Nigerian Journal of Pharmaceutical Research
Vol. 9 No 2 pp. 95 – 102 (September 2011)

PRELIMINARY STANDARDIZATION OF THE HYDRO-ALCOHOLIC LEAF EXTRACT OF *ANOGEISSUS LEIOCARPUS* (D.C.) GUILL AND PERR (COMBRETACEAE)

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Abstract

A study was conducted in order to standardize the hydro-alcoholic leaf extract of *Anogeissus leiocarpus* by investigating its moisture content, phytochemical constituents, acute toxicity effects, water and ethanol soluble extractives and its effect on smooth muscles. The moisture content was 7.43±0.16%. The phytochemical tests revealed the presence of flavonoids, tannins, cardiac glycosides, and alkaloids. The LD_{50} was 346.4 mg/Kg body weight. The ethanol and water soluble extractives were 12.70 and 8.12% respectively. The extract showed relaxation effect on rat duodenum and uterus.

Keywords: *Anogeissus leiocarpus*; acute toxicity; preliminary standardization.

INTRODUCTION

Anogeissus leiocarpus (D.C) Guill and Perr (Combretaceae) also known as Axle wood tree (common name); "In Nigeria the plant is known as Marke (Hausa), Ayin (Yoruba) Atara (Ibo)", is a tall evergreen tree native to savannah of tropical Africa. It is the sole West African species of the genius Anogeissus, a genus otherwise distributed from tropical central and east Africa through tropical Southeast Asia. It a graceful tree of the Sahel to forest zones, straight tapering boles branching from low down, often gregarious and effectively killing out grasses. The tree grows to about 15-18 m tall in Senegal, 22 m tall in Ghana and nearly 30 m tall in Nigeria. The bark is grey or pale brown and peels off in very thin patches and often curls at the edges. The leaves of the plant are 2.5-7.5 cm long and 12-30 mm broad. They are elliptic to ovate-lanceolate, acute or bluntly pointed, rounded at the base or broadly cuneate with the midrib projecting in short spine. The leaves are arranged alternately and the flowers which are bisexual are greenish yellow. In each flower, the calyx tube is fused to the ovary and resembles a stalk with 5 calyx teeth forming a shallow cup at the apex. It has ten free stamens and a short simple style. The slash of the wood is yellow with darker streak exuding a brown gum. The sapwood is rather wide, dirty-white or yellowish-grey and is susceptible to borers. The heartwood is dark dull brown, streaky, becoming

almost ebony black and is very hard, dense and fine textured. The plant fruits between August - January (Steentoft, 1988; Adejumobi, 2008).
The plant leaves and stem bark extracts have been reported in various African countries for the treatment and management of helminthosis, diarrhea, leprosy, schistosomiasis, sores, diabetic ulcers and boils (Adejumobi *et al.*, 2008, Mann[b] *et al.*, 2008; Kabone *et al*, 2009).

Many secondary metabolites such as alkaloids, glycosides, steroids, phenols, tannins, ellagic acid, anthraquinones, saponins and flavonoids, have been isolated from different parts of the plant (Mann[a] *et al.*, 2008) .

Many plant products possess useful and harmful components and, as a result, there is need to assess both the intrinsic toxicity of these components and the likely concentration to which an individual is liable to be exposed (Adeshina *et al.*, 2007).

During the past two decades, interest in traditional system of medicine and in particular, herbal medicines has increased substantially throughout the globe. In Africa, up to 60% of the population use traditional medicine for primary health care. In Nigeria, traditional medicine practice is a main source of livelihood for a significant number of the population who depend on it as their main source of income. High population growth rate (2.8% annually) and poverty, coupled with dwindling economic resources in the country, make our people resort to these cheap resources for their immediate needs. As the population increases, demand for traditional medicine will increase (NAFDAC, 2010).

As the trend rises, there is an urgent need for standardization of these plant substances. Standardization information required includes determination of contaminant, pharmacognostic standards, toxicological profile and efficacy. The guidelines do not apply to extemporaneous, which are preparations made by the practitioner and given to his/her clients on a one-one basis within the locality of its preparation. The guidelines are for manufactured products that are intended for distribution outside the locality of their production, i.e. to every wide area through other outlets and storage for considerable period of time. While National Agency for Food, Drugs Administration and Control (NAFDAC) regulates and controls the herbal products, the Ministry of Health through the adopted National Policy on Traditional Medicine regulates the practices of Traditional medicines, and will decide on the integration of herbal medicine into our health care delivery system as is done in some countries of the World (NAFDAC, 2010).

