Malaysian Journal of Microbiology, Vol 8(4) 2012, pp. 248-252

Antimicrobial evaluation of the methanol bark extracts of *Plumbago dawei* Rolfe, a local species used by the Samburu community, Wamba, Samburu District, Kenya for the treatment of diarrheal ailments

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Received 13 April 2012; Received in revised form 10 May 2012; Accepted 17 May 2012

ABSTRACT

Aims: The Samburu are a marginalized nomadic people in Kenya who have no access to conventional medical services thus they mainly depend on the medicinal plants for most of their medicare. Antimicrobial activity of the commonly used medicinal plant (*Plumbago dawei* Rolfe.) by the Samburu community was investigated to verify claims by locals of its medicinal properties.
Methodology and results: The antimicrobial bioassays of the methanol extracts of *P. dawei* Rolfe was carried out by the disc diffusion method against *Staphylococcus aureus* ATCC 20591, *Bacillus subtilis* local isolate, *Salmonella typhi* ATCC 2202, *Escherichia coli* STD-25922 and *Pseudomonas aeruginosa* ATCC 25852. By use of the micro dilution method MICs and MBCs were also determined. Preliminary phytochemical screening was done on the extracts. The methanol extracts were highly active against all the test strains. The inhibitory zones ranged from 16-25.66 mm. The zones of inhibition were not significantly different except for the *E. coli* (16.33 mm) at $p< 0.05$. The extract showed strong MIC and MBC against *S. typhi*, *S. aureus*, *E. coli* and *P. aeruginosa* (MIC = 9.38 mg/mL and MBC = 9.38mg/mL). Thus the extract was more of bactericidal than bacteriostatic in most test strains. Preliminary phytochemistry revealed presence of flavonoids, tannins and cardiac glycosides.
Conclusion significance and impact of study: The data suggests that methanolic extracts of *P. dawei* could be a rich source of antimicrobial agents. These results give scientific backing for the use of the *P. dawei* Rolfe, barks by the Samburu in the treatment of conditions associated with diarrhea and other associated infections caused by the test organisms.

Keywords: diarrhea, medicinal plants, phytochemicals, antimicrobial activity

INTRODUCTION

The Samburu community is one of those communities that are marginalized in Kenya in terms of 'HEALTH CARE FOR ALL' as a basic human right and prerequisite to social-economic development. The frequent use of medicinal plants by the Samburu for health care is as a result of the unavailability of health care services from the government in this remote region of Kenya (Bussmann, 2006; Omwenga *et al*., 2009). The problem is compounded by high poverty rate, poor sanitary conditions and inadequacy of clean water. For instance, pastoralism is a normal practice of the inhabitants' that leads to sharing of water with both domestic and wild animals. Since the region is neglected, the inhabitants use water without proper treatment as it is scarce most of the year. This has led to an increase in diarrhoreal diseases in the Samburu region (Omwenga *et al*., 2009).

The Samburu people contribute to the estimated 4.6 million people worldwide, including 2.5 million children, who die from diarrhoeal diseases every year particularly in developing countries (Bryce *et al.,* 2005). In the world diarrhea is the second killer of children after pneumonia related cases. The adults are also affected with an estimated incidence of 1.4 episodes/adult/year (Kosek *et al.,* 2003; Omwenga *et al*., 2009). Diarrhea is a killer disease worldwide and is amongst the symptoms of many other diseases (Amabeoku, 2009), which makes the need to control diarrhea very urgent.

In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobials in the treatment of infections (Amabeoku, 2009). It has been noted that microorganisms have the genetic ability to transmit and acquire resistance to antibiotics and have become a major global healthcare problem in the 21st century (Alanis, 2005).These situations are coupled with the undesirable side-effects of certain antibiotics and the emergence of previously uncommon infections. Hence this has forced scientists to search for new antimicrobial substances from various sources including plants (Karaman *et al*., 2003; Aliero *et al*., 2008).

The search for antimicrobials from higher plants is not in vain because it is estimated that over 75% of the antibacterial drugs in clinical use today are of natural origin (Khoobchandani *et al*., 2010). Plant derived

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 ISSN (print): 1823-8262, ISSN (online): 2231-7538

antimicrobial compounds have been observed to inhibit bacteria through different mechanisms and provide clinical value in the treatment of infections caused by resistant microbes (Stein *et al*., 2005). Therefore the plants and herbal extracts have found important position in modern medicine, due to their content in natural medicinal compounds. Their secondary metabolites represent a large reservoir of structural moieties which work together, exhibiting a wide range of biological activities. World Health Organization (WHO) estimates that more than 80% of the world's population is dependent (wholly or partially) on plant-based drugs (Kuete *et al*., 2008; Orwa *et al*., 2008). In East Africa, 90% of the population relies on traditional medicines and traditional health practitioners as the primary source of health care. There is need therefore to evaluate the herbs scientifically for their antimicrobial activity against the antibiotic-resistant microorganisms, in order to develop complementary phytochemical strategies (Simoes *et al*., 2009).

