



Phytochemical and Toxicological Studies of *Vitex doniana* (Black Plum) Crude Extracts Against Albino Rats

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ABSTRACT

The study was carried out to determine the phytochemical and toxicological studies of *Vitex doniana* extracts against Albino rats. The phytochemical screening of *Vitex doniana* leaf and stem-bark extracts was evaluated using standard qualitative methods. Acute toxicity (LD₅₀) was determined using oral administration methods. The phytochemical screening of all the crude extracts were found to have anthraquinones, flavonoids, glycosides, saponins, steroids, tannins and resins, while resins were absent in crude stem-bark extracts. The acute toxicity (LD₅₀) of the *Vitex doniana* leaf and stem-bark extracts against albino rats was greater than 5000 mg/kg body weight and could be safe for use in human or higher animals. The result has justified their utilization by traditional medicine practitioners for the treatment of diarrhoea and other related ailments due to the presence of bioactive ingredients.

Keywords: Phytochemical screening, Toxicological studies, *Vitex doniana*, Extract, Albino Rat.

INTRODUCTION

Plants that possess therapeutic properties or exert beneficial pharmacological effect on the human body are generally designated as medicinal plants (Melkamu *et al.*, 2018). Therefore, medicinal plant is that plant in which one or more of its organs contain active ingredients which are used for therapeutic purposes or contain active ingredients that can be used for synthesis of drugs (Muhammad *et al.*, 2020).

Medicinal plants are used by traditional medicine practitioners' in the form of decoction, concoction, infusion and tisane. Medicinal plants naturally synthesize and accumulate some secondary metabolites like alkaloids, steroids, tannins, terpenes, flavonoids, saponins, glycosides, cyanogenics, resins, lactones and carotenoids (Melkamu *et al.*, 2018). Plants have an elaborate array of natural products, many of which have antimicrobial activities (Kiyasar *et al.*, 2002).

In developing countries like Nigeria, Cameroon, Sudan, Niger, Republic of Benin and other Africa countries, more than 3.3 billion people use or utilize medicinal plant on regular basis for the treatment of various ailments such as malaria, typhoid fever, hepatitis, cancer, sexually transmitted diseases and others (Melkamu *et al.*,

2018). Moreso, World Health Organization (WHO), estimated that more than 80% of the world population still relies on traditional medicine for their primary health care needs (Sasidharan *et al.*, 2011). In recent years, medicinal plants have represented a primary health source for the pharmaceutical industry (Melkamu *et al.*, 2018). No less than 400 compounds derived from plants were reportedly used in the preparation of drugs such as vincristine and vinblastine used in the treatment of cancer, quinine and atemisinin used as antimalarial drugs (Muhammad *et al.*, 2020). The discovery of modern drugs such as quinine, vincristine, digoxin, digitoxin and artemisinin from medicinal plants signifies the huge potential that still exists for the production of more novel pharmaceuticals (Geyid *et al.*, 2005).

Vitex doniana commonly known as black plum and belongs to the *Verbernaceae* Family. The plant is called *Dinya* (Hausa), *Dinchi* (Nupe), *Orisi* or *Orinla* (Yoruba), *Efifi* (Igala) and *Oliti* (Etsako). It was also reported as a deciduous evergreen tree widely found in the middle belt of Nigeria particularly Niger, Kogi, Benue and parts

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of savannah regions of Kaduna, Sokoto, or Kano State (Mann *et al.*, 2003) (Figure 1).

According to the report of Arokiaraji *et al.* (2009), *Vitex doniana* has numerous applications in traditional medicine: Leaf sap was reported to be used as an eye drop for the treatment of conjunctivitis and other eye complaints. Leaf decoction of the plant is applied externally as a galactagogue and for the treatment of headache, stiffness, measles, body rash, fever and chicken pox and also internally as body tonic and anodyne (Dash *et al.*, 2005). Dash *et al.* (2005), also reported that pastes of pounded leaves and stem-bark are used for the treatment of wounds and burns, while a root decoction of the plant was reported to be administered orally to treat ankylostomiasis, rachitis, gastro-intestinal disorders and jaundice, as well as an anodyne.