The aim of this work is to attempt the standardization of herbal leaf extract of *Anogeissus leiocarpus*.

MATERIALS AND METHODS

Chemicals

Ethanol (BDH Chemical Ltd. England), Whatman filter paper No. 1 Acetycholine (BDH Chemical Ltd, England), sodium chloride (BDH Chemical Ltd. England), Stilboesterol, Ferric Chloride (BDH Chemical Ltd, England), hydroxide (Sigma-Aldrich, Germany) lead acetate(BDH Chemical Ltd, England), sodium hydroxide (Avondale lab, England),sodium hydrogen carbonate(Merck, Germany), acetone (Merck, Germany) Nitric acid (BDH Chemical Ltd, England), sodium hydrogen phosphate(Merck, Germany), glucose (Merck,Germany).

Methods

Collection and authentication of plant material

Anogeissus leiocarpus was collected from Buhit village in Buhit district of Bassa Local Government Area of Plateau state, Nigeria. The sample was compared with authentic plant material at the herbarium of the department of Forestry Technology, Federal College of Forestry, Jos, Nigeria by a taxonomist, where a voucher specimen (No 14) has been deposited.

Extraction of the plant material

The hydro-alcoholic (50%) extract was prepared by macerating 500 g of the leaf powder in 4.0 litres of the solvent and left for 7 days with daily intermittent shaking. The extract was filtrated using Whatman filter paper No 1 and was freeze-dried (Edward Freeze drier, USA). The dried extract (130 g) was kept in airtight container.

Acute toxicity study

White Albino mice (both sexes and weighing 18-25 g) were used. The animals were procured from the Animal House unit of the University of Jos, Jos, Nigeria. The animals were fed on standard mouse cube (PHOW) and water *ad libitum* under standard conditions of 12 hour/12hour light/darkness cycle.

. The study was divided into three phases as adapted from the methods of Locke as reported by Musa (2005). In the first phase, twelve white Albino mice of either sex were divided into three groups of three mice each. Group 1, II, III and IV received 10,100, and1000 mg/kg of the extract and 0.2 ml of normal saline intraperitoneally respectively. The animals were observed for signs of toxicity including mortality for 24 hours after treatment. In the second phase the same number of mice was divided into three groups of three mice each. Group I, II, III received 300, 600 and 900 mg/kg of the extract and 0.2 ml of normal saline respectively. The mice were also observed for 24 hours. In the third phase twelve white Albino mice were divided into three groups of four mice each. Group I, II and III received 400 and 500 mg/kg of the extract and 0.2 ml of normal saline. The final LD_{50} was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose i.e. the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded.

Moisture content determination

A 3 g quantity of the leaf powder was weighed out using a balance (Mettler, P 165) after air drying. The powder was dried at 105^0C in the drying cabinet (Gallenkamp, England) until no further loss of weight was recorded. The per cent moisture content was calculated using the formula below.

$$\% \text{ Moisture content} = \frac{\text{Initial weight - final weight at } 105\ ^0\text{C}}{\text{Final weight at } 105^0\text{C}} \times \frac{100}{1}$$

Ethanol soluble extractives

A 5 g quantity of the coarsely powdered air dried leaves of *A. leiocarpus* was weighed in a balance (Mettler, P 165). A 100 ml of ethanol (96%) was measured into a flat-bottom flask and the powdered leaves introduced and corked. This was allowed to macerate for 24 hrs with intermittent shaking for the first 6 hrs and then allowed to stand for 18 hrs. The mixture was filtered (Whatman filter paper No 1) thereafter with rapidity in order to guide against loss of ethanol. A 20 ml filtrate was evaporated to dryness in a drying cabinet (Gallenkamp, England) at 105^0C to constant weight. The percentage of ethanol soluble extractives was calculated with reference to the air dried drug as follows:

$$\frac{\text{Weight of drug after evaporation at } 105^0\text{C}}{\text{Weight of air dried drug}} \times \frac{100}{1}$$

Water soluble extractives

The above procedure for ethanol soluble extractives was followed

except that distilled water was used in place of ethanol.

Animals

Albino mice, and Albino rats of the Wistar strain were procured from the Animal House unit of the University of Jos, Jos, Nigeria. The animals were fed on standard mouse cube (PHOW) and water ad *libitum* under standard conditions of 12 hour/12hour light/darkness cycle.