Nature's biosynthetic engine produces innumerate secondary metabolites with distinct biological properties that make them valuable as health products or as structural templates for drug discovery (Kishore *et al*., 2009). In this study, ethnomedical information was collected for a commonly used medicinal plant, *P. dawei* Rolfe. This medicinal plant was chosen because it is widely available and used by the Samburu community at Wamba, Samburu District-Kenya in the management of various ailments among them being diarrhea related illnesses.

MATERIALS AND METHODS

Plant material collection, identification and extract preparation

The barks of *Plumbago dawei* Rolfe were collected from Namunyak conservancy-Wamba, Samburu District, Kenya (Figure 1), in September 2007 based on the ethnobotanical survey that was carried out (Omwenga, 2009). The plant was authenticated by a plant taxonomist from the Plant and Microbial Sciences Department, Kenyatta University, Nairobi, Kenya, in whose herbarium the voucher specimen was deposited. The barks were chopped into small pieces, shade dried and grounded using hammer type milling machine (Meecan, CM/L-1364548, India). The powdered material was transferred into and extracted in the soxhlet extractor using 80% methanol (MW-32.04) for 72 h (Aiyelaagbe and Osamudiamen, 2009). The extracts were filtered through a Whatmann filter paper No. 42 (125 mm) and concentrated using a rotary evaporator (VV 2000 Heidolph, Germany) with the water bath set at 40 °C (Edeoga *et al*., 2005), then dried in a dessicator over anhydrous $CuSO_4$. The powdered residue were transferred into vials and stored at 4 °C in airtight vials before the bioassays.

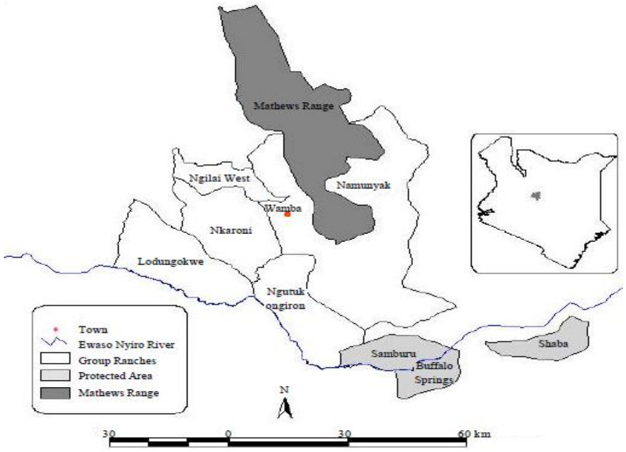

Figure1: Map of Kenya showing the location of Wamba Division and its conservancies.

Antimicrobial screening/ bioassay

Test cultures

Test cultures were obtained from Kenyatta National Hospital in Nairobi-Kenya, which included *Staphylococcus aureus* (Gram positive cocci) ATCC 20591, *Bacillus subtillis* (Gram positive spore forming bacilli) local isolate, *Salmonella typhi* (Gram negative rod) ATCC 2202, *Escherichia coli* (Gram negative rod) STD-25922 and *Pseudomonas aeruginosa* (Gram negative rod) ATCC 25852. All the microorganisms were maintained at 4 °C on nutrient agar slants. Some of the micro organisms were selected on the basis of their natural differences and cell wall properties, but others such as *E. coli* and *S. typhi* were identified in Samburu as actual causes of diarrhea (Omwenga *et al.,* 2009).

Disc diffusion method

The antimicrobial bioassay was performed by agar disc diffusion method for methanol extracts (Meite *et al.*, 2009). In the disc diffusion method, Mueller Hinton agar (Biotec) was prepared following the manufacturer's instructions and was inoculated with 100 µL of the inoculum that was prepared by diluting a 24 h culture of the bacterial type culture or clinical isolate to in normal saline solution to attain a 0.5 McFarland standard. Spread plate method was used to culture 0.1 mL of the microbial suspension that was introduced into the Petri dishes. Then a paper disc Whatmann No. 43 dry sterile disks (6 mm diameter) were soaked in the plant extract (made by dissolving 300 mg of the extracts in 1 mL of methanol) and placed on the spread plates at reasonable distances. Disks were impregnated with methanol and dried (negative control) and various positive controls (amoxicillin 250 mg) were used. The plates were then incubated at 35 °C for 24 h. The procedure was done in triplicate. Microbial growth was determined by measuring the diameter from the end of growth to the disc at one end to the beginning of growth

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

at the other end including the diameter of the disc. The experiment was repeated three times and the mean values recorded.

