

African Journal of Pharmacology and Therapeutics Vol. 1 No. 1 Pages 14-18, 2012

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Research Article

Antimicrobial activity of organic total extracts of three Kenyan medicinal plants

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Background: Traditional healers have used plants to prevent or treat infectious diseases since time immemorial. Natural products of plant origin are therefore a potential source of novel antimicrobial agents.

Objective: The objective of this study was to establish the antimicrobial properties of three selected medicinal plants as a basis for further research and possible development of herbal antimicrobial products.

Methods: Methanolic and Ethylacetate extracts prepared from different parts of three medicinal plant species - *Fuerstia africana*, *Pentas lanceolata* and *Sericocomposis hildebrandtii* - were screened for antibacterial and antifungal activities using the agar well diffusion method at a concentration of 100mg/ml. The Minimum Inhibitory Concentration (MIC) values were determined for *Fuerstia africana* extracts. Gentamycin sulphate (0.1 µg/ml) and nystatin (0.1 µg/ml) were used as reference drugs for antibacterial and antifungal activities, respectively.

Results: All the extracts tested exhibited antimicrobial activity with methanolic extracts of the aerial parts of *Fuerstia africana* and ethylacetate extracts of *Pentas lanceolata* roots showing the highest activity against *Klebsiella pneumonia*, with an inhibition zone measuring more than 17mm.

Discussion: These results partially support the reported traditional medicinal use of these plants and shows that they are potential sources of new antimicrobial agents.

Key words: *Fuerstia africana*, *Pentas lanceolata*, *Sericocomposis hildebrandtii*, antimicrobial activity

Received: February, 2012

Published: March, 2012

1. Introduction

In recent years, drug resistance to human pathogenic bacteria and fungi has increasingly been reported all over the world (Levy and Marshall, 2004; WHO 2004). Consequently, the increasing prevalence of multidrug-resistant strains of microorganisms raises an urgent need to search for new sources of antimicrobial agents (Sieradzki et al, 1999) alongside other strategies such as regulated and rational use of antibiotics (Hernandez, 2005).

In Africa, traditional healers have for centuries been the main providers of primary health care (Scheinman, 2002), extensively using herbal preparations in their practice. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids, many of which have been found to exhibit *in vitro* antimicrobial properties (Cowan, 1999; Lewis and Ausubel, 2006). Similarly, a number of Kenyan medicinal plants have been shown to possess antimicrobial properties (Matu and van Staden, 2003; Bii et al, 2008; Kareru et al, 2008).

The vast majority of traditionally used medicinal plants have not been adequately evaluated. This study was therefore undertaken to screen organic extracts obtained from three Kenyan medicinal plants for antibacterial and antifungal activity as a basis for further phytochemical studies. The plants to be studied were selected on the basis of ethnopharmacological reports of their use in traditional medicine; this approach is generally considered effective in the discovery of new bioactive agents from higher plants (Kloucek et al, 2005).

Pentas lanceolata (Forssk.) Defleors belongs to the family Rubiaceae. A decoction of the roots or leaves is used topically or taken orally to treat lymphadenitis or boils (Giday et al, 2009). An infusion of the leaves of *Fuerstia africana* T.C.E. Fr (Lamiaceae) is used to treat genital and oral thrush (TICHA 2008) while *Sericocomposis hildebrandtii* Schinz (Amaranthaceae) roots juice is drunk for purgative effect and to treat dysmenorrhea (Kokwaro, 1993).

2. Materials and Methods

2.1 Collection of plant materials

Plant materials (fresh roots and aerial parts) were collected from natural populations of the plants around Magadi, Kajiado District of Kenya in 2007. Authentication was carried out at the Department of Botany Herbarium, School of Biological Sciences, University of Nairobi, where voucher specimens were deposited. The voucher specimen numbers were assigned as follows: *Fuerstia africana* T.C.E. Friers (Lamiaceae): PGK 2007/1; *Pentas lanceolata* (Forssk.) Defleors (Rubiaceae): PGK 2007/2; *Sericocomposis hildebrandtii* Schinz (Amaranthaceae): PGK 2007/3.

2.2 Preparation of extracts

The plant materials were transported to Kenya Medical Research Institute (KEMRI) laboratories and washed thoroughly with running tap water, chopped into small pieces and then dried under shade for a period of 14 days. The dried plant materials were then ground into fine powders using a laboratory grinding mill. The powders were placed in sealed airtight bags, well labelled and stored in the dark at room temperature until extraction.

