

Research Article

Antimicrobial Properties of Some Medicinal Plants of the Luo Community of Kenya

Apollo O. Maima ^{a,*}, Stanley N. Ndwigah ^a, Grace N. Thoithi ^a, Franco N. Kamau ^a, and Isaac O. Kibwage ^a

^a *Department of Pharmaceutical Chemistry, School of Pharmacy, University of Nairobi,*

* **Corresponding author:** Department of Pharmaceutical Chemistry, School of Pharmacy, University of Nairobi, P.O. Box 19676-00202, Nairobi, Kenya. **Tel:** +254-72-2753156; **Email:** aomaima@yahoo.com

Background: The Luo community of Kenya rely mostly on ethno-medicine to manage human ailments. This study was set to survey, record and report the medicinal plant species they use to manage infectious conditions.

Objective of this study was to screen the plants used by this community to treat microbial infections, to demonstrate their *in-vitro* antibacterial and antifungal activities.

Methodology: Eight plants namely *Lannea stuhlmanii*, *Carissa edulis*, *Combretum fragrans*, *Conyza sumatrensis*, *Ormocarpum trichocarpum*, *Sida cuneifolia*, *Plumbago zeylanica*, and *Rhoicissus revoilii*, used by the Luo for treatment of microbial infections, were studied. Observations and semi-structured interviews were used to gather ethnobotanical data for each plant. About 3 kg of suitable specimens were harvested, with leaves pressed and preserved for identification at University of Nairobi's Department of Botany Herbarium. Voucher specimens were also deposited at the University's School of Pharmacy Herbarium and excess material powdered and kept dry. The pressed specimens were dried at 20 °C to 25 °C using plant blower. Their ethanolic extracts were screened for their antimicrobial activity against *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus pumulus*.

Results: Extracts from *Conyza sumatrensis*, *C. fragrans*, *C. edulis*, *S. cuneifolia*, *R. revoilii* and leaf *C. sumatrensis* had good activity against *E. coli*. Activity against *B. pumulus* was observed in all extracts except those of *L. stuhlmanii* bark and *R. revoilii* tubers. Good activity against *S. aureus* was observed with *C. fragrans*, *S. cuneifolia* and *L. stuhlmanii*. *Rhoicissus revoilii*, *L. stuhlmanii*, *C. fragrans* and *C. edulis* exhibited good antifungal activity against *Candida albicans*.

Conclusion: This work partially supports the traditional antimicrobial use of the various plants, and it is hoped that the results will form the basis for further research that could lead to isolation and/or development of antibacterial and antifungal medicines for use in primary health care. The results also confirm that plants are a potential source of antimicrobial compounds.

Key words: Luo; Antimicrobial; Ethanolic extracts; screening

Received: October, 2014

Published: December, 2014

1. Introduction

The Luo community of Kenya have traditionally used plants to treat diseases of microbial origin. These plants include *Lannea stuhlmanii*, *Carissa edulis*, *Combretum fragrans*, *Conyza sumatrensis*, *Ormocarpum trichocarpum*, *Sida cuneifolia*, *Plumbago zeylanica* and *Rhoicissus revoilii*. *Lannea stuhlmanii* Engl bark is usually mixed with *R. revoilii*, boiled and the decoction taken to treat pneumonia, diarrhoea and giardiasis.

Carissa edulis Forsk roots are mixed with *Ximenia americana* L. and *E. divinorum* then boiled and their decoction taken to treat gonorrhoea, boils and indigestion. *Combretum fragrans* F. Hoffm roots and bark are ground and extracted with warm water. This extract is used to treat typhoid and diarrhoea. *Conyza sumatrensis* leaves are dried and ground to make a cream or ointment which is used to treat pimples, *tinea versicolor* and tonsillitis. *Sida cuneifolia* Roxb leaves are dried and ground to a powder. This powder is applied

to cuts, wounds and tropical ulcers. *Ormocarpum trichocarpum* Hiern whole plant is powdered and formulated to a cream or ointment which is used to manage primary syphilitic sores (Obua et al, 2002; Eyhrquist et al, 2002; Kokwaro, 1976; Yenjai et al, 2004; Eloff, 1998; Ramezani, 2004; Setzer et al, 2004; Hoffman, 2004; Camporese et al, 2003; Goskel, 2003; Olivia et al, 2003; Mwangi et al, 2002).

