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PHARMACOGNOSTIC AND TOXICITY STUDY OF *LEPTADENIA HASTATA* (Pers.) Decne (Asclepiadaceae) ROOT

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ABSTRACT
Leptadenia hastata an African plant species used in traditional medicine as an anti-venom, antihypertensive, anti-diabetic, analgesic and also used for catarrh and skin disease. Despite the fact that the root of *Leptadenia hastata* have several medicinal properties, no standardization parameter has been assessed. The study was aimed at establishing a safety profile, evaluating phytochemical constituents and some Pharmacological properties of root extract of *L. hastata*. Evaluation of the powdered sample (chemomicroscopic, physicochemical parameters), qualitative and quantitative phytochemical analysis and acute toxicity study was carried out using standard methods. Chemomicroscopic characters present included; cellulose cell wall, lignified cell wall, tannins, starch, calcium oxalate and cutin. The physicochemical parameters evaluated included: moisture content (7.3%), total ash (10.33%), water soluble (7.0%), acid insoluble ash (5.37%), ethanol extractive value (13.33%) and water extractive value (10.33%).Trace metals which included Pb, Zn, Cd, Cu and Mn detected in *L. hastata* were below the FAO/WHO (1984) permissible limit for edible plants. Phytochemicals which include alkaloids, flavonoids, saponins, phenols, tannins, glycosides, carbohydrates and triterpenes were detected in both aqueous and methanolic extracts. Steroid was absent in both extracts. The quantitative phytochemical analysis showed that tannins (298 mg/g) was the highest phytochemical detected while the lowest was saponins (6.0 mg/g). LD_{50} was above 5000 mg/kg and did not cause mortality in all the tested rats. The results of this investigation may be useful for deriving doses that are safe for human consumption medicinally of *L. hastata*.

Keywords: L*eptadenia hastata*, Pharmacological, Chemomicroscopic characters, Phytochemicals

INTRODUCTION

Traditional medicine is the total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis improvement or treatment of physical and mental illness (WHO, 2013). Traditional use of herbal medicines refers to the long historical use of these medicines. Their use is well established and widely acknowledged to be safe and effective, and may be accepted by national authorities (WHO, 2013). In recent years, the treatments and remedies used in traditional African medicine have gained more appreciation from researchers in Western science. Developing countries have begun to realize the high costs of modern health care systems and the technologies that are required, thus proving Africa's dependence to it. Due to this, interest has recently been expressed in integrating traditional African medicine into the continent's national health care system (WHO, 2013). In Africa, the importance of traditional healers and remedies made from indigenous plants play a crucial role in the health of millions (WHO, 2013).

*Leptadenia hastata (*Pers.) Decne is a member of the Asclepiadaceae family, and a widely distributed tropical African herb used as vegetable (Thomas, 2013). *Leptadenia hastata* is edible non-domesticated vegetable and it is collected in wild throughout Africa. It is typically grown in tropical dry lands in sandy soil. Wild foods like *L. hastata* provide food security during seasonal changes and are used medicinally in many areas (Thomas, 2013). Decoction of the leaves of *L. hastata* with the bark of *Erythrina senegalensis* (coral tree) either taken orally or used as a medicinal bath to treat onchocercosis in Mali (Togola *et al.*, 2008). In Chad, the roots are used to treat scabies (Betti *et al.*, 2011). This plant is commonly used in Hausa-speaking communities in Nigeria as a spice and used in sauces (Ibrahim *et al.*, 2012). Also in Nigeria, local healers use the plant for hypertension, catarrh and skin diseases (Dambatta and Aliyu, 2011). In Burkina Faso, locally it is used for sexual potency (chewing leaves), trypanosomosis (decoction of leaves), skin diseases and wound-healing (application of latex) (Tamboura *et*

al., 2007). In Senegal, the leaves have been reportedly used for lactation and as a purgative by Kerharo and Adam, 1974, Arbonnier, 2000. Senegalese healers also use the *L. hastata* for prostate and rheumatism complaints (Mathieu and Meissa, 2007)**.** *L. hastata* has been used for wound healing and considered safe to use due to its high Lethal dose quotient value of 0.78 (Tamboura *et al.,* 2007). The aim of this study is to carry out pharmacognostic and acute toxicity studies on the root extract of *Leptadenia hastata.*

