permit movement of various molecules and ions in and out of the cell thus serving as a barrier to chemicals such as sesamin (Epand & Epand, 2009). The gram positive bacterium (*S. aureus*) on the other hand has a comparatively thick layer of peptidoglycan sheets composed of polymer that are porous to numerous substances, and thus delicate to most chemicals including sesamin (Malanovic & Lohner, 2016). Equally, lack of inhibition against *C. albicans* could be explained by the polysaccharides and proteins contained in the cell wall (Adejuwon *et al*., 2013). The protein present in *C. albicans*, serve as a transport system to selected materials and can eject wastes and chemicals that could be deemed lethal to the cell; This is known as efflux efflux and is a vital medical chracteristic (Niimi *et al*., 2010).

The compound 8- acetonyldihydrochelerythrine was active on the four microbes having MIC of 31.25 µg/ml on MRSA, 15.625 µg/ml on *S. aureus* and *E.coli* and 62.5 µg/ml on *C. albicans.* These results conform to the results obtained from same compound isolated from *Zanthoxylum rhetsa* roots which had strong inhibition on MRSA and moderate activity on *E. coli* (Tantapakul *et al*., 2012). Additionally, 8-acetonyldihydrochelerythrine isolated from *Zanthoxylum capense* showed significant inhibition on *Staphylococcus aureus*. The antimicrobial activity of 8-acetonyldihydrochelerythrine is attributed to two methoxyl groups present at carbon 7 and 8 of this compound (Tavares *et al*., 2014).

Arnottianamide recorded no activity. Elsewhere, a study on bioactivity of compounds isolated from *Zanthoxylum zanthoxyloides* recorded that arnottianamide was among the compounds isolated that failed to exhibit antifungal activity (Queiroz *et al*., 2006). In addition, antimicrobial studies of phytoconstituents of *Zanthoxylum capense* reported arnottianamide as one of the compounds that lacked antibacterial activity (Cabral *et al*., 2015).

The 10-methoxycanthin-6-one had important antimicrobial activity on the tested microbes with insignificant difference compared to omacilin (P≥0.05) against *E. coli*. The MIC values for each microbe were 3.906 µg/ml against MRSA, 1.95 µg/ml on *S. aureus*, 3.906 µg/ml on *E. coli* and 7.8125 µg/ml on *C. albicans*. This report corroborates with findings from other studies on antimicrobial activity of this compound where 10-methoxycanthin-6-one was active on *Bacillus cereus, Ralstonia solanacearum,* and *Pseudomonas syringae* (Li *et al*., 2019). Elsewhere, this compound exhibited best inhibitory activity against *Bacillus cereus, Bacillus subtilis, Ralstonia solanacearum,* and *Pseudomonas syringae* and significant antifungal activity against *Fusarium graminearum* (Zhao *et al*., 2016). The structure–activity relationship (SAR) for the antimicrobial activity shows that the high activity is conferred by the aliphatic ester derivatives (Dai *et al*., 2018).

Canthin-6-one showed high antimicrobial activity with no substantial variation compared to positive controls (P≥0.05) for all microbes. The MIC value was 0.97 µg/ml for MRSA, 0.48 µg/ml for *S. aureus*, 1.95 µg/ml for *E. coli* and 3.906 µg/ml against *C. albicans*. This study supports findings reported elsewhere on the activity of canthin-6-one. Soriano-Agatón et al. (2005) described the antifungal potential of canthin-6-one and their derivatives though, the structure-activity relationship (SAR) was not explained. Elsewhere in 2008, Lagoutte et al. studied the mode of action of canthin-6-one for the antifungal activity against *Saccharomyces cerevisiae* and explained that the compound has a high affinity for lipid droplets which affects the fungal metabolism by stimulation enzymes involved in alkyl chain desaturation. Additionally, canthin-6-one showed activity against *Mycobacterium* species MRSA (O'Donnell and Gibbons, 2007).

8-oxochelerythrine showed significant activity on the tested microbes with MIC of 62.5 µg/ml on MRSA, 31.25 µg/ml on *S. aureus*, 3.906 µg/ml on *E. coli* and 15.625 µg/ml on *C. albicans*. Reports on studies of antimicrobial activity of 8-oxochelerythrine which substantiates

this study have been recorded elsewhere. 8-oxochelerythrine was found to have exciting inhibition on *Clostridium sporogenes* and *Streptococcus pyogenes* (Cesari *et al.*, 2015). Moreover, 8-oxochelerythrine also showed stronger antibacterial activity against *S. aureus, E. coli* and *B. subtilis* (Zhang *et al*., 2014). Antimicrobial potential of 8-oxochelerythrine is ascribed to two methoxyl groups present in carbon 7 and 8 of this compound (Tavares *et al*., 2014).

6.4.4. Antimicrobial activity of the isolated compounds from *D. viscosa* coastal population

The compounds isolated from *D. viscosa* coastal population; hautriwaic acid lactone, 5, 7, 4', 5' -tetrahydroxy-3, 6, 2' -trimethoxyflavone, and hautriwaic acid were evaluated against four microbes, MRSA, *S. aureus, E. coli* and *C. albicans*. Three concentrations were used for each compound against each microbe 1000, 100 and 10µg/ml.

Hautriwaic acid lactone exhibited activity on the tested microbes. The activity on *S. aureus* and *E. coli* at 1000µg/ml was insignificantly different from omacilin at a similar concentration ($P \geq 0.05$). The MIC values for MRSA was 62.5 µg/ml while for *S. aureus* and *E. coli* was 1.95 µg/ml. The MIC value for *C. albicans* was 7.8125 µg/ml. The report of antimicrobial activity of hautriwaic acid lactone is in agreement with (Omosa *et al*., 2014) who found this compound to have a worthy antifungal activity on *S. cerevisiae* possibly owing to its lipophilic character.

5, 7, 4', 5' -Tetrahydroxy-3, 6, 2' -trimethoxyflavone **(10)** recorded no activity on the microbes tested and thus the MIC values for all microbes was ≥ 1000µg/ml. dodonic acid exhibited less inhibition on *S. aureus* and *E. coli* and was not active on MRSA and *C. albicans* in all the tested concentrations. The MIC for MRSA and *C. albicans* were ≥ 1000 µg/ml while for *S. aureus* and *C. albicans* were 500 µg/ml. This study is substantiated by (Omosa *et al*.,

2014) who found the dodonic acid was less active on *E. coli*, *S. aureus* and *B. pumilus* and *S. cerevisiae*.

6.4.5. Antimicrobial activity of compounds isolated from *D. viscosa* Nanyuki population

Catechin was the only compound isolated from *D. viscosa* Nanyuki population. Catechin exhibited activity on the tested microbes with no significant difference at 1000µg/ml in the inhibition of MRSA, *S. aureus* and *C. albicans* as compared to positive controls at similar concentration ($P \geq 0.05$). The mode of action of catechin is based on the OH groups at carbon 3 and 3' confirming the importance of hydroxyl groups in those carbons for antimicrobial activity in flavonoids. Findings from (Muthuswamy and Rupasinghe, 2007) who reported on selective antimicrobial activity of catechin substantiates this report.

6.5. CONCLUSION

Antimicrobial activity of the leaf, stem and root extracts of *Z. paracanthum, Z. holtzianum, Z. chalybeum, Z. usambarense and Z. gilletii* as well as leaf extracts of ten populations of *D. viscosa* showed that the root bark extracts were most active followed by leaf and finally stem bark extracts of *Zanthoxylum* species. *Z. paracanthum* root and stem extracts were most active in comparison to root and stem bark extracts of other species while *Z. usambarense* leaf extract was the most active compared to leaf extracts from other *Zanthoxylum* species.

Out of the ten populations of *D. viscosa*, coast, Makueni, Machakos, Karura, Nanyuki, Maimahiu, Narok, Loita Forest, Marigat and Homa bay tested, the Nanyuki population recorded the highest antimicrobial activity overall followed by coastal population while Homabay, Makueni and Machakos populations followed in that order. Leaf extract from Karura population was the least followed by Maimahiu and Marigat in that order.

The activity of eight compounds from *Z. paracanthum* indicated canthin-6-one (**7**) was the most active followed by 10-methoxycanthin-6-one (**6**) while Myristic acid (**1**) and

arnottianamide (**5**) failed to inhibit the tested microbes. On the other hand, the activity of compounds from *D viscosa* coastal population showed that hautriwaic acid lactone **(9)** was the most active compound while 5, 7, 4', 5' -tetrahydroxy-3, 6, 2’ -trimethoxyflavone **(10)** did not show any activity against the tested microbes. Catechin **(12)**, the compound fom *D. viscosa*, Nanyuki population showed high activity against all the tested microbes.

CHAPTER 7: ANTIPROLIFERATIVE ACTIVITY

ANTIPROLIFERATIVE ACTIVITY OF *ZANTHOXYLUM* AND *DODONAEA* COMPOUNDS

7.1. INTRODUCTION

Medicinal plants have been widely used traditionally and are embraced to be key foundations for drug inventions and improvement, although only a few have been scientifically investigated for their safety (Pereira *et al*., 2013). Phytotherapy has gained popularity as a therapeutic system to which majority of people rely on for their treatment. Plants serve as reservoir of drugs and Africa is one continent with abundance of herbs and medicinal plants. These herbs are used in various formulations and concoctions as treatment of various diseases and ailments (Kostova *et al*., 2010). This could be attributed to their availability, affordability and the belief that they are harmless. Different assays are used to determine proliferation and cytotoxicity of different chemicals as shown in the table below (Adan *et al*., 2016).

A lot of ethnic herbs are used traditionally across the world in management and treatment of cancer (Jaradat *et al*., 2016). Nevertheless, there is inadequate scientific backup on the safety of these plants (Saad *et al*., 2006). It is crucial to carry out toxicological evaluation to envisage toxicity and provide guidelines for safe dose selection.

7.1.1. Objective

To determine cytotoxicity of extracts and compounds from *Zanthoxylum* and *Dodonaea* species for anticancer potential against normal and multi drug resistant breast and prostate cancer cell lines.

7.2. MATERIALS AND METHODS

This study evaluated the antiproliferative activity of extracts and pure compounds from the root bark of *Z. paracanthum* and *D. viscosa* collected from Nanyuki and coast against on

human prostate cancer cell line (DU 145), human breast cancer cell line (HCC 1395) and African green monkey kidney normal cells (vero E6) cell lines obtained from Manassas, VA, USA. The study was conducted at Centre for Traditional Medicine and Drug Research (CTMDR), Kenya Medical Research Institute (KEMRI) Nairobi, Kenya.

7.2.1. Preparation of stock solution of test samples

DMSO was used to dissolve the test samples and diluted serially with minimal essential media to make 100, 33.33, 11.11, 3.70, 1.23, 0.41, and 0.14µg/ml as described in **table 28**.

Table 26: Dilution of test samples used in the assay

Well No.	A	B	C	D	E	F	G	H
Sample dilution	media and cells alone	50 µL from well C	50 µL from well D	50 µL from well E	50 µL from well F	50 µL from well G	50 µL from well H	150 µL test sample
Final concentration µg/mL	0.00	0.14	0.41	1.23	3.70	11.11	33.33	100

7.2.2. Cell culturing

The cells preserved in liquid nitrogen in vials were removed and thawed. This was followed by centrifuging the vial contents and transferring the supernatant into a growth minimum essential media (MEM) enriched using 10 v/v of Fetal Bovine Serum (FBS), 1v/v antibiotic and 1v/v L-Glutamine in a T75 culture flask and incubated for 48 hrs at 37˚C in 5 v/v CO_2 to reach confluence level (Mbugua *et al*., 2019).

7.2.3. Antiproliferative assay

After cells for all the cell lines attained confluence, they were cleaned with saline phosphate buffer and trypsinized. Active cells were calculated by determining cell density by exclusion method using the trypan blue in a haemocytometer. A portion of 100µl having 2.0

$\times 10^4$ cells/ml was seeded into 96-well plate and incubated for 24 h at 37°C in v/v CO_2. Fifteen µl of test samples was added from H to B in a 96 well plate where row H contained the highest sample concentration (100 µg/ml) while row B had the least sample concentration (0.14 µg/ml). Row A contained cells and medium only was used as negative control while doxorubicin was the positive control. The testing was replicated thrice and incubated for 48 hrs. Thereafter, 10 µl of MTT dye (5 mg/ml) was put into each well and incubated at 37 °C for 2 h in 5% CO_2. The enzyme mitochondrial dehydrogenase, a biomarker of live cells is known to interact with and reduce the yellow MTT dye to a purple insoluble formazan that correlates to the number of cells alive. Formazan development was solubilized with 50 µl of DMSO and confirmed using inverted light microscope. (Nemati *et al.*, 2013). The optical density (OD) was determined by use of calorimetric reader at 562 nm and 690 nm wavelength.

7.2.4. Data analysis

Cytotoxicity was determined by following the steps below (Wilson, 2000).

$$\% \text{ Cell viability} = (A_T - A_B) / (A_C - A_B) \times 100 \ldots \ldots \ldots (1)$$

Where, A_T = Absorbance of treated cells (test samples)

A_B = Absorbance of blank (media only)

A_C = Absorbance of control (untreated)

$$\%\text{Cytotoxicity} = 100 - \% \text{ cell viability} \ldots \ldots \ldots (2)$$

CC_{50} and IC_{50} was determined by use of Finney's Probit analysis using BioStat version 6.7. The values were then analysed using a Tukey's test to determine the samples with significantly similar IC_{50} values as well as those of positive control at probability level of 0.05.

Selectivity Index determination

Selectivity index (SI) indicates the capability of a treatment to selectively cause toxicity to cancerous cells and spare the normal cells was determined using (Mbugua *et al.*, 2019):

$$SI = \frac{CC_{50}}{IC_{50}}$$

Where;

CC_{50} – Concentration of sample that killed 50% of the normal cells

IC_{50}– Concentration of sample that killed 50% of cancerous cells

7.3. RESULTS

7.3.1. Antiproliferative activity of compounds isolated from *Z. paracanthum*

Eight compounds (myristic acid **1**, stigmasterol **2**, sesamin **3**, 8-acetonyldihydrochelerythrine **4**, arnottianamide **5**, 10-methoxycanthin-6-one **6**, canthin-6-one **7** and 8-oxochelerythrine **8**) isolated from root bark of *Z. paracanthum* were evaluated for antiproliferative activity on three cell lines DU 145 (human prostate cancer cell line), HCC 1395 (Human breast cancer cell line) and VERO E6 (African monkey kidney normal cells) at 0.14, 0.41, 1.23, 3., 11.11, 33.33 and 100 µg/ml. All the compounds showed concentration-dependent increase in cytotoxicity.

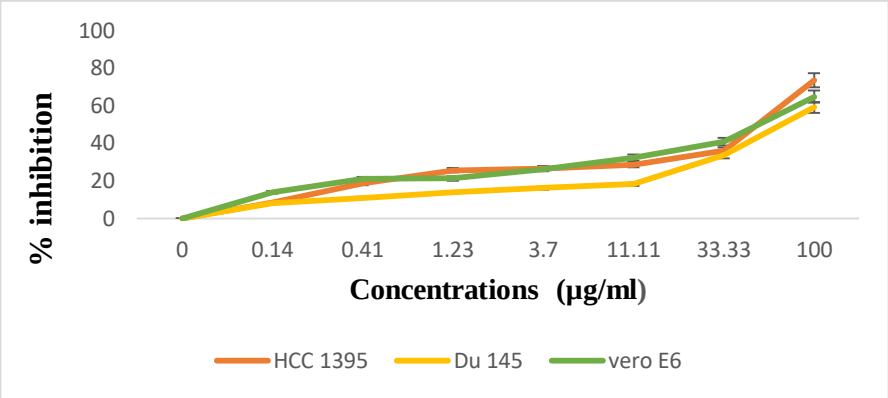


Figure 48; Dose response curves of percentage cell inhibition for myristic acid 1 against HCC 1395, DU 145 and Vero E6

Increase in concentration of myristic acid **1** led to increase in cytotoxicy of the selected cell lines. The overall cytotoxicity of DU 145 was the least followed by VERO E6 while HCC1395 was the highest. The CC_{50} value for VERO E6 was 64.86 µg/ml while the IC_{50}

values for HCC 1395 and DU 145 were 57.71 µg/ml and 80.24 µg/ml respectively. The selectivity index for HCC 1395 was 1.12 while that of DU 145 was 0.81 **(figure 48)**.

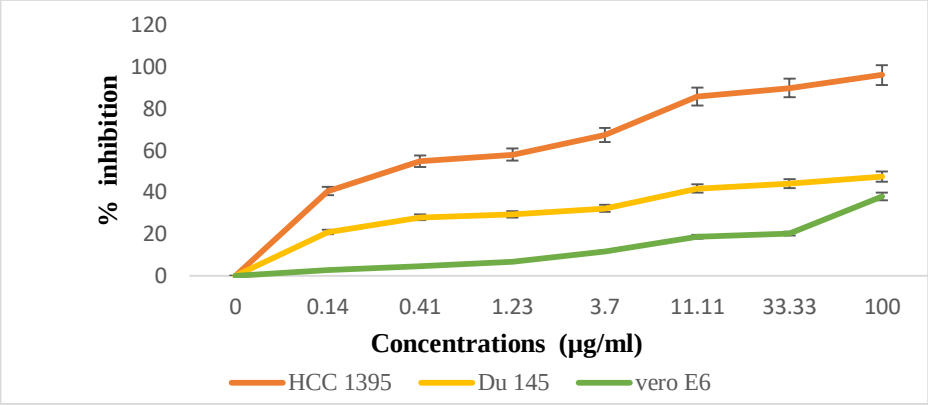


Figure 49: Dose response curves of percentage cell inhibition for stigmasterol 2 against HCC 1395, DU 145 and Vero E6

Increase in concentration of stigmasterol **2** led to increase in cytotoxicy of the selected cell lines. Stigmasterol **2** exhibited highest cytotoxicity on HCC1395 overall followed by DU 145 and VERO E6 was the least. The CC_{50} value for VERO E6 was 123.88 µg/ml while IC_{50} values for HCC 1395 and DU 145 were 0.42 µg/ml and 140.49 µg/ml correspondingly. Selectivity index for HCC 1395 was 294.94 while that of DU 145 was 0.88 **(figure 49)**.

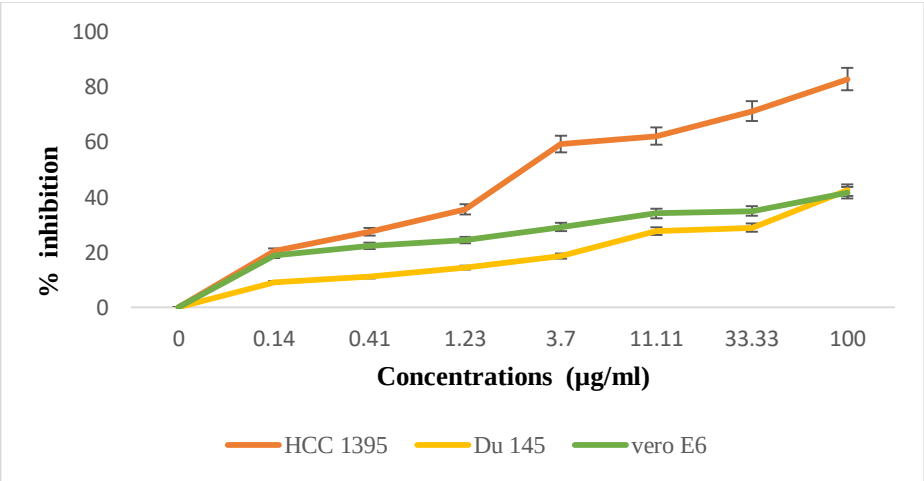


Figure 50; Dose response curves of percentage cell inhibition for sesamin 3 against HCC 1395, DU 145 and Vero E6

Increase in concentration of sesamin **3** led to increase in cytotoxicy of the selected cell lines. Sesamin exhibited highest cytotoxicity of HCC1395 overall followed by VERO E6 and DU 145 was the least. The CC_{50} value for E6 was 135.31 µg/ml while IC_{50} value for HCC 1395 was 3.39 µg/ml and DU 145 was 115.06 µg/ml respectively. The selectivity index for HCC 1395 was 39.97 while that of DU 145 was 1.18 **(figure 50)**.

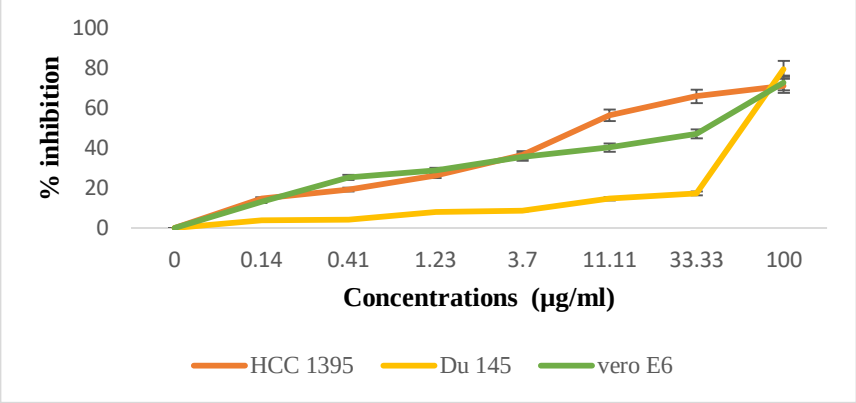


Figure 51: Dose response curves of percentage cell inhibition for 8-acetonyldihydrochelerythrine 4 against HCC 1395, DU 145 and Vero E6

Increase in concentration of 8-acetonyldihydrochelerythrine **4** led to increase in cytotoxicy of the selected cell lines. 8-Acetonyldihydrochelerythrine **4** exhibited highest cytotoxicity on HCC1395 overall followed by VERO E6 and DU 145 was the least. The CC_{50} value for E6 was 47.83 µg/ml while IC_{50} values for HCC 1395 and DU 145 were 9.99 µg/ml and 66.82 µg/ml in that order. The selectivity index for HCC 1395 was 4.79 while that of DU 145 was 0.72 **(figure 51)**.

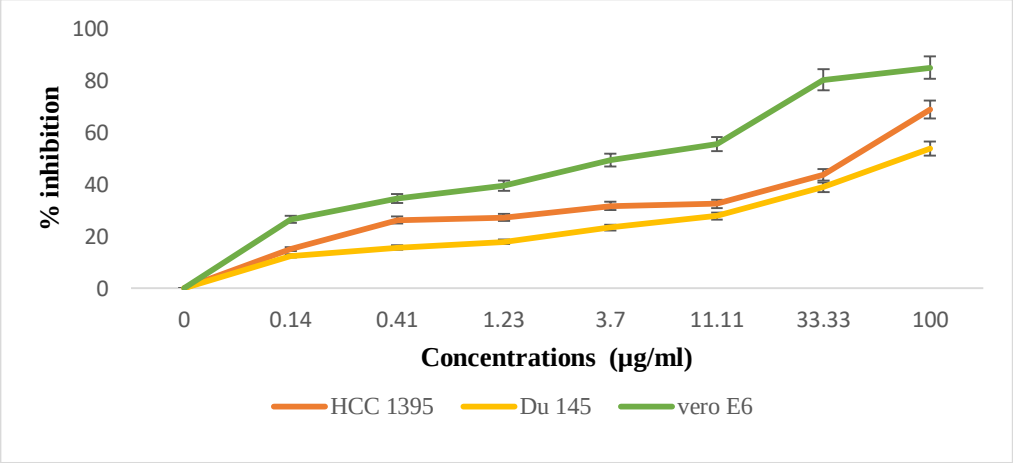


Figure 52: Dose response curves of percentage cell inhibition for arnottianamide 5 against HCC 1395, DU 145 and Vero E6

Increase in concentration of arnottianamide **5** led to increase in cytotoxicy of the selected cell lines. Overall, arnottianamide **5** exhibited highest cytotoxicity on VERO E6 followed by HCC1395 whle DU 145 was the least. The CC_{50} value for E6 was 2.77 µg/ml while IC_{50} values for HCC 1395 and DU 145 were 38.34 µg/ml and 84.31 µg/ml correspondingly. The selectivity index for HCC 1395 was 0.07 while that of DU 145 was 0.03 **(figure 52)**.

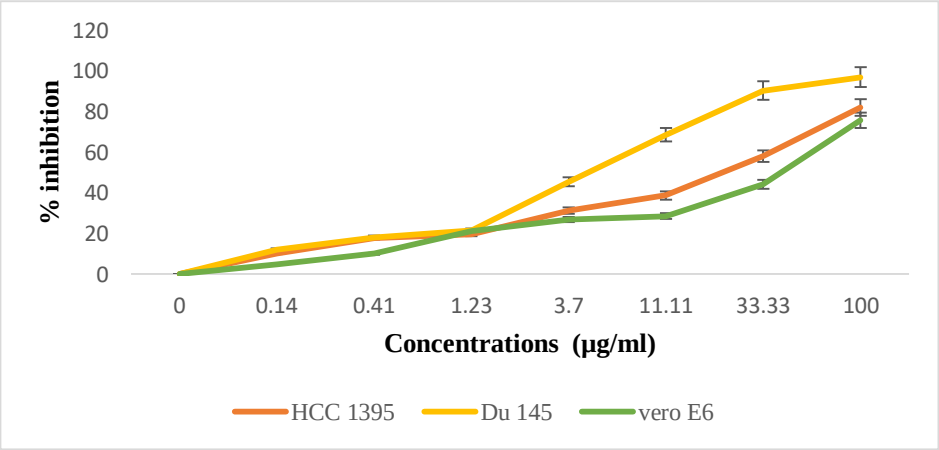


Figure 53: Dose response curves of percentage cell inhibition for 10-methoxycanthin-6-one 6 against HCC 1395, DU 145 and Vero E6

Increase in concentration of 10-methoxycanthi-6-one **6** led to increase in cytotoxicy of the selected cell lines. Overall, 10-methoxycanthi-6-one **6** exhibited highest cytotoxicity of DU

145 followed by HCC1395 and VERO E6 was the least. The CC_{50} value for VERO E6 was 53.95 µg/ml while IC_{50} values for HCC 1395 and DU 145 were 14.70µg/ml and 3.58 µg/ml correspondingly. The selectivity index for HCC 1395 was 3.67 while that of DU 145 was 15.09 **(figure 53)**.

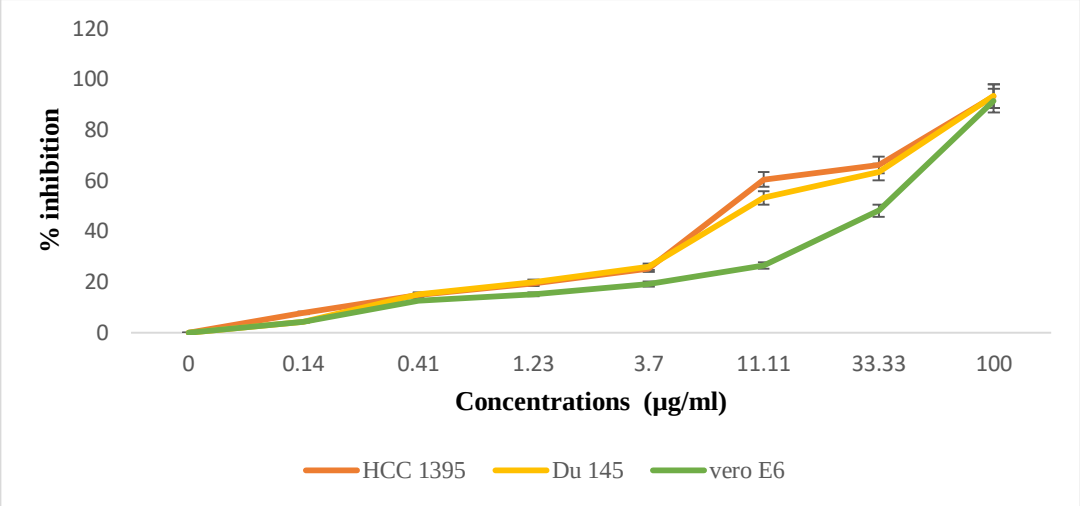


Figure 54: Dose response curves of percentage cell inhibition for canthin-6-one 7 against HCC 1395, DU 145 and Vero E6

Increase in concentration of canthin-6-one **7** led to increase in cytotoxicy of the selected cell lines. Overall, canthin-6-one **7** exhibited highest cytotoxicity on HCC1395 followed by DU 145 and VERO E6 was the least. The CC_{50} value for E6 was 41.81µg/ml while the IC_{50} values for HCC 1395 and DU 145 were 8.12 µg/ml and 9.43µg/ml correspondingly. The selectivity index for HCC 1395 was 5.15 while that of DU 145 was 4.43 **(figure 54)**.

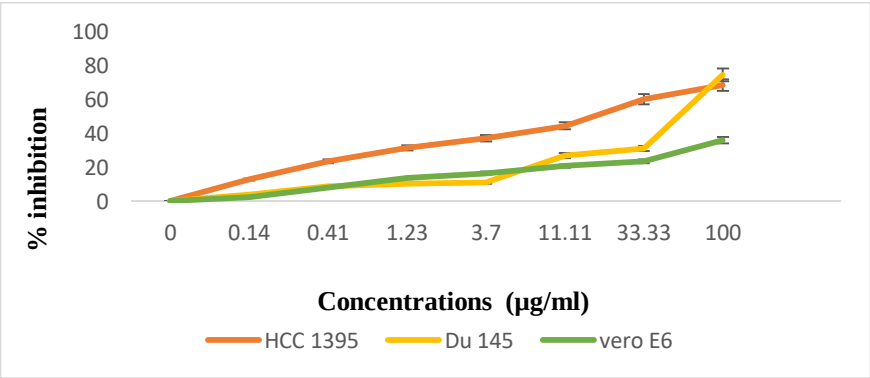


Figure 55: Dose response curves of percentage cell inhibition for 8-oxochelerythrine 8 against HCC 1395, DU 145 and Vero E6

Increase in concentration of 8-oxochelerythrine **8** led to increase in cytotoxicy of the selected cell lines. Overall, 8-oxochelerythrine **8** exhibited highest cytotoxicity on HCC1395 followed by DU 145 and VERO E6 was the least. The CC_{50} value for VERO E6 was 135.32 µg/ml while IC_{50} values for HCC 1395 and DU 145 were 14.09 µg/ml and 63.41µg/ml respectively. The selectivity index for HCC 1395 was 9.60 while that of DU 145 was 2.13 **(figure 55)**.

7.3.2. Antiproliferative activity of compounds isolated from *D. viscosa* coastal population

Hautriwaic acid **9** and hautriwaic acid lactone **11** from *D. viscosa* subsp *viscosa* were evaluated for antiproliferative activity on DU 145, HCC 1395 and VERO E6 (African green monkey kidney normal cells) at 0.14, 0.41, 1.23, 3.7, 11.11, 33.33 and 100 µg/ml. All the compounds showed a concentration-dependent increase in cytotoxicity.

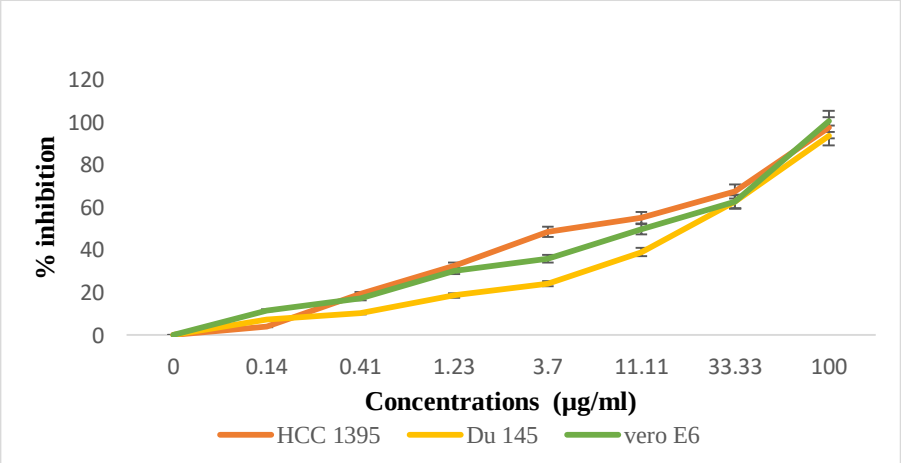


Figure 56; Dose response curves of percentage cell inhibition for dodonic acid 9 against HCC 1395, DU 145 and Vero E6

Increase in concentration of hautriwaic acid **9** led to increase in cytotoxicy of the selected cell lines. Overall, hautriwaic acid **9** exhibited highest cytotoxicity DU 145 followed by HCC1395 and VERO E6 was the least. The CC_{50} value forVERO E6 was 11.27µg/ml while

IC $_{50}$ values for HCC 1395 and DU 145 were 27.29 µg/ml and 11.62 µg/ml corresopndingly. The selectivity index for HCC 1395 was 0.41 while that of DU 145 was 0.97 **(figure 56)**.

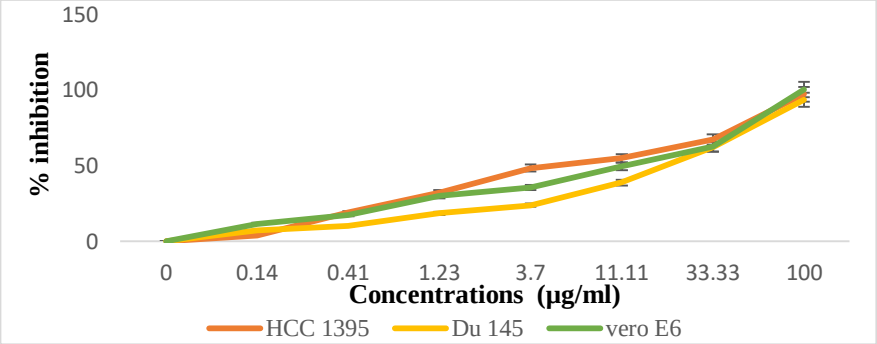


Figure 57: Dose response curves of percentage cell inhibition for hautriwaic acid lactone 11 against HCC 1395, DU 145 and Vero E6

Increase in concentration of hautriwaic acid lactone led to increase in cytotoxicy of the selected cell lines. Overall, hautriwaic acid lactone exhibited highest cytotoxicity on HCC1395 followed by VERO E6 and while DU 145 was the least. The CC $_{50}$ value for VERO E6 was 20.39µg/ml while IC $_{50}$ values for HCC 1395 and DU 145 were 5.21 µg/ml and 12.17 µg/ml respectively. The selectivity index for HCC 1395 was 3.92 while that of DU 145 was 1.68 **(figure 57)**.

