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Phytochemical screening and antimicrobial efficacy of the root bark of *Securidaca longipedunculata* extracts

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ABSTRACT

Background: In tropical Nigeria, plant parts are believed to possess phytochemicals that are capable of healing diverse ailments.
Objective: Phytochemical screening and anti-bacterial efficacy of the root bark of *Securidaca longipedunculata* were evaluated in five different solvent extracts.
Methods: Solvent extracts of the root bark of *S. longipedunculata* were prepared with hexane (HE), petroleum ether, methanol (ME), ethanol (ET), and water. Phytochemical screening was conducted on the extracts and the efficacies of the extracts were tested on *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Candida albicans* using agar well diffusion technique.
Results: Phytochemical analysis of the root bark showed the presence of saponins, terpenoids, tannins, and alkaloids. It was observed that the HE extract of the root bark was effective on only *K. pneumonia* and *P. aeruginosa* at a minimum inhibitory concentration (MIC) of 133 mg/ml. The ET extract of the root bark was effective on *P. aeruginosa* and *C. albicans* at a MIC of 80 mg/ml.
Conclusion: Phytochemical examination of the root bark of *S. longipedunculata* revealed the presence of some bioactive compounds. The HE and ET extracts produced considerable antimicrobial effects.

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Introduction

Outbreaks of multi-drug resistance to bacteria are increasing as a result of resistance to antibiotics [1]. In addition, the treatments of widespread of infectious diseases such as tuberculosis, malaria, typhoid fever and influenza are in poverty level in developing countries, including Nigeria. This has made investment and investigations on medical plants more attractive in human healthcare [2] as most of the available formulated medicines are too expensive for the poor populace. In the past decade, there has been renewed interest in the use of medicinal plants for management of several ailments [3]. Also, compounds of plant origin are potential sources of new antimicrobials.

Securidaca longepedunculata is a member of the Polygalaceae. It has oblancelate and obtuse broad leaves that is at apex. The flowers are purple or blue colored and the seeds are winged. They are commonly distributed in the Sudano–Zambezian zone [3]. Different medicinal values have been ascribed to this plant in North-central Nigeria. In Nigeria, the Hausa and the Nupe tribes use *S. longepedunculata*

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ethno-medically as a remedy for numerous ailments [4]. The aqueous extract of the root is used for treatment of ailments such as toothaches, coughs, gout, fevers, constipation, sleeping sickness, and some infection-related diseases [5]. The root is also reported to hold anti-inflammatory properties [6]. The root-methanol (ME) extracts of the plant with methyl salicylate are used as poison in hunt games in West Africa [7]. According to Dapar et al. [8], the aqueous root extracts of the plant are used as psycho-pharmacological agents. The leaf of the plant is used as sexual boost for men and for the management of several ailments such as rheumatism, tuberculosis, cancer, and venereal diseases [9,10]. This may be why the Hausas in Nigeria refer to the plant as *uwar magunguna* translated as "the mother of medicines." The ethno-medicinal usefulness of these plants may be ascribed to several compounds they hold [11]. The extracts from different parts of *S. longepedunculata* have been reported to contain numerous valuable compounds such as xanthones, benzyl benzoates, triterpene, alkaloids, phenols, flavonoids, terpenoids, anthraquinones, and saponin [12,13]. Fresh leaves of the plant can be eaten as vegetable or used to form paste in combination with the bark of *Gardenia erubescens* and *Jussiaea suffruticosa.* This combination is applied externally to manage skin cancer and skin infections [14]. The dried leaves when burnt and inhaled help to reduce and treat headaches. The leaves are sometimes chewed fresh to manage infertility and to expel the placenta [15]. Overall, this plant has relatively low toxicity [13].