MATERIALS AND METHODS

Collection, Identification and Extraction of Plant materials

Plants were collected in March, 2017 from Babura Local Government Area of Jigawa State and were conveyed for identification and authentication at the Herbarium unit of Department of Plant Biology, Bayero University, Kano by Malam Bahawuddeen Said was compared with a voucher specimen number of BUKHAN240.

Dried plant materials (50 g) were extracted using cold maceration with 500 ml of both distilled water and methanol (Evan, 2009). The contents were then filtered using a filter paper (Whatman no.1). Filtrate was concentrated to dryness using water bath which was kept in desiccator.

Chemo-microscopic Studies on the powdered root of *L. hastata*

Powdered sample (5g) of root was used for this study to detect the presence of cell wall materials and cell inclusions (WHO, 2011). Finely ground sample of root as cleared in a test tube containing 70% chloral hydrate solution. It was then boiled in water bath for about thirty minutes at 100ºC to remove obscuring materials. The cleared sample was mounted with dilute glycerol onto a microscope slide.

Test for Cell wall contents

Test for Cellulose

A drop or two of iodinated zinc chloride was added to the powdered sample and allowed for 2 minutes and observed under a microscope (WHO, 2011). It stained cellulose cell wall blue to blue- violet. The powdered root was moistened on a slide with a small volume of phloroglucinol and allowed to stand for two minutes (WHO, 2011). Drop of hydrochloric acid was added and viewed under a microscope. Pink stained or cherry red was observed for the presence of lignin. Two drops of Sudan red was added to the cleared sample and orange red or red colour was observed presence of suberin or cutin on the cell.

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 (WHO, 2011).

2. Cell Inclusions/ Cell Contents

Test for Starch grains

To a small portion of the cleared powder sample of the plant, two drops of N/50 odine was added (WHO, 2011). 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, two drops of HCl as 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 (WHO, 2011). 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 (WHO, 2011). To a small portion of the cleared powdered sample of the plant, two drops of 5% ferric chloride solution was added. Appearance of greenish black colour was considered as positive for tannins (WHO, 2011).

Determination of Physicochemical Parameters of the powdered root of *Leptadenia hastata*

Moisture Content

Powdered sample (3g) was accurately weighed and placed in an oven and heated at a temperature of 105ºC for 1 hour and then cooled in a desiccator and re-weighed (WHO, 2011). The weight loss when dry as computed following the formula below:

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

Total Ash Value

Two grams (2g) of powdered root was accurately weighed and placed separately in a crucible of known weight (WHO, 2011). It was heated gently and the heat gradually increased until it was white indicating the absence of carbon. It was cooled in a desiccator and weighed. The total ash value was determined as a percentage with the formula below

$$\% \text{ Ash Value} = \frac{\text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \text{ X } 100$$

Acid-insoluble ash

Dilute hydrochloric acid (25ml) was added to the crucible containing ash (WHO, 2011). It was covered with a watch glass and gently boiled for 5mins.The acid-insoluble ash will then be calculated as a percentage for each of the two plants with the formula

$$\% \text{ Acid insoluble Ash} = \frac{\text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \text{ X } 100$$

Water soluble ash

To the crucible containing the total ash, 25ml of water was added and boiled for 5 minutes (WHO, 2011). The insoluble matter was collected in a sintered glass crucible. It was then washed with hot water and ignited in a crucible for 15 minutes at 105ºC.

$$\% \text{ Water Soluble Ash} = \frac{\text{Weight of Total Ash} - \text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \text{ X } 100$$

Alcohol-Soluble Extractive Value

Powdered root (4g) was separately weighed in a conical flask. Ethanol (100 ml) was added and macerated for 24 hours, during

which the mixture was frequently shaken within the first 6hours using a mechanical shaker (WHO, 2011).

