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## Phytochemical Screening and Toxicological Studies of Aqueous Stem Bark Extract of *Anogeissus leiocarpus* in Rats

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### ABSTRACT

Despite the fact that *Anogeissus leiocarpus* has been in use for many decades in the treatment of many illnesses little is known about its phytochemistry and toxicological effect in mammals. This study sought to quantitatively determine its phytochemical constituents and its sub acute toxicological effects at certain doses with a view to recommending continual usage of the plant bark at a define concentration or otherwise and to suggest further research based on the outcome. The concentration of saponins was found to be higher (89.5 mg/100 g) than all other phytochemicals where as that of Glycosides were found to be the least (1.7 mg/100 g). Concentration of alkaloids, tannins, steroids, flavonoids and phenols were 26.7, 29.9, 10.6, 27.3, 5.2 mg/100 g, respectively in the aqueous extract. The activities of Alanine Amino Tranferases (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP) and levels of total and direct bilirubin were not significantly higher ( $p < 0.05$ ) in all the groups administered with dosage of up to 200 mg kg<sup>-1</sup> b.wt. of the aqueous extract of the bark. However, the studies showed that the bark extract results in no liver toxicity and that is safe for consumption at a dose of up to 200 mg kg<sup>-1</sup> b.wt.

**Key words:** *Anogeissus leiocarpus*, bark, aqueous extract, phytochemicals, liver enzymes, liver toxicity

### INTRODUCTION

Plant kingdom is a great source of various chemical substances, with potential therapeutic properties (Ghani, 1986).

*Anogeissus leiocarpus* is a tree of up to 30 m in height, typically 15-18 m with light green foliage. The base of the trunk is wider and occasionally striped. It has a dense crown and often drooping branches. The colour of the bark is grey and becomes blackish corresponding with the age and is fibrous with thin scales. It has a finely pubescent stems and alternate to sub-opposite, elliptical to oval leaves which are 2-8 cm long and 1.5-3.5 cm wide (Arbonnier, 2004).

The leaves are acuminate or micronate at the apex and cuneate at the base. The patiole is 1-6 mm long growing in drier areas tending to have smaller leaves and hairier

flowers. The inflorescence is a spherical, axillary and terminal cluster. The yellow green scented flowers are bisexual, apetalous, 5-6 mm in diameter and with 10 stamens (Arbonnier, 2004).

The phytochemical screening of the fractions of *A. leiocarpus* plant indicated presence of saponins and terpenes (Mann *et al.*, 2008). These compounds are known to have *in vitro* antimicrobial activity (Almagboul *et al.*, 1988; Sofowora, 1993; Adigun *et al.*, 2000, 2001; Mann *et al.*, 2008). *Anogeissus leiocarpus* has been shown to be active as antimicrobial agent against gram-positive and gram-negative bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* (Machido and Ado, 1999; Taiwo *et al.*, 1999; Adeleye *et al.*, 2003; Ibrahim *et al.*, 2005; Ndukwe *et al.*, 2005); antimycobacterial activity (Malcolm and Sofowora, 1969; Uba *et al.*, 2003; Johnbull and Abdu, 2006); trypanocidal activity (Atawodi *et al.*, 2003) and demonstrated activity against *Candida albicans* (Sanogo *et al.*, 1998; Sanogo, 2005; Chaabi *et al.*, 2006). The terpenoidal fractions from *A. leiocarpus* and *T. avicennioides* could be a potential source of chemotherapeutic agents (Mann *et al.*, 2009).

Many studies have demonstrated the antimicrobial; anti-carries, anti periopathic and antifungal properties of both aqueous and ethanolic extracts of various chewing sticks (Akande and Hayashi, 1998). The antimicrobial effect of its root extract on *Staphylococcus aureus* and *Pseudomonas aeruginosa* and also of its bark extract on *Bacteriodes gingivalis* and *Bacteriodes malaninogenicus* were documented, as well as significantly higher antibacterial activity of ethanol extract of *A. leiocarpus* root against *Staphylococcus aureus* and *Streptococcus pyogenes* was reported by Ndukwe *et al.* (2005).

