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PHYTOCHEMICAL COMPOSITION AND ACUTE TOXICITY EVALUATION OF AQUEOUS ROOT BARK EXTRACT OF *SECURIDACA LONGIPEDUNCULATA* (LINN)

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ABSTRACT
This study was carried out to determine the phytochemical constituents and acute toxicity of the aqueous root bark extract of Securidaca longipedunculata Linn. The result of phytochemical screening revealed the presence of some secondary metabolites of pharmacological significance in the aqueous root bark extract including alkaloids, cardiac glycosides, flavonoids, saponins and tannins with saponins and tannins in larger quantities. The extract was found to have an LD_{50} value of 771mg/kg body weight when it was orally administered to rats, which indicate the root bark to be slightly toxic to the experimental animals. The toxicity of the root bark is attributed to some of the phytochemicals present in the plant.
Key words: Securidaca longipedunculata, root bark, aqueous extract, phytochemicals, acute toxicity.

INTRODUCTION

Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as "secondary metabolities" of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids (Harborne, 1973; Okwu, 2004). These can act as agents to prevent undesirable side effects of the main active substances or to assist in the assimilation of the main substances (Anonymous, 2007). Opium juice, for example from *Papaver somniferum*, contain other chemical compounds in addition to morphine and reports show that it gives fewer side effects than morphine administered on its own (Anonymous, 2007).

Phytochemicals are present in a variety of plants utilized as important components of both human and animal diets. These include fruits, seeds, herbs and vegetables (Okwu, 2005). Diets containing an abundance of fruits and vegetables are protective against a variety of diseases, particularly cardiovascular diseases (Okigbo *et al.,* 2009). Herbs and spices are accessible sources for obtaining natural antioxidants (Okwu, 2004).

In contrast to synthetic pharmaceuticals based upon single chemicals, many medicinal and aromatic plants exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process. These synergistic pharmacological effects can be beneficial by eliminating the problematic side effects associated with the predominance of a single xenobiotic compound in the body (Tyler, 1999). The synergistic interactions that underlie the effectiveness of a number of Phytomedicines has been extensively documented (Kaufman *et al.,* 1999). Most of these phytochemical constituents are potent bioactive compounds found in medicinal plant parts which are precursors for the synthesis of useful drugs (Sofowora, 1993).

Toxicity is the degree to which a substance can damage an organism. Toxicity can refer to the effect on a whole organism, such as an animal, bacterium, or plant, as well as the effect on a substructure of the organism, such as a cell (cytotoxicity) or an organ (organotoxicity), such as the liver (hepatotoxicity). By extension, the word may be metaphorically used to describe toxic effects on larger and more complex groups, such as the family unit or society at large. A central concept of toxicology is that effects are dose-dependent and even water can lead to water intoxication when taken in large quantities/doses, whereas for even a very toxic substance such as snake venom there is a dose below which there is no detectable toxic effect (Cutler, 2010).

Toxicity can result from adverse cellular, biochemical, or macromolecular changes. Examples are: cell replacement, such as fibrosis, damage to an enzyme system, disruption of protein synthesis, production of reactive chemicals in cells and DNA damage. Some xenobiotics may also act indirectly by: modification of an essential biochemical function, interference with nutrition and alteration of a physiological mechanism (Cutler, 2010).

The toxicity of a substance depends on the following: form and innate chemical activity, dosage, especially dose-time relationship, exposure route, species, age, sex, ability to be absorbed, metabolism, distribution within the body, excretion and presence of other chemicals (Cutler, 2010).

Toxicity can be measured by its effects on the target (organism, organ, tissue or cell). Because individuals typically have different levels of response to the same dose of a toxin, a population-level measure of toxicity is often used which relates the probabilities of an outcome for a given individual in a population. One such measure is the LD_{50} (Mark, 1999).

Toxic effects are generally categorized according to the site of the toxic effect. In some cases, the effect may occur at only one site. This site is referred to as the specific target organ. In other cases, toxic effects may occur at multiple sites. This is referred as systemic toxicity. Following are types of systemic toxicity: Acute Toxicity, Subchronic Toxicity, Chronic Toxicity Carcinogenicity, Developmental Toxicity and Genetic Toxicity (somatic cells) (Cutler, 2010).

