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Pharmacognostic and Acute Toxicity Study of *Dalbergia Saxatilis* Stem Bark

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Abstract

The decoction of the stem bark and leaves of *Dalbergia saxatilis* is used in Traditional Medicine for various ailments such as cough, smallpox, skin lesions, bronchial ailments and toothache. The study was aimed at establishing a safety profile, evaluating phytochemical constituents and some Pharmacological properties of methanol stem bark extract of *Dalbergia saxatilis*.Evaluation of the powdered sample (chemomicroscopic, physicochemical parameters), qualitative and quantitative phytochemical analysis, and acute toxicity study was carried out using standard methods.Acute toxicity study was carried out as described by Lorke (1983) in rats. Chemomicroscopic characters present included; cellulose cell wall, lignified cell wall, tannins, starch, calcium oxalate and cutin. The physicochemical parameters evaluated included: moisture content (6.3%), total ash (15.0%), water soluble (9.0%), water insoluble acid (5.4%), ethanol extractive value (21.0%), and water extractive value (25.0%).Trace metals which included Pb and Mn detected in *Dalbergia saxatilis*were above the FAO/WHO (1984) permissible limit for edible plants. While Zn, Cd and Cu were found to be below the safety limit. Phytochemicals which include alkaloids, flavonoids, saponins, phenols, tannins, glycosides, carbohydrates and triterpenes were detected in both aqueous and methanolic extracts. Anthraquinones were detected in the methanolic extract but absent in aqueous extract of *Dalbergia saxatilis* stem bark. The quantitative phytochemical analysis showed that flavonoids (344 mg/g) was the highest phytochemical detected while the lowest was saponins (48 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 *Dalbergia saxatilis*.

Keywords: Phytochemicals, Pharmacological, Elemental analysis, Chemomicroscopic, Physicochemical

INTRODUCTION

Majority of the world population depend on traditional medicine such as herbs for treatment of various ailments. Present day medicine was derived from herbal traditions (Ezeonwumelu *et al.,* 2012). The use of traditional medicine is rapidly growing; most people are working in the field of ethnomedicine due to its accessibility and affordability. Hence, there is need for the establishment of toxicological profiles of these medicines (Salawu *et al.,* 2009).Plants used in traditional medicine are relatively safe, but some may have undesirable adverse effects which may be due to over dosage or effects of certain factors and these may lead to toxicity and death (Okigbo *et al.,* 2009). Several species of *Dalbergia* genus are widely used in traditional medicine systems and relatively few of these have been investigated from an evidence-based pharmacological approach. The species are used in traditional system of medicines all over the world in the treatment of various ailments like diarrhoea, pain, inflammation, pyrexia, leucoderma, dyspepsia, dysentery, syphilis, gonorrhoea, stomach ache, leprosy, eye diseases, scabies, pain, and ringworm (Saha *et al.,* 2013).

Dalbergia saxatilis is an African shrub 2-3m high, the flowers are white or pink borne in loose axillary or terminal panicles,15cm and is widely distributed in Northern Nigeria. It is native to Angola, Cameroon, Gabon, Ghana, Guinea, Guinea Bissau, Ivory Coast, Liberia, Nigeria, Senegal, Sierra Leone and Zaire and is used as decoction for treatment of cough, smallpox, skin lesions, bronchial ailments and toothache. Common parts used include the leaves, stem bark and roots (Saha *et al.,* 2013). Various studies have been carried out on the plant *Dalbergia saxatilis.* The describedpharmacological properties of the plant include repellant, insecticidal and antimicrobialeffects of dried leaves of the plant (Okwute *et al.,* 2009). The root solvent extract of theplant was shown to have uterine contractile activity, and contain

biologically active properties that act as a competitive inhibitor of the β2 - selective adrenoreceptoragoni stand enhanced α-adrenoreceptor function (Uchendu, 2000, 2003). The root solvent extract has also been shown to exhibit anticonvulsant effect as aqeous decoction of the root is used to treat convulsive disorders traditionally. The results obtained from the study conducted by Yemitan and Adeyemi, (2005) indicated that *Dalbergia saxatilis* may provide protection against absence andpartial seizures. Another study carried out by Yemitan and Adeyemi, (2003) on the rootextract indicated anxiolytic and muscle relaxant activities, as the decoction from theroot was used by herbal practitioners to produce sleep. (
The antioxidant activity of *Dalbergia saxatilis* has also been carried out using the radical scavenging method which indicates the presence of phenolic compounds. Antioxidants are relevant in prevention of pathologies such as arthritis, cancer, arteriosclerosis and heart diseases (Sofidiya *et al.,* 2006).

Materials and Methods

Study Area
The study was conducted at Pharmacognosy Department, Ahamdu Bello Univsersity, Zaria, Nigeria.

Collection and Identification of *Dalbergia saxatilis* Stem Bark
Fresh pieces of stem bark of *Dalbergia saxatilis* were collected from local farm at Falgore Game Reserve which lies in Tudun Wada, Doguwa and Sumaila Local government areas, Kano state, Nigeria. The pieces of the stem bark were taken to the Herbarium section of Department of Plant Biology, Bayero University, Kano for identification.