Antibiotics are drugs used to treat infectious diseases caused by bacteria [16]. Bacteria are becoming less susceptible to several formulated classes of these drugs as a result of prolonged use and growing misuse by patients [17]. Resistance created by bacteria involves several mechanisms such as efflux of antibiotics, destruction of antibiotics by bacterial enzymes, and the alteration of target proteins [18]. Mechanism to combat resistance requires two or more compounds combined so that they become synergetic as found in daptomycin and ceftaroline, or one potentiating the other as found in amoxicillin and clavulanic acid. Interest in the use of medicinal plants to destroy pathogenic organisms is increasing because these plants are composed of multiple bioactive compounds, which may be effective on these organisms. Akintobi et al. [19] demonstrated antimicrobial efficacy of *Zingiber officinale* (Ginger) extracts. Several other plants have been reported to have antimicrobial potentials [20,21]. Based on the medicinal use of *Securidaca longipedunculata*, this study was screening for phytochemicals and the antimicrobial efficacy of the plant extracts on some human pathogenic bacteria and fungi.

Materials and Methods

Sterilization of glassware

Glass wares used were properly washed with liquid soap and rinsed with tap water (AQ) then with distilled AQ. They were air dried and sterilized in hot air oven at 160°C for 1 hour. Autoclaving of heat destructible wares was done at 121°C for a time frame of 15 minutes. Inoculating wire loops and cock borers used were sterilized by heating red hot using a spirit lamp flame at the regular interval.

Sample preparation

Dried root bark of *S. longipedunculata* was purchased from the Central Market in Ibadan, Nigeria. It was pulverized and extracted with hexane (HE), petroleum ether (PE), ME, ethanol (ET), and AQ at a ratio 3-g root bark to 10-ml solvent. The extracts were concentrated with a rotary evaporator at 40°C and stored at 2°C for further use.

Phytochemical screening

Chemical tests were carried out on pulverized plant samples using standard procedures to identify the phyto-constituents as described by Sofowora [22] and Trease and Evans [23].

Preparation of potato dextrose agar

Potato dextrose agar (PDA) (Oxoid) was prepared according to instructions provided by the manufacturer. Briefly, 39 g of PDA was dissolved in 1 l of distilled AQ, the suspension was homogenized, and then heated in AQ bath for 15 minutes (95°C). The media were well capped in conical flask and sterilized using autoclave. The solution was allowed to cool to 45°C and 500-mg streptomycin was added to inhibit the growth of bacteria prior dispensing into sterile plates.

Preparation of nutrient agar

Nutrient agar (NA) (Himedia) was prepared in line with the manufacturer's instructions. Briefly, 28 g of the agar was dissolved in 1 l of distilled AQ. The suspension was homogenized, well capped in conical flasks, and sterilized using autoclave at 121°C for 15 minutes. The media were later cooled to 30°C and poured into sterile petri plates.

Test organisms

Clinical isolates, pure cultures of pathogenic bacteria obtained from the Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria (A Teaching Hospital): *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and the fungal isolate *Candida tropicalis* were subcultured. The bacterial isolates were cultured on NA and incubated overnight prior to use. The fungal isolate was cultured on PDA and incubated for 5–7 days prior to use. However, all the test strains were initially confirmed by cultural and biochemical characteristics at the Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria.

Antibacterial testing

Antibacterial testing of various solvent extracts of the root bark of *S. longepedunculata* was evaluated using AWD method described by Adeniyi and Ayepola [24]. Each bacterial isolate was cultured on solid NA covering the surface area of the petri dish. A sterile cork borer of 8 mm in diameter was used to bore uniform wells on each agar plate. Each of the wells was then filled with varying concentrations of the extract [25]. Each of the extraction solvent was also maintained on each plate and used as a negative control. The same procedure was carried out for the fungal isolate on PDA. The plates were allowed to remain for 5 minutes at the room temperature for proper diffusion of the extracts. The plates were then incubated for 24 hours at 37°C after which the zone of inhibition was measured and recorded in millimeter. The minimum inhibitory concentration (MIC) was determined by comparing the differences in concentrations of the extracts with the control. The fungal isolate was incubated at 25°C for 24–72 hours.

Antibiotic susceptibility test

Antibiotic susceptibility testing was done using antibiotic discs (Gram-positive and Gram-negative discs) using the disc diffusion method as reported by Bauer et al. [26]. Aseptically, using sterile forceps, each antibiotic disc was placed in the media surface after streaking the pathogenic bacterial isolate on each of the NA plates. The plates were then incubated at 37°C for 24 hours. Thereafter, the zones of inhibition were determined by measuring in millilitre.