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

A micro-dilution technique using 96 well micro-plates, (Andrews, 2001; Omwenga *et al*., 2009) was used to obtain MIC values of the crude extracts against all the test bacteria. Each plant extract was serially diluted to obtain a concentration that was ranging from 75 mg/mL to 18.75 mg/mL. Similar serial dilutions were performed for cefrodoxima (250 mg), as a positive control and nutrient broth was used as the negative control. The starting concentration for Cefrodoxima in the first well after the dilution was 75 mg/mL. An equal volume of 50 µL fresh bacterial cultures were added to each of the wells. Micro titre-plates were covered and incubated at 37 °C overnight. The MIC values were determined as the lowest concentrations of the extract showing no growth. All the wells where no growth (not turbid) was observed were sub cultured, and the lowest concentration of the plant extracts that did not yield any colony on the solid nutrient agar after sub-culturing and incubating for 12-24 h was taken as the MBC. All tests were performed in triplicates.

Phytochemical screening

Qualitative phytochemical analysis of the crude powder of the plant collected was determined by established methods (Edioga *et al*., 2005; Jigna and Sumitra, 2007).

Data analysis

Data was analyzed using the Minitab Statistical Software 13.20, © 2000 Minitab Inc. PA 16801-9928, USA. Among the groups, significance test was performed using the one-way ANOVA at 5% significance level.

RESULTS

Table 1 shows the disc diffusion results of the methanol extracts of *P. dawei* Rolfe. The extract was more active on the Gram positive bacteria than the Gram negative bacteria except for *P. aeruginosa* with very high zones of inhibition. The zones of inhibition were not significantly different except for the *E. coli* at $p< 0.05$ against those produced by the positive control. All the zones of inhibition were ≥9.00 mm which made it possible to proceed to MICs and the MBCs. For the negative control no zone of inhibition was observed (6 mm).

Table 2 shows the minimum inhibitory/bactericidal concentrations (MIC and MBC) of the methanolic extract of *P. dawei* Rolfe. against *S. typhi*, *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*. The extracts showed strong MICs and MBCs (9.38 mg/mL respectively) against *S. typhi*, *S. aureus*, *E. coli* and *P. aeruginosa*. Thus the extracts were more of bactericidal than bacteriostatic in all the test strains. The extracts produced lower inhibitory concentration than the positive control except for *P. aeruginosa* where the MIC and MBC concentration was the same (9.38 mg/mL respectively) as that of the positive control.

Table 1: Antimicrobial activity (diameter of inhibition zone in mm) of bark extracts of *P. dawei* Rolfe.

Microorganism	Extract	Positive control	Negative control
Staphylococcus aureus	25.66	18.66	6.00
Bacillus subtillis	24	17.33	6.00
Salmonella typhi	16.00	16.66	6.00
Escherichia coli	16.33	15	6.00
Pseudomonas aeruginosa	21.66	23.33	6.00

Positive controls: amoxicillin for *S. aureus*, *B. subtilis, S. typhi, E. coli* and *P. aeruginosa*. Negative control: dried methanol discs. Values are means of triplicates.

From the preliminary phytochemistry screening, the *P. dawei* extract tested positive for tannins, flavonoids and Cardiac glycosides (Table 3). Saponins, terpenoids and alkaloids were absent.

DISCUSSION

From the study, the activity of *P. dawei* Rolfe extracts against test strains showed that the plant contains pharmacologically active properties (Table 1). Usually a zone of inhibition ≥9.00 mm is an indication of strong antimicrobial activity (Rani and Khullar, 2004). Our data shows in general that the extract had strong antibacterial activity against both Gram-positive test and Gram-negative test cultures. This is because the extract produced zones of inhibition that are greater than 9.00 mm in diameter. The extract produced wider inhibition zones against *S. aureus* (25.66 mm) and *B. subtilis* (24 mm) than their counterparts i.e. Gram negative isolates. These zones of inhibition were not significantly different except for *E. coli* at $p< 0.05$. The activity of the extract could be ascribed to the cell wall properties i.e. unlike Gram positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major components in the outer surface of Gram negative bacteria and could have played a big role towards the general permeability of the two cell walls to the extracts (Govindarajan *et al.*, 2008).

