The antibacterial activity of some medicinal plants used in Meru Central District, Kenya

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Abstract

Five medicinal plants used by traditional medical health practitioners (TMP) in Meru central district namely: *Piliostigma thonningii*, *Ajuga remota, Ocimum suave*, *Erythrina abyssinica* and *Harissonia abyssinica* were investigated for their antibacterial activity against standard bacterial cultures namely; *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Pseudomonas aeruginosa*. The antibacterial activity of the methanolic and water extracts was determined using the minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC). Gram positive bacteria (*S. aureus and B. cereus*) were more susceptible to the plant extracts than Gram negative bacteria (*E. coli and P. aeruginosa*). The MIC and MBC of the positive control antibiotics (Ampicillin for gram positive and Gentamycin for gram negative) were less than 1mg/ml. The most susceptible bacteria was *S. aureus* followed by *B. cereus* while the most resistant was *E. coli* followed by *P.aeruginosa.* Methanolic extracts of *P. thonningii* stem and *Ocimum suave* leaves had the best antibacterial activity against the four bacterial species. There was no significant difference between the water and methanolic extracts of all the plants. These results justify the use of these plants by the traditional medical practitioners for management of bacterial conditions and further investigation on their safety and phytochemistry is needed.

Introduction

Plants have a long history of use in treatment and management of different diseases all over the world since ancient times and about 25% of current drugs are derived from plants(Wanyoike *et al*., 2004). In certain African countries up to 90% of the population relies exclusively on plants as sources of medicines (Hostetman *et al*., 2000).

The appearance of multidrug resistant pathogens and others with reduced susceptibility to available antimicrobials adds to the urgency for the search of new infection fighting strategies.

The side effects and toxicity of available antimicrobials and their high cost further complicates the current methods of fighting infections. Efforts are now directed towards finding new and innovative antimicrobial agents with novel mechanisms of actions (Bii, 2001; Janovska *et al*., 2003; Runyoro *et al*., 2006).

Natural products of higher plants are probable sources of antimicrobial agents which have added advantages of being safe and biodegradable (Adenisa *et al*., 2000). Plants may serve as natural blue prints in the development of new drugs or as phytomedicines to be used to treat disease (Abubakar *et al*., 2008). Infections associated with bacterial pathogens are among some of the indications for treatment with traditional remedies that include plant products (Njoroge and Busman, 2007).

The purpose of this study was to evaluate the antibacterial activity of some selected plants from Meru Central district used by the herbalist to manage bacterial infections.

Materials and methods

Selection and collection of plant materials

Ethnobotanical survey was carried out in Meru Central district and the herbal practitioners identified the 5 plants used to manage various infections in humans. These plants were collected and botanically identified in Department of Land Resource Management and Agricultural Technology (LARMAT), University of Nairobi. The information provided by the herbal practitioners was compared with available literature on the plants. The selected plants with their local names and their ethnobotanical uses are listed in table 1 below. The aerial parts of the selected parts for this study are shown in plate 1.

Table 1: The plants from Meru Central District selected for study on their antibacterial activity

Family	Plant Species	Local Name	Life form	Part used	Ethnomedical uses
CAESALPINIACEAE	*Piliostigma thonningii*	Mukuura	Tree	Stem bark, Leaves	Cough, colds, chest pains, stomachache, wounds, Menorrhagia
LABIATEAE	*Ajuga remota*	Kirurage	Herb	Leaves	Pneumonia, fever, toothache, dysentery, stomachache, eye infection, tongue infection
	Ocimum suave	Makuri	Tree	Leaves	Blocked nostrils, abdominal pains, sore eyes, ear infections, cough, disinfectant, insecticide
PAPILIONACEAE	*Erythrina abyssinica*	Muuti	Tree	Root bark, Stem bark	Anthrax, syphilis, gonorrhea, burns, body swellings, colic, antidote for poisoning
SIMAROUBACEAE	*Harrisonia abyssinica*	Mutagataga	Shrub	Whole plant	Stomachache, abdominal pains, fever, nausea, vomiting, plague, swollen testicles, dysentery, gonorrhea, tuberculosis

Plate 1: Photographs of aerial parts of *Harrisonia abyssinica* **(a)**, *Ocimum suave* **(b)**, *Erythrina abyssinica* **(c)**, *Ajuga remota* **(d)** and *Piliostigma thonningii* **(e)**

(a)

(b)

(c)

(d)

(e)

Preparation of plant extracts

The plants' parts (whole plant, stem bark, root bark, and leaves) were scrutinized for any foreign matter or moulds, cleaned with distilled water then chopped into small pieces and air dried under shade at the Department of Public Health, Pharmacology and Toxicology, University of Nairobi. The dried plant material was ground using a laboratory mill to fine powders in a fume chamber to protect from fumes and dust during the grinding. The powdered plant material obtained was packed in 500gram portions and placed in clean air tight polythene papers (Gakuya, 2001; Wagate, 2008).