Results

The phytochemical analysis of the root bark revealed the presence of saponins, terpenoids, tannis, and alkaloids.

The efficacies of the solvent extracts were tested on *E. coli*, *S. aureus*, *K. pneumonia*, *P. aeruginosa*, and *Candida albicans* using the AWD technique. From the results in Table 1, it was observed that the HE extract of the root bark was effective on *K. pneumonia* and *P. aeruginosa* at a MIC of 133 mg/ml. The ET extract of the root bark was effective on *P. aeruginosa* and *C. albicans* at a MIC of 80 mg/ml.

The antibiotic sensitivity on the test isolates is presented in Table 2. *Escherichia coli* was sensitive to ofloxacin, gentamicin, augmentin, and nitrofurantoin. *K. pneumoniae* was sensitive to ofloxacin and augmentin. *P. aeruginosa* and *S. aureus* were sensitive to augmentin (Table 2).

Discussion

Early studies by various researchers had revealed the presence of phytoconstituents with ranging pharmacological properties capable of exhibiting anti-plasmodial, anti-inflammatory, insecticidal, molluscicidal, and pesticidal activities [10,27–29]. We evaluated the antimicrobial property of root bark of *S. longipeidunculata* using different solvents as liquid of extraction with resultant exhibition of mild antiseptic activity against few selected isolates. This study showed that the aqueous extract of the root bark of *S. longipedunculata* was ineffective on the pathogenic isolates selected for this investigation. These pathogens are known to be the causative agents of various diseases. This is contrary to the reports of Ashidi et al. [30], substantiated by Ogunmefun and Gbile [31] that the aqueous extract of the root bark decoction of medicinal plants is able to cure diseases such as gonorrhea, fungal infections, and pneumonia. The antioxidant properties of the root bark extract of the plant has been reported to be much lower than in the leaves [32].

The methanolic, ethanolic, aqueous, and PE extracts of *S. longipedunculata* were ineffective on *K. pneumonia.* Fromtling et al. [33] reported that *Candida tropicalis* is taxonomically close to *C. albicans* and they share many pathogenic traits except that, in geographical distribution, the former predominates with wider occurrence. They are common cause of candidaemia, oral and vaginal thrush [34]. Only the ET extract of *S. longipedunculata* was effective on *Candida tropicalis* and was at a

Table 1. Effects the solvent extracts of the root bark of *S. longipedunculata* on microbial isolates.

Isolate Used	Solvent of Extraction	Undiluted sample 20g/100ml solvent i.e 200mg/ml	10ml sample + 5ml solvent i.e 133.3 mg/ml	10ml sample + 10ml solvent 1.e 100mg/ml	10ml sample + 15ml solvent i.e 80mg/ml
E. coli	HE	-	-	-	-
	Pet. Ether	-	-	-	-
	ME	-	-	-	-
	ET	-	-	-	-
	Aqueous	-	-	-	-
S. aureus	HE	-	-	-	-
	Pet ether	-	-	-	-
	ME	-	-	-	-
	ET	-	-	-	-
	Aqueous	-	-	-	-
K. pneumonia	HE	30	10	-	-
	Pet ether	-	-	-	-
	ME	-	-	-	-
	ET	-	-	-	-
	Aqueous	-	-	-	-
P. aeruginosa	HE	16	10	-	-
	Pet. Ether	-	-	-	-
	ME	-	-	-	-
	ET	36	26	25	12
	Aqueous	--	-	-	-
Candida albicians	HE	-	-	-	-
	Pet. Ether	-	-	-	-
	ME	-	-	-	-
	ET	20	18	15	11
	Aqueous	-	-	-	-

Diameter of zones of inhibition was measured in millimeter.

