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Toxicity Studies and Phytochemical Screening of Aqueous Extract of *Cissus aralioides* Plant

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Authors' contributions

This work was carried out in collaboration between all authors. Author NBC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AKK, ECN and UCS managed the literature searches. Author UCS managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Toxicity investigation are employed in animal models to examine the dose level for the treatment of different disease for drug efficiency while phytochemicals are naturally occurring components found in medicinal plants containing varying colour, flavor and smell which serves for plant's natural defense system. This study examined toxicity studies and phytochemical screening of aqueous extract of *Cissus aralioides* plant. The phytochemical assay was screened for alkaloid, saponin, tannin, flavonoid, steroid, terpenoid, cardiac glycoside, proteins and reducing sugar in plant extract of *C. aralioides* with standard procedure. Eighteen (18) of the animals were used for LD_{50} study of *Cissus aralioides* using Lork's method. Results of phytochemical screening of aqueous leaf extract revealed the presence of alkaloid, flavonoid, proteins, reducing sugar, saponin, terpenoid and cardiac glycoside. The acute toxicity showed LD_{50} greater than 5000 mg/kg body weight. Hence, the aqueous leaf extract of C. *aralioides* is relatively safe when administered through oral route in the wistar rats.

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1. INTRODUCTION

Medicinal plants are good sources of direct therapeutic agents and serves as models for generating new synthetic compounds. Plants are taxonomic marker for discovery of new drug agent. They act as raw material base for the elaboration of more complex semi synthetic chemical compounds for treating diseases [1]. The importance of medicinal plants and their immense contribution in phytomedicine to the well-being of a significant number of the world's population have attracted variety interest from different disciplines [2]. Medicinal plants have proven to be very vital in the tradiomedical practices and researches following the presence of phytochemicals and bioactive components in them. Subsequently, medicinal plants are useful in drug discoveries, synthesizes and development [3].

Cissus aralioides is a medium sized tendril climber with a length of approximately 25 m long whose stem are mostly fleshy but woody at the base of about 5m long. Its stems are often violet-purple or yellowish. Its leaves are greenish and slightly toothed, cylindrical and jointed. *C. aralioides* regenerates vegetatively from the individual internodes into which the stem may break up. Ecologically, *C. aralioides* is found in coastal and fringing forest in the Savanna zone. It is also found in the semi deciduous forest; disturbed forest; bush land; deep or narrow ravine areas; wet Acacia bush-land; grassland; granite rocks and termite mounds as well as around damp dense primitive woods and swampy forests [4].

C. aralioides is a forest plants with medicinal value, although, it is neglected by many due to the damages caused by these plants in farm lands in most part of Nigeria. Hence they are often uprooted and thrown by roadsides for vehicles to destroy them as the stems of the plant hardly die. Ethnobotany survey on this plant reveals that most farmers destroy these plants following its destructive abilities on crops and the health effects such as itching observable in them. Despite this envisaged problem, Assob et al. [5] maintains that *C. aralioides* plant extracts has several health benefits as noticed in Cameroon were it is commonly called 'Kindamina'. Meanwhile**,** in Congo, Burkill, [6] reported that *C. aralioides* stem were often used to prepare embrocation for fever pains. Also, in Tanzania, roots of *C. aralioides* were reported to be used in the treatment for fever and malaria. However, the seeds of *C. aralioides* are discarded as little or no literature accounts are available to support its nutritional values and other beneficial uses.

Toxicity studies are used in animal experimental designs for trial of different dose level of drugs in different disease conditions [7]. Toxicity studies can be classified into three (3) broad types, namely; acute, sub-acute and chronic toxicities. Acute toxicity connotes harmful effects in an organism through a single or short term exposure; sub-chronic toxicity relates to the ability of a toxic substance to cause effects for more than one year but less than the life time of exposed organism and chronic toxicity is concerned with the ability of a substance to cause harmful effects over an extended period or upon repeated and continuous exposure [8].

Phytochemicals generally are considered to be chemical compounds present in medicinal plants with normal metabolically processes [9]. Such chemicals are often called secondary metabolites such as; tannins, terpenes, reducing sugar, protein, flavonoids, coumarins, glycosides, saponin, gums, phenols, alkaloids and terpenoids [10]. When compared to synthetic pharmaceuticals based upon single chemicals, many medicinal plants exert their beneficial actions via synergistic or additive action of several chemical compounds acting which usually acts as single or multiple target sites that is associated with varying physiological process [9].

Sofowora [11], pointed out that most tropical rainforest are reservoir of beneficial phytomedicines. Wide variations in medicinal quality and content in phytopharmaceutical preparations have been observed by previous studies. Such variations could be as a result of cultivation period, variability accustomed from plant-to-plant medicinal content, season of collection of the plant, adulteration of preparation process of the medicinal plant, inadequate preparatory methods to ascertain the effectiveness of the production and standardization process of the plant, inability to understand the uniqueness of the plant physiology and its efficacy with human consumption as well as consumer fraud [12].