Many Combretaceae species are widely distributed in Nigeria and are used in traditional medicine for treatment of respiratory diseases (asthma, catarrh, chronic bronchitis, cough, hay-fever, hemoptysis, pneumonia, pulmonary disorders and tuberculosis) (Mann *et al.*, 2007) and other human diseases (Mann *et al.*, 2009).

The medicinal uses of *A. leiocarpus* can never be over-emphasized in Nigeria and other places. The plant is used medically for the treatment of ascariasis, gonorrhoea, general body pain, blood clots, asthma, coughing and tuberculosis (Mann *et al.*, 2008). Information obtained from the yorubas and south eastern people of Nigeria, indicated that the plant is also used as an antimicrobial agent against bacterial infections (Ogunyemi, 1979). The leaves of the plant are used externally as decoction in the eastern part of Nigeria for the treatment of skin diseases and the itching of psoriasis (Ogunyemi, 1979).

The powdered bark is applied to wounds, sores, boils, cyst and diabetic ulcers with good results (Ogunyemi, 1979). In an infusion or decoction form, the bark is used as a medicine for cough. The roots, when made as a pulp, it is used in the treatment of ulcers and is applied to wounds. The bark, when made as a powder, is used to relieve tooth ache on gums. The powder is also used as vermifuges and decoction of the leaves for fumigation and washing (Ibrahim *et al.*, 1997). Helminthiasis, trypanosomiasis, malaria and dysenteric syndrome are treated by traditional practitioners using the bark, trunk, roots and leaves parts of *A. leiocarpus* (Ibrahim *et al.*, 1997).

The inner bark of the tree is used as a human and livestock antihelmintic for treating worms and for treatment of a couple of protozoan diseases in animals, Nagana (an animal trypanosomiasis) and Babesiosis (Bohm and Koupai-Abyazani, 1994). In traditional medicine,

many ailments of livestock and man, such as helminthosis, schistosomiasis, leprosy, diarrhea and psoriasis are treated by the plant parts (Burkill, 1985; Ibrahim *et al.*, 1997; Onyeyili, 2000). Other medicinal uses include treatment for fever, cough, rheumatism, leprosy, wounds and skin diseases. Despite the fact that this plant has been in use for many decades in the treatment of many illnesses, little is known about its phytochemistry and toxicological effect in mammals. It is therefore important to determine its phytochemical constituents and its toxicological effects at the administered doses.

## **MATERIALS AND METHODS**

**Plant materials:** The bark of the *A. leiocarpus* was collected in May 2012, from within Kano metropolis, Kano. It was identified and authenticated at the specie level at the Herbarium unit of Biological Sciences Department, Faculty of Science, Bayero University, Kano. The specimen was deposited in the herbarium with voucher number 564. The bark was thoroughly washed and air dried for 2 weeks to a constant weight. The dried bark was pounded to fine powder with mortar and pestle and then stored in dried containers until needed.

**Experimental animals:** The protocol employed met the guidelines of Good Laboratory Practice (GLP) regulations of World Health Organisation and also the guidelines governing handling of laboratory animals as stipulated by Bayero University animal research ethics committee as well as the principles of laboratory animal care. Apparently healthy white albino rats (Wistar strain) of both sexes weighing between 180-260 g were used for the research and were obtained from the department of biological sciences, Bayero University Kano Nigeria. The rats were kept in well ventilated laboratory cages with 12 h day/night cycles. They were maintained on a ration containing commercial poultry feed (Vital Feeds<sup>®</sup>, Jos, Nigeria) made up of 54% carbohydrate, 20% protein, 2% minerals, 10% fibre, 1% vitamin and 13% fat. Water was also supplied *ad libitum*.

**Preparation of extract for animals' treatment:** The extracts of the bark of the plant were prepared by suspending 50 g of the bark powder separately in 100 cm<sup>3</sup> of distilled water and shaken intermittently with mechanical shaker for 6 h. The preparation was allowed to stand for 24 h and then filtered through a Whatmann's No. 1 (11 Cm) filter paper. The filtrate was concentrated to dryness at 40°C under reduced pressure on a rotary evaporator and stored in a refrigerator at -4°C until required.

### **Phytochemical screening**

**Quantitative test:** Sample (2 g) was defatted with 100 cm<sup>3</sup> of diethyl ether using soxhlet apparatus for 2 h after which the quantitative tests for phenols by spectrophotometric method as described by Henry (1974), alkaloids as described by Harborne (1973), tannins as described by Van Burden and Robinson (1981), saponins as described by Okwu (2001) and flavonoids as described by Bohm and Koupai-Abyazani (1994) were carried out.

