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Physicochemical screening and Acute Toxicity Study of *Anogeissus leiocarpa* Stem Bark

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Abstract

Anogeissus leiocarpa is a tree widely distributed in northern Nigeria and traditionally used in the treatment of diabetic ulcers, general body pain, blood clots, asthma, coughing, jaundice, pile and tuberculosis. The objective of the study was to establish some important pharmacognostic profile and safety margin of A. leiocarpa stem bark with the hope of assisting in its standardization for quality, purity and safety. Evaluation of the powdered sample of the stem bark was carried out to determine the chemomicroscopic, some physicochemical parameters of the plant and extracted with aqueous and methanol. The extracts were subjected to qualitative and quantitative phytochemical analysis and acute toxicity study. Chemomicroscopic characters present include; cellulose cell wall, lignified cell wall, tannins, starch, calcium oxalate and cutin. The physicochemical parameters evaluated include: moisture content (6.10%), total ash (10.47%), water soluble (7.83%), water insoluble acid (2.17%), ethanolextractive value (25.68%), and water extractive value (18.33%). The quantitative phytochemical analysis showed that alkaloids (412.0 mg/g) was the highest phytochemical detected in the stem bark while the lowest was saponins (4.0 mg/g).LD_{50} of both extracts 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 A. leiocarpus.

Keywords: *Anogeissus leiocarpus;* Standardization; Phytochemicals; Safety margin

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INTRODUCTION

Plant medicines are the most widely used medicines in the world today. Eighty-five percent (85%) of the world's population employs herbs as their primary medicines. Evidence for the use of plants for medicinal purposes dates as far back as 60,000 years ago in both developed and under-developed countries (Gossell-Williams *et al.,* 2006).The use of medicinal plants to cure many ailments has been a tradition in different parts of the world (Ayeni and Nuhu, 2018). Today with the present surge of interest in the phyto-therapeutics, the availability of genuine plant material is becoming scarce. Since crude plant drugs form the basis for the manufacture of numerous medicinal preparations, accurate determination of drug identity forms an essential part of its study. It becomes extremely important to make an effort towards standardization of the plant material as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies (Thomas *et al*., 2008; Ibrahim *et al*., 2017).

Anogeissus leiocarpa (DC.) Gill & Peer is adeciduous tree species that can grow up to 15 - 18 m of height and measure up to 1 m diameter (Ahmad, 2014). Bark greyish and scaly, branches often drooping and slender, leaves alternate, ovate–lanceolate in shape, 2 - 8 cm long and 1.3 - 5 cm across (Ouedraogo *et al*., 2013). The leaves are acute at the apex and attenuate at the base, pubescent beneath. Inflorescence globose heads, 2 cm across, yellow; the flowers are bisexual, petals absent. Fruits are globose, cone like heads; each fruit is broadly winged, dark grey, 3 cm across. It can reproduce by seeds as well as vegetative propagation (Ouedraogo *et al*., 2013; Mukhtar *et al.,* 2017). The plant is popularly known as African birch, Axle wood tree (Victor *et al*., 2013); "Marke", "Farin gamji" in Hausa, "Kojoli" in Fulfulde, "Kukunchi" in Nupe, "Annum" in Kanuri, "Ainy" or "Orin-odon" in Yoruba and "Atara" in Igbo language of Nigeria (Aliyu, 2006; Victor *et al.,* 2013). The plant has been reported to used traditionally for the treatment of different ailments including the treatment of diabetic ulcers, general body pain, blood clots, asthma, coughing, jaundice, pile and tuberculosis (El Ghazali *et al.,* 2003; Victor, 2013; Abubakar *et al.,* 2017), Malaria, Trypansomiasis, Helminthiasis and dysenteric syndrome (Okpekon, 2004) and also, it is used against stomach infections and fungal infections such as dermatitis and Mycosis (Batawila *et al*., 2005). Recent studies have revealed that the plant exhibits a variety of pharmacological activities including Antiplasmodial (Vonthron-Senecheau, 2003; Akanbi, 2012), Antioxidant and hepatoprotective (Olajide, 2011; Victor and Grace, 2013) Leishmanicidal (Shuaibu, 2008a) Anthelmintic (Agaie and Onyeyili, 2007; Ademola and Eloff, 2011) Trypanocidal (Atawodi, 2003; Shuaibu, 2008b) antimicrobial (Taiwo *et al.,* 1999; Elegami, 2002).