By convention, many toxicology studies conducted in the early to mid 20th century were designed to identify the median lethal dose, or the dose required to kill 50 percent of the test population, "LD_{50}" over a defined period of time. The most common LD_{50} test is the acute toxicity test, in which animals are given a single dose of chemical and the LD_{50} is determined over a 24-hour time period. Today, lethality studies are, conducted as a first step towards providing some insight into the relative potency of new chemicals (Mark, 1999).

Despite the widespread use of *S. longepedunculata* in all parts of Africa, not much has been reported in the literature about its mammalian toxicity. The few reports available are in connection with its trypanocidal and insect toxicity where it is used as a stored grain preservative (Atawodi *et al.,* 2003; Belmain *et al.,* 2001). When a herbal product is ingested, the body interacts with it in an attempt to get rid of any harmful toxins, especially if the body cannot convert the foreign substance into cellular components. These processes are commonly manifested by changes in enzyme levels and other cell components. The enzymes commonly involved include: aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), amylase, and alkaline phosphatase. Also, components like urea and uric acid are vital diagnostic tools for toxicity (Wannang *et al.,* 2005). In a previous study, it was found that aqueous extracts of the root bark of *S. longepedunculata* altered serum levels of these enzymes in rats; an indication of the potential toxicity of the plant which could as well result in tissue or organ damage. The vital organs that are commonly affected are heart, liver, pancreas, and kidney among others (Maxwell *et al.,* 2007). The present research was designed to carryout phytochemical and acute toxicity evaluation of the aqueous root bark extract of *S. longepedunculata.*

MATERIALS AND METHODS

Plant Identification and Collection

The plant was collected in July, 2011 from Ningi Local government, Bauchi state, Nigeria. It was authenticated at the Botany unit, Biological Sciences Department, Faculty of Science, Bayero University Kano, Nigeria.

Extraction of the Plant Materials

The root bark was collected and carefully washed in clean water, and then dried at room temperature. They were subsequently pulverized to coarse powder and distilled water ($120cm^3$) was added to 16g of the powdered root bark into a conical flask. The content of the flask was then shaken and the top was covered with aluminium foil and kept for 48hours at room temperature. The extract was then obtained by filteration using a whatman No1 filter paper and concentrated using vacuum evaporator.

Identification of Chemical Constituents

The phytochemical components of *S. longepedunculata* were determined using the methods of (Sofowora, 1993; Harbone, 1973; and Cuilei, 1994) with some minor modification.

Test for Tannins

$2.0cm^3$ of the aqueous extract was diluted with distilled water in a test tube. 2 – 3 drops of 5% ferric chloride solution was added. A green black or blue – black colouration would indicate the presence of tannins.

Test for flavonoids

0.5g of the plant material was shaken with petroleum ether to remove the fatty materials (lipid layer). The defatted residue was dissolved in $20cm^3$ of 80% ethanol and filtered. The filtrate was used for the following test:

$3cm^3$ of the filtrate was mixed with $4cm^3$ of 1% potassium hydroxide in a test tube and the colour was observed. A dark yellow colour indicated the presence of flavonoids.

Test for Alkaloids

To $10.0cm^3$ of the aqueous extract in 2 separate test tubes, 2 -3 drops of Dragendoff's and Mayers reagents were separately added. An orange red precipitate/ turbidity with Dragendoff's or white precipitates with mayer's would denote the presence of alkaloids.

Test for cardiac glycosides

$5cm^3$ of the aqueous extract was mixed with $2cm^3$ of glacial acetic acid containing one drop of ferric chloride (FeCl3) solution, followed by the addition of $1cm^3$ concentrated sulphuric acid. Brown ring was formed at the interface which indicated the presence of deoxysugar of cardenoloides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer.

Test for Saponins

0.5g of the powder was taken in a test tube. $5.0cm^3$ of water was added and vigorously shaken. A persistent froth that last for at least 15 minutes would indicate the presence of saponins.