Antibacterial Activity Testing

The methanolic and aqueous extracts were screened for their antibacterial activity. Broth dilution assay was used to screen the extracts for antibacterial activity. The gram positive bacterial strains used were *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 25922). The gram negative bacterial strains were *Escherichia coli* (ATCC 11778) and *Pseudomonas aeruginosa* (ATCC 27853). They were obtained from stock cultures at the Department of Public Health, Pharmacology, and Toxicology, University of Nairobi. The positive controls were antibiotics Ampicillin sodium powder (Flamingo Pharmaceuticals, India) for gram positive bacteria and Gentamycin sulphate (Shangdong Kangtai, China) for gram negative bacteria (Kisangau *et al*., 2007; Koshy *et al*., 2009; Pavithra *et al*., 2010). DMSO 2% was used as negative control (Botelho *et al*., 2007).

Bacteria quantification

Standard microorganisms, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureas* and *Bacillus cereus* were inoculated on blood agar plates from stock cultures and incubated at 37^0C for 24 hours. A colony from each BA plate of the standard microorganisms was emulsified in 3ml physiological saline solution in sterile tubes. Four rows of 10 tubes each with 9 ml of sterile physiological saline solution were made and each row labeled with the standard microorganism to be quantified. Ten fold serial dilutions of the standard microorganisms were made. TSA plates were inoculated with 1 ml from each tube and incubated at 37^0c for 24 hours. Colonies on the TSA plates were counted and quantified. The numbers of colony forming units were 2.2×10^8cfu/ml (Akinyemi *et al*., 2005; Koshy *et al*., 2007).

Determination of MIC and MBC

Test tube method was used in the procedure for determining the MIC and MBC. Standard microorganisms *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus cereus* were inoculated on BA plates and incubated at 37^0C for 24 hours. A colony of each standard microorganism was emulsified in 3ml of sterile physiological saline.

Plant extracts were weighed and mixed in 4ml of sterile Mueller Hinton broth to make a master dilution of 100mg/ml for gram positive bacteria and 250mg/ml for gram negative bacteria. Five (5) culture tubes containing 2ml Mueller Hinton broth each were arranged in four rows for each plant extract. Two fold serial dilutions were made from the master dilution. For gram positive bacteria, the concentrations were made as 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml. For gram negative bacteria, the concentrations were made as 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.625mg/ml and 7.8125mg/ml.

100µl of the test bacteria was inoculated into tubes in the respective rows. The tubes were incubated at 37^0C for 24hours. The culture tubes with the lowest dilution that showed no turbidity were recorded as MIC. This is the lowest concentration that inhibited any visible growth of bacteria. For determination of MBC, 100µl of broth from all tubes that showed turbidity was removed aseptically and then plated on TSA plates using pour plate method. The plates were incubated at 37^0C for 24 hours.

After incubation the lowest concentration of the plant extracts showing no bacteria growth was recorded as the MBC. The MBC is the lowest concentration at which 99.9% or more of the initial bacteria inoculums was killed. All the tests were performed in triplicate (Akinyemi *et al*., 2005; Botelho *et al*., 2007; Dewanjee *et al*., 2007; Pavithra *et al*., 2010; Wagate, 2008)

Results

The MIC and MBC of the test antibiotics were less than 1mg/ml. Overall the MIC and MBC of both the water and methanol plant extracts were not significantly different. When the MIC and MBC of the extracts against the bacteria were compared, there was significant difference between the bacteria species. Gram negative bacteria were more resistant to the plant extracts than gram positive bacteria (Table 2). There was no difference

in activity between the different plants parts used. Methanol extracts of *A. remota*, *O. suave,* *P. thonningii, E. abyssinica* (root bark)*, and H. abyssinica* showed activity against *S. aureus* and *B. cereus.* The water extracts of *E. abyssinica* root bark were active against *S. aureus* and *B. cereus.* However, both the water and methanolic extracts of *E. abyssinica* stem bark did not have activity against *S. aureus* and *B. cereus* at the concentrations tested.