The aim of the study was to screen *these plants* to demonstrate their *in-vitro* antibacterial and antifungal activities which can validate their traditional uses.

2. Materials and Methods

2.1 Plant collection, preservation and extraction

About 10 kg of the plants used in this study were collected from Rarieda, Bondo District, of Nyanza Province in Kenya, in October 2004. Plant identification was done at the Department of Botany Herbarium, University of Nairobi and voucher specimen deposited in the same herbarium. The plant material was oven dried at 45 °C, powdered and kept dry at room temperature until use.

2.2 Extraction procedure

About 25 g of each plant powder was weighed and extracted with 0.5 l ethanol for 3 hr by constantly stirring with a magnetic stirrer. The extract was filtered through filter paper and reduced *in vacuo* to dryness. The extract was then stored at 2-8 °C until used.

2.2 Culturing of test microorganisms

The fungus used was *Candida albicans* HG 392, while bacteria used were *Escherichia coli* NCTC 10418, *Staphylococcus aureus* NCTC 6571 and *Bacillus pumulus* NCTC 10073.

Standardised cultures of bacteria and fungi were sub-cultured overnight then harvested with 5 ml saline solution. The bacteria were sub cultured in tryptone soya agar (TSA) (12.2 g agar made to 100 ml in distilled water), while *C. albicans* was sub-cultured in Sabouraud's dextrose agar (SDA, i.e. 6.6 g agar made to 100 ml in distilled water). The two culture media solutions were sterilized at 121 °C for 15 min then allowed to cool in the laminar flow hood to 50 °C. The TSA solution was divided into three fractions of 100 ml for each organism. To each was added the harvested bacterial suspension. To the SDA solution was added the *Candida* suspension.

2.3 Testing for anti-microbial activity

The suspensions were plated out, 20 ml per plate and left to set. Reservoir wells (diameter 8 mm) of about 50 µl were then punched (6 per plate) at equal spacing round the circumference of the seeded agar. To each well was added 50 µl of test solution, each solution being in duplicate for each organism. The plates were incubated for about 20 hr after which the zones of inhibition (exhibition) were read. There was negative control using zero-dose preparation.

Agar diffusion assay method was used for both fungi and bacteria (Hugo and Russel, 1987; Hewit and Vincent, 1989). Standard concentrations of known antimicrobial agents (0.12 mg/ml nystatin and 0.3 mg/ml chloramphenicol) were used as positive controls.

Antimicrobial screening was done in duplicate for each plant specimen. About 500 mg of the ethanolic extract of each plant was triturated with 1 ml DMSO then made up to 5 ml in distilled water to give a test solution of 100 µg/µl concentration for each fraction. To each well was added 50 µl, giving a total of 5000 µg of extract per well.

Table 1: Zones of inhibition (in mm) of ethanol extracts against *C. albicans*, *E. coli*, *Staph. aureus* and *B. pumulus*

Sample of ethanol extract	Plant part	<i>C. albicans</i> (mm)	<i>E. coli</i> (mm)	<i>Staph. aureus</i> (mm)	<i>B. Pumulus</i> (mm)
<i>Conyza sumatrensis</i>	Leaves	9.8	13.96 ^a	0	16.78 ^a
<i>Conyza sumatrensis</i>	Roots	9.6	26.85 ^a	0	27.84 ^a
<i>Combretum fragrans</i>	Bark	20.0	13.78	14.51	14.41
<i>Carrisa edulis</i>	Roots	19.3	20.22 ^a	13.17 ^a	18.67 ^a
<i>Sida cuneifolia</i>	Whole	12.9	21.36 ^a	14.14 ^a	21.11 ^a
<i>Lannea stuhlmanii</i>	Bark	18.5	11.31	15.30	13.93
<i>Rhoicissus revoilii</i>	Rhizomes	17.5	12.82	12.50	12.18
<i>Plumbago zeylanica</i>	Whole	14.2	0	0	16.22 ^a
Nystatin 0.12 µg/µl in water		21.1	-	-	-
Chloramphenicol 0.3 µg/µl in water		-	13.67	20.76	15.80
Dimethylsulphoxide (DMSO)		0	0	0	0

All readings are means of duplicates. All extracts used at a concentration of 100 µg /µl.

^a: Zones of exhibition (growth enhancement).