$$\% \text{ Ethanol Extractive Value} = \frac{\text{Weight of Extract in 25ml X 4}}{\text{Original Weight of Powder}} \text{ X } 100$$

Water-Soluble Extractive Value

Same procedure as in alcohol-soluble extractive value was repeated here for the two plants, but solvent for extraction here was water.

$$\% \text{ Water Extractive Value} = \frac{\text{Weight of Extract in 25ml X 4}}{\text{Original Weight of Powder}} \text{ X } 100$$

Elemental analysis of *L. hastata* 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, 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 screening of *L. hastata* root extracts

The root extracts (aqueous and methanolic) 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 (Evans, 2009). A reddish colour at the interfacial ring indicates the presence of carbohydrate.

Tests for Saponins

Frothing test

About 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30seconds (Evans, 2009). The tube was allowed to kept in a vertical position and was observed for 30mins. A honeycomb froth that persists for 10-15mins indicates presence of saponins.

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 (Evans, 2009).

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, 2009).

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 (Evans, 2009). Concentrated sulphuric acid (1 ml) 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.

Test for Cardiac Glycoside

Kella-killiani's test

Portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution (Evans, 2009). 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 layer at the bottom. The mixture was 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.

Test for Tannins

Ferric chloride test

To a portion of the extract, 3-5 drops of ferric chloride was added (Evans, 2009). A greenish black precipitate indicates presence of condensed tannins while hydrolysable tannins give a blue or brownish blue precipitate.

Test for Anthraquinones

Borntrager's test

To a portion of the extract in a dry test tube, 5ml of chloroform was added and shaken for at least 5 minutes (Evans, 2009). 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.

Quantitative Phytochemical screening of *L. hastata* root extract

Sample (5 g) was weighed and 200 ml of 10 % acetic acid in ethanol were added, covered and allowed for four hours in vertical position (Harborne, 2009).

Root sample (10 g) was extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature (Bohm and Kocipal-Abyazan, 1994).The whole solution was filtered through Whatman filter upper No. 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

The method of Obadoni and Ochuko (2001) was used to determine the concentration of saponins. Powdered root (10 g) was weighed and 100 ml of 20% aqueous ethanol was added and heated for four hours at 55ºC. The mixture was re-extracted with another 200 ml ethanol and reduced to 40 ml at about 90ºC, 20 ml diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n – butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride

Root sample (500 mg) was weighed and 50 ml of distilled water was added and shaken for 1hour on a mechanical shaker (Van-Burden and Robinson, 1981) to determine concentration of tannins .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 ferrocyanide. The absorbance was measured 120 mm within 10 min.

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 (Oyedemi *et al.,* 2012) bout 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 also added. This was measured at 505nm.

Acute toxicity studies of methanol extract of *L. hastata* root
Lethal Dose (LD_{50}) Determination

Determination of the lethal dose is known as LD_{50}. 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 order to test the safety of the extract at lower doses. Then the rats 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, rats were orally given 1,500, 2,200, 3250 and 5,000mg/kg dose levels of the crude extract.

RESULTS AND DISCUSSION

RESULTS

Chemo-microscopical examination of powdered root of *L. hastata* revealed the presence of cellulose, tannins, starch, lignin, calcium oxalate, suberin, aleurone grain and mucilage but calcium carbonate was absent (Table 1).
The average moisture contents in the powdered plant material using loss on drying method was calculated to be 7.3%, the total ash was 10.33% while acid insoluble and water soluble were 5.37% and 7.0% respectively. The alcohol and water extractives values were obtained to be 13.33% and 10.33% respectively (Table 2).
Trace metals which include Fe, Mn and Ni detected in *Leptadenia hastata* 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 (Table 3).
Phytochemical screening of aqueous and methanol extracts revealed the presence of alkaloid, flavonoids, glycoside, triterpenes, tannins, saponins, phenols, anthraquinones and carbohydrates while steroid was absent in both extracts (Table 4).

