



Research Article

Acute Toxicity and Antidiabetic Study of *Anogeissus leiocarpus* (Dc.) Guill. and Perr. (Combretaceae) Ethanol Stem Bark Extract

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ABSTRACT

Anogeissus leiocarpus is a medicinal plant used locally for its anti-microbial, anti-helminthic including anti-diabetic effect among others. The research aimed to evaluate the phytochemical constituents and anti-diabetic effect of the stem bark of *Anogeissus leiocarpus* against alloxan-induced diabetic rats. The fresh stem bark of the plant which was extracted using ethanol by reflux extraction method was screened for phytochemicals, determines its median lethal dose (LD₅₀) and evaluated for its anti-diabetic potential on alloxan-induced diabetes in albino rats using standard protocols. The phytochemical screening revealed the presence of flavonoids, saponins, carbohydrate, cardiac glycosides, terpenoids, tannins and cardenolides. The oral and intraperitoneal LD₅₀ were 3,807 mg/kg and 1,265 mg/kg respectively. The extract at doses of 200 mg/kg, 400 mg/kg and 800 mg/kg exert insignificant percentage (%) inhibition of hyperglycaemia when compared with insulin at 0.75 units/kg exert significant percentage (%) inhibition of hyperglycaemia at 78.68 %. The ethanol stem bark extract of *Anogeissus leiocarpus* does not imply any significant anti-diabetic.

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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 because of the high cost of access to modern drugs. Evidence for the use of plants for medicinal purposes dates as far back as 60,000 years ago in both developed and underdeveloped countries (Gossell-Williams *et al.*, 2006). The therapeutic properties of plants have been recognised since time immemorial. Many pathological conditions have been treated using plant-derived medicines. These medicines are used as concoctions or concentrated plant extracts without isolation of active compounds. The active chemical components of plant are known as phytochemicals, they include; flavonoids, tannins, saponins, cardiac glycosides, Terpenoids etc. They are considered as the agents that help man in fighting against several diseases (Yakubu *et al.*, 2022a). There are however a lot of global health challenges with diseases such as cancer, degenerative diseases, HIV/AIDS and diabetes, of which modern medicine is struggling to provide cures (Thomford *et al.*, 2018). Today, with the present surge of interest in phytotherapeutics, 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 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).

Today, the effectiveness of this therapy is proven and its benefits for human health have allowed natural medicine to enter in our daily habits. Even the most developed countries are not left behind. Traditional medicine is therefore

as an alternative to the economically disadvantaged populations, especially in sub-Saharan African countries.

Diabetes mellitus (DM), is commonly referred to as diabetes, is a group of metabolic disease in which there is high blood sugar level over a long period. Symptoms of high blood sugar include frequent urination, increased thirst and increased hunger if left untreated diabetes can cause many complications World Health Organizations (WHO, 2014; Cooke *et al.*, 2008).

Diabetes is due to either the pancreas not producing enough insulin or the walls of the body not responding properly to the insulin produced (Kumar and Clark, 2002).

Acute complication includes diabetic ketoacidosis and non ketotic hyperosmolar coma. Serious long term complication includes cardiovascular disease, stroke, chronic kidney failure, foot ulcer and damage to eyes (commonly known as: nephropathy, neuropathy and retinopathy) (Aurell, 1992).

The disease burden related to diabetes is high and rising in every country, fueled by the global rise in the prevalence of obesity and unhealthy lifestyles. The latest estimates show a prevalence rate of 11.1 % with diabetes in 2019, expected to rise to 13 % by 2045 in Northern America and Caribbean regions (Saeedi *et al.*, 2019). The prevalence rate is most pronounced in the Middle East and North African regions which are predicted to rise 13.9 % by 2045. The lowest prevalence rate (4.7 %) is observed in Africa which is expected to increase to 5.2 % in 2045 (Saeedi *et al.*, 2019).

Term consequences or elevated blood glucose levels, high blood pressure and dyslipidaemia. Treatment focuses on discovering the most effective method to lower blood glucose levels, whether it is lifestyle modifications, insulin therapy, oral agents, or any combination of these factors (Kahn *et al.*, 2006).