Chemo-microscopic Studies on the Powdered Stem Bark of *Dalbergia saxatilis*
Powdered sample (5g) of plant species were 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 boiled in 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 stem of *Dalbergia saxatilis*
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
Moisture content of the powdered sample was 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 105^{o}C 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:

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

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 was 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:

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

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

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

Water Soluble Ash

To the crucible containing the total ash, 25ml of water was added and boiled for 5 minutes. 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 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).

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

Alcohol-Soluble Extractive Value

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

$$\text{Alcohol-Soluble Extractive Value (\%)} = \text{Weight of Residue in 25ml extract} \times 4 \text{Initial weight of sample} \times 100$$

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.

$$\text{Water-Soluble Extractive Value (\%)} = \text{Weight of Residue in 25ml extract} \times 4 \text{Initial weight of sample} \times 100$$

Elemental Analysis of *Dalbergia saxatilis* Powdered Stem Bark

Acid Digestion of the Samples

0.5 grams of the powdered plant materialwas weighed into 10 different beakers each of 50 ml, to which 2.5ml of hydrochloric acid (HCl) and 7.5ml Nitric Acid (HNO_3) were added to each beaker. The 10 beakers used were placed in an open space for 2 hours and mixture of hydrochloric acid (HCl) and nitric acid (HNO_3) in 1:1 ratio was added to each beaker. It was kept on a hot plate at 100^{o}C-170^{o}C for 1- 4 hours. After the contents in beakers is about to dried; 5 ml of Hydrochloric acid (HCl) was added to each beaker and be kept on the hot

plate until the entire liquid content in the beakers got evaporated. Then, 5 ml of de-ionized water was added to each beaker and the solutions were poured in sterile bottles and tested for the quantification of the metals. The concentration of Fe, Mg, Zn, Cu was read using the flame atomic absorption spectrophotometer (FAAS), AA 500 model, Atomic Emission Spectrophotometer, HACH Spectrophotometry (DR/4200) and Atomic Absorption Spectrophotometer were used for other elements detected The elemental analyses of theplant materialswere carried out in Ahmadu Bello University Zaria, Multi-user Research Laboratory. The mineral elements estimations indicated the amount of macro, trace elements and heavy metals present in the Plant samples.The mineral elements detected include; Zinc (Zn), Magnesium (Mg), Lead (Pb), Manganese (Mn), Selenium (Se), Copper (Cu), Iron (Fe), Cadmium (Cd), Arsenic, Nickel and these were done by Spectrophotometric methods. 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 *Dalbergia saxatilis* StemBark

The plant extracts 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 deoxysugars 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 *Dalbergia saxatilis*stem 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.

Flavanoid 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 and weighed to a constant weight.

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 55^{o}C. 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 90°C. 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 ferrocyanide. 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 minutes for colour development. This was measured at 505nm.

Thin Layer Chromatography Profile of Crude Extracts

TLC aluminum sheet of 20 x 20cm silica gel pre-coated plate using the one way ascending techniques was employed for this analysis. The plates were cut into sizes of 5 x 10 cm. The extract was dissolved in the initial extraction solvents and spots were applied manually on the cut plate using capillary tubes, after which the plates were dried and developed in different solvents ratios of: Hexane :Ethyl acetate (8:2) in the chromatographic tank. Developed plate was sprayed using general detecting reagents (*p*-anisaldehyde/H_2SO_4, 10% H_2SO_4 in methanol) and specific detecting reagents: Borntragers, Dragendoff, ferric chloride, Libermann-buchards and aluminum chloride (it was viewed under UV 365nm) and heated at 110° C for 2 minutes where applicable. Number of spots and retardation factors (Rf values) for each of the spots were determine and recorded while the chromatograms was scanned accordingly (Gennaro, 2000; Stahl, 2005).

Acute toxicity studies of methanol extract of *Dalbergia saxatilis*stem bark

Lethal Dose (LD_{50}) 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 dosesof 10, 100 and 1,000 mg/kg of the crude extract, to three different groups of three adultwister albino rats. In a fourth group, three adult male wister albino rats wereadministered with equivalent/volume of distilled water to serve as control. All theanimals were orally administered the extract using a curved needle to which acatheter had been fixed. The animals were monitored closely every 30 minutes forthe 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 furtherobservation.

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 animalswere left for observation as in stage one.

RESULTS

Chemo-microscopical studies on stem bark of *Dalbergia saxatilis*was found to have cellulose cell wall, lignin, calcium oxalate crystals, tannins, starch and calcium carbonate, gum and mucilage.

Table 1: Chemomicroscopical studies of *Dalbergia saxatilis*powdered stem bark

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 *Dalbergia saxatilis* powderedstem bark

Parameters	Values (%w/w) ± SEM*
Moisture content	6.30±0.00
Ash content	15.00±0.58
Acid insoluble ash	5.40±0.14
Water soluble ash	9.00±0.00
Water extractive value	25.00±0.00
Ethanol extractive vale	21.00±0.58

*Average values of three determinations.