7.3.3. Antiproliferative activity of compounds isolated from *D. viscosa* Nanyuki population

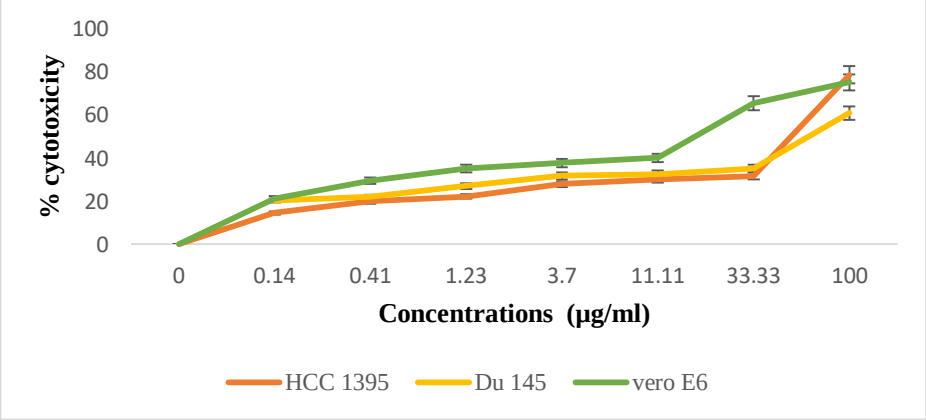


Figure 58; Dose response curves of percentage cell inhibition of catechin 12 against HCC 1395, DU 145 and Vero E6

Increase in concentration of catechin **12** led to increase in cytotoxicy of all the selected cell lines. Overall, catechin exhibited highest cytotoxicity of VERO E6 followed by DU 145 and HCC1395 was the least. The CC_{50} value for VEERO E6 was 34.74 µg/ml while IC_{50} values for HCC 1395 and DU 145 were 53.58 µg/ml and 72.05µg/ml correspondingly. The selectivity index for human breast cancer cell line, HCC 1395 was 0.65 while that of human prostate cancer cell line, DU 145 was 0.48 **(figure 58)**.

7.3.4. Antiproliferative activity of crude extracts

Like the pure compounds reported above root bark of *Z. paracanthum* and leaf extracts of *D. viscosa* (coastal and Nanyuki populations) were evaluated for antiproliferative activity. All the extracts showed concentration dependent increase in cytotoxicity as shown in **figure 59.**

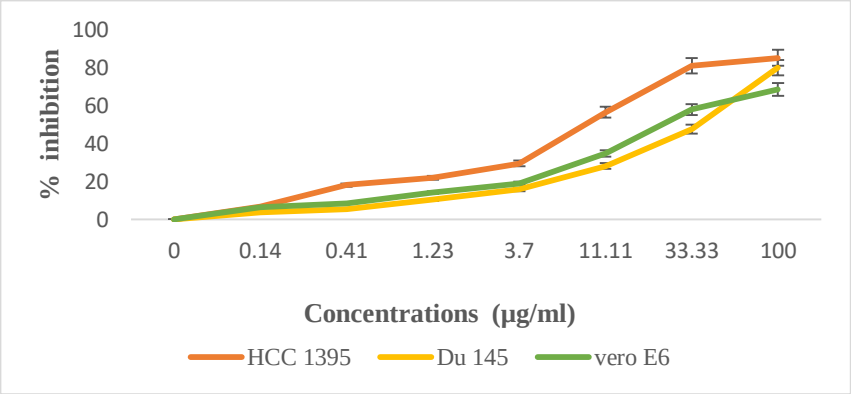


Figure 59: Dose response curves of percentage inhibition of *Z. paracanthum* root extract against HCC 1395, DU 145 and Vero E6

Increase in concentration of *Z. paracanthum* root extract led to increase in cytotoxicy in all the selected cell lines. Overall, this extract exhibited highest cytotoxicity of HCC1395 followed by VERO E6 and DU 145 was the least. The CC_{50} value for VERO E6 was 28.28 µg/ml while IC_{50} values for HCC 1395 and DU 145 were 7.27 µg/ml and 53.21 µg/ml correspondingly. The selectivity index for HCC 1395 was 3.89 while that of DU 145 was 0.53.

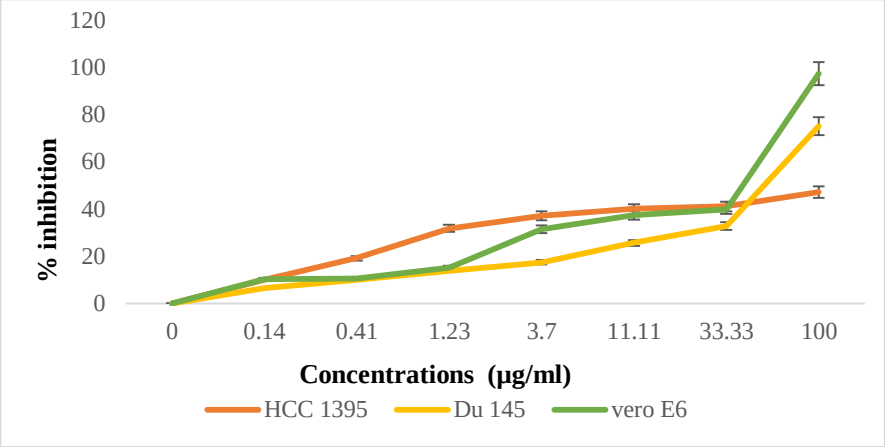


Figure 60; Dose response curves of percentage inhibition of *D. viscosa* coastal population against HCC 1395, DU 145 and Vero E6

Increase in concentration of *D. viscosa* susp *viscosa* leaf extract led to increase in cytotoxicy in all the selected cell lines. Overall, this extract exhibited highest cytotoxicity on HCC1395 followed by VERO E6 and DU 145 was the least. The CC_{50} value for VERO E6 was 35.12 µg/ml while IC_{50} values for HCC 1395 and DU 145 were 98.15 µg/ml and 60.95 µg/ml correspondingly. The selectivity index for HCC 1395 was 0.36 while that of DU 145 was 0.58 **(figure 60)**.

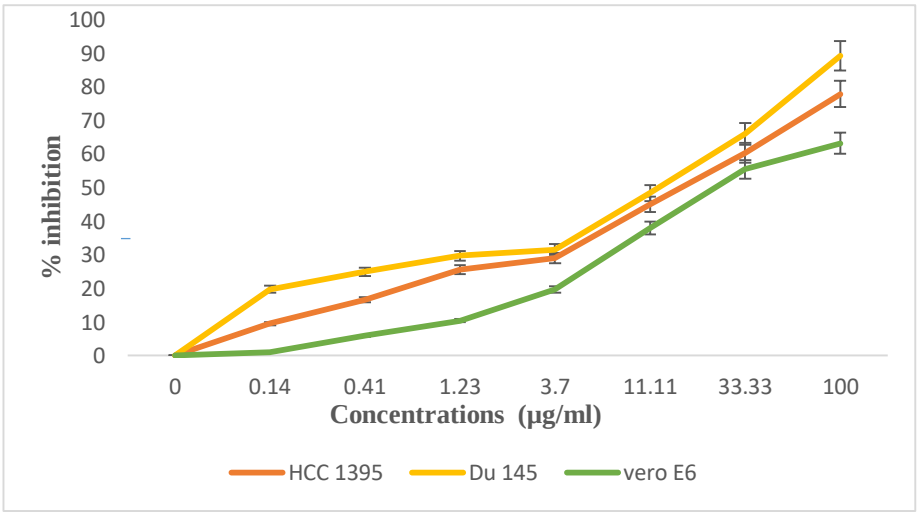


Figure 61: Dose response curves of percentage cell inhibition for D. viscosa, Nanyuki population against HCC 1395, DU 145 and Vero E6

Increase in concentration of *D. viscosa* susp *angustifolia* leaf extract led to increase in cytotoxicy in all the selected cell lines. Overall, this extract exhibited highest cytotoxicity on

DU 145 followed by HCC1395 and VERO E6 was the least. The CC_{50} value for VERO E6 was 33.33 µg/ml while IC_{50} values for HCC 1395 and DU 145 were 22.28 µg/ml and 13.52 µg/ml in that order. The selectivity index for HCC 1395 was 1.5 while that of DU 145 was 2.47 **(figure 61)**.

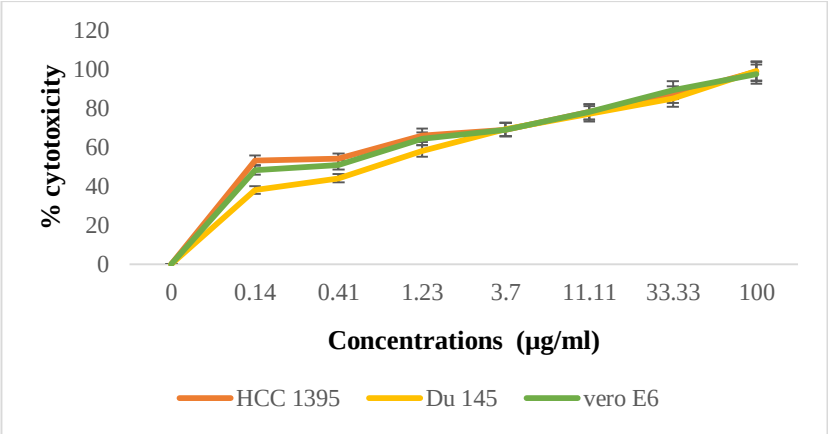


Figure 62; Dose response curves of percentage cell inhibition for doxorubicin against HCC 1395, DU 145 and Vero E6

Increase in concentration of doxorubicin (positive control) led to increase in cytotoxicy in all the selected cell lines. Overall, doxorubicin exhibited highest cytotoxicity on HCC1395 followed by VERO E6 and DU 145 was the least. The CC_{50} value for VERO E6 was 0.30 µg/ml while the IC_{50} values for HCC 1395 and DU 145 were 0.21µg/ml and 0.59 µg/ml respectively. The selectivity index for HCC 1395 was 1.41 while that of DU 145 was 0.51 **(figure 62)**. The IC_{50} values of the all the samples tested are shown in **table 27** while their selectivity indices are in **table 28**

Samples	VERO E6/ NORMAL	HCC 1396	DU 145
Myristic acid (**1**)	64.86 ± 0.51^{c}	57.71 ± 1.2^{b}	80.24 ± 0.12^{d}
Stigmasterol (**2**)	123.88 ± 0.00^{b}	0.42 ± 0.1^{n}	140.49 ± 1.27^{a}
Sesamin (**3**)	135.31 ± 0.12^{a}	3.39 ± 1.0^{m}	115.06 ± 0.03^{b}
8-acetonyldihydrochelerythrine (**4**)	47.83 ± 1.15^{e}	9.99 ± 0.6^{i}	66.82 ± 0.58^{f}
Arnottianamide (**5**)	2.77 ± 0.12^{m}	38.34 ± 0.1^{d}	84.31 ± 0.64^{c}
10-Methoxycanthin-6-one (**6**)	53.95 ± 0.38^{d}	14.70 ± 0.5^{f}	1.58 ± 0.00^{n}
Canthin-6-one (**7**)	41.81 ± 0.64^{f}	8.12 ± 0.6^{j}	9.43 ± 0.01^{m}
8-Oxochelerythrine (**8**)	135.32 ± 0.12^{a}	14.09 ± 0.3^{h}	63.41 ± 1.10^{g}
Hautriwaic acid (**9**)	11.27 ± 0.02^{l}	27.29 ± 0.3^{f}	11.62 ± 0.02^{l}

Samples	VERO E6/ NORMAL	HCC 1396	DU 145
Hautriwaic acid lactone (**11**)	20.39 ± 0.00^{k}	5.21 ± 0.0^{l}	12.17 ± 0.12^{k}
Catechin (**12**)	34.74 ± 0.36^{h}	53.58 ± 0.1^{c}	72.05 ± 0.03^{e}
Z. paracanthum root bark	28.28 ± 0.34^{j}	7.27 ± 0.0^{k}	53.21 ± 1.21^{i}
D. viscosa (coastal)	35.12 ± 0.04^{g}	98.15 ± 0.2^{a}	60.95 ± 0.03^{h}
D. viscosa (Nanyuki)	33.33 ± 0.69^{i}	22.22 ± 0.6^{g}	13.52 ± 0.01^{j}
Doxorubicin	$\mathbf{0.30\pm0.12^{n}}$	$\mathbf{0.21\pm0.2^{n}}$	$\mathbf{0.59\pm0.01^{n}}$

Table 27: IC50/CC50 values in µg for the tested samples against the three cell lines.

Inhibition concentrations of extracts and isolated compounds from *Z. paracanthum* and *D. viscosa* from Nanyuki and coast collections that killed 50% of human breast and prostate cancereous and normal cells

Values are expressed as Mean±SEM. Values sharing a letter (superscript) in each column are not significantly different from each other ($p \leq 0.05$).

Table 28; Selectivity indices for different samples against selected cancer cell lines

Samples	Selectivity index for HCC 1395	Selectivity index for DU 145
Myristic acid (**1**)	1.12	0.81
Stigmasterol (**2**)	294.94	0.88
Sesamin (**3**)	39.97	1.18
8-acetonyldihydrochelerythrine (**4**)	4.79	0.72
Arnottianamide (**5**)	0.07	0.03
10-Methoxycanthin-6-one (**6**)	3.67	15.09
Canthin-6-one (**7**)	5.15	4.43
8-Oxochelerythrine (**8**)	9.60	2.13
Hautriwaic acid (**9**)	0.41	0.97
Hautriwaic acid lactone (**11**)	3.92	1.68
Catechin (**12**)	0.65	0.48
Z. paracanthum root bark	3.89	0.53
D. viscosa (coastal)	0.36	0.58
D. viscosa (Nanyuki)	1.5	2.7
Doxorubicin	**1.41**	**0.51**

7.4. DISCUSSION

All the samples showed a concentration-dependent increase in cytotoxicity. VERO E*6* cells were used as a normal cell line in this study because it has a wide application in virology, toxicology as well as pharmacology research, and development of diagnostic components and vaccines (Menezes *et al*., 2013). Specifically, VERO E*6* cells have applications in toxicity assays for different chemicals including natural products (Matskevich *et al*., 2009).

The antiproliferative potential of the samples was grouped established on medium inhibition concentration (IC_{50}). The criteria for *in vitro* cytotoxicity after the exposure time of 72 hours as established by the U.S. National Cancer Institute (NCI): an IC_{50}< 20µg/ml for crude extracts and IC_{50} < 4µg /ml for pure compounds is considered to be highly antiproliferative. An IC_{50} value less than 30µg /ml is considered to be antiproliferative and an IC_{50} value between 30µg/ml and 100µg /ml is considered to be moderately antiproliferative while above 100µg/ml is considered inactive (Boik, 2001). The selectivity of the samples was determined as selectivity index (SI). SI values reveal the ability of a drug to target the cancerous cells without harming the normal cells. Plants with SI values ≥2 are taken to be very selective and vice versa (Badisa *et al.*, 2009).

7.4.1. Antiproliferative activity of compounds isolated from *Z. paracanthum*

All the eight compounds from *Z. paracanthum* showed a concentration dependent increase in cytotoxicity. The overall cytotoxicity of myristic acid was the least on DU 145 followed by VERO E6 and highest on HCC1395. Based on the criterion established by the US National cancer Institute for describing the level of antiproliferative activity of samples, myristic acid **1** can be termed as moderately antiproliferative as the median inhibitory concentration values for the three cell lines are between 30µg/ml and 100µg /ml. Additionally, myristic acid was less selective in both HCC 1395 and DU 145 as their SI values were ≤ 2. This study confirms a report made on cytotoxicity of myristic acid to the breast cancer cells (Wongtangtintharn *et al*., 2004). Elsewhere, it was found out that there was significant reduction of myristic acid synthesis among breast cancer patients (Aro et al., 2000). The anticancer property of fatty acids is associated with their chain length (Koseki *et al*., 2016).

Stigmasterol **2** exhibited highest cytotoxicity on HCC1395 overall followed by DU 145 while VERO E6 was the least. This therefore means that stigmasterol lacked antiproliferative activity against VERO E6 and DU 145 as the CC_{50}/ IC_{50} values for both cell lines were found

to be >100 µg/ml while on the other hand the compound was highly antiproliferative against HCC 1395 as the IC_{50} < 4µg /ml. Stigmasterol was highly selective against HCC 1395 and less selective against DU 145. This compound forms the best candidate for further research on control of breast cancer as it has a very low IC_{50} value against HCC1395 as well as a very high selectivity index (Frankfurt & Krishan, 2003). Stigmasterol inhibited proliferation of skin cancerous cells in Swiss albino mice due to its antioxidant and antigenotoxic potential (Ali *et al*., 2015).

Sesamin **3** exhibited highest cytotoxicity on HCC1395 overall followed by DU 145 and VERO E6 was the least. The selectivity index for HCC 1395 was very high (39.97) confirming that it was highly selective and less selective against DU 145 where the SI value was 1.18. This study is in agreement with studies that reported good tumour chemopreventative potency of sesamin (Hirose et al., 1992) as well as ability to suppress development of mammary tumours (Lee *et al*., 2011; Yokota *et al*., 2007). Elsewhere, sesamin has bee reported to iduce autophagy in colon cancer cells (Tanabe *et al*., 2011) and cell cycle arrest and apoptosis in human hepatocellular carcinoma cell line HepG2 (Deng *et al*., 2013).

8-Acetonyldihydrochelerythrine **4** exhibited cytotoxicity on HCC 1395 overall followed by VERO E6 while DU 145 was the least. The CC_{50} value for VERO E6 and DU 145 showed that 8-acetonyldihydrochelerythrine had moderate antiproliferative activity against VERO E6 and DU 145 cell lines. The 8-acetonyldihydrochelerythrine was very selective against HCC 1395 and less selective against DU 145. This is the first report on antiproliferative activity of 8-Acetonyldihydrochelerythrine. However, reports on antiproliferative activity of phenanthridine compounds shows that the compounds exhibit significant antitumour activity (Lamoral-Theys *et al*., 2009; Tsukamoto *et al*., 2011).

Overall, arnottianamide **5** exhibited highest cytotoxicity of VERO E6 followed by HCC1395 while DU 145 was the least. The antiproliferative activity of VERO E6 was high

with evidence of moderate antiproliferative activity of this compound against both HCC 1395 and DU 145. From the SI values, it was evident that arnottianamide showed less selectivity against both HCC 1395 with SI value of 0.07 and DU 145 with SI value of 0.03. The lack of specificity of this compound in different cell lines has been reported (Chang *et al.*, 2003; Sreelekha *et al*., 2014).

Increase in concentration of 10-methoxycanthin-6-one **6** led to increase in cytotoxicy percentage of the selected cell lines. This compound showed moderate activity against VERO E6 and antiproliferative activity against HCC 1395 and high antiproliferative activity against DU 145. 10-methoxycanthin-6-one was very selective for both HCC 1395 and DU 145. This is the first report on antiproliferative activity of 10-methoxycanthin-6-one.

Canthin-6-one **7** exhibited highest cytotoxicity on HCC1395 followed by DU 145 while VERO E6 was the least. Based on IC_{50}/CC_{50} values, canthin-6-one was found to have a moderate activity against VERO E6 and antiproliferative activity against both HCC 1395 and DU 145. The SI values indicated that the compound was highly selective against both HCC 1395 and DU 145. Reports on antiproliferative potential of this compound corroborate with findings from this study. (Dai *et al*., 2016) reported that canthin-6-one has a broad anticancer activity this has made the compound be used as an anticancer agent (Murakami *et al*., 2004). Elewhere, this compound was shown to prompt cell death, cell cycle arrest and differentiation in human myeloid leukemia (Omosa *et al*., 2019; Torquato *et al*., 2017). Strong antiproliferative activity of canthin-6-one was recorded in seven cancer cell lines (Dejos *et al*., 2014).

Increase in concentration of 8-oxochelerythrine **8** led to increase in cytotoxicy percentage of the selected cell lines. Overall, 8-oxochelerythrine exhibited highest cytotoxicity on HCC1395 followed by DU 145 while VERO E6 was the least. The CC_{50} value for VERO E6 implyed that the compound was inactive against VERO E6. Conversely, the compound was

found to be toxic against HCC 1395 and moderately toxicity against DU 145. 8-Oxochelerythrine was higly selective against HCC 1395 and DU 145. This is the first report on antiproliferative activity of 8-oxochelerythrine. However, reports on antiproliferative activity of phananthridine compounds shows that the compounds exhibit significant antitumour activity in cancer cells (Lamoral-Theys *et al*., 2009; Tsukamoto *et al*., 2011).

7.4.2. Antiproliferative activity of compounds isolated from *D. viscosa* coastal population

Overall, dodonic acid **9** exhibited highest cytotoxicity on human prostate cancer cell line (DU 145) followed by human breast cancer cell line (HCC1395) and Vero E6 was the least. Hautriwaic acid showed antiproliferative activity against all the three tested cell lines SI values showed that the compound was less selective against the two cancer cell lines tested with SI values indicating that the compound was not selective in bth HCC 1395 and DU 145. The inability of hautriwaic acid to select the cancer cells has been reported elsewhere (Bajaj *et al*., 1986; Jolad *et al*., 1982).

Hautriwaic acid lactone **11** exhibited cytotoxicity on HCC1395 followed by VERO E6 while DU 145 was the least. From IC_{50}/ CC_{50} values, it is clear that this compound showed antiproliferative activity against all the tested cell lines. The selectivity index for HCC 1395 and DU 145 showed that hautriwaic acid lactone was more selective against HCC 1395 as compared to DU 145. This is the first report on antiproliferative activity of hautriwaic acid lactone.

7.4.3. Antiproliferative activity of compounds isolated from *D. viscosa,* Nanyuki population

Catechin **12** showed highest cytotoxicity on VERO E6 followed by DU 145 while HCC1395 was the least. This therefore means that catechin had moderate antiproliferative

activity against the three cell lines. The SI values for HCC 1395and DU 145 indicated that the compound was not selective. Studies on cytotoxicity of catechin against cancer cells have been reported elsewhere (Manikandan *et al*., 2012; Suganuma *et al*., 2011).

7.4.4. Antiproliferative activity of crude extracts

The extract of *Z. paracanthum* root bark exhibited highest cytotoxicity on HCC 1395 followed by VERO E6 while DU 145 was the least. There was some antiproliferative activity against VERO E6 and HCC 1395 and moderate antiproliferative activity against DU 145. The extract was more selective on HCC 1395 and less selective on DU 145. This is the first report on antiproliferative activity of *Z. paracanthum.* Reports on antiproliferative activity of other *Zanthoxylum* species are in a greement with this study (Singh *et al*., 2015) for example, a substantial antiproliferative activity was reported on breast cancer cell lines by extracts of *Z. armatum* (Alam *et al*., 2017).

Increase in concentration of *D. viscosa,* coastal population leaf extract led to increase in cytotoxicy percentage in all the selected cell lines. Overall, this extract exhibited cytotoxicity on HCC 1395 followed by VERO E6 while DU 145 was the least. There was less selectivity of this extract on HCC 1395 and DU 145. *D.* viscosa, Nanyuki population leaf extract exhibited highest cytotoxicity on DU 145 followed by HCC1395 while VERO E6 was the least. This report concurs with studies conducted elsewhere, Shafek et al. (2015) showed high cytotoxicity of *Dodonaea viscosa* on breast carcinoma cell line (MCF7). (Rautenbach *et al*. (2014) also reported the anticancer potential of *D. viscosa* on MCF-7 breast adenocarcinoma cell line while a patent on treatment of breast cancer using *D. viscosa* exists (Makunga & Engelbrecht, 2019). However, reports on selectivity index of *D. viscosa* are missing and therefore, this is the first report showing the selectivity index of the anticancer potential of this plant.

7.5. CONCLUSION

All compounds isolated from the root bark of *Z. paracanthum* (myristic acid **1**, stigmasterol **2**, sesamin **3**, 8-Acetonyldihydrochelerythrine **4**, arnottianamide **5**, 10-methoxycanthin-6-one **6**, canthin-6-one **7** and 8-oxochelerythrine **8**) had antiproliferative activity against all the cell lines selected. Based on IC_{50} and selectivity index values, stigmasterol showed the best activity against HCC 1395. Other compounds from this plant which showed potential antiproliferative activity were canthin-6- one **7**, 10-methoxy canthin-6-one **6**, 8-acetonyldihydrochelerythrine **4**, sesamin **3** and 8-oxochelerythrine **8**. Hautriwaic acid lactone **11** isolated from *D. viscosa*, coastal collection and crude extracts from root bark of *Z. paracanthum* as well as leaf extract of *D.* viscosa, Nanyuki population showed antiproliferative potential.

In the order listed below, stigmasterol **2**, Sesamin **3**, 8-oxochelerythrine **8**, canthin-6-one **7**, 8-acetonyldihydrochelerythrine **4**, hautriwaic acid lactone **11**, root bark extract of *Z. paracanthum* and 10-methoxycanthin-6-one **6** showed high antiproliferative potential against HCC 1395. While antiproliferative potential against DU 145 was highest in 10-methoxycanthin-6-one **6** followed by canthin-6-one **7**, 8-oxochelerythrine **8** and leaf extract of *D. viscosa*, Nanyuki collection.

CHAPTER EIGHT: GENERAL DISCUSSION AND CONCLUSION

8.1. GENERAL DISCUSSION

Zanthoxylum species and *D. viscosa* populations are widely distributed in Kenya with wide usage in herbal medicine in the world. Irrespective of the wide usage of members of these genera in Kenya, their taxonomy confusion continues to exist due to use of old methods such as morphology to classify these groups of plants. This study focused on classifying members of these groups of plants using chemical markers as well as evaluating their potential in fighting growth of microbes of economic importance and different cancer cell lines representing the leading cancer types in Kenya.

In testing for relatedness of *Zanthoxylum* species, (*Z. chalybeum, Z. gilletii , Z. holtzianum, Z. paracanthum and Z. usambarense*) twelve discriminants were identified as the cause of the differences in the five species. The dendogram composed of the five species of *Zanthoxylum* resulted into two groups *Z. usambarense*, *Z. gilletii* and *Z. Paracanthum* and that of *Z. chalybeum* and *Z. holtzianum*. The relationship of Kenyan *Zanthoxylum* species showed in this study is supported by a report by Appelhans et al. (2018) who showed that *Z. gilletii* and *Z. holtzianum* were rooted to a different ancestor using molecular phylogenetics and (Beentje *et al*., 1994) based on morphology. It may therefore be argued that the relationship of Kenyan *Zanthoxylum* species in this study is not influenced by environmental conditions but evolution as species like *Z. holtzianum* and *Z. paracanthum* were collected from same locality (Mrima Hills) and seem to appear in different groups based on the metabolites present.

The chemosystematics of the five groups of populations of *D. viscosa* resulted to a dendogram composed of 3 clusters (cluster A composed of Machakos and Nanyuki, cluster B composed of the coastal and cluster C composed of Nairobi and Narok populations). The coastal population was closely related to Machakos and Nanyuki populations. Nairobi and Narok populations are attached on the same root from the ancestor showing that they are closely

related. The two populations (Nairobi and Narok populations) seem to arise from a different lineage from that of Nanyuki, Machakos and coastal. This is the first report on classification of different *D. viscosa* populations.

Reports on antimicrobial activity of the crude extracts showed that root bark extracts were more active followed by leaf and finally stem bark extracts of *Zanthoxylum* species. *Z. paracanthum* root and stem barks were found to be more active compared to root and stem bark extracts of other species while *Z. usambarense* leaf extract was the most active compared to leaf extracts from other *Zanthoxylum* species. On the other hand, Nanyuki population recorded the highest antimicrobial activity overall followed by Coastal population while Homabay, Makueni and Machakos populations followed in that order. Leaf extract from Karura population was the least in terms of antimicrobial activityfollowed by Maimahiu and Marigat in that order.

The myristic acid **1** had no activity at all against all the tested microbes with MIC values of more than 1000µg/ml. Studies to investigate the antimicrobial activities of saturated fatty acids and fatty amines against MRSA found that there was no activity of myristic acid against MRSA (Kitahara *et al*., 2004). On the other hand, myristic acid **1** showed antiproliferative activity to the cell lines tested. The CC_{50} value for E6 was 64.86 µg/ml while the IC_{50} values for HCC 1395 and DU 145 were 57.71 µg/ml and 80.24 µg/ml correspondingly. The selectivity index for HCC 1395 was 1.12 while that of DU 145 was 0.81. Myristic acid **1** was therefore less selective on the two cancer cell lines used in this study as their SI values were ≤ 2. Cytotoxicity of myristic acid on breast cancer cell lines was recorded elsewhere (Wongtangtintharn *et al*., 2004). Stigmasterol **2** had no activity on MRSA and *C. albicans* but showed moderate activity against the growth of *S. aureus* and *E. coli* with MIC values of 62.5 µg/ml and 15.625 µg/ml respectively. Elsewhere, a study on antimicrobial activity of stigmasterol **2** reported limited activity on *Streptococcus mutans* and *S. sobrinus* strains

(Laggoune et al., 2008; Taleb-Contini et al., 2003). On the other hand, stigmasterol **2** exhibited highest cytotoxicity on HCC1395 overall followed by DU 145 while VERO E6 was the least. The CC_{50} value for VERO E6 was 123.88 µg/ml while IC_{50} values for HCC 1395 and DU 145 were 0.42 µg/ml and 140.49 µg/ml respectively. The selectivity index for HCC 1395 was 294.94 hence stigmasterol was highly selective against HCC 1395 and less selective against DU 145 where the SI value was 0.88. This compound forms the best candidate for further research on control of breast cancer as it has a very low IC_{50} value against HCC1395 as well as a very high selectivity index (Frankfurt & Krishan, 2003).

Sesamin **3** had no activity against MRSA, *E. coli* and *C. albicans* but showed inhibition against the growth of *S. aureus* with a MIC value of 500 µg/ml. Elsewhere, studies on antimicrobial properties of sesamin **3** revealed little antibacterial activity (Zhou *et al.*, 2004). Additionally, sesamin failed to inhibit the growth of MRSA because it is a resistant strain of *S. aureus* (Enright *et al*., 2002). On the other hand, sesamin exhibited highest cytotoxicity on HCC1395 overall followed by DU 145 and least on VERO E6. This study is in agreement with reports where sesamin has been shown to act as a strong chemopreventative agent against mammary tumors in rats (Hirose *et al.*, 1992) and to suppress cell cycle progression and angiogenesis in different mammary cancer models (Lee *et al*., 2011; Yokota *et al.*, 2007).

The 8- acetonyldihydrochelerythrine **4** inhibited the growth of all the four microbes tested. These results conform to the results obtained from same compound isolated from *Zanthoxylum rhetsa* roots which exhibited strong activity against MRSA and moderate activity against *E. coli* (Tantapakul *et al*., 2012). Results on antiproliferative activity showed that 8-acetonyldihydrochelerythrine **4** exhibited highest cytotoxicity on HCC 1395 overall followed by VERO E6 and DU 145 was the least. This is the first report on antiproliferative activity of 8-acetonyldihydrochelerythrine. However, reports on antiproliferative activity of

phenanthridine compounds shows that the compounds exhibit significant antitumour activity in cancer cells (Lamoral-Theys *et al*., 2009; Tsukamoto *et al*., 2011).

Arnottianamide **5** did not inhibit the growth of all the microbes tested resulting to MIC values of each microbe to be more than 1000 µg/ml. Other studies elsewhere support this report where arnottianamide **5** failed to exhibit ny antimicrobial activity against the microbes tested (Queiroz *et al*., 2006; Cabral *et al.*, 2015). The antiproliferative activity was highest on VERO E6. There was also evidence of moderate antiproliferative activity of this compound against both HCC 1395 and DU 145 with no specificity. The lack of specificity of this compound in different cancer cells has been reported (Chang *et al*., 2003; Sreelekha *et al*., 2014).

The 10-Methoxycanthin-6-one **6** showed significant antimicrobial activity against all the tested microbes with no significant difference compared to omacilin ($P \geq 0.05$) against *E. coli*. Elsewhere, this compound exhibited best inhibitory activity against *Bacillus cereus, Bacillus subtilis, Ralstonia solanacearum*, and *Pseudomonas syringae* and *Fusarium graminearum* (Zhao et al., 2016) with structure–activity relationship (SAR) showing the high antimicrobial activity was conferred by the aliphatic ester derivatives (Dai et al., 2018). The 10-Methoxycanthin-6-one **6** recorded moderate activity against VERO E6 and antiproliferative activity against HCC 1395, and high antiproliferative activity against DU 145. 10-methoxycanthin-6-one was highly selective on the two cancer cell lines with SI values for HCC 1395 as 3.67 while that of of DU 145 was 15.09. This is the first report on antiproliferative activity of 10-methoxycanthin-6-one.

Canthin-6-one **7** showed high antimicrobial activity of all the tested microbes with no significant difference compared to positive controls ($P \geq 0.05$) for all the microbes. Soriano-Agatón et al. (2005) showed the antifungal activity of canthin-6-one on pathogenic fungi however, the structure-activity relationship (SAR) for the antifungal activity was unclear. Based IC_{50}/CC_{50}values, canthin-6-one **7** was found to have a moderate activity against VERO

E6 vero cell line and antiproliferative activity against both HCC 1395 and DU 145. The SI values indicated that the compound was highly selective against both HCC 1395 and DU 145. Dai et al. (2016) reported that canthin-6-one has a broad anticancer activity which has made the compound be used as an anticancer agent (Murakami *et al*., 2004).

8-Oxochelerythrine **8** showed significant antimicrobial activity against all the tested microbes. Reports on studies of antimicrobial activity of 8-oxochelerythrine which substantiates this study have been recorded elsewhere. 8-Oxochelerythrine **8** was found to have very interesting inhibition against *Clostridium sporogenes* and *Streptococcus pyogenes* (Cesari et al., 2015). The compound was found to be toxic against HCC 1395 and moderately toxic against DU 145. 8-Oxochelerythrine **8** was found to be highly selective against HCC 1395. This is the first report on antiproliferative activity of 8-oxochelerythrine.

Dodonic acid **9** exhibited less activity against *S. aureus* and *E. coli* with no inhibition activity against the growth of MRSA and *C. albicans* in all the tested concentrations. This study is substantiated by (Omosa *et al*., 2014) who found the compound to be less active against *E. coli, S. aureus* and *B. pumilus* and *S. cerevisiae*. Dodonic acid **9** showed antiproliferative activity against all the three tested cell lines. The SI values showed that the compound was less selective on the two cancer cell lines tested. The inability of hautriwaic acid to select the cancer cells has been reported elsewhere (Bajaj *et al*., 1986; Jolad *et al*., 1982).

The hautriwaic acid lactone **11** exhibited antimicrobial activity against all the tested microbes with no significant difference between the inhibition of *S. aureus* and *E. coli* at 1000µg/ml with that of omacilin at a similar concentration ($P \geq 0.05$). The report of antimicrobial activity of hautriwaic acid lactone **11** is in agreement with (Omosa *et al*., 2014) who found this compound had a good antifungal activity against *S. cerevisiae*. This compound was more selective against HCC 1395 as compared to DU 145. This is the first report on antiproliferative activity of hautriwaic acid lactone **11**.

It was found that catechin **12** exhibited antimicrobial activity against all the tested microbes with no significant difference at 1000µg/ml in the inhibition of MRSA, *S. aureus* and *C. albicans* as compared to positive controls at similar concentration ($P \geq 0.05$). Findings from (Bais *et al.*, 2002; Veluri *et al.*, 2004; Muthuswamy and Rupasinghe, 2007) on antimicrobial activity of catechin **12** substantiate this report. The antiproliferative activity of catechin **12** indicated that it was less selective to the two cancer cell lines compared to the normal cells. Reports on cytotoxicity of catechin against cancer cells has been reported elsewhere (Manikandan *et al.*, 2012; Suganuma *et al.*, 2011).