Anogeissus leiocarpus (Figure 1) is a medicinal plant which belongs to the family Combretaceae.

It is a typical element of woodlands and savannas of the woodlands and savannas of the Sudanian regional centre of endemism (Ahmad, 2014). It has a large ecological distribution ranging from the borders of Sahara up to the outer humid tropical forests, in West Africa, from Senegal to Cameroon and extends to Ethiopia and East Africa. It grows in dry forests and gallery forests (Ouedraogo *et al.*, 2013; Hennenberg, 2005).

The plant is commonly called African Birch or Axle-wood in English, “Marke” in Hausa, “Atara” in Igbo, “Orin-odon” in Yoruba and “Annum” in Kanuri languages. Medicinally, the bark, leaves and roots of *Anogeissus leiocarpus* are all used in traditional medicine usually in the form of decoctions. They have antimicrobial and anthelmintic activities and are usually taken as decoctions (Omeje *et al.*, 2016; Namadina *et al.*, 2021). Extracts of the stem, root bark and of the leaves have shown antifungal activity against a number of pathogenic fungi. A moderate antibacterial activity of the bark was also demonstrated. Extract of the plant have exhibited *in-vitro* activity against chloroquine-resistant *Plasmodium falciparum* strains (Burkil, 1985; Ibrahim *et al.*, 2017; Namadina *et al.*, 2021). A decoction of the leaves or leafy twigs is used against yellow fever, jaundice, asthma, tuberculosis, common cold and headache (Burkil, 1985; Adesina *et al.*, 2016). Externally, a leaf decoction is applied against haemorrhoids and skin diseases. The root-bark is considered to be aphrodisiac and stimulant. In some regions the bark is used as a febrifuge in hot lotions and infusions. The powdered bark and a bark

decoction are used externally to treat wounds, eczema, psoriasis, anthrax, carbuncles, boils and several kinds of ulcers (Burkil, 1985). The bark decoction is also known as a muscular tonic. The bark and the exuding gum have been shown to prevent and cure dental caries and toothache and are commonly used in Africa (Burkil, 1985). The gum obtained from the bark is a laxative, it can be used in pharmacy as an emulsifying agent. The bark itself is chewed to obtain the gum (Burkil, 1985).

Preliminary phytochemical screening of *Anogeissus leiocarpus* stem bark for the major secondary constituents revealed, the plant was rich in tannins and having appreciable quantities of flavonoids, terpenes and saponins. However, it was devoid of alkaloids and anthraquinones (Elegami, 2002; Salau *et al.*, 2013).

The increasing challenge of managing diabetes mellitus, the poor potency of present drugs, the high cost of access to modern drugs and increasing demand for total cure have created a quest for alternative drugs; a necessity which makes the evaluation of the plant stem bark of *Anogeissus leiocarpus* (Guill and Perr.) for anti-diabetic activity imperative. This plant bark is used in North-east sub region of Nigeria by traditional medical practitioners (TMP) to as a concoction with *Boswellia dalzielii* and *Senna siamea* to treat diabetes mellitus. The plants *Boswellia dalzielii* and *Senna siamea* have been scientifically proven to possess antidiabetic effects (Yakubu *et al.*, 2020a,b; 2022b). Therefore, it is essential to evaluate and validate the folkloric use of the stem bark to ascertain its individualistic antidiabetic effect.



Figure 1: *Anogeissus leiocarpus* plant in its natural habitat (Plantnet, 2009)

MATERIALS AND METHODS

Sample Collection, Identification and Preparation

The Fresh stem bark of *Anogeissus leiocarpus* was collected from a farmland in Damaturu,

Yobe State, Nigeria and was identified and authenticated by Prof. S. S. Sanusi of Biological Sciences Department, Faculty of Science, University of Maiduguri, Maiduguri, Borno State. A plant specimen voucher number

(UMM/FPH/COB/003) was given and deposited at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Borno State.