Trace metals which include Pb and Mn detected in *Dalbergia saxatilis*were above the FAO/WHO (1984) permissible limit for edible plants. While Zn, Cd and Cu were found to be below the safety limit.

Table 3: Elemental analysis of *Dalbergia saxatilis* powdered stem bark

Elements	Concentration (ppm)	FAO/WHO (1984) limit* (ppm)
Iron(Fe)	6.357	20.00
Copper (Cu)	0.085	3.00
Lead (Pb)	0.538	0.43
Zinc (Zn)	0.154	27.40
Nickel (Ni)	0.188	1.63
Manganese (Mn)	2.583	2.00
Aluminum (Al)	5.370	-
Cadmium (Cd)	0.008	0.21
Selenium (Se)	0.161	-
Chromium (Cr)	0.027	-
Arsenic (As)	0.201	-

Phytochemicals which include alkaloids, flavonoids, saponins, phenols, tannins, glycosides, carbohydrates and triterpenes were detected in both aqueous and methanolic extracts. Anthraquinones were detected in the methanolic extract but absent in aqueous extract of *Dalbergia saxatilis* stem bark

Table 4: Qualitative Phytochemical screening of aqueous and methanolic extract of *Dalbergia saxatilis* stem bark

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

Table 5: Quantitative Phytochemical screening of aqueous and methanolic extract of *Dalbergia saxatilis* stem bark

Metabolite	Quantity (mg/g)
Alkaloids	234.00±0.33
Flavonoids	344.00±0.58
Saponins	48.00±0.33
Tannins	194.00±0.33
Phenols	49.00±0.88

TLC Profile of *Dalbergia saxatilis*methanol extract using solvent system Butanol: Acetic acid: water (6:2:2). Four spots were detected with *p-Anisaldehyde* spray and the Rf values were shown alongside the spots.

Table 6: TLC results of Methanolic extract of *Dalbergia saxatilis* stem bark

Extract	Solvent system	Number of Spots	Distance of spots	RF-Value
Dalbergia saxatilis (Methanol)	BU:AA: water (6:2:2)	4	5.7	0.35, 0.47, 0.61, 0.88

Key: BU (Butanol), AA (Acetic acid)

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 (LD50) for the aqueous and methanol stem bark-extract of *Dalbergia saxatilis* was therefore estimated to be greater than 5000mg/kg and no sign of behavioural changes were also observed.

Table 7: Acute toxicity studies of aqueous and methanolic extracts of *Dalbergia saxatilis*stem bark

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 stem bark of *Dalbergia saxatilis*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). Macro and microscopical evaluation of crude drugs are targeted at identification of precise variety and search for contaminants in plant materials (WHO, 1996).

Chemo-microscopical examination of the powdered stem bark of *Dalbergia saxatilis*revealed the presence of cellulose, tannins, starch, lignin, calcium oxalate, suberin, aleurone grain, mucilage and calcium carbonate. 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 *Dalbergia saxatilis*stem bark determined include the moisture content, total ash value, acid insoluble ash, water soluble ash, alcohol (ethanol) extractives value and water extractives value. 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 15.0%, acid insoluble ash 4.0% and water soluble 8.43%. 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 21.0% and 25.0% respectively. It was observed that water had a higher extractive value (25.0%). This is because 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). Despites alcohol's low 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.

The preliminary phytochemical screening and thin layer chromatography of themethanol leaf extract of *Dalbergia saxatilis* revealed the presence of several constituents. These constituents are known to be responsible for several pharmacological activities. Flavonoids were reported as prostaglandin synthetase inhibitors (Watanebe *et al.,* 2000). Prostaglandins are known to be involved in the pain perception (Helms and Barone, 2008). This suggests that reduced availability of the prostaglandins by flavonoids might have been responsible for their analgesic activity. Saponins possess a wide range of therapeutic actions in the body including antiinflammatory, 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 stem bark extract of *D. saxatilis* 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 ≤ 5 mg/kg, toxic if > 5 mg/kg but ≤ 50 mg/kg, harmful if > 50 mg/kg but ≤500 mg/kg, and non-toxic or not harmful if > 500 mg/kg or ≤ 2000 mg/kg (Walum, 1998). Based on this classification, the oral median lethal dose obtained for rats found to be above 5000 mg/kg, is relatively safe orally.

CONCLUSION

The established pharmacognostic standards for the powder of *D. saxatilis*stem bark 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. *D. saxatilis*extracts have some secondary metabolites namely alkaloids, tannins, flavonoids, cardiac glycosides and saponins. These were confirmed by thin layer chromatography profiles. These contribute in the effect of the plant. The Acute toxicity (LD50) of the stem bark extracts of *D. saxatilis* (Aqueous and Methanol) was found to be greater than 5000 mg /kg and is considered safe for use.

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