Z. paracanthum showed antiproliferative activity against VERO E6 and HCC 1395 and moderate antiproliferative activity against DU 145. The extract was highly selective against HCC 1395 and less selective against DU 145. On the other hand, the leaf extract of *D. viscosa* collected from coast resulted to moderate antiproliferative activity against the three cell lines. There was less selectivity of this extract on both HCC 1395 and DU 145. Conversely, leaf extract of *D. viscosa* from Nanyuki was more selective on DU 145 compared to HCC 1395. Shafek et al. (2015) reported that *Dodonaea viscosa* possessed strong cytotoxic activity on breast carcinoma cell line.

8.2 GENERAL CONCLUSION

This study confirmed that there were differences in the types and concentrations of secondary metabolites produced within *Zanthoxylum* species and *D. viscosa* populations in Kenya and the the extracts and compounds isolated from *Zanthoxylum* species and *D. viscosa* varieties had antimicrobial and antiproliferative activities. Analysis of relatedness of five *Zanthoxylum* species resulted in two clusters which conformed to the current morphological classification of *Zanthoxylum* species. Conversely, this study did not agree with the current classification of five *D. viscosa* populations because, Machakos, Nanyuki and coastal collections were closely related irrespective of the coastal population being represented by *D.*

viscosa subsp *viscosa* different from the two which were made of *D. viscosa* subsp *angustifolia*. The Narok and Nairobi populations were also closely related and different from Machakos and Nanyuki collection which are all composed of *D. viscosa* subsp *angustifolia*. This study has also generated chemical profiles (diversity, variation and abundance) of compounds occuring in the Kenyan species of *Zanthoxylum* and *D. viscosa* populations that may provide an alternative criterion to help increase the taxonomic delineation leading to better inter- and intra-specific resolution in the two groups of plants.

Antimicrobial analysis showed that root bark extracts were the most active followed by leaf and finally stem bark extracts in *Zanthoxylum* species. The *Z. paracanthum* root and stem barks were more active than the corresponding parts of the other species while *Z. usambarense* leaf extract was the most active of all leaf extracts from *Zanthoxylum* species. This could be attributed to high concentration of chemicals such as canthin-6-one, 8-oxochelerythrine and 10-methoxycathin-6-one in the root bark extracts.

In regard to *D. viscosa* populations, Nanyuki population recorded the highest antimicrobial activity overall followed by coastal collection while Karura population exhibited the least antimicrobial activity against all the tested microbes. This may be attributed to the high concentration of the compounds catechin and hautriwaic acid lactone occurring in the Nanyuki and coastal populations respectively. Catechin and hautriwaic acid lactone isolated from Nanyuki and coastal popuations exhibited high antimicrobial activity against *S. aureus*, *E. coli*, MRSA and *C. albicans*. This therefore has established a scientific basis for the utilization of members of these genera in traditional medicine by linking the identified active compounds therein to their related pharmacological activities.

This work evaluated extracts and characterized chemicals which showed significant potency against cancer cell lines, with higher selectivity index compared to doxorubicin (a cancer chemotherapy drug). Stigmasterol, sesamin and root bark extract of *Z. paracathum* were

the most potent against human breast cancer cell line while canthin-6-one, 10-methoxycanthin-6-one and 8-oxochelerythrine were most active against both human breast and prostate cancer cell lines. The leaf extract of *D. viscosa* was active against human prostate cancer cell line. These extracts and compounds could lead to discovery of new drugs that can be used to fight the two types of cancer.

8.3. RECOMMENDATIONS

The scope for the chemosystematics study needs to be increased to factor in all *Zanthoxylum* species and *D. viscosa* populations in the world. Further omics studies (including genomics, transcriptonomics and proteonomics) are recommended to determine the genes and biochemical pathways responsible for the production of the chemical markers/discriminants found in this study as well as revision of the current classification of *D. viscosa*. Further more, other modern systematics techniques such as molecular phylogeny should be carried out on these groups of plants to determine the relationship amongst their members.

Additionally, in vivo studies involving different models of the most potent chemicals for both antimicrobial (canthin-6-one, methoxycanthin-6-one, catechin and hautriwaic acid lactone and anticancer (stigmasterol, sesamin, canthin 6-one and 10-methoxy canthin-6-one) assays should be studied. An increased scope to cover other microbes of economic importance and other cancer cell lines should be carried out to determine the potency of these samples across the board. This will lead to discovery of new drugs that will help counter the multiple drug resistance in the fight of microbial and cancer diseases.

Conservation of plants investigated in this study is recommended especially the *Zanthoxylum* species as they have been exploited widely to the verge extinction. This can be done through awareness creation on selective harvesting of these plants as well as domestication and development of mass propagation technologies of this genus.

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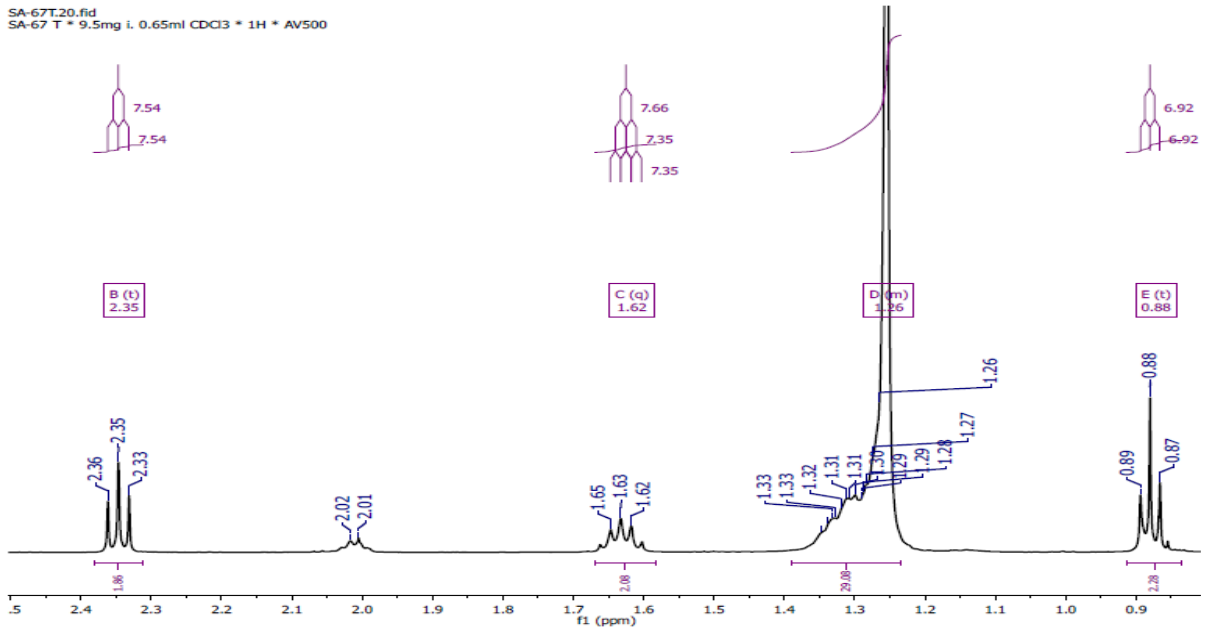
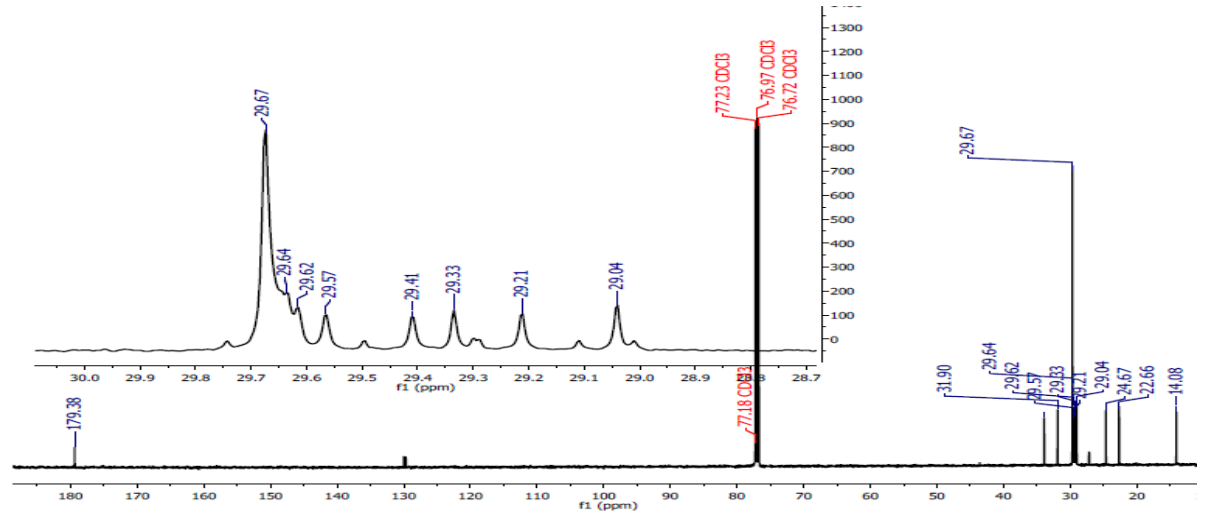
APPENDICES

Appendix 1; NMR and mass spectra for myristic acid (1)

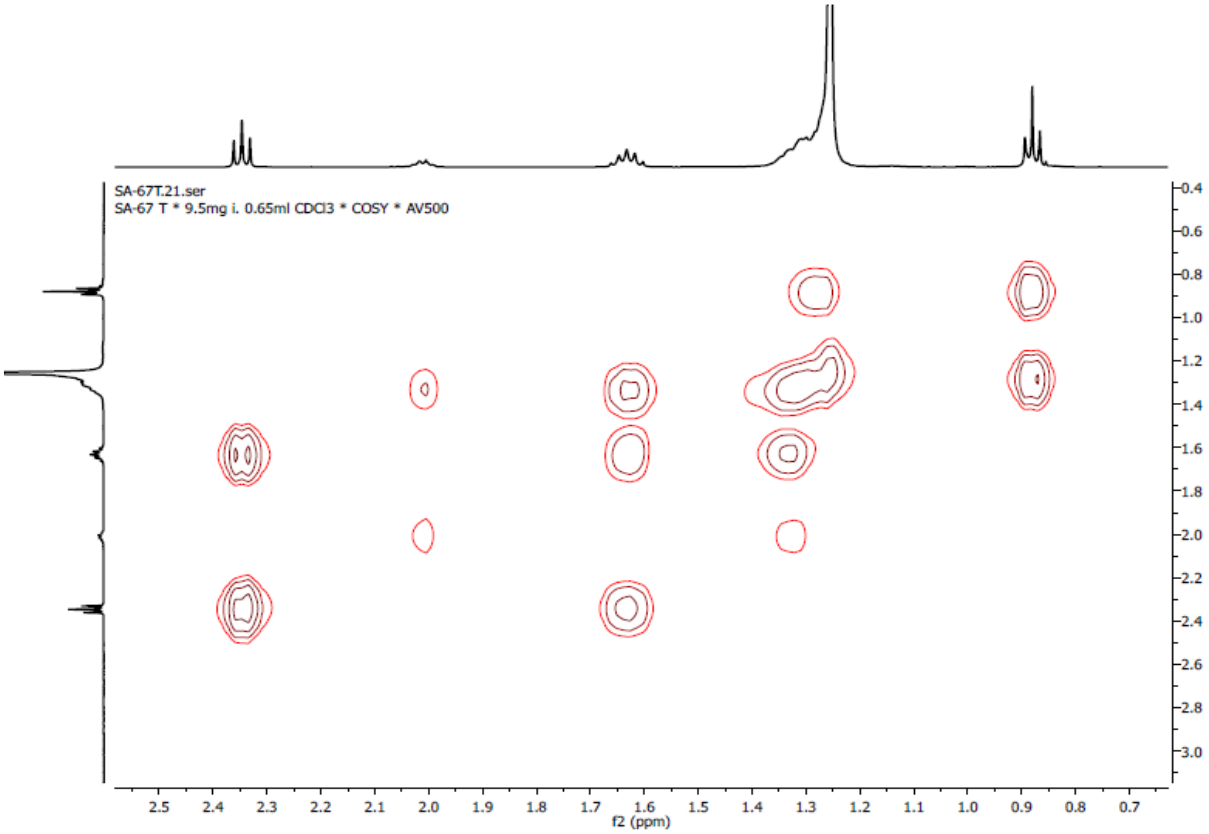
Appendix 1A. The ^{1}H NMR spectrum of myristic acid (**1**) observed at 500 MHz for $CDCl_3$ solution at 25 °C.



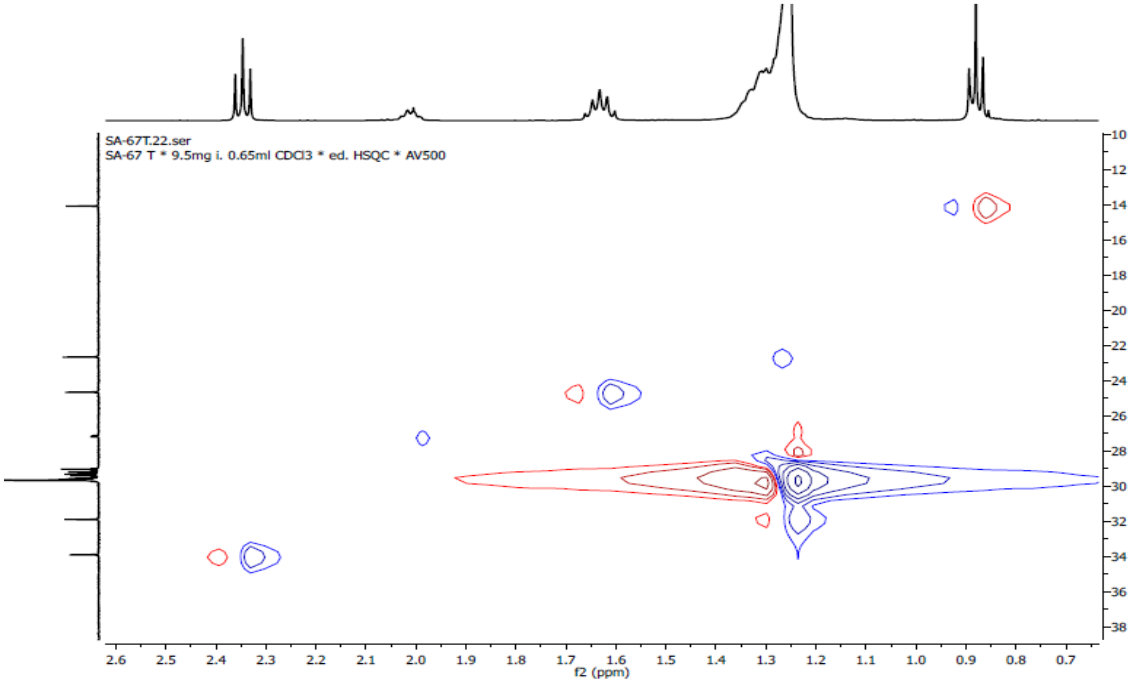
Appendix 1B. The ^{13}C NMR spectrum of myristic acid (**1**) observed at 125 MHz for $CDCl_3$ solution at 25 °C.



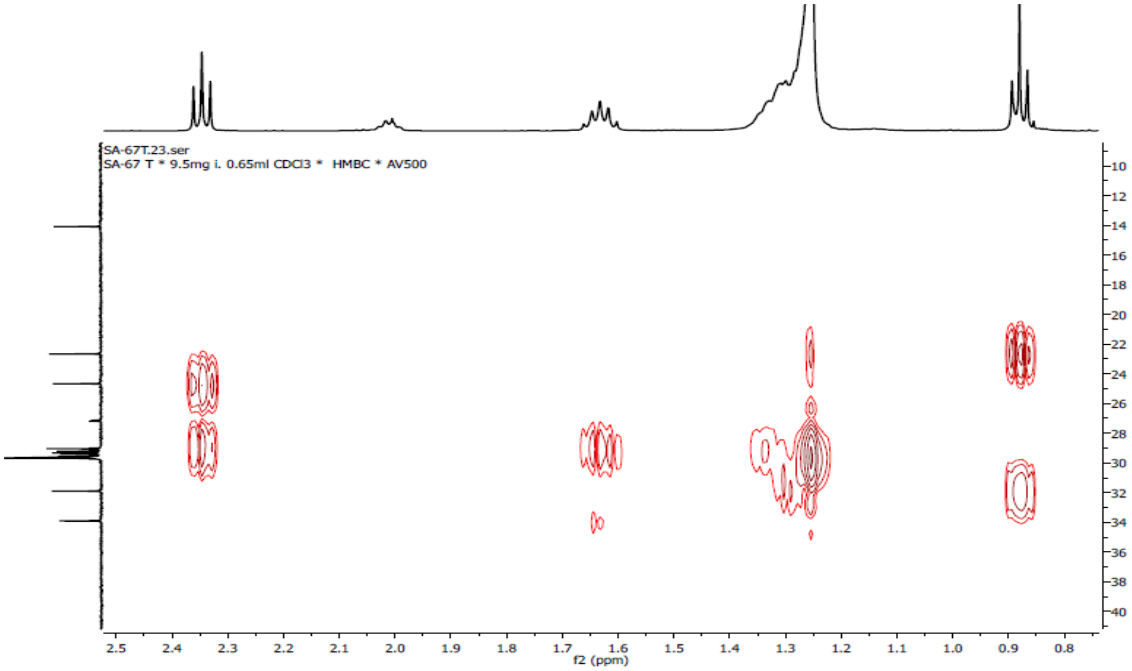
Appendix 1C. The ^{1}H-^{1}H COSY spectrum of myristic acid (**1**) observed at 500 MHz for $CDCl_3$ solution at 25 °C.



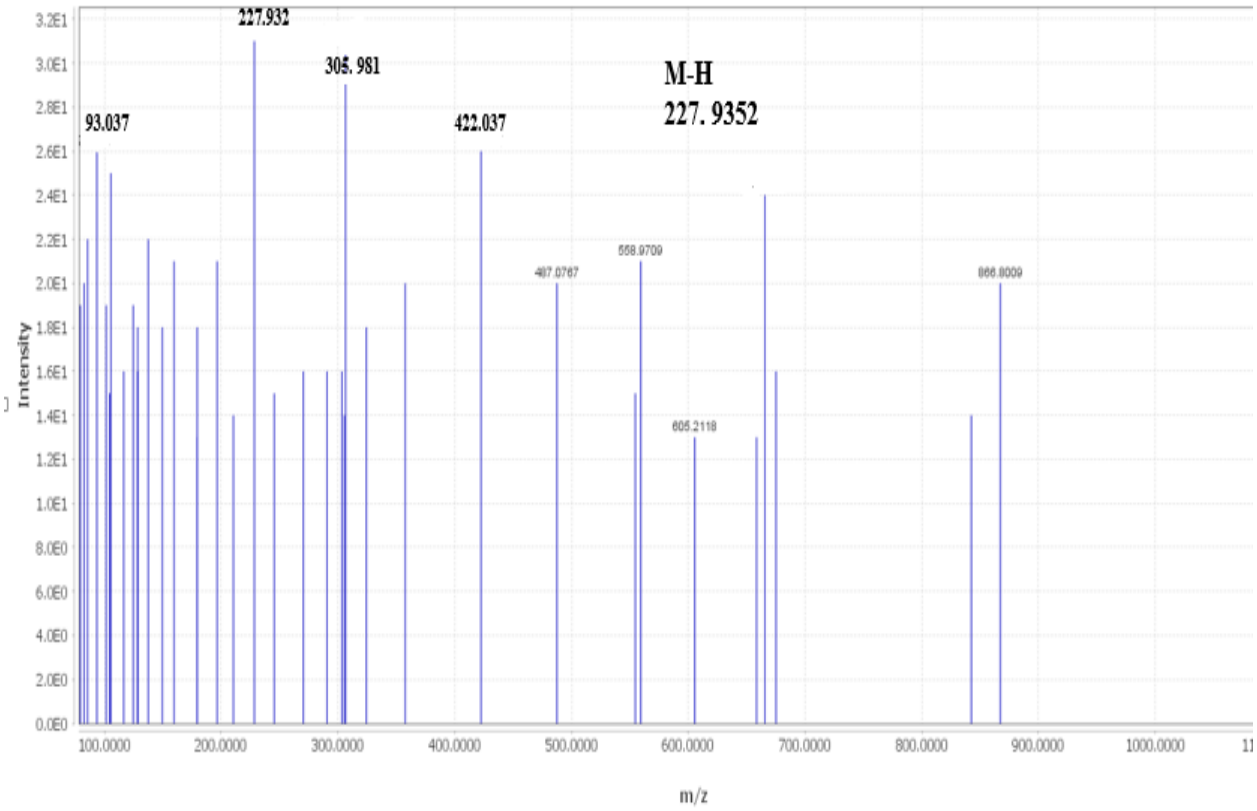
Appendix 1D. The ^{1}H-^{13}C HSQC NMR spectrum of myristic acid (**1**) observed at 500 and 125 MHz for $CDCl_3$ solution at 25 °C.



Appendix 1E. The ^{1}H-^{13}C HMBC NMR spectrum of myristic acid (**1**) observed at 500 and 125 MHz for $CDCl_3$ solution at 25 °C.

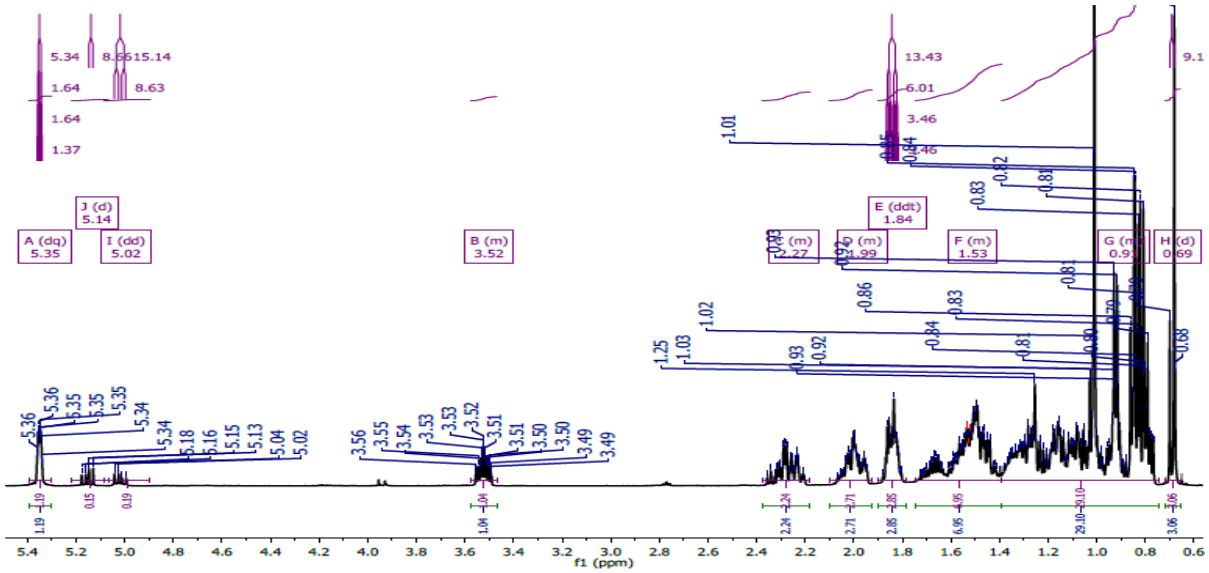


Appendix 1F. The ESIMS spectrum for myristic acid (**1**).

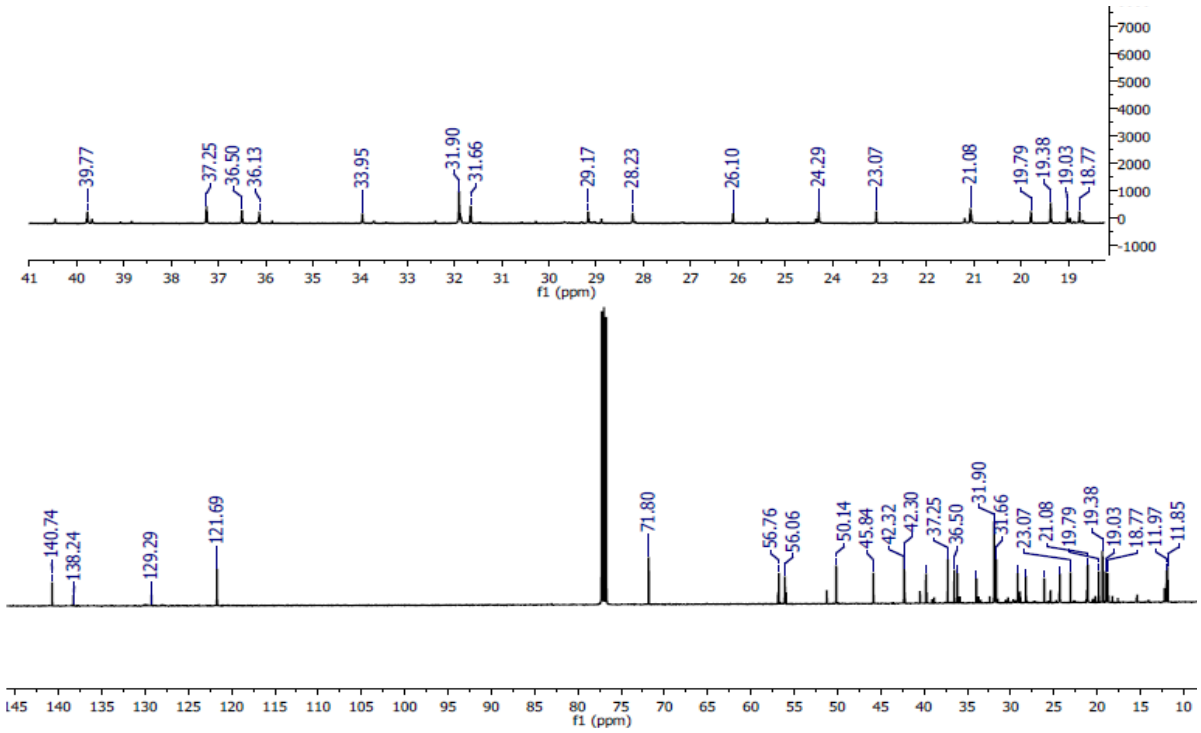


Appendix 2; NMR and mass spectra for stigmasterol (2)

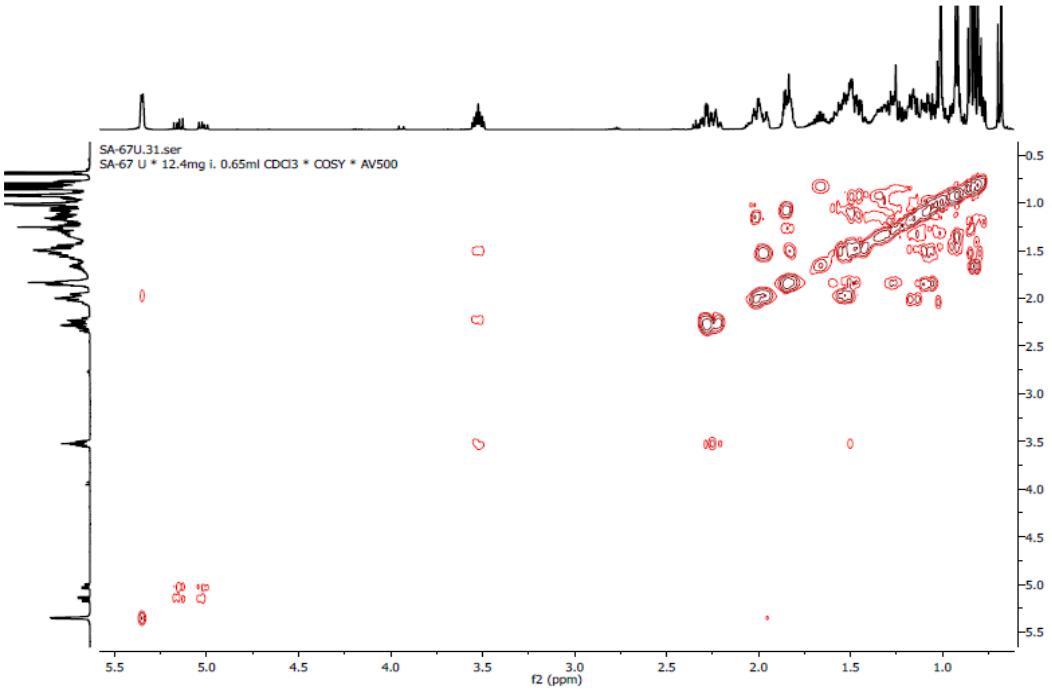
Appendix 2A. The ^{1}H NMR spectrum of stigmasterol (**2**) observed at 500 MHz for $CDCl_3$ solution at 25 °C.



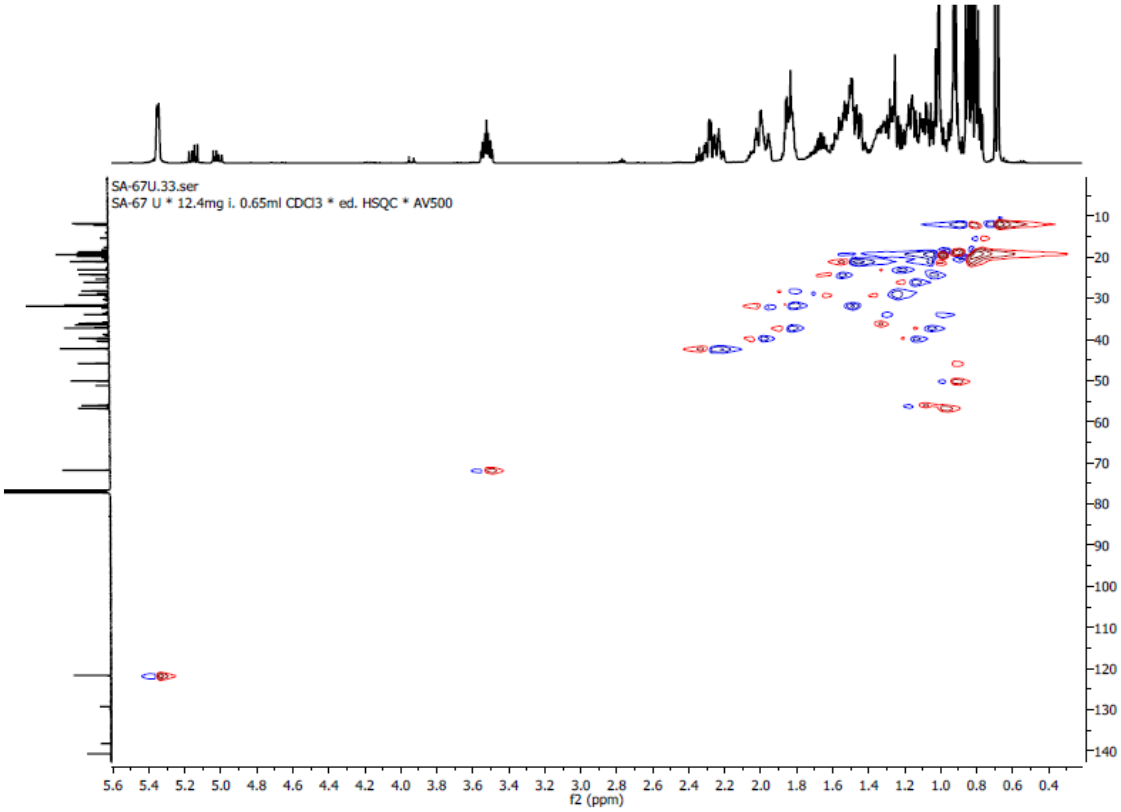
Appendix 2B. The ^{13}C NMR spectrum of stigmasterol (**2**) observed at 125 MHz for $CDCl_3$ solution at 25 °C.



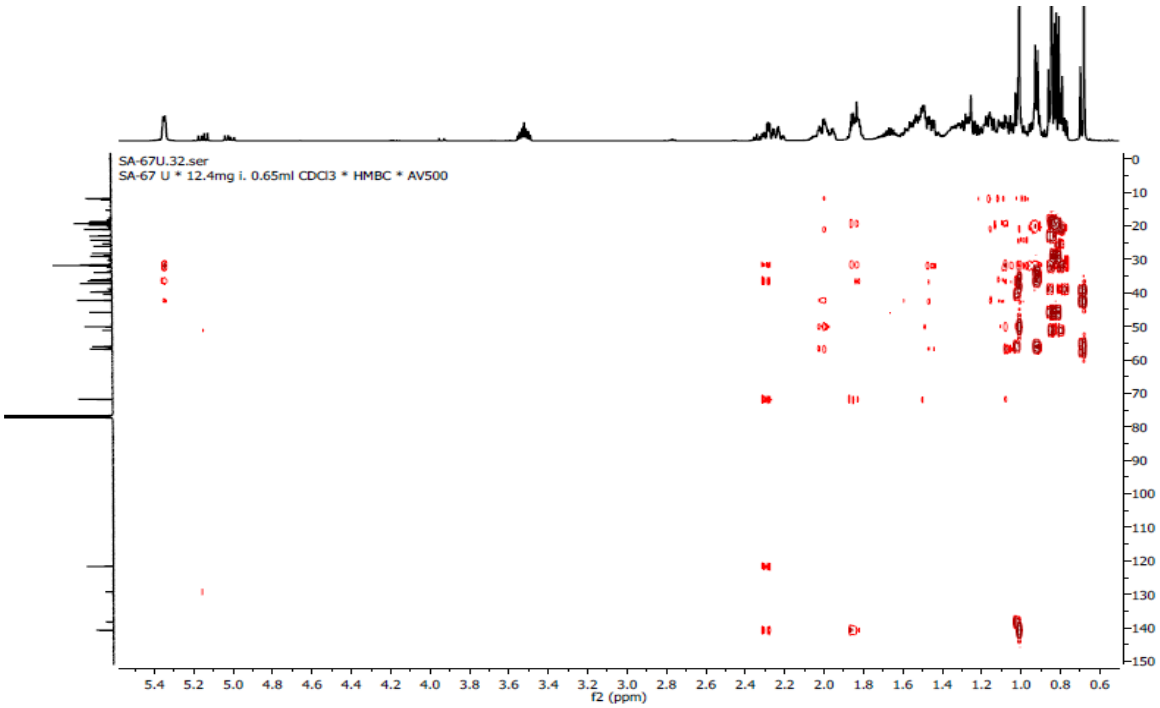
Appendix 2C. The ^{1}H-^{1}H COSY spectrum of stigmasterol (**2**) observed at 500 MHz for $CDCl_3$ solution at 25 °C.



