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Phytochemical screening, acute toxicity and anti-ulcer activity of the stem bark of *Anogeissus leiocarpus* (DC.) Guill. & Perr. (Combretaceae)

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Abstract
Peptic ulcer disease constitutes a major health challenge resulting in morbidity and in some cases, mortality. This study evaluated the phytochemistry, acute toxicity, and anti-ulcer activity of the methanol extracts of the stem bark of *Anogeissus leiocarpus* **(**DC.) Guill. & Perr. Flavonoids, tannins and saponins are some phytochemicals found in the extracts. From the results, the LD_{50} of the aqueous and methanol extract was >5000 mg/kg and 3807 mg/kg. The mean ulcer indices for the aqueous extract was 0.38 ± 0.07, 0.38 ± 0.10 and 0.36 ± 0.07 respectively for the 100, 200 and 400 mg/kg), while those of the methanol extract were 0.32 ± 0.09, 0.10 ± 0.03 and 0.12 ± 0.10 respectively for 100, 200 and 400 mg/kg). These values were not significant at $P>0.05$ when compared with control. However, the percentage ulcer inhibition for methanol extract in group treated with 200 and 400 mg/kg, were both significantly ($p<0.05$) higher compared with 58.8% from omeprazole (20 mg/kg) treated group. These findings showed that the methanol extract at 200 mg/kg and 400 mg/kg. significantly ($p<0.05$) increased the anti-ulcer activity, suggesting that *A. leiocarpus* possesses anti-ulcer property which could be useful in the treatment of peptic ulcer disease.

Keywords: *Anogeissus leiocarpus;* Acute toxicity; Anti-ulcer; Medicinal plants, Peptic ulcer

INTRODUCTION

Herbal medicines are extensively used across the globe presently and have played significant roles in the prevention and management of numerous diseases [1]. About 80% of the African population depend on traditional medicine for their healthcare needs, while a growing number of patients in the developed world rely on alternative medicine [2].

Peptic ulcer disease constitutes a major health challenge in the world today, resulting in morbidity and in some cases, mortality. About 10% of the world's population develop peptic ulcer at some point in their life [3]. Peptic ulcer is prolonged mucosal erosion in the gastric or duodenal mucosa, affecting the duodenum or stomach [4]. Excessive gastric acid secretion resulting in high acidity is a significant aspect of the pathophysiology of peptic ulcer disease. The pathophysiology of

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this gastro-intestinal disorder is viewed as an imbalance between mucosal defensive factors such as bicarbonate, prostaglandin, nitric oxide, peptides, growth factors and injurious factors like acid, pepsin [5,6] It also involves chronic infection with *Helicobacter pylori* [7] Orthodox therapy employed to treat the ailment is associated with numerous difficulties which include: prolong duration of usage, high cost of drugs and adverse effects such as impaired calcium absorption seen in histamine$_2$–antagonists like cimetidine and ranitidine, cimetidine is also associated with hyper-oestrogenism which leads to impotence and gynaecomastia in some men who use it for a long time, arrhythmias, arthralgia, hypergastrinemia and haemopoeitic changes are also serious side effects seen with anti-ulcer drugs [8]. There is need therefore, to look into the use of medicinal plants for reduction of gastric acid secretion in ulcer management. The plant *A. leiocarpus* (DC.) Guill. & Perr, is of the family Combretaceae. It is a small to medium sized tree species whose height ranges from 15–30 m and can measure up to 1 m diameter, with grayish to pale brown bark which darkens with age. Various traditional uses have been documented for the plant. The stem and root bark, leaves and roots have been reported to be used in traditional medicine frequently in the form of decoctions for the treatment of numerous conditions, including helminthic and microbial infections [9]. Extracts of the stem and root bark and of the leaves have been used in treatment of fungal infections like dermatitis and mycosis in some countries [10]. It has been reported that a decoction of the leaves or leafy twigs is used in the treatment of jaundice, hepatitis, common cold and headache [9]. Externally, it is applied on haemorrhoids and skin diseases. The root-bark is used externally to treat wounds, eczema, psoriasis, carbuncles, boils and several kinds of ulcers. The barks and the exuding gum are commonly used in Africa to prevent and cure dental caries and toothache. The seeds have a wide bactericidal and fungicidal activity in humans and animals [10].

An ethnobotanical survey carried out in Sokoto with respect to plants used in the management of peptic ulcer disease showed that the plant *A. leiocarpus* is one of those used in that region for the treatment of ulcer by traditional healers [11]. There appears to be no scientific reports on studies conducted to evaluate the anti-gastric secretory activity of *A. leiocarpus*. Therefore, the aim of the study was to evaluate the in vivo anti-gastric acid secretory activity of the methanol and the aqueous extracts of the stem bark of *A. leiocarpus*.