Some of the phytochemicals present in medicinal plants are discussed as follows:

1.1 Flavonoids

Flavonoids such as; quercetin and kaempferol glycosides are pigments often found in medicinal plants. They are polyphenolic compounds with a base structure that consists of two aromatic rings joined with a three-carbon chain – the so-called 'C_6-C_3-C_6' carbon skeleton. Over 2000 different components of flavonoids are found in medicinal plants in either the free-state or glycosides. According to Trease and Evans, [13] three different methods are used to confirm the presence of flavonoids in medicinal plant extracts of *C. aralioides*. Manikandan et al. [14] confirmed that flavonoids are significantly recognized for their anti-oxidant, anti-carcinogenic, anti-microbial and anti-tumor properties.

1.2 Saponin

Saponnin is another class of chemical compound found in particular abundance in various plant species. Specifically, they are amphipathic glycosides. The persistent frothing test for saponin is used in examining for its presence in *C. aralioides.* Saponins lower the cholesterol levels; in addition, they have both anti-diabetic and anti-carcinogenic properties. Okwu, [15] opted that saponins are expectorants, cough suppressants and for haemolytic activities.

1.3 Terpenes or Terpenoids

Terpenes often at times referred to as terpenoids are phytochemicals that are present in *C. aralioides;* they have anti-hepatoxic properties which help to prevent liver damages such as; cirrhosis. Consequently, terpenes equally have both anti-microbial and anti-septic properties. The Salkowski tests are often used in conducting this test where a reddish brown colouration of the inter-face shows positive result for its presence.

1.4 Steroids

Steroids are present in stems of *C. aralioides.* They generally regulate carbohydrate and protein metabolism as well as possess anti-inflammatory properties. 2 mL of acetic anhydride are added to 0.5 g ethanolic extract of each sample with 2 mL of H_2SO_4 while colour changes from violet to blue often indicate the presence of steroids in plants.

1.5 Alkaloids

Alkaloids are phytochemicals that posses both anti-spasmodiac, analgesic and bactericidal properties [10]. Drangendoff's reagent tests are often used to investigate the presence of alkaloids in *C. aralioides* plant as described in the technique adopted by Harborne [16]. The formation of orange precipitate indicates the presence of alkaloids in the plant [17].

1.6 Tannins

Tannins are phytochemicals that are present in *C. aralioides* plant extracts; they possess both anti-oxidant and anti-microbial properties. Tannins also help in soothing relief, skin regeneration, anti-inflammatory and diuretics [10].

1.7 Cardiac Glycosides

Cardiac glycosides are phytochemicals that acts on the heart muscles and increase renal flow (diuresis). Keller-Killani tests are often used to investigate the present of cardiac glycoside in *C. aralioides* plants, whereas, the browning of the interface indicates a deoxysugar characteristic (cardenolides). However, below the 'brown' colour is a violet ring while in the acetic acid layer is the presence of a greenish ring colouration.

2. METHODOLOGY

2.1 Identification of Plant Extract

Fresh species of *C. aralioides* were collected from farms in Nnewi, Anambra State of Nigeria. The plant specimen was identified by a plant taxonomist with voucher number V1291 and specimen was deposited in the herbarium of this University of Benin for future reference.

2.2 Phytochemical Screening of the Plant Extract

Aqueous extracts of *C. aralioides* was tested for the determination of phytochemical constituents, specifically qualitative screening using classic methods of Guessan et al. [18] and Edeoga et al. [19] as shown below;

Test for proteins: Crude extract of *C. aralioides* was mixed with 2 ml of Millon's reagent; result yielding a white precipitate after heating indicates the presence of protein.

Test for carbohydrates: Equal volume of Fehling A and Fehling B reagents were mixed together and 2 ml of it was added to crude extract of *C. aralioides* and gently boiled. A brick red precipitate appeared at the bottom of the test tube signifies the presence of carbohydrate.

Test for phenols and tannins: Crude extract of *C. aralioides* was mixed with 2 ml of 2% solution of $FeCl_3$. Result yielding a blue-green or black colouration implies the presence of phenols and tannins.

Test for flavonoids: Crude extract of *C. aralioides* was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Result yielding pink scarlet colouration after few minutes indicates the presence of flavonoids.

Test for saponins: Crude extract of *C. aralioides* was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam signifies the presence of saponins.

Test for cardiac glycosides: Crude extract of *C. aralioides* was mixed with each of 2 ml of chloroform and 2 ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A colour change from violet to blue to green indicates the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Test for steroid: Crude extract of *C. aralioides* was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2 ml of chloroform. Then 2 ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicates the presence of steroids.

Test for terpenoids: Crude extract of *C. aralioides* was dissolved in 2 ml of chloroform and evaporated to dryness. To this, 2 ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour signifies the presence of terpenoids.

