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Research Article

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Phytochemical screening and antimicrobial activity of solvent fractions of *Securidaca longepedunculata* (Fresen) root bark methanol extract

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ABSTRACT

The exhaustive extraction of Securidaca longepedunculata (Fresen) root bark harvested few days after rainy season with methanol gave an extract yield (19.82 %), which was then exhaustively fractionated with solvents of increasing polarity namely- n-hexane, chloroform, and ethyl acetate. The remaining residue after solvent fractionation was regarded as methanol fraction. These four fractions were analyzed for their antimicrobial activity against some multidrug resistant pathogenic microorganism and phytochemical screening was carried out to determine the secondary metabolites in each fraction. The antimicrobial analysis revealed that the chloroform fraction was the most active with mean zone of inhibition (23 mm) followed by methanol fraction (21 mm), ethyl acetate fraction (19 mm) and hexane fraction (15.5 mm). The chloroform fraction was highly potent against methicillin resistant-Staphylococcus aureus (MRSA), Streptococcus pyogenes, vancomycin-resistant Klebsiella pneumonia and multidrug resistant Pseudomonas aeruginosa and Pseudomonas fluorescens. Phytochemical screening revealed that cardiac glycosides, flavonoids, tannins, and saponins are present in the entire fractions, alkaloids were present in methanol and ethyl acetate fractions only, tannins are present in all the fractions except hexane fraction and anthraquinones were absent in the entire fractions. The presence of these secondary metabolites accounts for the antimicrobial activities of the fractions obtained from the root bark extract of this plant and also, justified the claimed traditional medicine use in the treatment of bacterial infections.

Keywords: *Securidaca longipedunculata*, Agar well method, solvent fractions, MRSA, antibiotic resistant.

INTRODUCTION

Antibiotics are drugs used to treat infections caused by bacteria, fungi, protozoan and other harmful microorganisms. Due to the ease of production of antibiotics in standardized forms, it has become increasingly popular worldwide for the treatment of different kinds of diseases. However, the downside of using antibiotics is that many bacteria, which were previously susceptible to the antibiotics, have now become resistant, especially *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* [1,2], *Klebsiella pneumoniae* [3], methicillin resistant-*Staphylococcus aureus* particularly the strain implicated in nosocomial infections [4,5] and *Escherichia coli*, *Streptococcus pneumoniae*, *Haemophilius influenzae* [6]. This incidence of antibiotic resistant of some pathogenic microorganisms arises due to prolong use and growing misuse of antibiotics by patient [7,8]. Therefore, to remedy this problem of inability of antibiotics to successfully kill pathogenic bacteria and fungi, people in both developed and developing countries are now not only embracing herbal medicine but are using it as effective drugs for their primary healthcare needs [9,10].

Based on the foregoing disclosure, clinical microbiologists, natural product chemist and other scientists worldwide are continually contributing to the development of plant based antimicrobial agents. To this end the aim of this paper is to report the research findings of the effectiveness of *Securidaca longepedunculata* as a plant with potential antimicrobial agents capable of treating drug resistant strains of some pathogenic bacteria, which include methicillin resistant- *Staphylococcus aureus* (MRSA). *Securidaca longepedunculata* belongs to the family Polygalaceae and it is extensively distributed in West and East Africa. Also, it is found in the Zambezian zone of Africa. In East Africa because of over harvesting the plant is almost going into extinction [14,15]. The plant is called *Uwar maguguna* by the Hausa speaking people in Nigeria and other countries in tropical West Africa. The literal meaning of *Uwar maguguna* is mother of all medicine. It is used to treat ailments like headaches, rheumatism, diabetes, cancer, tuberculosis, veneral diseases, abortifacients and so many diseases. The plant parts mainly used to treat diseases are roots, stem bark and leaves [15,16]. Previously, the antimicrobial activity of the root bark of this plant has been investigated with the extraction solvent used that include 70 % ethanol or aqueous ethanol [11,12], ethanol, acetone, and distilled water [13]. Solvent fractions of the pure acetone extract, which include hexane, dichloromethane, ethyl acetate, n-butanol, aqueous methanol and water were collected. However, only the antimicrobial activities of dichloromethane, hexane, n-butanol and ethyl acetate fractions were investigated and reported [13]. This present study reports findings of the antimicrobial activity and phytochemical screening of fractions obtained from the fractionation of the methanol extract of *S. longepedunculata* root bark.