EXPERIMENTAL METHODS

Collection, preparation and extraction of plant materials. The plant *Anogeissus leiocarpus* was collected in October, 2018 from the wild in Jos North Local Government Area of Plateau state and identified by a taxonomist Mr. J.J. Azila in the Federal College of Forestry, Jos. The plant was given the voucher number UJ/PCG/HSP/16CO2 in the Herbarium of the Department of Pharmacognosy, University of Jos. The stem bark was air dried at room temperature for 3 weeks. The dried stem bark was reduced to powder using a wooden mortar and pestle. About 10 g of the powder was used for phytochemical and pharmacognostic evaluation, while 240 g of the powder was measured and extracted by maceration for 48 hours in 80% methanol at a volume of 200 mL. In similar manner, 240 g of the powdered stem bark was measured and extracted with aqueous solvent and then concentrated on a water bath at 60ºC until a constant weight was achieved. The percentage yield for both extracts were determined using the formula;

$$Percentage\ Yield = \frac{Weight\ of\ dried\ extract}{Weight\ of\ \ dried\ plant\ sample} X\ 100$$

The extracts were freshly prepared on the days of the experiment; they were dissolved in normal saline and administered orally. Vehicle

(normal saline) was administered in a volume of 10 mL/kg/body weight.

Animals. Male albino (Wistar) rats were purchased from the Animal Experimental Unit, Department of Pharmacology, University of Jos, Nigeria, after receiving ethical clearance from the Institutional Animal Ethics Committee with reference number REF/UJ/PCL/AEU/2 on 03/03/2018. The animals were kept in plastic cages under standard environmental conditions of temperature and humidity and allowed access to food and water *ad libitum*. The animals were handled in accordance with standard guidelines and the institutional Animal Ethics Committee approved all procedures. The drugs and extract were administered orally during the study with the use of an oral cannula 16-18 gauge.

Phytochemical tests. Phytochemical tests were carried out for both methanol and aqueous extracts using standard method as prescribed by Sofowora [1], Evans [26] and Harborne [27].

Acute toxicity tests. Acute toxicity test was conducted for both extracts using adult male Wistar rats which weighed between 200 -250 g, following Lorke's method [12]. The test was done in two phases.

Phase 1: nine animals were used for each extract. They were divided into three groups, with three animals in each group. For both methanol and aqueous extracts, the first group was administered 10 mg/kg of the extract orally, while the second and third groups of rats received 100 mg/kg and 1000 mg/kg respectively. The animals in each group were then placed under observation for 24 hours to monitor for behavioral changes and mortality.
Phase 2 involved the use of three animals for each extract which were distributed into three groups of one animal each. Higher doses of extract (1600 mg/kg, 2900 mg/kg and 5000 mg/kg) were administered to the first, second and third animals respectively and the animals were observed for 24 hours for behavioral changes as well as mortality. Then the LD_{50} for each extract was calculated by the formula:

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where D_0 = Highest dose that gave no mortality;

D_{100} = Lowest dose that produced mortality

Determination of anti-ulcer activity. The method of Datta *et al.* [13] was used; for each extract of *A. leiocarpus* stem bark, 25 adult male albino rats were fasted (denied access to food but not water) for 36 hours, and were divided randomly into five groups of five rats each. The first group which was the control was administered normal saline at a dose of 10 mL/kg orally. Omeprazole (20 mg/kg) was administered to the second group. The animals in group 3, 4 and 5 were administered the extract at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg respectively by oral gavage. The animals were allowed to stay for 1 hour, after which, 1 mL of 90% ethanol was administered to each animal orally. The animals were sacrificed an hour later by cervical dislocation. Their stomachs were isolated, cut open along the greater curvature and rinsed with normal saline, after which they were pinned on a soft board and examined macroscopically using a hand lens (at a magnification of 10) for the presence of mucosal lesions. The ulcer lesions observed were scored, according to severity, using the scores given by Kulkarni [14] as follows:

0 = Normal stomach 0.5 = Red coloration
1 = Spot ulcers 1.5 = Haemorrhagic streaks
2 = Ulcer > 3 mm but < 5 mm 3 = ulcers > 5 mm

Mean ulcer score for each group was obtained by dividing the sum of ulcer scores in the group by the number of animals in that group. The mean ulcer index for each group and the percentage ulcer inhibition were calculated, using the method adapted by Falang *et al*. [15], as follows:

$$Mean\ Ulcer\ Index = \frac{Mean\ Ulcer\ Score}{Magnification}$$

$$Percentage\ Ulcer\ Inhibition = \frac{MUI\ of\ Control - MUI\ of\ Treatment}{MUI\ of\ Control} X\ 100$$

Where MUI = Mean Ulcer Index.