Fifty grams of the powdered material was then soaked in 500ml of ethyl acetate or methanol, macerated for 24 hr and then filtered using Whatman No.1 filter paper. The filtrates were then dried under reduced pressure using a rotatory evaporator, yielding sticky brown residues that were further dried under a stream of nitrogen for 24 hr and used for the biological assays. For the antimicrobial assay, the extracts were reconstituted using DMSO to give a concentration of 100mg/ml which was the test concentration.

2.3 Test microorganisms and culture media

The following microbial strains were used as test cultures: *Escherichia coli* ATCC 25922, *Staphylococcus aureus*, ATCC 29737, *Klebsiella pneumonia*, ATCC 15380,

Bacillus subtilis, ATCC 441, *Bacillus pumillus*, ATCC 14884 and a fungal strain, *Candida albicans* ATCC 53324. The cultures were obtained from the National Quality Control Laboratory, Ministry of Health, Nairobi, Kenya. Tryptone soy agar and Sabourauds Dextrose agar (Oxoid, Hampshire, England) were used as nutrient media for the growth of bacteria and fungi respectively.

2.4 Sterilization

All the glassware used in the antimicrobial activity studies was sterilized in a Memmert Universal oven (Memmet GmbH and Co, KG, Schwabach, Germany) using dry heat at 150° C for 1 hr while the nutrient media and distilled water were sterilized using a portable autoclave (Dixons surgical Instruments Ltd, Sussex, UK) at 121° C for 15 min before use. The bacteriological wire loop and cork borer were sterilised by flaming using a Bunsen burner flame. All bench work involving use of micro-organisms was carried out in a Bioflow laminar flow cabinet (Vermeulen, L.J. BVBA, Westmalle, Belgium) while a Freezer-1 incubator (Analis, Suarlee, Belgium) was used for incubation of the micro-organisms.

2.5 Antimicrobial assay

Antimicrobial activity was determined using the agar diffusion method (Rojas et al, 2006). The test microorganisms were sub-cultured for 18h in their prescribed nutrient media to obtain working cultures. Nutrient media for growth of the test microorganisms were prepared as per the manufacturer's instructions, sterilized and left to cool to around 50°C. Each of the cultured microorganisms were suspended in 5 ml sterilized distilled water and the suspension inoculated into the respective growth medium so as to produce inoculated agar with about 10⁶ colony forming units/ml. The inoculated nutrient media were then rapidly but carefully poured into 90mm Petri dishes using a 100ml measuring cylinder in such a manner as to deliver 20ml of the seeded agar with a uniform thickness of 3mm in each Petri dish. The seeded agar was allowed to cool so as to set into a firm gel.

Using a symmetric paper template with 6 circles drawn in a hexagonal array, six cylindrical wells were punched in the seeded media using a cork borer (7mm, diameter). The test solutions of the extracts were applied into the wells using a fixed-volume micropipette set to deliver 50 µl per well. A pre-diffusion period of 1 hr was allowed to facilitate diffusion of the applied solutions into the inoculated media before the Petri dishes were incubated for 18 hr at 37 °C for bacterial stains and 35 °C for *Candida albicans*. Gentamycin sulphate (0.1 µg/ml) and Nystatin (0.1 µg/ml) were used as the standard reference drugs for antibacterial and antifungal activities respectively (Hewitt and Vincent, 1989). Negative controls comprised of 50 µl DMSO per well.

Antimicrobial activity was estimated by measuring the zones of growth inhibition (clear zone) surrounding the wells. Diameters of the zones of inhibition were measured using a hand-held electronic digital Vernier Caliper with a precision of 0.1 mm. All tests were replicated three times and the results are given as the means and standard error of the means (SEM) of the

diameters of the zones of growth inhibition. An inhibition zone of 14 mm or greater (including the diameter of the well) was considered as high antimicrobial activity (Phillip et al, 2009).

F. africana extracts were also evaluated to determine the Minimum Inhibitory Concentration (MIC) using the agar well diffusion technique. Serial dilutions of the test extracts were prepared in DMSO to yield solutions of 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 and 0.39 mg/ml. Fifty μ l aliquots of each dilution were introduced into wells in nutrient agar plates already seeded with the standardised inoculums of *Staphylococcus aureus* (Gram-Positive bacterial strain), *Escherichia coli* (Gram-negative bacteria strain) and *Candida albicans* (a fungi). The test plates were incubated under the same condition as the screening stage. The lowest concentration of each extract showing a clear zone of inhibition was taken as the MIC.