The objective of the present investigation is to establish some important pharmacognostic profile and safety margin of *A.leiocarpa*stem bark with the hope of assisting in its standardization for quality, purity and safety.

MATERIALS AND METHODS

Collection of Sample

The plant material was collected at Babura Local Government Area of Jigawa State. The plant was taxonomically authenticated at the Herbarium unit, Department of Plant biology, Bayero University Kano, Nigeria with Voucher specimen number BUKHAN29. The stem bark was dusted, cleaned and all foreign matter removed, it was then air-died and comminuted to powder form, stored in an air-tight container for subsequent use.

Laboratory Procedure

Various laboratory procedures were carried out for further analyses of the plant material. The histochemical detection of cell walls and contents of the powdered stem bark such as cellulose

cell wall, lignin, starch, cutin, tannins and calcium oxalate, calcium carbonate etc. was carried out using standard method (Evans, 2009; WHO, 2011).

Physicochemical Parameters

Powdered sample was subjected to physicochemical analysis such as water and alcohol soluble extractives, total ash, acid insoluble ash, water soluble ash and moisture content were determined (WHO, 2011).

About 50 g of the powder sample of *A.leiocarpa* stem bark was macerated with 500 ml of aqueous and methanol successively. The extracts was evaporated to dryness on water bath.

Qualitative Phytochemical screening of the aqueous and methanol extracts of *Anogeissus leiocarpa* stem bark

The aqueous extracts was subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the standard phytochemical reagents and procedures (Sofowora, 2006; Evans, 2009).

Preparation of Sample

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

Alkaloid Determination

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 (Harborne, 1973).

Flavonoid Determination

About 10 g 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 (125 mm). The filtrate was transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Bohm and Kocipal – Abyazan, 1994).

Saponin Determination

The method of Obadoni and Ochuko (2001) was used. Out of the grinded samples 10 g was weighed for each and put into a conical flask and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55^{o}C. The mixture was filtered and the residue re-extracted with another 200 ml, 200% ethanol. The combined extracts were reduced to 40ml over water bath at about 90^{o}C. The concentrate was transferred into a 250 ml separating funnel and 20 ml 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 60 ml of n – butanol was added. The combined n-butanol extracts were washed twice with 10 ml 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

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 (Van-Burden and Robinson, 1981).

Acute toxicity studies of aqueous and methanol extracts of *Anoigeissus leiocarpa*stem bark
The lethal dose was determined by Lorke's method. Phase I: Nine wistar rats were used. They were divided into three groups of three animals each. Each groups of animals were administered different doses (10, 100, and 1000 mg/kg) of the extracts and then observed for 24 hours to monitor their behavior as well as mortality. Phase II: Three animals were used. They were divided into three groups of one animal each. The animals were administered higher doses (1600, 2900 and 5000 mg/kg of the extracts and observed for behavior as well as mortality (Lorke, 1983). The oral median lethal dose (LD_{50}) was calculated as the geometric mean of the minimum toxic dose and maximum tolerated dose.

RESULTS AND DISCUSSION

Chemomicroscopical examination of the powdered stem bark of *A. leiocarpus*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).

Table 1: Chemomicroscopical Examination of *Anoigeissus leiocarpus* Powdered Stem bark

Constituents	Inference
Starch	+
Gum and Mucilage	+
Cellulose cell walls	+
Lignin	+
Aleurone grain	+
Calcium oxalate crystals	+
Calcium carbonate	-
Cutin	+
Inulin	+

Key: + Present, - Absent

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 6.10% and the percentage yield of total ash, acid insoluble and water soluble matter were recorded in percentage values as 10.47%, 2.17% and 7.83% respectively. The extractives obtained were 18.33% and 25.68% for alcohol and water solvents respectively (Table 2). 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 (5.53 %) 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 (8.4 %) 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. The acid insoluble ash values (1.2 %) 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 & 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 lower extractive value (25.6 % w/w) compared to water which had extractive value of 32.4 % w/w.

