

ANTIMICROBIAL AND PHYTOCHEMICAL EVALUATION OF SELECTED MEDICINAL PLANTS IN MERU COMMUNITY OF KENYA

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Abstract

The aim of the study was to evaluate antimicrobial potentiality and establish phytochemical profiles of ten medicinal plants collected from the rural communities of Chuka, Tharaka Nithi County of Kenya. Plant samples were collected, dried, pulverized into a fine powder and extracted with distilled water. Phytochemical screening was carried out qualitatively on the aqueous extracts using standard established procedures. Filter-paper disc-agar diffusion procedure was used to determine the plant extract activity on four bacterial strains and a fungus. *Albizia anthelmintica*, *Entada leptostachya* and *Warbugia ugandensis* extracts were active against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*. *E. coli* was the most susceptible bacteria against all the plant extracts tested except *Harissonia abyssinica*. *Vernonia lasiopus* and *Uvariadendron anisatum* were the least active extracts. Phytochemical investigation revealed the presence of terpenoids, tannins and reducing sugars in all the plants except one. Saponins were found to be present in *A. anthelmintica*, *E. leptostachya*, *Rapona rhododendroides*, and *Warbugia ugandensis*. Steroids were present in seven plants while alkaloids were present in five plants. Condensed tannins, terpenoids and saponins have been reported to have antimicrobial as well as antihelmintic properties, and their presence in most plant extracts investigated in this evaluation could be attributable to them. Susceptibility against *E. coli* and *C. albicans* was significantly comparable to benzathine penicillin and streptomycin. There exist a correlation between antimicrobial activity and phytoconstituents present in the plants evaluated in this study. The type of ailments the plants are claimed to treat by the particular communities can be justified to some extent by the presence of various classes of phytochemicals such as terpenoids associated with antimalarial, tannins and saponins as antibacterial and anthelmintics. In conclusion, the plants evaluated were found to be active against the microorganisms tested. Further investigation for the active phytoconstituents present in these plants can lead to lead compounds for antimicrobial drug development.

Key words: Antimicrobial evaluation, phytochemical profile, medicinal plants, multi-drug resistant strains

1.0 Introduction

Antibiotics are the main basis for therapy provision of bacterial and fungal infections (Khan *et al.*, 2009). Since their discovery, it was believed that that this would eventually lead to the eradication of infectious diseases. On the contrary, overuse and indiscriminate use of antibiotics has led to the emergence of multi-drug resistant strains of several groups of microorganisms (Harbottle *et al.*, 2006; Khan *et al.*, 2009; Wagate *et al.*, 2009), and this has become a global concern (Parekh and Chanda, 2007). This emergence of multidrug-resistant pathogens threatens the clinical efficacy of many existing antibiotics (Parekh and Chanda, 2007). *Escherichia coli*, *Klebsiella pneumoniae*, *Haemophilus* and many other β -lactamase producers have emerged and become a major therapeutic problem in the world today. Multi-drug resistant strains of *E. coli* and *K. pneumoniae* are widely distributed in hospitals (Khan and Musharraf, 2004). These strains are increasingly being isolated from community acquired infections (Akram *et al.*, 2007). *Candida albicans*, also a nosocomial pathogen, has been reported to account for 50-70% cases of invasive candidiasis (Paula *et al.*, 2006). This rapid global spread of resistant clinical isolates implies that the need to find new antimicrobial agents is of paramount importance. In addition, the life expectancy of antimicrobial agents remains a challenge to researchers. Widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (Coates *et al.*, 2002). Hence, this has led to an increasingly shift of attention by researchers to herbal products in search of new

leads to develop better drugs against multiple drug resistant pathogenic microbe strains of clinical origin (Parekh and Chanda, 2007).

