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Phytochemical And Cytotoxicity Study Of *Vitex doniana* Stem Bark And Leaves

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ABSTRACT
It is obvious that plants and herbs contain a variety of phyto-chemicals that have healing properties and a majority of modern medicine are derived from plants. The high cost and substandard production of the modern medicine is driving peasant people away from modern medicine and leading them to patronize the traditional medicine more. However the scientific facts on the phytochemicals and safety of some of the plant product are still not enough. On these bases, this study aimed to assess the phytochemicals and cytotoxicity of *Vitex doniana* stem bark and leaves. The stem bark and the leaves were extracted using the maceration method with methanol and analyzed using standard phytochemical screening method. The extracts were also assessed for their cytotoxicity using the brine shrimp lethality bioassay. The study reveals the presence of alkaloids, flavonoids, glycosides, saponins, tannins, phenols, terpenoids and anthraquinones in the stem bark and of alkaloids, flavonoids, saponins, tannins, phenols, and anthraquinones. The cytotoxicity assay shows an LC50 of 175 µg/ml and 260 µg/ml for the stem bark and the leaves extracts respectively. The findings implies that the plant, vitex doniana contain variety of phytochemicals and also possess cytotoxicity property. This justify the claims bt traditional medicine practitioner that the plant can be used to cure some ailments as well as an antitumor based on the cytotoxic activity revealed.
Keywords: Phytochemicals, Cytotoxicity, *Vitex doniana*, Extraction, Maceration

INTRODUCTION
Plants have been playing a vital role in treatment of various ailment since time immemorial. Most the present modern medicine are all of plant origin. Sometimes, some modern medicines are said to be not effective on some diseases however this may be attributed to standard of the medicine in question. Now a days there are a lots of medicine preparation which are much substandard and hardly their production companies can be traced. It is because of this reason among others like high cost the modern medicine, traditional medicine is getting more affiliation (Agbafor and Nwachukwu, 2011) and also the emergence of bacterial antimicrobial resistance has made the choice of empirical therapy more difficult and expensive. As result, regular screening of causative organism and determination of susceptibility pattern of such organisms to commonly used antibiotics is needed for empirical treatment of infections (Gambo, *et al.,* 2018)
Vitex doniana is not left out of the plants that are used as medicinal plant in many communities in Nigeria, Africa and the world at large (Bangou, *et al.,* 2019). *Vitex doniana* which a time are referred to black plum is called Dinya in hausa. *Vitex doniana* is a deciduous tree commonly consumed as a delicacy, medicine or spice (Kolawale, *et al.,* 2020). It is much widespread in the areas of savannah it produces

black sweet fruits which is consumed. *Vitex doniana* have been used to treat a range of human ailments, particularly related to insects, fungi, bacteria, and diseases associated with menstruation and gynecological problems (Bangou, *et al.,* 2019). In Nigeria *Vitex domiana* have been used in the management and treatment of several disorders which include rheumatism, hypertension, diarrhea, jaundice, anemia, mental illness, dysentery, gastroenteritis cancer, wound infection and inflammatory diseases (Gambo, *et al.,* 2018 and Abdulrahman, *et al.,* 2007). However, the information is lacking on the application of *Vitex* genus in human clinical trials, toxicology and safety, marketed products, and patents (Nurkhalida, 2022).

The use of the medicinal plants in many cases have not been backed by scientific proves, therefore it is against this the present study aimed at investigating the biochemical and cytotoxicity study of *Vitex doniana* leaves and stem bark

MATERIAL AND METHODS

Collection of Sample and Preparation

Fresh stem bark plus the leaves were harvested and identified in the herbarium unit, of the Department Biological Science, Gombe State University. After the identification the stem bark was collect in more quantity and dried under shade. The dried sample was the crushed in to powder using mortar and pestle.

Extraction

The crushed stem bark of the *Vitex doniana* was extracted with ethanol using maceration method as described by James, *et al.,* (2014) with slight modification. 150 g of the each of the crushed sample were placed separately in 500 ml sterilized conical flask. To each of the flasks, 300 ml of ethanol was added, shake and covered with cork. These were kept at ambient temperature for 72 hours (3 days and 3 nights). After 72 hours, the content of the flask were filtered and the filtrates were concentrated using rotary evaporator and dried in a desiccator.

Phytochemical screening of the cured extracts

Phytochemical screening was carried out as described by Soforowa (1984) and Harborne (1973). This was aimed to determine the presence of alkaloid, tannin, saponin, steroid, terpenoid, anthraquinone, flavonoid, glycoside, and reducing sugar in the methanol extracts of the stem bark and leaves of *Vitex doniana*.

Test for alkaloids

About 0.5 g of each extract was stirred with 5 ml of 1 percent aqueous hydrochloric acid on water bath and filtered. 1 ml of the filtrate was treated with a few drops of Mayer's reagent and second 1 ml portion was treated with Dragendorff's reagent. Turbidity or precipitation with either of these reagents indicates the presence of alkaloids. To 1 ml third portion of the filtrate, 3 drops of Wagner's reagent (Iodine in Potassium iodide) was added. Formation of reddish brown precipitate indicates the presence of Alkaloids.

