

## Vitamin Content of Ethanolic Extract of *Vitex doniana* Leaf

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### ABSTRACT

*This work investigated the vitamin contents of ethanolic extract of vitex doniana using the modified methods described by Association of official Analytical chemists. Vitex doniana leaves were obtained, which is further air dried for a week, sorted and pulverized with a laboratory blender. Then mixed with ethanol for some days, and then the samples was filtered with muslin cloth, to obtain the extract which was used for the investigation. Then the sample was subjected to the water bath to dry the extract for further analysis. The vitamin content of the ethanolic extract of vitex doniana was determined using the standard method of Association of official Analytical Chemist. The result indicated that vitex doniana extract has 1.52+0.61 of vitamin A, 1.23+ 0.57 of*

*vitamin., 0.73+ 0.03 of vitamin B2, 0.60 + of vitamin B30.19 ±0.01 of vitamin B6, 0.15+0.11 of vitamin B9, 1.45+0.1 of vitamin C, 0.71+0.04 of vitamin D, 0.25+0.01 of vitamin E, 0.11+ 0.32 of vitamin K, and 0.89 + 0.02 of beta. Carotene. The result obtained have shown that Vitex doniana ethanol extract contains significant amount of vitamins at varying proportion. In accordance with above figures, vitamin A among other gave the highest percentage followed by vitamin C, then comes others. Therefore, the present finding suggest that the ethanolic extract of vitex doniana a considerable source of vitamin and also the leaves in diet and may have health and economic benefits due to its fibre, minerals, phenolics content and it antioxidant activities*

*Keywords: Vitamin, content, ethanolic, extract, leaf.*

### INTRODUCTION

The use of plant in the management and treatment of diseases started with life. In more recent years, with considerable research, it has been found that many plants do indeed have medical value [1]. Some medical plants used in Nigeria include *Garcina kola* used in the treatment of asthma, carica papaya, used as a remedy for hypertension, *Ocomum basilicum*, a cure for typhoid fever and *cola nitida*, for treatment of pile. *Vitex doniana* (vebenaceae), commonly called black plum, is widely distributed in the eastern and western parts of Nigeria. Various parts of the plants are used by traditional medicine practitioners in Nigeria in the management and treatment of several disorders which include rheumatism, hypertension, cancer and inflammatory disease [2]. The vitamins and phytochemicals are bioactive compounds found in plant that work with nutrients and dietary fibre to protect against disease. They

are non-nutritive compounds (secondary metabolities) that contribute to Flavour colour, [3]. Many vitamins have antioxidant activity and reduce the risk of many oxidant stress diseases, vitamin C and E find in (carrots) and vitamin A, found in water melon and cucumber [4]. Reactive oxygen-free radicals (Ros) have been implicated in many diseases and in aging process. These free radicals, which cause tissue demands via oxidative stress, are generated by aerobic respiration, inflammation, and lipid peroxidation.

*Vitex doniana* sweet, (family verbanaceae) is a perennial shrub widely distributed in tropical west Africa, and some East African countries including Uganda, Kenya and Tanzania; and high rainfall areas. It is found in the middle belt of Nigeria particularly Kogi, Benue, and Parts of the Savannah regions of Kaduna, Sokoto and Kano state [5]. It is variously called *vitex*

(English). Dinya (Hausa), dinchi (Ggbogyi), uchakoro (Igbo), oriri (Yoruba) ejiji, (Igala) and olih (Etsak) [6]. *Vitex doniana* is employed locally in the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhoea, dysentery and diabetes indicating that the plants leaves may possess anti diabetic properties [7]. The roots and leaves are used for nausea, Colic and epilepsy [8]. In North-Central and Eastern parts of Nigeria, the young leaves are used as vegetables or sauces and porridge for meals especially for diabetic patients. *Vitex doniana* is known to help in production of serotonin, which help to ward off depression as well as improving mood. Riboflavin niacin and vitamin E that helps preventing cataract in the eyes while vitamin C help to slow bone loss and decrease the risks of fractures. It also allows the body to make up collagen, which is the major component of cartilage, which aids in joint support and flexibility [8]. Vitamin in general serves as cofactor that help to enhance metabolic activities in the system (eg) vitamin B6 pyridoxine, which is converted into its active form (PLP) pyridoxal phosphate which function as a cofactor in all of the enzyme that carry out the transamination reaction required for the synthesis and catabolism of amino acid and also vitamin B3 niacin which is further converted to (NAD) and (NADP) and they are involved in various metabolic reaction, lactate dehydrogenase, and pyruvate dehydrogenase.

