Phytochemical analysis and acute oral toxicity study of acetone leaves extracts of *Anogeissus leiocarpus* (Axle wood) in Wistar rats

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**ABSTRACT**

This study was performed to determine the qualitative phytochemical constituents of *Anogeissus leiocarpus* and its oral acute toxicity (*LD₅₀*) in Wistar rats. The phytochemical analysis was carried out using acetone as a solvent and the acute toxicity study was carried out using Lørke’s method. Twelve (12) adult rats weighing 180–230 g body weight were used. The rats were divided into three groups of three rats each in phase 1 and one rat each in phase 2, respectively. The extract was administered to the rats orally at concentrations of 10, 100, and 1,000 mg/kg in phase 1. In phase 2, the rats were given the extract at concentrations of 140, 225, and 600 mg/kg. Signs of toxicity were observed within 24 hours interval in both phases. Also, mortality was observed only in group 3 of phase 1 where all the rats died. Based on this study, it can be concluded that the leaves of this plant should be used with caution for medicinal purposes.

**ARTICLE INFO**

*Article history:*
Received 14 June 2019
Received in revised form XX
Accepted 18 August 2019
Published XX
Available online XX

**KEYWORDS**

Acute toxicity
*Anogeissus leiocarpus*
Acetone
Phytochemical analysis

**1. Introduction**

Medicinal plants are of great importance to the health of man and animals and the value of these plants lies in some chemical substances contained in them that produce a definite physiological action (Olajide et al., 2013). Plants represent a rich source of a wide variety of secondary metabolites and many of the conventional drugs that are presently used are from herbs (Subramanian et al., 2018). Farnsworth (1984) report that out of the 119 plant-derived drugs listed by WHO study, 74% were discovered as a result of chemical studies in isolating the phytochemical compounds responsible for their use in traditional medicine. Medicinal plants have their use as medicament simply based on a traditional folk use that has been adopted along several generations, where rural and urban dwellers with small income depend on medicinal plants for the treatment of one form of ailment or the other without really knowing the active constituent of these plants (Sule et al., 2009). Despite the safety of plant drugs remedies, many unsafe and fatal side effects have been reported (Izzo, 2004; Whitto et al., 2003). Because of this renewed interest in herbal remedies, there is a need for thorough scientific safety evaluation of the medicinal plants (Sofowora, 1982). The plant *Anogeissus leiocarpus* belongs to the family Combretaceae. It is the sole West Africa species of...
the genus *Anogeissus* (Ouedraogo, 2013); the plant is used for the treatment of diabetic ulcers, general body pain, blood clots, asthma, coughing, and tuberculosis (Victor, 2013). The inner bark is used as a chewing stick in Nigeria and extracts of the bark show antibacterial properties (Mann et al., 2008; Victor, 2013). The decoction and maceration of the stem bark are used against various ailments such as anorexia, constipation, malaria, jaundice, itching, wounds, eczema, psoriasis, carbuncles, boils, and ulcers (Kerharo et al., 1974; Nacoulma, 1996). Ivory Coast traditional practitioners use the plant for treatments against Malaria, Trypanosomiasis, Helminthiasis, and dysenteric syndrome (Okpekton, 2004). In Togolese traditional medicine, it is used against fungal infections such as dermatitis and Mycosis, also the decoction of leaves is used against stomach infections (Batawila, 2005). This study aims at determining the qualitative phytochemical constituents as well as *in vivo* acute oral toxicity study (LD$_{50}$) of *A. leiocarpus* acetone leaves extracts in Albino rat.

### 2. Materials and Methods

#### 2.1. Plant collection and identification

The leaves of *A. leiocarpus* were collected from a rural community in Lassa, Askira/Uba Local Government Area of Borno State, Nigeria. The plant was identified and tagged by a Botanist from Department of Biological Sciences, University of Maiduguri and deposited in the herbarium of Veterinary Physiology and Biochemistry with herbarium voucher number Vet. Specimen 208A.