Test for flavonoids

Lead acetate test: Extracts were treated with few drops of lead acetate solution. The formation of yellow colored precipitate indicates the presence of flavonoids.

Test for glycosides

To about 1 ml of the extract, about 10 ml of 50% Tetraoxosulphate (VI) acid was added in a test tube and the mixture was heated gently for 15 minutes followed by addition of 10ml of Fehling solution and boiling. Formation of brick red precipitate indicated the presence of glycosides.

Test for Cardiac glycoside

Salkwowski test: 0.5 g of each of the extracts was dissolved in 2 ml of chloroform. Tetraoxosulphate (iv) acid was carefully added to for a lower layer. A redish-brown colour at the interface indicates the presence of steroidal ring (aglycone portion of the cardiac glycoside).

Test for saponins

The ability of saponins to produce frothing in aqueous solution and to haemolyse red blood cells used to test for the saponins. About 0.5 g of each of the powdered sample was dispensed in distilled water in a

test tube and shaken vigorously. Persistent froth (foam) that lasted for about 10 minutes on warming indicated the presence of saponin.

Test for steroids

To 2ml of the sample, about 2 ml of acetic acid was added and the solution was kept under ice for cooling for few minutes. Then 2 ml of concentrated Tetraoxosulphate (VI) acid was added carefully. Color changes, from violet to blue/bluish green indicated the presence of steroids.

Test for tannin

Gelatin test: To 2ml of the extract, 1% gelatin solution containing sodium chloride was added. The formation of white precipitate indicated the presence of tannins.

Test for phenol

Ferric chloride test: Extracts were treated with 5 drops of ferric chloride solution. The formation of bluish black color indicated the presence of phenols.

Test for terpenoid

Salkowski test: About 5ml of extract was added with 2ml of chloroform and 3ml of concentrated Tetraoxosulphate (VI) acid. Reddish brown colour at the interface indicates the presence of terpenoids.

Test for Anthraquinones

Borntrager's test. About 2ml of extract was added into a test tube, 5ml of benzene was added and shaken, then 5ml of 10% Ammonia solution was also added followed by shaking. The formation of pink/red/violet color in the lower phase is positive for Anthraquinone.

For combined anthraquinones, 5 g of each of the extracts was boiled with 10 ml aqueous tetraoxosulphate (iv) acid and filtered while hot. The filtrate was shaken with 5 ml benzene and benzene layer separates. To few drops of the benzene layer, ammonia solution was added. A pink, red or violet colouration in the ammonia phase indicates the presence of anthraquinones.

Cytotoxicity Using Brine Shrimp

The Brine Shrimp Lethality Test (BSLT) is a protocol that is used to detect a wide spectrum of bioactivity (cytotoxic activity) in plants crude extracts. Brine shrimp lethality bioassay is a simple, inexpensive bioassay used for testing the efficacy of phytochemical present in the plant extracts. It is based on the killing ability of test compounds on a simple zoological organism-brine shrimp.

The methods described by Kabubiia, *et al.,* (2015), Kamanja, *et al.,* (2018) and Gunda, *et al.,* (2016) were adopted with some modifications.

Hatching of Brine shrimp nauplii.

Thirty five grams of salt is weighed into a conical flask using electronic balance. Distilled water was added with continues stirring to ensure complete dissolution of the salt. The volume of the solution was then made to one litre. The salinity of the salt solution was also adjusted to 33 ppt. The saline solution was then placed in a rectangular box which was partitioned into two chambers. One chamber was dark while the other was illuminated by a 40 watt electric bulb. 50 grams of brine shrimp eggs were sprinkled with a spatula into the box. Five grams of yeast was added that was the feed for the naupli. After 48 hours the nauplii which are attracted by light were collected from the illuminated chamber and used for the brine shrimp lethality test.

Brine Shrimps Lethality Assay.

5 milligrams of each of the extracts was separately dissolved in 1000 ml DMSO. The extract solutions were then prepared in various concentrations of 5 µg/ml, 10 µg/ml, 25 µg/ml. 50 µg/ml, 100 µg/ml and 200 µg/ml and 300 µg/ml. 1 ml of the each of the various concentrations were introduced in labelled vial and drops of saline water containing the live brine shrimps were drown by means of Pasture pipette then introduced into a clean sterilized vials and the solutions were made to 5ml with brine. The brine shrimp nauplii were then counted macroscopically under lighted background. A drop of dry yeast suspension was added as food to each vial. All the vials were incubated and maintained under illumination. The some nauplii were also added to the same volume of the artificial sea water and DMSO to serve as a negative control. The surviving nauplii were counted with the aid of a 3x magnifying glass after 6, 12, and 24

hours. The exercise was carried in triplicate. The mean mortality at the six dose levels for each extract was determined.

The Percentage mortality (M %) was calculated by dividing the number of dead nauplii by the total number, and then multiply by 100%.

$$\text{Percentage of Death} = \frac{\text{Total nauphlii} - \text{Alive nauphlii}}{\textit{Total nauphlii}} \; x\ 100$$

LC50 values were obtained from the graph plotted of the percentage mortality against concentration of the crude extracts by determining the concentration at which 50% of the brine shrimp were dead.