Medicinal plants have been recognized as potential sources of new compounds for therapeutic use (Wagate *et al.*, 2009). Findings from researchers and pharmaceutical entrepreneurs have shown and pointed out that ethnobotanically derived compounds have greater activity than compounds derived from random screening and thus a greater potential for novel products developed (Flaster, 1996; Njoroge and Bussmann, 2006). Natural products as pure compounds or standardized plant extracts provide an unlimited opportunities for new drug leads due to their unmatched availability of chemical diversity (Parekh and Chanda, 2007). Natural products have been used in traditional medicine all over the world for thousands of years and they predate the introduction of antibiotics and other modern drugs (Balunas and Kinghorn, 2005; Kareru *et al.*, 2007; Khan *et al.*, 2009; Parekh and Chanda, 2007). Plants are rich in a wide variety of secondary metabolites called phytochemicals such as tannins, alkaloids, and flavonoids that have been found to have antimicrobial properties (Lewis and Ausubel, 2006). Medicinal plants have been reported to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections (de Boer *et al.*, 2006). For example, the essential oil and eugenol purified from *Ocimum gratissimum* has been reported to treat pneumonia, diarrhea and conjunctivitis (Nakamura *et al.*, 1999). These evidences contribute to support and quantify the importance of screening natural products. The aim of the present study was to investigate the antibacterial and antifungal activity of aqueous and methanolic extracts of some selected plants used in the rural communities of Chuka in Meru-South District of Kenya. The extracts were tested against four bacterial strains and *Candida albicans*, a fungus.

2.0 Materials and Methods

2.1 Collection and Preparation Plant Materials

Ten medicinal plant samples (Table 2) were collected from Marembo Village in Chuka, Meru-South District of Tharaka Nithi County in Kenya based on ethno-pharmacological use through interviews with traditional local herbalists and old people of 70 years and above in the area. Information gathered included vernacular names, part used and the method of preparation and administration of herbal remedies. Botanical identity of the plants was done by a botanist from the Department of Botany, Jomo Kenyatta University of Agriculture and Technology, (JKUAT), Kenya. The samples were then chopped into small pieces after thorough washing in running water and air-dried on the laboratory bench at room temperature ranging from 23 to 26°C for four weeks. The dry plant samples were ground into a fine powder using a laboratory mechanical mill.

2.2 Extraction of Plant Material

Extraction was carried out using distilled water and methanol as extraction solvents to obtain respective aqueous and methanolic extracts. Aqueous extraction was carried out by soaking 5g of the plant powder of every plant sample in 100mL hot distilled water for 2 hours. The aqueous extracts obtained were filtered in a Whatman Filter Paper No. 1 and analysed immediately. Methanol extraction on the other hand was carried out by weighing 5g of the fine powders of every plant sample and macerating in 200mL methanol for 4 days at room temperature. The methanol extracts were filtered using Whatman Filter Paper No. 1 and concentrated using a Rota evaporator at 40 °C. The crude extracts were then left to dry in the fume chamber, after which they were stored at 4°C for analysis.

2.3 Phytochemical Analysis

The screening for phytochemicals was carried out qualitatively on the aqueous and methanol extracts using standard established procedures for identifying plant constituents as described by Sofowora (1982) and Harbone (1973). An aliquot of every plant extract was analysed for the presence of saponins, alkaloids, terpenoids, steroids, cardiac glycosides, anthraquinones, tannins and reducing sugars.

2.4 Antimicrobial Analysis

Filter-paper disc-agar diffusion procedure, known as the Kirby-Bauer method (Bauer *et al.*, 1966) was used to determine the drug susceptibility of *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus*

aureus and *Candida albicans*. These microorganisms obtained from the Department of Botany, Jomo Kenyatta University of Agriculture and Technology (JKUAT).

2.5 Media Preparation

Mueller-Hinton nutrient agar was prepared by boiling 56g to dissolve in 2L of distilled water. The agar was autoclaved at 121°C for 15 minutes and left to cool down. It was then dispensed in sterile petri dishes and allowed to solidify. The solid agar was incubated at 37°C for 24 hours. Paper disks were punched from filter papers and autoclaved at 121°C for 15minutes.

2.6 Preparation of Cultures

Bacteria strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans* were sub-cultured in nutrient broth and incubated at 37°C for 24 hours.

