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Pharmacognostic and Toxicity study of *Securidaca longipedunculata* root

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Abstract
Despite the fact that roots of *S. longipedunculata* have several medicinal properties, no standardization parameter has been assessed. Due to lack of standard parameters, proper identification and ascertaining quality and purity in the events of adulteration has been thwarted. The objective of the study was to establish some important pharmacognostic profile and safety margin of *Securidaca longipedunculata* root with the hope of assisting in its standardization for quality, purity and safety. Elemental analysis was carried out using acid digestion method and phytochemical composition of the plants was evaluated using standard method. Acute toxicity was achieved using Lorke method to determine the LD_{50}. Chemomicroscopical evaluation revealed the presence of cellulose, tannins, starch, lignin, calcium oxalate, suberin, aleurone grain and mucilage with the exception of calcium carbonate. The average moisture contents, total ash, acid insoluble, water soluble ash, alcohol extractive value and water extractive values in the powdered plant material were 6.30%, 9.76%, 4.0%, 8.47%, 16.0% and 10.0% respectively. In addition, Fe, Mn, Ni, Pb, Zn, Cd and Cu were found to be within the safety limit. Phytochemicals which include alkaloids, flavonoids, saponins, tannins, carbohydrates and triterpenes were detected in both aqueous and methanolic extracts. The LD_{50} of *Securidaca longipedunculata* was found to be greater than 5000 mg /kg and could be considered safe for consumption.

Keywords: Elemental analysis, *Securidaca longipenduculata*, Pharmacognostic, Phytochemical

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Introduction

Securidaca longipedunculata is a plant commonly known as Violet tree in English and Krinkhout in Afrikaans. In Nigeria, the Hausa people call it Uwar magunguna while the Ibos call it Ezeogwu, Fulani name is 'aalali', Yoruba call it ipeta. *Securidaca longipedunculata* is a medium size tree measuring 8 to 9 m height with visible violet or white flowers, pale smooth bark, common in North-Central Nigeria and is generally

widespread in hot temperate part of Africa. When in flower the plant is distinctly ornamental. The fruits are round, with a characteristic membranous wing up to 45 mm, purplish green when young, becoming pale straw colored between April and August (Alqasim, 2013). The genus *Securidaca* comprises about 80 species, characterized by papillionaceous purplish flowers and mostly scandent shrubs and lianas, which produce compounds known as securixanthones with antimicrobial and antioxidant properties (Da Costa *et al.,* 2013). *S. longipedunculata* stem bark and roots are still found amongst the most traded medicinal plants in Africa (Tabuti *et al.,* 2012).

The root extracts are used for treating venereal diseases, skin cancer, skin infections, flu; they are also used for contraceptive purposes, abortion, constipation, coughs and fever. Other uses of the root extracts are sexual boost, toothache, tuberculosis, rheumatism, pneumonia and as blood purifier and it is also used as an aphrodisiac for men (Mongalo *et al.,* 2015). It is used in treating infections related to nervous and circulatory system, dysentery, malaria, typhoid and frequent stomach ache (Maroyi, 2013; Mustapha, 2013a, b). Traditionally, the root and bark are taken orally either powdered or as infusion for abortion, infertility, venereal diseases, headache among other diseases (Mongola *et al.,* 2015). In Limpopo, the Venda people mix the powdered root with maize and sorghum beverages for sexually weak men (Togun and Egbunike, 2006). A root decoction may be drunk in beer as an aphrodisiac, for sexual impotence, toothache, fungal infections and malaria among other diseases (Maroyi, 2013; Ogunmefun and Gbile, 2012; Mongalo *et al.,* 2015). Furthermore, extracts have antimicrobial, antioxidant, antiparasitic, anti-diabetic, anti-inflammatory, antimalarial, insecticidal, pesticidal, and anticonvulsant properties. The powdered stem bark has antimicrobial activity against a variety of organisms including *Neisseria gonorrhea*, *Candida albicans*, *Trichomonas vaginalis* and the agent for syphilis (Hedimbi and Chinsembu, 2012).The aqueous root and ethanol extracts yielded alkaloids, cardiac glycosides, flavonoids, saponins, tannins, volatile oils, terpenoids and some steroids (Haruna *et al.,* 2013a; Auwal*et al.,* 2012; Gbadamosi, 2012) while chloroform and ethanol extracts indicated flavonoids, saponins, coumarins, tannins and alkaloids (Adebayo and Osman, 2012). The aqueous root bark extract was slightly toxic to albino rats with an LD_{50} of 0.771 g/kg (Auwal *et al.,* 2012), while Agbaje and Adekoya (2012) reported an LD_{50} of 3.16 g/kg when administered orally to rats.

