



Pharmacognostic and Acute Toxicity Study of *Burkea Africana* Root

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ABSTRACT: *Burkea africana* is a plant that belongs to then family Fabaceae; it is widely spread in tropical Africa including Nigeria. It is of valuable in ethnomedicine especially in the treatment of antidote for venomous stings and bites, cutaneous and sub cutaneous parasitic infection, convulsion and pulmonary troubles. Despite the fact that roots of *Burkea africana* 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 *Burkea africana* 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 LD₅₀. 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 3.8%, 7.5%, 4.43%, 8.07%, 25.0% and 20.33% respectively. In addition, Fe, Mn, Ni, Pb, 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 LD₅₀ of *Burkea africana* was found to be greater than 5000 mg /kg and could be considered safe for consumption.

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Ever before the advent of orthodox medication, plants and plant parts in forms of squeezed, aqueous (cold, warm or hot) and illicit gin extracts have served as dependable sources of medicine in traditional African societies and beyond. Indeed, according to WHO (2002) report, traditional medicine using plant extracts will continue to provide health coverage for over 80% of the world's population, particularly the developing countries. The emergence of hitherto unknown disease causing microbes is of immense public health concern. This requires renewed strategies on treatment and prevention, essentially through the development of new antimicrobials (Iwu *et al.*, 1999). Thus, further drug research and development from any viable source is imperative, and the investigation of indigenous

plants may yield positive results. A number of medicinal plants have been found and put into use in ethnomedicine by traditional healers in the management of many ailments for many years (Sofowora, 1993). Many medicinal plants are believed to be important source of new chemical compounds with potential therapeutic effects (Eisner, 1990). The stem bark of *Burkea africana* is used as antidote for venomous stings and bites, cutaneous and sub cutaneous parasitic infection, convulsion, pulmonary troubles. The plant is indigenous to Tropical Africa including Nigeria and commonly known as Bakin makarho and Apasa among the Hausas and Yoruba, respectively (Burkill, 1985). Previous works revealed that the stem bark extract of the plant possess

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pharmacological properties which include antioxidant and weak antimicrobial activity (Mathisen *et al.*, 2002); antiarrhoeal activity (Tanko *et al.*, 2011); anticonvulsant activity (Yaro *et al.*, 2010; Mahdi *et al.*, 2013) and antioxidative stress of the polyphenolic-rich stem bark fractions in cell lines (Cordier *et al.*, 2013). The aim of this work is to carry out pharmacognostic standardization and toxicity analysis on *Burkea africana* root.

MATERIALS AND METHODS

Collection and Identification of Plant material: *Burkea africana* root was collected in March, 2017 from Babura Local Government Area of Jigawa State and conveyed for identification and authentication at the Herbarium unit of Department of Plant Biology, Bayero University, Kano, Nigeria.

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

Chemo-microscopic Studies on the powdered root of *Burkea Africana*: 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 *Burkea africana*: 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: Exactly 3 g 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°C for 1 hour, then cooled in a desiccator 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:

$$\%MC = \frac{W_1 - W_2}{W_1} \times 100$$

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Where %MC = Percentage Moisture content; W_1 = initial weight of powder; W_2 = Final weight of powder
Total Ash Value: Exactly 2 g 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

$$\%AV = \frac{\text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \times 100$$

Where AV = Percentage Ash Value

Acid-insoluble ash: This was determined for the powdered plant material. 25 ml 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 5 ml 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, 2009).

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

$$\%AIA = \frac{\text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \times 100\%$$

Where %AIA = percentage acid insoluble ash

Water soluble ash: To the crucible containing the total ash, 25 ml 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°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).

$$\%ASA = \frac{W_1 - W_2}{W_3} \times 100\%$$

Where %ASA = percentage acid insoluble ash; W_1 = weight of total ash; W_2 = weight of residual ash and W_3 = original weight powder

Alcohol-Soluble Extractive Value: 4 g of each of the plant material was separately weighed in a conical flask. 100 ml of ethanol was added and macerated for 24 hours, during which the mixture was frequently shaken within the first 6 hours using a mechanical shaker. It was filtered and 25 ml 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

$$\%EEV = \frac{\text{Weight of Extract in 25ml} \times 4}{\text{Original Weight of Powder}} \times 100\%$$

Where %EEV = percentage acid insoluble ash

Water-Soluble Extractive Value: Same procedure as in alcohol-soluble extractive value was repeated here for the sample, but solvent for extraction here was water.

$$\%WEV = \frac{\text{Weight of Extract in 25ml} \times 4}{\text{Original Weight of Powder}} \times 100\%$$

Where %WEV = Percentage Water Extractive Value

Elemental analysis of the Powdered Root of Burkea Africana: 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, Mn, 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 Burkea africana 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 Saponins: 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 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 (Evan, 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. 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 (Evans, 2009).

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, 2009).

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, 2009).

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 Burkea africana 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 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 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 FeCl₃ 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 and weighed to a constant weight.

Acute toxicity studies of methanol extract of *Burkea africana* 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 AND DISCUSSION

Chemo-microscopical examination of powdered root of *Burkea africana* revealed the presence of cellulose, tannins, starch, lignin, calcium oxalate, suberin, aleurone grain, calcium carbonate and mucilage. (Table 1). The average moisture contents in the powdered plant material using loss on drying method was calculated to be 3.8%, the total ash was 7.5% while acid insoluble and water soluble ash were 4.43%

and 8.07% respectively. The alcohol and water extractives values were obtained to be 25.0% and 20.33% respectively (Table 2). Trace metals which include Fe, Mn and Ni detected in *Burkea africana* powdered root were below the FAO/WHO (1984) permissible limit for edible plants. While others, Pb and Zn were found to exceed the safety limit (Table 3). Phytochemical screening of aqueous and methanol extracts revealed the presence of alkaloid, flavonoids, saponins, tannins, anthraquinones and steroid (Table 4). 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 (table 5). The oral median lethal dose (LD₅₀) for the methanol root-extract of *Burkea africana* was therefore estimated to be greater than 5000mg/kg and no sign of behavioural changes were also observed (Table 6).