Therefore, mean ulcer score and mean ulcer index were the major parameters considered from this experiment for both the methanol and aqueous extracts of the stem bark of the plant *A. leiocarpus*.

Data analysis. Results obtained were analyzed and expressed as Mean ± Standard Error of Mean using the one-way Analysis of Variance (ANOVA) compared to the control, at a significant level of $p < 0.05$.

RESULTS

The percentage yield of the extracts is 10.27% for methanol extract and 14.6% for aqueous extract. Results of the phytochemical tests are shown in Table 1. Results of the acute toxicity tests as presented in Table 2. Acute toxicity study was carried out to establish the safety of the drug for consumption and to determine the therapeutic indices (LD_{50}) of the extracts. The results of the *in-vivo* anti-ulcer evaluation carried out using Wistar rats are as shown in Tables 3 and 4.

DISCUSSION

Phytochemical tests carried out on the aqueous extract of the stem bark revealed the presence of saponins, carbohydrates, free anthraquinones and cardiac glycosides, and like the powder and methanol extracts it showed that it was rich in tannins and flavonoids.

Table 1: Preliminary phytochemical screening results for *A. leiocarpus* stem bark

Metabolite	Test	Stem bark powder	Methanol extract	Aqueous extract
Saponins	Frothing reaction test	+	+	+
Tannins	Ferric chloride	+	+	+
Flavonoids	Lead acetate: sodium hydroxide	+	+	+
Carbohydrates	Molisch's	+	+	+
Steroids	Salkowski's test	+	+	+
Terpenes	Leibermann-Burchard's Test	-	-	-
Free anthraquinones	Borntrager's test	+	-	+
Alkaloids	Dragendorff's / Mayer's	-	-	-
Cardiac glycosides	Keller- Kiliani's test	+	+	+

+ = present - = absent

Table 2: Acute toxicity results for both aqueous and methanol extracts of *A. leiocarpus* stem bark

Phase	Group	Dose (mg/kg)	Number of deaths	
			Methanol extract	Aqueous extract
1	1	10	0	0
1	2	100	0	0
1	3	1000	0	0
2	1	1600	0	0
2	2	2900	0	0
2	3	5000	1	0

LD_{50} for Aqueous Extract of A. *leiocarpus* is > 5000 mg/kg since there was no death observed. For Methanol extract $LD_{50} = \sqrt{2900 \times 5000} = 3807.89$ mg/kg.

Table 3: Effects of aqueous extracts of the stem bark of *A. leiocarpus* on ethanol-induced ulcer in Wistar rats

Treatment	Dose	Mean ulcer score	Mean ulcer index ± S.E.M*	% ulcer inhibition
Normal saline	10 mL/kg	4.8 ± 0.20	0.48 ± 0.02	0
Omeprazole	20 mg/kg	2.6 ± 0.98	0.26 ± 0.10	45.83
AEAL	100 mg/kg	3.8 ± 0.73	0.38 ± 0.07	20.83
AEAL	200 mg/kg	3.8 ± 0.97	0.38± 0.10	20.83
AEAL	400 mg/kg	3.6 ± 0.68	0.36 ± 0.07	25.0

Values were expressed as mean ± S.E.M of (n = 5). AEAL= Aqueous Extract of *Anogeisus leiocarpus*

Table 4: Effects of methanol extracts of the stem bark of *A. leiocarpus* on ethanol-induced ulcer in Wistar rats

Treatment	Dose	Mean ulcer score	Mean ulcer index ± S.E.M*	% ulcer inhibition
Normal saline	10 mL/kg	3.4± 0.68	0.34 ± 0.07	0
Omeprazole	20 mg/kg	1.4 ± 0.40	0.14 ± 0.04	58.8*
MEAL	100 mg/kg	3.2 ± 0.92	0.32 ± 0.09	5.88*
MEAL	200 mg/kg	1.0± 0.32	0.10 ± 0.03	70.6*
MEAL	400 mg/kg	1.2 ± 0.97	0.12 ± 0.10	70.6*

Values were expressed as mean ± S.E.M of (n = 5). * $p < 0.05$ as compared to Normal saline group. MEAL= Methanol Extract of *A. leiocarpus*

Some researchers reported in their study that flavonoids, saponins, tannins, cardiac glycosides, steroids, carbohydrates and alkaloids were present in the aqueous stem bark extract but anthraquinones were absent [16]. Others carried out phytochemical tests on the aqueous extracts of the stem bark and it revealed the presence of a high concentration of saponins and then tannins, alkaloids, steroids, glycosides, flavonoids and phenols [18]. There are notable similarities observed from the studies noted or carried out by these authors and the finding from this study, with the exception of the presence of alkaloids in some of the studies which was absent in this study. This could be as a result of climatic and soil variations of the different geographic locations where the study was carried out.