#### **Statement of Problem**

Nigeria is a country with different

ecological zones that support production of different kinds of food crops and forest resources [9]. However, according to findings, hunger and malnutrition are still found in many rural and urban areas. The quality and quantity of nutrients present in the food consumed by the people in the rural area is very low compare to the actual requirement from a balance diet for normal growth. Intake of vitamin enriched food among the rural dwellers is grossly insufficient and this is because they are simply unavailable or too expensive for them to procure. Consequently, sickness and even death in young children are very common due to malnutrition. The use of tree leaf vegetables to supply these nutrients has declined over the years and the material and knowledge of its uses are decreasing at an alarming rate. Inadequate documented scientific information on indigenous Africa vegetable species is a major factor that influences people to choose exotic vegetable over indigenous types, thus promoting consumption of indigenous green leafy vegetables among people is a key to improving the health of many. Man, more than ever before needs a re-orientation on the sustainable use of his natural resources particularly tree leaf vegetables for the provision of balance nutrition. So knowledge of indigenous plant use needs urgent scientific investigation and documentation before it is irretrievably lost to future generation [10].

#### **Aims and Objectives**

The objective is to investigate the vitamin contents of the ethanolic extract of *Vitex doniana* leaf.

Fig 1: Picture of *Vitex doniana* leaf



## MATERIALS AND METHODS

### Plant Source

Fresh *Vitex doniana* was collected from Obe in Nkanu West Local Government Area, Enugu State in July, 2018 and was conveyed to ESUT biochemistry laboratory in a polythene bag.

### Extraction of *Vitex doniana*

Fresh leaves of *V. Doniana* were collected and air-dried. Then the sample was pulverized and separated into two bottles, container one 250g of sample with 750ml of ethanol and the other 200g of the sample with 500ml of ethanol, at room temperature (28+ 2°C) with occasional shaking [11]. Then which was later filtered with muslin clothe. The filtrates were collected on a beaker and the filtrate was evaporated to dryness on a hot water bath and after evaporation, the plant extracts (residues) were stored in a clean sterile container for further use.

**Methods for determining the vitamin content of *Vitex doniana***

The vitamin contents of the *Vitex doniana* extract were determined using the modified method of AOAC (2005).

### Determination of vitamin A (Retinol)

Concentration 0.5g of sample was macerated with 100ml of petroleum ether for 10mm, and allowed to stand for 1 hour with intermittent shaking at every 1 minute. The mixture was centrifuged for 5 minutes and ml of the supernatant in the test tube was evaporated to dryness and the residue re-dissolved with 0.2ml of acetic assnhydride/chloroform (1:1) and 2ml of 50% trichloroacetic acid (TCA) in chloroform. The absorbance of the resulting solution was taken at wavelength of 620nm at 15 seconds and 30 seconds against the corresponding blank.

Concentration of vitamin A (mg)

=  $\frac{\text{Abs} \times \text{DFx pathlength}}{\text{E}}$

E

Where Abs = absorbance DF = dilution factor, E = extraction coefficient

#### **Determination of beta-carotene**

Concentration sample 2.5g was weighed into the test tube and 20ml of petroleum spirit was added and shaken for 5 min. The supernatant was decanted into another test-tube and the absorbance read at 450nm.