According to WHO (1996a and b, 1992), standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion. Attention is normally paid to such quality indices such as: Macro and microscopic characters, Foreign organic matter, ash values (total ash, sulphated ash, water soluble ash and acid insoluble ash etc.), moisture content, extractive values, chemical evaluations (qualitative and quantitative), Chromatographic examination (chemical constituents as markers) and Toxicological studies (to determine the pesticide residues, potentially toxic elements, safety studies in animals like LD_{50} and Microbial assay).The aim of this work is to carry out pharmacognostic standardization and toxicity analysis on *S. longipenduculata* root.

Materials and Methods

Chemo-microscopic Studies on the powdered root of *S. longipedunculata*

Powdered sample (5g) of plant species was used for this study to detect the presence of cell wall materials and cell inclusions. Finely ground sample of plant was cleared in a test tube containing 70% chloral hydrate solution. It was then be boiled on a water

bath for about thirty minutes to remove obscuring materials. The cleared sample was mounted with dilute glycerol onto a microscope slide. Using various detecting reagents the presence of cell wall materials and cell inclusions was detected in accordance to WHO (2011) guidelines.

Cell wall Materials

Test for Cellulose

A drop or two of iodinated zinc chloride was added to the powdered sample and allowed to stand for a few minutes and observed under a microscope. It stained cellulose cell wall blue to blue- violet.

Test for Lignin

The powdered plant material was moistened on a slide with a small volume of phloroglucinol and allowed to stand for about two minutes or until almost dry. A drop of hydrochloric acid was added and viewed under a microscope. Pink stained or cherry red was observed for the presence of lignin.

Test for Suberized or Cuticular cell walls

A drop or two of Sudan red was added to the cleared powdered sample and allowed to stand for few minutes and observed under a microscope. Orange red or red colour was observed presence of suberin or cutin on the cell.

Test for Gum and Mucilage

To a small portion of the cleared powdered sample of the plant, a drop of ruthenium red was added. Appearance of pink coloration was considered positive for gums and mucilage.

Cell Inclusions/ Cell Contents

Test for Starch grains

To a small portion of the cleared powder sample of the plant, N/50 iodine was added. Appearance of blue-black or reddish-blue coloration on some grains would be considered positive for starch.

Test for Calcium oxalates and Calcium Carbonates

To a small portion of the cleared powdered sample of the plant, HCl was added, dissolution of crystals in the powdered drug without effervescence was considered positive for calcium oxalate while slow dissolution with effervescence was considered positive for calcium carbonate.

Inulin

A drop of 1-naphthol and that of sulphuric acid was added to the powdered sample and viewed under the microscope. Spherical aggregations of crystals of Inulin turned brownish red and dissolve.

Test for Tannins

To a small portion of the cleared powdered sample of the plant, 5% ferric chloride solution was added. Appearance of greenish black colour was considered as positive for tannins.

Determination of Physicochemical Constants of the powdered root of *S. longipedunculata*

Some physicochemical parameters of the powdered sample of the plant such as moisture content, total ash, acid-insoluble ash, water-soluble ash, alcohol and water extractive values was determined as described in the updated edition of quality control methods for medicinal plant materials (WHO, 2011).

Moisture Content

This is the quantity of moisture present in a plant material. Moisture content of the powdered sample will be determined by loss on drying method.

3.0g each of the powdered sample was accurately weighed and placed in some clean, dried evaporating dishes of known weights. They were placed in an oven and heated at a temperature of 105ºC for 1 hour, then cooled in a dessicator and re-weighed. Heating and weighing were repeated until a constant weight was obtained. The weight loss on drying was computed following the formula below:

$$\% \text{ Moisture content} = \frac{\text{Initial Weight of Powder} - \text{Final Weight of Powder}}{\text{Initial Weight of Powder}} \times 100$$

Total Ash Value

2g of powdered plant materials was accurately weighed and placed separately in a crucible of known weight. It was heated gently and the heat gradually increased until it is white indicating the absence of carbon. It was allowed to cool in a desiccator and

weighed; this was repeated until a constant weight was obtained. The total ash value was determined as a percentage with the formula below

Total ash value (%) =

$$\frac{\text{Total ash value (\%)}}{\text{Initial Weight of Sample x 100}}$$

Acid-insoluble ash

This is the residue that remains after boiling the total ash with dilute hydrochloric acid.

