

Full Length Research Paper

## Phytochemical and antibacterial activity of *Securidaca longepedunculata* on selected pathogens

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Accepted 20 August, 2013

*Securidaca longepedunculata* family Polygalaceae is a tropically distributed medicinal plant. Antibacterial activity of chloroform, methanol and aqueous extracts of the roots and leaves of the plant against some selected microorganisms were shown using standard Kirby-Bauer disk diffusion method. The plants extracts showed inhibitory activity against the tested organisms. The diameter of zones of inhibition exhibited by all the extracts was between 15 and 20 mm. The methanol and the chloroform extracts of the leaves compared favorably with ampiclox capsule used as a standard control. The minimum inhibitory concentrations (MICs) of the extracts ranged from 0.591 to 6.25 mg/ml while the minimum bactericidal concentrations (MBCs) ranged from 1.56 to 6.25 mg/ml. Chromatography of methanol and aqueous extracts of the leaves revealed two major spot. The phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, saponins, tannins, cardiac glycosides, anthraquinones, steroids, balsams and reducing sugars. The study scientifically validates the use of this plant in traditional and ethnomedicine and these extracts may be a potential source of future antibacterial drugs against enteric organisms.

**Key words:** Phytochemical, antibacterial activity, *Securidaca longepedunculata*.

### INTRODUCTION

Resistance to antibiotics has been so tremendous that, incidences of outbreaks of multi-drug resistant bacteria in the past decades increased dramatically with no effective antibiotics to treat them (Walsh and Ames, 2004). In addition, widespread of diseases such as cancer, tuberculosis, typhoid fever, malaria, influenza, skin rashes and cardiovascular diseases coupled with high poverty level in developing countries like Nigeria has made investment and investigations on herbal plants an attractive endeavour in human healthcare. This is because most of the available synthetic medicines are too expensive for most

patients (Eisenberg et al., 2005). In the past decade, there has been renewed attention and interest in the use of traditional medicine globally (WHO, 2002). Traditional medicine was a source of many important medical pharmaceuticals (Gilani and Atta-ur-rahman, 2005). Recently, plant derived compounds offer an additional potential source of new antimicrobial, anticancer and anti-HIV agents (Gurib-Fakim et al., 2005).

*Securidaca longepedunculata* is a shrub of about 10 cm high, 2 to 9cm long and 0.5 to 2.5cm broad leaves commonly found in the entire Sudano – Zambebian zone.

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The plant belongs to the family Polygalaceae. Its leaves are oblanceolate and obtuse at apex. The flowers are purple or blue in colouration and the seeds are winged (Abdullahi et al., 2003). In northern Nigeria, the Nupe and the Hausa tribes utilize *S. longepedunculata* ethnomedicinally as a remedy for numerous human and animal ailments (Deeniand and Sadiq, 2002). According to Dapar et al. (2007), the aqueous extracts of its roots are used as psychopharmaceutical agents. It is also used as a sexual boost for men (Menecke and Mulhall, 1999; Nair and Chanda, 2006). This plant is also used for the treatment of every conceivable ailment such as headache, rheumatism, tuberculosis, cancer, venereal diseases, diabetes as well as abortifacient (Avhurengwi and Walter, 2006) and probably that is why the Hausas refer to it as “uwar magunguna” (the mother of all medicines). Therefore, the objective of this study was to authenticate the claims of the traditional healers on this plant which will form the basis for further research.

## MATERIALS AND METHODS

### Collection and preparation of plant materials

The leaves and roots of the plant were collected from the Abattoir, behind Ahmadu Bahago Secondary School in Bosso Local Government Area, Minna, Niger State. Identification was done by Professor Z. I. E. Ezenwa of School of Agriculture and Agricultural Technology, Department of Soil Science, Federal University of Technology, Minna. The samples were washed with distilled water to remove earthy materials, dried at ambient temperature in the laboratory to avoid heat destruction of the active components before powdering. The powdered samples were then put in clean dried cellophane bags and kept in a cool dry place for further use.

### Plant extraction

100 g of each powdered material was weighed and extracted with 300 cm<sup>3</sup> of chloroform by refluxing for six hours and filtered. The marc was extracted with 300 cm<sup>3</sup> of methanol in the same way as that of the chloroform. After drying, the marc was extracted with distilled water using reflux. The various extracts obtained were evaporated to dryness on a steam bath. The dried extracts were weighed, kept in well labeled sterile specimen bottles and stored in a refrigerator at 4°C until required.