Concentration of  $\beta$ -carotene (mg)

$$= \frac{\text{Abs} \times \text{DF} \times \text{pathlength}}{E}$$

#### **Determination of vitamin C (Ascorbic acid)**

Concentration 0.25g of each sample was macerated with 20ml of 0.4% oxalic acid for 10 min and centrifuged for 5 min. The supernatant (1ml) was transferred into test tubes to which 9ml or 2,6-dichlorophenol indophenols (12ml/L) had been shaken and then mixed thoroughly by shaking. The absorbance of the resulting solution was taken at 520nm at 15 seconds and 30 seconds against corresponding blank.

Concentration of vitamin C (mg)

$$= \frac{\text{Ab} \times \text{DF} \times \text{pathlength}}{E}$$

#### **Determination of vitamin E**

( $\alpha$ -tocopherol) concentration 0.5 gram of each sample was macerated with 20ml of petroleum ether for 10min and allowed to stand for 1 hour with intermittent shaking at every 1 min, and centrifuged for 5mm supernatant (3ml) was transferred into triplicate test tubes; evaporated to dryness and the residue re-dissolved with 2ml ethanol and shaken. A known volume. 1ml of 0.22% ferric chloride in ethanol and 1ml of 0.5%  $\alpha$ -dipyridyl in ethanol were added to the resulting solution and then made up to 5ml with ethanol. The mixture was thoroughly shaken and the absorbance taken at a wavelength of 520nm against corresponding blank.

Concentration of vitamin E (mg)

$$= \frac{\text{Ab} \times \text{DF} \times \text{pathlength}}{E}$$

#### **Determination of Vitamin B1 (thiamine) concentration**

Sample 0.5gram was homogenized with 50ml of ethanol sodium hydroxide solution and filtered into a 100ml flask. Filtrate (10ml) was pipette into a beaker and 10ml potassium dichromate added for colour development. A blank sample was prepared and the absorbance was taken at 560nm. The

concentration of each sample was extracted from a standard curve.

Concentration vitamin B1 (mg)

$$= \frac{\text{Ab} \times \text{DF} \times \text{pathlength}}{E}$$

#### **Determination of Vitamin B2 (Riboflavin concentration)**

Each sample 1.25 was extracted with 5ml of 50% hydrogen peroxide and allowed to stand for 30min. More after, 2ml of 40% sodium sulphate was added to make up to 50ml mark. The absorbance and a wavelength of 510nm was read in a spectrophotometer.

Concentration of vitamin B2 (mg)

$$= \frac{\text{Ab} \times \text{DF} \times \text{pathlength}}{E}$$

#### **Determination of Vitamine B3 (Niacin)**

Each sample (5g) was added 50ml sulphuric acid and shaken for 30min. Thereafter, 3 drops of ammonia solution was added to the mixture and filtered. Potassium cyanide (5ml) was added to 10ml volumetric flask and the mixture acidified with 0.02m H<sub>2</sub>SO<sub>4</sub>. The absorbance was read at a wavelength of 470nm in a spectrophotometer.

Concentration of vitamin B3 (mg)

$$= \frac{\text{Ab} \times \text{DF} \times \text{pathlength}}{E}$$

#### **Determination of Vitamin B6 (pyridoxine Concentration)**

0.5g of each sample was extracted with 225ml of distilled water for 1 hour and filtered. Then, 2ml of distilled water 0.5ml of 50% sodium acetate, 0.1ml of diazotized reagent and 0.2ml of 5.5% sodium carbonate was added to 1ml of the filtrate and mixed thoroughly. The absorbance of the solution was read at a wavelength of 540nm.

Concentration of vitamin B6 (mg)

$$= \frac{\text{Ab} \times \text{DF} \times \text{path length}}{E}$$

#### **Determination of Vitamin B9 (Folic acid) Concentration**

One gram of each sample was weighed into beaker and extract with 100ml of distilled water with slight heat. The mixture was shaken thoroughly and filtered after cooling. The absorbance of the filtrate was read spectrophotometrically at a wavelength of 325nm

Concentration of vitamin B9 (mg)

$$= \frac{\text{Ab} \times \text{DF} \times \text{path length}}{E}$$

**Determination of Vitamin K concentration**

One gram of each sample was dissolved in 10ml of distilled water and filtered to 1ml of the filtrate, 2ml of distilled water and 1ml of 0.04% in 1:5 hydrochloric acid were added. The mixture was heated in boiling water for 45 min and

cooled. The mixture was then diluted with 10ml 1:3 ammonium hydroxide and the absorbance of the mixture read wavelength of 635nm against a blank.