#### 2.2. Acetone Extract preparation

The crude aqueous acetone extract was prepared following the method of Mann et al. (2014). Briefly, fresh leaves of *A. leiocarpus* were air-dried at room temperature under a shade and pulverized into fine powder using mill hammer. Soxhlet extraction was used for the extraction. The pulverized plant material was macerated by adding 2 l of acetone to 220 g of the plant material in a 5 l round bottom flask. This was then filtered using Whatman (No. 1) filter paper. The filtrate was then transferred to evaporating dish in order to concentrate the extract in a hot air oven at 40°C–50°C. The product (yield) obtained was then weighed and stored at 4°C for future use.

#### 2.3. Phytochemical analysis

##### 2.3.1. Test for carbohydrates

Molisch’s test was used to test for carbohydrates contents of the extract. Few drops of Molisch’s reagent were added to 0.5 g of the extract dissolved in distilled water. This was followed by addition of 1 ml of concentrated tetraoxosulphate (VI) acid by the side of the test tube so that the acid forms a layer beneath the aqueous layer. The mixture was then allowed to stand for 2 minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet color at the interphase of the two layers indicates a positive test according to Trease and Evans (2002).

##### 2.3.2. Test for cardiac glycosides

Cardiac glycosides were screened using Salkowski’s and Liebermann–Burchard’s tests. Briefly, for Salkowski’s test, 0.5 g of the powdered leaves extract was added into 2 ml of chloroform and tetraoxosulphate (VI) acid was carefully added by the side of the test tube to form a lower layer. Appearance of a reddish-brown color or yellow at the interphase was an indication for the presence of a steroidal ring (i.e., aglycone portion of cardiac glycoside) or methylated sterols (Silver et al., 1998). In order to determine the presence of steroidal nucleus using Liebermann–Burchard test; 2ml of acetic acid anhydride was added onto the powdered form (0.5 g) of the plant leaves extract and allowed to dissolve and cool on ice packs. Concentrated tetraoxosulphate (VI) acid was carefully added to observe for color change. Color change from violet to blue or bluish-green indicates the presence of a steroidal ring (that is aglycone portion of cardiac glycoside) (Silver et al., 1998).

##### 2.3.4. Test for terpenoids

A little of the extract (0.5 g) was dissolved in ethanol and 1 ml of acetic anhydride was then added followed by the addition of concentrated tetraoxosulphate (VI) acid. A color change from pink to violet indicates the presence of terpenoids (Silver et al., 1998).

##### 2.3.5. Test for flavonoid

##### 2.3.5.1. Shinoda’s test. The powdered leaves (0.5 g) of the aqueous extract of *A. leiocarpus* was dissolved in ethanol, warmed, and filtered. Three pieces of magnesium chips were added to the filtrate and then followed by a few drops of concentrated hydrochloric acid (HCl). A pink, orange, or red to purple coloration indicated the presence of flavonoids Markham (1987).

##### 2.3.5.2. Ferric chloride test. The extract was boiled with distilled water and filtered. Few drops of 10% ferric chloride solution were added to 2 ml of the filtrate. A green-blue or violet coloration indicated the presence of a phenolic hydroxyl group (Trease and Evans, 2002).
2.3.5.3. Lead ethanoate test. A small quantity of the extract (0.5 g) was dissolved in water and filtered. Three (3 ml) of lead ethanoate solution was then added to 5 ml of the filtrate. Appearance of a buff-colored precipitate indicated the presence of flavonoids (Brain and Turner 1975).

2.3.5.4. Sodium hydroxide test. A small quantity of the extract (0.5 g) was dissolved in water and filtered, and then 2 ml of 10% aqueous sodium hydroxide was added and produced a yellow coloration. To this, a dilute hydrochloric acid was added; a change of color from yellow to colorless indicated the presence of flavonoids (Trease and Evans, 2002).

2.3.6. Test for tannins

Zero point five grams (0.5 g) of the extract was stirred with 10 ml of distilled water and filtered. The filtrate was used for the following tests: 1 to 2 ml of the filtrate, few drops of 1% ferric chloride solution was added, the occurrence of a blue-black, green, or blue-green precipitate showed the presence of tannins. A mixture of an equal volume of 10% lead ethanoate was added to 2 ml of the filtrate. Formation of a white precipitate was an indication for the presence of tannins. The filtrate of the extract was boiled with three drops of 10% HCl and one drop of methanol. A red precipitate was taken as evidence for the presence of tannins (Sofowora, 1993; Trease and Evans, 2002).