Test for alkaloids: Crude extract of *C. aralioides* was mixed with 2 ml of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

2.3 Lethal Dose (LD_{50}) Toxicity Test

Aqueous plant extracts of *C. aralioides* extract from cold maceration filtration method was administered to determine the LD_{50} using the methods of Lorke [20]. The test was conducted in two phases. Phase I group involved 9 rats. They were grouped into 3 groups of 3 rats each; that is; group I received 800 mg/kg of the extract p.o; group II received 1600 mg/kg; while; group III received 2900 mg/kg. The animals were constantly monitored for 1 hour, intermittently for the next 3 hours and finally over a period of 24/hours for mortality and behavioural changes.

From the result of the Phase I, then, Phase II was carried out. In this phase, a total of 9 rats were used. They were grouped into 3 groups of 3 rats per group. Group I received 3200 mg/kg p.o; group II received 4100 mg/kg p.o; group III received 5000 mg/kg p.o respectively. The animals were monitored as in phase I for mortality and behavioural changes. Also, they were observed for the same changes and signs of toxicity and/or possible death as well as the latency of death in phase II. The LD_{50} was calculated as follows: $LD_{50} = \sqrt{a \times b}$; where: *a* = least dose that kills a mouse and *b* = highest dose that does not kill any mouse.

3. RESULTS

Results obtained from the phytochemical screening of aqueous extract of *C. aralioides* plant extract as shown in Table 1 revealed the presence of various secondary metabolites that are moderately present such as; alkaloid, flavonoid, protein and reducing sugar. More so, it was observed that saponin, terpenoid and cardiac glycoside were mildly present. However, there was absence tannin and steroid in the plant.

The results obtained from LD_{50} studies using Dietrich Lorke method showed no acute toxicity at higher doses as shown in Table 2 below:

Table 1. Qualitative analysis of phytochemicals in *Cissus aralioides*

Samples	Result
Alkaloid	++
Saponin	+
Tannin	-
Flavonoid	++
Steroid	-
Terpenoid	+
Cardiac Glycoside	+
Protein	++
Reducing Sugar	++

Key: -- Absent
+- Midly present/trace
++- Moderately present/trace
+++- Abundantly present

Table 2. Result of acute toxicity (LD_{50})

Phase	Dose (mg/kg)	Death	Behaviour
I	800	$^0/_3$	Normal
	1600	$^0/_3$	Normal
	2900	$^0/_3$	Normal
II	3200	$^0/_1$	Normal
	4100	$^0/_1$	Normal
	5000	$^0/_1$	Normal

÷ LD_{50} > 5000mg/kg

4. DISCUSSION

In all cultures and throughout history, herbal medicine or traditional medicine is the oldest form of health care known to man. Much of the medicinal use of plants seems to have developed by trial and error and or through observations of wild animals. Medicinal plants have been identified traditionally and have been used in the treatment of various diseases. According to documents the use of the herbs has a long history in order to fertility regulation [21]. Many plants/plants extracts have been used as fertility agents in folklore and traditional medicines without producing apparent toxic effects [22,23]. On this accounts, many plant derived chemicals that influence endocrine activities in both humans and animals have received a great deal of attention due to their possible benefits as well as adverse effects.

The preliminary phytochemical analysis showed the presence of alkaloid, flavonoid, protein, reducing sugar, saponin, terpenoid and cardiac glycoside. The results indicate that the leaves of these plants posses some biologically active compounds which could serve as potential sources of drugs. The result of phytochemical screening obtained from this study has revealed that the plant *C. aralioides* contains many bioactive as well as toxic agents that can affect the regulation of conception and reproduction. Moreover, literature by Okon and Etim, [24] shows that presence of saponins, flavonoids, tannins, alkaloids possess anti-fertility activity. According to Edeoga et al. [19] and Yakubu et al. [25] bioactive components such as; alkaloids and flavonoids have been shown to reduce the regulation of conception and reproduction. This was also consistent with the findings from previous reports that indicate the presence of flavonoids, alkaloids [26], saponins and glycosides and terpenoids [27] in medicinal plants with contraceptive or pregnancy interceptory effects.

The results of the acute toxicity study revealed that aqueous leave extract of *C. aralioides* is relatively non-toxic since no treatment-related signs of toxicity were noticed in the animals throughout the observation period. Although, the animals showed some signs of weaknesses at 5000mg/kg body weight, they became more active afterward. The LD_{50} of more than 5000mg/kg body weight observed in this study is in line with the findings of Kennedy et al. [28] who reported that some active substances in medicinal plants are relatively non-toxic. The safety of *C. aralioides* extract was further supported by the absence of observable adverse effects on the respiratory, nervous activities as observed over the period of administration of the LD_{50} studies; thus, this is an indication that the extract might be safe orally at the stipulated dose.

5. CONCLUSION

In this present study, the screening of aqueous leave extract of *C. aralioides* for qualitative phytochemicals revealed that alkaloid, flavonoid, protein, reducing sugar, saponin, terpenoid and cardiac glycoside were present. Consequently, the result of the acute toxicity (LD_{50}) test of the aqueous leaf extracts of *Cissus aralioides* showed no toxicity on the Wistar rats; hence, the aqueous leaf extract of C. *aralioides* is relatively safe when administered through oral route.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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