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Proximate, Vitamin, Mineral and Anatomical Studies on *Vitex chrysocarpa* Planch ex Benth. (Verbenaceae)

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Authors' contributions

This work was carried out in collaboration between all authors. Author CVI designed the study. Authors CVI and EE wrote the protocol and wrote the first draft of the manuscript. Author EE managed the literature searches, analyses of the study and managed the experimental process. Author CVI supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

Proximate, vitamin, mineral and anatomical studies were carried out on various parts (Leaf, stem, root and petiole) of *Vitex chrysocarpa* using standard techniques. Analysis of Variance (ANOVA) was employed in data analysis. The proximate study indicated that the various parts contained all the nutrients assayed but in varied quantities. Crude protein and fat were highest in the leaf (6.93+0.00% and 5.06+0.00% respectively). Crude fibre, ash and moisture were highest in the root (10.91+0.05%, 10.32+0.66%, and 29.23+0.01% respectively). The result revealed also that the various parts contained vitamins and minerals in varied proportions. The leaf contained significantly the highest proportions of zinc, phosphorus, iron, vitamin A and vitamin C (0.38±0.00 mg/100 g, 24.91±0.01 mg/100 g, 10.18±0.00 mg/100 g, 45.06+ 3.24 µg/g, and 13.81 + 3.09 mg/100 g, respectively). Anatomical study showed uniseriate epidermis, non-glandular trichomes in all the parts, presence of diffuse vessels and rays in mature stem. This work, confirmed the plant's potential uses. These parts could therefore be used as natural food and also extracted for

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manufacture of food supplements and drugs. Anatomical study is an additional aid to the plant taxonomic characterization and identification.

Keywords: Anatomical; proximate; mineral; vitamin; Vitex chrysocarpa.

1. INTRODUCTION

No scientific work has been published on anatomy, proximate, vitamin and mineral studies on *Vitex chrysocarpa*. But the extracts have been an important source of traditional medicine and nutrient in our community. *Vitex* is a genus of flowering plants in the family Verbenaceae. The genus has been used extensively in traditional medicine. In Nigeria, several species of *Vitex* are found including *Vitex chrysocarpa*. *Vitex chrysocarpa* is a West African plant. It is usually a shrub but sometimes reaching the size of a small tree. The leaflets are densely but very shortly hairy beneath and the fruits are velvety when young [1]. In Anambra East Local Government Area of Anambra State, Nigeria, the leaves are locally used for the cure of malaria, diabetes, venereal diseases and as food. Seeds are taken to reduce fever and headache. Plants contain many nutrients which are important for proper functioning of human body system. These nutrients are protein, ash, fat and oil, moisture, minerals, vitamins [2]. Anatomy of plants revealed the internal organization of the cells organelles, tissues and their function. The size, shape and arrangement of most cells in the epidermis, sclerenchyma etc. have aided the studying the wood formation in plant [3].

Vitex chrysocarpa has been a source of traditional medicine and nutrient in our community and, no scientific study has been done on it. In order to determine whether this plant truly possesses these potentials there is need for the present study. Accordingly, the problem and focus of this research is to provide detailed nutritional composition and anatomical description of its various parts.

2. MATERIALS AND METHODS

2.1 Area of Study, Collection and Identification of Plant Materials

The experiments were carried out in the Botany Laboratory, University of Nigeria, Nsukka. The species *Vitex chrysocarpa* was collected from a forest along Omambala/Ezu River bank in Aguleri, Anambra East Local Government Area of Anambra State, Nigeria. The species was authenticated at Bio-diversity Development and Conservation Center Enugu. The Voucher Specimens were deposited at the Department of Botany Herbarium, Nnamdi Azikiwe University, Awka.

2.2 Samples Preparation

Leaf, stem and root of *Vitex chrysocarpa* were washed and oven dried at 65℃ for 7 h. The samples were ground into powder and stored in an air tight container at room temperature until required for use.

2.3 Proximate, Mineral and Vitamin Studies

2.3.1 Materials used

The following materials were used for the nutrients analylsis: Dessicator, muffle furnace, spectrometer, silica dish, kjeldahl flask, funnel, soxhlet apparatus, filter paper, thimble, electric oven, grinder, retort stand, test tube and test tube rack, crucible, weighing balance, petri dish. The chemicals used include: Tetrahydrosulphate (vi) acid, Boric acid indicator solution, Sodium hydroxide, Hydrochloric acid, Petroleum ether, Potassium hydroxide, Acetone, Phenolphthaline indicator, Ammonia, Dithezone solution, Carbon tetrachloride, Hydroquinoline, Phenonthroline, Vanado Molybidic acid, Selenium oxide.