2.3.7. Test for cardenolides

The extract (0.2 g) was dissolved in three drops of pyridine and a drop of recently prepared 0.5% Sodium nitroprusside in water was added. Then four drops of 0.2 N NaOH were added. Deep-red colorations indicate the presence of cardenolide-aglycone (Silva et al., 1998).

2.4. Acute toxicity study

A total of 12 adult Wistar rats weighing between 180 and 230 g sourced from the Department of Biochemistry, University of Maiduguri animal house were used for this study; they were kept in the laboratory of Veterinary physiology and Biochemistry, University of Maiduguri for 7 days for acclimatization prior to the commencement of the experiment. The acute toxicity study was performed using the method as described by Lorke's (1983), briefly, the rats were divided into two groups A and B, group A consists of 9 rats divided into three groups containing three rats each per group, they were administered 10, 100, and 1,000 mg/kg bw orally of the plant extract to the respective group as phase I, the rats were placed under observation for 24 hours to monitor signs of toxicity and mortality. The group B consist of three rats, divided into three groups containing one rat each per group received 140, 225, and 600 mg/kg bw of the extract orally and observed for 24 hours to monitor signs of toxicity and mortality as the phase II; also, the \( LD_{50} \) was determined using the following formula:

\[
LD_{50} = \sqrt{A \times B}
\]

\( A \) = dose that causes mortality in phase one
\( B \) = dose that did not cause mortality in phase two

3. Results

3.1. Qualitative phytochemical constituents

The pulverized leaves of A. leiocarpus at 60°C, using acetone as extracting solvent, produced a dark brown, sticky semisolid substance with a yield of 65% (11 g) from the starting material. Phytochemical analysis revealed the presence of the following phytochemical constituents: cardiac glycosides, terpenoids, saponin glycosides, tannins, flavonoids, carbohydrates, and cardenolides (Table 1).

3.2. Acute toxicity

The results of the acute toxicity studies showed clinical signs at doses of 10, 100, and 1,000 mg/kg. The signs of toxicity were observed for 2 hours after extract administration. The clinical signs include: abdominal stretching, restriction movement, piloerection, lethargy, and droopy eye. Death of all the rats was recorded at the highest dose of 1,000 mg/kg in the first phase. In the second phase of extract administration of 140, 225, and 600 mg/kg, there was no mortality recorded within 24 hours. The calculated value is \( LD_{50} 774.60 \) mg/kg (Table 2).

<table>
<thead>
<tr>
<th>Phytochemical Constituents</th>
<th>Result</th>
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<tbody>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Cardenolites</td>
<td>+</td>
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</tbody>
</table>

Present = +, Absence = –.
Table 2. The acute toxicity.

<table>
<thead>
<tr>
<th>Phase 1</th>
<th>Doses in mg/kg body weight (mortality)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>10 mg/kg</td>
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<tr>
<td></td>
<td>(0/3)</td>
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<tr>
<td>Phase 2</td>
<td>140 mg/kg</td>
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<tr>
<td></td>
<td>(0/3)</td>
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LD$_{50} = \sqrt{A \times B}$,

$A =$ dose that killed the rats (3/3) in phase one,
$B =$ dose that all rats are alive (0/3) in phase two,

LD$_{50} = \sqrt{1,000 \times 600} = 774.60$ mg/k.

4. Discussion

The present study shows the LD$_{50}$ and bioactive constituents contained in the acetone leaves extract of *A. leiocarpus*. The leaves, roots, and barks of the plant have many bioactive phytoconstituents (Arbab, 2014). Based on this study, flavonoids, tannins, cardiac glycosides, terpenoids, saponins, carbohydrates, and cardenolide were present from the leaves extract. Cardiac glycosides are a group of plant metabolites that comprises the most drug-like molecules subjected to several investigations and they were proved to be productive in developing potential drugs (Shaker et al., 2010). Cardiac glycosides occur in small amounts in different plants parts of wide geographical distribution (Nagy, 2017). The most important use of the cardiac glycosides is its effects in the treatment of cardiac failure, or congestive heart failure, antitumor activity (Doskotch et al., 1972), and inhibitory activity against rhinovirus (Kamano, 1988). Tannins are polyphenols (Haslam, 1989) widely distributed in many species of plants, where it plays a role in protection from predation, and also in plant growth regulation (Elfadil et al., 2013). Tannins can be used in the prevention and treatment of diabetes, heart diseases (Rai et al., 1997), antimicrobial (Hatano et al., 2006), and antitumor activities (Yoshizawa et al., 1987).

