Phytochemical Analysis and Acute Toxicity Studies on the Methanol Extract of Securidaca longepedunculata (Fresen) Root Bark in Mice

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ABSTRACT

The methanol extract of Securidaca longepedunculata root bark was screened phytochemically and its intraperitoneal acute toxicity evaluated in mice. The phytochemical screening was carried out based on standard methods. The Median Lethal Dose (LD₅₀) was determined using Lorke's method while the Maximum Tolerated Dose (LD₀) was determined by the method described by Mosser and Padilla. The root bark extract revealed the presence of carbohydrates, terpenoids, cardiac glycosides, saponins and flavonoids. The extract produced intraperitoneal LD₅₀ and LD₀ values of 6.92 mg/kg and 6.0 mg/kg, respectively. Based on the research findings, the methanol extract of S. longepedunculata root bark was found to contain important phytochemicals which may be attributed to its enormous use in traditional medicine, but it was a highly toxic extract in mice with intraperitoneal LD₅₀ and LD₀ values of 6.92 mg/kg and 6.0 mg/kg, respectively.

Keywords: Securidaca longepedunculata, phytochemical screening, LD₅₀, LD₀, mice

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INTRODUCTION

Phytomedicine also known as herbal medicine entails the use of plants and plant parts such as leaves roots, barks, flowers, stems and seeds in the prevention and management of various diseases [1, 2]. Medicinal plants are sources of bioactive phytochemicals or bionutrients which could play an important role in drug development for the prevention and treatment of chronic diseases like cancer, diabetes and coronary artery disease which make it very necessary for plants to be screened phytochemically. The bioactive substances are further studied and characterized to ascertain their mechanisms of action and the potentials in the prevention and treatment of various ailments [3]. The value of a medicinal plant in phytomedicine lies in the presence of bioactive phytochemical constituents of the plant that is able to initiate a physiologic response in a living cell with alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, phenolic compounds being some of the most important bioactive phytochemical constituents [4, 5]. Phytochemical constituents are natural bioactive compounds found in plants that form part of the plant's defense mechanism against various diseases and for survival adaptation. Based on the functions in plant metabolism, phytochemical constituents could be primary (sugar, amino acids, protein, chlorophyll) or secondary (alkaloids, terpenoids, phenolic compounds such as tannins, phlobatannins, anthraquinines and flavonoids), others include cardiac glycosides and saponins [6].

Phytochemically, extracts from various parts of S. longepedunculata especially the root bark contains numerous valuable compounds including xanthones, some benzyl benzoates and triterpene saponins amongst others. The aqueous root and ethanol extracts yielded alkaloids, cardiac glycosides, flavonoids, saponins, tannins, volatile oils, terpenoids and some steroids [7, 8, 9], while chloroform and ethanol extracts indicated the presence of flavonoids, saponins, coumarins, tannins and alkaloids [10]. The crude methanol root bark extract was highly toxic to mice with an intraperitoneal LD<sub>50</sub> of 11.0 mg/kg [11]. The aqueous root bark extract was slightly toxic to albino rats with an LD<sub>50</sub> of 0.771 g/kg [8], while Agbaje and Adekoya [12] reported an LD<sub>50</sub> of 3.16 g/kg when administered orally to rats. Toxicity studies by Ngulde et al., revealed an intraperitoneal LD<sub>50</sub> of 14.14 mg/kg in rats [13], while Maxwell et al., reported an LD<sub>50</sub> 37.0 mg/kg when administered intraperitoneally to albino rats [14]. This study is however aimed at screening the phytochemical constituents of the methanol extract of Securidaca longepedunculata root bark as well as evaluating its intraperitoneal acute toxicity in mice.

MATERIALS AND METHODS

Plant collection, identification, preparation and extraction

Fresh root bark of S. longepedunculata was obtained alongside its leaves, stem and flowers from Ngulde district of Askira/Uba Local Government Area of Borno State in the northern part of Nigeria. The plant was identified and authenticated by Professor S.S Sanusi of the Department of Biological Sciences, University of Maiduguri and a voucher sample (Vet212A2) was preserved at the Veterinary Pharmacology Laboratory herbarium, University of Maiduguri Nigeria. The root bark was air-dried and pulverized in a clean wooden mortar using pestle to obtain its powdery form as described by Junaidu et al. [15]. The extraction was done at 1:5 (w/v) of the dry powder of the plant to solvent (methanol). Therefore, 1kg of the plant powder was extracted using 2,500 ml of methanol in a soxhlet extractor as described by Tiwari et al. [16] to obtain its methanol extract. The product was then concentrated in an aluminum tray in hot air oven at 40-45°C until constant weighed was achieved and then stored at 4°C.
Experimental animals
Adult mice (20.1-32.5 g) of both sexes bred at the Animal house of the Faculty of Veterinary Medicine, University of Maiduguri were used for the experiments. The animals were housed in clean aluminum cages with soft wood shavings as bedding. All animals were fed with pelletized commercial feed (poultry grower feed) manufactured by Livestock feeds Plc. at No. 1, Henry Carr street, Ikeja, Lagos and tap water was provided ad libitum. All procedures and techniques used in this study were in accordance with the National Institute of Health guidelines for the care and use of laboratory animals [17]. Ethical clearance was obtained from the Animal Research Ethics Committee, Faculty of Pharmacy, University of Maiduguri with approval number REF/FP/092019/PGVP/01.