Appendix 2D. The ^{1}H-^{13}C HSQC NMR spectrum of stigmasterol (**2**) observed at 500 and 125 MHz for $CDCl_3$ solution at 25 °C.

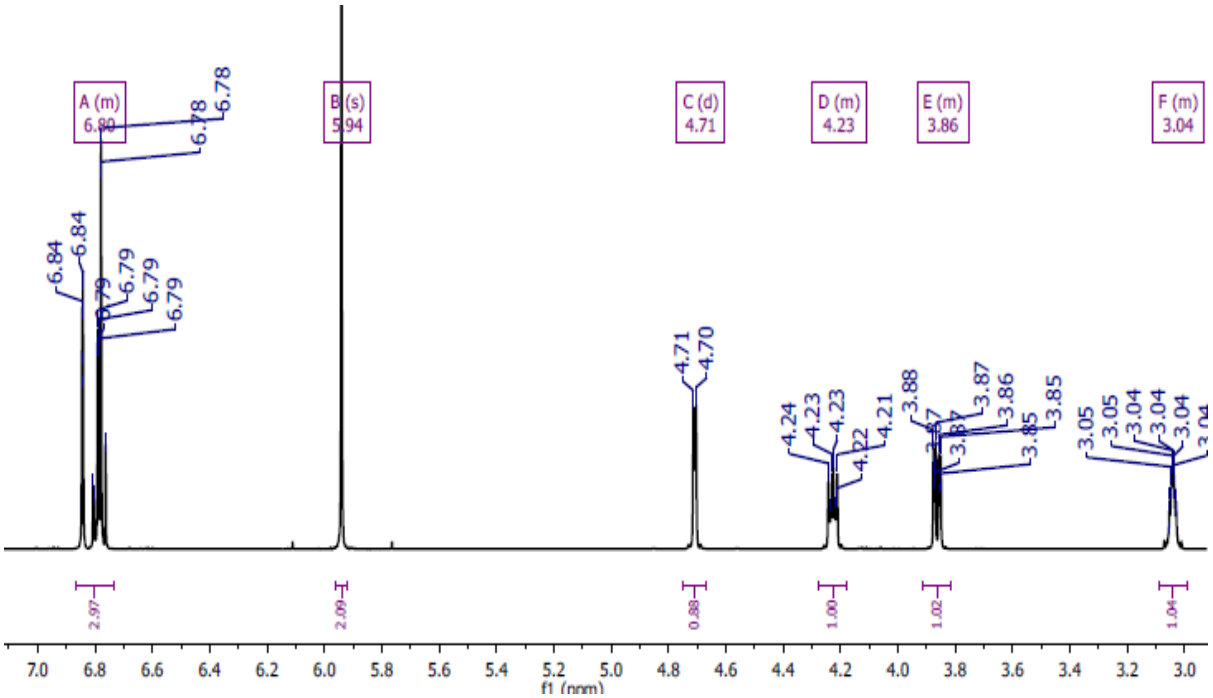


Appendix 2E. The ^{1}H-^{13}C HMBC NMR spectrum of stigmasterol (**2**) observed at 500 and 125 MHz for $CDCl_3$ solution at 25 °C. Assignment is given in Table S2.

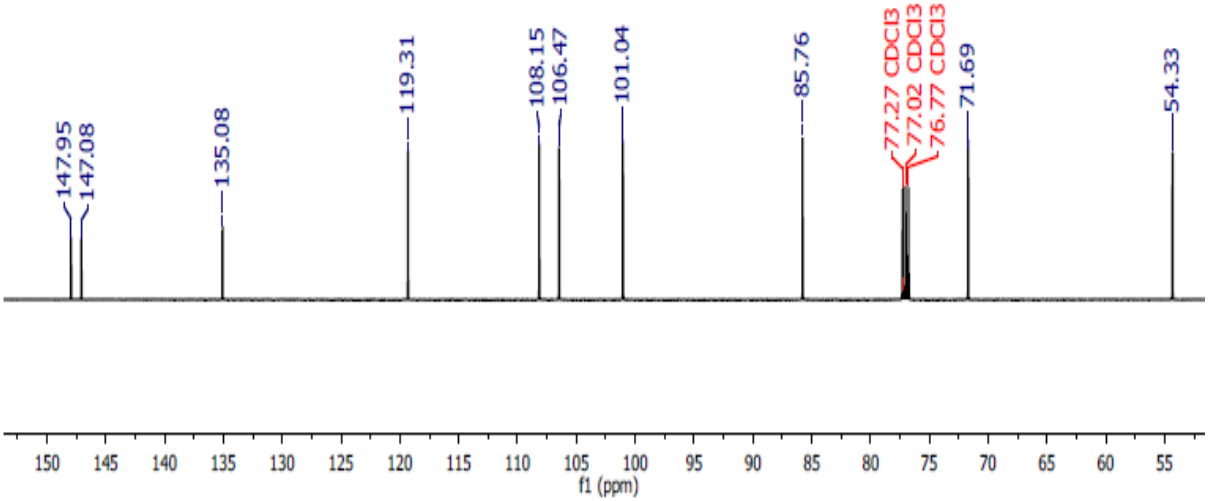


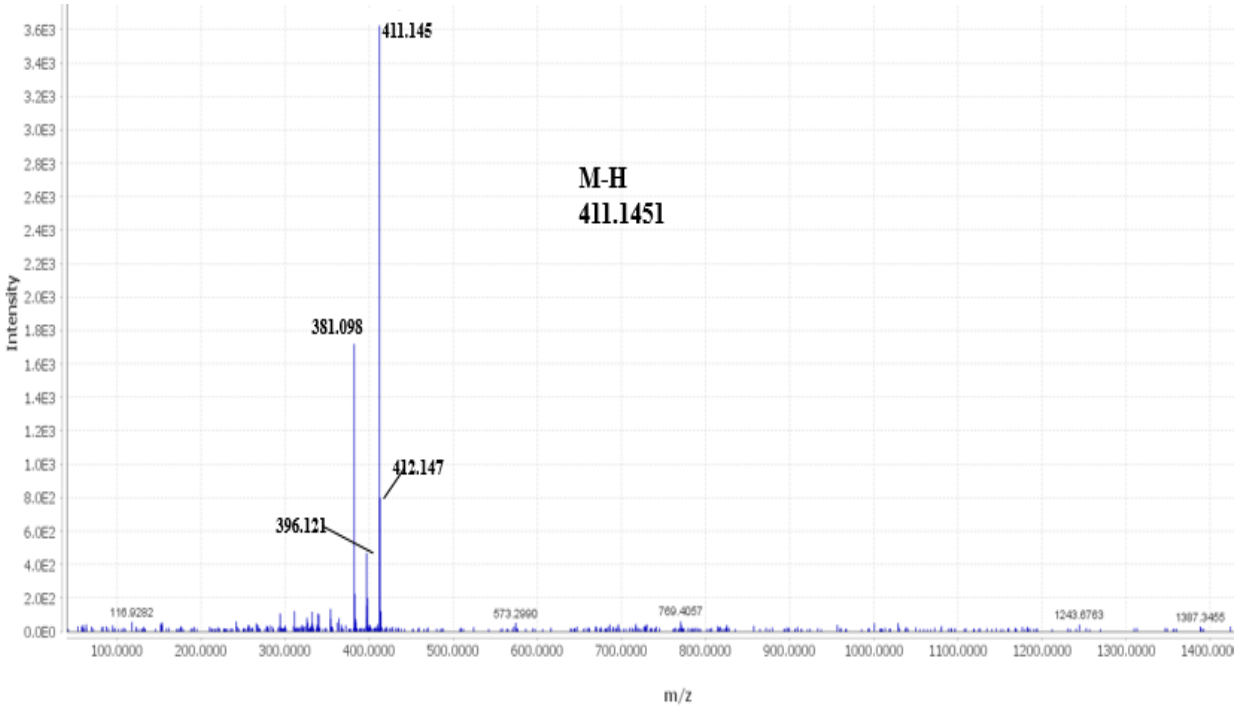
Appendix 3; NMR and mass spectra for sesamin (3)

Appendix 3A. The ^{1}H NMR spectrum of sesamin (**3**) observed at 500 MHz for $CDCl_3$ solution at 25 °C.

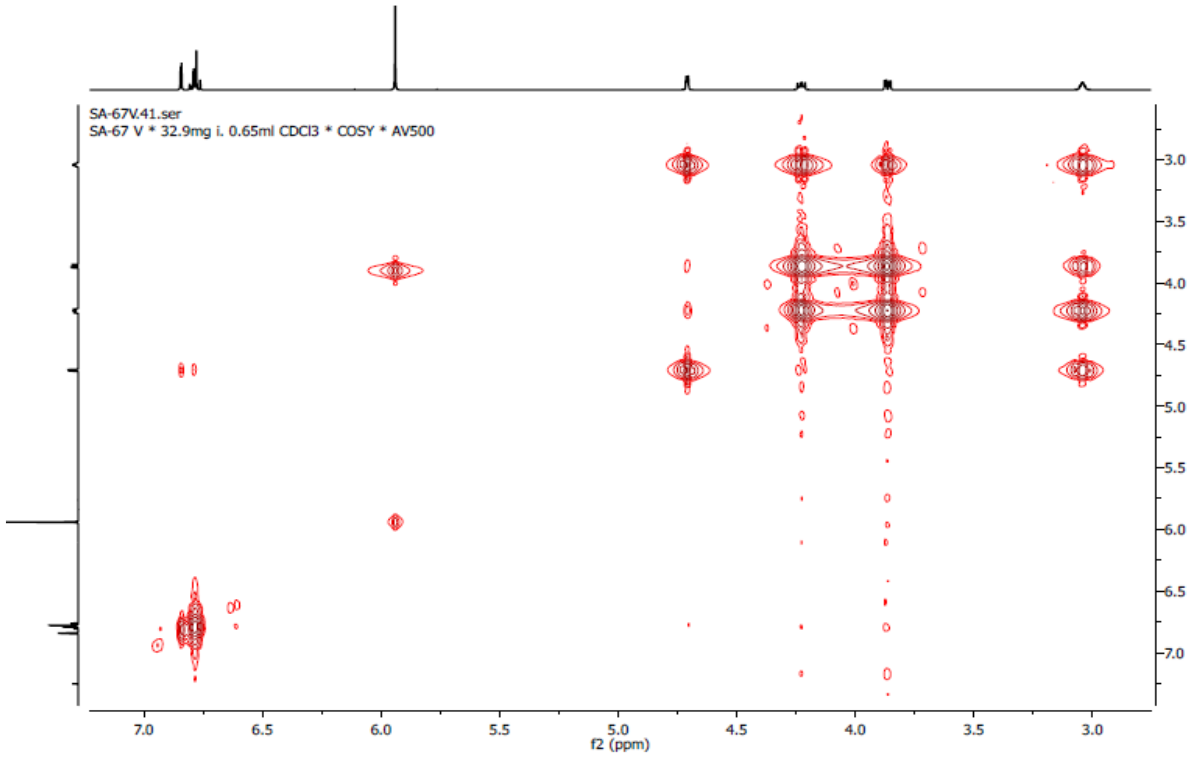


Appendix 3B. The ^{13}C NMR spectrum of sesamin (**3**) observed at 125 MHz for $CDCl_3$ solution at 25 °C.

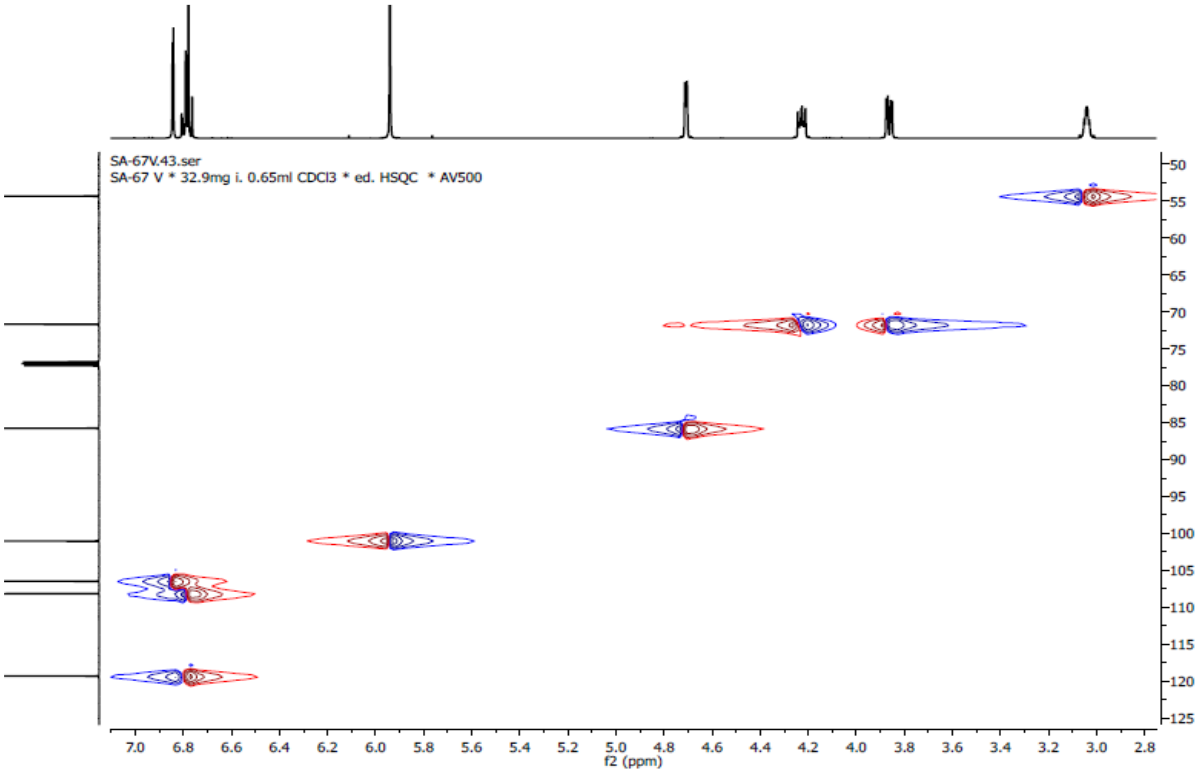




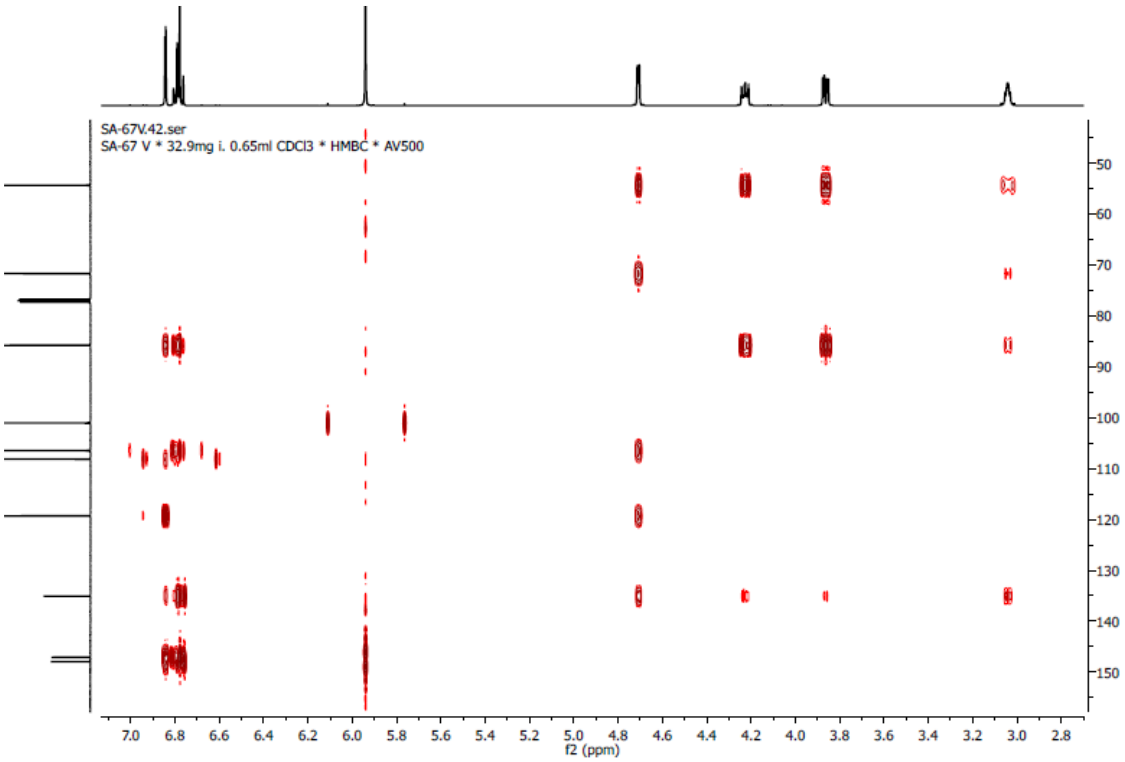
Appendix 3C. The ^{1}H-^{1}H COSY spectrum of sesamin (**3**) observed at 500 MHz for $CDCl_3$ solution at 25 °C.



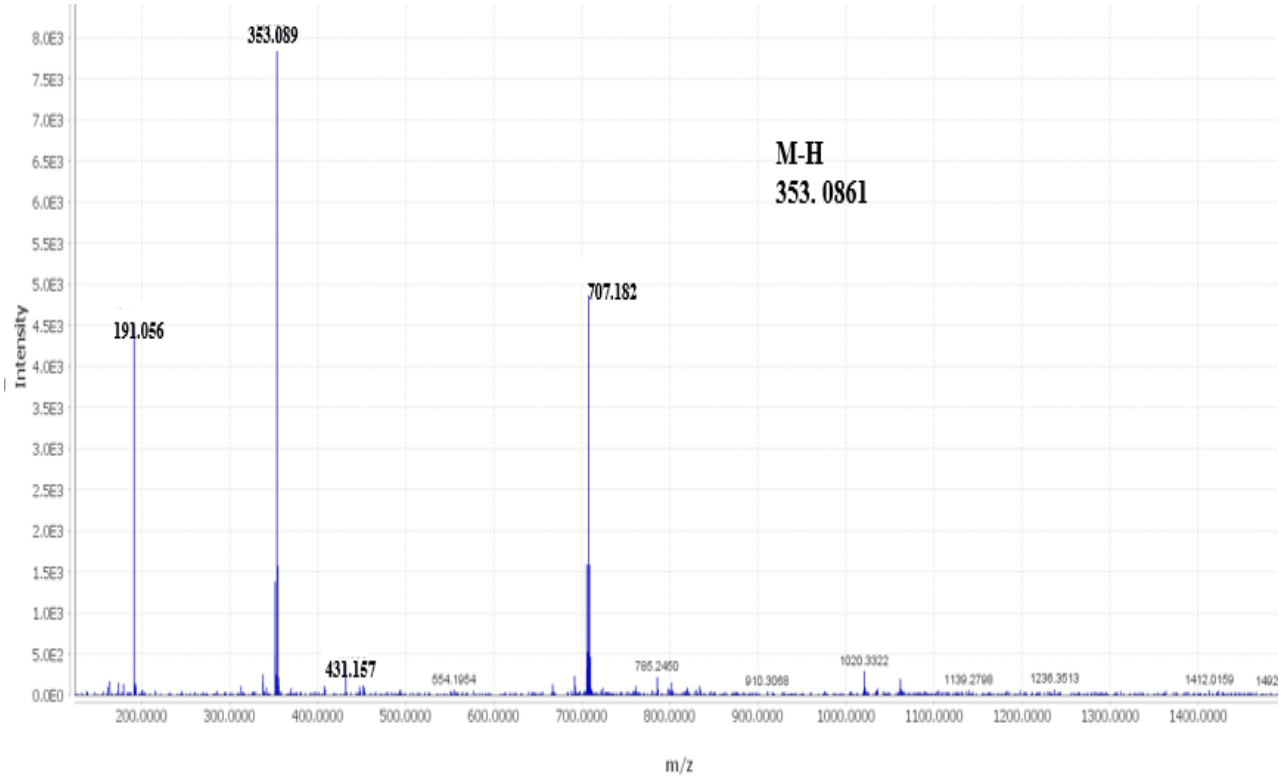
Appendix 3D. The ^{1}H-^{13}C HSQC NMR spectrum of sesamin (**3**) observed at 500 and 125 MHz for $CDCl_3$ solution at 25 °C.



Appendix 3E. The ^{1}H-^{13}C HMBC NMR spectrum of sesamin (**3**) observed at 500 and 125 MHz for $CDCl_3$ solution at 25 °C.

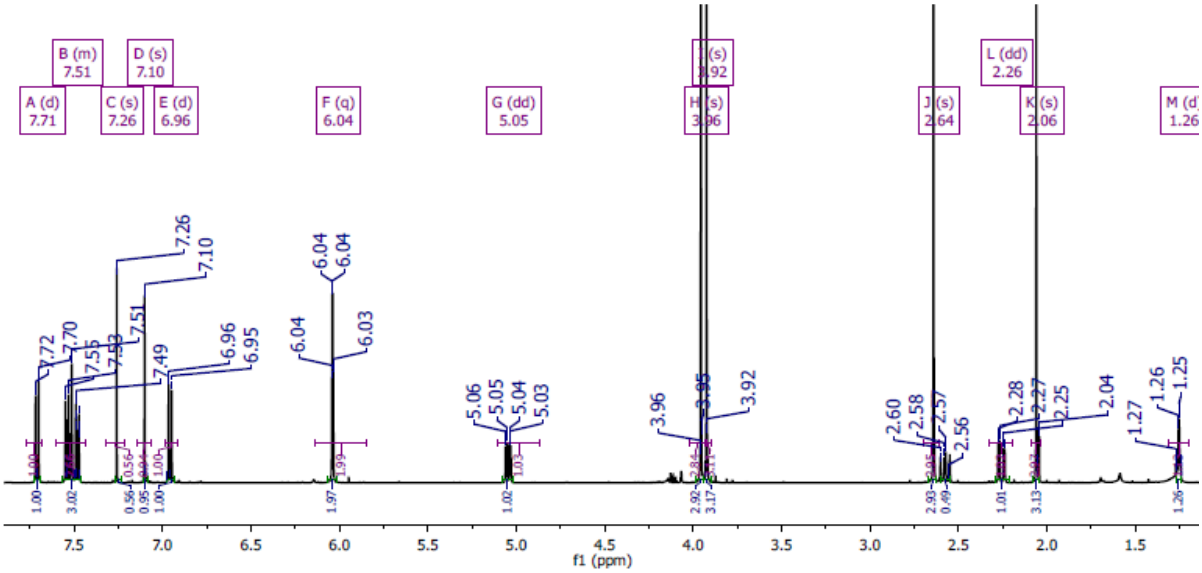


Appendix 3F. The ESIMS spectrum of sesamin (**3**).

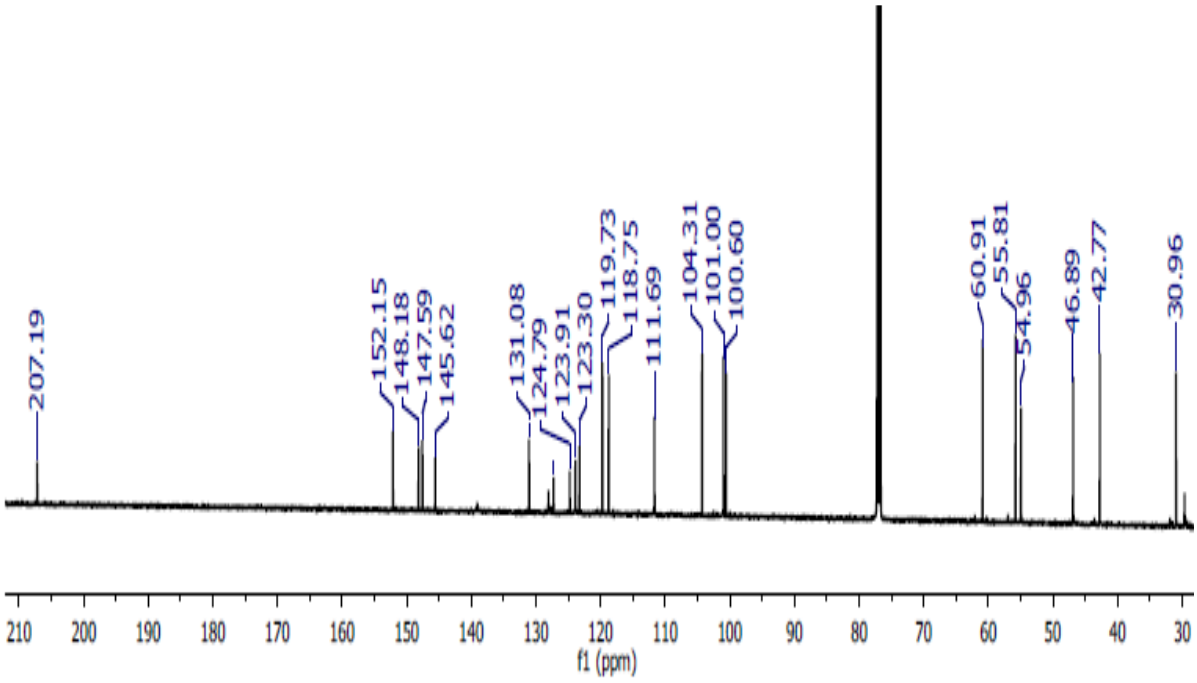


Appendix 4; NMR and mass spectra for 8-acetonylhydrochelerythrine (4)

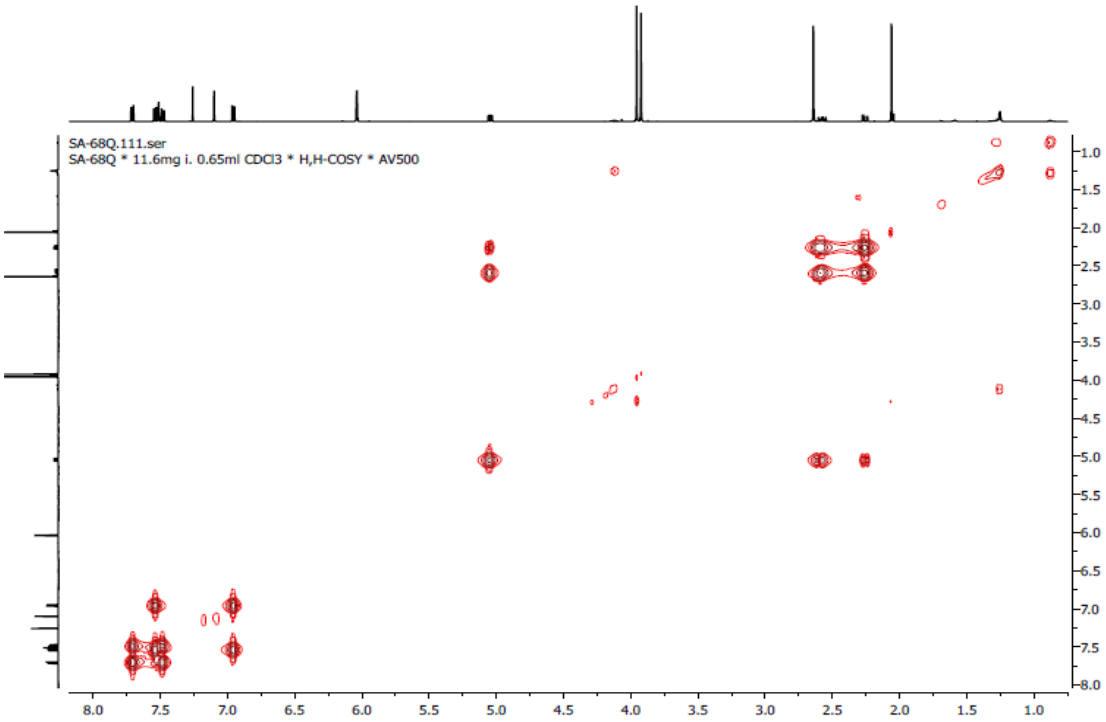
Appendix 4A. The ^{1}H NMR spectrum of 8-acetonyldihydrochelerythrine (**4**) observed at 500 MHz for $CDCl_3$ solution at 25 °C.



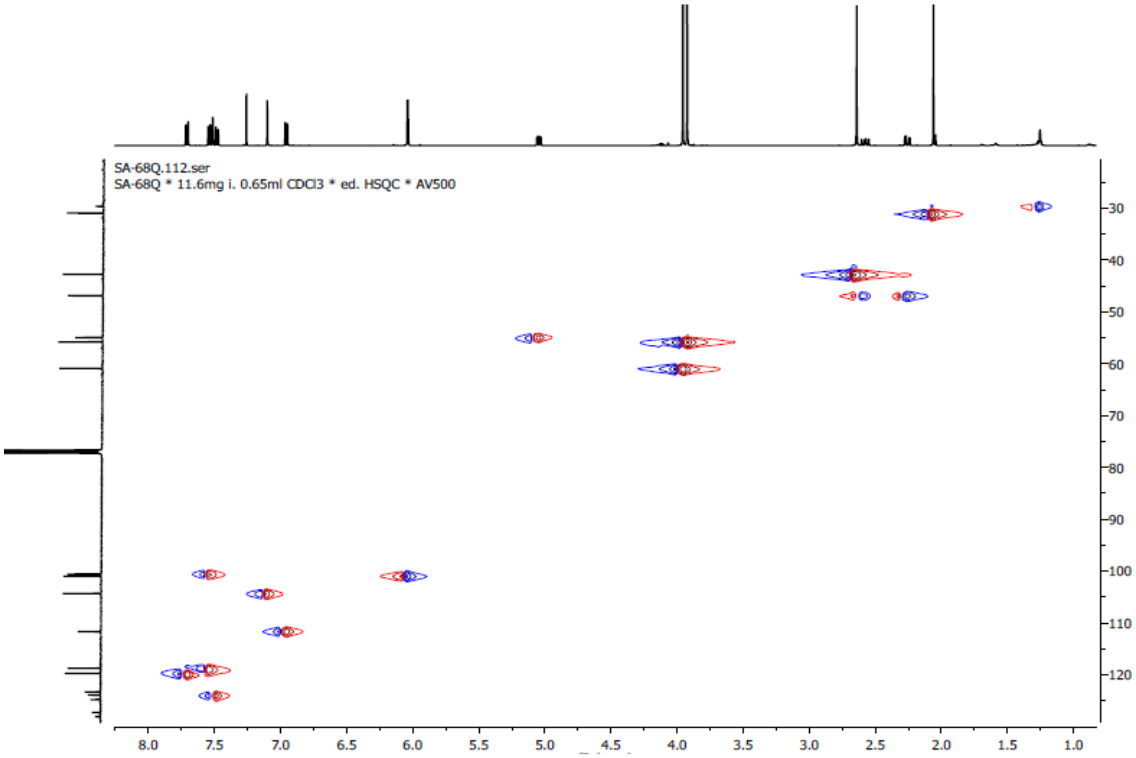
Appendix 4B. The ^{13}C NMR spectrum of 8-acetonyldihydrochelerythrine (**4**) observed at 125 MHz for $CDCl_3$ solution at 25 °C.



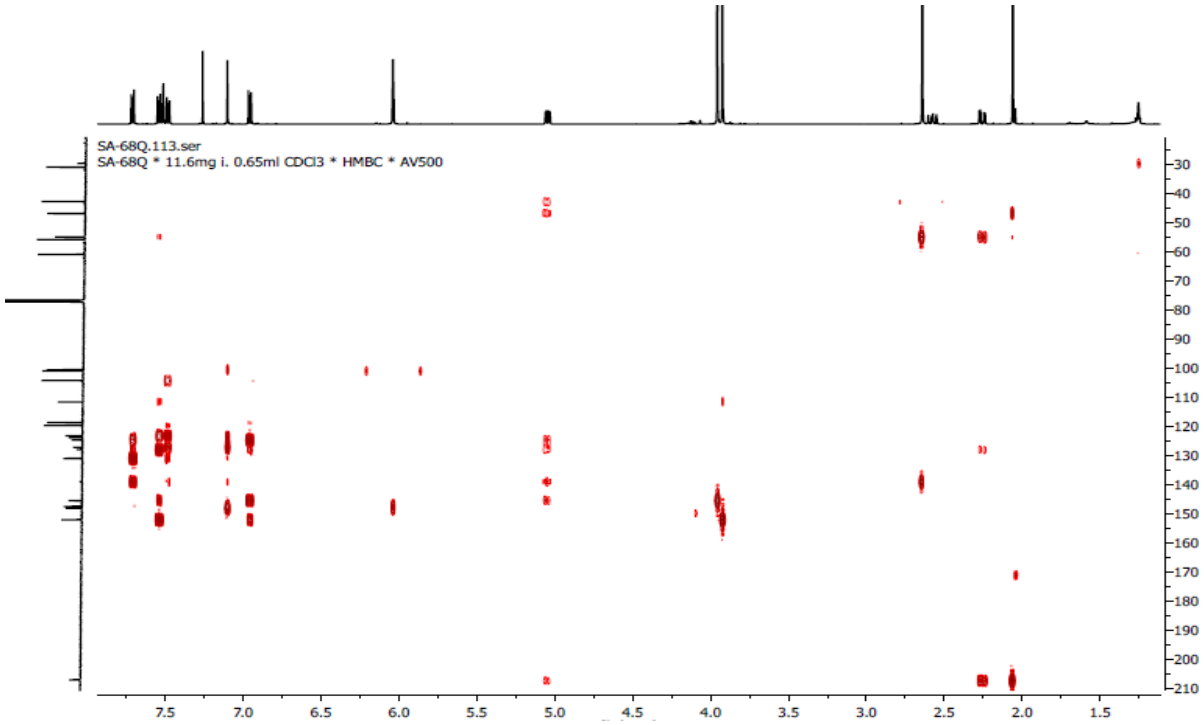
Appendix 4C. The ^{1}H-^{1}H COSY spectrum of 8-acetonyldihydrochelerythrine (**4**) observed at 500 MHz for $CDCl_3$ solution at 25 °C.