Table 2: Zones of inhibition (mm) of *Combretum fragrans* extracts against *Escherichia coli* and *Bacillus pumilus*

Sample	Zone of inhibition in <i>Escherichia coli</i> (mm)	Zone of inhibition in <i>Bacillus pumilus</i> (mm)
Methanol Extract	13.01	14.49
Chloroform Extract	9.11	7.34
Ethanol extract	18.25	17.71
Ethyl acetate partition of ethanol extract	11.47	10.25
Chloramphenicol 0.3 µg/µl in water	21.12	21.04
Dimethylsulphoxide (DMSO)	None	None

All readings are means of duplicates. All extracts used at a concentration of 100 µg /µl.

3. Results

Zones of inhibition readings (in mm) for *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus pumilus* for the test solutions of all the specimen were recorded as in **Table 1**.

From the results, it was observed that *C. fragrans* bark ethanol extract had the highest and broadest antimicrobial activity. *Combretum fragrans*, *C. edulis*, *L. stuhlmanii* and *R. revoilii* extracts (100 µg/ µl) had very satisfactory anti-fungal activity, comparable with that of the standard drug (Nystatin 0.12 µg/µl in water) while those of *S. cuneifolia* and *P. zeylanica* had fair activity. *C. sumatrensis* had the lowest activity. Against *E. coli*, *C. sumatrensis*, *C. edulis* and *S. cuneifolia* enhanced growth. *C. fragrans* had better inhibitory activity than the standard drug. *L. stuhlmanii* and *R. revoilii* had fair activity while *P. zeylanica* had no activity at all.

Against *S. Aureus*; *C. fragrans* and *L. stuhlmanii* had good inhibitory activity, *R. revoilii* had fair activity, *C. sumatrensis* and *P. zeylanica* had no activity, while *C. edulis* and *S. cuneifolia* enhanced growth. Against *B. Pumulus*; *C. sumatrensis*, *C. edulis*, *P. zeylanica* and *S. cuneifolia* all had exhibitory activity. Compared with the standard, *C. fragrans*, *L. stuhlmanii* and *R. revoilii* had favourable inhibitory activity.

Combretum fragrans was selected for further study. The results of bacterial growth inhibition (**Table 2**) showed the highest inhibition with the ethanol extract, followed by the methanol, ethyl acetate and chloroform extracts. The antimicrobial activity of the ethanol extract compared well with the activity of chloramphenicol standard. This means the antimicrobial compound(s) in *C. fragrans* bark powder are fairly polar. However, the methanol, ethanol, ethyl acetate and acetone extracts of *C. fragrans* all formed dark brown insoluble precipitates on exposure to heat and light, making further work on them impossible under the circumstances. The chloroform extract was chosen for further evaluation.

4. Discussion

These results compare very well with the known antimicrobial activities of methanol extracts of *Artemisia* species of Iran (Ramezani et al, 2004) and

Terminalia chebula (Shahidi, 2004a; Shahidi, 2004b) both of the *Combretaceae* family.

Of the 8 plants specimen studied, good justification was found for the use of *R. revoilii*, *L. stuhlmanii*, *C. fragrans* and *C. edulis* in fungal infections caused by *Candida albicans*. Although the use of *P. zeylanica* in the treatment of tonsillitis and pharyngitis by chewing has not been validated through this work, the uses of the other listed plants by the Luo community seem very reasonable. This work has, especially, validated the use of a mixture of *L. stuhlmanii* and *R. revoilii* (in the treatment of diarrhoea and bacterial pneumonia), *C. sumatrensis* (in pimples, tinea versicolor and tonsillitis), *R. revoilii* (in tonsillitis) and *C. sumatrensis* (in cuts, wounds, tropical ulcers and sore throat).

S. cuneifolia and *C. edulis* enhanced bacterial growth generally. This is possibly because they contain significant amounts of nutrients like amino acids and, especially, vitamins. Could they have found use in traditional medicine because of this, or do they enhance immunity? These are questions that need further research to answer. *C. edulis* is traditionally used in conjunction with other herbs, which may have antimicrobial activity. However, *S. cuneifolia* is used alone, as powder, applied on wounds. This is potentially dangerous.

Different *C. fragrans* extracts were screened for their antibacterial activity against *Escherichia coli* and *Bacillus pumilus*. Ethanol extract showed the highest activity, followed by the methanol extract, ethyl acetate extract and chloroform extract, in that order.