Proximate (ash, crude protein, crude fat, crude fibre and moisture), minerals (zinc, iron and phosphorus) and vitamins (vitamin A and C) contents were carried out to ascertain the nutrient compositions present in the plant extracts. Moisture content, total ash and protein were determined according to the method of [4]. Crude fat and carbohydrate were determined using the method of [5] while crude fibre was done by solvent extraction gravimetric method described by [6]. Vitamins and minerals were done following the methods of [7].

2.4 Anatomical Study

The following materials were used for the anatomical study: Photomicroscope, a staining jar, a wash bottle, a sledge microtome, a beaker, Carmel's hair brush, Zeiss light microscope with the serial No. 4F8662206, MC'35 Camera for 53mm film. The reagents and stains were 97% alcohol, absolute alcohol, 50/50 alcohol, /xylene, xylene, safranin, fast green and Canada balsam as mountant.

Anatomical study was carried out at the Anatomy Laboratory of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka using Reichert sledge microtome. Transverse sections were made from middle part of fully grown leaves, midpoint of petiole, centre of an internode of young and mature stem and mature root. This was done using standard procedure as described by [8,9]. Photomicrographs of the specimens were taken with Zeiss light microscope with MC'35 Camera for 53mm film.

2.5 Statistical Analysis

Results were presented in mean±standard deviation and were subjected to analysis of variance (ANOVA) using Duncans Multiple Range Test (DMRT) at 5% probability to separate the treatments. Difference in mean value were considered significant.

3. RESULTS AND DISCUSSION

Results are shown in Tables 1- 3 and Figs. 1- 8.

The result indicated that the nutrients were present in all the parts of the *Vitex chrysocarpa* investigated but in varied amounts. The leaf contained the highest percentage of Crude protein (6.93±0.00) and fat (5.06±0.00).Crude fibre, ash and moisture compositions were highest in the root (10.91±0.05, 10.32±0.66 and 29.23±0.01 respectively) (Table 1). The result has indicated that *Vitex chrysocarpa* has some nutritional potential that can be exploited in diet. Fat is high energy nutrient and does not add to the bulk of the diet [10]. Proteins are used for building and repairing of body tissues, regulation of body processes and formation of enzymes and hormones [11]. Generally, fibre aids and speeds up the excretion of waste and toxins from the body, preventing them from accumulating in the intestine or bowel for too long, which could cause a build-up which or azotaemia [12].

The various parts of *Vitex chrysocarpa* contained all the minerals assayed but in varied quantities. The leaf contained significantly the highest percentage of Zinc, Phosphorus and Iron (0.38±0.00, 24.91±0.01 and 10.18±0.00 respectively) (Table 2). The higher compositions of zinc, phosphorus and iron in leaf imply that it will serve as a better source of zinc, phosphorus and iron than stem and root. Iron is an important constituent of haemoglobin found in blood. Iron serves as a carrier of oxygen to the tissues from the lungs by the red blood cell, haemoglobin as a transport medium for electrons within cells and as integrated part of important enzyme systems in various tissues [13]. Phosphorus is essential for the process of bone mineralization as well as a role in the structure of cellular membranes, nucleic acids and nucleotides, including adenosine triphosphate while zinc is an essential trace element needed in the body for boosting the immune system and preventing lower respiratory infections [14].

The result revealed also that the various parts of *Vitex chrysocarpa* contained all the vitamins but in varied amounts. The leaf contained significantly the highest composition of vitamin A and C (45.06± 3.24 and 13.81 ± 3.09 respectively) (Table 3). This implies that the leaf of *Vitex chrysocarpa* will serve as a better source of vitamin A and C than the root and stem. Vitamins A enhance vision while vitamin C activates the cell functions. Vitamin C is a powerful antioxidant. It favours the absorption of iron in the intestine, protects against infections, neutralizes blood toxins and intervenes in the healing of wounds [13].

Table 1. Percent proximate composition of the stem, leaf and root of *Vitex chrysocarpa*

Plant part	Percentage proximate composition (%)				
	Crude protein	**Crude fibre**	**Ash**	**Fat**	**Moisture**
Stem	$3.52±0.00^b$	$10.62±0.43^b$	$9.67±0.28^a$	$5.02±0.00^b$	$10.61±0.00^c$
Leaf	$6.93±0.00^c$	$8.86±0.08^a$	$8.93±0.05^a$	$5.06±0.00^c$	$18.06±0.01^a$
Root	$3.23±0.00^a$	$10.91±0.05^b$	$10.32±0.66^a$	$5.01±0.00^a$	$29.23±0.01^b$
p-value	**	**	**	**	**

*Results are mean ±SD, *Columns followed by the same letter are not significantly different, significant difference exist at **p<.05*