Saponins are phytochemical compounds that are produced mainly by plants, and also by some insects and marine organisms (Thakur et al., 2011); they mostly occur as glucosides of steroids or polycyclic triterpenes (Kensil, 1996). Saponins have been attributed to a number of pharmacological actions, among the most important ones are, lowering of serum cholesterol levels (Francis et al., 2002), cytostatic and cytotoxic effects on malignant tumor cells (Bachran et al., 2008), immunomodulatory and antiviral properties for vaccines as immunostimulatory complexes (Sjolander et al., 1998), stimulation of luteinizing hormone release leading to abortifacient properties (Francis et al., 2002), and synergistic enhancement of the toxicity of immunotoxins (Heisler et al., 2005). Flavonoids are natural occurring polyphenolic compounds that contained two benzene rings linked with heterocyclic pyrone ring (Kandakumar and Manju, 2017); they are mostly present in aromatic plants, medical herbs, fruits, and vegetables (Rice-Evans, 2001). Plant rich in flavonoids may be used to reduce the incidence of existence diseases (Katan, 1997; Steinberg, 1989). Terpenoids are structurally most diverse group of secondary metabolites among plants which functions as phytoalex-ins in plant direct defense or as signals in indirect response (Ai-Xia et al., 2007). They have been reported to have anti-cancer properties (Roslin Thoppil and Anupam Bishayee, 2011) anti-oxidative properties (Grassman, 2005; Karl-Heinz and Ibrahim, 2003), and also used in the treatment of cardiovascular diseases (Karl-Heinz and Ibrahim, 2003). Carbohydrates serve as structural constituents (cellulose), components of energy (ATP), DNA, and RNA (Suman et al., 2008).

Previous studies have supported the use of this plant in traditional medicines (Arbab, 2014) and the findings of this study revealed that the acetone leaves extract of *A. leiocarpus* exerts acute toxicity at a dosage of 774.60 mg/kg. This finding does not agree with that of Ahmad and Wudil (2013), who reported acute toxicity at a dosage of 3,200 mg/kg using aqueous leaves extract. This variation could be due to differences in the solvents used for preparing the extract as positioned by earlier researchers Seddon and Downey (2008); Harbertson and Downey, (2009) who suggested that different extraction solvents have the propensity of preferentially extracting different classes of heterogeneous compounds with varied biological activities, especially when the phytochemicals are present at different concentrations. This work is also not in congruence with the result of the sub-acute toxicological study of the aqueous stem bark extract of this plant by Ahmad and Wudil (2013), which showed no significant toxicological effects of the extract on the liver. However, it is important to note that different parts of the plant, extraction solvent, and geographical location for the plant source could be the main source of these variations. The LD$_{50}$ observed in this study is lower than the reports of Ahmad et al. (2014) following intravenous and intraperitoneal administration in mice. This variation is obviously due to differences in the routes of administration as intravenous and intraperitoneal routes have the ability to cause profound effects on the body systems, since the extract is directly released into the circulation.
Conclusion

The leaves of *A. leiocarpus* seem to be potential in various activities, so it can be further explored to find an application in the control of human or animal disorder. According to Gadanyav et al. (2011), any compound with oral LD50 (rat) of 5,000 mg/kg or more should be considered as practically harmless. However, this study revealed the oral LD50 of *A. leiocarpus* to be 774.6 mg/kg; therefore, it should be used with caution.

References


Farnsworth NR. How can a well be dry when it is filled with water? Econ Bot 1984; (38):4–13.


Morsy N. Cardiac glycosides in medicinal plants, aromatic and medicinal plants—back to nature. Hany A. El-Shemy. Intech Open, 2017; doi:10.5772/65963

Nacoulma OG. Plantes médicinales traditionnelles au Burkina Faso. Cas du plateau central.