

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

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 LD₅₀ 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 LD₅₀ 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.

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 the treating diseases (Akerele, 1992). 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 (Biapa *et al*, 2007). 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 (Phapale and Misra-Thukur, 2010).

35 *C. aralioides* is a medium sized tendril climber with a length of approximately 25m
36 long whose stem are mostly fleshy but woody at the base of about 5m long. Its stems are
37 often violet-purple or yellowish. Its leaves are greenish and slightly toothed, cylindrical and
38 jointed. *C. aralioides* regenerates vegetatively from the individual internodes into which the
39 stem may break up. Ecologically, *Cissus aralioides* is found in coastal and fringing forest in
40 the Savanna zone. It also found in the semi deciduous forest; disturbed forest; bush land; deep
41 or narrow ravine areas; wet Acacia bush-land; grassland; granite rocks and termite mounds as
42 well as around damp dense primitive woods and swampy forests (Castarini, Havik and
43 Roneiras, 2016).

44 *Cissus aralioides* is a forest plants with medicinal value, although, it is neglected by
45 many due to the damages caused by these plants in farm lands in most part of Nigeria. Hence
46 they are often uprooted and thrown by roadsides for vehicles to destroy them as the stems of
47 the hardly die. Ethnobotany survey on this plant reveals that most farmers destroy these
48 plants following its destructive abilities on crops and the health effects such as itching
49 observable in them. Despite this envisaged problem, Assob *et al.*, (2011) maintains that *C.*
50 *aralioides* plant extracts has several health benefits as noticed in Cameroon were it is
51 commonly called 'Kindamina'. Meanwhile, in Congo, Burkill, (2000) reported that *C.*
52 *aralioides* stem were often used to prepare embrocation for fever pains. Also, in Tanzania,
53 roots of *C. aralioides* were reported to be used in the treatment for fever and malaria.
54 However, the seeds of *C. aralioides* are discarded as little or no literature accounts available
55 to support its nutritional values and other beneficial uses.

56 Toxicity studies are used in animal experimental designs for trial of different dose
57 level of drugs in different disease conditions (Hodgson, 2010). Toxicity studies can be
58 classified into three (3) broad types, namely; acute, sub-acute and chronic toxicities. Acute

59 toxicity connotes harmful effects in an organism through a single or short term exposure; sub-
60 chronic toxicity relates to the ability of a toxic substance to cause effects for more than one
61 year but less than the life time of exposed organism and chronic toxicity is concerned with
62 the ability of a substance to cause harmful effects over an extended period or upon repeated
63 and continuous exposure (Schrager, 2006).

64 Phytochemicals generally are considered to be chemical compounds present in
65 medicinal plants with normal metabolically processes (Okigbo *et al*, 2009). Such chemicals
66 are often called secondary metabolites such as; tannins, terpenes, reducing sugar, protein,
67 flavonoids, coumarins, glycosides, saponin, gums, phenols, alkaloids and terpenoids (Okwu,
68 2004). When compared to synthetic pharmaceuticals based upon single chemicals, many
69 medicinal plants exert their beneficial actions via synergistic or additive action of several
70 chemical compounds acting which usually acts as single or multiple target sites that is
71 associated with varying physiological process (Okigbo *et al*, 2009).

72 Sofowora (1993) pointed out that most tropical rainforest are reservoir of beneficial
73 phytomedicines. Wide variations in medicinal quality and content in phytopharmaceutical
74 preparations have been observed by previous studies. Such variations could be as a result of
75 cultivation period, variability accustomed from plant-to-plant medicinal content, season of
76 collection of the plant, adulteration of preparation process of the medicinal plant, inadequate
77 preparatory methods to ascertain the effectiveness of the production and standardization
78 process of the plant, inability to understand the uniqueness of the plant physiology and its
79 efficacy with human consumption as well as consumer fraud (Nalawade and Tsay, 2004).
80 Some of the phytochemicals present in medicinal plants are discussed as follows:

81 **Flavonoids:**

82 Flavonoids such as; quercetin and kaempferol glycosides are pigments often found in
83 medicinal plants. They are polyphenolic compounds with a base structure that consists of two
84 aromatic rings joined with a three-carbon chain – the so-called ‘C₆-C₃-C₆’ carbon skeleton.
85 Over 2000 different components of flavonoids are found in medicinal plants in either the
86 free-state or glycosides. According to Trease and Evans, (1989) three different methods are
87 used to confirm the presence of flavonoids in medicinal plant extracts of *C. aralioides*.
88 Manikandan *et al.*, (2006) confirmed that flavonoids are significantly recognized for their
89 anti-oxidant, anti-carcinogenic, antimicrobial and antitumor properties.