This was determined for the powdered plant material. 25ml of dilute hydrochloric acid was added to the crucible containing ash. It was covered with a watch glass and gently boiled for 5mins. The watch glass was rinsed with 5ml of hot water and the liquid added to the crucible. The insoluble matter was collected on an ash less filter-paper and washed with hot water until the filtrate is neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, dried in an oven and ignited to a constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes and then weighed without delay (Evans, 2002).

The acid-insoluble ash will then be calculated as a percentage for each of the two plants with the formula

Acid-Insoluble Ash (%) =

$$\frac{\text{Weight of Residual Ash}}{\text{Initial Weight of Sample x 100}}$$

Water soluble ash

To the crucible containing the total ash, 25ml of water was added and boiled for 5 minutes. The insoluble matter was collected in a sintered glass crucible. It was then be washed with hot water and ignited in a crucible for 15 minutes at 105^{o}C. The weight of the residue was subtracted from the weight of the total ash. The content of water soluble ash per air dried powdered sample was calculated and recorded (WHO, 2011).

Alcohol-Soluble Extractive Value

This is the amount of extraction in percentage of a plant sample with alcohol.

4g of each of the plant material was separately weighed in a conical flask. 100ml of ethanol was added and macerated for 24 hours, during which the mixture was frequently shaken within the first 6hours using a mechanical shaker. It was filtered and 25ml of the filtrate transferred into an evaporating dish of known weight and evaporated to dryness on a water bath. It was dried to a constant weight, the percentage of alcohol-soluble extractive value was then determined for the plant as

Alcohol-Soluble Extractive Value (%) = Weight of Residue in 25ml extract x 4 x 100 / Initial weight of sample

Water-Soluble Extractive Value

This is the amount of extraction in percentage of a plant sample with water. Same procedure as in alcohol-soluble extractive value was repeated here for the two plants, but solvent for extraction here was water.

Water-Soluble Extractive Value (%) =

$$\frac{\text{Weight of Residue in 25ml extract x 4}}{\text{Initial weight of sample x100}}$$

Elemental analysis of the powdered root

The elemental analyses of the plant materials were carried out in Ahmadu Bello University Zaria, Multi-user Research Laboratory. Powdered plant material was digested using 2.5ml of hydrochloric acid (HCl) and 7.5ml Nitric Acid (HNO3). The concentration of Fe, Mg, Zn, Cu was read using the flame atomic absorption spectrophotometer (FAAS), AA 500 model, Atomic Emission Spectrophotometer. Atomic Absorption Spectrophotometer were used for other elements. Before determining the concentration of any element in the sample, calibration curve of the element in the sample was prepared using prepared standard stock solutions for the elements as reported by AOAC, 2000; 2005; Akpabio and Ikpe (2013).

Qualitative Phytochemical screening of the aqueous and methanolic extract of *S. longipedunculata* root

The plant extracts (aqueous and methanol) were subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the method described below.

Tests for carbohydrates

Molish's (General) Test for Carbohydrates: To 1 ml of the filtrate, 1 ml

of Molish's reagent was added in a test tube, followed by 1 ml of concentrated sulphuric acid down the test tube to form a lower layer. A reddish colour at the interfacial ring indicates the presence of carbohydrate (Evans, 2009).

Tests for Saponin

Frothing test: About 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30seconds. The tube was allowed to stand in a vertical position and was observed for 30mins. A honeycomb froth that persists for 10-15mins indicates presence of saponin.

Test for Flavonoids

Shinoda Test: A portion of the extract was dissolved in 1-2ml of 50% methanol in the heat metallic magnesium chips and few drops of concentrated hydrochloric acid were added. Appearance of red color indicates presence of flavonoids (Evan, 1996).

Test for Alkaloid

Wagner's Test: Few drops of Wagner's reagents were added to a portion of the extract, whitish precipitate indicates the presence of alkaloid (Evans, 1996).