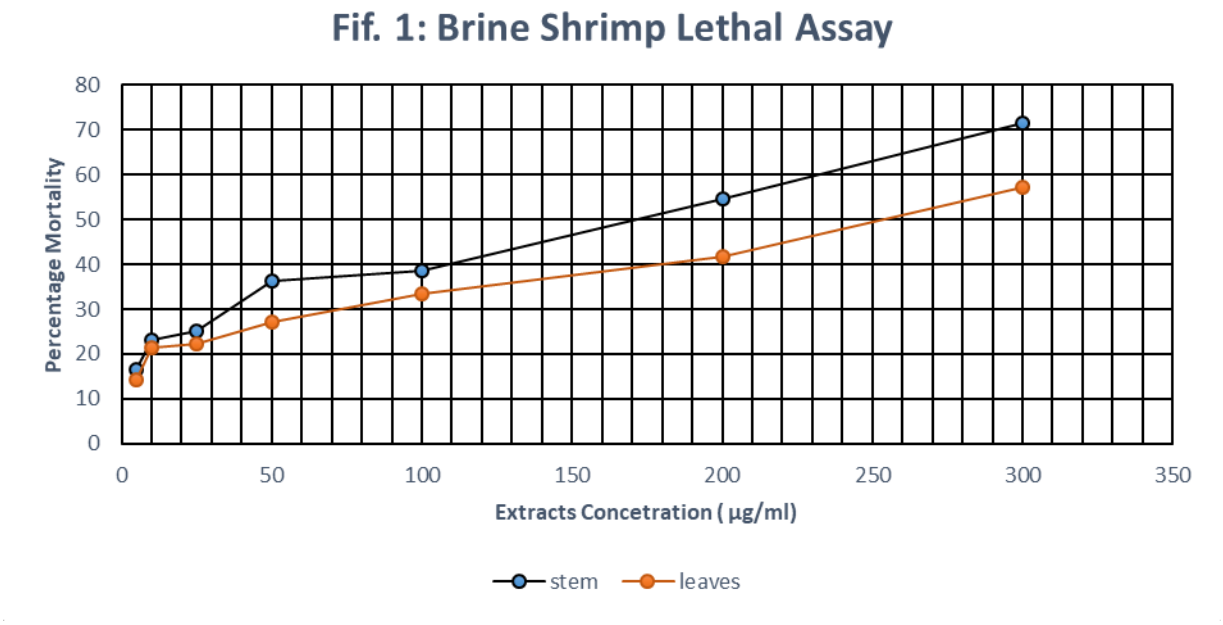
RESULTS AND DISCUSSION

The methanol extracts of *Vitex doniana* stem bark and leaves subjected to phytochemical screening revealed that, the stem bark contain alkaloids, flavonoids, glycosides, saponins, tannins, phenols, terpenoids and anthraquinones. While the leaves revealed the presence of alkaloids, flavonoids, saponins, tannins, phenols, and anthraquinones. This findings is in agreement with Ushie *et al.,* (2022) and Nurkhalida *et al.,* (2022). The phytochemicals and other chemical constituents of medicinal plants are claimed by many researcher account for their medicinal value (Gambo, *et al., 2018;* Amuzat *et al.,* 2020). For example, saponins are having hypotensive and cardio depressant properties, while anthraquinones possess astringent, purgative, anti-inflammatory, moderate antitumor, and bactericidal effects. Cardiac glycosides are naturally cardio active drugs used in the treatment of congestive heart failure and cardiac arrhythmia. Table 1 summarizes the phytochemical screening result.

The cytotoxicity study of the stem bark and leaves of the *Vitex doniana* shows percentage mortality of 16.66 - 71.42% and 14.28 - 57.14% for stem bark and leaves respectively within the concentration range of 5-300 µg/ml of extracts as can be observed in figure 1. The LC50 for the stem bark and the leaves extracts traced from the graph were 175 µg/ml and 260 µg/ml respectively. Moshi, *et al.,* (2010) states that, brine shrimp lethality assay with LC50 of plants extracts above 100 µg/ml is considered non-toxic. Therefore, the observed lethality of the stem bark and leaves of *Vitex doniana* indicated the plant has anticancer and antitumor agents which can be attributed to the phytochemicals detected.

Table 1: Phytochemical Analysis

S/No.	Phytochemicals	Stem bark extract	Leaves Extract
1	Alkaloids	++	+
2	Flavonoids	++	+
3	Glycosides	+	-
4	Cardiac glycosides	-	-
5	Saponins	++	+
6	Steriods	-	+
7	Tannins	+	++
8	Phenols	++	++
9	Terpenoids	+	_
10	Anthraquinones	+	+



CONCLUSION AND RECOMMENDATIONS

In conclusion, this study revealed the presence of some important phytochemicals which shows that *Vitex doniana* have the potentials to cure some ailments as claimed by the traditional medicine practitioners. The cytotoxic activity of the *Vitex doniana* stem bark and leaves detected is a prediction of the acute toxicity of the plant, hence it is recommended to investigate the acute toxic activity of the plant, *Vitex doniana*.

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