Effect of extract on the isolated rat duodenum preparation

An Albino rat weighing 240 g was sacrificed by a blow on the head and exanguinated after an overnight fast. A transverse incision was performed on mid abdominal section through the peritoneum to expose the duodenum. A 3 cm segment was sectioned and suspended in an organ bath containing Tyrode Physiological Salt Solution [(NaCl (8 g), KCl (0.2 g), $CaCl_2$ (0.2 g) $NaHC0_3$ (1 g), NaH_2 $P0_4$ (0.5 g), $MgCl_2$ (0.1 g), Glucose (1.0 g), distilled water (1L)] at 35-37^o C, aerated with 5% co_2 and 95% o_2. The effect of the extract (25 mg/ml) was tested alone, and with acetylcholine.

Extract effect on the uterus

A female albino rat (250 g) was pre-treated with 41mg/kg dose of stilboesterol to sensitize the uterus, the rat was stunned by a gentle blow on the back of the head and immediately exanguinated. The uterine strip was isolated and mounted in Dejalon physiological salt solution [NaCl (9 g), KCl (0.42 g), $CaCI_2$ (0.069 g), $NaHco_3$ (0.5 g), Glucose (0.5 g), Distilled water (1L)], and aerated with 95% o $_2$+ 5% co_2)]. The action of the extract (25 mg/ml), was tested in the absence and presence of acetylcholine (1x $10g^{-5}$ /ml), using Normal Saline as solvent for both. Contractions were recorded using a student's kymograph.

Phytochemical screening of the leaf powder

The presence or absence of the following constituents: Alkaloids, saponins, cardiac glycosides, tannins, anthraquinones and flavonoids were tested for using standard methods (Trease and Evans , 1986).

RESULTS

Acute toxicity studies

The intraperitoneal LD_{50} of the lead extract was found to be 346.4 mg/kg. The animals showed weakness prior to death.

Moisture content

The moisture content of the dry powdered leaf was 7.43±0.16%.

Ethanol and water soluble Extractives

The ethanol soluble extractive was 12.7% while that of water was 8.12%.

Extract effect on the duodenum

The extract at 0.5 mg/ml showed no contraction of the small intestine.

Extract effect on uterus

The extract at 0.5mg/ml. showed relaxation of the uterus.

Phytochemical screening

Phytochemical screening of the leaf extract revealed the presence of alkaloids, cardiac glycosides, tannins and flavonoids (Table 1).

DISCUSSION

The organoleptic properties of the leaf extract of *A. leiocarpus* include bitterness to taste, offensive smell, and coarseness when felt , and its greenish yellow colour. The microscopy of the powdered leaves revealed cellular inclusion such as rosette , cluster, prism crystals, numerous glandular trichomes. Also found were spiral

vessel , fibre (raphid) and a helium which serve as important qualities in assessment and identification of various plants.

The acute toxicity study of the extract was carried out in order to assess its safety status for the purpose of medication. The LD_{50} value of the aqueous ethanolic extract of *A. leiocarpus* was 346.4 mg/kg which

Table 1: Phytochemical Properties of *Anogeissus leiocarpus* leaf

Properties	Remark
Organoleptic	Bitter, offensive Odour, Golden Yellow in colour and coarse powder.
Alkaloid	+
Cardiac glycoside	+
Tannins	++
Anthraquinones	_
Saponin	_
Flavonoids	++

Present = +, Absent = -

Table 2: LD_{50} results

No	Dose (mg/kg)	No of animals in a group	No of death
1	10	3	0
2	100	3	0
3	300	3	0
4	400	3	1
5	500	3	3
6	600	3	3
7	900	3	3
8	1000	3	3

NB: No death occurred from the control groups.

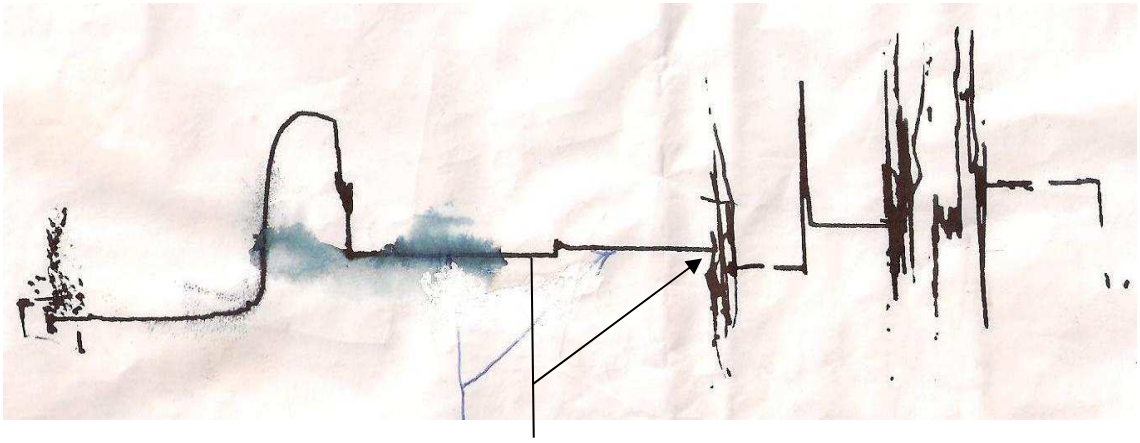

Figure 1: The Effect of Leaf Extracts on an Albino Rat Uterus strip (weight of animal = 250 g)