EXPERIMENTAL SECTION

Collection of plant materials: The fresh roots of *Securidaca longepedunculata* was dug out from the soil near Kufena mountain, Wusasa, Zaria in October, 2010. The plant was identified by Mallam Mohammed Sule Gallah of the Herbarium Unit of the Department of Biological Science, Ahmadu Bello University, Zaria – Nigeria by comparison with a voucher specimen number 900149 of the plant deposited in the herbarium unit. The bark of the roots was peeled off with a table knife and debris removed with a small brush and then air-dried for about 4 days. The dried root bark was grinded with a pestle and mortar into coarse powder and stored in the dark with a big air-tight glass container until when used.

Extraction of plant materials: The coarse powdered root bark of *Securidaca longepedunculata* (1 kg) was extracted with (4 dm^3 x 3) methanol at ambient temperature for 12 days (each 4 dm^3 of methanol was used for 4 days). After each extraction period, the extract was filtered with a Whatman 1 filter paper and then combined. The combined extract was concentrated in vacuum with a rotary evaporator at 40 ^oC. The extract was then dried further in a desiccator to constant weight.

Fractionation of the crude methanolic extract: The methanol extract (190 g) of *Securidaca longepedunculata* root bark was defatted by soaking it in n-hexane (500 cm^3 x 4) at ambient temperature and allowed to stand for 2 days to give n-hexane fraction, which was filtered with a Whatman 1 filter paper and concentrated under vacuum with a rotary evaporator. The air dried residue from n-hexane solvent fractionation was further fractionated with (500 cm^3 x 4) each of the following solvents of increasing polarities namely- n-hexane, chloroform, and ethyl acetate, in the same manner as described for n-hexane fraction . The air dried residue from the preceding solvent fractionation is used for the next solvent to ensure very good fractionation efficiency. The remaining air-dried residue after n-hexane, chloroform and ethyl acetate fractionation had been carried out is taken as methanol fraction.

Antimicrobial Activity Test

Preparation of test samples: Each of the hexane, chloroform, ethyl acetate and methanol fractions of the plant root bark was dissolved in dimethylsulfoxide (DMSO) solvent. As a precaution not to miss trace amount of antimicrobial agent for preliminary screening, a relatively high concentration of 200 mg/ ml of each fractions were prepared for bioassay.

Test microorganisms: The hexane, chloroform, ethyl acetate and methanol fractions of the plant were screened against multidrug resistant six clinical bacteria isolates, which include Gram positive bacteria namely- methicillin resistant-*Staphylococcus aureus* (MRSA), *Streptococcus pyogenes* and Gram negative bacteria namely- *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. *Candida albicans* was the only fungus screened in this experiment. The clinical bacteria isolate and fungus were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Shika, Zaria – Nigeria. The bacteria were rejuvenated on Mueller Hinton agar medium (MHM, Merck, Germany) and subculture as needed.

Agar well diffusion method: Mueller Hinton agar (blood agar) was the medium used as the growth medium and was prepared according to manufacturer's instructions, the medium was dissolved into sterile distilled water in conical flask capped with cotton wool and boiled to dissolved on Bunsen burner, the medium was sterilized at 121

^{o}C for 15 min, cooled to 45 ^{o}C and then 20 cm^3 of the sterilized medium was poured into sterile Petri-dishes and then allowed to cool and solidify. These were then used for antimicrobial activity bioassay.

For the bioassay experiment, bacteria or fungus suspension of approximately 1.5 x 10^8 cfu/ cm^3 in sterile normal saline were prepared as described in a previous standard procedure [17] as follows. About 1.5 cm^3 of the bacteria or fungus suspension was uniformly seeded on Mueller Hinton agar in 12 x 1.2 cm glass Petri dishes, left to stand for 15 minutes and excess of suspension was then drained and discarded properly. Wells of 6 mm diameter and about 2 cm apart were punctured in the culture media using sterile borers. Respective concentrations of the medicinal plant fractions were administered to fullness in each well. Culture plates were incubated at 37 ^{o}C for 48 hours and then after 48 hours bioactivity was determined by measuring the diameter of inhibition zones in millimeters (mm) using a transparent ruler. Bioassay experiment against each bacteria or fungus was done in triplicates and the mean of the diameter of inhibition zones was calculated. Controls included use of solvent without test fractions, although no antibacterial activity was noted in the solvent used to prepare the plant solvent fractions solution used for the experiment.

Phytochemical Screening: a qualitative chemical analysis of hexane, chloroform, ethyl acetate and methanol fractions was carried out to detect the presence of some classes of secondary metabolites or natural products like alkaloids, tannins, flavonoids, saponins, anthraquinones and cardiac glycosides using standard procedure as follows. Wagner reagent test for alkaloids, ferric chloride test for tannins, ammoniacal silver nitrate test for flavonoids, Salkowski and Liebermann-Burchardt's test for saponins, Borntrager's test for free and combined anthraquinones, Legal test/ sodium nitroprusside test for cardiac glycosides, and Benedict and Fehling's test for reducing sugars [18,19].