Phytochemical constituents are chemical compounds that occur naturally in plants. They are the secondary metabolites (plant by-product) which act as a protective mechanism against

environmental stressors and the more environmental stressors; the more phytochemicals are produced by plants (Muhammad *et al.*, 2020). The study was aimed to determine the phytochemical constituents responsible for the therapeutic potency of the leaf and stem-bark of *Vitex doniana* extracts and also to determine the acute toxicity (LD₅₀) of crude extracts of *Vitex doniana*.

METHODOLOGY

Study Area

The study was carried out in Bida Niger State, Nigeria between July and December, 2022. Bida is the headquarters of Bida local Government. Niger State is located in the central part (middle belt) of Nigeria. The state lies on latitude 08⁰ to 11⁰30 North and longitude 30⁰ to 07⁰40 east. It has a land mass of 76,363km² with a population of 4,082,558. Below are the maps Niger State showing the sampling site (Figure 1).

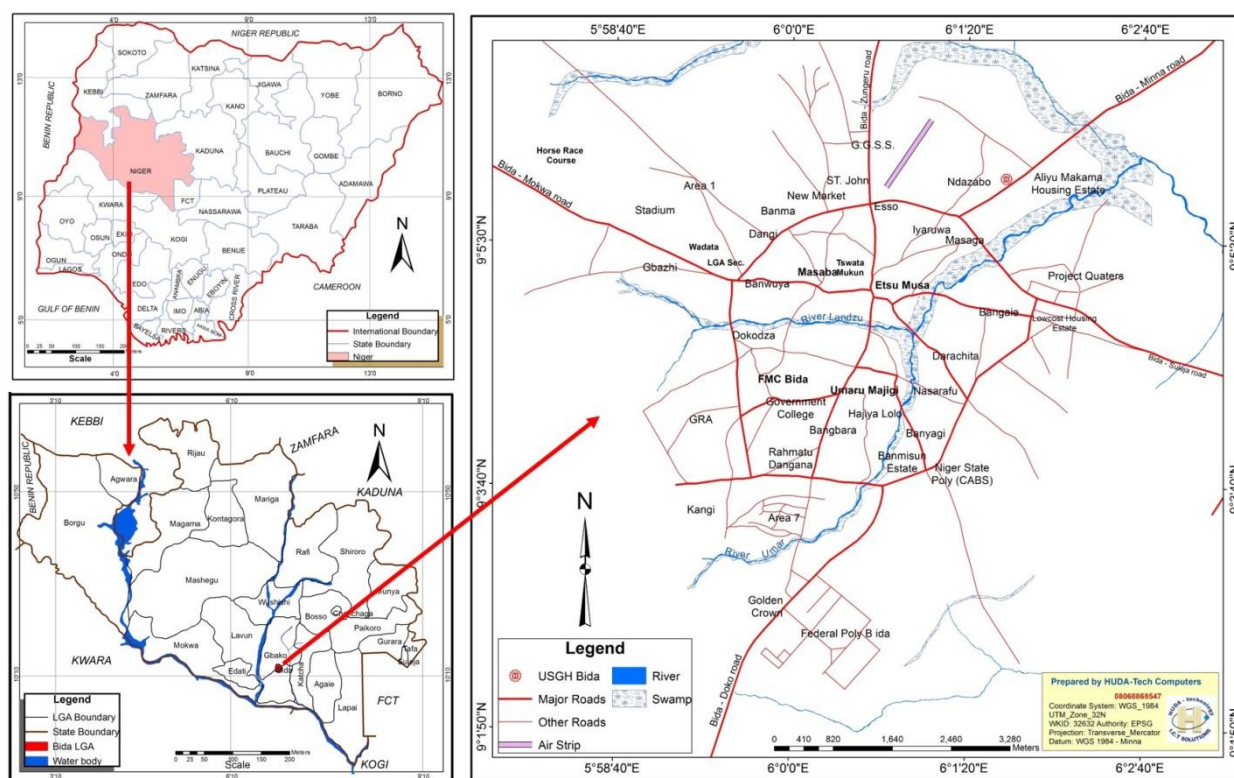


Fig 1: Map of Niger State showing Bida town and the sampling sites

Collection and Identification of Plant Samples

Fresh leaves and stem-bark of *Vitex doniana* were collected within Bida Metropolis and were identified and authenticated at the Department of Biological Sciences, Ibrahim Badamasi Babangida University Lapai, Niger State. A

voucher specimen number VD/0145 was deposited at the Departmental Herbarium. The identification was done based on the literature and Atlas of medicinal plants by Mann *et al.* (2003).