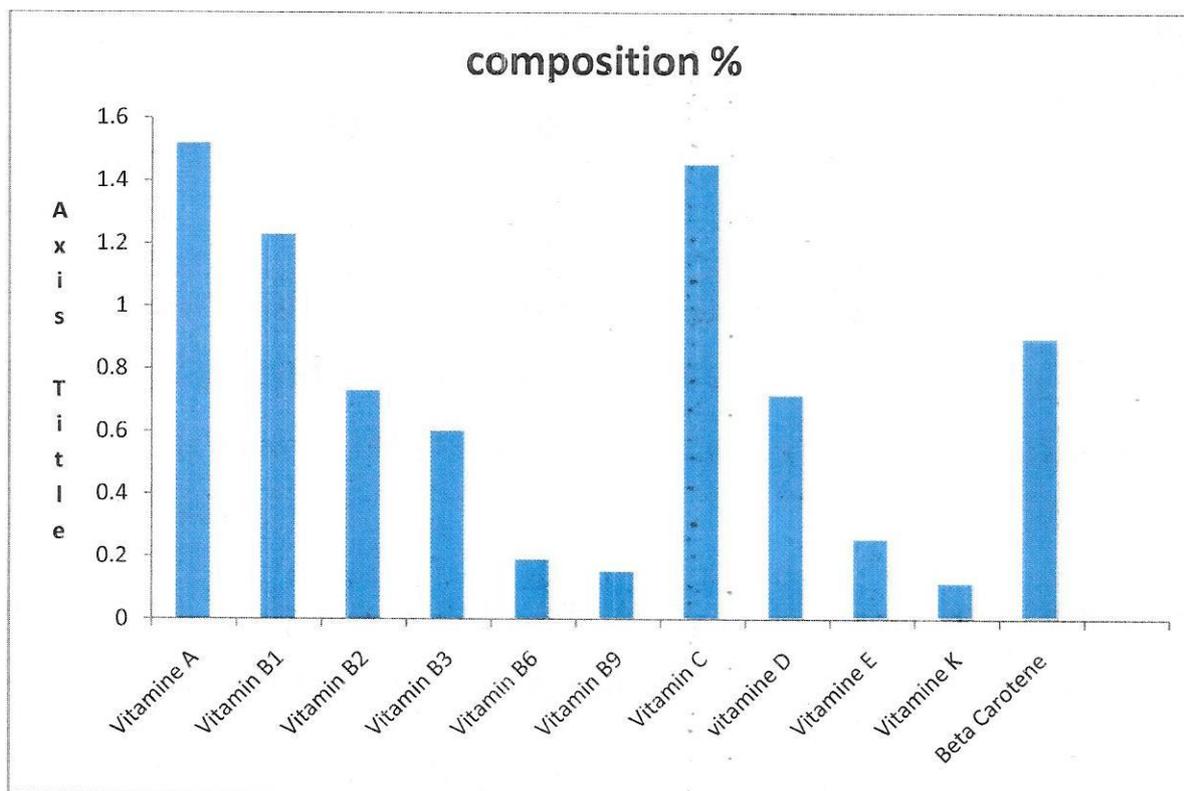
$$\text{Concentration of vitamin K (mg)} = \frac{\text{Ab} \times \text{DF} \times \text{wavelength}}{\text{E}}$$

**RESULTS**

Table 1: The values of the vitamin content of the leaf extract of *Vitex doniana*.