RESULTS AND DISCUSSION

In this research work the initial bulk extraction with methanol as solvent gave a methanol extract of 19.82 % yield, which was higher compared with the 1.45 % yield of methanol extract reported previously [20], 1.74 % yield of acetone extract [21], 5.0 % yield, after 2 hours extraction with methanol [22], and 10.90 – 12.59 % yield, after 48 hours extraction with methanol [23]. This development is likely attributable to the differences in time of the season when the root was harvested and/ or whether only the root bark was used or it was the complete root (which is made up of the inner cortex and root bark) that was used. The high percentage yield of the methanol extract obtained in this research work is likely due to debarking of the root and then the root bark was used for the extraction. Also, it could likely be due to the harvesting of the plant roots in month of October, 2010, few days after rainy season in Zaria, Nigeria. In addition, the duration of extraction period, plays a significant role in improved extract yield because exhaustive extraction was carried out for up to 4 days in this study. However, this yield is lower compared to when distilled water (35.80 % yield) was used as reported in the literature [24], because distilled water normally extract mucilage and resins in addition to the secondary metabolites that other non-polar and polar solvents would usually extract. The use of distilled water is strongly discouraged since it could lead to the decomposition of precious secondary metabolites with immense therapeutic value, especially, if it is concentrated on a steam bath at high temperatures [25].

The methanol extract of *S. longepedunculata* root bark was subjected to solvent fractionation with solvents of increasing polarities in order to reduce the complexities of the extract to facilitate ease of compound isolation and also give a better understanding of the polarities of not only the solvent fractions but also that of the isolated compounds. It also facilitates understanding of the most bioactive fraction polarity. The result of this experiment showed that the methanol fraction, which is the most polar was obtained in the highest yield, while the hexane fraction has the least yield (see Table 1). This is an indication that the methanol fraction contains more components compared to the other three fractions.

Table 1. Weight of fractions from fractionation of initial crude methanol extract

Fractions	Weight (g)	% Yield
Hexane	5.23	2.75
Chloroform	9.81	5.16
Ethyl acetate	12.85	6.7
Methanol	162.07	85.3

In the antimicrobial activity assay, the entire six pathogenic bacteria assayed were susceptible to the four fractions while the fungus *Candida albicans* was resistant to the entire fractions tested as shown in Table 2. Interestingly, it can be observed that chloroform and methanol fractions inhibit methicillin resistant – *Staphylococcus aureus* (MRSA) by the same degree or extent because both fractions gave zone of inhibition of 28 mm (see Table 3). The

ethyl acetate fraction gave a comparable result to that of chloroform and methanol fractions, which is 27 mm. However, the hexane fraction gave the least zone of inhibition value, which is 14 mm. Based on these findings, the chloroform, ethyl acetate, and methanol fractions were very effective in inhibiting the growth of MRSA. The reason is because their zones of inhibition exceed 15 mm, which is the ideal effective zone of inhibition for crude fractions [26]. The same trend for MRSA is applicable to three other drug resistant bacteria tested, which are *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Despite this identical trend, there is slight variation as observed in the *Pseudomonas aeruginosa* assay result, particularly in its activity against hexane fraction, which is 16 mm. This value exceeds the ideal zone of inhibition of 15 mm set for this research study with regards to crude fraction. Therefore, the entire four fractions are very effective in inhibiting the growth of the bacteria tested. Considering the values obtained by averaging the zone of inhibition obtained for each fraction against all the tested bacteria. It could be observed that chloroform fraction has the highest average zone of inhibition (23 mm) followed by methanol fraction (21 mm), ethyl acetate (19 mm) and hexane extract (15.5 mm) as compared from the values in each column of Table 3. The extents of activities of the solvent fractions recorded in this experiment are more potent compared to previous experiment done only on the 70 % ethanol fraction of this plant [11].