Table 2. Mineral composition of the stem, leaf and root of *Vitex chrysocarpa*

Plant part	Mineral composition (mg/100 g)		
	Zinc	Phosphorus	Iron
Stem	0.22±0.00^{a}	23.95±0.06^{b}	1.37±0.00^{a}
Leaf	0.38±0.00^{c}	24.91±0.01^{c}	10.18±0.00^{c}
Root	0.32±0.00^{b}	3.69±0.00^{a}	3.25±0.00^{b}
p-value	**	**	**

*Results are mean ±SD *Columns followed by the same letter are not significantly difference. **p<.05*

Table 3. Vitamin composition of the stem, leaf and root of *Vitex chrysocarpa*

Plant parts	Percentage vitamin composition	
	Vitamin A (µg/g)	Vitamin C (mg/100 g)
Stem	23.00±2.05^{c}	7.25±3.04^{b}
Leaf	45.06±3.24^{a}	13.81±3.09^{a}
Root	32.02±2.00^{b}	6.12±0.02^{c}
P-value	**	**

*Results are mean ±SD *Columns followed by the same letter are not significantly different, significant difference exist at **p<.05*

3.1 Anatomical Results

The transverse section of leaf (Fig. 1) showed non-glandular trichomes mostly on the abaxial surface; epidermis, cortex, and well arranged vascular bundle. The transverse section of petiole (Fig. 2) was somewhat oblong to round. The abaxial surface was convex while the adaxial surface markedly concave. There were non-glandular trichomes on the abaxial surface. The vascular bundle was in a dome form and well arranged. The transverse section of primary stem (Fig. 3) was circular and the epidermis covered with trichomes. The cortex was well arranged; vessels were diffuse- porous and not occluded by tyloses. In the transverse section of secondary stem (Fig. 4) the vessels were diffuse-porous and not occluded by tyloses. The pores were oval in shape. Multiseriate and uniseriate rays were present. In the transverse section of root (Fig. 5) the vessels were diffuse- porous and not occluded by tyloses. The pores were oval in shape. There were also pronounced secondary rays. Tangential longitudinal section of stem (Fig. 6) showed multiseriate, heterocellular and procumbent rays, vessels were not occluded by tyloses. The radial longitudinal section of stem (Fig. 7) showed the vascular rays which appeared in uniseriate and multiseriate forms. Vessels not occluded by tyloses. The result has revealed similar features among the various parts and some dissimilarity in the arrangement and distribution of vascular bundle. The above result is in line with anatomical observations of most dicotyledonous plants. Also, in line with the work of [12, 15] who reported similar results among the various parts of *Gomphrena celosioides and Piper guineense*.

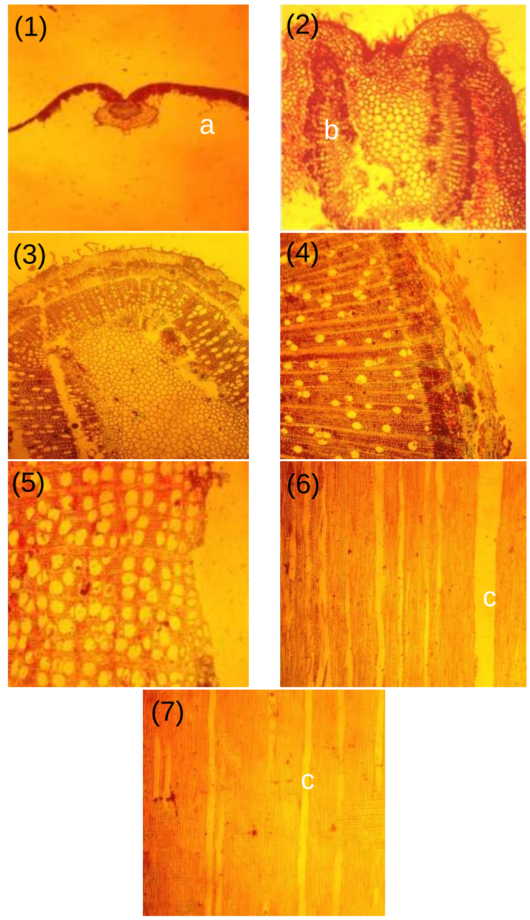

Fig. 1-7. T/S of leaf, petiole, primary stem, secondary stem, root, TLS and RLS of stem respectively. (a - c) trichome, vessel, procumbent ray respectively (Fig. 1-2 (x40), 3-7 (x 100) respectively)

Fig. 8. *Vitex chrysocarpa* plant in its natural habitat

4. CONCLUSION

This work, confirmed the plant's potential uses. These parts could therefore be used as natural food and also extracted for manufacture of food supplements and drugs. Anatomical study is an additional aid to the plant taxonomic characterization and identification.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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