### Phytochemical screening

The crude extracts of the samples were subjected to phytochemical tests to determine their chemical constituents using standard methods described by Evans and Trease (1989) and Sofowora (1982). The tannins were determined by suspending 3 g of each extract in 6 cm<sup>3</sup> of distilled water after which it was filtered and iron (III) chloride reagent was added. For the cardiac glycosides, Keller-Killiani's test (Trease and Evans, 1989) was adopted by taking 0.5 g of the extract and adding 2 cm<sup>3</sup> of acetic acid plus H<sub>2</sub>SO<sub>4</sub>. The alkaloids were tested for by taking 0.5 g of the aqueous extract in 5 cm<sup>3</sup> 1% HCl. This was boiled, filtered and Mayer's reagent added (Trease and Evans, 1989) while for saponins, the extracts were subjected to frothing test. Haemolysis test was performed on the frothed extracts in water to remove false positive results (Sofowora, 1993). Anthraquinones were

tested for by treating 5.0 g of each extract with 10 cm<sup>3</sup> of benzene, filtered and ammonia solution was added (Sofowora, 1993). The presence of flavonoids were determined using Shinoda's test for flavonoids by dissolving 0.5 g of each extract in 5 cm<sup>3</sup> of ethanol, warmed and filtered. This was followed by the addition of magnesium chips to the filtrate and few drops of concentrated HCl (Trease and Evans, 1989). The steroidal constituents were determined when 2.0 g of each extract was treated with 2 cm<sup>3</sup> of acetic acid, warmed and cooled in ice followed by careful addition of concentrated H<sub>2</sub>SO<sub>4</sub> (Sofowora, 1993). The presence of reducing sugar was established by Fehling's test for reducing sugar. For each extract, 0.5 g was dissolved in distilled water and filtered. The filtrate was heated with 5 cm<sup>3</sup> of equal volumes of Fehling's solutions A and B (Sofowora, 1993).

### Test bacteria

The test bacteria used in this study were isolates of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhii* obtained from their stock cultures in Microbiology Department Laboratory, Federal University of Technology, Minna. The bacterial isolates were cultured on nutrient agar and incubated at 37°C for 24 h. These were repeatedly sub-cultured in order to obtain pure isolates while morphological and biochemical tests were carried out to ensure proper identification. In this case, a loopful of test organism was inoculated on nutrient broth and incubated for 24 h. A volume of 0.2 ml from the previously cultured organism was dispensed into sterile nutrient broth and incubated for 3 h to standardize the culture to 1.0 × 10<sup>6</sup> cfu/ml (Oyeleke et al., 2008).

### Antibacterial activity

The standard Kirby-Bauer disk diffusion method described by NCCLS (2002) was adopted for the antibacterial activities of the extracts. Stock solutions of 25 mg/ml were prepared from the dried extracts. Test bacteria were sub-cultured onto brain-heart infusion agar (Becton Dickinson Comp., USA) and incubated at 37°C for 24 h after which 3 colonies were isolated with inoculating loops, transferred to three tubes of sterile saline and vortex thoroughly. The bacterial suspensions were compared and adjusted to 0.5 McFarland standards to prepare culture stocks (about 10<sup>6</sup> cfu/cm<sup>3</sup>). Within 15 min, sterile cotton swabs were dipped into the bacterial suspensions and streaked over sterile plates containing nutrient agar and left for a while to set. Sterile filter paper (6 mm in diameter) was impregnated with 15 µl of extract (7.5 mg/disc) from previously prepared stock of 25 mg/ml. After 20 min, the plates were gently turned upside down and incubated at 37°C for 24 h. The diameters of inhibition zones (in mm) were measured and recorded. Commercial antibiotic (ampiclox) was applied as positive control.

### Determination of minimum inhibitory concentration (MIC)

MICs of the extracts were recorded as the lowest concentrations of the extracts that inhibited the growth of the microorganisms.

### Determination of minimum bactericidal concentration (MBC)

MBCs of the extracts were determined by sub-culturing the test tubes that showed no visible turbidity after the incubation of the batch test tubes in the MICs determination on nutrient agar plates which were then incubated at 37°C for 24 h. The concentration that showed no visible growth after incubation was taken as the MBC (Suffredini et al., 2004; Doughari et al., 2007).

