

## Phytochemical Constituents and Toxicological Study of Vitex Doniana Leaf

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**Abstract:** The continuous emergence of multidrug resistance by microorganisms to antibiotics has led to increased use of medicinal plants. Therefore it is needful to ascertain the presence of the phytochemical constituents and toxicity of Vitex doniana leaf. The phytochemical screening was performed using standard qualitative procedure. The acute toxicity effect of ethyl acetate Vitex doniana leaf extract was determined by Lorke's method. The experiments involved oral administration of different doses of the plant extract on mice per body weight. The leaf extract of V. doniana contained alkaloids, flavonoids, cardiac glycosides, terpenes, steroids and resins. The presence of these secondary metabolites indicates the medicinal importance of the leaf extract of V. doniana. The acute toxicity of ethyl acetate V. doniana leaf showed LD<sub>50</sub> to be greater than 5000mg/kg body weight and there was no significant weight increase ( $P \geq 0.05$ ) in both body weight and organs of the test mice. This suggests that there is safety in the use of V. doniana leaf extract in treatment of infections.

**Key words:** Vitex doniana, phytochemical constituents, toxicity

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### I. Introduction

There has been increasing challenges with the use of synthetic drugs [1] partly because microorganisms continue to exhibit resistance against these drugs and also the cost of most effective synthesised drugs the third generation antibiotics are not within the reach of the poor populace. Thus, there has been gradual shift from these synthetic drugs to plants products of medicinal value.

In spite the importance of medicinal plants, there is need for toxicological test to determine their safety. However, most of the concoctions of the medicinal plant products are not screened for their safety consumption and to worsen the situation overdose is a common phenomenon.

The plant Vitex doniana is commonly called black plum and belongs to the family Lamiceae. It is called "ladeep" in Pan, "dinya" (Hausa), "ucha koro" (Igbo) and "cori-nla" in Yoruba. The genus Vitex has 250 species [2]. V. doniana has been reported to be used in treatment of various diseases [3]. The authors further quoted the work done on the V. doniana, such as hot aqueous extracts of the leaves being used in the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhea and dysentery [4,5]. The roots and leaves are also used in curing nausea, colic and epilepsy [6,7]. In some parts of this nation (Nigeria) the ripe fruits are eaten and foliage leaves are used as salad and in compounding local spice in Pan – land.

The use of V. doniana stem bark extract in treating hypertension has also been reported [8]. The extracts of the stem bark have also shown effectiveness in trypanocidal activity against Trypanosoma brucei in vitro [9]. Again, aqueous methanol extract has been documented to demonstrate anti – diarrhoeal activity [10]. The bark extract of V. doniana has been used in treating stomach complaints and kidney troubles [11]. Locally, dried and fresh fruits are eaten against diarrhea and as source of vitamin A and B [12, 13]. Thus, the aim of this present study was to identify the secondary metabolites present and the toxicity of the V. doniana leaf so as to encourage its usage.

### II. Methodology

#### 2.1 Collection and Identification of Plant Samples

The plants Vitex doniana was collected from Doemak in Quaan-Pan L.G.C of Plateau State and confirmed in the herbarium of the Federal College of Forestry, Jos, Plateau State.

#### Ethyl Acetate Cold Extraction of Plant Extracts

The procedure of Ndip et al., [14] was used for the extraction of the plant extract with slight modification by using the water bath instead of rotorvapor to concentrate the plant extract. The leaves of V. doniana plant was room dried to prevent the ultra violet rays from inactivating the chemical constituents. The dried leaves were then pulverized in a mortar using pestle.

Analytical grade 99.5% ethyl acetate was used for the cold extraction. Pulverized V. doniana (527g) was macerated in the solvent in extraction bottle with the level of the solvent above that of the plant material and allowed to stay for 2-3 days. The liquid was then removed and filtered through Whatman No.1 filter paper. The extraction solvent was again added to the re plant extract and the same process repeated until exhausted cold extraction was reached. The filtrate from the mixture was then concentrated over thermostatic water

Cabinet (model HH-W420, XMTD-204 and TT42D Multipurpose use. Techmel & Techmel, USA) at 100°C. The plant extract recovered was transferred into beaker and allowed to stand on the bench to allow for total evaporation of the residual solvent. The pulverized *V. doniana* yielded 34g extract. The dried plant extract was preserved until required for use.

## **2.2 Screening of phytochemical components of crude extracts**

The phytochemical screening of the ethyl acetate extract of *Vitex doniana* extract was carried out using standard qualitative procedure [15,16]. The crude extract of the plant was subjected to various tests as stated below to indicate the presence or absence of the various phytochemical constituents.

### **2.2.1 Test for alkaloids**

Few drops of the Dragendorff reagent were added to 2.0ml of extract and observed for orange colouration to indicate that alkaloid is present [16].