Plant	Part	Extract	Bacteria	MIC (mg/ml)	MBC (mg/ml)
Ocimum suave	Leaves	Water	*P. aeruginosa*	250	250
Ocimum suave	Leaves	Methanol	*S. Aureus*	6.25	12.5
			E. coli	31.25	62.5
			P. aeruginosa	31.25	62.5
Piliostigma thonningii	Leaves	Methanol	*S. aureus*	12.5	25
			E. coli	62.5	125
			P. aeruginosa	62.5	125
Ajuga remota	Leaves	Methanol	*S. aureus*	25	50
			E. coli	250	250
Ajuga remota	Leaves	Water	*B. cereus*	25	50
			E. coli	250	250
			P. aeruginosa	62.5	125
Erythrina abyssinica	Root bark	Methanol	*S. aureus*	3.125	6.25
			B .cereus	50	100
			P. aeruginosa	125	250
Erythrina abyssinica	Root bark	Water	*S. aureus*	3.125	3.125
			B. cereus	12.5	25
			E. coli	250	250
			P. aeruginosa	125	250
Piliostigma thonningii	Stem bark	Methanol	*S. aureus*	3.125	6.25
			B. cereus	3.125	3.125
			E. coli	31.25	3.125
			P. aeruginosa	15.625	31.25
Harissonia abyssinica	Whole plant	methanol	*S. aureus*	6.25	12.5
			B. cereus	6.25	12.5
			E. coli	250	250
			P. aeruginosa	62.5	125

Table 2: MIC/MBC levels of aqueous and methanolic extracts of the selected plants obtained in Meru Central District

Water extracts of *O. suave, A. remota and E. abyssinica* roots were active against *P. aeruginosa. E. coli* was the most resistant bacteria. The most active plant extract against the bacteria species was the stem bark of *P. thonningii* while *E. abyssinica* stem bark showed no activity against the test bacteria and at the given concentrations.

Discussion

The MIC and MBC of the antibiotics were less than 1mg/ml. Overall the MIC and MBC of both the water and methanol plant extracts were not significantly different. This supports the use of the plants by the herbalists since many use water as solvent when needed. There was significant difference between the bacteria species. Gram positive bacteria were more susceptible to the plant extracts than gram negative bacteria. There was no difference in activity between the different plants parts used.

All the plants' parts tested had activity against the test bacteria except the water and methanolic extracts of stem bark of *Erythrina abyssinica*. Wagate, (2008), was also not able to detect antibacterial activity of *Erythrina abyssinica*. However further tests are needed to confirm whether the stem bark of *Erythrina abyssinica* has any antimicrobial activity.

Of importance is that 64.3% of the extracts had activity against *Pseudomonas aeruginosa* which is a major cause of opportunistic infections especially in hospitals. *Pseudomonas aeruginosa* is usually resistant to many antibiotics. The activity of these plants towards *Pseudomonas aeruginosa* should be investigated further. The stem bark of *Piliostigma thonningii* was the most active against all the test bacteria. Ibewuke *et al* (1997) showed that the methanol extracts of *Piliostigma thonningii* inhibited the activity of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis*. This study corroborates those findings and further research is needed to study the antibacterial spectrum of this plant.

Conclusions

Due to the increase in antimicrobial resistance to the available conventional drugs, researchers are now turning to plants and other natural products as sources of drugs. The new drugs are expected to have minimal side effects, be less toxic and provide novel mechanisms of action. Despite the fact that plants form components of every system of medicine in the world, less than 5% of over 250,000 species in the world have undergone any meaningful analysis.

Folklore medicine is a major starting point for this research as it provides a guide to the assumed properties of the plants and other natural products. For research in herbal medicine to be effective, collaboration between the herbal practitioners and conventional health care givers is necessary. The greatest shortcoming of herbal medicine in Kenya is that very few natural products have undergone any scientific validation. Most of the natural products are not well understood with regard to their actions. Besides, the use of these products has not been evaluated in long term use.

Plants contain several constituents whose concentration is what determines the action of the plant. It is possible that toxicity can arise due to synergism of these constituents. Research ought to be carried out to determine the accepted levels of the plants constituents that will not harm the users. This can be possible if studies of the plants are carried out in animal models to show the overall effect of the plant and that of each of its constituents.

All the plants in this study had activity against some or all the bacteria tested. The greatest activity was shown by the methanolic stem bark of *Piliostigma thonningii*. However it is worth noting that the medicinal action of the plants may not be confined to one constituent of a plant.

Even though these plants were active against the test bacteria, their usage against general bacterial infections can only be advocated for after further investigations. For now, the study was able to show some proof that the plants have antibacterial activity and that the herbalists can use them for the specific infections caused by the bacteria that were shown to be susceptible. The herbalists should be guided by use of more scientific evaluations and studies on the plants that showed significant antibacterial activity.

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