Test for Steroid and Triterpenes

Liebermann-Burchard's test: To a portion of the extract, equal volume of acetic acid anhydride was added and mixed gently. 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. A colour change observed immediately and later indicates the presence of steroid and triterpenes. Red, pink or purple colour indicates the presence of Triterpenes while blue or blue green indicates steroids (Trease and Evans, 1996).

Test for Cardiac Glycoside

Kella-killiani's test: A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Observed carefully at the interphase for purple-brown ring, this indicates the presence of deoxy sugars and pale green colour in the upper acetic acid layer indicates the presence of cardiac glycosides (Evans, 1996).

Test for Tannins

Ferric chloride test: To a portion of the extract, 3-5 drops of ferric chloride was added. A greenish black precipitate indicates presence of condensed tannins while hydrolysable tannins give a blue or brownish blue precipitate (Evans, 1996).

Test for Anthraquinones

Bontrager's test: To a portion of the extract in a dry test tube, 5ml of chloroform was added and shaken for at least 5mins. This was filtered and the filtrate shaken with equal volume of 10% ammonium solution, bright pink colour in the aqueous upper layer indicates the presence of free anthraquinones (Evans, 1996).

Quantitative Phytochemical screening of the methanol extract of *S. longipedunculata* root

Preparation of Fat free Sample

About 2g of the sample was weighed and defatted with 100ml of diethyl ether using a soxhlet apparatus for 2hours.

Alkaloid Determination using Haborne (1973) Method

About 5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol were added and covered and allowed to stand for 4hours. This was filtered and the extract is concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation is completed. The whole solution was allowed to settle and the precipitates were collected and wash with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Flavonoid Determination by the Method of Bohm and Kocipal – Abyazan (1994)

About 10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter upper No. 42 (125mm). The filtrate was later be transferred into a crucible and

evaporated into dryness over a water bath

Saponin Determination

The method of Obadoni and Ochuko (2001) was used. Out of the grinded samples 10g was weighed for each and put into a conical flask and 100ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4hours with continuous stirring at about 55ºC. The mixture was filtered and the residue re-extracted with another 200ml, 200% ethanol. The combined extracts were reduced to 40ml over water bath at about 90ºC. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60ml of n – butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Tannin Determination by Van-Burden and Robinson (1981) Method

About 500mg of each sample was weighed into a 50ml plastic bottle and 50ml of distilled water was added and shaken for 1hour on a mechanical shaker. This was filtered into a 50ml volumetric flask and made up of the mark. Then 5ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1M HCl and 0.008M potassium ferro-cyanide. The absorbance was measure at 120mm within 10min.

Determination of Total Phenols by Spectrophotometric Method

The fat free sample was boiled with 50ml of ether for the extraction of the phenolic component for 15minutes. About 5ml of the extract was pipetted into a 50ml flask, and then 10ml of distilled water was added. About 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were added also. The sample was made up to mark and allowed to react for about 30 and weighed to a constant weight.

minutes for colour development. This was measured at 505nm.

Acute toxicity studies of methanol extract of *S. longipedunculata* root

Lethal Dose (LD50) Determination

This is the determination of the lethal dose known as LD50. The method of Lorke (1983) was employed. The phase I involved the oral administration of three different doses of 10, 100 and 1,000 mg/kg of the crude extract, to three different groups of three adult wister albino rats. In a fourth group, three adult male wister albino rats were administered with equivalent/volume of distilled water to serve as control. All the animals were orally administered the extract using a curved needle to which acatheter had been fixed. The animals were monitored closely every 30 minutes for the first 3 hours after administration of the crude extract and hourly for the next 6hours for any adverse effects. Then the animals were left for 72 hours for further observation.

When no death occurred, the phase II was employed, only one animal was required in each group. Groups 1–4 , animals were orally given 1,500, 2,200, 3250 and 5,000mg/kg dose levels of the crude extract. All the animals were left for observation as in stage one.

Results

Chemo-microscopical studies on root of *Securidaca longipedunculata* were found to have cellulose cell wall, lignin, calcium oxalate crystals, tannins, starch and mucilage but calcium carbonate was absent and is presented in Table 1. The result of average moisture contents using loss on drying method was calculated to be 6.30% and the percentage yield of total ash, acid insoluble and water soluble matter were recorded in percentage values as 9.76%, 4.0% and 8.47% respectively. The extractives obtained were 16.0% and 10.0% for alcohol and water solvents respectively (Table 2).