3. Results

The antimicrobial effects of the tested organic extracts at a concentration of 100mg/ml are presented in **Table 1**. All the evaluated extracts demonstrated antimicrobial activity against the selected bacterial and fungal strains with inhibition zones greater than 7 mm (diameter of the well). The extracts that exhibited high

antimicrobial activity (zone of inhibition \geq 14 mm) were methanolic extracts of the aerial parts of *F. africana* against all the bacterial strains tested, ethylacetate extracts of *F. africana* aerial parts against *Klebsiella pneumonia* and ethylacetate extracts of *P. lanceolata* roots against all the tested bacterial stains.

Methanolic extracts of *F. africana* aerial parts had the highest activity against *Klebsiella pneumonia* with a zone of inhibition measuring 17.21 ± 0.22 mm, followed by ethylacetate extracts of *P. lanceolata* roots with a zone of inhibition of 17.06 ± 0.28 mm. The antibacterial standard drug Gentamycin ($0.10 \mu\text{g/ml}$) had a zone of inhibition of 23.88 ± 0.01 mm.

Generally, *S. hildebrandtii* extracts showed lower antimicrobial effects compared to the other plant extracts as none of the extracts produced a zone of inhibition more than 12 mm for all the tested microorganisms.

The zones of inhibition produced by the tested extracts against *Candida albicans* ranged from 9.73 ± 0.14 mm to 12.41 ± 0.35 mm. The widest zone of inhibition was produced by methanolic root extracts of *P. lanceolata* while methanolic root extract of *S. hildebrandtii* had the smallest zone of growth inhibition (**Table 1**).

Table 1: Antimicrobial activity^a of *F. africana*, *P. lanceolata* and *S. hildebrandtii* organic extracts at a concentration of 100 mg/ml

Plant species	Plant part	Test extracts	Mean inhibition zone diameters (mm) \pm SEM					
			Kp	Ec	Bs	Bp	Sa	Ca
<i>F. africana</i>	AP	Methanol	17.21 \pm 0.22	14.24 \pm 0.35	15.35 \pm 0.63	15.4 \pm 0.41	15.18 \pm 0.42	11.62 \pm 0.74
<i>F. africana</i>	AP	Ethylacetate	14.55 \pm 0.46	13.17 \pm 0.2	13.9 \pm 0.72	12.16 \pm 0.44	13.09 \pm 0.28	10.62 \pm 0.62
<i>F. africana</i>	R	Methanol	13.10 \pm 0.26	10.4 \pm 0.74	11.64 \pm 0.65	11.45 \pm 0.68	12.09 \pm 0.64	11.77 \pm 0.31
<i>F. africana</i>	R	Ethylacetate	10.96 \pm 0.08	12.08 \pm 0.26	12.32 \pm 0.60	11.18 \pm 0.15	11.39 \pm 0.6	11.37 \pm 0.29
<i>P. lanceolata</i>	AP	Methanol	9.15 \pm 0.18	9.63 \pm 0.11	9.30 \pm 0.08	9.70 \pm 0.12	9.43 \pm 0.05	10.73 \pm 0.43
<i>P. lanceolata</i>	R	Methanol	11.49 \pm 0.11	10.6 \pm 0.07	10.55 \pm 0.13	11.06 \pm 0.06	10.40 \pm 0.05	12.41 \pm 0.35
<i>P. lanceolata</i>	AP	Ethylacetate	10.32 \pm 0.06	9.44 \pm 0.32	9.84 \pm 0.07	9.94 \pm 0.09	9.89 \pm 0.01	10.33 \pm 0.41
<i>P. lanceolata</i>	R	Ethylacetate	17.06 \pm 0.28	14.29 \pm 0.03	15.82 \pm 0.34	15.7 \pm 0.01	14.28 \pm 0.23	12.17 \pm 0.14
<i>S. hildebrandtii</i>	R	Ethylacetate	11.28 \pm 0.09	10.33 \pm 0.06	11.18 \pm 0.20	11.58 \pm 0.28	10.66 \pm 0.18	11.30 \pm 0.30
<i>S. hildebrandtii</i>	AP	Ethylacetate	9.64 \pm 0.05	9.66 \pm 0.18	10.08 \pm 0.09	10.20 \pm 0.05	9.78 \pm 0.02	11.35 \pm 0.20
<i>S. hildebrandtii</i>	R	Methanol	10.38 \pm 0.08	9.98 \pm 0.06	9.85 \pm 0.05	9.34 \pm 0.05	10.16 \pm 0.09	9.73 \pm 0.14
<i>S. hildebrandtii</i>	AP	Methanol	10.69 \pm 0.20	10.32 \pm 0.29	10.37 \pm 0.06	11.52 \pm 0.11	10.39 \pm 0.10	10.01 \pm 0.06
Gentamycin			23.88 \pm 0.01	23.68 \pm 0.12	23.55 \pm 0.04	22.72 \pm 0.01	25.9 \pm 0.01	-
Nystatin			-	-	-	-	-	22.21 \pm 0.02