90 **Saponin:**

91 Saponnin is another class of chemical compound found in particular abundance in
92 various plant species. Specifically, they are amphipathic glycosides. The persistent frothing
93 test for saponin is used in examining for its presence in *C. aralioides*. Saponins lower the
94 cholesterol levels; in addition, they have both anti-diabetic and anti-carcinogenic properties.
95 Okwu, (2005) opted that saponins are expectorants, cough suppressants and for haemolytic
96 activities.

97 **Terpenes or terpenoids:**

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

103 **Steroids:**

104 Steroids are present in stems of *C. aralioides*. They generally regulate carbohydrate
105 and protein metabolism as well as possess anti-inflammatory properties. 2mL of acetic

anhydride are added to 0.5g ethanolic extract of each sample with 2mL of H₂SO₄ while colour changes from violet to blue often indicate the presence of steroids in plants.

Alkaloids:

Alkaloids are phytochemicals that possess both anti-spasmodic, analgesic and bactericidal properties (Okwu and Okwu, 2004). Drangendoff's reagent tests are often used to investigate the presence of alkaloids in *C. aralioides* as described in the techniques adopted by Harborne (1973). The formation of orange precipitate indicates the presence of alkaloids.

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 (Okwu and Okwu, 2004).

Cardiac glycosides:

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

Methodology

Identification of plant extract:

Fresh species of *Cissus 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.

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.*, (2009) and Edeoga *et al.*, (2005) as shown below;

Test for proteins: Crude extract of *C. aralioides* was mixed with 2ml 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 2ml 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 2ml of 2% solution of FeCl₃. 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 5ml 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 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H₂SO₄ 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 2ml of chloroform and concentrated H₂SO₄ 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 2ml of chloroform. Then 2ml of each of concentrated H₂SO₄ 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 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H₂SO₄ 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 2ml 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.

Lethal Dose (LD₅₀) toxicity test:

Aqueous plant extracts of *C. aralioides* extract from cold maceration filtration method was administered to determine the LD₅₀ using the methods of Lorke (1983). 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 800mg/kg of the extract p.o; group II received 1600mg/kg; while; group III received 2900mg/kg. The animals were constantly monitored for 1hour, intermittently for the next 3hours 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 3200mg/kg p.o; group II received 4100mg/kg p.o; group III received 5000mg/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 LD₅₀ 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.

182

183

184 **Results**

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

190 **Table 1: Qualitative analysis of phytochemicals in *Cissus aralioides***

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

191 **Key:**

192 - - Absent
 193 + - Mildly present/trace
 194 ++ - Moderately present/trace
 195 +++ - Abundantly present
 196

197 The results obtained from LD₅₀ studies using Dietrich Lorke method showed no acute
 198 toxicity at higher doses as shown in Table 2 below:

199 **Table 2: Result of Acute Toxicity (LD₅₀)**

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

200 ÷ **LD₅₀ > 5000mg/kg**

201 **Discussion:**

202 In all cultures and throughout history, herbal medicine or traditional medicine is the
 203 oldest form of health care known to man. Much of the medicinal use of plants seems to have
 204 developed by trial and error and or through observations of wild animals. Medicinal plants
 205 have been identified traditionally and have been used in the treatment of various diseases.
 206 According to documents the use of the herbs has a long history in order to fertility regulation
 207 (Talukder *et al.*, 2011). Many plants/plants extracts have been used as fertility agents in
 208 folklore and traditional medicines without producing apparent toxic effects (Vasudeva and
 209 Sharma, 2007; Singh and Singh, 2009). On this accounts, many plant derived chemicals that
 210 influence endocrine activities in both humans and animals have received a great deal of
 211 attention due to their possible benefits as well as adverse effects.

212 The preliminary phytochemical analysis showed the presence of alkaloid, flavonoid,
 213 protein, reducing sugar, saponin, terpenoid and cardiac glycoside. The results indicate that the
 214 leaves of these plants posses some biologically active compounds which could serve as
 215 potential sources of drugs. The result of phytochemical screening obtained from this study
 216 has revealed that the plant *Cissus aralioides* contains many bioactive as well as toxic agents
 217 that can affect the regulation of conception and reproduction. Moreover, literature by Okon
 218 and Etim, (2014) shows that presence of saponins, flavonoids, tannins, alkaloids possess anti-
 219 fertility activity. According to Edeoga *et al.*, (2005) and Yakubu *et al.*, (2005) bioactive
 220 components such as; alkaloids and flavonoids have been shown to reduce the regulation of
 221 conception and reproduction. This was also consistent with the findings from previous reports
 222 that indicate the presence of flavonoids, alkaloids (Ravichandra *et al.*, 2007), saponins and

glycosides and terpenoids (Uchendu, 2000) 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 LD₅₀ of more than 5000mg/kg body weight observed in this study is in line with the findings of Kennedy *et al* (1986) 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 LD₅₀ studies; thus, this is an indication that the extract might be safe orally at the stipulated dose.

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 (LD₅₀) 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.

Ethical Disclaimer:

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

Consent: NA

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