Table 1. Chemomicroscopical studies of *Securidaca longipedunculata* powdered root

Constituents	Inference
Starch	+
Gum and Mucilage	+
Cellulose cell walls	+
Lignin	+
Aleurone grain	+
Calcium oxalate crystals	+
Calcium carbonate	-
Suberized/Cuticular cell wall	+
Inulin	+

Key: + Present, - Absent

Table 2. Physicochemical Constants of *Securidaca longipedunculata* powdered root

Parameters	Values (%w/w) ± SEM*
Moisture content	6.30±0.00
Ash content	9.76±0.33
Acid insoluble ash	4.00±0.27
Water soluble ash	8.47±0.00
Water extractive value	10.00±0.00
Ethanol extractive vale	16.00±0.00

*****Average values of three determinations.

Elemental analysis of *Securidaca longipedunculata* powdered root is presented in Table 3. Trace metals which include Fe, Mn and Ni detected in *Securidaca longipedunculata* powdered root were below the FAO/WHO (1984) permissible limit for edible plants. While others, Pb, Zn, Cd and Cu were found to be within the safety limit. Phytochemicals which include alkaloids, flavonoids, saponins, tannins, glycosides, carbohydrates and triterpenes and steroid were detected in both aqueous and methanolic extracts. Anthraquinones and phenols were detected in the methanolic extract but absent in aqueous extract of *Securidaca longipedunculata* as presented in Table 4. Table 5 shows the results for the quantitative phytochemical content of the root of *Securidaca longipedunculata*. The tannins (194.0 mg/g) was the highest phytochemical detected in the plant while the lowest was saponins and phenol (4.0 mg/g). Alkaloid and flavonoids was also seen in moderate quantity.

No death was recorded in the first phase of the study in rats (Table 6). In the second phase, doses of 1500, 2250, 3250 and 5000mg/kg were used and no death was also recorded. The oral median lethal dose (LD50) for the methanol root-extract of *Securidaca longipedunculata* was therefore estimated to be greater than 5000mg/kg and no sign of behavioural changes were also observed.

Discussion

The studies carried out on the root of *Securidaca longipedunculata* have established some pharmacognostic standards that will guide its utilization as crude drug in pharmacy and other fields. The anatomical features of the internal structures of plant drugs provide salient diagnostic characteristics for the identification of both entire and powdered crude drugs and detection of adulterants in plant materials (Ghani, 1990). Chemo-microscopical examination of the powdered root of *Securidaca longipedunculata* revealed the presence of cellulose, tannins, starch, lignin, calcium oxalate, suberin, aleurone grain and

Table 3: Elemental analysis of *Securidaca longipedunculata* powdered root

Elements	Concentration (ppm)	FAO/WHO (1984) limit* (ppm)
Iron(Fe)	1.933	20.00
Copper (Cu)	0.039	3.00
Lead (Pb)	0.328	0.43
Zinc (Zn)	0.251	27.40
Nickel (Ni)	0.282	1.63
Manganese (Mn)	1.951	2.00
Aluminium (Al)	1.319	-
Cadmium (Cd)	0.003	0.21
Selenium (Se)	0.008	-
Chromium (Cr)	0.017	-
Arsenic (As)	0.095	-

Table 4. Qualitative Phytochemical screening of aqueous and methanolic extract of *Securidaca longipedunculata* root

Metabolite	Inference	
	Aqueous	Methanolic extract
Alkaloid	+	+
Flavonoid	+	+
Saponins	+	+
Cardiac glycoside	+	+
Tannins	+	+
Steroid	+	+
Triterpenes	+	+
Phenol	-	+
Anthraquinones	-	+
Carbohydrate	+	+

Table 5. Quantitative Phytochemical screening of methanolic extract of *Securidaca longipedunculata* root

Metabolite	Quantity (mg/g)
Alkaloids	106.00±0.33
Flavonoids	142.00±0.22
Saponins	4.00±0.12
Tannins	194.00±0.33
Phenols	4.00±0.88

Table 6 Acute toxicity studies of methanolic extracts of *Securidaca longipedunculata* root

Plant species	Group	Number of Animals	Dose (mg/kg)	Mortality recorded after 24hrs
Phase I	I	3	10	0/3
	II	3	100	0/3
	III	3	1000	0/3
Phase II	I	1	1500	0/1
	II	1	2250	0/1
	III	1	3250	0/1
	IV	1	5000	0/1

mucilage but calcium carbonate was absent (Table 1). The chemo-microscopic features are most valuable in the identification of powdered drug as their identification is largely based on the form, the presence or absence of certain cell types and cell inclusions. These are very important diagnostic pharmacognostic parameters for the identification and authentication of crude drugs especially in powdered plants (Chanda, 2011).