Preparation of Plant Sample

The fresh leaves and stem-bark of *Vitex doniana* were washed with tap water, rinsed with distilled water and then air dry at room temperature (25°C) for two weeks (Muhammad *et al.*, 2020). The dried plant parts were pulverized using sterile laboratory mortar and pestle to obtain the plant powder. Mesh sieve of 0.05 mm was used in sieving the pounded powder to obtain a fine powder. The fine powders were stored in airtight sterile bottles at ambient temperature (30°C) until required for further analysis (Hassan *et al.*, 2005).

Extraction of Pulverized Plant Sample

Extraction of plant samples was carried out using Soxhlet extractor. Acetone, water, hexane and methanol were used as solvents of extraction. Methanol being a polar solvent has capacity of extracting hydrophilic compounds, while hexane (non-polar) has capacity of extracting lipophilic compounds.

Twenty-five grams (25 g) of the powdered leaves of *Vitex doniana* were weighed and wrapped in a plain paper and placed in a Soxhlet extractor and extracted with 250 mL of water, acetone, hexane and methanol respectively for the period of 16 hours. The filtrate was transferred into a Porcelain dish and then allowed to dry. The extract obtained was labelled, weighed and kept for further analysis. The same procedure was followed for stem-bark sample (Hassan *et al.*, 2005).

Qualitative Preliminary Phytochemical Screening of Plant Extracts

The crude aqueous, acetone, hexane and methanolic extracts were subjected to phytochemical screening for alkaloids, anthraquinones, flavonoids, glycosides, resin, saponins, steroid and tannins.

Test for alkaloids

To 3 mL of extract, 1 mL of 1% of HCl was added. The mixture was treated with two drops of Meyer's reagent. A creamy white precipitate indicated the presence of alkaloids. Absence of white precipitate indicated negative result (Ogukwe *et al.*, 2004).

Test for anthraquinones

To 4 mL of extract, 4 mL of 100% ammonia solution was added. Pink violet or red colour in the ammoniacal layer (lower layer) indicated the presence of anthraquinones, while yellow colour indicated negative result (Danlami, 2009).

Test for flavonoids

To 1 mL of extract, 3 drops of ammonia solution was added. Half (0.5) mL of conc. HCl was further added to the mixture. A pale brown coloration indicated the presence of flavonoids, while white color indicated a negative result (Odebiyi *et al.*, 2012).

Test for glycosides

To 1 mL of the extract, 2 mL of acetic acid was added and then cooled in an ice bath at 4°C. To the mixture, 1 mL of conc. H₂SO₄ was added drop wise. Oil layer formed on top of the solution indicated the presence of glycosides, while absence of oil layer formation indicated a negative result (Ogukwe *et al.*, 2004; Odebiyi *et al.*, 2012).

Test for resins

To 5 mL of extract, 5 mL of copper acetate solution was added. The mixture was shaken vigorously and then allowed to separate. A reddish brown precipitate indicated the presence of resins, while a pale blue indicated a negative result (El- Mahmood *et al.*, 2009).

Test for saponins

To 2 mL of the extract, 5 drops of olive oil was added. The mixture was vigorously shaken, stable emulsion forms in the extract indicated the presence of saponins, while absence of emulsion form indicated a negative result (Hassan *et al.*, 2005).

Test for steroids

To 1 mL of extract, 1mL of conc.H₂SO₄ was added. A red coloration indicated the presence of steroids, while a yellow colour indicated a negative result (Hassan *et al.*, 2005).

Test for tannins

Two drops of 5% FeCl₃ was added to 1mL of extract and a dirty green precipitate indicated presence of tannins, while absence of green precipitate indicated a negative result (Ogukwe *et al.*, 2004).