Quantitative Determination of Phytochemicals

Phytochemicals were determined by the method of El – Olemyl *et al.* (1994).

Alkaloids

The plant extract ($10cm^3$) was transferred into a $250cm^3$ separatory funnel followed by dilute H_2SO_4 ($5cm^3$) and H_2O ($5cm^3$). The extract was shaken twice with $CHCl_3$ ($10cm^3$) and the combined $CHCl_3$ extract was transferred to a second separatory funnel containing dilute H_2SO_4 ($5cm^3$) and H_2O ($10cm^3$). The $CHCl_3$ layer was discarded and the aqueous acidic layer was transferred to the contents of the first separating funnel. The extract was then made alkaline with ammonia and shaken for about half a minute. The alkaloids were extracted completely by successive portions of $CHCl_3$ each of $20cm^3$. (Complete extraction was tested using Mayer's reagent). The combined $CHCl_3$ extract was shaken with H_2O ($5cm^3$). The extract was run through a plug of cotton wool, previously moisten with $CHCl_3$ and covered with a little anhydrous sodium sulphate (for complete dehydration). The sodium sulphate was then washed with $CHCl_3$ ($5cm^3$). The combined $CHCl_3$ extract was received in to a $250cm^3$ dry conical flask. The $CHCl_3$ was then completely distilled and neutral alcohol ($5cm^3$) was added and evaporated on to a boiling water bath. The residue was further heated on the boiling water bath for 10 minutes (to remove volatile bases). The residue was then dissolved in $CHCl_3$ ($2cm^3$), and N/50 H_2SO_4 ($20cm^3$) added and warmed on a water bath to remove the $CHCl_3$ completely and cooled. The excess acid was then titrated with N/50 NaOH using methyl red as indicator till the first drop of N/50 NaOH caused color change from pink to yellow.

Flavonoids

The extract ($5cm^3$) was transferred in to a small flask and then hydrolysed by heating on a water bath with 10% H_2SO_4 ($10cm^3$) for 30minutes. The original volume was reduced to half and the mixture was cooled on ice for 15minutes where the flavonoids were precipitated. The cooled solution was then filtered and the residue was dissolved by pouring warm 95% ethanol ($50cm^3$) and further made to $100cm^3$ with 95% ethanol. Aliquot ($5cm^3$) was pipetted into a $25cm^3$ volumetric flask and diluted to volume with 50% ethanol. The absorbance of the resulting solution was measured at 370nm against 50% ethanol blank. The flavonoid concentration was finally calculated using a reference curve of pure quercetin.

Tannins

Plant extract ($5cm^3$) was transferred to a stoppered conical flask and 0.1N Iodine ($25cm^3$) and 10% 4% NaOH were added. This was mixed and kept in the dark for 15minutes. The mixture was diluted with water and acidified with 4% H_2SO_4 ($10cm^3$). The mixture was then titrated with 0.1N Sodium thiosulphate solution using starch solution as indicator. The volume (cm^3) of 0.1N Iodine used corresponds to the sum of tannins and pseudotannins [A]. Another $25cm^3$ of the extract was mixed with gelatin solution ($15cm^3$) in a $100cm^3$ measuring flask and complete to volume with water and filtered. To the filtrate ($20cm^3$), 0.1N Iodine ($25cm^3$) and 4% NaOH ($10cm^3$) were added. This was mixed and kept in the dark for 15minutes. The mixture was then diluted with water ($10cm^3$) and acidified with 4% H_2SO_4 ($10cm^3$) and then titrated with 0.1N sodium thiosulphate using starch as indicator.The volume (cm^3) of 0.1N Iodine used corresponds only to pseudotannins content [B]. Additionally, a blank experiment was carried out using distilled water.