The physicochemical constants of *Securidaca longipedunculata* root determined include the moisture content, total ash value, acid insoluble ash, water soluble ash, alcohol (ethanol) extractives value and water extractives value (as seen in Table 2). These values are useful as criteria to evaluate the identity and purity of crude drugs (Evans, 2009; WHO, 1996). It also indicates the presence of various inorganic materials like carbonate, oxalate and silicate in plant materials. The average moisture content of the powdered plant material using loss on drying method was found to be 6.30%, and this value is within the permissible limits because B. H. P, (1990) and WHO, (2011) recommend the percentage of moisture content in any crude drug to be within 12-14 %. Low or permissible moisture in crude drugs may discourage the growth of bacteria, yeast, mould and fungi and will stand for long period of time during storage without spoilage or suggesting better stability against degradation of product (WHO, 1996). Ash values obtained include total ash as 9.76%, acid insoluble ash 4.0% and water soluble 8.47%. These Ash values indicate the presence of various impurities such as carbonate, oxalate, sand and silicate in plant materials (Kaneria and Chanda, 2011).

From the ash values mentioned above, the total ash value represented both the physiological and non-physiological ash from the crude drugs upon incineration. The non-physiological ash is the inorganic residues in water soluble ash after the plant drug is burnt while the acid insoluble ash indicated that the plant was in good physiological condition and it contained little extraneous matters compared to the total ash content. The total ash value is used as a standard to assess the identity and purity of crude drugs (WHO, 1996, WHO, 2011). The alcohol and water extractive values were 16.0% and 10.0% respectively (Table 2). It was observed that water had a higher extractive value (16.0%). This is because alcohol extraction capacity, it is sometimes more preferred than water especially in researches that deals with natural products because it serves as preservative against microbial growth and easy to evaporate and handle. Water is a universal solvent that has high polarity and is able to extract more phytochemical constituents than alcohol that has less polarity. This verified why water is mostly used as solvent by traditional medical practitioner and individuals in preparation of dosage forms (Ajazuddin and Shailendra, 2010).

The elemental analysis revealed some of the elements that are present in the root of *Securidaca longipedunculata* (Table 3). The elements are rich sources of macro and minor elements that aid in the growth of plants, and as well in human body functions such as muscle contraction, bone formations, growth, metabolism, osmotic balance, regulatory processes activation and other organic bimolecular activities (Rabia *et al.*, 2012). The concentrations of elements gotten from this study were below FAO/WHO (1984) permissible limits for edible plants. Zinc (Zn) is an essential component of a number of enzymes present in animal tissue including alcohol dehydrogenase, carbonicanhydrase, procarboxypeptidase and aids in normal growth, reproduction, tissue repair and wound healing. Zinc deficiency causes growth retardation and skin lesions (Chatterjee and Shinde, 1995)

Manganese (Mn) is often found in minerals in combination with iron. It is helpful in carbohydrate metabolism and served in the body as a co-factor for the enzymes involved in hydrolysis, phosphorylation, transamination and decarboxylation. It

promotes the activities of transferases such as superoxide dismutase and aids as antioxidant to scavenge damaging particles (superoxide) known as free radicals in the body (Dias, 2012). Low levels of manganese can cause infertility, bone malformation weakness and seizures.

The main function of iron is the transport of oxygen to the tissues (haemoglobin) and also in cough associated with angiotensin-converting enzyme (ACE) inhibitors, haemopoietic and cell mediated immunity (Faizul *et al.,* 2012). The deficiency of iron has been related to anemia and described as the most prevalent nutritional deficiency. The systemic decrease in Copper levels causes iron deficiency. Therefore it is antianaemic and essential for the formation of iron and haemoglobin. Copper (Cu) play important role in treatment of chest wounds and prevent inflammation in arthritis and similar diseases (Faizul *et al.,* 2012).