Preparation and Extraction of the Stem Bark of *Anogeissus leiocarpus*

The method of extraction employed in this experiment was reflux extraction. The dirt and other extraneous materials were removed from the fresh stem bark of the plant by hand pick, size reduced and allowed to dried naturally (under shade) for two (2) weeks. It was further pulverized into fine powder using mortar and pestle. The sample was kept in a cool dried place until required for use. 500 g of plant material was introduced into a 5 L round bottom flask, 1.8 L of 97 % ethanol by soxhlet extraction method for 6 h. The mixture obtained was filtered using Whatman filter papers (grade 42:2.5 μm) to remove the debris. The filtrate was concentrated using rotary evaporator at 40-50 °C and stored in an air-tight container until required for further analysis.

Phytochemical Analysis

Ethanol stem bark extract of *Anogeissus leiocarpus* was screened for phytochemical constituents using standard methods as described by Brain and Turner, 1975; Vishnoi, 1979; Markham *et al.*, 1987; Silva *et al.*, 1998; Sofowora, 2008 and Evans, 2009 and adopted and by Yakubu *et al.*, 2016.

Experimental Animal and Acclimatization

Adult albino rats of both sexes weighing between 100-276 g were used for both the acute toxicity studies (LD_{50} determination) amounting to a total number of 25 rats were used. These rats were purchased from Animal House Section of Faculty of Pharmaceutical Science, Ahmadu Bello University (ABU), Zaria, Kaduna State, Nigeria.

The animals were housed in a standard wire meshed plastic cages in the animal section of Physiology, Pharmacology Laboratory of

Veterinary Medicine Faculty, University of Maiduguri, Borno State. At standard condition of temperature, light and humidity for a period of two weeks to allow them acclimatizes to laboratory condition. These animals were allowed free access to drinking water and standard livestock feed (Grand Cereals and Oils Mills Ltd.) Bukuru, Jos, Plateau State, Nigeria. All the studies were carried out in the animal section of Physiology, Pharmacology Laboratory of Veterinary Medicine Faculty, University of Maiduguri, Maiduguri, Borno State, Nigeria. The animals were handled according to the International Guiding Principle for Biomedical Research involving animals (CIOMS and ICLAS, 2012). Ethical approval (Reference ID: HREC:2022-010) was granted by the Health Research Ethics Committee, University of Maiduguri, Maiduguri, Borno State

Determination of Acute Toxicity (LD_{50})

The acute toxicity of the ethanolic stem bark extract of *Anogeissus leiocarpus* was determined using standard conventional procedure described by Lorke (1983). In this study, two routes of administration were considered, that is, oral and intraperitoneal routes. This comprises two phases which include:

Phase I: The rats were divided into three groups of three rats each route, they were then administered with the *Anogeissus leiocarpus* stem bark extract at doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight orally and intraperitoneally (*i.p*) and were observed for signs of acute toxicity and mortality for 24 h.

Phase II: Three groups of two rats were used based on the result of phase I after 24 h for the oral route. Three rats were given the *Anogeissus leiocarpus* stem bark extract the doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg respectively. For the intraperitoneal route, four dose levels were used based on the result of the phase I that is, four rats were given the dose of the leaf extract which were 1000 mg/kg, 1600 mg/kg, 2900 mg/kg and 5000 mg/kg

respectively giving a total of seven rats for this phase. These rats were then observed for 24 h for signs of toxicity and death after which the LD₅₀ was calculated appropriately.

Antidiabetic Study of Ethanol Stem Bark Extract of *Anogeissus leiocarpus* on Alloxan Induced Diabetes Albino Rats

The method described by Yakubu *et al.* (2020a) was adopted for this study. In this study, Diabetes was induced by injecting alloxan at dose of 150 mg/kg body weight intraperitoneally to overnight fasted animals. It was injected in single dose in total volume of 0.5mL distilled water prepared freshly. A total number of fifteen (15) albino rats of both sexes weighing between 100-276 g were used. The rats were denied feed for 24 h but were provided with

water *ad libitum*. The rats were divided into five groups of three rats each, that is, groups A, B, C, D and E. Group A was given 2mL of distilled water orally and serve as the negative control. Groups B, C and D were treated orally with 200 mg/kg, 400 mg/kg and 800 mg/kg of the extract respectively while group E was given Insulin Intraperitoneally (IP) and it served as the positive control as it is a standard antidiabetic drug.

