

A comparative evaluation of *in vitro* growth inhibitory activities of different solvent extracts of some medicinal plants in Northern Ghana against selected human pathogens

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ABSTRACT

Bacterial resistance to conventional antibiotics justifies the need to explore alternative remedies from medicinal plants since they represent a rich source of antimicrobial agents. We present a comparative evaluation of *in vitro* antibacterial activities of different solvent extracts of seven medicinal plants used to treat bacterial infections amongst the tribes of Northern Ghana. In the present study, extracts of the plants were obtained using solvents of different polarities and their growth inhibitory activity against *Salmonella typhi* and *Escherichia coli* evaluated *in vitro*. Phytochemical screening revealed the presence of alkaloids, saponins, tannins, coumarins and flavonoids in most of the extracts. These secondary metabolites have been implicated as antibacterial agents in several reports. The extracts from the seven plants showed varied antibacterial activity against the test organisms. Of the bacteria tested, *Salmonella typhi* was the least susceptible to most of the extracts. The ethanol extracts of the plants generally demonstrated superior growth inhibitory activity at 100 and 200mg/ml concentrations while the aqueous extracts were the least active at similar concentrations. The maximum antibacterial activity was recorded for *Khaya senegalensis* ethanol stem bark extract against *Escherichia coli* (zone of inhibition = 20.10mm) and *Salmonella typhi* (zone of inhibition = 17.10mm) at 200mg/ml. The result presents the basis for which these plants have been used for treatment of bacterial infections in folkloric medicine. The results further reveal that ethanol stem bark extract of *K. senegalensis* demonstrated the greatest activity and thus can be very useful in the search for novel antibacterial agents.

Keywords: Solvent extracts, polarity, medicinal plants, antimicrobial assay, higher plants, phytochemistry

1. INTRODUCTION

The use of medicinal plants as a source of for relief from various illnesses has been with mankind since time immemorial. Several cultures across the world have relied on medicinal plants for primary health care. The medicinal value of plants has been attributed to the presence of secondary metabolites in plant extracts. Depending on the type of plant and illness, leaf, bark, roots and sometimes whole plant extracts are used in treatment. Herbal medicine is widely used in Ghana both by rural and urban dwellers. The primary reason is attributed to their proclaimed efficacy, accessibility and affordability compared to conventional drugs.

In recent times, the research community has witnessed increased scientific investigations with results that seem to support the use of plants in folkloric medical settings. In spite of the ever increasing efforts by researchers to discover medicinal potentials of plants, the potentials of many higher plants as source for new drugs is still largely unexplored.

Bacterial infections can be treated with a wide range of antibiotics. Most of these antibiotics are either too expensive or not readily accessible by many people particularly those living in rural communities. Further to this, the development of bacterial resistance to many existing antibiotics is a major concern. These concerns justify the urgent need to discover novel drugs for treatment of bacterial infections.

Medicinal plants represent rich sources of antimicrobial agents [1]. A wide range of extracts from medicinal plant parts are used as raw drugs as they possess varied medicinal properties. While some these raw drugs are collected in smaller quantities by the local communities and folk healers for local use, many other raw drugs are collected in larger quantities and traded on the markets as raw materials for herbal industries [2]. Although, hundreds of plant species have constantly been investigated for potential antibacterial activity, the vast majority of plant species have not been adequately evaluated [3].

Securidaca longepedunculata, *Ficus gnaphalocarpa*, *Blighia sapida*, *Ficus platyphylla*, *Ficus thonningii*, *Khaya senegalensis* and *Pseudocedrela kotschy* are medicinal plants widely used by several tribes in Northern Ghana for the treatment of a wide range of bacterial infections.

P. kotschy (*Schweinf.*) Harms belongs to the Meliaceae family. It is widespread in savannah woodland [4] [5]. *P. kotschy* is a tree of up to 20 metres high with a crown, fissured bark and fragrant white flowers [5]. The bark is bitter and exudes a dark-coloured gum. The root bark of *P. kotschy* is used in Togo as a febrifuge and in the treatment of gastro-intestinal diseases and rheumatism [4]. In Ghana, the twigs and leaves are of value in the treatment of malaria and stomach aches [6]. The twigs are also used as chewing stick in several West African countries. The antimicrobial activity of the stem has been reported [7]. The antibacterial activity of leaf extract has also been reported [8].

F. thonningii, *F. gnaphalocarpa* and *F. platyphylla* are all species of *Ficus*. They are native to tropical Africa and belong to the Moraceae family. The trees grow up to a height of 10 metres with a thick brown stem [9]. The plants are reported to possess some medicinal properties. The leaves, roots fruits and flowers of *F. thonningii* are used in Northern Ghana for the treatment of conditions like diarrhea and vomiting. *F. thonningii* is also used in coastal Nigeria to treat epilepsy. The ethanolic extract of leaves of *F. thonningii* has been reported to exhibit analgesic activity [10]. The antimicrobial effects of the methanol stem bark extract of *F. thonningii* has also been reported [11]. Gastrointestinal activity of *F. platyphylla* was reported by Amos et al. [12].