Besides macro and trace elements, heavy metals were also present in the root of *S. longipedunculata* but in negligible concentrations that will not cause harm when consumed or ingested as prescribed by WHO, (2007). Though, it is advisable not to be taken for a long period of time to prevent untoward effects. Cobalt (Co) is required in very small amounts in all mammals and is used to treat several different types of cancer in humans and to treat anaemia, but the intake of its high amount can cause heart diseases (Faizul *et al.,* 2012). Lead (Pb) is toxic and a non-essential element for human body, it causes a rise in blood pressure, kidney damage, miscarriages and subtle abortion, brain damage, declined fertility of men through sperm damage, diminished learning abilities of children and disruption of nervous systems (Khan *et al.,* 2011 and Obiajunwa *et al.,* 2002). Concentration of elements in plants varies from region to region due to factors such as environmental, atmospheric, pollution, season of collection of sample, age and soil conditions in which plant grows (Faizul and Rahat, 2011).

The preliminary phytochemical screening of the methanol root extract of *Securidaca longipedunculata* revealed the presence of several constituents as shown in table 4. These constituents are known to be responsible for several pharmacological activities. Flavonoids were reported as prostaglandin synthetase inhibitors (Watanebe *et al.,* 2000). This suggests that reduced availability of the prostaglandins by flavonoids might have been responsible for their analgesic activity. The presence of tannins and saponins possibly might have given rise to the observed anti-inflammatory property and contributed to the anti-pyretic activity of the plant extract.

Saponins possess a wide range of therapeutic actions in the body including anti-inflammatory, expectorant, diuretic, anti-malarial and haemolytic effects on red blood cells, while tannins are used in compress for cuts and wounds, haemorrhoids, varicose veins and in medicine for diarrhoea, catarrh, heavy menstrual flows and inflammatory conditions of the digestive tract (Evans, 1989). Cardiac glycosides increase the force of myocardial contraction and reduce conductivity within the atrioventricular (AV) node. They are used in the treatment of supraventricular tachycardias, especially for controlling ventricular response in persistent atrial fibrillation (Prassas and Diamandis, 2008).

The oral median lethal dose value for the methanol root extract of *Securidaca longipedunculata* obtained in rats was found to be above 5000mg/kg. This suggests that the plant extract is non-toxic as no death was recorded. Acute toxicity studies are usually carried out to determine the dose that will cause death or serious toxic manifestations when administered singly or severally at few doses in order to establish doses that should be used in subsequent studies (Wanda *et al.,* 2002). The Organization for Economic Cooperation and Development (OECD), Paris, France, recommended chemical labelling and classification of acute systemic toxicity based on oral median lethal dose values as: very toxic if $\leq$ 5 mg/kg, toxic if $>$ 5 mg/kg but $\leq$ 50 mg/kg, harmful if $>$ 50 mg/kg but $\leq$ 500 mg/kg, and non-toxic or not harmful if $>$ 500 mg/kg or $\leq$ 2000 mg/kg

(Walum, 1998). The LD50 was found to be greater than 5000 mg/kg when administered orally in rats (Table 6) and all the animals remain alive and did not manifest any significant visible signs of toxicity at these doses. These studies showed the extracts *S. longipedunculata* root of are practically non-toxic when administered using the oral route. This is based on the toxicity classification which states that substances with LD50 values of 5000 to 15,000 mg/kg body weight are practically non-toxic (Loomis &Hayes, 1996).

Conclusion

The established pharmacognostic standards for the powder of *Securidaca longipedunculata* root could be used as a diagnostic tool for the standardization and identification of this medicinal plant for its purity and quality in the future and hence, inclusion into the pharmacopoeia for official use. *Securidaca longipenduculata* extracts have some secondary metabolites namely alkaloids, tannins, flavonoids, cardiac glycosides and saponins. The values of Fe, Mn and Ni in the plant were below the FAO/WHO (1984) permissible limit for edible plants. However, Pb, Zn, Cd and Cu were found to be within the safety limit. The Acute toxicity (LD50) of the methanolic root extract of *S. longipenduculata* was found to be greater than 5000 mg /kg and is considered safe for use. Nonetheless, further studies are encouraged to evaluate toxicity at much higher doses.

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