Another few drops of Wagner reagent were added to 2.0ml of extract and observed for the formation of deep brown precipitate to indicate the presence of alkaloid [16] as a complimentary test.

**2.2.2 Test for flavonoids** Two ml of extract was added to 10% lead acetate solution and observed for either cream or light yellow coloration confirming the presence of flavonoid [16].

### **2.2.3 Test for tannins**

One ml of extract was diluted with 4.0ml water (in a ratio of 1:4) and few drops of 10% ferric chloride solution were then added. The mixture was observed for blue or green precipitate or coloration which would indicate the presence of tannins [15].

### **2.2.4 Test for cardiac glycosides**

Two ml of conc. H<sub>2</sub>SO<sub>4</sub> was carefully added down the side of the tube containing 2.0ml of the extract and observing for the formation of a layer of interphase of reddish brown colour indicative of cardiac glycoside (Salkowski Test) as described by Sofowora [16].

### **2.2.5 Test for saponins**

One ml of extract was added to 4.0ml of distilled water in a test tube and the tube was stoppered and shaken vigorously for about 30 seconds and was then allowed to stand for half an hour. A honey comb-froth formation was an indication of the presence of saponins [16].

### **2.2.6 Test for terpenes and steroids**

Two ml of the extract was mixed with 1.0 ml acetic anhydride followed by the addition of 1.0ml conc. H<sub>2</sub>SO<sub>4</sub> carefully down the side of the test tube while observing for the formation of interphase layer of reddish brown colour which showed the presence of terpenes and steroids [16].

### **2.2.7 Test for phenols**

Few drops of chloroform and 2.0ml of FeCl<sub>3</sub> were added to 2.0ml of extract, a deep bluish green colouration indicated the presence of phenol [16].

### **2.2.8 Test for resin**

Small amount of the plant extract was dissolved in acetic anhydride and 1 drop of conc. sulphuric acid was added. Development of a purple or violet colour indicated presence of resin [17].

### **2.2.9 Test for balsam**

Three drops of alcoholic ferric chloride was added to 2.0ml of the extract, and then warmed. Formation of a dark green colouration formed showed the presence of balsam [15].

## **2.3 Toxicological Analysis of V. doniana Extract**

The toxicity of the *V. doniana* leaf extract was determined using standard method of Lorke [18]. White albino mice (*Mus sp*) of both sexes weighing between 16.8g – 26.7g were used for this work. They were purchased from a colony of mice kept at the animal house of the University of Jos and treated according to animal ethics. The first batch of the experiment had 11 mice divided into 5 groups with first three groups consisting of 3 mice each while fourth and fifth group (control) has one mouse each. The *V. doniana* extract was dissolved in dimethylsulphoxide. Group 1, 2 and 3 were administered orally with 10 mg, 100 mg and 1000 mg of *V. doniana* extract respectively and per body weight of the mouse. The group 4 and 5 were administered

dimethylsulphoxide and water respectively. The mice were observed for 48 hours and then sacrificed and the weights of their livers and kidneys were taken.

The second batch of the experiment which was done after the first batch consisted of 4 groups with one mouse each. Group 1, 2, 3, and 4 were given 1500mg, 2500 mg, 3500 mg and 5000 mg of the plant extract respectively and according body weight. They were then observed for number of death.

### 2.4 Statistical Analysis

The student t-test was used to ascertain the significant difference between mean body weight of mice and the control and also between the mean weight of organs and that of the control

## III. Results

The phytochemical screening of the extract of the Vitex doniana leaf showed the presence of alkaloids, flavonoids, cardiac glycosides, terpenes and steroids and resins but tannins, saponins, balsam and phenols were absent (Table 1). The acute toxicity test showed that the weight of the mice increased with the increased concentration of the Ethyl acetate Vitex doniana extract however not significantly different ( $P \geq 0.05$ ) (Table 2). No death was recorded even at the highest dose of 500mg/kg (Table 4). Also, there was no significant increase ( $P \geq 0.05$ ) in the weight of the organs used for all the doses used as compared with the control (Table 3). The DMSO which was the solvent in which the plant extract was dissolved showed no adverse effect on the mice (Table 2, 3 and 4).