Blood was withdrawn from the tail veins at defined time intervals and plasma glucose level was measured using accu-check on test strips at the interval of 0 h, 1 h 3 h, 6 h, 8 h, 12 h and 18 h respectively, the result was recorded at the end of the experiment.

Percentage protection (%) was calculated using the formula:

$$\% \text{protection} = \frac{\text{Mean glucose level of control} - \text{Mean glucose level of treated group} \times 100}{\text{Mean glucose level of control}}$$

Statistical Analysis

The results (data) were analysed using Graph Pad Instant soft wire and presented as Mean \pm Standard Deviation (S.D). Analysis of variance (One-Way ANOVA) followed by Dunnett multiple comparison was used to test between means at which $p < 0.05$ was considered significant.

RESULTS

Percentage Yield and Physical Appearance of the Extract

The weight, colour, texture and the yield of the ethanolic stem bark extract of the *Anogeissus leiocarpus* from reflux extraction are presented in Table 1. The weight was 46.55 g, colour of the extract was brown, its texture was a solid mass while the yield was 9.31 %.

Phytochemical screening of the *Anogeissus leiocarpus* Stem Bark Extract

The phytochemical profile of ethanol extract from the stem bark of *Anogeissus leiocarpus* presented in Table 2. The chemical constituent revealed the presence of flavonoids, cardiac

glycosides, terpenoids, carbohydrates, tannins, cardenolides and saponins.

Acute Toxicity Studies of Ethanolic Stem Bark Extract of *Anogeissus leiocarpus*

The result of the LD₅₀ study obtained using Lorke's method is as shown in Table 3. The oral LD₅₀ is 3,807 mg/kg as there was a record of mortality at that same dose (3,807 mg/kg). The intraperitoneal LD₅₀ was 1,265 mg/kg as there was also a record of mortality at same dose (1,265 mg/kg).

Anti-Diabetic Effect of Ethanol Stem Bark Extract of *Anogeissus leiocarpus* on Alloxan Induced-Diabetic Albino Rats

The result (Table 4) obtained after 18 h of intraperitoneal administration of *Anogeissus leiocarpus* stem bark extract showed that the control Group A, (negative control) which was pretreated with distilled water yielded a normal plasma glucose level (random blood glucose test) [70 mg/dl – 100 mg/ dl] with mean plasma glucose level \pm S.D. of 85.33 ± 5.03 and percentage protection of 0.00 %, Group B, 200

mg/kg of the *Anogeissus leiocarpus* stem bark extract had mean plasma glucose level \pm S.D. of 259.33 ± 225.75 and percentage protection of -0.02 %, Group C, 400 mg/kg of the *Anogeissus leiocarpus* stem bark extract yielded mean plasma glucose level \pm S.D. of 460.67 ± 78.62 and percentage protection of -0.04 %, Group D, 800 mg/kg of the *Anogeissus leiocarpus* stem bark extract yielded mean plasma glucose level \pm S.D. of 290.00 ± 192.87 and percentage

protection of -0.03 %, and finally, Group E, Insulin (standard) [positive control] yielded mean plasma glucose level \pm S.D. of 86.33 ± 5.51 with the highest percentage protection of -0.01 % than the other treatment groups (B, C and D). The extract reduced the plasma glucose level time dependent but there was no significant difference within the group and significance across the groups. ($p < 0.05$) when compared with the positive control (insulin standard).