**Sub acute toxicological studies:** Forty white albino rats were divided into four groups of 10 rats each. Groups 2, 3 and 4 were orally given daily doses of 100, 150, 200 mg kg<sup>-1</sup> b.wt., respectively

of the aqueous bark extract by gastric tube for 4 weeks after which they were sacrificed and blood samples collected. Group 1 was not administered with any extract. The rats were sacrificed humanely by jugular decapitation 24 h after the last administration of the extract and blood samples collected. The samples were allowed to clot and centrifuged at 3500 rpm for 10 min and serum collected for analysis.

**Methods:** Serum collected from the blood samples at the end of the treatment was used to assay for alanine aminotransferase (ALT), aspartate aminotransferase (AST) as described by Reitman and Frankel (1957) using commercial reagent kits (Randox Laboratories Limited, United Kindom). Determination of serum alkaline phosphatase activity, total and direct bilirubin were conducted as described by Chawla (1997).

**Statistical analysis:** Statistical analysis was carried out using Students' t-test to compare the measured parameters with those obtained in the control group the differences were accepted when  $p < 0.05$ . the analysis was carried out using in STAT. 3.

## RESULTS

Table 1 presents the result of quantitative estimation of phytochemicals present in the aqueous bark extract of the *A. leiocarpus*. The concentration of saponins (89.5 mg/100 g) was higher than all other phytochemicals present in the bark extract. The mean concentration of tannins is 29.9 mg/100 g where as that of alkaloids, steroids, glycosides, flavonoids and phenols were 26.7, 10.6, 1.7, 27.3, 5.2 mg/100 g, respectively.

The results of the liver enzymes and total and direct bilirubin were presented in Table 2. The results of all the liver enzymes tested namely ALT, AST and ALP were found to have no significant increase in activity at  $p < 0.05$  in all the groups indicating absence of liver toxicity at dosage of up to 200 mg kg<sup>-1</sup>.

Mean serum total and direct bilirubin levels, were found not to have been significantly ( $p < 0.05$ ) different when compared with control, indicating no effect to the liver at the dose of up to 200 mg kg<sup>-1</sup>.

Table 1: Quantitative analysis of phytochemicals in aqueous bark extract of *A. leiocarpus*

Alkaloid (mg dL <sup>-1</sup> )	Tannins (mg dL <sup>-1</sup> )	Steroids (mg dL <sup>-1</sup> )	Glycosides (mg dL <sup>-1</sup> )	Saponins (mg dL <sup>-1</sup> )	Flavonoids (mg dL <sup>-1</sup> )	Phenol (mg dL <sup>-1</sup> )
26.7±0.36	29.9±0.21	10.6±0.67	1.7±0.06	89.5±0.57	27.3±1.39	5.2±0.17

Values are Mean±SD

Table 2: Effect of administration of aqueous bark extracts of *A. leiocarpus* on some liver function indices in rats

Group	AST (U L <sup>-1</sup> )	ALT (U L <sup>-1</sup> )	ALP (U L <sup>-1</sup> )	Total bilirubin (mg dL <sup>-1</sup> )	Direct bilirubin (U L <sup>-1</sup> )
Group 1 (Control)	8.60±4.40	7.2±3.630	82.80±43.19	0.18±0.08	0.04±0.05
Group 2 (100 mg kg <sup>-1</sup> )	11.00±2.00	10.60±2.79	87.60±7.920	0.18±0.08	0.08±0.04
Group 3 (150 mg kg <sup>-1</sup> )	11.00±1.00	10.60±2.41	90.40±50.23	0.42±0.13	0.10±0.07
Group 4 (200 mg kg <sup>-1</sup> )	11.80±1.10	12.00±1.22	110.40±43.80	0.44±0.11	0.12±0.04

n = 5, in each group values are Mean±SD

## DISCUSSION

The concentration of saponins (89.5 mg/100 g) was higher than all other phytochemicals present in the bark extract and this may be the reason why the plant is used as anti-cancer medication (Akande and Hayashi, 1998). Saponins found in beans interfere with the replication of cell DNA thereby preventing the multiplication of cancer cells.