**Table 1. Phytochemical Constituents of Ethyl Acetate Leaf Extract of Vitex doniana**

Chemical Components	Vitex doniana
Alkaloids	+
Flavonoids	+
Tannins	-
Saponins	-
Cardiac glycosides	+
Terpenes & Steroids	+
Balsam	-
Phenols	-
Resins	+

### Key

- + = Positive/present
- = Negative/Absent

**Table 2. Effect of Ethylacetate Vitex doniana Extract on Weight of Mice**

	Dose (mg/kg b.w)	Weight(g) before	Weight (g) after 48hrs
<b>Phase 1</b>	10	18.85	16.85 ± 4.08
	100	17.70	17.6 ± 3.35
	1000	23.77	25.0 ± 0.90
	DMSO	22.4	25.1
	Control	20.2	22.90
<b>Phase 2</b>		<b>Before</b>	<b>After 24hrs of Administration</b>
	1500	21.60	23.50
	2500	22.70	23.40
	3500	22.70	23.90
	5000	20.40	21.90

Values are express as mean ± S.D

**Table 3. Effect of Ethyl acetate Vitex doniana Extract on liver and Kidney of Mice**

Weight of Organ (g)			
Dose (mg/kg b.w)	Liver	Left kidney	Right kidney
10	1.32 ± 0.05	0.17 ± 0.01	0.19 ± 0.01
100	1.05 ± 0.15	0.15. ± 0.04	0.13 ± 0;01
1000	1.48 ± 0.99	0.18 ± 0.00	0.19 ± 0.03
DMSO	1.53	0.16	0.16
Control	1.09	0.14	0.19

Values are express as mean ± S.D

**Table 4. Effect of Median Lethal Dose of Ethyl acetate Vitex doniana Extract on Mice**

Dose (mg/kg b.w)	Number of Animals Per Group	Lethality	Survival	% Lethality
10	3	0/3	3/3	0
100	3	0/3	3/3	0
1000	3	0/3	3/3	0
1500	1	0/1	1/1	0
2500	1	0/1	1/1	0
3500	1	0/1	1/1	0
5000	1	0/1	1/1	0
DMSO	1	0/1	1/1	0

#### IV. Discussion

The presence of alkaloids, flavonoids, cardiac glycosides and steroids, but absence of saponins agrees with the finding of Dauda [3] who reported the presence of these bioactive compounds in *V. doniana* extracts. Generally, the antimicrobial activity of plant extracts is attributed to the presence of various secondary metabolites (bioactive compounds) like saponins, tannins, flavonoids, phenolic compounds and essential oil [19].

The antimicrobial activity of the *V. doniana* could also be due to contributory effect from alkaloids, flavonoids, steroids and tannins. This assertion is in line with those of earlier researchers who reported the use of alkaloids in medicine and flavonoids exhibiting cytotoxic, antifungal and anti-inflammatory activities [20, 21].

Also, flavonoids and steroids are said to show affinity for hydroxyl radicals and superoxides anion radicals, as a result, plants with these constituents help in improving health [22]. Antibacterial potentials of naturally occurring flavonoids and polyphenolic compounds have also been reported [23,24,25]. Nyenje and Ndip [26] have also documented antimicrobial activity of plant extracts due to the presence of hydrolysable tannins. These tannins are generally believed to be non-selective enzyme inhibitors in microorganisms as a result of the tannins polyphenolic groups. Earlier authors further explained that tannins are known antimicrobial agents that could prevent the growth of microorganisms by precipitating the microbial protein thus deprive them of the proteins needed for their growth and development (27). In addition, it has been suggested that antidiarrhoeic activity may be associated with the property of phenolics [28].

Medicinal plants are widely used in West Africa and some parts of Nigeria but most of them are without toxicological analyses data. One danger of using plants as alternative remedies is that most of the time, definite doses are not prescribed thus resulting in overdose [29]. The progressive increase in weight of the mice though not significant could be as result of the nutritive value of the *V. doniana* leaf extract which has been reported elsewhere that the *V. doniana* young leaves are highly rich in nutrients [30]. It had been reported that the absolute organ weight has been considered as a relative sensitive indicator of nephrotoxicants and therefore defined nephrotoxicity as increased kidney weights [31]. Thus, the ethyl acetate *V. doniana* exerted no toxic effect on the kidneys and livers since there was no significant difference ( $P \geq 0.05$ ) between the weights of the organs and that of control. One of the toxicological indices accepted for determination of the safety of drugs is lethal dose 50% ( $LD_{50}$ ) which is the amount of acute dose of drug required to kill half of the test population. Since there was no death recorded during acute toxicity experiment by oral administration it is evident that the median lethal dose  $LD_{50}$  greater than 5000mg/kg body weight [32]. This then means that *V. doniana* leaf extract is assumed to be safe (non-toxic) as suggested by Lorke [18]. Additionally the absence of death among the treated mice at all does strongly support the claim [33]. Again, these reporters [33] recommended that young leaves of *V. doniana* are good for human consumption for the sustenance of health and vitality. However, it is unlike its bark stem which was reported to be moderately toxic via intraperitoneal administration [34].

In conclusion, the non-toxicity of *Vitex doniana* leaf and the presence of various bioactive compounds may present *V. doniana* as source of raw material in drug formulation.

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