K. senegalensis is a genus of seven species of trees in the mahogany family Meliaceae, native to Tropical Africa and Madagascar. It is found in many countries across West and Central Africa. The bark is bitter and used in the treatment of common cold in Northern Ghana. It has also been reported to be used for treating diarrhea, syphilis, pyrexia and malarial fever [13]. The seed oil has insecticidal properties [14].

B. Sapida is a member of the Sapindaceae family and native to tropical West Africa. The tree grows up to 12 metres high. The dried seeds, fruit bark and used as herbal remedies in folkloric medical practice. Immature and overripe fruits can be poisonous. Ethnomedical information revealed that members of this family are commonly used for the treatment of boils, ulcers, pain dermatological troubles, wound healing, diarrhea and dysentery [15], [16], [17].

S. longepedunculata belongs to the family Polygalaceae and also known as violet tree. It is commonly used in traditional medicine in many parts of Africa. The violet tree is a small to medium sized tree that grows up to 6 metres high with characteristic pale grey, smooth bark. The root bark are taken orally powdered or as infusions for treating chest complaints, headache, inflammation, tuberculosis, infertility problems and constipation in many communities of Northern Ghana. Some traditional healers also recommend chewing the root to relieve toothache. The antibacterial potential of the root bark of *S. longepedunculata* has been reported [18], [19].

Despite reports on the use of these plants in Ghanaian folk medicine together with reports on medicinal potentials of these plants, information on the comparative evaluation of antibacterial activity of different solvent extracts of these plants are unavailable to the best of our knowledge. Since the amount and quality of active principles in medicinal plants vary according to location, origin and prevailing weather conditions, the current research will reveal the antibacterial potentials of these plants found in Northern Ghana. The overarching aim of this research is therefore to evaluate and compare the antibacterial activities of different solvent extracts of *S. longepedunculata*, *F. gnaphalocarpa*, *B. sapida*, *F. platyphylla*, *F. thonningii*, *K. senegalensis* and *P. kotschy* found in Northern Ghana using *S. typhi* and *E. coli* as test organisms. The information will further reveal the extract with superior antibacterial activity against common human pathogenic bacteria.

2. MATERIALS AND METHODS

2.1 Selection of plant materials

Seven medicinal plants viz., *Securidaca longepedunculata*, *Ficus gnaphalocarpa*, *Blighia sapida*, *Ficus platyphylla*, *Ficus thonningii*, *Khaya senegalensis* and *Pseudocedrela kotschy* were selected based on ethnomedicinal importance. Various parts of the plants were collected around the Tamale municipality in Northern Ghana in December. The parts of plants used, the family name and ethnomedicinal uses are presented in table 1.

Plant name	Family	Local name	Part used	Some uses
<i>S. longepedunculata</i>	Polygalaceae	Paliga	Root bark	Chest pains, headache, inflammation, tuberculosis, infertility, constipation and toothache
<i>F. gnaphalocarpa</i>	Moraceae	Nayutia	Root	Gastro-intestinal activity, wound healing
<i>F. platyphylla</i>	Moraceae	Galinyawu	Root	Gastro-intestinal activity, wound healing
<i>F. thonningii</i>	Moraceae	Gulungon	Root	Diarrhea, vomiting and analgesic
<i>K. senegalensis</i>	Meliaceae	Kuywa	Stem bark	Diarrhea, syphilis, pyrexia and malaria
<i>P. kotschy</i>	Meliaceae	Siyirili	Root bark	Febrifuge, gastro-intestinal diseases and rheumatism
<i>B. sapida</i>	Sapindaceae	Gmaapihiga	Fruit	Boils, ulcers, pain dematological troubles, wound healing, diarrhea and dysentery

Table 1. List of plants under investigation

2.2 Test organisms

Two standard strains of bacteria were purchased at the Komfo Anokye Teaching Hospital (KATH), Kumasi. The bacteria were *E. coli* (ATCC25922) and *S. typhi* (ATCC 700931).

2.2 Extraction from plant material

The selected medicinal plant materials such as root, stem bark and fruits were washed and dried. Adequate quantities were then milled to powder using blender. About 250g of each powdered was accurately weighed and extracted successively with water, ethanol, chloroform, petroleum ether and methanol using soxhlet extractor for 48 hours. Extracts were filtered through Whatman No. 1 filter paper. The extracts were then concentrated using rotary evaporator and then freeze dried until further use. All extracts were screened for phytochemicals and were also subjected to antibacterial activity assay.