The tannins (298 mg/g) was the highest phytochemical detected in the plant while the lowest was saponins (6.0 mg/g) (Table 5).
No death was recorded in the first phase of the study in rats. 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 (LD_{50}) for the aqueous and methanol root-extract therefore estimated to be greater than 5000mg/kg and no sign of behavioural changes were also observed (Table 6).

Table 1: Chemomicroscopical studies of *L. hastata* Powdered root

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

Key: + Present , - Absent

Table 2: Physicochemical parameters of *L. hastata* powdered root

Parameters	Values (%w/w) ± SEM*
Moisture content	7.30±0.58
Ash content	10.33±0.17
Acid insoluble ash	5.37±0.38
Water soluble ash	7.00±0.06
Water extractive value	10.33±0.33
Ethanol extractive vale	13.33±0.33

*****Average values of three determinations.

Table 3: Elemental analysis of *L. hastata* Powdered root

Element	Concentration (ppm)	FAO/WHO (1984) limit* (ppm)
Iron(Fe)	8.848	20.00
Copper (Cu)	0.082	3.00
Lead (Pb)	0.304	0.43
Zinc (Zn)	0.616	27.40
Nickel (Ni)	0.137	1.63
Manganese (Mn)	0.910	2.00
Aluminum (Al)	6.182	-
Cadmium (Cd)	0.006	0.21
Selenium (Se)	0.178	-
Chromium (Cr)	0.029	-
Arsenic (As)	0.166	-

Table 4. Qualitative Phytochemical screening of aqueous and methanolic extracts.

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

Table 5. Quantitative Phytochemical screening using methanolic extract

Metabolite	Quantity (mg/g)
Alkaloids	30.0±0.11
Flavonoids	52.0±0.33
Saponins	6.0±0.33
Tannins	298±0.33
Phenols	8.0±0.88

Table 6 Acute toxicity studies of aqueous and methanolic extract

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

DISCUSSION

The studies carried out on the root of *L. hastata* have established some pharmacognostic standards that will guide its utilization as crude drug in pharmacy and other fields. These 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). Macro and microscopical evaluation of crude drugs are targeted at identification of precise variety and search for contaminants in plant materials (WHO, 1996). Chemomicroscopical examination of the powdered root of *L. hastata* revealed the presence of cellulose cell wall, lignified cell wall, tannins, starch, calcium oxalate and cutin while calcium carbonate was found to be absent (Table 1). The microscopic structures 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 (Jeremiah *et al.,* 2019). 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 *L. hastata* root determined include the moisture content, total ash value, acid insoluble ash, water soluble ash, alcohol (ethanol) extractives value and water extractive value (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 within the permissible limits because British Herbal Pharmacopeia (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 10.89%, acid insoluble ash 3.17% and water soluble 7.83%. These Ash values indicate the presence of various impurities such as carbonate, oxalate, sand and silicate in plant materials (Kaneria and Chanda, 2011). The total ash value is used as criteria to judge the identity and purity of drugs (WHO, 2011; Prasad *et al.,* 2012). Extractive value is determined when a given amount of plant material is extracted with a particular solvent. When the crude drug is extracted with a particular solvent, it produces a solution that contains several constituents (Evans, 2009 Vyry *et al*., 2013). The nature of the crude drug and the solvent used determines the constitution of the phyto-constituents present (Rajurkar & Damame, 1997; Nuhu *et al.,* 2016). It also helps to determine if the crude drug is debilitated or not (Tatiya *et al.,* 2012; Adekunle *et al.,* 2014). This study indicated that ethanol gave higher extractive value compared to water. The elemental analysis revealed some of the elements that are present in the root of *L. hastata* (Table 3). The elements are rich sources of macro and minor elements that aid in the growth of plants as well as 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 within FAO/WHO (1984) permissible limits for edible plants.
In this study, methanol and aqueous extracts have similar composition of phytochemical constituents (i.e. alkaloids, cardiac glycosides, saponins, flavonoid, tannins, phenols and triterpenes) but in varied concentrations (Table 4 and 5) and is