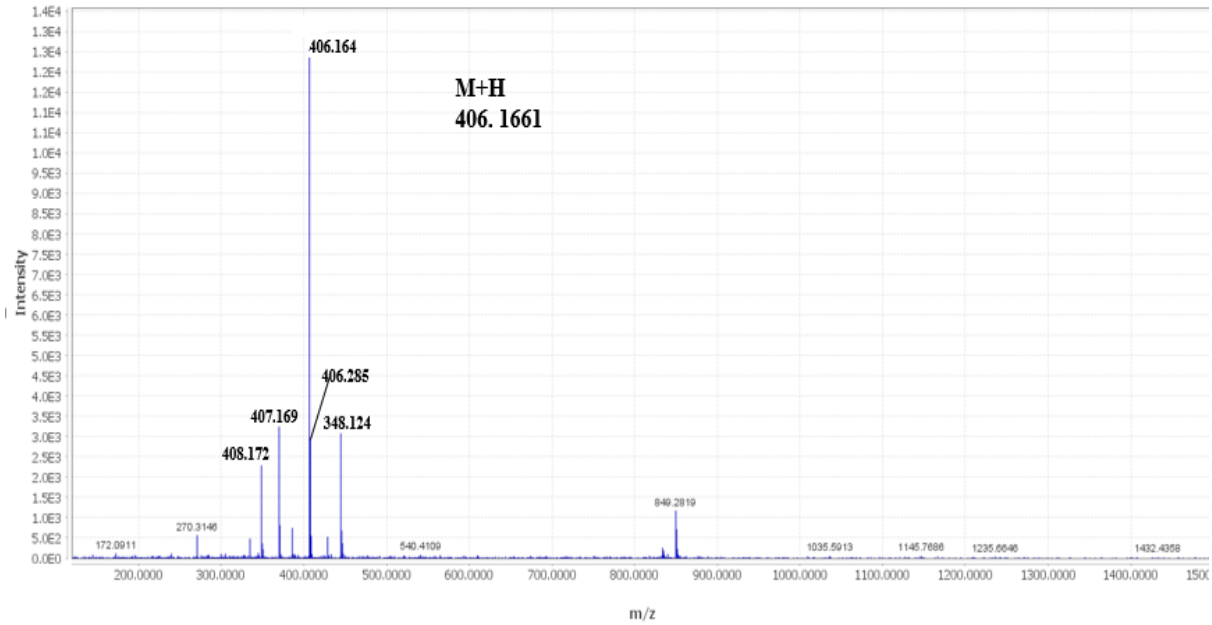
Appendix 4D. The ^{1}H-^{13}C HSQC NMR spectrum of 8-acetonyldihydrochelerythrine (**4**) observed at 500 and 125 MHz for $CDCl_3$ solution at 25 °C.



Appendix 4E. The ^{1}H-^{13}C HMBC NMR spectrum of 8-acetonyldihydrochelerythrine (**4**) observed at 500 and 125 MHz for $CDCl_3$ solution at 25 °C.

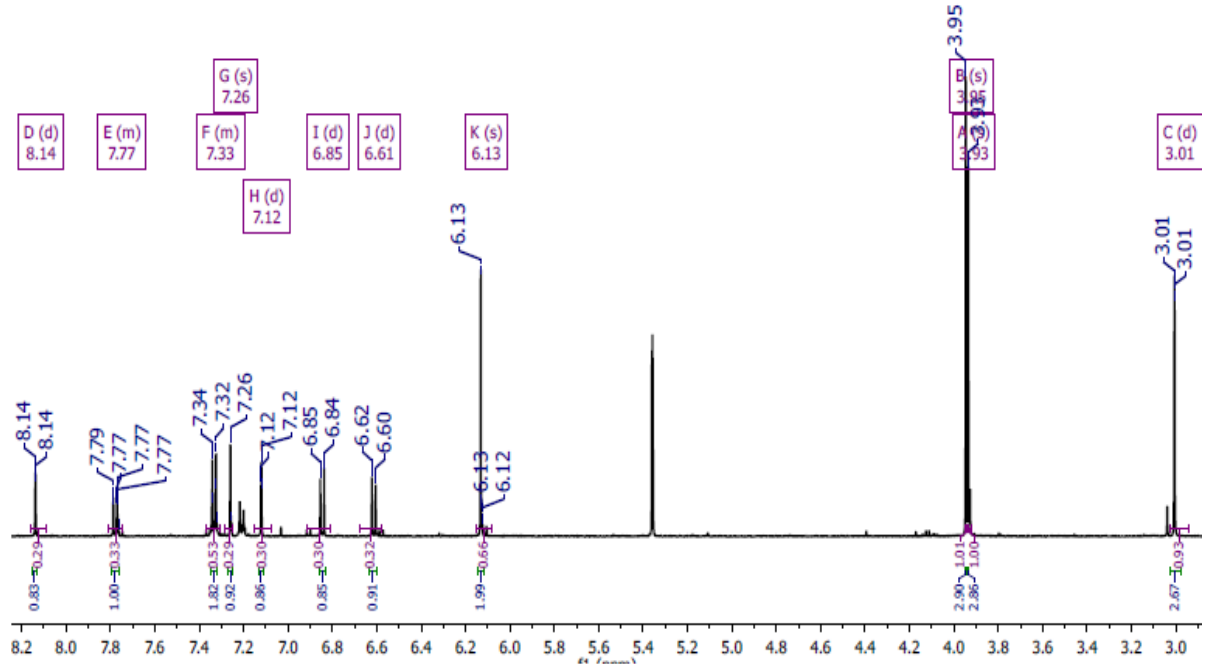


Appendix 4F. The ESIMS spectrum of 8-acetonyldihydrochelerythrine (**4**).

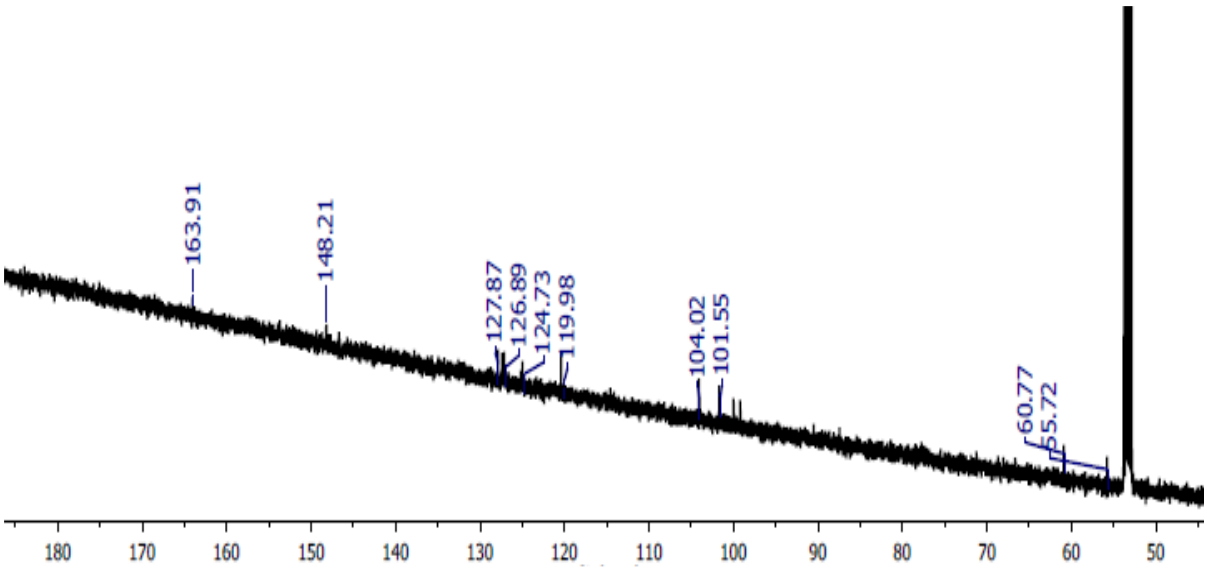


Appendix 5; NMR and mass spectra for arnottianamide (5)

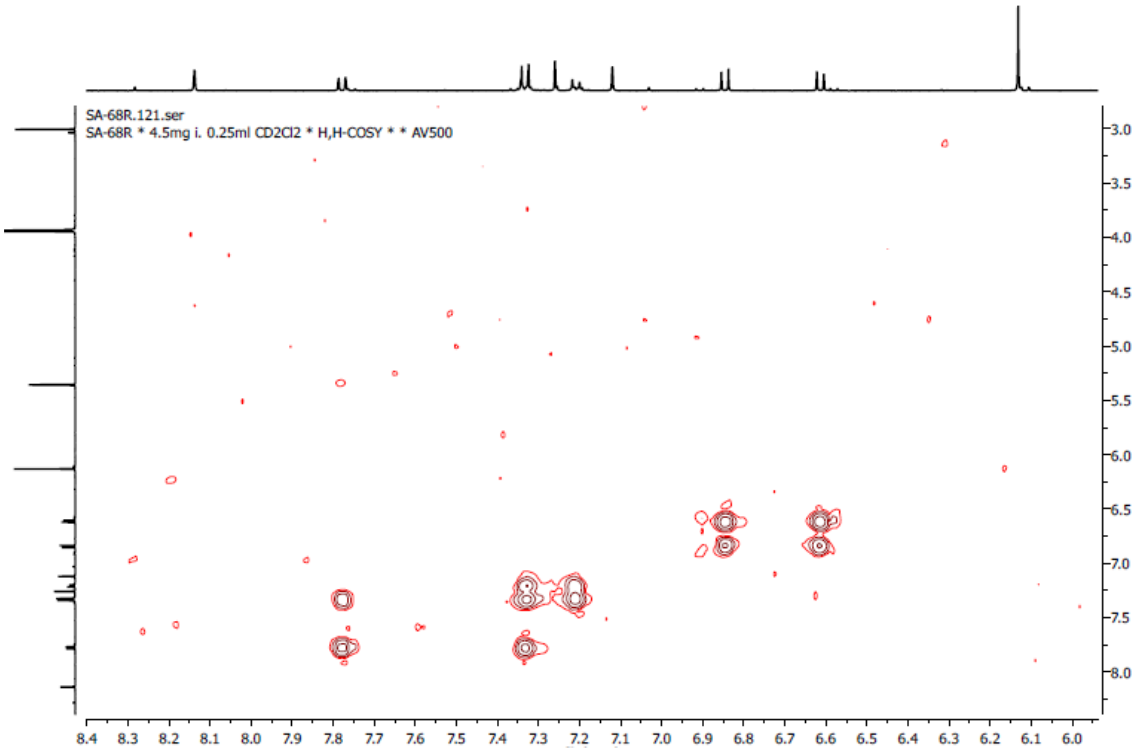
Appendix 5A. The ^{1}H NMR spectrum of arnottianamide (**5**) observed at 500 MHz for CD_2Cl_2 solution at 25 °C.



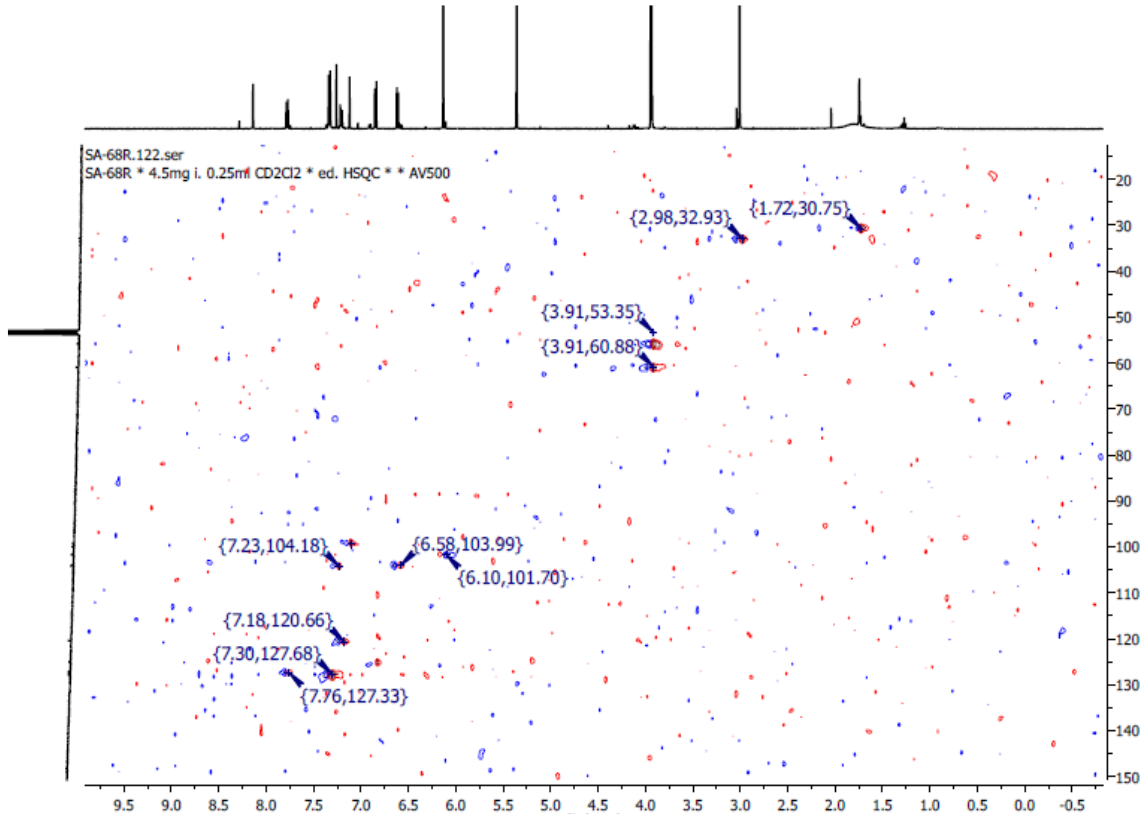
Appendix 5B. The ^{13}C NMR spectrum of arnottianamide (**5**) observed at 125 MHz for CD_2Cl_2 solution at 25 °C.



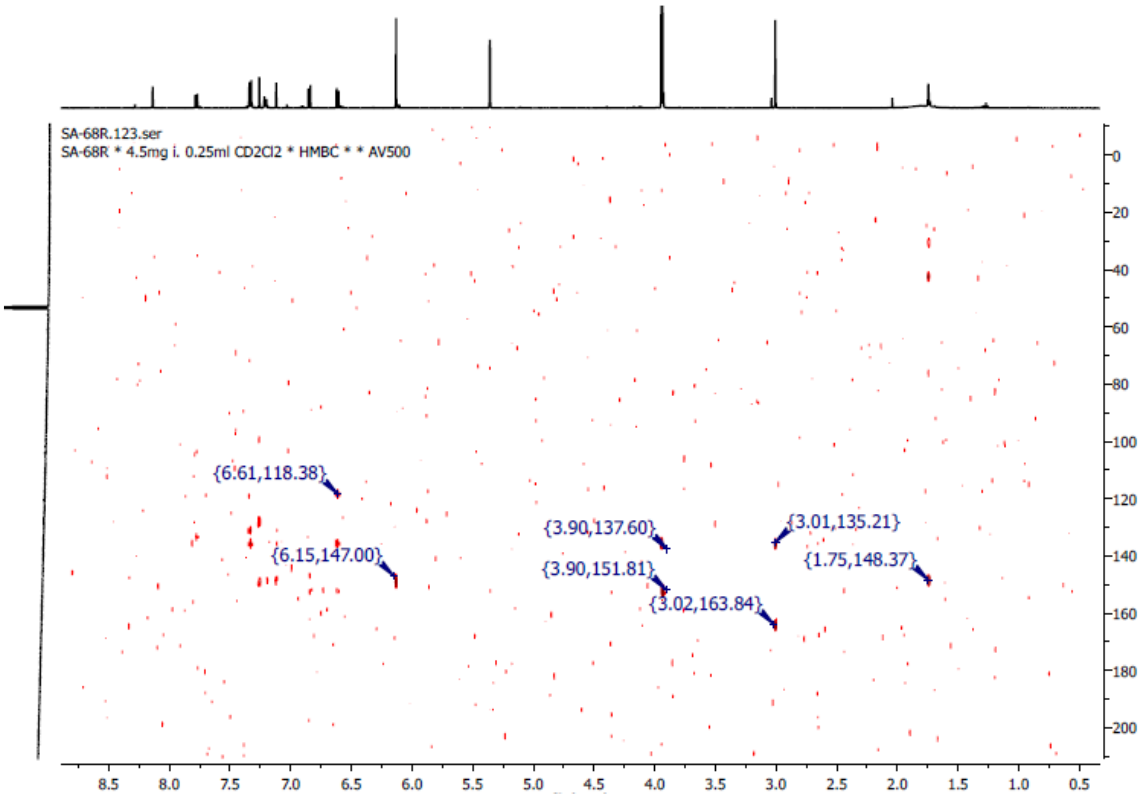
Appendix 5C. The ^{1}H-^{1}H COSY spectrum of arnottianamide (**5**) observed at 500 MHz for CD_2Cl_2 solution at 25 °C.



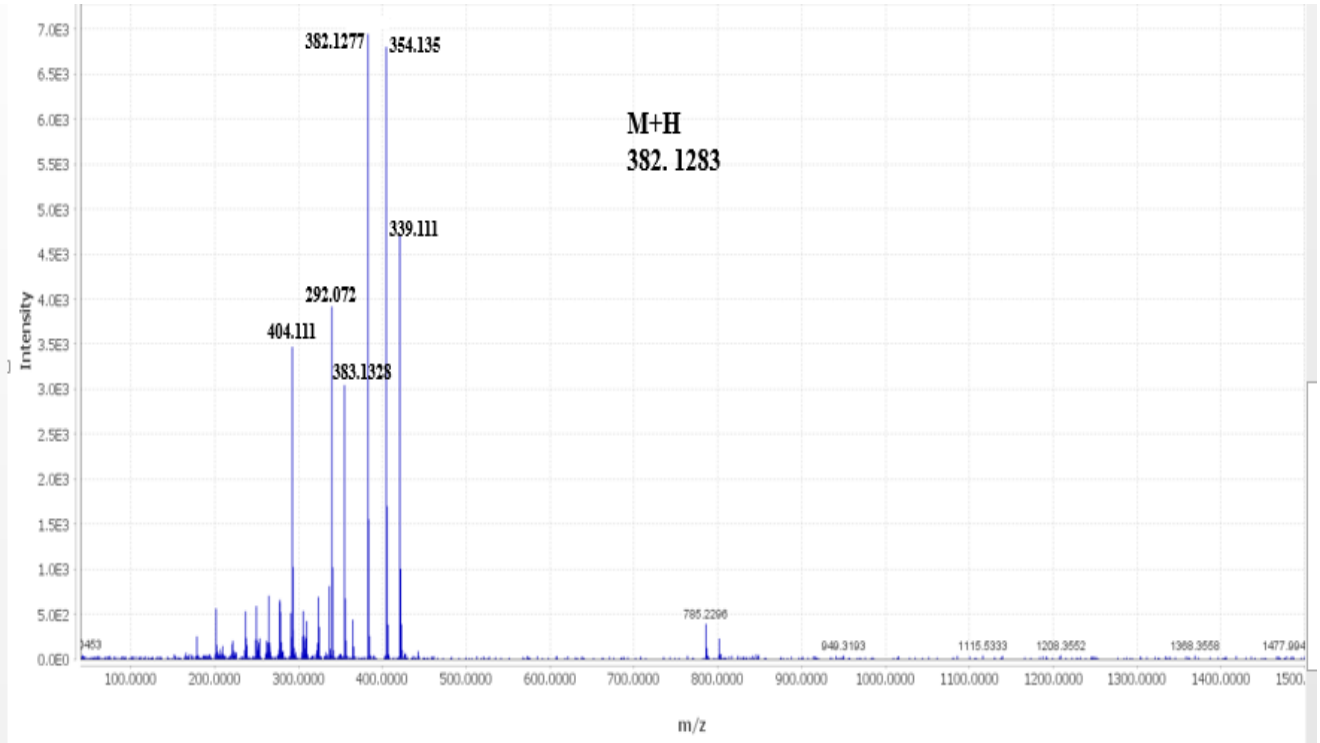
Appendix 5D. The ^{1}H-^{13}C HSQC NMR spectrum of arnottianamide (**5**) observed at 500 and 125 MHz for CD_2Cl_2 solution at 25 °C.



Appendix 5E. The ^{1}H-^{13}C HMBC NMR spectrum of arnottianamide (**5**) observed at 500 and 125 MHz for CD_2Cl_2 solution at 25 °C.

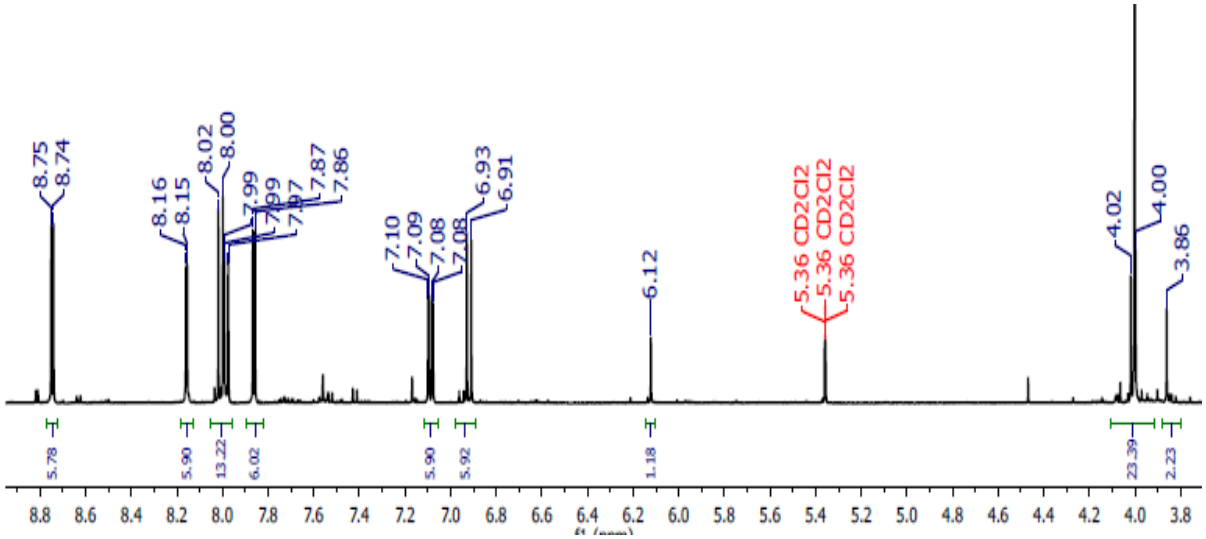


Appendix 5F. The ESIMS spectrum for arnottianamide (**5**).

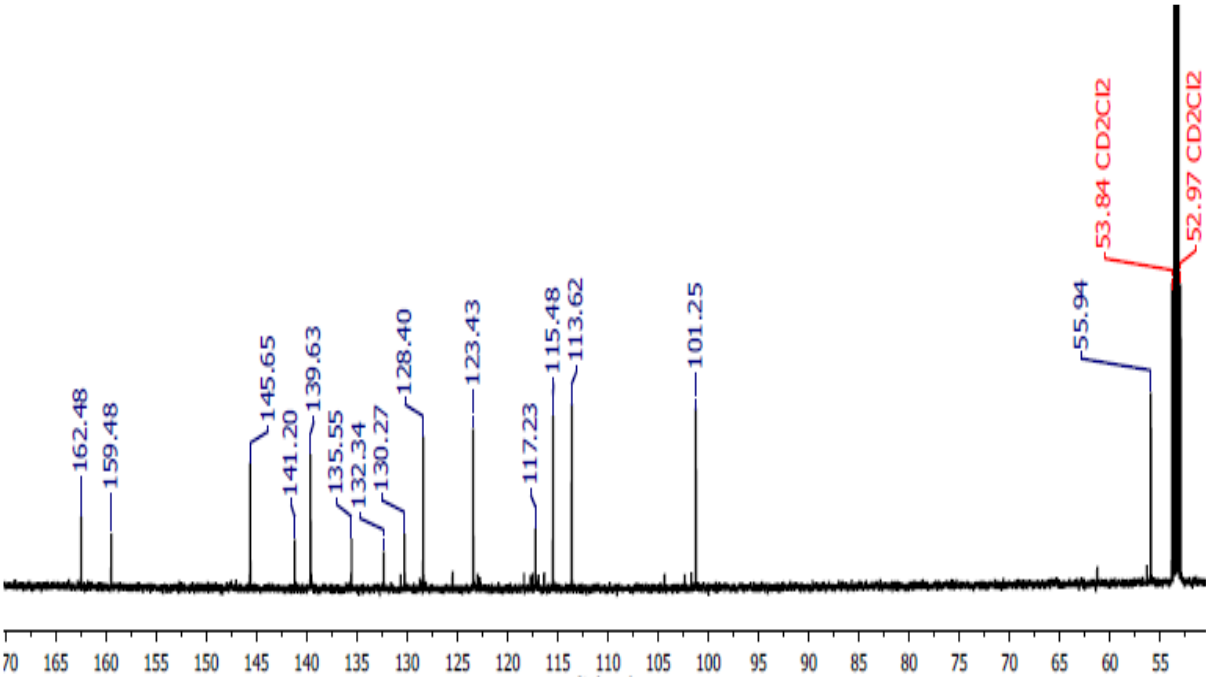


Appendix 6; NMR and mass spectra for 10-methoxycanthin-6-one (6)

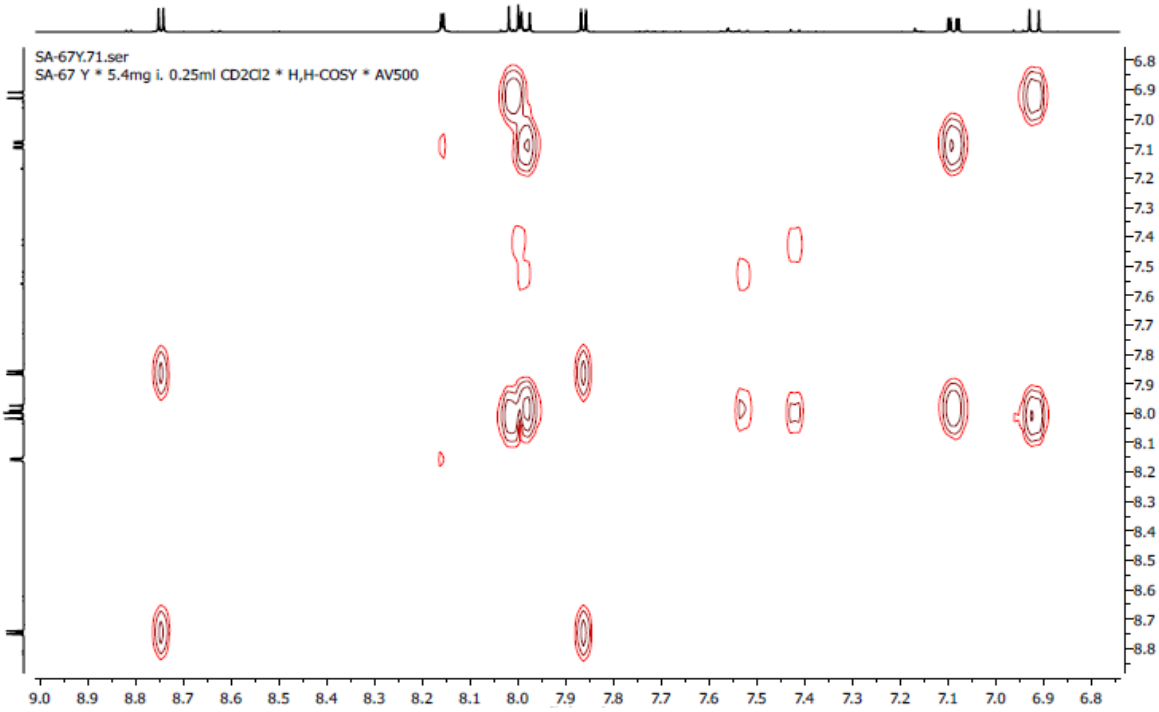
Appendix 6A. The ^{1}H NMR spectrum of 10-methoxycanthn-6- one (**6**) observed at 500 MHz for CD_2Cl_2 solution at 25 °C.



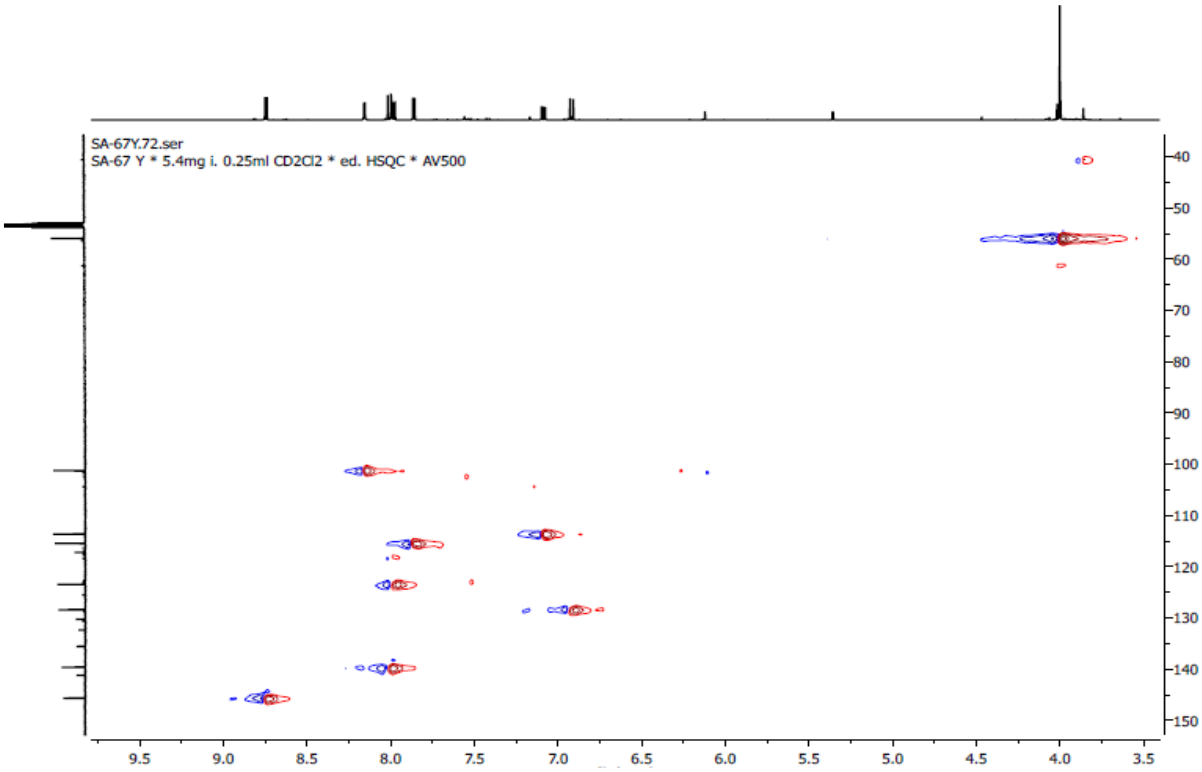
Appendix 6B. The ^{13}C NMR spectrum of 10-methoxycanthn-6- one (**6**) observed at 125 MHz for CD_2Cl_2 solution at 25 °C.



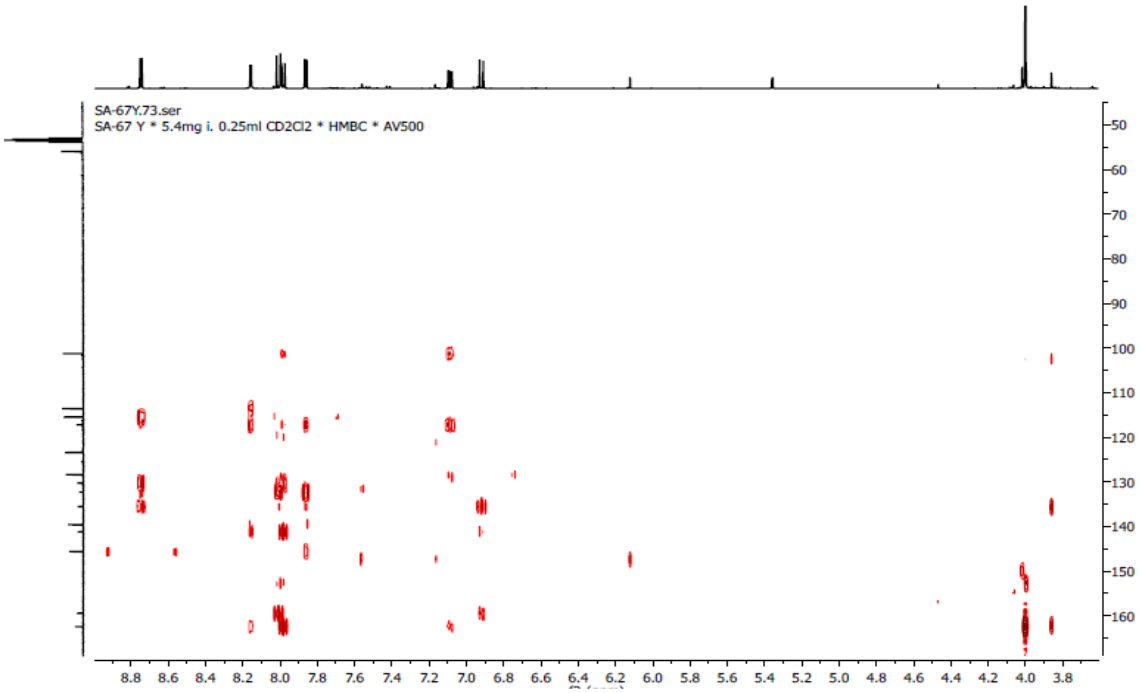
Appendix 6C. The ^{1}H-^{1}H COSY spectrum of 10-methoxycanthn-6- one (**6**) observed at 500 MHz for CD_2Cl_2 solution at 25 °C.



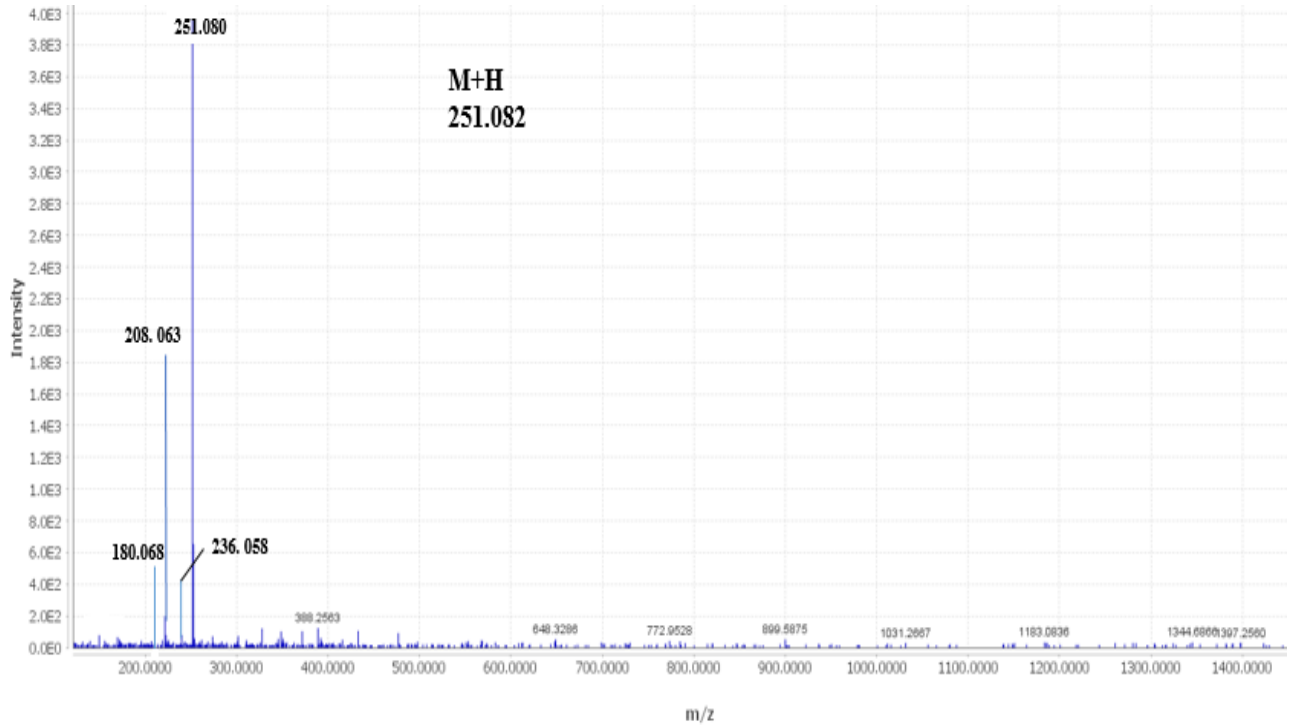
Appendix 6D. The ^{1}H-^{13}C HSQC NMR spectrum of 10-methoxycanthn-6- one (**6**) observed at 500 and 125 MHz for CD_2Cl_2 solution at 25 °C.