2.7 Procedure

Using a pipette, 0.1mL of a 24 hour culture was drawn and placed in the nutrient agar and spread using a spreader. Paper discs were then soaked in each plant extract using a sterile forceps and placed in duplicate on the bacteria in the nutrient agar. They were then incubated for 24 hours, after which zones of inhibition were then measured in millimetres.

3.0 Results

Table 1: Inhibitory potential of aqueous plant extracts against microbial organisms

Plant	Zone of inhibition diameter (mm)				
	Gram positive bacteria		Gram negative bacteria		Fungi
	<i>S. aureus</i>	<i>B. Subtilis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>C. albicans</i>
<i>Vernonia lasiopus</i> --	--		9	10	--
<i>Albizia anthelmintica</i>	11.5	8	10	9	10
<i>Entada leptostachya</i>	11	12.5	8.5	9	7.5
<i>Rapanea rhododendroides</i> 11		7	--	10	--
<i>Warbugia ugandensis</i>	9	9	8.5	9	9.5
<i>Carissa edulis</i>	9	--	8.5	7	--
<i>Senna didymobotrya</i>	--	7	9	7	9.5
<i>Uvariadendron anisatum</i>	9	--	--	9.5	--
<i>Harissonia abyssinica</i>	10	9.5	8.5	--	7
<i>Zanthoxylum usambarensis</i>	--	8	8.5	10.5	8
Benzathine penicillin	38	21	52	11.5	19
Streptomycin	44	40	44	30	16
Distilled water	--	--	--	--	--

Key:

Diameter of disc (6mm), (--) no inhibition, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*

Antimicrobial evaluation findings (Table 1) shows that *Albizia anthelmintica*, *Entada leptostachya* and *Warbugia ugandensis* extracts were active against all the bacterial strains tested (*Staphylococcus aureus*, *Bacillus subtilis*,

Pseudomonas aeruginosa, *Escherichia coli*) and the fungus *Candida albicans*. *Escherichia coli* was found to be the most susceptible bacteria against all the plant extracts under investigation except *Harissonia abyssinica*. *Pseudomonas aeruginosa* was the second most susceptible bacteria. However, *Vernonia lasiopus* and *Uvariadendron anisatum* were the least active extracts. The activity of all the plant extracts tested revealed antimicrobial activity against *E. coli* and *C. albicans* that was significantly comparable to that of benzathine penicillin and streptomycin, the conventional antibiotics used as positive control in our investigations.

On the other hand, the results of phytochemical screening (Table 2) exhibited positive reactions for terpenoids, tannins and reducing sugars in all the plants except *Senna didymobotrya*, *Harissonia abyssinica* and *A. anthelmintica* respectively. Saponins were found to be present in *V. lasiopus*, *A. anthelmintica*, *E. leptostachya*, *Raponae rhododendroides* and *Warbugia ugandensis*. Steroids were found to be present in *A. anthelmintica*, *E. leptostachya*, *W. ugandensis*, *U. anisatum*, *H. Abyssinica* and *Zanthoxylum usambarensis*, while *V. lasiopus*, *A. anthelmintica*, *E. leptostachya*, *R. rhododendroides* and *usambarensis* contained alkaloids. Anthraquinones were found to be present only in *V. lasiopus*, *R. rhododendroides* and *U. anisatum*.

Table 2: Phytochemical profiles of the aqueous plant extracts

Plant species	Phytochemical constituents						
	Saponins Reducing sugars	Alkaloids	Terpenoids	Steroids	Anthraquinones	Tannins	
<i>Vernonia lasiopus</i>	++	+	+	-	+	+	+
<i>Albizia anthelmintica</i>	+++	+	+	+	-	+	-
<i>Entada leptostachya</i>	+++	+	+	+	-	+	+
<i>Raponae rhododendroides</i>	+++	+	+	-	+	+	+
<i>Warbugia ugandensis</i>	+	-	+	+	-	+	+
<i>Carissa edulis</i>	-	-	+	+	-	+	+
<i>Senna didymobotrya</i>	-	-	-	-	-	+	+
<i>Uvariadendron anisatum</i>	-	-	+	+	+	+	+
<i>Harissonia abyssinica</i>	-	-	+	+	-	-	++
<i>Zanthoxylum usambarensis</i>	-	+	+	+	-	+	++