attributable to their closeness in polarity. This agrees with the findings of Abubakar *et al*., 2015 and Alhassan *et al.,* 2014 where similar phytochemicals were reported in the extracts. These primary and secondary metabolites in plants have numerous functions. Plants have the ability to produce a large varieties of secondary metabolites such as saponins, tannins, phenols, alkaloids, triterpens and phytosterols that can protect against chronic diseases. The presence of the secondary metabolites in the crude extracts of this plant may be responsible for some of the biological activities observed (Musa *et al*., 2005). Phenolic compounds such as flavonoids and tannins which are presents in this plant are one of the largest and most ubiquitous groups of plant metabolites (Adejumomi *et al*., 2008). They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han *et al*., 2007). This could explain the vast usage of this plant to manage infectious disease in folklore medicine. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Krings and Berger, 2001). Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. This therefore implies that this plant could possess antiaging, anticarcinogenic properties (Ali *et al*., 2008). Saponins are known to produce inhibitory effect on inflammation and as such, the presence of saponins in the crude extracts of this plant shows that this plant could be used as an anti-inflammatory agent (Just *et al*., 1998). Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al*., 2000; Okwu, 2004). Glycosides are known to lower the blood pressure according to many reports (Del-Rio *et al*., 1997). The results obtained in this study suggest that, the identified phytochemical compounds may be the bioactive constituents and this plant is proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit (Marjorie, 1999). Tannins reduce the risk of coronary heart diseases (Just *et al*., 1998). Tannins may be employed medicinally in antidiarrheal, haemostatic, and antihemorroidal compounds.

In order to determine the safety margin of drugs and plant products for human use, toxicological evaluation was carried out in experimental rats using Lorke's method to predict toxicity and to provide guidelines for selecting a "safe'' dose in animals and also used to estimate the therapeutic index (LD_{50}/ED_{50}) of drugs (Olson *et al.,* 2000; Rang *et al*., 2012). In this study, median lethal dose (LD_{50}) of the extracts (aqueous and methanol) of the *L. hastata* root was carried out orally in rats. The LD_{50} was found to be greater than 5000 mg/kg when administered orally in rats (Table 6) and all the animals remained alive and did not manifest any significant visible signs of toxicity at these doses. The outcome of the study of Alhassan *et al.,* 2014 gave an LD_{50} of 2000 mg/kg and this guided our choice of dose used (5000 mg/kg). The LD_{50} was found to be greater than 5000 mg/kg body weight orally and this suggested that the extract has low acute toxicity when administered orally. This may be attributed to the incomplete absorption brought about by inherent factors limiting absorption in the gastro intestinal tract (Dennis, 1984). The present study agrees with the work done by Prasanth *et al*., (2015); Ugbogu *et al*., (2016); Kofi *et al*., (2014) and Adesegun *et al.* 2016; Bruce, (2006) reported that any substance with LD_{50} estimated to be greater than 2000-5000 mg/kg body weight given orally could be considered to be of low toxicity and safe. Similarly, the chemical labelling and classification of acute systemic toxicity based on oral LD_{50} values recommended by the organisation of Economic Cooperation and Development (OECD, Paris, France) and (Walum, 1998) are as follows: less than 5 mg/kg: very toxic, greater than 5 but less than 50 mg/kg: toxic, greater than 50 but less than 500 mg/kg: harmful and, greater than 500 but less than 2000 mg/kg: no label. The very high LD_{50} observed is not a conclusive finding about the safety of the extracts of *L. hastata*, higher doses could be tested for better understanding of its effects if used or a long period of time and for proper recommendation on its future utilization (Olson *et al.,* 2000; Rang *et al.,* 2001)

CONCLUSION

The established pharmacognostic standards for the powder of *L. hastata* 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. *L. hastata* extracts have some secondary metabolites namely alkaloids, tannins, flavonoids, cardiac glycosides and saponins. These contribute in the effect of the plant. The Acute toxicity (LD50) of the root extracts of *L. hastata* (Aqueous and Methanol) was found to be greater than 5000 mg /kg and is considered safe for use.

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