^a Antibacterial activity depicted by the mean of inhibition zone diameter(mm) \pm Standard Error of the Means (SEM)

AP-Aerial Parts; R-Roots

- Not done

Kp-Klebsiella pneumonia, Ec-Escherichia coli, Bs-Bacillus subtilis, Bp-Bacillus pumillus, Sa- Staphylococcus aureus, Ca-Candida albicans

The MIC values of *F. africana* extracts are shown on **Table 2**. The lowest MIC value (0.39 mg/ml) was exhibited by ethylacetate extracts of the roots against all the tested micro-organisms as well as the methanolic extracts of the aerial parts against *Candida albicans*.

Table 2 Minimum Inhibitory Concentrations (MIC) of *F. africana* extracts against selected bacteria and fungi

Plant part	Type of Extract	MIC values (mg/ml)		
		<i>Sa</i>	<i>Ec</i>	<i>Ca</i>
Aerial parts	Ethylacetate	1.56	1.56	3.125
Aerial Parts	Methanol	6.25	3.125	0.39
Roots	Ethylacetate	0.39	0.39	0.39
Roots	Methanol	0.78	0.78	6.25

Sa- *Staphylococcus aureus*, *Ec*-*Escherichia coli*, *Ca*-*Candida albicans*

4.0 Discussion

Ethnopharmacological reports indicate that the medicinal plants evaluated in this study are traditionally used to treat infections of microbial nature (TICHA 2008, Giday et al, 2009). The results obtained in this study partially support these claims as all the tested extracts demonstrated some antimicrobial effects. These findings demonstrate the potential of these plant species as sources of novel antimicrobial compounds, a potential supported by a recent study that showed that organic extracts of the three tested plant species had low cytotoxicity levels ($CC_{50} > 20\mu\text{g/ml}$) against Vero cell line (Kigonde et al, 2011). An earlier study had also shown that organic extracts of *F. africana* and *P. lanceolata* had low cyto-toxicity levels ($CC_{50} > 20\mu\text{g/ml}$) against cultured KB cells (Koch et al, 2005).

There are no previous reports on antimicrobial effects of the three plant species investigated in this study. Nevertheless, previous pharmacological studies demonstrated *in vitro* antiplasmodial effects of *F. africana* (Koch et al, 2005; Muthaura et al, 2007; Muganga et al, 2010; Kigonde et al, 2011), *P. lanceolata* and *S. hildebrandtii* (Koch et al, 2005; Kigonde et al, 2011).

Phytochemical studies and isolation of bioactive compounds from the plants investigated have been limited. However, an antimalarial abietane diterpene, ferruginol, has been isolated from *F. africana* (Koch et al, 2006). In other studies, chemical investigation on the leaf of *P. lanceolata* revealed the presence of asperuloside, an iridoid monoterpene and a series of iridoid glucosides (Inouye et al, 1988; Schripsema et al, 2007).

There is therefore still a need to conduct detailed bioassay-guided phytochemical studies in the bid to identify and characterize bioactive secondary metabolites which can be used as templates for new drugs development programs or as markers for standardisation of antimicrobial herbal remedies. In

particular, the high activities exhibited by *F. africana* and *P. lanceolata* extracts demonstrate the potential of these plants species as a source of novel, alternative antimicrobial agents.

In conclusion, the results obtained in this study provide a partial rationale for the use of these medicinal plants in traditional medicines. Further studies to establish the safety profiles of the bioactive extracts in an animal model and identification of their chemical constituents which can be used as markers for standardization of antimicrobial herbal remedies are ongoing.

Conflict of Interest declaration

The authors declare no conflict of interest.

Acknowledgements

The authors acknowledge Hannington Mugo for technical assistance with the antimicrobial assays and Patrick Mutiso Kyalo for identification of plant species. The authors also gratefully acknowledge the International Foundation for Science (IFS) for funding this work through project grant F/4199-1 (P.G.K.).

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