Minimum inhibitory concentrations and minimum bactericidal concentrations produced by the extract against various bacterial test cultures showed strong antimicrobial activity also (Table 2). The findings clearly demonstrates that the methanol extract has more of the bactericidal properties than bacteriostatic since the extract inhibited growth of the test strains at similar concentrations of both MIC and MBC (9.38 mg/mL respectively) except for *B. subtillis.* This concentration was similar to that produced by the positive

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 2: Minimum inhibitory/bactericidal concentrations (mg/mL) of the bark extracts of *P. dawei* Rolfe.

Microorganism	Extract		Positive controls		Negative control	
	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus	9.38	9.38	18.75	18.75		
Bacillus subtilis	9.38	18.75	18.75	18.75		
Salmonella typhi	9.38	9.38	18.75	18.75	Growth observed in all tubes	
Escherichia coli	9.38	9.38	18.75	18.75		
Pseudomonas aeruginosa	9.38	9.38	9.38	9.38		

Positive controls: cefrodoxima for *S. aureus*, *B. subtillis*, *S. typhi*, *E. coli* and *P. aeruginosa*. Negative control: nutrient agar

Table 3: Phytochemical screening test result.

Phytochemical	Concentration
Tannins	++
Saponins	-
Flavonoids	++
Terpenoids	-
Cardiac glycosides	+
Alkaloids (Wagner's test)	-

control against the test isolates. These results suggest that the *P. dawei* Rolfe. could be very promising in the treatment of bacterial related illnesses. Among the Gram negative test strains, the highest activity was observed in *P. aeruginosa*. This was a good finding as *P. aeruginosa* is known to be difficult to be controlled by the commonly used antibiotics because of the cell wall properties (Omwenga *et al*., 2009). Similar activity of the extract and the positive control was observed for *S. typhi* and *E. coli* test cultures. These were also a good finding since these test cultures have been found to be the main pathogens for diarrhea in the Samburu community (Omwenga *et al*., 2009). It clearly demonstrates that the extract could be having modes of action which may help to combat some of the diseases that are caused by these Gram negative bacteria.

This activity of the *P. dawei* may be due to the presence of the screened phytochemicals and those that may be present but whose presence is not known (Table 3). The extract was found to possess flavonoids, tannins and cardiac glycosides among the screened phytochemicals. Flavonoids have been known to form complexes with bacterial cell wall, therefore inhibiting microbial growth (Kuete *et al*., 2008). Also flavonoids (catechins) have been reported to have antibacterial activity by inhibiting the action of DNA polymerase (Chakraborty and Chakraborti, 2010). On the other hand tannins have been reported to have bacteriostatic or bactericidal activities against Gram positive and Gram negative bacteria (Akiyama *et al*., 2001). The tannins do disrupt the cell membranes of the microorganisms by denaturing, hence inhibiting their growth (Akiyama *et al*., 2001). The activity of the extract may also be due to the synergistic activity of the phytochemicals that were found to be present and those that are yet to be known (Ruttoh *et al*., 2009). The ability of *P. dawei* to be sensitive to both Gram positive and Gram negative bacteria is a clear indication of its broad spectrum antimicrobial activity.

CONCLUSION

The findings of this study therefore, back the Samburu community for usage of the *P. dawei* in the treatment of diarrhoeal diseases since the extracts were active against the test cultures especially the ones known to cause diarrhea in the community. However, the mechanism of action of the constituents of *P. dawei* extracts may be difficult to speculate irrespective of the fact that they are likely to provide biologically active constituents which may serve as alternatives to the presently less effective antimicrobials. Thus further studies on the *in vivo* activity, isolation and structural elucidation of the active component(s) and toxicological studies of the plant extract are recommended.

ACKNOWLEDGEMENT

The authors are grateful to the Earthwatch Institute for sponsoring the project and the Earthwatch volunteers from many parts of the world who took their time to join in the collection of information. Most sincere gratitude also goes to local informants and healers like Margaret Nawamarrian, Samuel Legalgoroule, Benjamin Lekarkaraule, and Lekutuka amongst many who shared their knowledge on the use of medicinal plants with us; as well as Mr. Tom Owuor who assisted in plant collection as an intern student. We are also grateful to Philip Loitore a Laboratory Technologist at Wamba Catholic Hospital for the enormous assistance he gave to the project. Special thanks finally go to Wamba Catholic Hospital, Samburu for accepting and giving us bench space for the laboratory work and to Kenyatta University where finer details of the work were concluded.

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