Table 1: Extraction Profile of *Anogeissus leiocarpus* Stem Bark

Parameter	Ethanol Stem Bark Extract
Weight	46.55 g
Colour	Brown
Texture	Solid mass
% yield (w/w)	9.31 %

Weight of the ground stem bark = 500 g

Weight of the extract = 46.55 g

Percentage (%) yield = $\frac{46.55 \text{ g}}{500 \text{ g}} \times 100 = 9.31 \%$ w/w

Table 2: Phytochemical Constituents of *Anogeissus leiocarpus* Stem Bark Extract

Phytochemical	Inference
Alkaloids	-
Flavonoids	+
Saponins	+
Carbohydrate	+
Cardiac glycosides	+
Terpenoids	+
Tannins	+
Anthraquinones	-
Cardenolides	+

Keys: + = present

- = absent

Table 3: Oral and Intraperitoneal Acute Toxicity Test of the Crude Ethanol Stem Bark Extract of *Anogeissus leiocarpus* on Albino Rats

S/N	Phase	No. of Rats	Route	Dose (mg/kg)	Clinical sign	Mortality
1	1	3	Oral	10	None	0/3
2	1	3	Oral	100	None	0/3
3	1	3	Oral	1000	None	0/3
4	2	1	Oral	1600	None	0/1
5	2	1	Oral	2900	None	0/1
6	2	1	Oral	5000	Death	1/1
7	1	3	Intraperitoneal	10	None	0/3
8	1	3	Intraperitoneal	100	None	0/3
9	1	3	Intraperitoneal	1000	None	0/3
10	2	1	Intraperitoneal	1000	None	0/1
11	2	1	Intraperitoneal	1600	None	0/1
12	2	1	Intraperitoneal	2900	Death	1/1
13	2	1	Intraperitoneal	5000	Death	1/1

Where: a= Highest dose that kills the animal, b=Least dose that did not kill the animal

$$\text{Oral LD}_{50} = \sqrt{a \times b}$$

$$\sqrt{5000 \times 2900}$$

$$= \sqrt{14,500,000}$$

$$\text{LD}_{50} = 3,807 \text{ mg/kg}$$

$$\text{I.P. LD}_{50} = \sqrt{a \times b}$$

$$\sqrt{1600 \times 1000}$$

$$= \sqrt{1,600,000}$$

$$\text{LD}_{50} = 1,265 \text{ mg/kg}$$

Table 4: Effect of the Ethanol Stem Bark Extract of *Anogeissus leiocarpus* on Alloxan Induced-Diabetic Albino Rats

Extract/ Dose (mg/kg)	Plasma Glucose Level (Mean \pm SD)							Percentage (%) Protection
	0 hour	1 hour	3 hour	6 hour	8 hour	12 hour	18 hour	
Distilled H ₂ O 2ml (-ve control)	87.67 \pm 5.13	86.33 \pm 4.73	87.33 \pm 4.51	86.67 \pm 5.86	86.67 \pm 4.16	88.33 \pm 5.03	85.33 \pm 5.03	2.67
200	217.67 \pm 19.66 ^a	275.00 \pm 58.81 ^b	301.33 \pm 78.24 ^c	270.33 \pm 15.04 ^d	363.00 \pm 99.82 ^e	359.00 \pm 79.07 ^e	259.33 \pm 225.75	-19.14
400	419.33 \pm 10.07 ^a	427.33 \pm 11.02 ^b	447.33 \pm 28.31 ^c	441.33 \pm 35.23 ^c	466.00 \pm 28.16 ^d	481.67 \pm 18.93 ^e	460.67 \pm 78.62 ^d	-9.86
800	281.33 \pm 70.54 ^a	379.33 \pm 23.12 ^b	425.00 \pm 63.17 ^c	428.00 \pm 62.22 ^c	491.00 \pm 98.27 ^d	405.67 \pm 107.22	290.00 \pm 192.87	-3.08
Insulin* 0.75unit/kg (+ ve control)	405.00 \pm 83.82 ^a	314.00 \pm 89.92 ^b	166.67 \pm 51.32 ^c	123.00 \pm 32.51 ^d	105.00 \pm 13.00 ^e	94.00 \pm 3.46 ^f	86.33 \pm 5.51 ^g	78.68