Experimental Animals (Albino Rats)

Twelve (12), three months male Wistar albino rats with an average body weight (190–225g) were used for this study. They were obtained from the Animal House Unit of the Department of Biochemistry, Ibrahim Badamasi Babangida University Lapai, Niger State. They were housed in a polypropylene cage and maintained at 30–37°C for 12 hours' dark/light cycle. The animals were acclimatized to laboratory condition for 7

days prior to experiment. They were fed with standard mash and water ad-libitum. For hygienic purposes, the litter to the cage was cleaned twice a week.

Acute Toxicity Studies (Determination of LD₅₀)

The method of Hassan *et al.* (2007) and Dawang (2015) were used for an acute toxicity study of the plant extracts. Water (aqueous) extract of *Vitex doniana* leaf (1000, 2000, 3000, 4000 and 5000 mg/kg) was administered to two (2) groups (six Wister albino rat per group) of rats (one after the other at a grace observation of 48 hours) in single oral dose by using a feeding needle. The control rat received distilled water. The same procedure was followed with aqueous *Vitex doniana* stem-bark extracts respectively.

Observations of toxic symptoms were made and recorded systematically at one, two and six hours after administration. The number of survivors was noted after 48 hours for each animal (rat). The toxicological effect was assessed on the basis of mortality, which was expressed as LD₅₀ and was calculated using the fixed dose.

Ethical Clearance

Ethical approval was obtained from research animal ethic care and uses committee of Ibrahim Badamasi Babangida University, Lapai before the commencement of experimental study.

RESULTS

The result of preliminary phytochemical screening of *Vitex doniana* extracts are presented in Table 1. The *Vitex doniana* crude aqueous leaf extracts indicated the presence of anthraquinones, saponins and steroids. Hexane leaf extract contained alkaloids, anthraquinones, resins, saponins and steroids, while methanol leaf extract indicated the presence of anthraquinones, glycosides, saponins, tannins and steroids.

As for the stem-bark extract, the acetone stem-bark contained anthraquinones, flavonoids, glycosides, saponins and tannins, the aqueous stem-bark contained all phytochemicals tested except resins. The hexane stem-bark extract contained only anthraquinones and tannins, while the methanol stem-bark extract contained all phytochemical constituents tested except steroids and saponins.

The results of an acute toxicity against albino are presented in Table 2. The acute toxicity test of *Vitex doniana* leaf and stem-bark extracts showed no toxic effect at 1000-4000 mg/kg but exhibited behavioural change like slow movement indicating weakness of the body at 5000 mg/kg.

Table 1: Phytochemical Constituents of Leaf and Stem-Bark Crude Extracts of *Vitex doniana*.

Extract	Phytochemical constituents							
	Alkaloids	Anthraquinones	Flavonoids	Glycosides	Resins	Saponins	Steroids	
VDALF	-	+	-	-	-	+	+	-
VDQLE	-	-	+	-	-	+	+	-
VDHLE	+	-	-	-	+	+	+	-
VDMLE	-	+	-	+	-	+	+	+
VDASBE	-	+	+	+	-	+	-	+
VDQSBE	+	+	+	+	-	+	+	+
VDHSBE	-	+	-	-	-	-	-	+
VDMSE	+	+	+	+	+	+	-	+

Key: VDALE=*Vitex doniana* acetone leaf extract; VDASBF=*Vitex doniana* acetone stem-bark extract; VDHLE=*Vitex doniana* hexane leaf extract; VDHSBE=*Vitex doniana* hexane stem-bark extract; VDMLF=*Vitex doniana* methanol leaf extract; VDMSE=*Vitex doniana* methanol stem-bark extract; VDQLF=*Vitex doniana* aqueous leaf extract; VDQSBE=*Vitex doniana* aqueous stem-bark extract; + = Present; - = absent.

Table 2: Acute Toxicity (LD₅₀) of Crude Aqueous Leaf and Stem-Bark Extracts of *Vitex doniana* against Albino Rat.

