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Evaluation Of The Proximate And Phytochemical Compositions Of The Leaf Of Vitex Doniana

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Abstract
This study presents the proximate and phytochemical analysis of the leaf of Vitex doniana. *The proximate composition revealed the presence of moisture (13.08%), protein (11.27%), ash (5.21%), crude fiber (18.44%), fat and oil (3.96%), and carbohydrates (48.08%). The qualitative phytochemical analysis indicated that ethanol extracts contained significant amounts of alkaloids (++), saponins (+++), flavonoids (++), tannins (+++), and phenols (+), while water extracts showed lower presence of alkaloids (+), saponins (+++), tannins (+), and absence of flavonoids, steroids, glycosides, and terpenoids. The quantitative analysis further quantified these compounds as follows: alkaloids (3.46%), saponins (1.08%), flavonoids (4.13%), phenols (0.14%), and tannins (23.39 mg/100g). These findings suggest that* Vitex doniana *leaves are rich in carbohydrates and crude fiber, and contain various phytochemicals, which may contribute to their medicinal properties.*

Keywords: *Vitex doniana, Proximate analysis, Phytochemical analysis, Medicinal properties*

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I. Introduction

Research has shown that collectively, plants produce a remarkably diverse array of over 500,000 low molecular mass natural products known as secondary metabolites (Al-Khayri *et al*., 2023). Medicinal plants are therefore, plants that contain these substances in one or more organs (root, stem, bark and flower) which can be used for therapeutic purposes and treatment of ailments. . Some medicinal plants used in Nigeria include *Garcina kola*, used in the treatment of asthma, *Carica papaya*, used as a remedy for hypertension, *Ocimum basilicum*, a cure for typhoid fever, and *Cola nitida*, for treatment of pile (Agbafor & Nwachukwu, 1996). *Vitex doniana* (Verbenaceae), commonly called black plum, is widely distributed in the eastern and western parts of Nigeria. Various parts of the plant are used by traditional medicine practitioners in Nigeria in the management and treatment of several disorders which include rheumatism, hypertension, cancer, and inflammatory diseases (Abubakar *et al.*, 2022). Plant chemicals useful in medicine are utilized mainly by incorporating them into medicines. Secondary metabolites are also used as precursors for the manufacture of new or synthetic drugs and to help in the elucidation of the physiological mechanisms in drug development or testing. For example, medicinal plants with anti-inflammatory activity are widely employed in the traditional treatment of several disorders. The inflammatory response involves a complex array of enzyme activation, mediator release, cell migration, tissue breakdown and repair (Abdulkhaleq *et al.,* 2018). Some important groups of these phytochemicals include: alkaloids, glycosides, steroids, flavonoids, fats, phenols, resins, saponins, tannins and terpenes

Vitex doniana, commonly known as black plum, is a plant of significant ethnobotanical interest, found primarily in tropical Africa. Its leaves, in particular, have been traditionally used in various medicinal and nutritional applications, prompting scientific inquiry into their chemical composition and potential health benefits. The evaluation of the proximate and phytochemical compositions of *Vitex doniana* leaves is crucial for understanding their nutritional value and therapeutic potential.

Proximate analysis typically involves determining the moisture, ash, crude protein, crude fiber, crude fat, and carbohydrate content of plant materials. These components provide insight into the nutritional profile of the leaves and their suitability for use as a food source or dietary supplement. Previous studies have indicated that *Vitex doniana* leaves are rich in essential nutrients, suggesting their potential to contribute to dietary requirements and overall health (Ayodele, 2014).

Phytochemical analysis, on the other hand, focuses on identifying the bioactive compounds present in the leaves, such as alkaloids, flavonoids, tannins, saponins, and phenolic compounds. These phytochemicals are known for their diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, and

anticancer properties. Research has demonstrated that *Vitex doniana* leaves contain significant levels of these compounds, which may underlie their traditional medicinal uses (Okwu & Josiah, 2006).

This study systematically evaluated the proximate and phytochemical compositions of *Vitex doniana* leaves to provide a comprehensive understanding of their nutritional and medicinal potential. Such information could support the development of functional foods and nutraceuticals derived from this plant, contributing to improved health outcomes and the preservation of traditional knowledge.

II. Material And Methods

Plant Material Collection

Leaves of *Vitex doniana* were collected from mature trees in the wild in Botanical garden of Alex ekwueme Federal University Ndufu Alike Ikwo Ebonyi State, Nigeria. The leaves were identified and authenticated by a Taxonomist Godwin Ikedichi at PRODA Enugu State.

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Sample Preparation

The leaves were washed with distilled water to remove any dirt and then air-dried at room temperature for 7-10 days until a constant weight was achieved. The dried leaves were then ground into a fine powder using an electric blender and stored in airtight containers at 4°C until further analysis.

Proximate Analysis

The proximate composition of the leaf powder was determined according to the standard methods of the Association of Official Analytical Chemists (AOAC, 2016).