Steroids were found to be 10.6 mg/100 g. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones (Okwu, 2001). This may be the reason why *Anogeissus leiocarpus* is used as aphrodisiacs, since steroidal structure could serve as potent starting material in synthesis of sex hormones (Okwu, 2001).

Flavonoids was found in a high concentration in the plant extract. An 8 year study found that three flavonoids-kaempferol, quercetin and myricetin were associated with a reduced risk of pancreatic cancer of 23% (Ute *et al.*, 2007). This may be the reason why the plant is used as anti-cancer.

The concentration of saponins was found to be higher in the root and stem extract of the plant and found phenols as the least in concentration (Adejumobi *et al.*, 2008). This finding however, corroborates the findings of the recent study. The concentration of tannin in the stem and root extracts were found be 32.5 and 71.9 mg/100 g, respectively (Adejumobi *et al.*, 2008). However, this study found tannin in the bark extract to be lesser in concentration (29.9 mg/100 g). Tannic acid is directly applied to the affected area to treat cold sores and fever blisters, diaper rash and brickly heat, poison ivy, ingrown toenails, sore throat, sore tonsils, spongy or receding gums and skin rashes and to stop bleeding (Covington, 1996).

The higher concentration of these phytochemicals in the root extract of *A. leiocarpus* may have been responsible for a relatively higher antimicrobial activity (Adejumobi *et al.*, 2008). Previous reports have indicated that the root of the plant is often used as chewing stick (Akande and Hayashi, 1998; Ndukwe *et al.*, 2005). Result from the present studies provides an insight why the root rather than the stem of *A. leiocarpus* has been utilized as chewing stick (Adejumobi *et al.*, 2008).

Aspartate aminotransferase and alanine aminotransferase are diagnostic enzymes used as sensitive indicators of liver disease, although AST is known to be distributed widely in other tissues like kidney (Kaplan *et al.*, 1988). The AST and ALT play important role in the conversion of amino acids to ketoacids and they are major markers of liver damage caused by exposure to toxic substances (Chawla, 1999). Serum levels of AST will increase due to necrosis and inflammation of heart, liver and muscle tissues where as ALT levels can be expected to increases during liver damage (Chawla, 1999). However, primary and secondary hepatic tumours cause an elevation of both enzymes with AST higher than ALT. Based on this study, one can therefore, say there was no necrosis and inflammation of the heart, liver and muscle tissues as well as primary and secondary hepatic tumours, since there is no significant ( $p < 0.05$ ) increase in both the AST and ALT.

The study of unconjugated bilirubin is used to assay for the synthetic and detoxification function of the liver. Unconjugated bilirubin is conjugated with glucuronic acid in hepatocytes to increase its water solubility and is then rapidly transported into bile. The serum conjugated bilirubin level does not become elevated until the liver has lost at least one half of its excretory capacity (Bosma *et al.*, 1995). The results of this study indicated that there was no significant ( $p < 0.05$ ) difference in the conjugated bilirubin between all the groups administered with the

( $p < 0.05$ ) difference in the conjugated bilirubin between all the groups administered with the extract (group 2-4) and group 1 (Control), following the administration of the aqueous stem bark extract of the *A. leiocarpus* at a dose of up to 200 mg kg<sup>-1</sup>. However, there was no significant ( $p < 0.05$ ) elevation in bilirubin level, following the administration of the aqueous extract at a dose of up to 200 mg kg<sup>-1</sup>.

Increase in the level of these parameters in the serum after exposure of the experimental animals to xenobiotics, such as drugs and medicinal plants extract, indicate an injury to the liver (Finlayson *et al.*, 1995). Therefore, this study showed that no damage was done to the liver, since there was no significant ( $p < 0.05$ ) in the parameters assayed for, in all the experimenting and control groups.

## CONCLUSION

In conclusion, it was found that saponins is much higher (89.5 mg/100 g) than the other phytochemicals found and glycosides is the least (1.7 mg/100 g) in concentration. However, the studies showed that the activities of alanine amino transferases (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and levels of total and direct bilirubin were not significantly higher ( $p < 0.05$ ) in all the groups indicating no liver toxicity at dosage of up to 200 mg kg<sup>-1</sup> b.wt.

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