**Table 1.** Phytochemical constituents of extracts from roots and leaves of *S. longepedunculata*.

Chemical compound	Chloroform extract		Methanol extract		Aqueous extract	
	Roots	Leaves	Roots	Leaves	Roots	Leaves
Saponins	-	-	+	++	++	++
Tannins	-	+	-	+++	-	+++
Flavonoids	+	+	+	+	+	+
Steroids	-	+	+	-	-	-
Cardiac glycosides	-	+++	-	+++	-	+++
Alkaloids	-	-	+	+	+	+
Anthraquinones	-	-	++	++	-	++
Reducing sugars	+	+	+	+	+	+

**Table 2.** Antibacterial activities of the crude extracts of *S. longepedunculata* (showing zones of inhibition in mm).

Bacteria	Chloroform extract		Methanol extract		Aqueous extract		Control (Ampiclox)
	Roots	Leaves	Roots	Leaves	Roots	Leaves	
<i>Escherichia coli</i>	-	17	-	15	20	15	23
<i>Salmonella typhi</i>	-	16	-	18	-	15	23
<i>Pseudomonas aeruginosa</i>	-	18	-	19	-	-	18

- = Not active.

### Thin layer chromatographic determination

The  $R_F$  values of the separated components obtained by chromatographic separation of the crude extracts were determined using mini thin layer plates prepared by using microscope slides while the macro-plates were prepared using 20 × 10 cm glass slides. A mobile phase made up of the mixture of ethyl acetate, methanol and distilled water in the ratio of 12:1.5:1.0 was made, put in a glass tank, closed and allowed to stand for about 10 min. The plates were inserted into the tank with the origin of spots towards the bottom of the tank but above the solvent and covered tightly in order to allow the solvent get close to the top. They were removed, dried in the oven and the distances moved by the solvent and the extracts measured. The separations obtained by the macro-plates were scraped, dissolved in appropriate solvents, evaporated and packaged in airtight containers for further use.

### RESULTS AND DISCUSSION

Table 1 shows the phytochemical constituents of the extracts. While flavonoids and reducing sugars were fairly present in all the extracts, tannins, were highly present only in the methanolic and aqueous leaves extracts. Other constituents were present in the methanolic roots and leaves extracts except tannins, steroids and cardiac glycosides.

The presence of alkaloids and flavonoids in both methanol and aqueous extracts (Table 1) revealed the efficacy of the plant against the diseases that they are used locally for. The presence of flavonoids in the extracts also confirms the assertion of Ingrid and Mathias (2006) who said that *S. longepedunculata* is a highly antidiabetic plant. The antidiabetic activity of this plant is probably due to the ability of flavonoids to inhibit  $\alpha$ -amylase activity which

regulates the amount of glucose in the blood. The presence of saponins and glycosides in reasonable quantities also justified the traditional use of the plant in the treatment of tuberculosis and diabetes (Abdullahi et al., 2003).

Table 2 shows the zones of inhibition of the crude extracts against the test organisms. The chloroform and methanolic roots extracts showed no activity and the aqueous roots extract was only active against *E. coli*. The chloroform, methanolic and aqueous leaves extract showed varied activities against the test organisms.

The strong activity of the roots and leaves extracts suggested that this plant could be used for the treatment of infections caused by the test organisms except *P. aeruginosa* that was resistant to activity of the aqueous leaves extract (Table 2). The root extracts of the three solvents used were inactive against the test organisms except *E. coli* while the control (ampiclox) had the highest inhibitory activity against any of the test organisms.

These results were however, higher than the respective 14.0 and 8.0; 10.0 and 10.0 mm reported by Yahaya et al. (2012) as the zones of growth inhibition of *Salmonella typhi* by the methanolic (stem extract) and methanolic or aqueous (leaves extracts) of *Combretum glutinosum*. In addition, none of these values was lower than the 7 mm reported for *Andrographilis paniculata* although they were lower than the 23 mm reported for *Euginia jambolana* (Muhamed et al., 2010). However, the recorded zones in this study for *P. aeruginosa* were lower than the respective 18 mm reported for *A. paniculata* and *E. jambolana* by Muhamed et al. (2010) but similar to the 15

**Table 3.** The minimum inhibitory concentrations (MIC) in mg/ml of the crude extracts of *S. longepedunculata*.

Bacteria	Chloroform extract		Methanol extract		Aqueous extract	
	Roots	Leaves	Roots	Leaves	Roots	Leaves
<i>Escherichia coli</i>	-	1.563	-	1.563	6.25	1.563
<i>Salmonella typhi</i>	-	0.591	-	0.591	-	6.25
<i>Pseudomonas aeruginosa</i>	-	0.591	-	1.563	-	-

- = Not active.