Appendix 6E. The ^{1}H-^{13}C HMBC NMR spectrum of 10-methoxycanthn-6- one (**6**) observed at 500 and 125 MHz for CD_2Cl_2 solution at 25 °C.

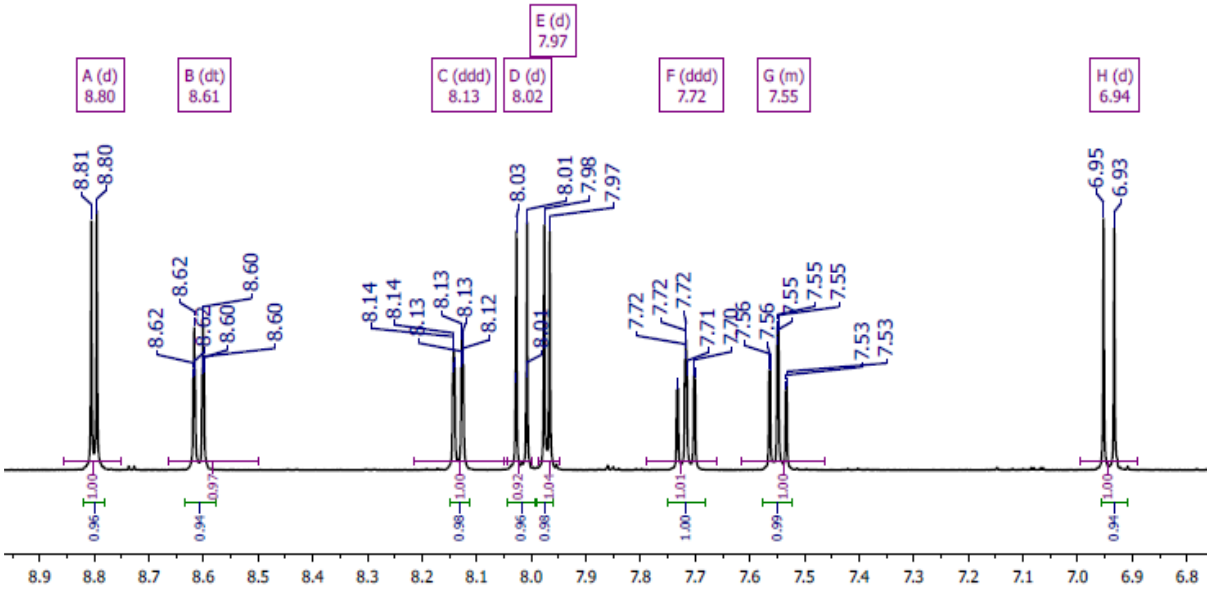


Appendix 6F. The ESIMS spectrum for 10-methoxycanthin- 6- one (**6**).

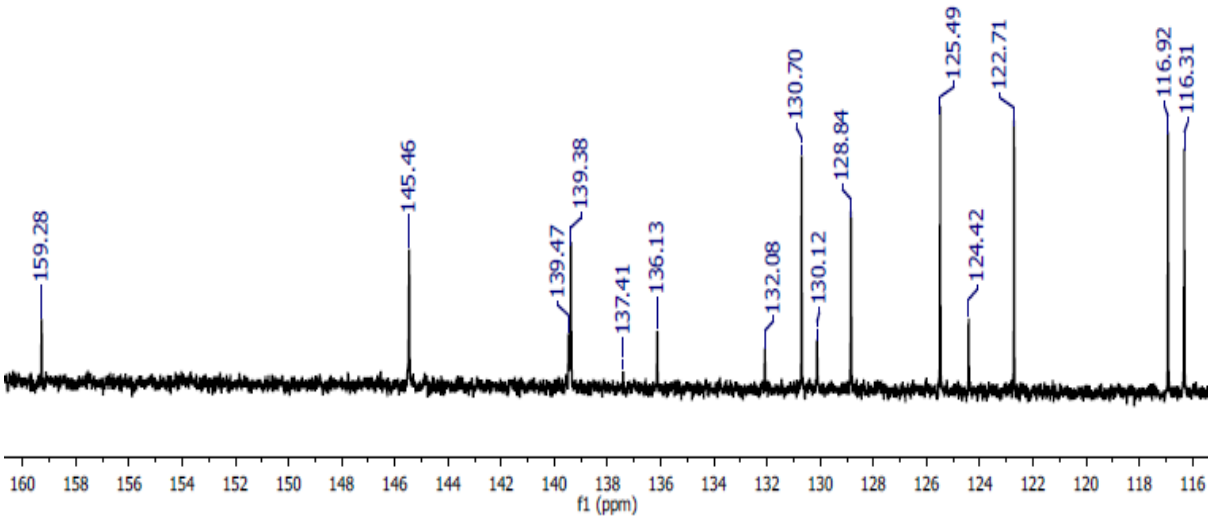


Appendix 7; NMR and mass spectra for canthin-6-one (7)

Appendix 7A. The ^{1}H NMR spectrum of canthin-6-one (**7**) observed at 500 MHz for CD_2Cl_2 solution at 25 °C.



Appendix 7B. The ^{13}C NMR spectrum of canthin-6-one (**7**) observed at 125 MHz for CD_2Cl_2 solution at 25 °C



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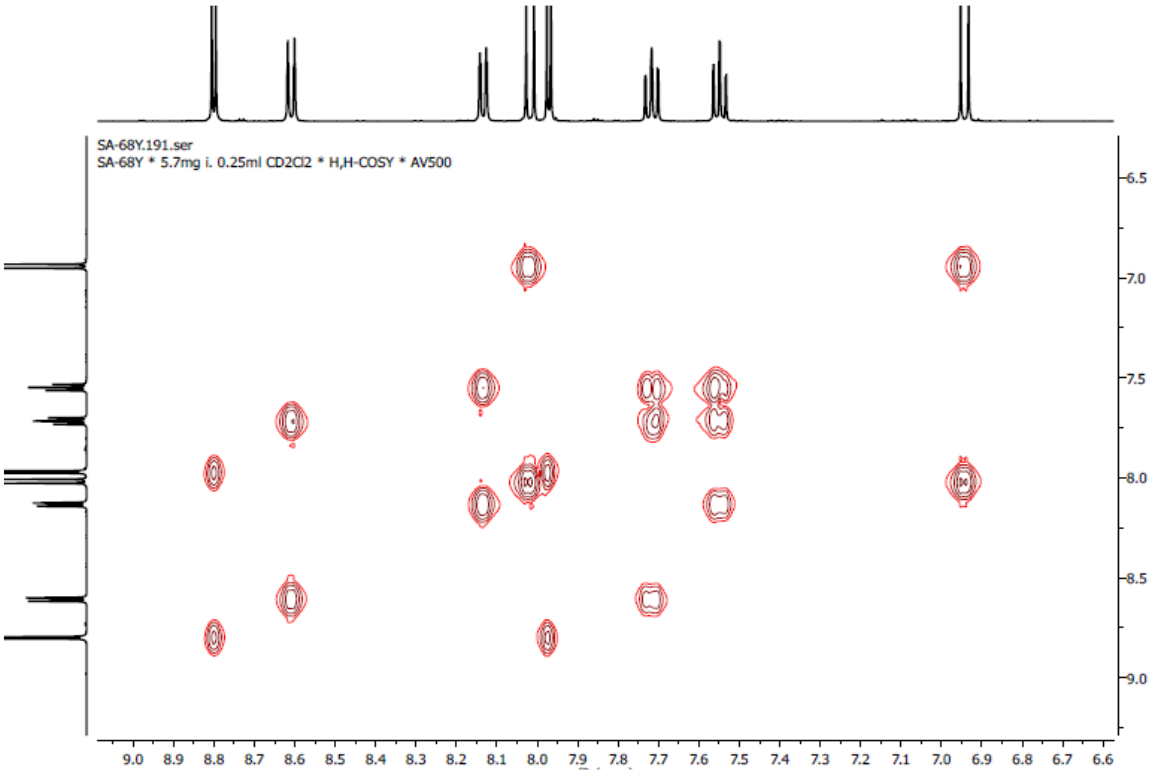
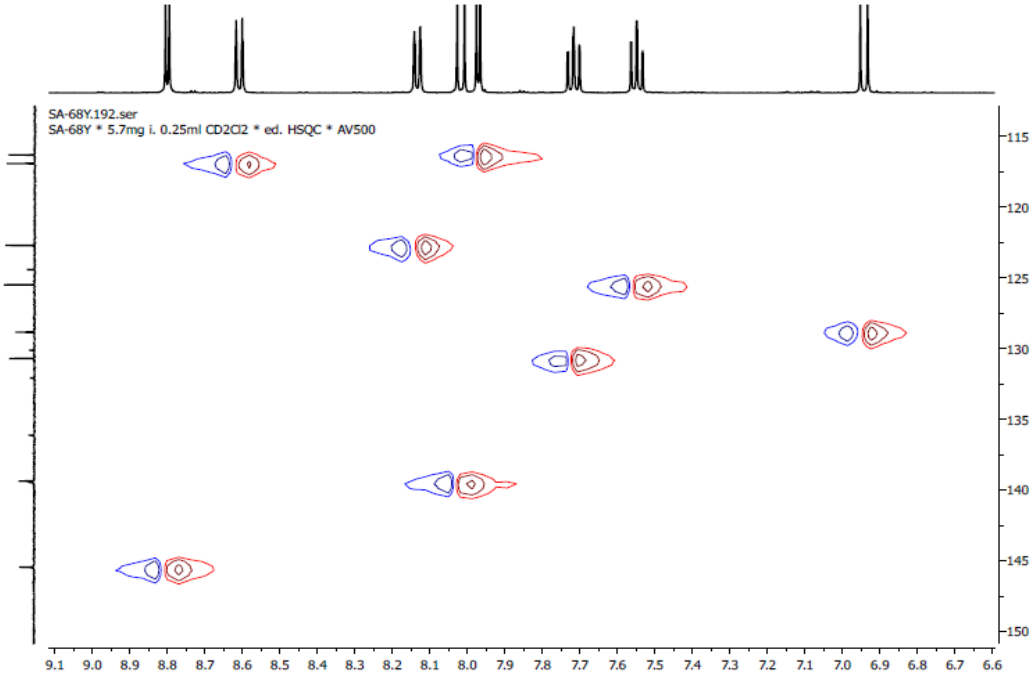
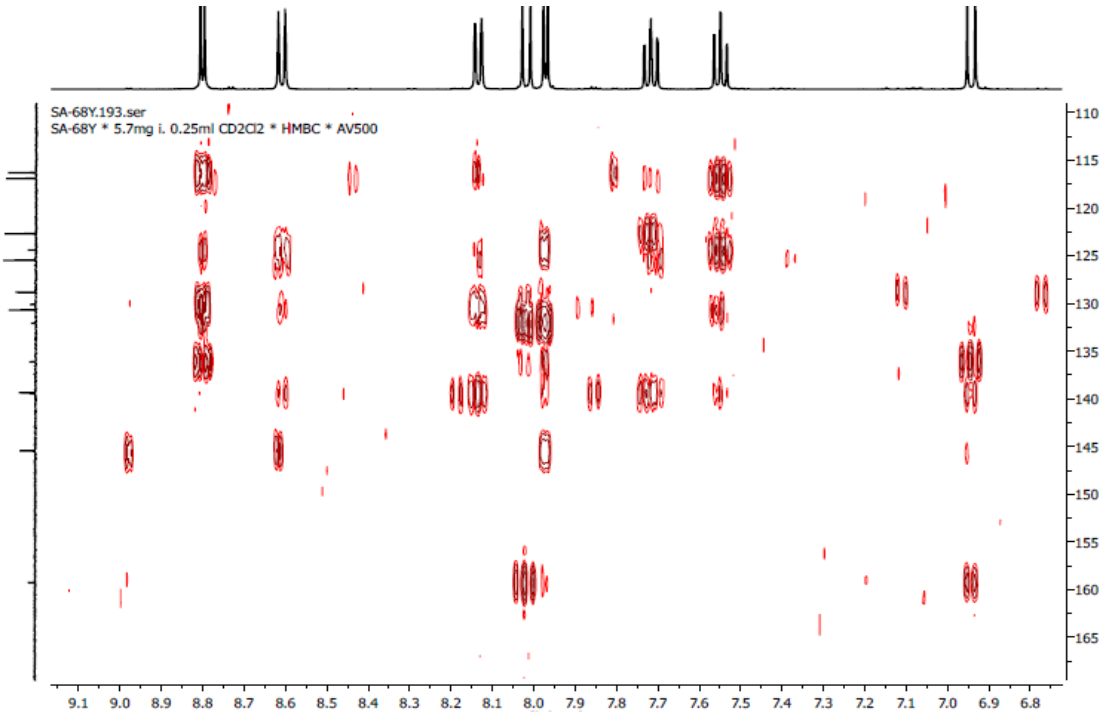
Appendix 7C. The ^{1}H-^{1}H COSY spectrum of canthin-6-one (**7**) observed at 500 MHz for CD_2Cl_2 solution at 25 °C.



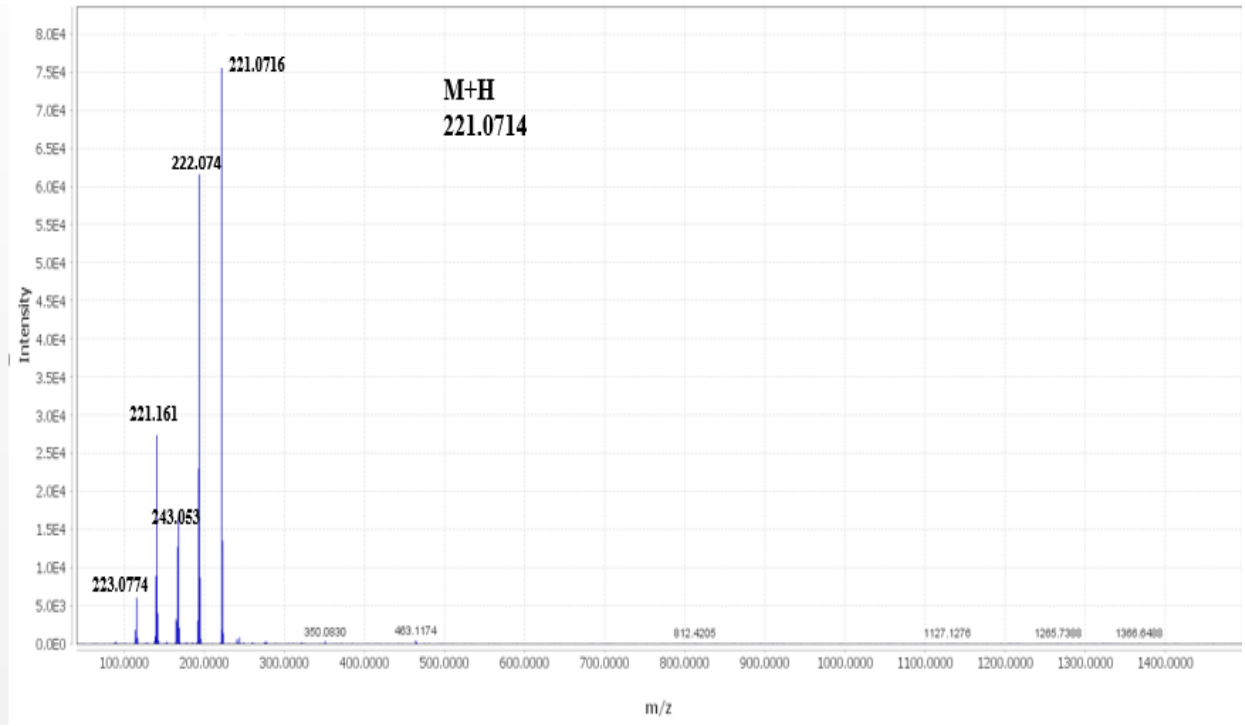
Appendix 7D. The ^{1}H-^{13}C HSQC NMR spectrum of canthin-6-one (**7**) observed at 500 and 125 MHz for CD_2Cl_2 solution at 25 °C.



Appendix 7E. The ^{1}H-^{13}C HMBC NMR spectrum of canthin-6-one(**7**) observed at 500 and 125 MHz for CD_2Cl_2 solution at 25 °C.

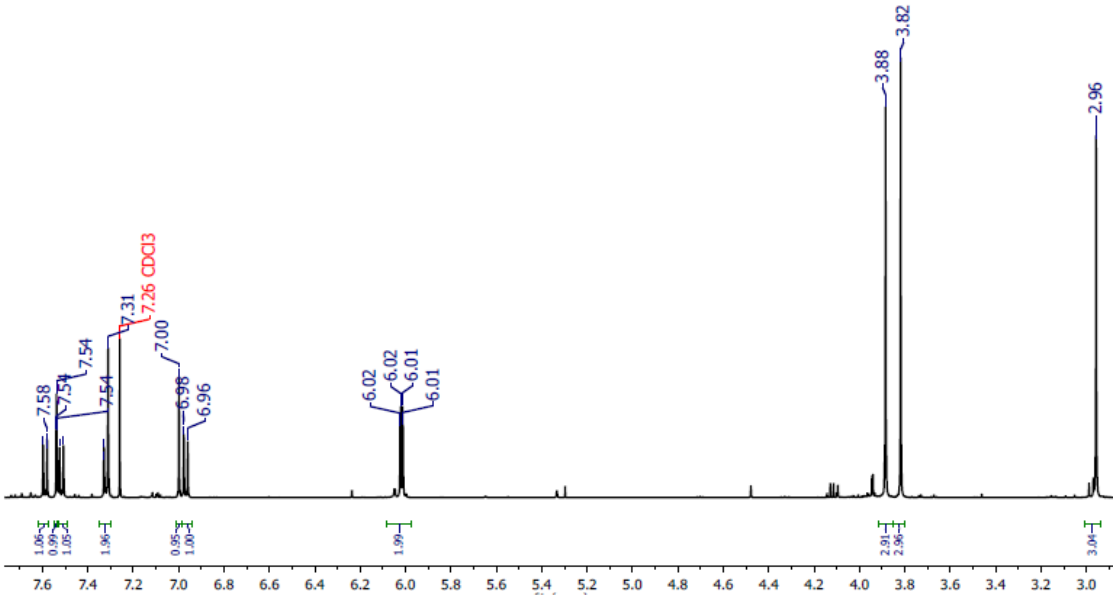


Appendix 7F. The ESIMS spectrum of canthin- 6- one (**7**).

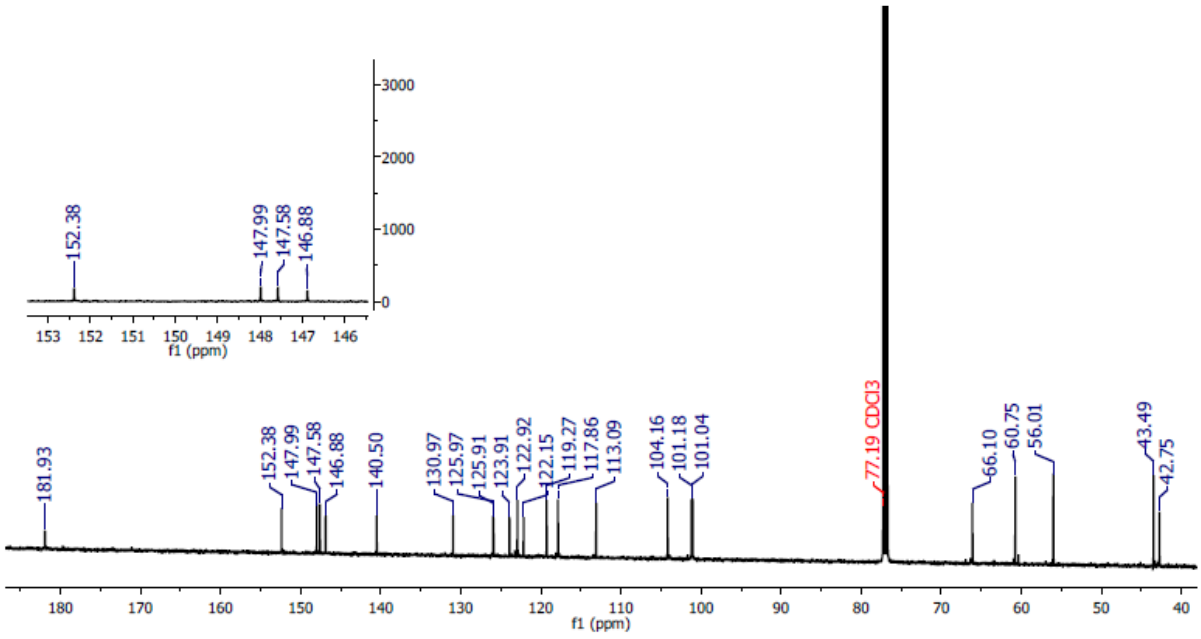


Appendix 8; NMR and mass spectra for 8-oxochelerythrine (8)

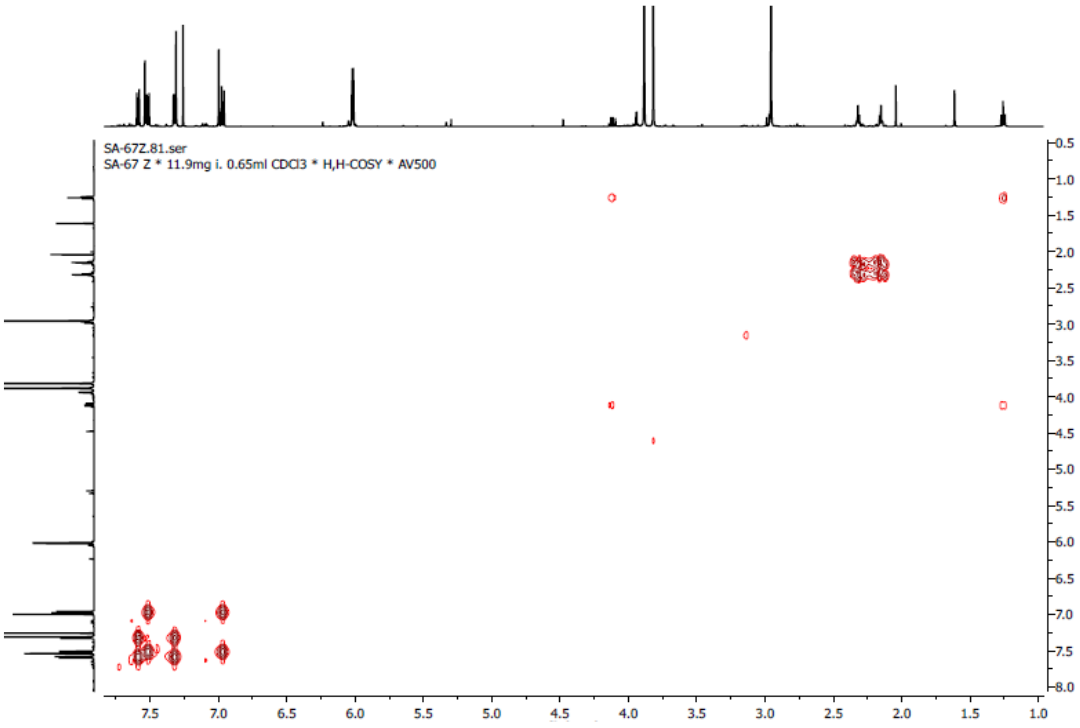
Appendix 8A. The ^{1}H NMR spectrum of 8-oxochelerythrine (**8**) observed at 500 MHz for $CDCl_3$ solution at 25 °C.



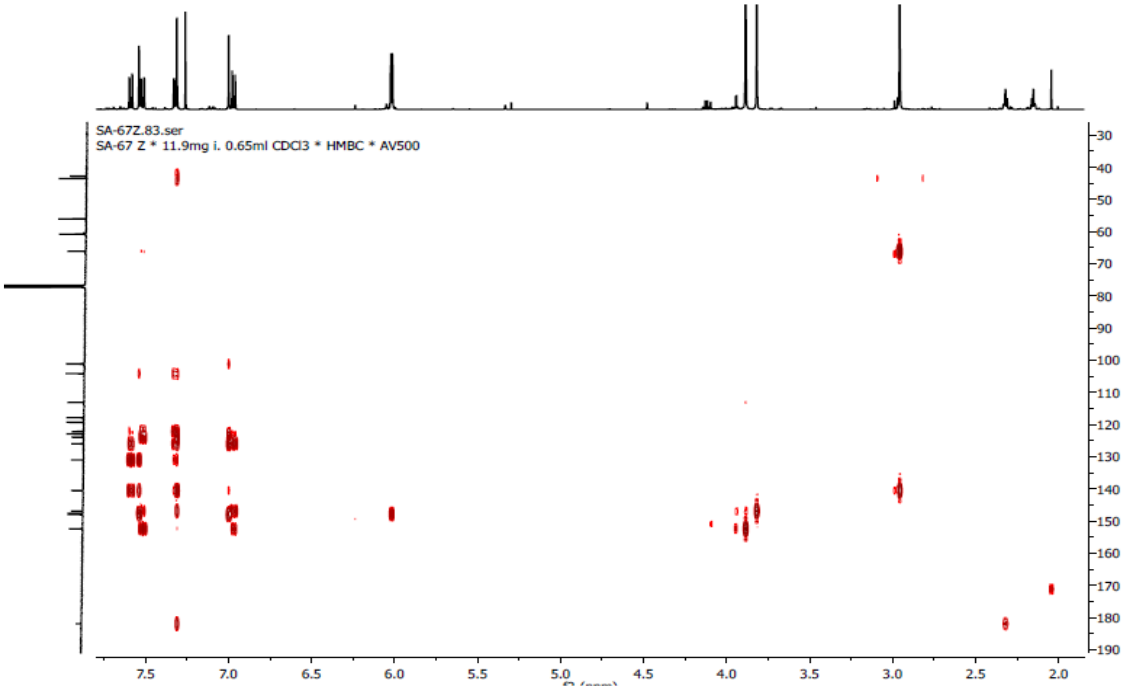
Appendix 8B. The ^{13}C NMR spectrum of 8-oxochelerythrine (**8**) observed at 125 MHz for $CDCl_3$ solution at 25 °C.



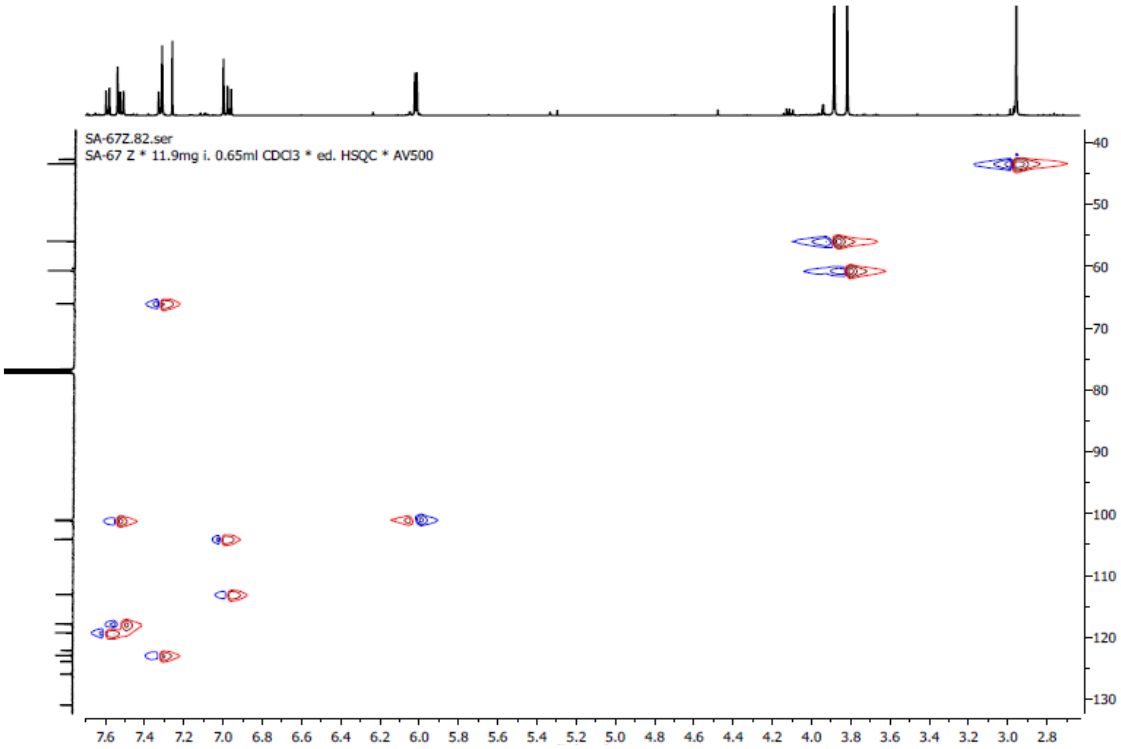
Appendic 8C. The ^{1}H-^{1}H COSY spectrum of 8-oxochelerythrine (**8**) observed at 500 MHz for $CDCl_3$ solution at 25 °C.



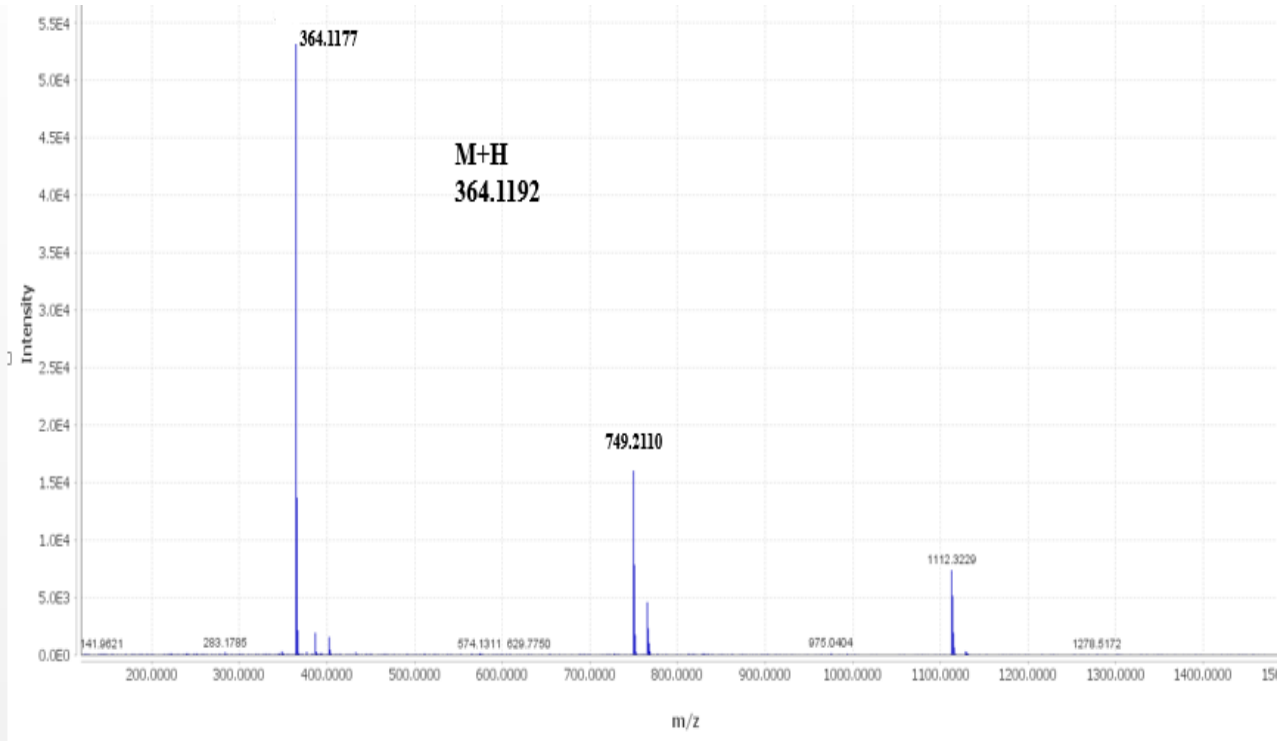
Appendix 8D. The ^{1}H-^{13}C HSQC NMR spectrum of 8-oxochelerythrine (**8**) observed at 500 and 125 MHz for $CDCl_3$ solution at 25 °C.



Appendix 8E. The ^{1}H-^{13}C HMBC NMR spectrum of 8-oxochelerythrine (**8**) observed at 500 and 125 MHz for $CDCl_3$ solution at 25 °C. Assignment is given in Table S8.

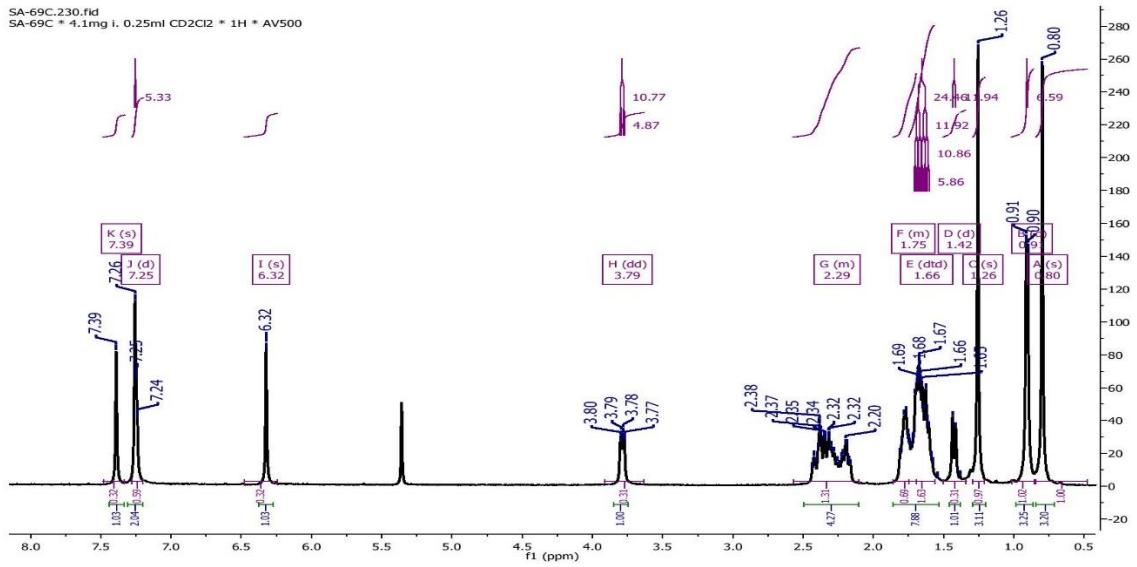


Appendix 8F. The ESIMS spectrum for 8-oxocheerythrine (**8**).