Chemicals and diluents
Methanol manufactured by Sigma-Aldrich Inc., St. Louis, MO, USA and Normal saline manufactured by Fidson Healthcare Plc. at Km. 38, Lagos-Abeokuta Expressway, Sango-Ota, Ogun State, Nigeria were used during the experiment for extraction and reconstitution of the extract, respectively.

Phytochemical analysis
The methanol extract of *S. longepedunculata* root bark was screened for the presence of primary and secondary metabolites such as: carbohydrates (Molisch's test, Barfoed's test, Fehling's test, Selivanoff's test), soluble starch, terpenoids, tannins (Ferric chloride test, lead sub acetate test), phlobatansins, cardiac glycosides (Lieberman's, Keller-kiliani, Salkowski's tests), alkaloids (Mayer's and Dragendorff's reagents tests), saponin (Foam test), anthraquinones and flavonoids (Shinoda, Alkaline reagent, Ferric chloride, Lead acetate tests) using methods described by Velavan and Evans [18, 19].

Determination of median lethal dose (LD$_{50}$)
The acute toxicity (LD$_{50}$) of the methanol extract of *S. longepedunculata* was determined using Lorke's method [20] with slight modifications. The experiment involved two phases: first and second phases. In the first phase, a group comprising of 3 mice was administered with the extract of *S. longepedunculata* intraperitoneally at 10 mg/kg (dosage was selected based on literature and pilot studies), signs of toxicity and death was observed within the period of 24 hours. In the second phase, four groups of one mouse each were administered with 2, 4, 6 and 8 mg/kg doses of the extract of *S. longepedunculata*. The doses administered in the second phase were determined by the outcome of the first phase, signs of toxicity and death within 24 hours were also observed in the second phase. Mice were kept for 14 days to observe for any symptom of delayed toxicity.

The LD$_{50}$ value was calculated using the formula:

$$LD_{50} = \sqrt{a \times b}$$

Where;

- $a$ = least dose that killed the mouse while
- $b$ = highest dose that did not kill any mouse

Determination of maximum tolerated dose (MTD or LD$_{0}$)
Maximal Tolerated Dose (MTD or LD$_{0}$) of methanol extract of *S. longepedunculata* was determined in mice by the method described by Moser and Padilla [21] with slight modifications. In determining the pilot dose range, a single group comprising of 3 mice was used for single intraperitoneal doses each. The first mouse received the extract at 6 mg/kg, while the second and the third received 8 and 10 mg/kg respectively. The MTD was considered as the highest dose that produced clear observable signs of toxicity without any lethality. Subsequently, MTD was verified by administering the extract at 4, 6 and 8 mg/kg in
RESULTS

Phytochemical constituents of the methanol extract of S. longepedunculata root bark

The result of the phytochemical analysis of methanol extract of *S. longepedunculata* root bark revealed the presence of carbohydrates, terpenoids, cardiac glycosides, saponin, and flavonoids whereas soluble starch, tannins, phlobatannins, alkaloids and anthraquinones were absent as shown in Table 1.

Table 1: Phytochemical constituents of the methanol extract of *S. longepedunculata* root bark

<table>
<thead>
<tr>
<th>Component</th>
<th>Test</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Molisch’s</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Barfoed’s</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Fehling’s</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Combined reducing sugar</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Selivanoff’s</td>
<td>+</td>
</tr>
<tr>
<td>Soluble starch</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Lead sub acetate</td>
<td>-</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>Lieberman’s</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Keller-Kiliani</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Salkowski</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Mayer’s</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Dragendorff’s Reagents</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td>Foam</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Alkaline Reagent</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Ferric chloride</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Lead acetate</td>
<td>+</td>
</tr>
</tbody>
</table>

+= present  
-= not detected

Results of the median lethal dose (LD₅₀)

The result of the acute toxicity test of the methanol extract of *Securidaca longopedunculata* root bark in mice using Lorke’s method is presented in Table 2. The intraperitoneal administration of the extract at 1 and 2 mg/kg produced 0% mortality with no observable signs of toxicity whereas 6 mg/kg also produced 0% mortality but with clear observable toxic sign. Dosages of 8 and 10 mg/kg produced 100% mortality with clear observable toxic signs. Dosage of 8 mg/kg was found to be the least dose that kill the mouse while 6 mg/kg was the highest dose that did not kill the mouse and hence the intraperitoneal median lethal dose (LD₅₀) was calculated to be = 6.92 mg/kg.
Table 2: Intraperitoneal median lethal dose (LD₅₀) of the methanol extract of *S. longepedunculata* root bark in mice