2.4 Phytochemical analysis

Phytochemical analysis of all extracts were done for the presence or absence of secondary metabolites such as flavanoids, saponins, coumarins, tannins and alkaloids following the procedures of Harborne (1998) [20].

2.5 Antibacterial activity assay

Antibacterial activity of the various solvent extracts of the plants under investigation was determined by the agar well diffusion method on nutrient agar medium [21]. Wells were made in nutrient agar plate using cork borer (5mm) and inoculums containing 10^6 CFU/ml of bacteria were spread on the solid media with a sterile swab moistened with the bacterial suspension. The dried solvent extracts were reconstituted in dimethylsulfoxide (DMSO) to a concentration of 100mg/ml. About 100 μ l of solvent extract of each plant was placed in the wells made in the inoculated plates. The plates were made in triplicate. Also, DMSO was placed in the wells separately as negative control. The antibiotic Gentamycine (1mg/ml) was used as positive control. The plates were incubated for 24 hours at 37⁰C and the zone of inhibition if any, was measured in millimeter according to reported procedures [22], [23], [24].

3. RESULTS AND DISCUSSIONS

3.1 Phytochemical screening

Preliminary phytochemical screening of the extracts revealed varied composition of phytochemicals in the extracts (Table 2). Of all the plants screened, solvent extracts of SL demonstrated the presence of most of the phytochemicals tested. All solvent extracts of SL revealed the presence of flavonoids, saponins, coumarins, tannins and alkaloids except for the

aqueous and pet ether extracts. Saponins were absent in both aqueous and pet ether extracts of SL while coumarins were absent only in the aqueous extract of SL. The result shows that the phytochemicals present in SL are moderately non-polar. This is in agreement with previously published works [25], [26].

Extracts of FT and PK showed the absence of most of the phytochemicals tested. Alkaloids featured more prominently in most of the extracts than any other phytochemical investigated. Alkaloids have been implicated as antibacterial agents in several reports [27], [28]. Further to this, tannins and saponins which were also found to be present in the extracts have been reported for their antibacterial activity [29]. The result thus, supports the antimicrobial potentials of the plants under study as reported in folkloric medicine.

Of the solvents used, the ethanol extracts generally showed the presence of phytochemicals while the aqueous extracts showed absence of most of the phytochemicals. This result may be attributed to the polarity of ethanol compared with the other solvents used.

3.2 Antibacterial activity

Most of the extracts showed significant antibacterial activity against the test organisms at concentrations of 100mg/ml and 200mg/ml (Table 3). Generally, ethanol extracts demonstrated higher antibacterial activity than extracts of the other solvents. Aqueous extracts of most of the plants showed the least antimicrobial activity. The result is consistent with the results of the phytochemical screening since the ethanol extracts showed the presence of most of the phytochemicals tested.

Of the bacteria tested, *E. coli* was the most susceptible to the extracts. The ethanol stem bark extract of KS was the most effective against the tested organisms. It recorded the highest zone of inhibition of 20.10mm against *E. coli* and 17.10mm against *S. typhi* at 200mg/ml. This is significant compared with the activity of the standard Gentamycin which showed zones of inhibition of 22.30mm and 21.20mm against *E. coli* and *S. typhi* respectively at 40mg/ml. Pet ether and aqueous extracts of PK, pet ether extract of KS, aqueous extract of FT and pet ether extract of FG however did not show any activity against both bacteria at 100mg/ml and 200mg/ml concentrations. These extracts, although contain some of the phytochemicals tested for; their inactivity may be as a result of low concentrations of phytochemicals present. In the crude form, these extracts may also contain other components which hinder their activity against the organisms.

4. CONCLUSION

Medicinal plants are a valuable and readily accessible resource for primary health care. Although large numbers of plants are consistently screened for their antibacterial potentials, many species of plants of medicinal value are yet to be explored. The present study reveals the phytochemical constituents and antibacterial potentials of different solvent extracts of seven higher plants found in Northern Ghana. These plants were selected based on their ethnomedicinal use. Ethanol, water, chloroform, pet ether and methanol were used to extract various parts of *Securidaca longepedunculata*, *Ficus gnaphalocarpa*, *Blighia sapida*, *Ficus platyphylla*, *Ficus thonningii*, *Khaya senegalensis* and *Pseudocedrela kotschyi*. The antibacterial activities of the extracts were evaluated against *E. coli* and *S. typhi* and compared with the standard antibacterial Gentamycin. Although the extracts showed varying degrees of activity against the tested organisms, the ethanol stem bark extract of *K. senegalensis* was found to be the most active against the bacteria used. We conclude that bioactive substances from ethanol extracts of the plants particularly, the stem bark extract of *K. senegalensis* are valuable in the search for novel antibacterial compounds. Further investigations may be carried out on solvent fractions of this extract in order to narrow down to the lead molecule(s) in the stem bark extract of *K. senegalensis*.