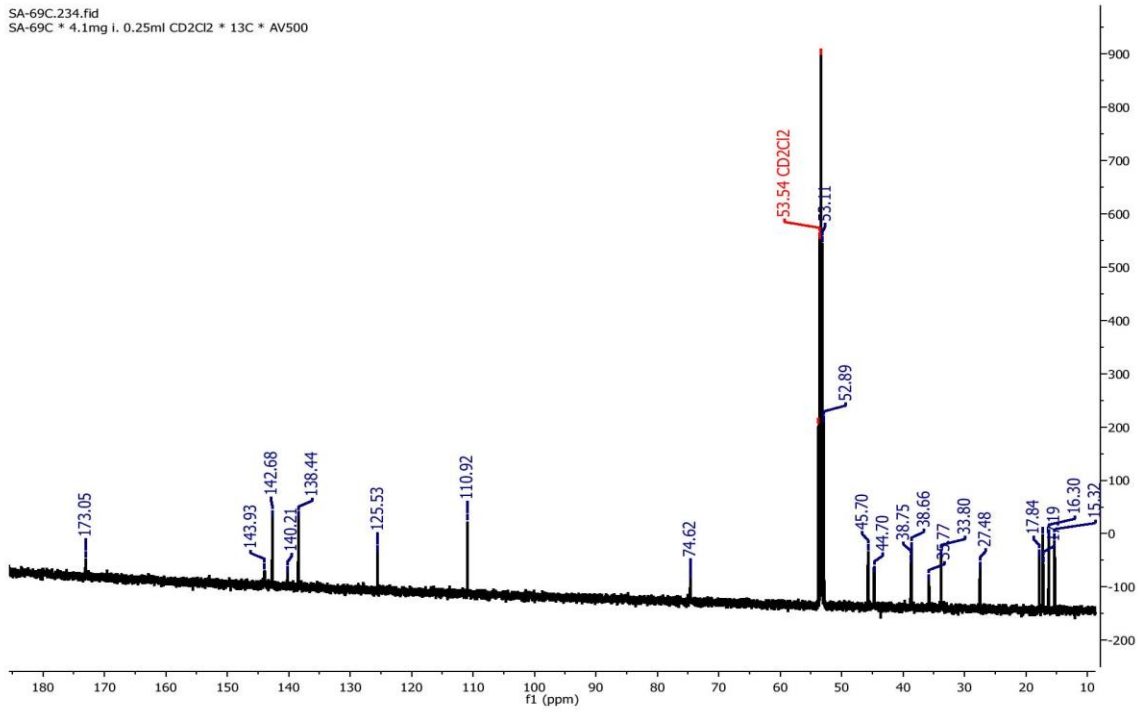


Appendix 9; NMR and mass spectra for dodonic acid (9)

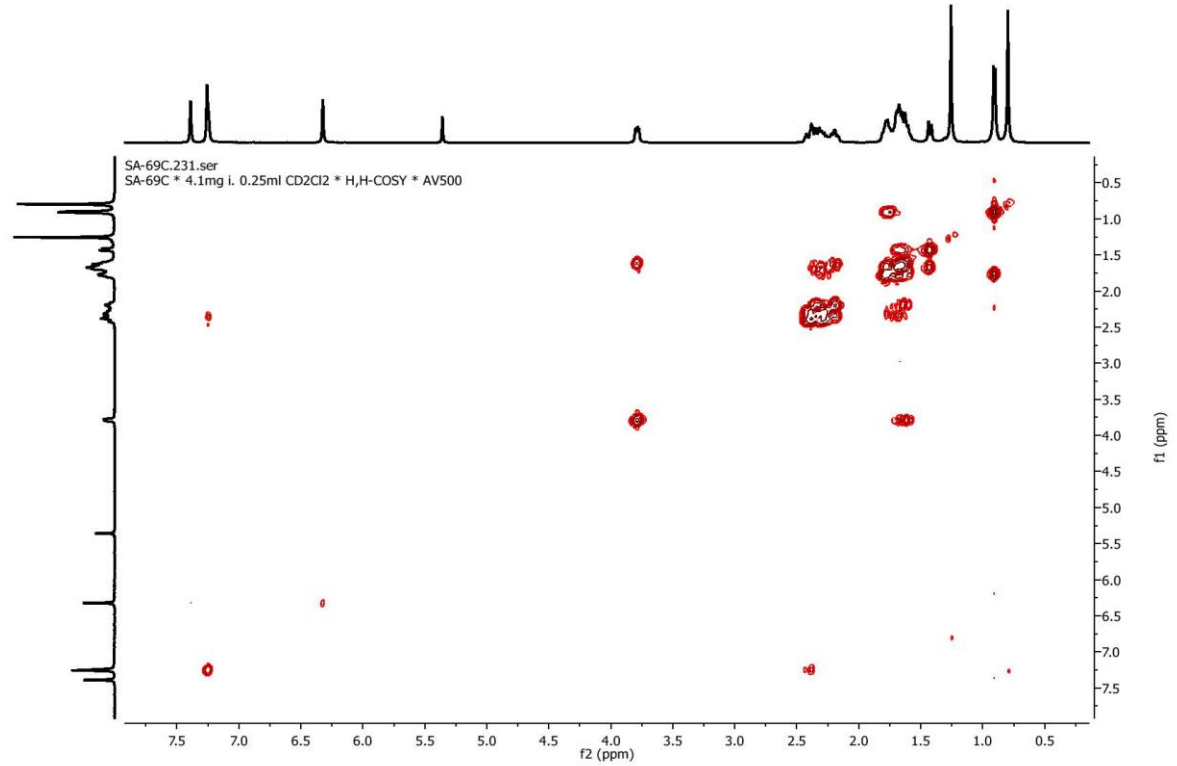
Appendix 9A. The ^{1}H NMR spectrum of dodonic acid (**9**) observed at 500 MHz for CD_2Cl_2 solution at 25 °C.



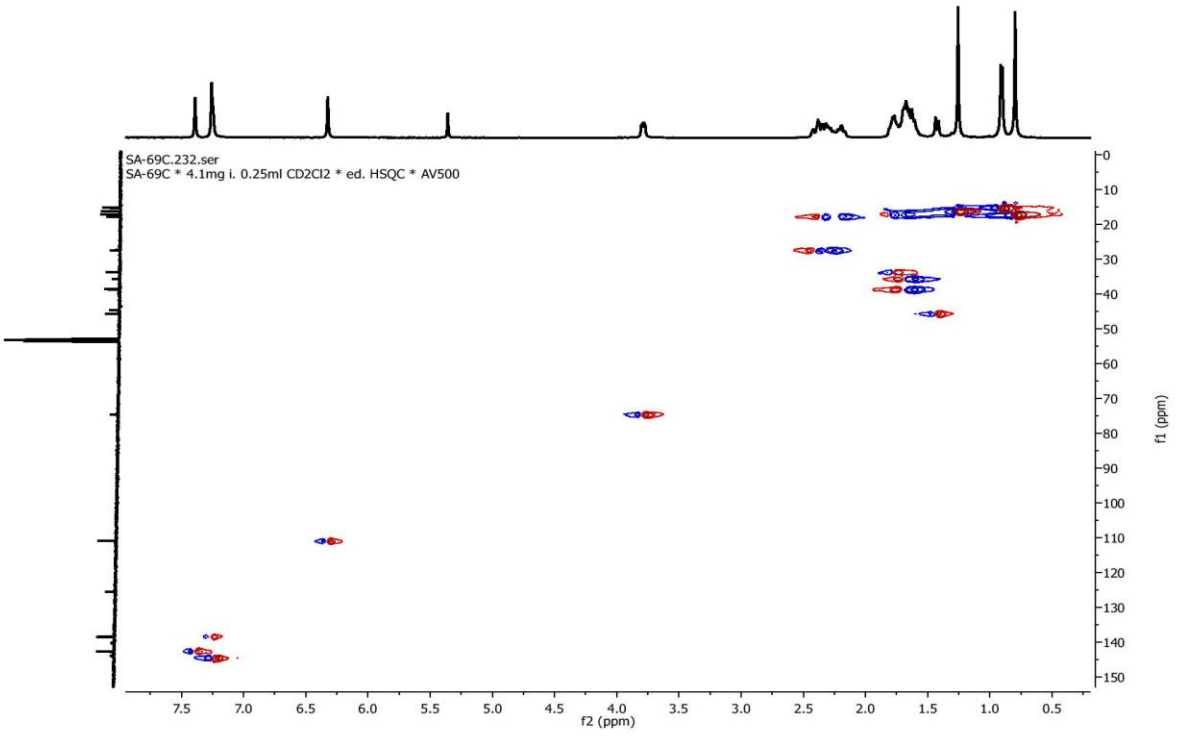
Appendix 9B. The ^{13}C NMR spectrum of dodonic acid (**9**) observed at 500 MHz for CD_2Cl_2 solution at 25 °C.



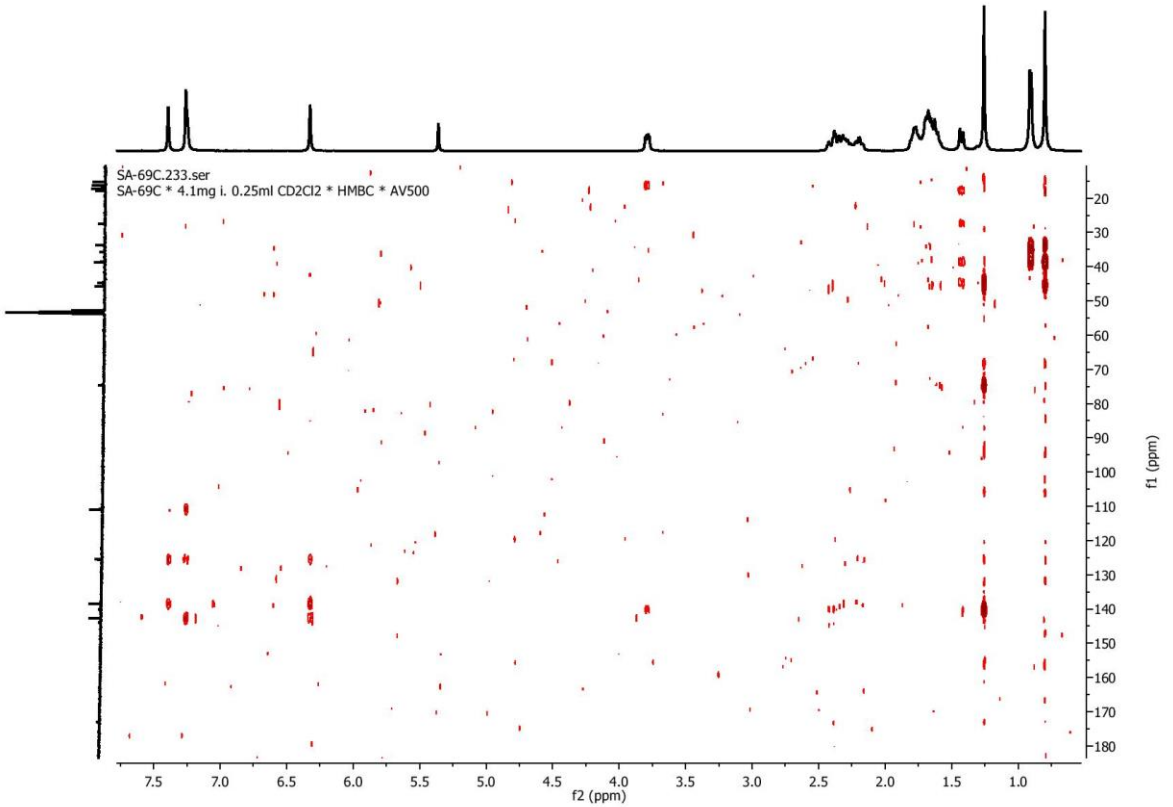
Appendix 9C. The ^{1}H-^{1}H COSY NMR spectrum of dodonic acid (**9**) observed at 500 MHz for CD_2Cl_2 solution at 25 °C.



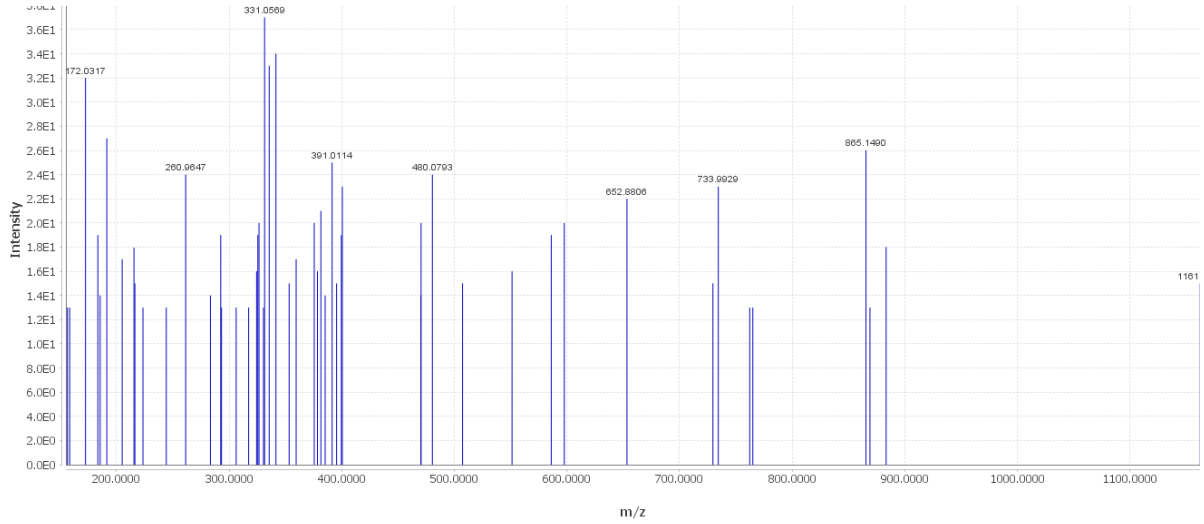
Appendix 9D. The ^{1}H-^{13}C HSQC NMR spectrum of dodonic acid (**9**) observed at 500 MHz for CD_2Cl_2 solution at 25 °C.



Appendix 9E. The ^{1}H-^{13}C HMBC NMR spectrum of dodonic acid (**9**) observed at 500 MHz for CD_2Cl_2 solution at 25 °C.

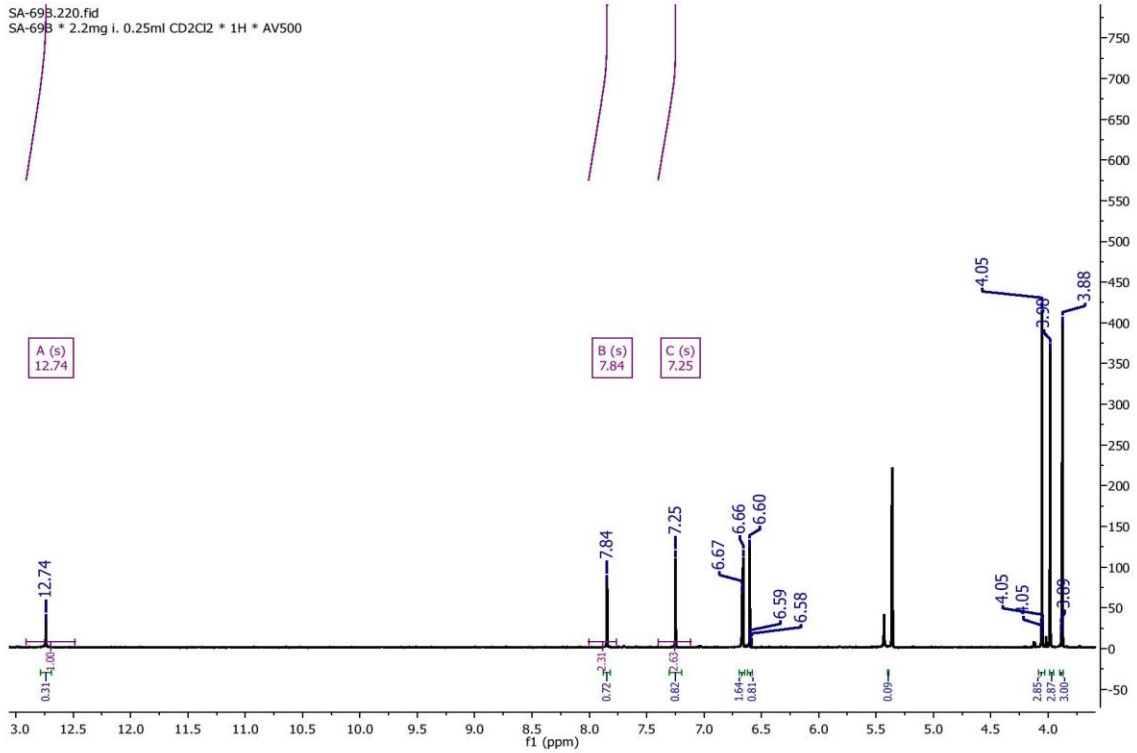


Appendix 9F. ESIMS spectrum for dodonic acid (**9**)

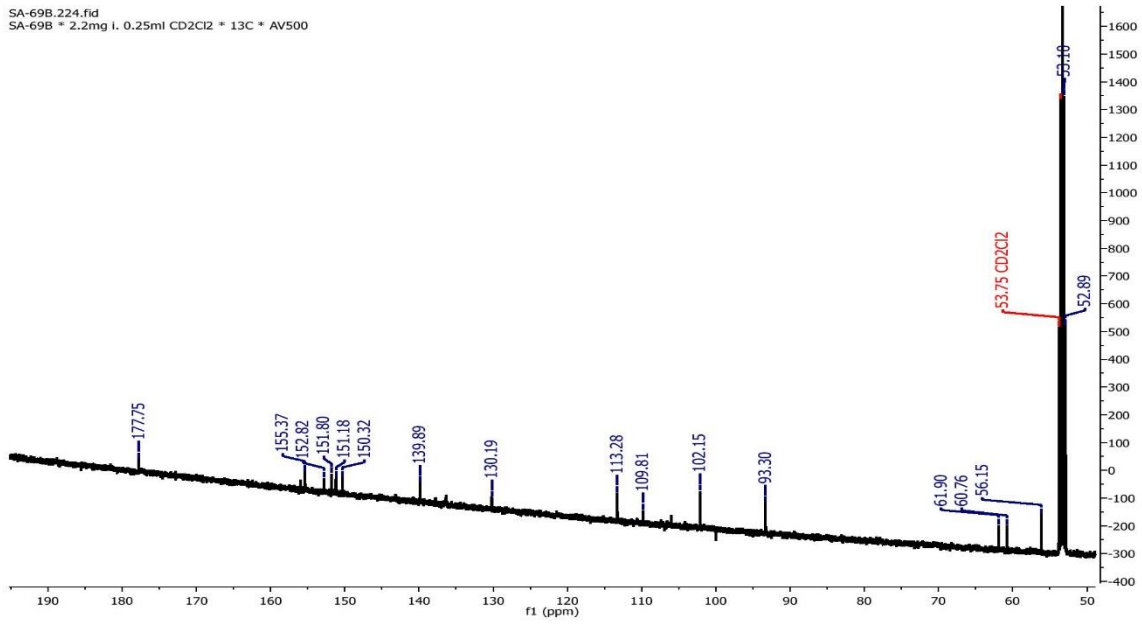


Appendix 10; 1H and 13C NMR for 5, 7, 4', 5' -Tetrahydroxy-3, 6, 2’ -trimethoxyflavone (10)

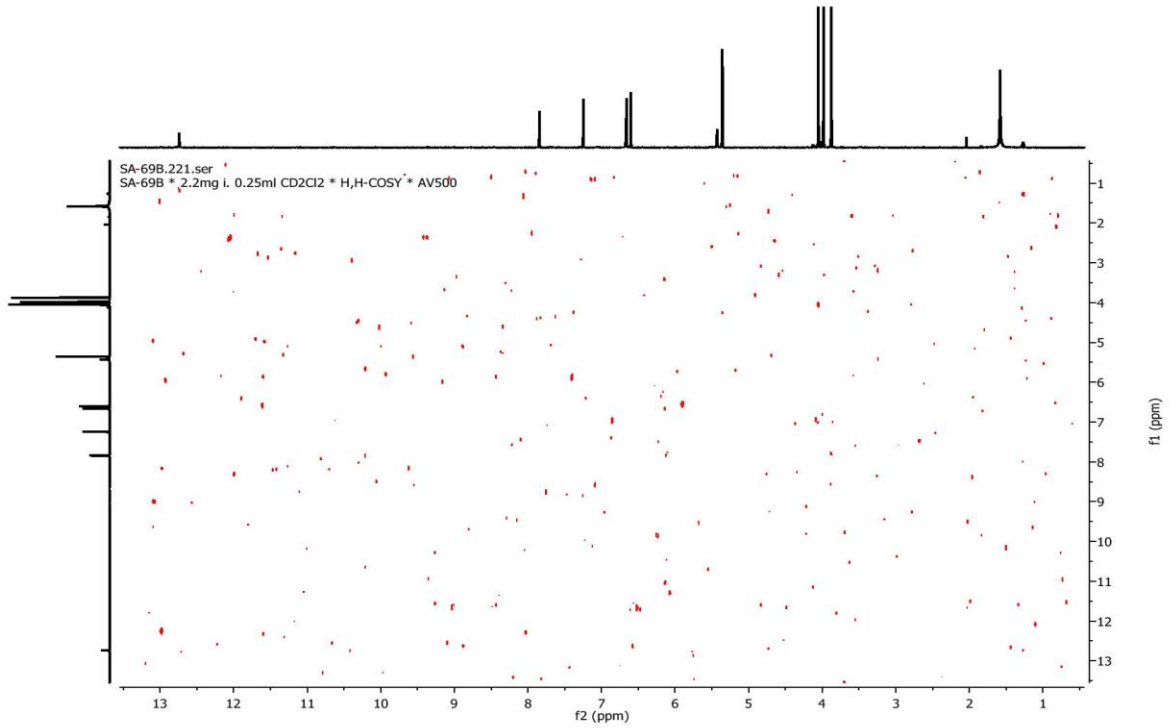
Appendix 10A. The ^{1}H NMR spectrum of 5, 7, 4', 5' -tetrahydroxy-3, 6, 2’ -trimethoxyflavone (**10**) observed at 500 MHz for CD_2Cl_2 solution at 25 °C



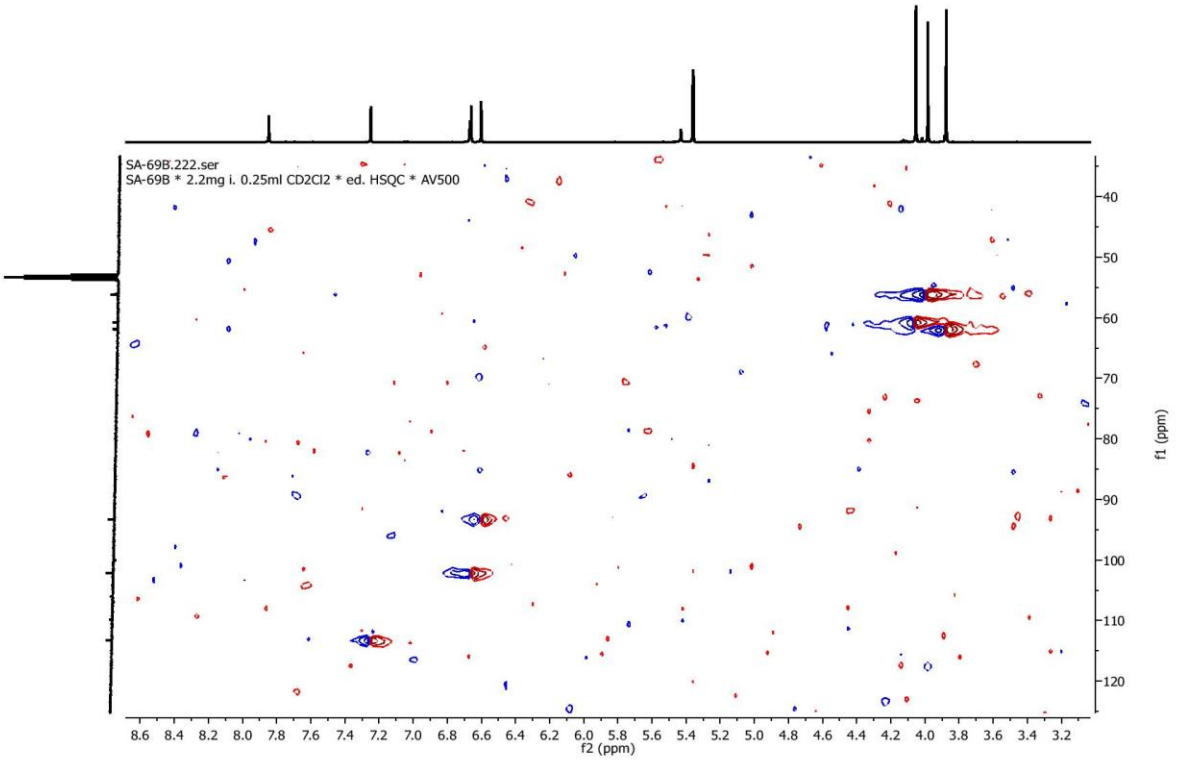
. **Appendix 10B.** ^{13}C NMR spectrum of 5, 7, 4', 5' -tetrahydroxy-3, 6, 2’ -trimethoxyflavone (**10**) observed at 500 MHz for CD_2Cl_2 solution at 25 °C.



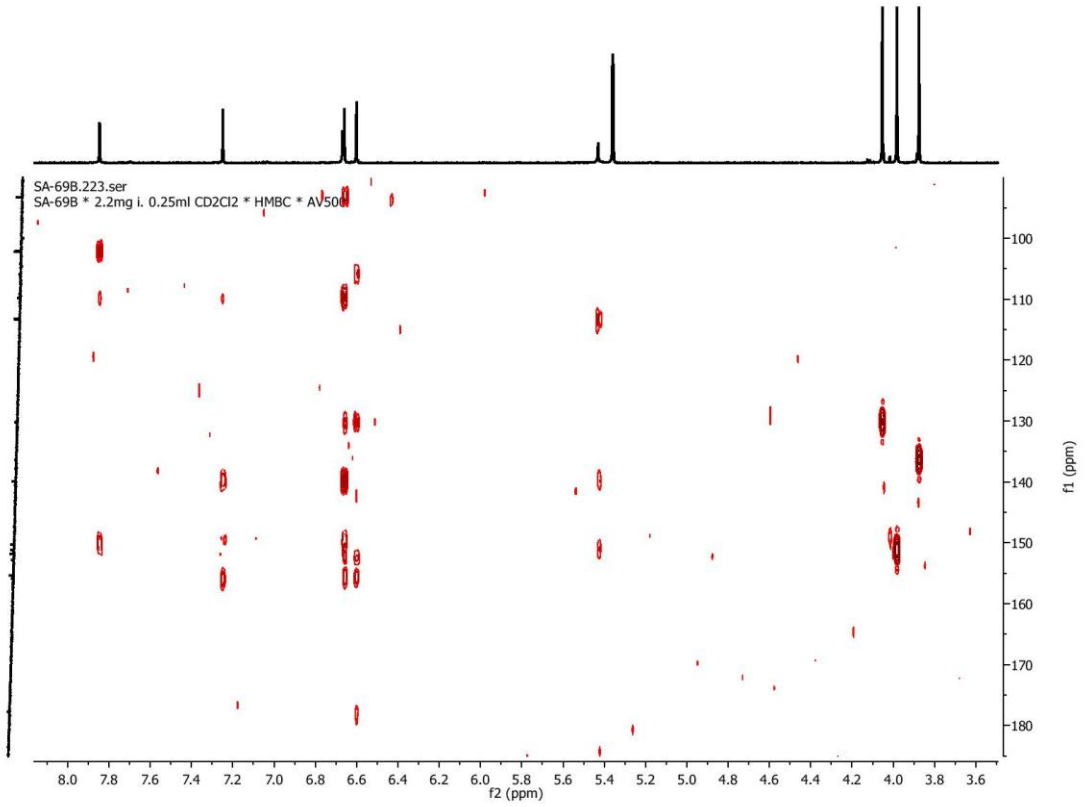
Appendix 10C. ^{1}H-^{1}H COSY NMR spectrum of 5, 7, 4', 5' -tetrahydroxy-3, 6, 2' -trimethoxyflavone (**10**) observed at 500 MHz for CD_2Cl_2 solution at 25 °C.



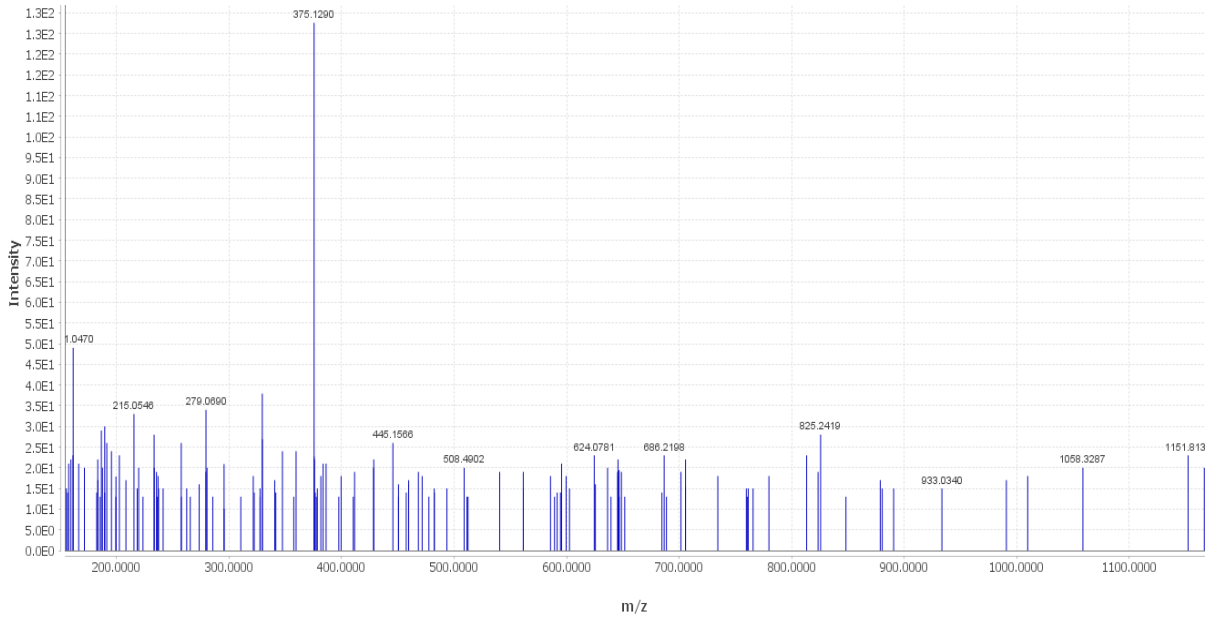
Appendix 10D. ^{1}H- ^{13}C HSQS spectrum of 5, 7, 4', 5' -Tetrahydroxy-3, 6, 2' -trimethoxyflavone (**10**) observed at 500 MHz for CD_2Cl_2 solution at 25 °C.