<table>
<thead>
<tr>
<th>Phase</th>
<th>Dosage (mg/kg)</th>
<th>Sign of toxicity</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>Depression, incoordination</td>
<td>3/3 (100)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>None</td>
<td>0/1 (0)</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>None</td>
<td>0/1 (0)</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>Depression</td>
<td>0/1 (0)</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>Depression</td>
<td>1/1 (100)</td>
</tr>
</tbody>
</table>

\[ \text{LD}_{50} = \sqrt{a \times b} = \sqrt{6 \times 6} = \sqrt{4 \times 6} \]

\[ \text{LD}_{50} = 6.92 \text{ mg/kg} \]

**Results of the maximum tolerated dose (MTD or LD₀)**

The result of the maximum tolerated dose (MTD or LD₀) of methanol extract of *S. longepedunculata* root bark in mice using the method described by Moser and Padilla is presented in Table 3. Three groups A, B and C comprising of a mouse each were administered with the extract intraperitoneally at 6 mg/kg, 8 mg/kg and 10 mg/kg respectively. All the three groups exhibited clear observable signs of toxicity, groups B and C recorded 100% mortality while group A recorded 0% mortality. The experiment was verified using three larger groups D, E and F comprising of 3 mice each which were administered with the extract intraperitoneally at 4, 6 and 8 mg/kg respectively. Groups E and F exhibited clear observable signs of toxicity while group D did not exhibit any observable sign of toxicity. Groups D and E recorded 0% mortality while group F recorded 100% mortality. The maximum tolerated dose (LD₀) which is the highest dose that produced clear observable signs of toxicity without any lethality was found to be 6.0 mg/kg. Toxic signs observed include tremor, incoordination and depression.

Table 3: Intraperitoneal maximum tolerated dose (MTD) of the methanol extract of *S. longepedunculata* root bark in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dosage (mg/kg)</th>
<th>Toxicity signs</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>Depression</td>
<td>0/1 (0)</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>Depression</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>Depression, incoordination</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>None</td>
<td>0/3 (0)</td>
</tr>
<tr>
<td>E</td>
<td>6</td>
<td>Depression</td>
<td>0/3 (0)</td>
</tr>
<tr>
<td>F</td>
<td>8</td>
<td>Depression</td>
<td>3/3 (100)</td>
</tr>
</tbody>
</table>

\[ \text{LD}_0 = 6.0 \text{ mg/kg} \]
DISCUSSION
The phytochemical screening of the methanol extract of *S. longepedunculata* root bark in the present studies revealed the presence of carbohydrates, terpenoids, cardiac glycosides, saponins, and flavonoids. The results obtained supports earlier scientific findings [7, 8, 9, 22, 23]. The presence of the above phytochemical constituents may be responsible for its role in African traditional medicine. Scientific research has shown that a considerable number of phytochemical constituents possesses significant therapeutic properties [24].

Research findings appear to suggest that flavonoids are one of the most important phytochemicals that is responsible for reduced mortality rate observed in people consuming high level of plant-based foods [25]. Many phytochemicals have been reported to have antioxidant activities which helps in protecting the living cells against oxidative damage hence reducing the risk of cancer development [26]. Bioactive phytochemical constituents are essential as absence of some vital bioactive phytochemical constituents in processed foods could result in increased incidence of many preventable diseases [26, 27]. Flavonoids have been reported to possess antimicrobial, anticancer, antiallergic and antitumour properties. Owing to antioxidant potentials of flavonoids, it has been envisaged that it could limit neurodegeneration as well as reverse deterioration in cognitive performance [28, 29]. Reports revealed that saponins are potent bioactive phytochemicals present in many medicinal plants with anti-inflammatory, antifungal, antitumour, antiparasitic, antiviral, hemolytic, coagulative and cholesterol binding properties [30, 31, 32]. Mild astringent and detergent properties were reported in tannins [33].

The acute toxicity studies revealed an intraperitoneal LD₅₀ of 6.92 mg/kg and LD₅₀ of 6.0 mg/kg. A research by Okoli *et al.* [11] reported an intraperitoneal LD₅₀ of 14.14 mg/kg and 37 mg/kg in rats respectively, but all fall within the same class of toxicity with this result. Based on the LD₅₀ value, toxic substances are classified into 6 classes as extremely toxic, highly toxic, moderately toxic, slightly toxic, practically non-toxic and relatively harmless. Any substance which has LD₅₀ value ranging from 5 - 50 mg/kg is considered as highly toxic [34, 35]. Therefore, the extract could be considered as highly toxic when given intraperitoneally to mice.

Conclusion
The findings of this study revealed the presence of carbohydrates, terpenoids, cardiac glycosides, saponin, and flavonoids in the methanol extract of *S. longepedunculata* root bark which may be responsible for its traditional use in the management of many conceivable ailments and contribute to its toxicity. The root bark of *S. longepedunculata* has a low margin of safety if administered intraperitoneal due to its high toxicity and therefore, it should be used with caution to avoid organ/system damage. The isolation of the active principles, subacute and chronic toxicity studies is however recommended to clearly substantiate the reported bioactive phytochemicals and the high toxicity. Pesticides could also be harnessed from the high toxicity of the extract.

Conflict of Interest
We wish to state that there is no conflicting financial interest among the authors.

Acknowledgement
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