concentration of 80.0 mg/ml. The other solvent extracts of *S. longipedunculata* were ineffective on this isolate. Similar to the work by Fernandes et al. [27], antiparasitic property of the aqueous extract of root bark of the plant was ineffective on *Trichomonas vaginalis* at about 10 mg/ml MIC. Our results are in agreement with some other reports that the ME extract and acetone extract of the root bark of the plant inhibit the growth of *C. albicans* at MICs of 40 and 25 mg/ml [32,35,36]. The MIC is defined as the lowest concentration at which about 75% or more of the inocula are rendered in-active. This our current study shows that about 50% of the isolates used reacted with HE and ET extracts of *S. longipedunculata* at 100 mg/ml and 80 mg/ml MIC, respectively. In this investigation, the aqueous extract of *S. longipedunculata* showed no inhibition. This contradicts the reports of Ndamitso et al. [10] that the aqueous extract of the root of *S. longipedunculata* actively inhibited *Salmonella typhii* at 75 mg/ml MIC. Similarly, studies have independently shown that ME and HE extracts from the bark of *S. longipedunculata* inhibited *S. aureus*, *Streptococcus* pyogenes, *Pseudomonas fluorescens,* and *K. pneumonia* at 75 and 100 mg/ml MIC [37,38]. Musa et al. [38] opined in his conclusion that the ability of plant extract to inhibit microorganism in AWD *in vitro* assay is dependent on a number of factors

Table 2. Antibiotic sensitivity of bacterial isolates.

S/N	Antibiotics	Code of the disk	Concentration	Isolate used			
				E. coli	*K. pneumonia*	*P. auruginosa*	*S. aureus*
1.	Ofloxacin	Ofl	5 µ	16	17	R	R
2.	Gentamycin	Gen	10 µg	12	R	R	R
3.	Augumentin	Aug	30 µg	-	-	-	-
4.	Nalixidic acid	Nal	30 µg	R	R	R	R
5.	Nitrofurantin	Nit	200 µg	-	R	R	R
6.	Cotrimoxazole	Cot	25 µg	R	R	R	R
7.	Amoxicillin	Amx	25 µg	R	R	R	R
8.	Tetracycline	Tet	25 µg	R	R	R	R
9.	Cloxacillin	Cot	25 µg	R	R	R	R
10.	Erythromycin	Ery	5 µg	R	R	R	R
11.	Streptomyscin	Str	10 µg	R	R	R	R
12.	Chloramphenicol	Chl	10 µg	R	R	R	R
13.	Zinnacel	Z	20 µg	R	R	R	R

Diameter of zones of inhibition/clearance was measured in millimeter; R = Resistance.

such as concentration of inocula number, type of culture media used, solvent of extraction, and diffusion ability of the extract.

The antiseptic properties possessed by the root bark of this plant using HE or ET as solvent of extraction showed that half (50%) of the bacterial isolates used were sensitive to the bio-constituents of the extracts, a result that made it difficult to agree with the anti-bacterial efficacy of the aqueous extract. The combination powdered extract of root bark of *S. longipedunculata* with powdered extract from other plants as antibacterial cannot be over-emphasized. Mustapha [14] reported that the dry root bark of *S. lonpenduculata* boiled with root bark of *Anona senegalensis* in AQ can be used traditionally to cure pneumonia, a pulmonary lung inflammatory disease traceable to be caused by *K. pneumoniae* and *Streptococcus pneumonia.* Also, if dry root bark of *S. longipendunculata* is soaked in AQ with *Citrus aurantifolia*, the resulting juice taken orally for 3 days can be used to treat constipation, severe headache, cough, and pneumonia. The results from this research study failed to establish the anti-bacterial properties of the aqueous extract of the root bark of the plant. This is not in association with the claim by earlier works that supported the lone use for folk medicine. However, the traditional application of the mixture calls for further studies on the antimicrobial efficacies of the phyto-constituents of other plants that can be combined with it.

Conclusion

This study shows that the root bark of *S. longipedunculata* contains phytochemicals such as saponins, terpenoids, tannis, and alkaloids. The HE extract of the root bark of *S. longipedunculata* was effective on *K. pneumonia* and *P. aeruginosa* at a MIC of 133 mg/ml, while the ET extract of the root bark was effective on *P. aeruginosa* and *C. albicans* at a MIC of 80 mg/ml. The aqueous extract does not possess antimicrobial efficacy.

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