Plant extract	Group	Albino Rat	Weight of animals (g)	Dosage (mg/kg)	Vol. of extract administered (g/mL)	Mortality
Leaf	Grp A	A1	190	1000	0.2	None
		A2	195	2000	0.4	“
		A3	198	3000	0.6	“
		A4	195	4000	0.8	“
		A5	200	5000	1.0	“
		Control	225	0.00	1.0 mL dw	“
Stem-bark	Grp B	B1	198	1000	0.2	None
		B2	200	2000	0.4	“
		B3	205	3000	0.6	“
		B4	215	4000	0.9	“
		B5	220	5000	1.1	“
		Control	225	0.00	1.0mL dw	“

Key: LD₅₀=Median Lethal Dose; dw=Distilled water; g=gram; mL=milliliter; kg=Kilogram mg=Milligram; vol=Volume

DISCUSSION

The phytochemical screening of *Vitex doniana* leaf extracts revealed the presence of anthraquinones, steroids and saponins. This could be attributed to the season of plant collection and also due to phytochemicals shift. Also, trace elements of alkaloids, flavonoids, glycosides and resins observed may be attributed to different extractants used for extraction. This disagrees with the findings of Dauda *et al.* (2011) who reported the presence of cardiac glycosides, flavonoids, anthraquinones, steroids and resin in their studies on phytochemical and *in-vitro* antibacterial investigation of *Vitex doniana* leaf, stem-bark and root extracts. This also disagrees with the findings of Agbonlahor and Nwachukwu (2011), who reported the presence of anthraquinones, saponins, flavonoids and terpenoids and tannins in their studies of phytochemical analysis and antioxidant properties of leaf extracts of *Vitex doniana* and *Mucuna pruriens*. The findings are as well contrary to the report of Ferguson (2001) who documented the presence of resins, tannins and saponins in his study on phytochemical and antimicrobial assessment of hexane leaf extract of *Vitex doniana*. This could be attributed to hexane used as extractants which is capable of extracting lipophilic compounds only. The finding however agrees with that of Dawang (2015) who reported the presence of flavonoids, cardiac glycosides, steroids and resin in his studies of constituents and toxicological analysis of *Vitex doniana* leaf extracts.

This study revealed the presence of anthraquinones, tannins, flavonoids and glycosides in the stem-bark extracts of *Vitex doniana*. The reason may be attributed to the period of plant collection. This report is similar to the findings of Emmanuel *et al.* (2015) who reported the presence of anthraquinones, tannins, flavonoids, glycosides and alkaloids in their studies on phytochemical and antimicrobial screening of the stem-bark extracts of *Vitex doniana*. It also agrees with the findings of Ali and Ibrahim (2017), who reported the presence of anthraquinones, tannins, glycosides and phenol, in their studies on the assessment of antibacterial activity and phytochemical screening of *Vitex doniana* extracts on clinical isolates of *Salmonella* Typhi.

However, this is contrary to the findings of Olusola *et al.* (1997) who reported the presence of alkaloids, anthraquinones, saponins, tannins, resins and glycosides in their studies on the effect of *Vitex doniana* stem-bark extract on blood pressure. This could be attributed to ethyl acetate used as an extractants, being polar aprotic solvent which is capable of extracting both hydrophilic and lipophilic compounds.

The result of an acute toxicity test (LD₅₀) of *Vitex doniana* extracts on the albino rats in the present study indicated that all albino rats tested survived at 5000 mg/kg body weight. No mortality was recorded and one of the toxicological indices accepted for the determination of the safety of

drug/substances/extract is lethal dose of 50% (LD₅₀), which is the amount of acute dose required to kill half of the test population (Dawang, 2015). However, since there was no death recorded during acute toxicity experiment by oral administration, then it is evidence that the median lethal dose (LD₅₀) is greater than 5000 mg/kg weight (Assob *et al.*, 2011). This implies that *Vitex doniana* extract is safe (non-toxic). The result in this study agrees with the finding of Dawang (2015) who reported that all albino rats tested against *Vitex doniana* ethanol leaf extract survived at 5000 mg/kg. This is also in conformity with the findings of Abdulrahman *et al.* (2007).

Conclusion

All the crude extracts of the plant were found to have anthraquinones, flavonoids, glycosides, saponins, steroids, tannins and resins except resin which was not detected in the stem-bark extract. The lethal dose (LD₅₀) of the plant extracts against albino rats was greater than 5000 mg/kg, indicating that the plant is safe for consumption.

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