Table 2. Physicochemical Constants of *Anogeissus leiocarpa* powdered stem bark

Parameters	Values (%w/w) ± SEM*
Moisture content	6.10 ± 0.11
Total ash content	10.47 ± 0.11
Acid insoluble ash	2.17 ± 0.83
Water soluble ash	7.83±0.60
Water extractive value	18.33±0.88
Ethanol extractive vale	25.68±0.88

*Average values of three determinations, SEM (Standard error mean)

Preliminary phytochemical screening gives a brief idea about the qualitative nature of active phytochemical constituents present in plant extracts, which will helps the future investigators regarding the selection of the particular extract for further investigation or isolating the active principle (Mishra *et al.,* 2010). Phytochemical analysis of the stem-bark extracts had revealed the presence of some secondary metabolites namely carbohydrate, alkaloids, tannins, flavonoids, cardiac glycosides, saponins, steroid/triterpenes and absences of anthraquinones (Table 3); this result is in agreement with the finding of Mann *et al*. (2010) and Aliyu and Sani (2011). The information on the presence or absence and the type of phytochemical constituents especially the secondary metabolites are useful taxonomic keys in identifying a particular species and distinguishing it from a related species, thus helping in the delimitation of taxa (Jonathan and Tom, 2008).

Table 3: Qualitative Phytochemical screening of aqueous and methanolic stem bark extract of *Anogeissus leiocarpa*

Metabolite	Inference	
	Aqueous Extract	Methanol Extract
Alkaloid	+	+
Flavonoid	+	+
Saponins	+	+
Cardiac glycoside	+	+
Tannins	+	+
Steroid/ Triterpenes	+	+
Anthraquinones	-	-
Carbohydrate	+	+

Table 4 shows the results for the quantitative phytochemical content of the stem bark of *A. leiocarpa*. The alkaloids (412.0 mg/g) was the highest phytochemical detected in the plant while the lowest was saponins (4.0 mg/g). Tannins and flavonoids was also seen in moderate quantity. These phytochemicals are known to exhibit medicinal activity as well as pharmacological activity. Alkaloids have a wide range of pharmacological activities including antimalarial (e.g., quinine), anticancer (e.g., homoharringtonine) (Kittakoop *et al.,* 2014), antibacterial (e.g., chelerythrine) (Cushnie *et al.,* 2014), and antihyperglycemic activities (e.g., piperine) (Qiu *et al.,* 2014). Tannin is one of the major active ingredients found in plant based medicines (Haslam, 1996); they are used in the dyestuff industry as caustics for cationic dyes

(tannin dyes), and also in the production of inks (iron gallate ink), textile dyes, antioxidants in beverages, and coagulant in rubber production as well as possessing antiviral, antibacterial, and antitumor activity (Haslam, 1996; Khanbabaee & Van Ree, 2001). Tannin has been reported to selectively inhibit HIV replication (Kashiwada *et al*., 1992). Flavonoids are known to have antioxidant effects and have been shown to inhibit the initiation, promotion, and progression of tumors (Kim *et al*., 1994); reduction of coronary heart disease has been reported to be associated with intake of flavonoid (Hertog *et al*., 1993).

Table 4. Quantitative Phytochemical screening of methanolic extract of *Anogeissusleiocarpa* stem bark

Metabolite	Quantity (mg/g)
Alkaloids	412 ± 0.11
Flavonoids	223 ± 0.22
Saponins	4.00 ± 0.12
Tannins	243.00 ± 0.33

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 (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 *A. leiocarpus* stem bark was carried out orally in rats. The LD_{50} was found to be greater than 5000 mg/kg when administered orally in rats (Table 5) and all the animals remain alive and did not manifest any significant visible signs of toxicity at these doses. These studies showed the extracts *A. leiocarpus* stem bark of are practically non-toxic when administered using the oral route. This is based on the toxicity classification which states that substances with LD_{50} values of 5000 to 15,000 mg/kg body weight are practically non-toxic (Loomis &Hayes, 1996).
Table 5: Acute toxicity studies of aqueous and methanol extracts of *Anogeissusleiocarpa* stem bark when administered orally to Wistar Rats

Experiment	Dose(mg/kg)	Number of dead rat after 24 hours	
		Aqueous Extract	Methanol Extract
Phase 1	10	0/3	0/3
	100	0/3	0/3
	1000	0/3	0/3
Phase 2	1600	0/1	0/1
	2900	0/1	0/1
	5000	0/1	0/1

CONCLUSION

From the results obtained, .*A. leiocarpa* possess secondary metabolites which include alkaloids, tannins, flavonoids, cardiac phenols and saponins. The values of Fe, Mn and Ni in the plant were below the FAO/WHO (1984) permissible limit for edible plants. However, Pb, Zn, Cd and Cu were found to be within the safety limit. The Acute toxicity (LD50) of the methanolic leaf extract of *A. leiocarpa* was found to be greater than 5000 mg /kg and is considered safe for use. Nonetheless, further studies are encouraged to evaluate toxicity at much higher doses.

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