Appendix 10E. ^{1}H- ^{13}C HMBC spectrum of 5, 7, 4', 5' -Tetrahydroxy-3, 6, 2' -trimethoxyflavone (**10**) observed at 500 MHz for CD_2Cl_2 solution at 25 °C

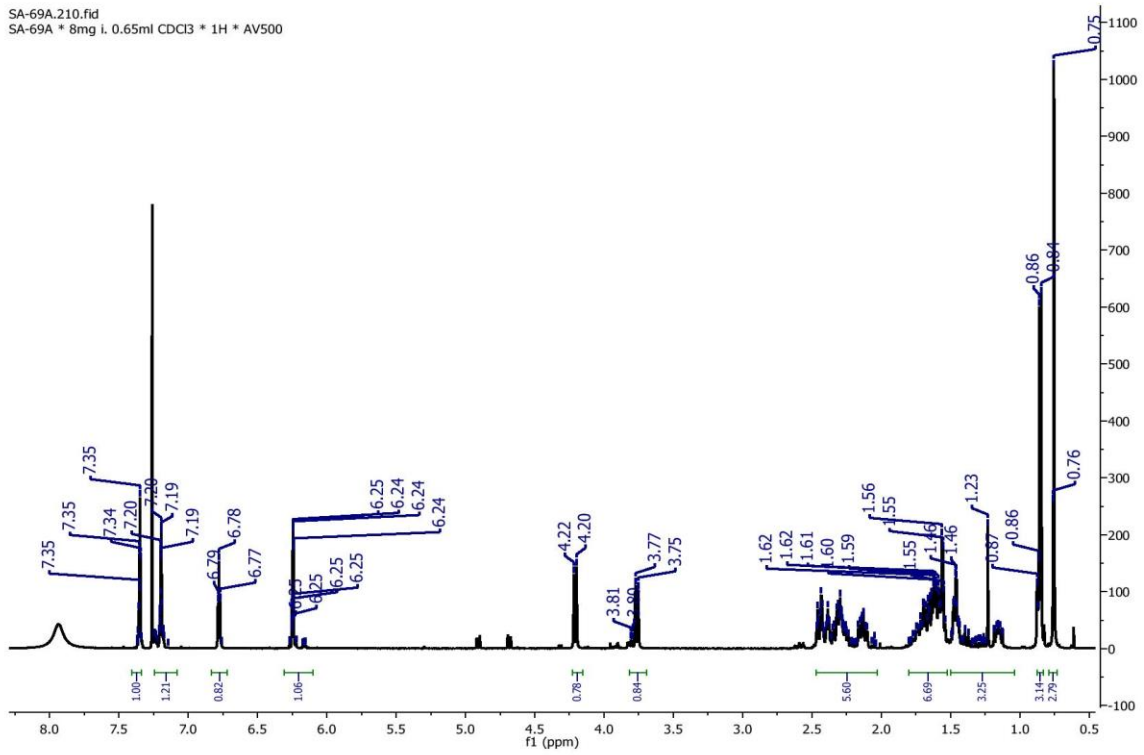


. **Appendix 10F.** ESIMS spectrum for 5,7, 4', 5' -Tetrahydroxy-3, 6, 2' -trimethoxyflavone (**10**)

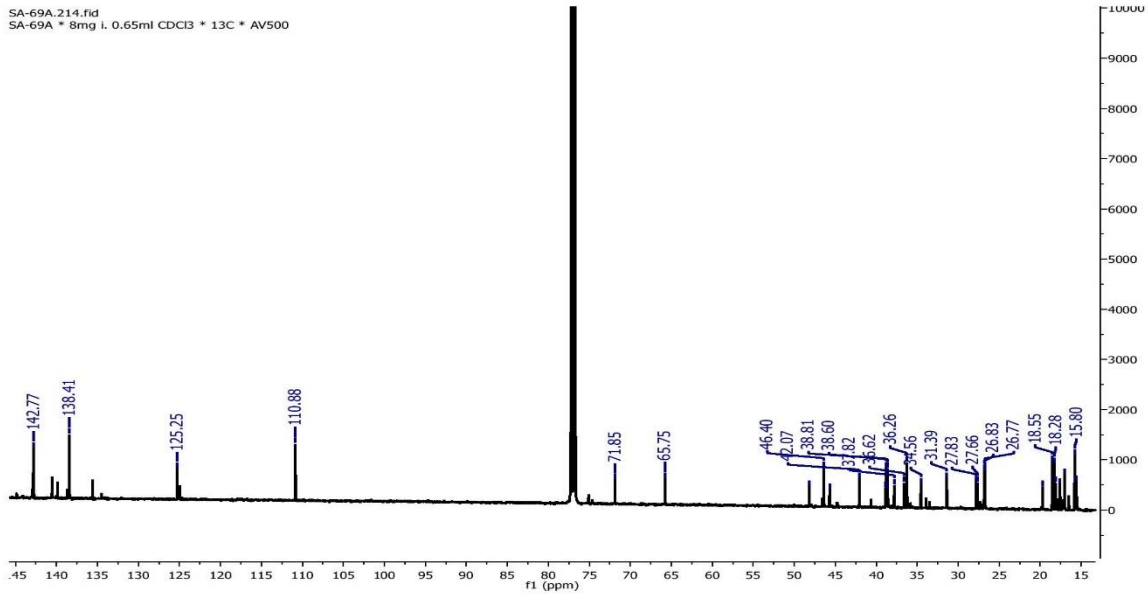


Appendix 11; NMR and mass spectra for hautriwaic acid lactone

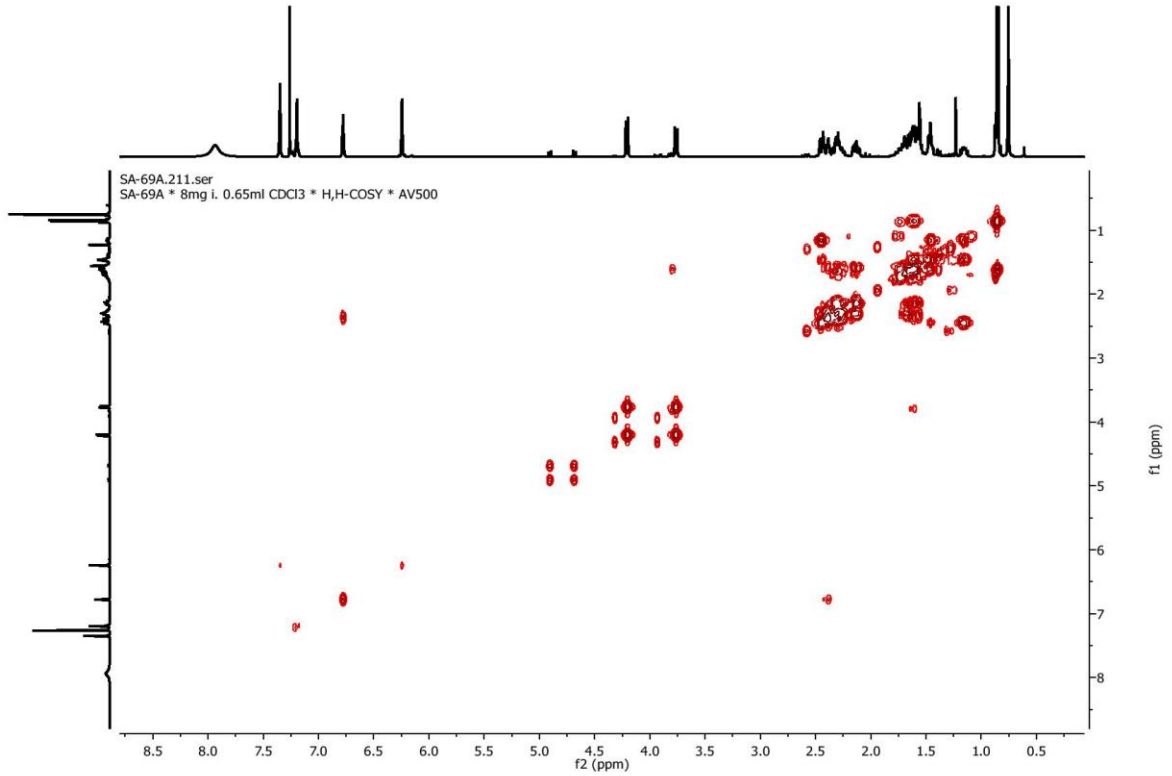
Appendix 11A. The ^{1}H- NMR spectrum of hautriwaic acid lactone (**11**) observed at 500 MHz for $CDCl_3$ solution at 25 °C.



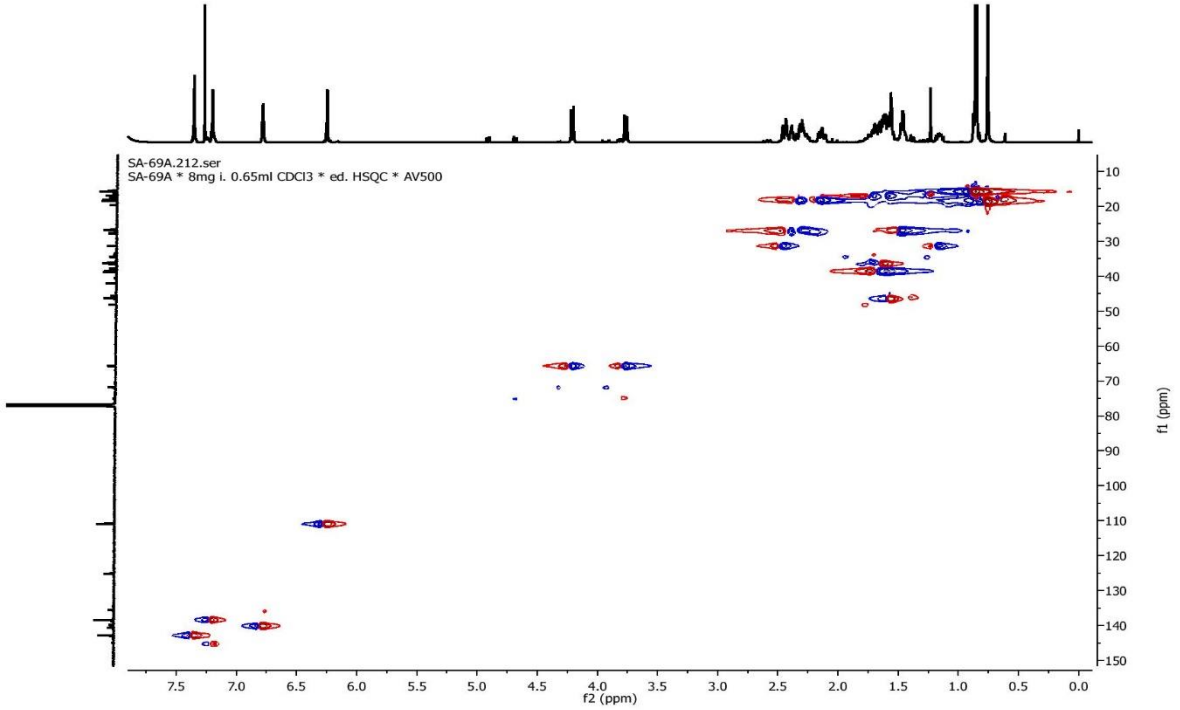
Appendix 11B. The ^{13}C- NMR spectrum of hautriwaic acid lactone (**11**) observed at 500 MHz for $CDCl_3$ solution at 25 °C.



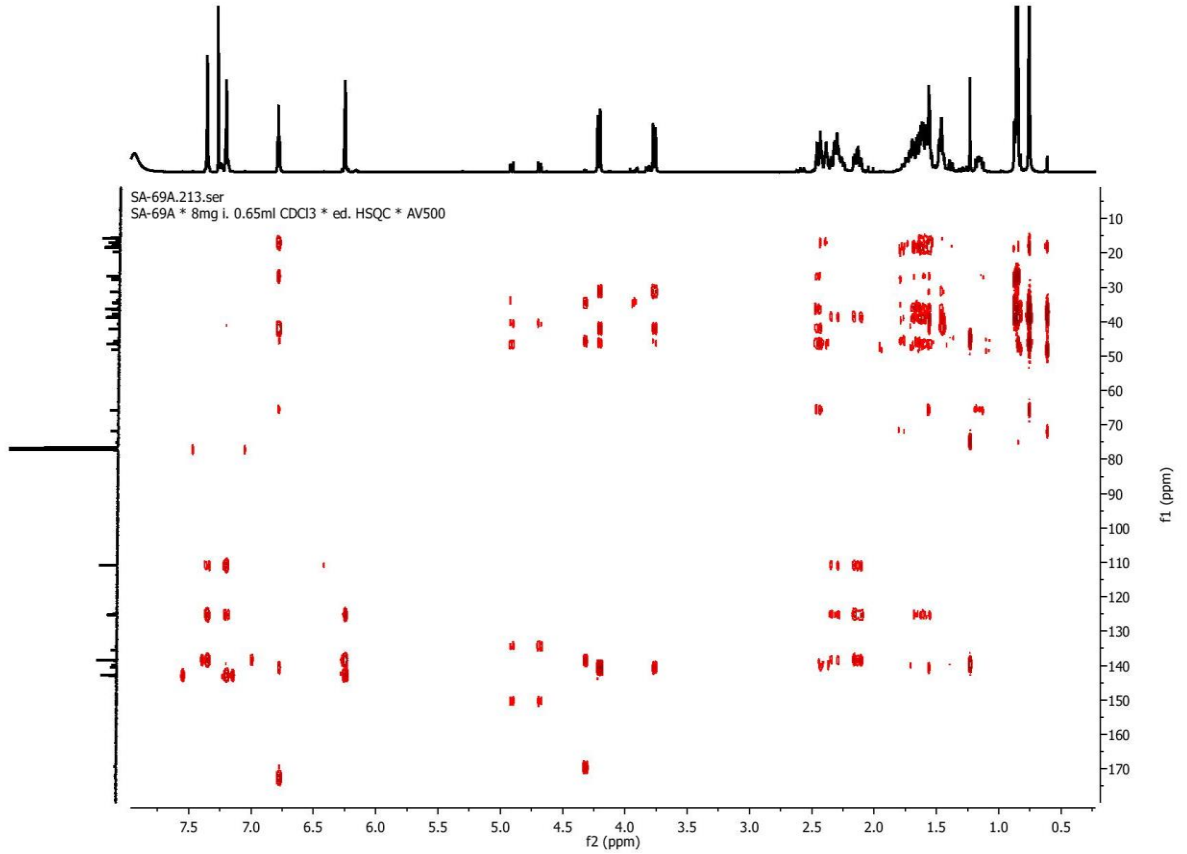
Appendix 11C. The ^{1}H- ^{1}H COSY- NMR spectrum of hautriwaic acid lactone (**11**) observed at 500 MHz for $CDCl_3$ solution at 25 °C.



Appendix 11D. The ^{1}H- ^{13}C- HSQS NMR spectrum of hautriwaic acid lactone (**11**) observed at 500 MHz for $CDCl_3$ solution at 25 °C.



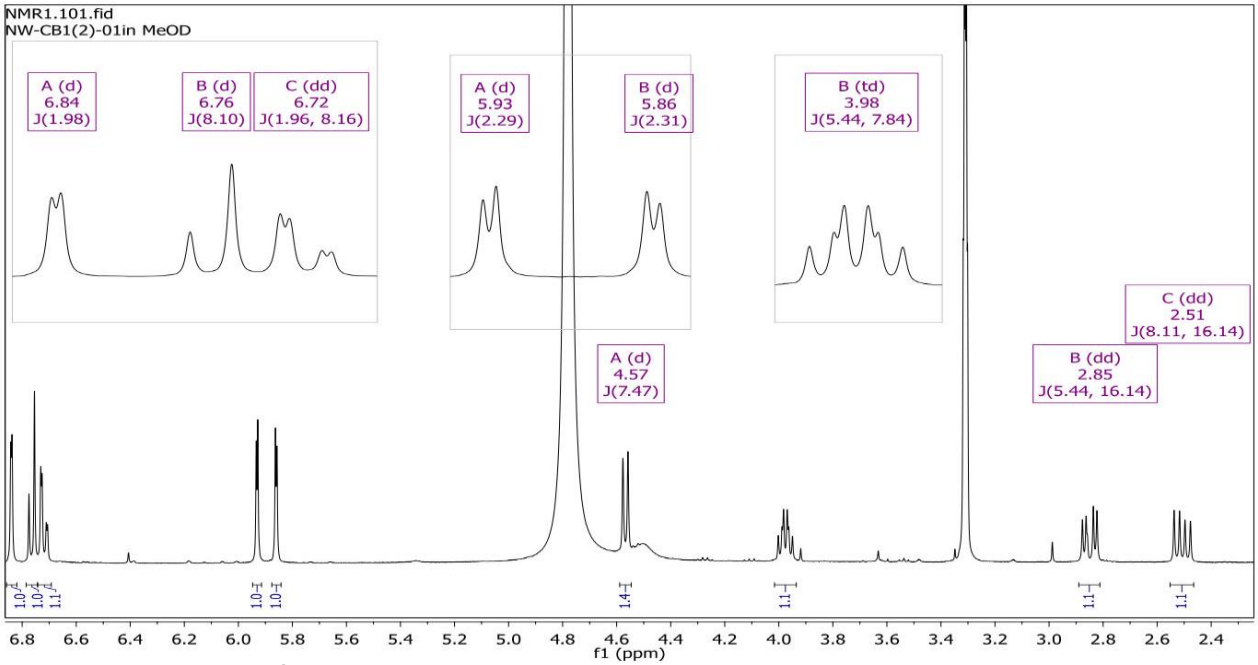
Appendix 11E. The ^{1}H- ^{13}C- HMBC NMR spectrum of hautriwaic acid lactone (**11**) observed at 500 MHz for $CDCl_3$ solution at 25 °C.



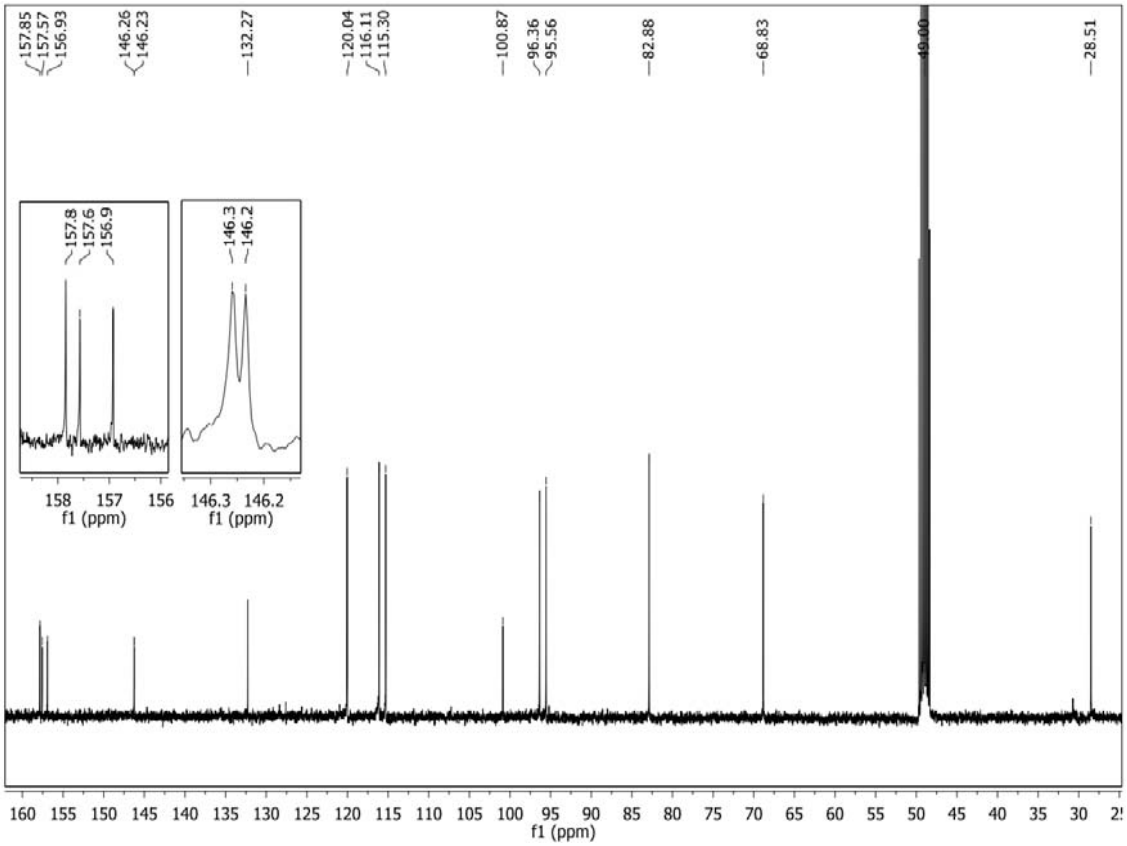
Appendix 11F. ESIMS spectrum for hautriwaic acid lactone (**11**)

Appendix 12; NMR and mass spectra for catechin (12)

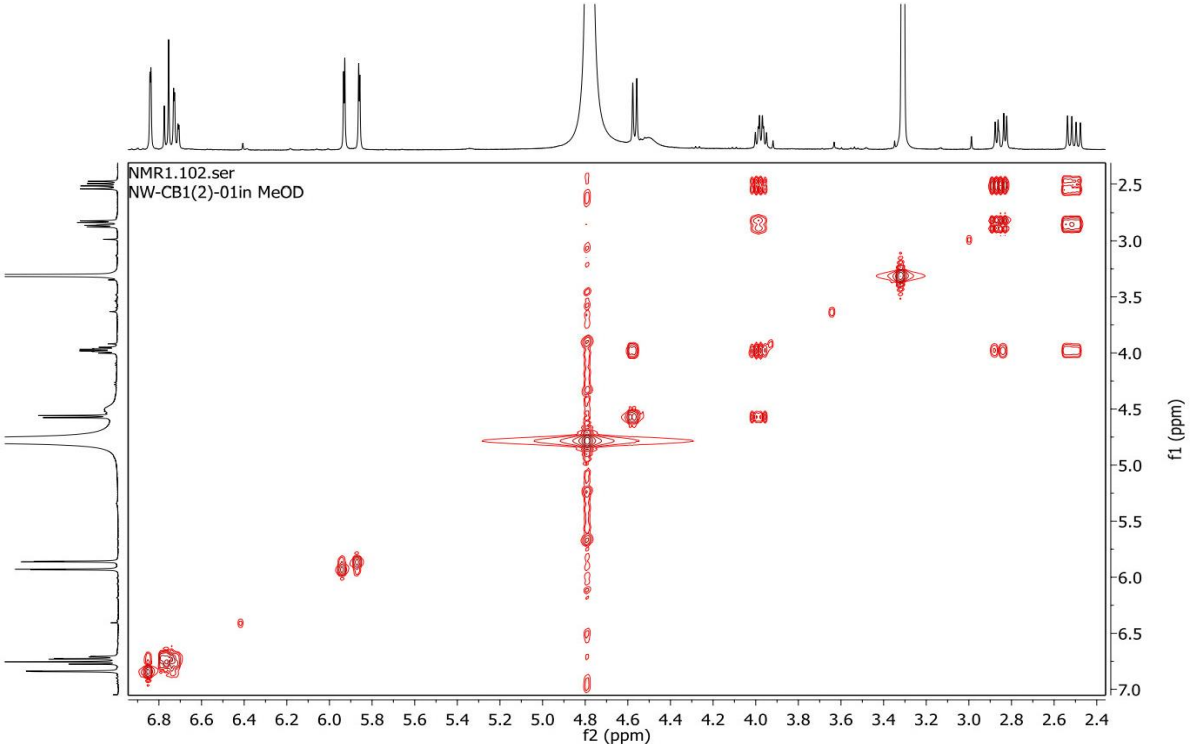
Appendix 12A. The ^{1}H NMR spectrum of catechin (**12**) observed at 500 MHz for MeOD solution at 25 °C.



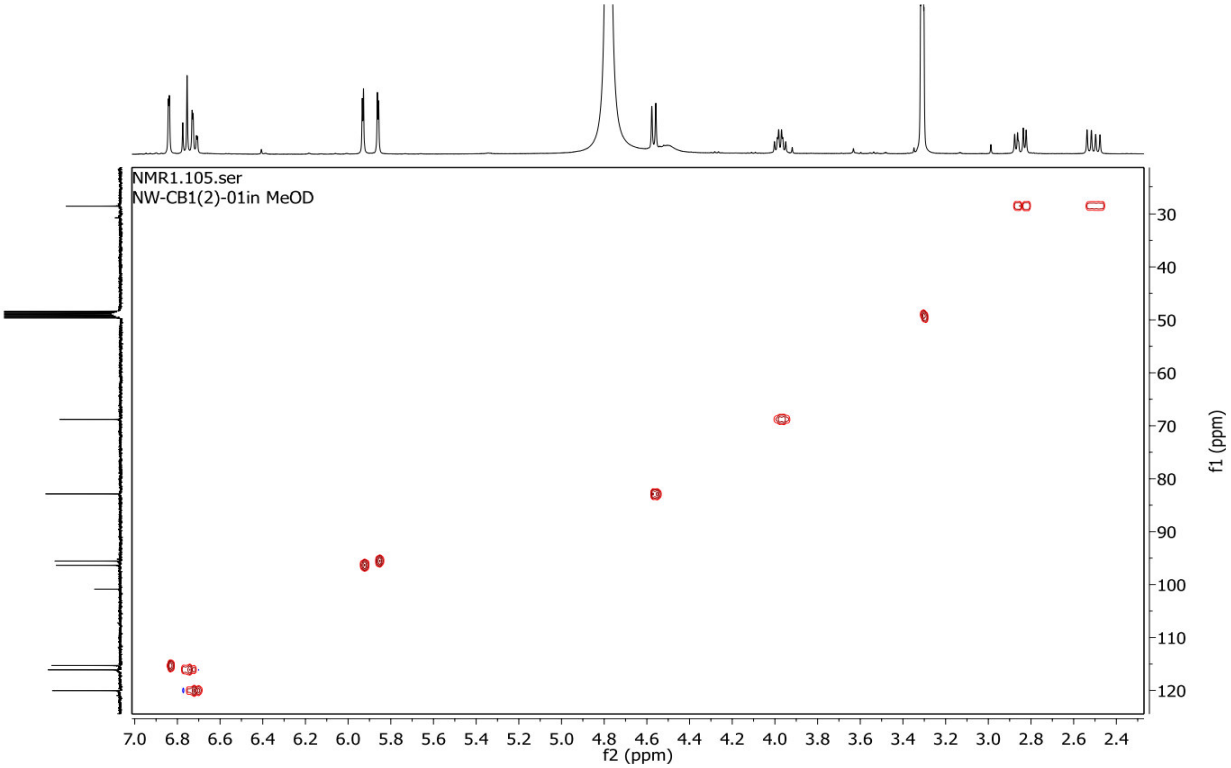
Appendix 12B. The ^{13}C NMR spectrum of catechin (**12**) observed at 500 MHz for MeOD solution at 25 °C.



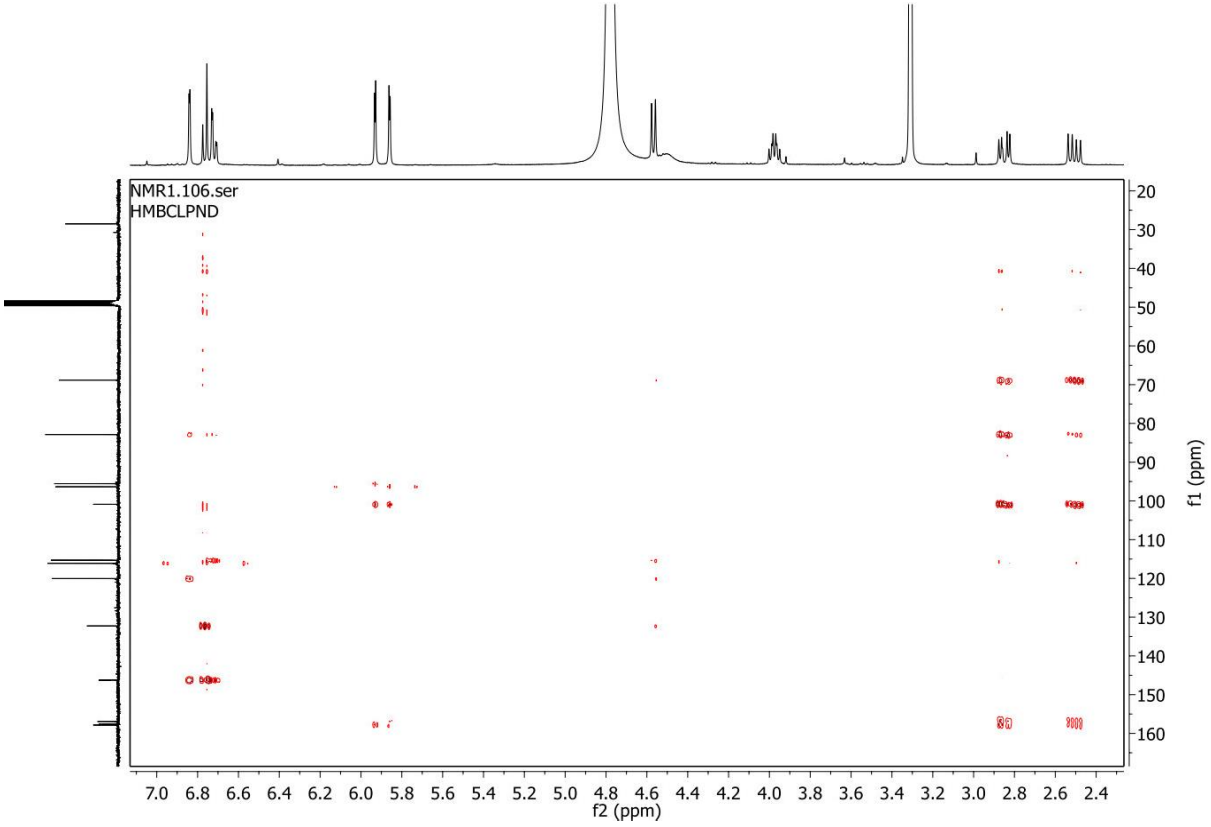
Appendix 12C. The ^{1}H-^{1}H COSY spectrum of catechin (**12**) observed at 500 MHz for MeOD solution at 25 °C.



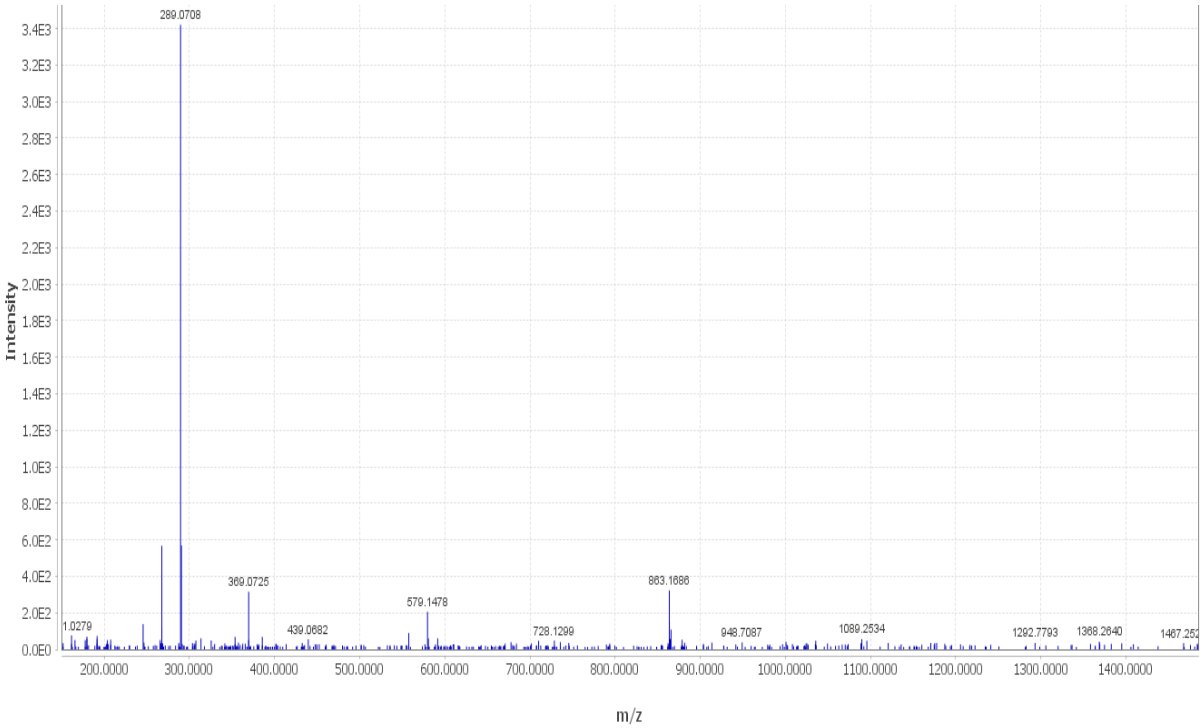
Appendix 12 D. The ^{1}H-^{13}C HSQC NMR spectrum of catechin (**12**) observed at 500 and 125 MHz for MeOD solution at 25 °C.



Appendix 12E. The ^{1}H-^{13}C HMBC NMR spectrum of catechin (**12**) observed at 500 and 125 MHz for MeOD solution at 25 °C.



Appendix 12F. ESIMS spectrum for catechin (**12**).



Appendix 13; Publications from this PhD work.

Publication 1

plants

an Open Access Journal by MDPI

Indexed in: PubMed | CITESCORE 1.8 SCOPUS | IMPACT FACTOR 2.762

CERTIFICATE OF PUBLICATION

Certificate of publication for the article titled:

In Vitro Antimicrobial and Antiproliferative Activities of the Root Bark Extract and Isolated Chemical Constituents of *Zanthoxylum paracanthum* Kokwaro (Rutaceae)

Authored by:

Magrate M. Kaigongi; Catherine W. Lukhoba; Souaibou Yaouba; Nokwanda P. Makunga; Joseph Githiomi; Abiy Yenesew

Published in:

Plants 2020, Vo ...

MDPI Academic Open Access Publishing since 1996

Basel, September 2020

Publication 2

molecules

an Open Access Journal by MDPI

Indexed in: PubMed | CITESCORE 4.1 SCOPUS | IMPACT FACTOR 3.267

CERTIFICATE OF PUBLICATION

Certificate of publication for the article titled:

LC-MS-Based Metabolomics for the Chemosystematics of Kenyan *Dodonaea viscosa* Jacq (Sapindaceae) Populations

Authored by:

Magrate M. Kaigongi; Catherine W. Lukhoba; Purity J. Ochieng'; Malcolm Taylor; Abiy Yenesew; Nokwanda P. Makunga

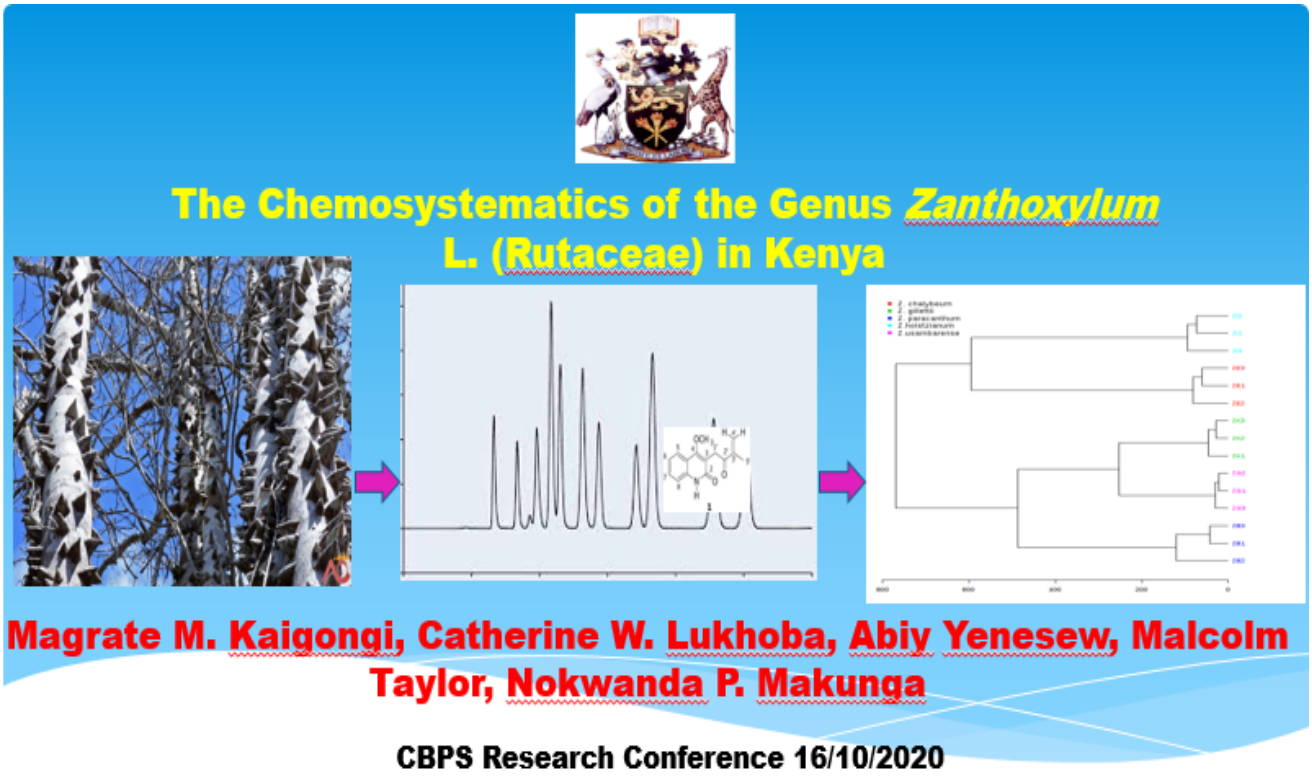
Published in:

Molecules 2020, Volume 25, Issue 18, 4130

MDPI Academic Open Access Publishing since 1996

Basel, September 2020

Publication 3



Publication 4

Kaigongi, M.M., Lukhoba, C. W., Yenesew, A., Taylor, M., and Makunga, N.P. (2020). The Metabolomics of the Five *Zanthoxylum* Species (Rutaceae) in Kenya, *African Journal of Physical Sciences* (In preparation).

Publication 5

Kaigongi, M.M., Lukhoba, C. W., Wachira, P., and Yenesew, A. (2021). Metabolic Profiling and In vitro Antimicrobial Activity of Five *Zanthoxylum* species in Kenya, *Journal of Ethnopharmacology* (In preparation).