Moisture Content: The moisture content was determined by drying 2 g of the leaf sample in an oven at 105°C until a constant weight was achieved.

Ash Content: Ash content was determined by incinerating 2 g of the sample in a muffle furnace at 550°C for 5 hours.

Crude Protein: The crude protein content was estimated using the Kjeldahl method, where the nitrogen content was multiplied by a conversion factor of 6.25.

Crude Fat: Crude fat was extracted using the Soxhlet extraction method with hexane as the solvent.

Crude Fiber: The crude fiber content was determined by acid-base digestion method.

Carbohydrate Content: Carbohydrate content was calculated by difference, using the formula: Carbohydrate = 100 − (Moisture + Ash + Protein + Fat + Fiber)

Phytochemical Screening

Phytochemical analysis was conducted to identify the presence of various bioactive compounds using standard qualitative methods as described by Harborne (1998) and Sofowora (1993).

Alkaloids

Dragendorff's and Mayer's reagents were employed to detect alkaloids, with a positive result shown by the formation of an orange-red precipitate for Dragendorff's reagent and a white precipitate for Mayer's reagent.

Flavonoids: Flavonoids were detected using the alkaline reagent test.

Saponins

The froth test was used to identify saponins, with persistent froth formation for at least 10 minutes after shaking the extract in distilled water indicating their presence.

Steroids

Precisely 5 drops of sulfuric acid were added to 1 ml of the plant crude extract, resulting in a red to dark coloration that indicated the presence of steroids.

Flavonoids

The presence of flavonoids was confirmed by a yellow coloration formed when the extract was treated with a few drops of concentrated hydrochloric acid and a magnesium ribbon, known as the Shinoda test.

Glycosides

The method described by Ajayi (2018) was employed to determine the cyanogenic glycoside content in the plant extract. Plant samples were weighed into a 250 cm^3 round bottom flask, and approximately 200 cm^3 of distilled water was added to 1 g of each dry wood powder sample. The mixture was allowed to stand for 2 hours to facilitate autolysis. Complete distillation was then performed in a 250 cm^3 conical flask containing 20 cm^3 of

2.5% NaOH (sodium hydroxide) and an antifoaming agent (tannic acid). The distillate, containing cyanogenic glycosides, was mixed with 8 cm^3 of 6 M NH_4OH (ammonium hydroxide) and 2 cm^3 of 5% KI (potassium iodide). The resulting mixture was titrated with 0.02 M $AgNO_3$ (silver nitrate) using a microburette against a black background, with the endpoint indicated by continuous turbidity. The cyanogenic glycoside content in the sample was then calculated using the formula:

Mg= (titre value (cm^3) × 1.08 × extract value) / (aliquot volume (cm^3) × sample weight)

Tannin

The ferric chloride test was utilized to detect tannins, with a blue-black or greenish-black coloration indicating their presence when the extract was mixed with a 5% ferric chloride solution.

Terpenoid

This was determined using Salkowski Test**.** Formation of a reddish-brown or violet ring at the chloroform-sulfuric acid interface indicates terpenoids.

Phenols

The Folin-Ciocalteu reagent method was used for both qualitative and quantitative determination of total phenolic content, with the presence of phenolic compounds indicated by the development of a blue color upon reacting the extract with the reagent.

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using SPSS software, employing one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to determine significant differences between means at $p < 0.05$.

III. Results

Proximate Analysis

The proximate analysis of the phytochemical composition of the leaf of *Vitex doniana* is presented in Table 1. The leaves contain 13.08% moisture, indicating a relatively moderate water content, which can influence the shelf life and storage conditions of the plant material. The crude protein was 11.27%. The ash content, which is 5.21%, represents the total mineral content in the leaves, indicating the presence of essential minerals. The leaves have a high crude fibre content of 18.44%. The fat and oil content is 3.96%, showing that the leaves have a low lipid content, which is beneficial for a low-fat diet. Carbohydrates make up 48.08% of the leaves, providing a significant source of energy.

Table 1: Proximate Analysis of Phytochemical Composition of the Leaf of *Vitex Doniana*

PARAMETERS	VALUES IN (%)
Moisture	13.08
Protein	11.27
Ash	5.21
Crude fibre	18.44
Fat and oil	3.96
Carbohydrate	48.08

Qualitative Phytochemical Analysis

The qualitative phytochemical analysis of the leaf extracts of *Vitex doniana* using ethanol and water as solvents is shown in Table 2. The analysis indicates: The presence of alkaloids was strongly indicated (++ for ethanol, + for water). A high presence of saponins was detected (+++ for both solvents). Steroids were not detected (- for both solvents), suggesting their absence or very low levels in the leaves. The ethanol extract showed a presence of flavonoids (++), while the water extract did not show any (-). Glycosides were not detected in either solvent (- for both), indicating their absence in the leaves. The presence of tannins was strongly indicated (+++ for ethanol, + for water). Terpenoids were not detected (- for both solvents), suggesting their absence in the leaves. The ethanol extract showed a slight presence of phenols (+), while the water extract did not show any (-).