The results from this study partially support the traditional antimicrobial use of *C. fragrans*, and it is hoped that the results will form the basis for further research that could lead to isolation and/or development of antibacterial and antifungal medicines for use in primary health care. The results also confirm that plants are a potential source of antimicrobial compounds.

Conflict of Interest declaration

The authors declare no conflict of interest.

Acknowledgements

The authors are grateful to the Deans Committee, University of Nairobi, for funding this study (Grant No. 500 - 655 - 499), Dr. H. Chepkwony of National Quality Control Laboratory for allowing us to use their laboratory facilities, the late Joan Dina Awuor (Nyar Magwambo) for pointing out the medicinal plants studied.

References

- Camporese A, Balick MJ, Arvigo R, Esposito RG, Morsellino N, Simone FD, and Tubaro A (2003). Screening of anti-bacterial activity of medicinal plants from Belize (Central America). *J. Ethnopharmacol.* **87**: 103-107.
- Eloff JN (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? *J. Ethnopharmacol.* **60**: 1-8
- Eyhrquist P, Mwasumbi L, Haeggstrom CA, Vuorela H, Hiltunen R, and Vuorela P (2002). *J. Ethnopharmacol.* **79**: 169-77.
- Goksel K (2003). The antimicrobial activity of essential oils of *Hypericum scabrum*, *Hypericum scabroides* and *Hypericum triquetrifolium*. *Phytother. Res.* **18**: 339-341.
- Hewit W, and Vincent S (1989). Theory and Application of Microbiological Assay, Academic Press Inc., San Diego, USA. Pp. 4-6, 10, 14, 72.
- Hoffman BR (2004). Screening of antibacterial and antifungal activities of ten medicinal plants from Ghana, Pharmaceutical Biology. *Int. J. Pharmacogn.* **42**: 13-17.
- Hugo WB, Russell AD, Eds (1987). Pharmaceutical Microbiology 4th Edition, Blackwell Scientific Publications, London, UK. Pp. 163-170, 204-214.
- Kokwaro JO (1976). Medicinal Plants of East Africa, East African Literature Bureau, Nairobi, Kenya. Pp 17, 22-26, 36, 58, 64-67, 85, 122-129, 156, 177, 203-227, 251-280.
- Mwangi JW, Thoithi GN, Kibwage IO, Zygadlo JA, Lopez ML, Olivia MM, Demo MS, Toyota M and Chalchat JC (2001). Constituents of the essential oil of *Cymbopogon afronardus* Stapf. *East Cent. Afr. J. Pharm. Sci.* **4**: 43-47.
- Obua C, Odyek O, Anokbonggo WW, Apio SK, Waako P, and Ogwal-Okeng JW (2002). *East Cent. Afr. J. Pharm. Sci.* **5**: 33-37.
- Olivia MM, Demo MS, Malele RS, Mutayabarwa CK, Mwangi JW, Thoithi GN, Kibwage IO, Faillaci SM, Scrivanti RL, Lopez AG and Zygadlo JA (2003). Essential oil of *Brachylaena hutchinsii* Hutch from Tanzania: Antimicrobial activity and composition. *East Cent. Afr. J. Pharm. Sci.* **6**: 61-63.
- Ramezani M, Fazli-Bazzaz BS, Saghafi-Khadem F, and Dabaghian A (2004). Antimicrobial activity of four Artemesia species of Iran, *Fitoterapia* **75**: 201-203.
- Setzer WN, Vogler B, Schmidt JM, Leahy JG, and Rives R (2004). Antimicrobial Activity of *Artemisia douglasiana* leaf essential oil. *Fitoterapia* **75**: 192-200.
- Shahidi G, and Bonjar H (2004). Inhibition of clotrimazole-resistant *Candida albicans* by plants used in Iranian folkloric medicine, *Fitoterapia* **75**: 74 -76.
- Shahidi, G, and Bonjar H (2004). Antibacterial screening of plants used in Iranian folkloric medicine, *Fitoterapia* **75**: 231-235.
- Yenjai C, Prasanphen K, Daodee S, Wongpanich V, and Kittakoop P. (2004). Bioactive flavonoids from *Kaempferia parviflora*. *Fitoterapia* **75**: 89-92.