Saponins

The plant extract ($50cm^3$) was placed in a $500cm^3$ flask, 50% alcohol ($300cm^3$) was added and boiled under reflux for 30minutes and filtered while hot through a coarse filter paper. Charcoal (2g) was added to the filtrate, boiled and filtered again while hot. The filtrate was cooled and an equal volume of acetone was added to completely precipitate the saponin. The precipitated saponin was collected by decantation and dissolved in small amount of boiling 95% alcohol and filtered while hot. The filtrate was cooled to room temperature to separate the saponins in a relatively pure form. The clean supernatant fluid was decanted and the saponins suspended in alcohol ($20cm^3$) and filtered. The filter paper was then transferred to a dessicator containing anhydrous calcium chloride and left to dry and weighed.

Cardiac glycosides

Plant extract ($8cm^3$) was transferred to a $100cm^3$ volumetric flask and H_2O ($60cm^3$) and 12.5% lead acetate ($8cm^3$) was added, mixed and filtered. The filtrate ($50cm^3$) was transferred into another $100cm^3$ flask and 4.7% Na_2HPO_4 ($8cm^3$) was added to precipitate excess Pb^{++} ions. This was mixed and completed to volume with water. The mixture was filtered twice through some filter paper to remove excess lead phosphate. Purified filtrate ($10cm^3$) was transferred into clean Erlyn – meyer flask and treated with Baljet reagent ($10cm^3$). A blank titration was carried out using distilled water ($10cm^3$) and Baljet reagent ($10cm^3$). These were allowed to stand for one hour for complete color development. The color intensity was measured colimetrically at 495nm.

Experimental Animals

Fourteen male white albino rats (weighing 150 to 240g) were purchased from the Animal Room of the Biological Sciences Department, Bayero University, Kano. The rats were maintained under standard laboratory conditions and were allowed free access to both food and water throughout the period of the experiment.

The volume (cm^3) of the root bark aqueous extract given to each rat was determined by its weight and required dose as follows:

$$\text{Volume administered } (cm^3) = \frac{\text{weight of rat (kg)} \times \text{Required dose (mg/kg)}}{\text{Concentration of the extract (mg/ } cm^3)}$$

Where concentration of the aqueous root bark extract = 100mg/ cm^3

Percentage yield (w/w) of the extract = $\frac{\text{amount produced}}{\text{amount dissolved}} \times \frac{100}{1}$

Pharmacological Study

Acute Toxicity (LD_{50}) Testing

Determination of acute toxicity (LD_{50}) was carried out using the method of Lorke (1983), Fourteen rats were used for acute toxicity testing for the determination of LD_{50} in two phases (Lorke, 1983). In the initial phase, the rats were divided into three groups of 3rats each. They were orally treated with 10, 100 and 1000mg/kg of the extract orally. The rats were observed for 24hours for any mortality. In the second phase, the rats were grouped into five of one rat each and orally treated with the extract at varying doses (250, 400, 600, 700 and 850mg/kg). The animals were observed for 24hours and the final LD_{50} value was determined from the minimum concentration (full death) and maximum concentration (no death) of the dose.

Statistical Analysis

Comparison between groups was performed using Student's paired t-test. Data are given as the mean±Standard deviation. Statistical significance was accepted at a level of P□0.05.

RESULTS

The percentage yield (w/w) of the extract was found to be 62.5% at room temperature.

Identification of Chemical Constituents

Various phytochemical constituents from the aqueous root bark extract of *S. longipedunculata* Linn. were detected and the results were summarized in Table 1.

Quantitative Determination of Phytochemicals

The various phytochemicals identified from the aqueous root bark extract of *S. longipedunculata* Linn. were quantified and the results were summarized in Table 2.

Pharmacological Study

Acute Toxicity Study

The LD_{50} value in the rats was determined to be 771mg/kg body weight intraperitoneally in Table 3 (a – b).