*= Standard antidiabetic agent

N=5= Number of rats in each group within columns

Values with the same alphabetical superscripts across column not different statistically ($P>0.05$) while values with different alphabetical superscripts across rows are different statistically ($p<0.05$) between groups

DISCUSSION

The phytochemical constituents of the ethanol stem bark extract of *Anogeissus leiocarpus* detected in these current studies conform to the studies described by Elegam (2002) and Salau *et al.* (2013). Moola (2014) reported that these chemical compounds have been associated with various kinds of bioactivities such as antimicrobial, antimalarial; antidiabetic, antidiarrhoeal, etc. The presence of saponins in *Anogeissus leiocarpus* stem bark supports its traditional use for procuring abortion since they are linked to the sex hormone, oxytocin involved in controlling the onset of labour (Njoku and Akumefula, 2007). The presence of tannins suggested the growth inhibitory effect of this stem bark extract on bacteria (Sharall *et al.*, 2013).

The toxicity study of crude ethanol extract of *Anogeissus leiocarpus* stem bark in albino rats showed the intraperitoneal (i.p) and oral LD₅₀ to be 3,807 mg/kg and 1,265 mg/kg respectively. According to Clarke and Clarke (1977), when classifying toxicity of substances according to LD₅₀ values, substances with oral LD₅₀ values of ≥ 1000 mg/kg body weight and intraperitoneal LD₅₀ values of ≥ 500 mg/kg body weight can be said to be of low toxicity and therefore safe. However, the intraperitoneal LD₅₀ (1,265mg/kg) and oral LD₅₀ (3,807 mg/kg) values suggest the *Anogeissus leiocarpus* stem bark extract was of low toxicity via both *i.p* and oral routes. Therefore, it can be deduced from both studies that, the crude extract of *Anogeissus leiocarpus* stem bark extract is significantly safe or non-toxic owing to their high LD₅₀ values, which implies that, the higher the LD₅₀ value, the safer the extract and vice versa and the wide range of LD₅₀ denoted the safety effect of the extract which confers with the study of Adamu *et al.* (2022) who reported an Oral LD₅₀ of the methanol stem bark from Biu, Borno State, Nigeria to as ≥ 5000 mg/kg. The slight difference in the reports could be due to the different

extraction methods (soxhlet vs maceration), polarities of the solvents used for the extract or geographical locations of the sampling areas.

The result obtained from the alloxan-induced diabetes revealed that, the stem bark extract of *Anogeissus leiocarpus* reduced the plasma glucose level with time when compared within group at different time intervals. Thus the values were insignificant ($p > 0.05$) across the groups compared with positive control (insulin standard) and time dependently reduced the plasma glucose level within groups, Hence the plasma glucose level reduced with time within groups are significant ($p < 0.05$). Rao *et al.* (2021) reported that alkaloids and flavonoids has been associated with antioxidant effect which plays a vital role as antidiabetic agent. These compounds are present but the dose dependent effect was not significant which may be as a result of differences in geographical location, difference in time or season of sample collection or the solvent used and method of extraction which resulted to minute quantity of compounds responsible for antidiabetic effect and hence not sufficient to produce any significant effect.

CONCLUSION

The toxicity study revealed that the plant could be a safe remedy when taking via oral route than the *i.p.* route, hence lower dose should be used when administering via *i.p.* route. The study also revealed that the stem bark extract of *Anogeissus leiocarpus* does not possess antidiabetic effect scientifically and this has not supported the claim of its use in traditional medicine as an antidiabetic agent as there are alternatives (the roots and leaves extract of *Anogeissus leiocarpus*). Although, since the plant is used locally as a combination with other plants, their ability to inhibit hyperglycaemia could be as a result of a synergistic effect, hence the other alternatives should be used as this particular part (stem bark) is devoid of antidiabetic effect as claimed when used individualistically.

CONFLICT OF INTEREST

The authors declared no conflicts of interest.

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