This qualitative analysis demonstrates that the leaves of *Vitex doniana* contain significant amounts of alkaloids, saponins, flavonoids, and tannins, particularly when extracted with ethanol.

Table 2: Qualitative Phytochemical Analysis of the Leaf of *Vitex Doniana*

PARAMETERS	ETHANOL	WATER
Alkaloid	++	+
Saponin	+++	+++
Steroid	-	-

Flavonoid	++	-
Glycoside	-	-
Tannin	+++	+
Terpenoids	-	-
Phenol	+	-

Key + = Present in Low quantity
++ = Present in Moderate quantity
+++ = Present in Excess quantity
- = Absent

Quantitative Phytochemical Analysis

The quantitative analysis of the phytochemical constituents of the leaf of *Vitex doniana* is presented in Table 3. The results are as follows:

The leaves contain 3.46% alkaloids, saponin content of 1.08%, flavonoid content of 4.13% and tannin content is 23.39 mg/100g. The phenol content was 0.14%.

Table 3: Quantitative Phytochemical Analysis of the Leaf of *Vitex Doniana*

PARAMETER	VALUES IN (%) EXCEPT TANNIN
Alkaloid	3.46
Saponin	1.08
Flavonoid	4.13
Tannin (mg/100g)	23.39
Phenol	0.14

IV. Discussion

The proximate analysis of the leaf of *Vitex doniana* (Table 1) reveals significant nutritional and functional properties. The moisture content of 13.08% is within an acceptable range for dried plant materials, suggesting good potential for storage stability and minimal risk of microbial growth, which is crucial for the shelf life of the leaf powder (Onwuka, 2018). The protein content, at 11.27%, indicates that the leaves could serve as a supplementary protein source, which is essential for various bodily functions including enzyme activity and muscle repair (FAO, 2019).

The ash content, representing the total mineral content, is 5.21%. This relatively high value suggests that the leaves are a good source of essential minerals necessary for metabolic processes (Afolayan & Jimoh, 2009). The crude fibre content is particularly high at 18.44%, which can aid in digestive health and prevent constipation, making the leaves beneficial for maintaining gastrointestinal health (Alkhatib *et al*., 2017).

The fat and oil content is low at 3.96%, aligning with the needs of low-fat diets and reducing risks associated with high-fat consumption, such as cardiovascular diseases (Mensink, 2016). Carbohydrates constitute the largest portion of the leaves at 48.08%, providing a significant energy source, which is essential for daily activities and overall metabolism (Slavin, 2013).

The qualitative phytochemical analysis (Table 2) of the leaf extracts using ethanol and water reveals a varied presence of bioactive compounds. Alkaloids were detected in both extracts (++ in ethanol, + in water), signifying their therapeutic potential, including analgesic and antimicrobial properties (Cushnie *et al*., 2014). The high presence of saponins (+++ in both extracts) is noteworthy as these compounds exhibit beneficial properties such as cholesterol reduction, immune system enhancement, and anti-cancer activities (Man *et al*., 2010).

Steroids were absent in both extracts, suggesting that these particular compounds are either not synthesized by the plant or are present in undetectable amounts. Flavonoids were present in the ethanol extract (++) but not in the water extract, highlighting the importance of solvent choice in phytochemical extraction. Flavonoids are known for their antioxidant properties and their role in reducing inflammation and oxidative stress (Panche *et al*., 2016).

The absence of glycosides in both extracts indicates that these compounds are not significant constituents of *Vitex doniana* leaves. The presence of tannins (+++ in ethanol, + in water) underscores their potential astringent and antimicrobial properties, which can be useful in treating diarrhea and wound healing (Hider *et al*., 2017). Terpenoids were not detected, which aligns with the quantitative analysis. The slight presence of phenols in the ethanol extract (+) suggests some antioxidant activity, albeit at a lower concentration (Rice-Evans *et al*., 1996).

The quantitative phytochemical analysis (Table 3) provides a more detailed understanding of the bioactive compounds in *Vitex doniana* leaves. Alkaloids are present at 3.46%, which is substantial and supports their potential medicinal uses in treating a variety of ailments (Gurib-Fakim, 2006). The saponin content is 1.08%, reinforcing their role in enhancing immune functions and acting as natural antibiotics (Price *et al*., 1987).

Flavonoids are quantified at 4.13%, reflecting their significant presence and corroborating their role as potent antioxidants that can help in preventing chronic diseases (Hollman, 2001). Tannins are present at 23.39 mg/100g, indicating a considerable amount that could contribute to the plant's astringent, antimicrobial, and

antioxidant properties (Scalbert, 1991). The phenol content is relatively low at 0.14%, yet even in small amounts, phenols can provide substantial antioxidant benefits (Shahidi & Naczk, 2004).

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