Table 1 Chemomicroscopical Studies of Powdered Root of *Burkea africana*

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

Chemomicroscopical examination of the powdered root of *Burkea africana* revealed the presence of cellulose cell wall, lignified cell wall, tannins, starch, calcium oxalate, cutin and calcium carbonate. 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; Chanda. 2011). The physicochemical parameters including the moisture content, acid insoluble ash, water soluble ash, alcohol extractives value, water extractives and total ash values were determined from the powdered leave of the plant (Kokate, 2003).The result of average moisture contents using loss on drying method was calculated to be 3.8% and the percentage yield of total ash, acid insoluble and water soluble ash were recorded in percentage values as 7.5%, 4.43% and 8.07% respectively. The extractives obtained were 18.33% and 25.68% for alcohol and water solvents respectively. These are values that are very important as basis to judge the identity, purity and in detecting adulterants in a crude drug (Evans, 2009; WHO, 2011). Moisture content was not high which indicated

less chances of microbial degradation of the drug during storage. The general requirement of moisture content in crude drug is recommended not to be more than 14 % (Peter, 1990) and the value obtained in this research work was within the accepted range. Determination of the moisture content helps preventing degradation of drug during storage.

The lower the value, the less likelihood of degradation of drug and suggests better stability of the product. Moisture is considered an adulterant because of its added weight as well as the fact that excess of moisture promotes mold and bacterial growth (Prasad *et al.*, 2012; Nuhu *et al.*, 2016). Total ash value represents both the physiological and non-physiological ash from the plant. The non-physiological ash is an indication of inorganic residues after the plant drug is incinerated.

Table 2 Physicochemical Constants of *Burkea africana* Powdered Root

Parameters	Values (%w/w) ± SEM*	EP (2011)
Moisture content	3.80±0.58	10-12%
Ash content	7.50±0.29	6-19%
Acid insoluble ash	4.33±0.23	>1
Water soluble ash	8.07±0.22	
Water extractive value	20.33±0.33	
Ethanol extractive value	25.0±0.58	

*Average values of three determinations.

Table 3: Elemental Analysis of *Burkea africana* Powdered Root

Element	Concentration (ppm)	FAO/WHO (1984) limit* (ppm)
Iron(Fe)	6.025	20.00
Copper (Cu)	0.435	3.00
Lead (Pb)	0.678	0.43
Zinc (Zn)	29.485	27.40
Nickel (Ni)	0.245	1.63
Manganese (Mn)	0.529	2.00
Aluminum (Al)	7.415	-
Cadmium (Cd)	0.000	0.21
Selenium (Se)	0.044	-
Chromium (Cr)	0.029	-
Arsenic (As)	0.038	-

Table 4. Qualitative Phytochemical Screening of Aqueous and Methanolic root extract of *Burkea africana*

Chemical compound	Inference aqueous	Methanolic extract
Alkaloid	+	+
Flavonoid	+	+
Saponins	+	+
Cardiac glycoside	+	+
Tannins	+	+
Steroid	+	+
Triterpenes	+	+
Phenol	+	+
Carbohydrate	+	+
Anthraquinones	+	+

Key; + Presence, -absence

The acid insoluble ash values obtained in this study indicated that the plant was in good physiological condition and contained little extraneous matter such as sand, silica and soil. 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 and 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 (25.0% w/w) compared to water which had extractive value of 20.33 % w/w.

Table 5. Quantitative Phytochemical Screening of Methanolic Extract of *Burkea africana* Root

Chemical compounds	Quantity (mg/g)
Alkaloids	186.0±0.33
Flavonoids	230.0±0.58
Saponins	49.0±0.33
Tannins	81.0±0.33
Phenols	49.00±0.58

Table 6. Acute Toxicity Studies of Methanolic Root extract of *Burkea africana*

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

The elemental analysis revealed some of the elements that are present in the root of *Burkea africana*. 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 with exception of zinc and lead. 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)

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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 *Burkea africana* 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) which exceed the permissible limit, 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 aqueous and methanol root extract of *Burkea africana* 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). 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). In order to determine the safety margin of drugs and plant products for human use, toxicological evaluation was carried out in experimental animals 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 (LD₅₀/ED₅₀) of drugs (Olson *et al.*, 2000; Rang *et al.*, 2012). In this study, median lethal dose (LD₅₀) of the extracts (aqueous and methanol) of the *Burkea africana* root was carried out orally in rats. The LD₅₀ was found to be greater than 5000 mg/kg when administered orally in rats and all the animals remain alive and did not manifest any significant visible signs of toxicity at these doses. These studies showed the extracts *Burkea africana* 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 and Hayes, 1996).

Conclusion: The established pharmacognostic standards for the powder of *Burkea africana* root could be used as a diagnostic tool for the standardization and identification of this medicinal plant for its purity, quality and compilation of a suitable monograph on *Burkea africana*. Methanolic root extract of *Burkea africana* was found to be practically safe and is considered safe for use. Nonetheless, further studies are encouraged to evaluate toxicity at much higher doses.

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