**Table 4.** The minimum bactericidal concentrations (MBC) of the crude extracts of *S. longepedunculata* in (mg/ml).

Bacteria	Chloroform extract		Methanol extract		Aqueous extract	
	Roots	Leaves	Roots	Leaves	Roots	Leaves
<i>Escherichia coli</i>	-	15.63	-	15.63	62.5	15.63
<i>Salmonella typhi</i>	-	5.91	-	5.91	-	62.5
<i>Pseudomonas aeruginosa</i>	-	5.91	-	15.63	-	-

- = Not active.

mm reported for the acetone extract of *Aloe vera* by Arun and Muthuselvam (2009). These antibacterial effects of *S. longepedunculata* were attributable to the presence of the active phytochemicals like tannins, saponins, alkaloids and glycosides in the extracts (Enwerem et al., 2001, 2003).

Dapar et al. (2007) reported that tannins form complexes with proteins through hydrogen and covalent bonds as well as other hydrophobic effects which inhibit cell protein synthesis. The presence of these phytochemicals therefore has been adduced for the significant antibacterial activity of the extracts. Also, the protein-precipitating and vasoconstriction effects of tannins help in preventing ulcer development (Dahiru et al., 2006) and this is one of the ailments that this plant is used to treat locally in the study area.

The diuretic and antibacterial activity of plant extracts containing flavonoids have been documented (Enwerem et al., 2001, 2003). The activities of the methanolic extracts and chloroform fractions of the leaves were not significantly different and this showed that both extracts can be exploited for antibacterial actions.

Tables 3 and 4, respectively, show the minimum inhibitory and minimum bactericidal concentrations of the extracts. The test bacteria were inhibited at concentrations ranging from 0.591 to 6.25 mg/ml while the minimum bactericidal concentrations ranged from 5.91 to 62.50 mg/ml. Just in line with the observations of Karaman et al. (2003), the aqueous root extracts had the highest MIC values but these were all lower than the 256 mg/ml reported as the minimum inhibitory concentration for the ethanolic extract of *Iresine herbstii* by Bussmann et al. (2010) but were higher than the respective 16, 32 and 32 µg/ml reported as the MIC values for *E. coli*, *P. aeruginosa* and *S. typhi* by Hassan et al. (2009) for *Polygonum hydropiper*. The lowest MBC of 5.91 mg/ml recorded for *P. aeruginosa*

in this work was higher than the 256 µg/ml reported as the MIC of *Dioscorea bulbifera* extracts against this organism (Victor et al., 2012).

Table 5 showed the zones of inhibition of the chromatographic fractions of the extracts against the test organisms. The chloroform and methanolic roots fractions showed no activity while the aqueous roots fractions were only active against *E. coli*. The leaves extracts of the three solvents showed various degrees of activity against the test organisms.

The TLC fractions obtained from the leaves had  $R_F$  value of 0.66 for the aqueous extract while that obtained from the methanol extract of the leaves was 0.78. The roots and leaves chloroform extracts as well as the roots methanol and aqueous extracts had no noticeable spots in the solvent mixture but chlorophylls were observed at the solvent front. The scrapped fractions exhibited antibacterial activities against the test organisms and one of the fractions from the aqueous leaves extracts had the highest effect against *S. typhi*. The fractions however, showed lower antibacterial activities against the test organisms (Table 5) than the crude extracts (Table 2). The lower antibacterial activities of the fractions than the crude ones might have been due to the synergic effects of the active components in the crude extracts thus agreeing with the reports of Harborne (1984) and Oyeleke et al. (2008) who said that activities of plant extracts could change after fractionation making the obtained pure component to lack the activity of the original crude extract.

## Conclusion

It could be inferred that these plant extracts could be useful in the industrial manufacture of drugs used in the

**Table 5.** Antibacterial activities of the crude chromatographic fractions of *S. longepedunculata* showing zones of inhibition in (mm).

Bacteria	Chloroform extract		Methanolic extract		Aqueous extract		Control (Ampiclox)
	Roots	Leaves	Roots	Leaves	Roots	Leaves	
<i>Escherichia coli</i>	-	13	-	15	16	17	23
<i>Salmonella typhi</i>	-	17	-	17	-	16	23
<i>Pseudomonas aeruginosa</i>	-	12	-	16	-	-	18

- Not active

chemotherapy of some microbial infections. Thus, the present study provides some information on the phytochemical and antibacterial investigation of *S. longepedunculata* which paves way for further research to identify the active compounds responsible for the biological activity of the plant.

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