Key:

(-) = absent; (+) = present; (++) = moderately present, (+++) = present in large amount

Table 3: Botanical identification uses and parts of medicinal plants

Plant species	Family	Trivial name	Part used	Therapeutic use
<i>Warbugia ugandensis</i>	Muthiga		Bark	Gonorrhea, tooth ache, malaria, anaplasmosis

<i>Uvariadendron anisatum</i>	Ntonga	Whole	Stomach ache, anaplasmosis
<i>Carissa edulis</i>	Mukawa	Roots	Malaria
<i>Albizia anthelmintica</i>	Mubarwa	Bark	Worms
<i>Harissonia abyssinica</i>	Mutagata	Roots	Malaria, pneumonia, stomach
<i>Zanthoxylum usambarensis</i>	Mugucwa	Bark,	Malaria
<i>Senna didymobotrya</i>	Mwinu	Leaves	Malaria
<i>Vernonia lasiopus</i>	Mucatha	Leaves	Worms
<i>Rapanae rhododendroides</i>			Worms
<i>Entada leptostachya</i>	Mwaritha	Bark/Roots	Worms, chases snakes
<i>Rapanea rhododendroides</i>	Mugeta	Bark	Worms

4.0 Discussion

There is no scientific data on the bioactivity and levels of safety of traditional medicinal plants that are in use today including Kenya, or even how they are likely to affect each other when used as combinations in medicines (Mwitari *et al.*, 2013), yet they constitute an effective source of both traditional and modern medicines (Akinyemi *et al.*, 2005). In addition, research that has been done on their mechanisms of action is scanty, given that their mode of administration is oral (Mwitari *et al.*, 2013). Plants have been utilized as medicines for thousands of years. Initially, they took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations. The knowledge of the specific plants used and the methods of application for particular ailments were passed down the family lineage orally (Balunas and Kinghorn, 2005). Qualitative phytochemical analysis is an important step in drug discovery; it gives information about the presence of any particular class of primary or secondary metabolite(s) in plant extracts that is (or are) of clinical significance (Ganatra *et al.*, 2012). The presence of any phytoconstituents of any significant bioactivity are isolated and investigated further for their activity as lead compounds (Ganatra *et al.*, 2012). In addition, the presence of tannins in all the plants except *H. abyssinica* can be associated with their various antimicrobial properties since the class of condensed tannins have been known to have antimicrobial as well as anthelmintic properties (Athanasiadou *et al.*, 2001). Saponins have also been reported to have antibacterial properties (Avato *et al.*, 2006). Thus, the antimicrobial properties exhibited by *Albizia anthelmintica*, *Entada leptostachya* and *Warbugia ugandensis* extracts can be attributed to saponins found to be present in these. Warburganal and muzigadial isolated from *W. ugandensis* exhibit very potent antifungal properties (Mwitari *et al.*, 2013). Antibacterial properties of *Trichosanthes cucumerina* has been reported to be as a result of carotenoids, flavonoids, lycopene, phenolics and β -carotene present in its seed. The dried seeds of the plant are used as antihelmintic and antidiarrhoeal, and also have the seeds have anti-bacterial, anti-spasmodic and insecticidal properties in India (Reddy *et al.*, 2010).

5.0 Conclusion

There exists a correlation between antimicrobial activity observed, and phytoconstituents present in the plants evaluated in this study. *Albizia anthelmintica*, *Entada leptostachya* and *Warbugia ugandensis* extracts that were found to be active against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and the fungus *Candida albicans*, contained saponins and tannins. The presence of reducing sugars in the three plants implies that the particular classes of saponins and tannins present could be bonded through a glycosidic bond to a

sugar molecule. The various types of ailments the plants are claimed to treat by the particular community can be justified by the presence of various classes of phytochemicals that are known to possess those properties such as terpenoids associated with antimalarial, tannins and saponins as anthelmintics. The antimicrobial potentiality exhibited by these plants evaluated implies and justifies the need for further intensive investigation of these plants in addressing the escalating trend of microbial resistance towards existing antibiotics.

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