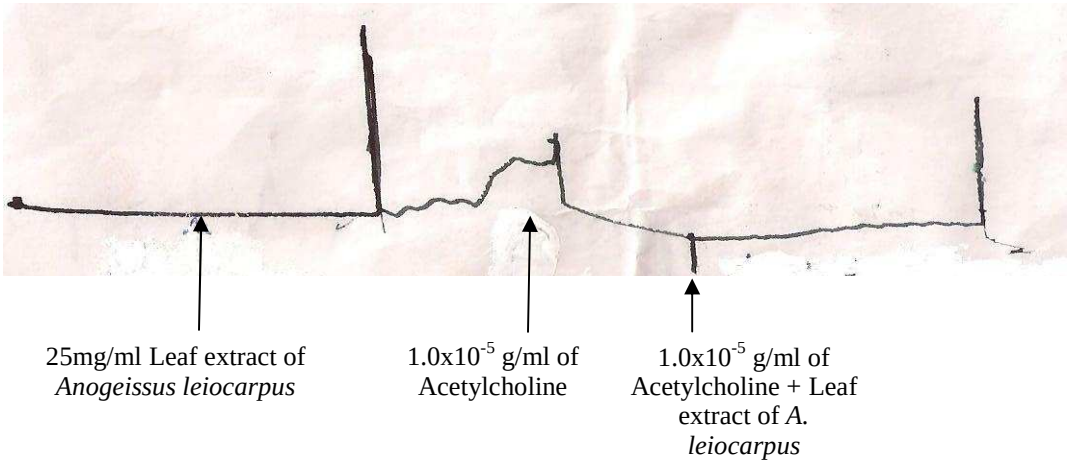

Figure 2: The Effect of leaf extracts on isolated rat duodenum (weight of animal = 240 g)

implies that it is very toxic when administered intraperitoneally (Musa *et al*, 2005; Otimenyin and Uguru, 2006; Agaie *et al.,* 2007). If the animals were treated orally, the LD_{50} would have increased because drug toxicity is manifested best by intraperitoneal route (Musa *et al.,* 2005).

Acute toxicity studies conducted by Agaie *et al* (2007) with the aqueous leaf extract of *A. leiocarpus* using both the oral and intraperitoneal route supported the above claim that intraperitoneal route manifests drug toxicity than the oral route. Their studies revealed that aqueous lead extract of *A. leiocarpus* given orally showed no death at 3200 mg/kg and the intraperitoneal route gave LD_{50} of 1400 mg/kg. This present study was carried out with mice, and also hydro-alcoholic solvent was used for the extraction, and it is expected that more ingredients would extracted than for aqueous or alcoholic solvent alone. It means that route of administration, animal species and types of solvent used in the extraction may have the possibility of influencing lethal dose. According to the toxicity scale of Hodge and sterner, any compound with an oral LD_{50} of between 50-500 mg/kg is considered as non-toxic while Clarke and Clarke reported that any substance with an intraperitoneal LD_{50} of above 1000 mg/kg should be regarded as safe (Agaie *etal*, 2007).

The extract (0.5 mg/ml) showed anti-cholinergic effect on the duodenum by antagonizing the effect of acetylcholine ($1x10^{-5}$ g/ml). The extract also relaxed the uterus of albino rat after sensitization with stilboesterol for 24 hrs. It then means that it has a relaxation effect on smooth muscles.

The moisture content of the extract was 7.43±0.16% and this meets the limit of 8%/g (BP, 1993). Therefore the extract when formulated into tablet might not require a desiccant.

The extractive values showed that ethanolic solvent extracted more constituents than the aqueous solvent which may have influenced the lethal dose as reported earlier.

The phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, and cardiac glycosides. These are less than those revealed by the stem and bark (Adejumobi, 2008; Mann[a] *et al.,* 2008).

CONCLUSION

The preliminary standardization investigations so far have not exhaustively handled the standardization of the leaf of *A. leicarpus*. More work is needed to be done on the leaf and the entire plant for full standardization and generation of monograph.

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