Table 1: Phytochemical constituents of the aqueous root bark extract of *Securidaca logipedunculata*

Constituents	Inference
Alkaloids	+
Cardiac glycosides	+
Flavonoids	+
Saponins	+
Tannins	+

Key : + = present

Table 2: Quantitative estimation (mean) of some Phytochemical constituents from the aqueous root bark extract of *Securidaca longipedunculata*

Constituents	Quantity (mg/kg)
Alkaloids	14.69±0.13
Total tannins	338.63±0.29
Pseudo tannins	157.37±0.22
True tannins	181.50±0.16
Flavonoids	31.12±0.18
Saponins	270.17±2.38
Cardiac glycosides	24.37±0.14

Results are expressed as mean ± SD

Table 3 (a-b): LD_{50} (Oral) determination of the aqueous root bark extract of *Securidaca longipedunculata*

(a)

Doses (mg/kg)	Result of first phase (mortality) n = 3
10	0/3
100	0/3
1000	3/3

(b)

Doses (mg/kg)	Result of second phase (mortality) n = 3
250	0/1
400	0/1
600	0/1
700	0/1
850	1/1

n = number of rats per group

$$LD_{50} = \sqrt{700 \times 850}$$

LD_{50} (Oral) = 771 mg/kg

DISCUSSION

The result of the phytochemical screening of the aqueous root bark extract of *S. longipedunculata* showed the presence of alkaloids, cardiac glycosides, flavonoids, saponins and tannins (Table 1). Saponins and tannins were found to be present in larger quantities than alkaloids, cardiac glycosides and flavonoids (Table 2). The reason may be due to the greater solubility of saponins and tannins in aqueous solution (Tailang and Sharma, 2009).

The presence of these chemical constituents in the aqueous root bark extract of *S. longipedunculata* is an indication that this plant if properly screened would yield drugs of plant origin with pharmacological significance. This is better supported by the fact that, the plant family (Polygalaceae) to which *S. longipedunculata* belongs, is known to be involved in ethnomedicine in the management of some ailments notably epilepsy (Mathias, 1982). They are also used as anti snake venom and as pugativ(Chhabra *et al.,* 1991). In furtherance to this, the presence of alkaloids, cardiac glycosides, flavonoids, saponins and tannins in this plant is in compliance with an already documented literature which stated that, plant families (Polygalaceae), Moraceae, and Cannabinaceae are known to contain glycosides, triterpenes, resins and higher fatty acids in their stem and root bark (Evans, 1996). It is also important to note that, the plant species has varied biological activities when various solvents extract from its root bark were tested previously (Kamba and Hassan, 2010). This is clear indication that such biological activities are easily obtainable from this part of the plant, by considering the various chemical constituents that were detected. Thus the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Furthermore, these tests facilitate the quantitative estimation and qualitative separation of pharmacologically active chemical compounds (Mallikharjuna *et al.,* 2007).

Pharmacologically, a median lethal dose; LD_{50} (Oral) value of 771mg/kg obtained is an indication that the plant is slightly toxic to the experimental model (albino rats) used. This is in accordance with toxicity classification/scale of toxic substances (Hodge and Sterner, 2005).

It has been argued that even if LD_{50} values could be measured exactly and reproducibly, the knowledge of its precise numerical value would barely be of practical importance, because an extrapolation from the experimental animals to man is hardly possible (Lorke, 1983). However, it still serves a great purpose as a first pointer to the safety or toxic potential of a substance whose toxicity profile is not yet known (Kagbo and Ejebe, 2010).

The LD_{50} value of the root bark of *S. longipedunculata* was found to be lower than the LD_{50} (Oral) of *Secuninega virosa*; 5000mg/kg (Magaji *et al.,* 2008). This indicates higher toxicity of the root bark of *S. longipedunculata* compared to that of this plant.

CONCLUSION

The result obtained in this research, indicated the presence of all the phytochemicals tested in the aqueous root bark extract of *S. longipedunculata*. Saponins and tannins were found to be present in larger quantities than alkaloids, flavonoids and cardiac glycosides.

Acute toxicity evaluation of the extract classified it as slightly toxic to the experimental animals.

Recommendations

The root bark extract of this plant should be use in lower doses especially when use as a regimen for long term treatment of chronic illnesses in view of the possible toxicities that might result at high doses as indicated by the acute toxicity testing. It will be of great important to carry out sub-acute toxicity and histopathological evaluation of the different parts of this plant in order to assess its toxic effects on vital organs including liver, lungs and kidney. Further studies would be required to isolate the specific component(s) of the plant responsible for the toxicity in order to standardize the plant preparation for maximum therapeutic benefit.

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