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Table 2. Result of phytochemical screening of solvent extracts

Drug	Part used	Solvent	Phytochemicals				
			Flavonoids	Saponins	Coumarins	Tannins	Alkaloids
S L	Root bark	Ethanol	++	++	+	+	+
		Water	+	-	-	+	+
		Chloroform	+	+	+	+	+
		Pet Ether	+	-	+	+	+
		Methanol	+	+	+	+	+
F G	Root	Ethanol	++	++	++	+	+
		Water	+	+	-	+	-
		Chloroform	-	+	+	-	+
		Pet Ether	+	-	-	+	+
		Methanol	+	+	+	+	+
B S	Friut	Ethanol	+	++	++	+	+
		Water	-	-	+	+	+
		Chloroform	+	+	+	+	+
		Pet Ether	+	+	-	-	-
		Methanol	-	-	+	+	+
F P	Root	Ethanol	+	+	++	+	+
		Water	+	-	-	+	-
		Chloroform	-	+	+	-	+
		Pet Ether	+	+	+	+	+
		Methanol	-	+	-	+	+
F T	Root	Ethanol	+	+	+	-	-
		Water	-	+	-	-	-
		Chloroform	-	-	+	-	+
		Pet Ether	+	+	+	-	-
		Methanol	-	+	+	+	+
K S	Stem bark	Ethanol	++	++	+	+	+
		Water	+	+	+	-	+
		Chloroform	+	-	-	+	+
		Pet Ether	+	-	+	-	-
		Methanol	+	-	+	+	+
P K	Root bark	Ethanol	-	+	++	-	-
		Water	-	+	+	-	-
		Chloroform	-	+	+	+	+
		Pet Ether	-	-	-	+	+
		Methanol	-	+	+	+	+

++ = Abundantly present + = Present - = Absent

SL = *Securidaca longepedunculata*, **FG** = *Ficus gnaphalocarpa*, **BS** = *Blighia sapida*, **FP** = *Ficus platyphylla*, **FT** = *Ficus thonningii*, **KS** = *Khaya senegalensis*, **PK** = *Pseudocedrela kotschy*

Table 3. Zones of inhibition of extracts against test organisms

Drug	Part used	Solvent	Zones of inhibition (mm)			
			E. coli		S. typhi	
			100mg/ml	200mg/ml	100mg/ml	200mg/ml
S L	Root bark	Ethanol	15.10	18.20	12.00	15.00
		Water	6.00	8.00	0.00	0.00
		Chloroform	13.30	16.00	10.00	14.30
		Pet Ether	8.30	12.00	6.50	10.40
		Methanol	11.80	15.20	12.40	16.60
F G	Root	Ethanol	11.80	17.20	10.30	16.30
		Water	5.30	6.80	0.00	0.00
		Chloroform	0.00	4.30	0.00	0.00
		Pet Ether	0.00	0.00	0.00	0.00
		Methanol	12.60	15.80	9.00	14.40
B S	Friut	Ethanol	10.80	18.80	12.60	17.00
		Water	8.80	12.00	4.60	7.20
		Chloroform	13.30	16.80	10.20	16.10
		Pet Ether	0.00	4.00	0.00	0.00
		Methanol	5.30	8.00	0.00	0.00
F P	Root	Ethanol	9.40	14.60	10.60	16.90
		Water	4.30	6.20	0.00	0.00
		Chloroform	6.50	10.00	7.00	12.80
		Pet Ether	11.40	19.90	10.00	15.70
		Methanol	5.30	9.20	0.00	0.00
F T	Root	Ethanol	8.00	10.00	7.00	9.30
		Water	0.00	0.00	0.00	0.00
		Chloroform	4.00	6.40	0.00	0.00
		Pet Ether	6.80	10.20	6.20	9.20
		Methanol	5.40	9.60	7.40	11.30
K S	Stem bark	Ethanol	14.20	20.10	12.30	17.10
		Water	8.20	12.40	6.50	10.20
		Chloroform	5.10	8.60	0.00	0.00
		Pet Ether	0.00	0.00	0.00	0.00
		Methanol	6.20	8.10	5.90	7.30
P K	Root bark	Ethanol	5.40	9.80	6.20	10.00
		Water	0.00	0.00	0.00	0.00
		Chloroform	6.00	8.40	4.60	8.00
		Pet Ether	0.00	0.00	0.00	0.00
		Methanol	12.10	14.00	6.00	10.00
Gentamycin (40mg/ml)			22.30		21.20	
DMSO			0.00	0.00	0.00	0.00

SL = *Securidaca longepedunculata*, FG = *Ficus gnaphalocarpa*, BS = *Blighia sapida*, FP = *Ficus platyphylla*, FT= *Ficus thonningii*, KS = *Khaya senegalensis*, PK = *Pseudoceadrela kotschy*

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