Table 2. The Antimicrobial susceptibility test of *Securidaca longepedunculata* root bark solvent fractions

Test microorganisms	Hexane	Chloroform	Ethyl acetate	Methanol
Staphylococcus aureus (MRSA)	S	S	S	S
Streptococcus pyogenes	S	S	S	S
Pseudomonas aeruginosa	S	S	S	S
Pseudomonas flourescens	S	S	S	S
Klebsiella pneumoniae	S	S	S	S
Salmonella typhi	S	S	S	S
Candida albicans	R	R	R	R

Key: S = sensitive R = resistant

Table 3. Zone of inhibition (mm) of *Securidaca longepedunculata* root bark solvent fractions

Test microorganisms	Hexane	Chloroform	Ethyl acetate	Methanol
Staphylococcus aureus (MRSA)	14	28	27	28
Streptococcus pyogenes	17	20	19	20
Pseudomonas aeruginosa	16	28	17	18
Pseudomonas flourescens	14	20	17	16
Klebsiella pneumoniae	16	17	16	20
Salmonella typhi	16	27	18	23
Candida albicans	0	0	0	0

In order to account for the reason why the fractions exhibited significant antimicrobial activities, phytochemical test was carried out to determine the secondary metabolites present in each fraction. Table 4 shows the results of phytochemical screening, that indicates the presence of many secondary metabolites as follows. Alkaloids were detected in both ethyl acetate and methanol fractions. This is in agreement with the result obtained for Malawian variety of the plant [13]. However, it was previously detected in the crude water extract but absent in ethanolic extract [12]. This is the first time that alkaloids was reported to be present in the ethyl acetate and methanol fractions of the solvent-solid partitioned methanolic extract. The presence of alkaloids may account for the antimicrobial activities of the plant [27].

Anthraquinones were absent in the entire fractions investigated. Although, it was previously reported to be present in both ethanolic and crude water extracts [12]. The absence of anthraquinones in this research work could probably be due to either the solvent (methanol) used for the initial extraction could not extract it or may be it has not formed in the plant as of the time of plant collection or harvest. The plant was collected from the wild in October, 2010 for this research work, while in the research of [12], it was collected in April, 2010. Cardiac glycosides are present in all the fractions with exception of hexane fraction. Its presence has not been reported previously. Cardiac glycosides present in the plant fraction accounts for its antibacterial activity especially, if its aglycone portion is steroidal [28]. However, saponins have been detected in the entire fractions tested. Each fraction contains saponins which are both steroids and terpenoids in nature, because they gave positive response for both Salkowski and Liebermann-Burchardt's test. Saponins have been reported to be present in both ethanol and crude water fraction [12,13]. However, this research work has shown that the saponins are distributed in the root of this plant as non-polar and polar in nature. The presence of saponins may account for the potent antimicrobial activities of the fractions of this plant. Recently, it was pointed out that saponins, especially steroidal saponins have very potent antimicrobial activity. This claim is corroborated by works of other researchers [29,30,31].

Flavonoids are present in the entire fractions with the exception of hexane fraction. Previously, it was reported that flavonoids are present in the ethanolic and crude water extracts [12]. The result of this research work showed that

the plant root possesses both polar and non-polar flavonoids. The presence of flavonoids may account for the antimicrobial activity of the roots of this plant [27]. Reducing sugars are present in the entire fractions tested with the exception of hexane fraction. This is an indication that the hexane fraction does not possess secondary metabolites, which are glycosides in nature. The absence of cardiac glycosides in the hexane fraction is in line with the absence of reducing sugar in it. The other fractions possess free sugars which are reducing and/ or secondary metabolites which have reducing sugars bound to the aglycone unit.

Table 4. Phytochemical screening of *Securidaca longepedunculata* root bark solvent fractions

Secondary metabolites	Plant fractions			
	Hexane	Chloroform	Ethyl acetate	Methanol
Alkaloids	-	-	+	+
Anthraquinones	-	-	-	-
Cardiac glycosides	-	+	+	+
Flavonoids	+	+	+	+
Reducing sugars	-	+	+	+
Tannins	-	+	+	-
Saponins	+	+	+	+

Key: + = positive response, - = negative response

CONCLUSION

This study has shown that higher yield of methanol extract can be obtained few days after the rainy season, especially in the tropics and it also showed that the solvent fractions obtained from the methanol extract of the root bark of *Securidaca longepedunculata* are highly potent antimicrobial agents with remarkable activities against pathogenic Gram positive and Gram negative bacteria, significantly against multidrug resistant bacteria, which include methicillin resistant-*Staphylococcus aureus* (MRSA) and vancomycin resistant-*Klebsiella pneumoniae*. Interestingly, the fractions were highly active against *Pseudomonas fluorescens* a bacterium, which is also difficult to treat because only few antibiotics are available in the market for treatment of diseases it causes and are now resistant. In addition, the secondary metabolites responsible for the antimicrobial activities of the fractions were identified. Further research is required in order to isolate the active compounds responsible for the antimicrobial activity of the solvent fractions. In addition, the structure of the isolated pure compounds should be identified with spectroscopic techniques, which include IR, UV, NMR and Mass spectrometry. It is recommended that toxicity test should be carried out on the crude extracts, fractions and isolated pure compounds.

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