The results from acute toxicity studies showed that in the first phase, for the two extracts, no death was recorded. However, in the second phase, death of one animal was recorded at the highest dose of 5000mg/kg only with methanol extract and the LD_{50} obtained for methanol extract was 3807mg/kg body weight. No death was recorded for the aqueous extract even at the highest dose of 5000 mg/kg and there was no visible sign of toxicity. There was no mortality in both the first and second phase of drug administration, from the acute toxicity study of the aqueous extract suggesting that LD_{50} of the leaf extract is higher than 5000 mg/kg body weight orally in mice [12]. Our observations from the acute toxicity studies also indicate that the aqueous extract is practically nontoxic up to a dose level of 5,000 mg/kg body weight via oral route. The methanol extract is also safe at an oral LD_{50} of 3807 mg/kg. Some [16] in their research, also carried out acute toxicity studies on both the aqueous and methanol extracts of the plants and recorded no death at all in all the animals who were administered the extract, even with doses as high as 5000 mg/kg. There was no visible sign of toxicity recorded either. The difference between the acute toxicity for methanol and aqueous extract in this study could arise from phytochemical variations or the extract preparations. Overall, the results showed that the extracts of the stem bark of the plant *Anogeissus leiocarpus* were safe even at relatively high doses.

The results of the anti-ulcer acid activity show that mean ulcer index and percentage ulcer inhibition were the parameters used to assess the effect of the controls and extracts on stomach lesions in the animals. The data analysis was carried out on the values for mean ulcer index and the results showed that there was no significant difference ($p>0.05$) in mean ulcer index between the extract and the negative control and normal saline. However, on calculation of percentage ulcer inhibition using the formula given in the method above, the values obtained for methanol extract were significantly different from that of the control group. Interestingly, the percentage inhibition at 200 mg/kg and 400 mg/kg, which were both 70.6%, were higher than that of the standard drug, Omeprazole, which was 58.8%, revealing that *A. leiocarpus* possesses active phytochemicals worthy of further investigation. The phytochemical studies showed that the stem bark has rich phytochemical constituents that could be responsible for its therapeutic properties such

as tannins, flavonoids and saponins which is also reported by various researchers [16, 17, 18]. Even though there is no recorded comparative phytochemical quantitative study of the methanol and aqueous extracts, there is a possibility that the methanol extract contained a greater measure of the active principles than the aqueous extract.

Saponins are known to possess anti-ulcer and anti-secretory properties, Fernando & Pellison [19] documented that Saponins possess anti secretory properties: they lower the pH of gastric juice, inhibit acid secretion, and total acid output. Tannins also elicit some inhibitory effect on acid secretion. A research done [20] showed that some tannins are competitive inhibitors of gastric H^+-K^+-ATPase pump, thereby inhibiting acid secretion. Other authors [21] have also mentioned the anti-secretory effect of tannins. Flavonoids also play an inhibitory role on gastric acid secretion. A study carried out by [22] to investigate the effect of some flavonoids in parietal cell acid production and H^+-K^+-ATPase activity revealed that the flavonoids inhibited acid production in isolated parietal cells in response to histamine and dibutyryl cAMP stimulation and inhibited H^+-K^+-ATPase activity. The anti-peptic ulcer activity of saponins and flavonoids was also reported [23], although the exact anti-ulcer mechanism of these phytochemicals is not fully understood, however, the authors suggest an increase in prostaglandin which play a role as a cytoprotective agent in the stomach in addition to the stimulation of the secretion of mucus and bicarbonate ions which preserves the gastric membrane may be responsible for the anti-ulcer activity [24]. *A. leiocarpus* is also known to possess antibacterial effects [25], this could be useful in eradication of H. pylori and consequent cytoprotective effects. Further studies need to be carried out to ascertain the anti-ulcer mechanism of *A. leiocarpus*. Findings from this research show that the plant drug has a high safety profile. The results obtained showed that there was no significant difference (p>0.05) in mean ulcer index between the extracts (methanol and aqueous) and the negative control which was normal saline. However, the values obtained for percentage ulcer inhibition for the 200 mg/kg and 400 mg/kg methanol extracts were higher than that of omeprazole. This can serve as scientific evidence to aid in justifying the plant's use in peptic ulcer therapy [22].

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