<b>Vitamin A</b>	<b>1.52 ±0.61</b>
<b>Vitamin B1</b>	<b>1.23 ±0.37</b>
<b>Vitamin B2</b>	<b>0.73 ± 0.03</b>
<b>Vitamin B3</b>	<b>0.60 ±0.04</b>
<b>Vitamin B6</b>	<b>0.19 ±0.01</b>
<b>Vitamin B9</b>	<b>0.15 ±0.11</b>
<b>Vitamin C</b>	<b>1.42 ±0.1</b>
<b>Vitamin D</b>	<b>0.71 ±0.04</b>
<b>Vitamin E</b>	<b>0.25 ± 0.01</b>
<b>Vitamin K</b>	<b>0.11 ±0.32</b>
<b>Beta, carotene</b>	<b>089 + 0.02</b>

The data are mean values ± standard deviation (SD) of three replicates: n =3



### Vitamins

**Fig 2: A bar chart showing the concentration of vitamins present in *Vitex doniana* leaf.**

### DISCUSSION

Table 1 shows the result of vitamin concentration of ethanolic extract of *Vitex doniana*. The vitamin A ( $1.52 \pm 0.61$ ) B1 ( $0.73 \pm 0.03$ ) B2 ( $0.73 \pm 0.03$ ) B3 ( $0.60 \pm 0.04$ ) B6 ( $0.19 \pm 0.01$ ) B9 ( $0.15 \pm 0.11$ ) vitamin C ( $1.45 \pm 0.1$ ) D ( $0.71 \pm 0.04$ ) E ( $0.25 \pm 0.01$ ) K ( $0.11 \pm 0.32$ ) Beta carotene ( $0.89 \pm 0.02$ ).

Vitamin A was highest in concentration followed by vitamin C which is also high in concentration and also Beta carotene which is also high. While vitamin K has the lowest concentration.

It can be concluded from this study that leaves of *Vitex doniana* contains vitamin which include vitamin A (Retinol) vitamin B (thiamine), vitamin B2 (Riboflavin), vitamin B6 (pyridoxine), vitamin B9 (folic acid), vitamin C (ascorbic acid), vitamin D, vitamin E,

### RECOMMENDATION

Since the leaves of the ethanol extract investigated in this study contained considerable amount of important nutrients, it is suggested that they should be taken as food or added to food as condiments to supplement

The value obtained for vitamins in the sample investigated revealed that the values shows a close agreement with these obtained by [12].

The sample which contain B1, B2, and B3 have been reported to be highly essential for micronutrient metabolism while vitamin C is used for protein metabolism and collagen synthesis [13]. Vitamin A and beta carotene while vitamin E is essential for the production of enzymes and hormones needed for proper growth and development [14].

### CONCLUSION

vitamin K and beta carotene. The leaves contain high level of active biological substance such as (flavonoid, tannins, saponins) which has great potential use. Vitamins extracted from *Vitex doniana* are found to have antioxidant properties.

minerals and vitamins especially among the rural dwellers with low income. It is also recommended that government and cooperate bodies should embark on plantation establishment of these species for sustainable production.

REFERENCES

1. Keay, R.W.J (2011). Trees of Nigeria. Oxford university of press, New York, USA 191 pp.
2. Lagnika, L.G. and Hanlt, R.Z. (2012). Use of vegetable as n nutritional food role in human health. *Journal of Agric Biology Science* 1: 18-28
3. Madisa, M.E; Assefa, Y. And Obofule, M. (2010). Crop diversity extension services and marketing of vegetable in botsiwana. *Egypt Acad J. Biology Science* 1(11): 13-22.
4. Mohammed, M.I. and Sharif, N (2011): mineral composition of some leafy vegetables consumed in Kano Nigeria. *Nigeria Journal of basic of basic and Applied Science*. 19(2), 208-211.
5. Muzychkina, R.A (2007). Natural anthraquinones: Biological and physiological properties, G.A. tolstixov, Ed. Phasis, Moscow, Russia.
6. Olaleye, M.T. (2007). "Cytotoxicity and antibacterial activity of ethanolic extract of vitex doniana leaf", *Journal of Medical Plants Research* 1, 1.9-13.
7. Ononugbu, I.C (2010): Lipids in human existence 1<sup>st</sup> Edition A.P express Publishing company. Nsukka. Nigeria. 1-15.
8. Pottarat, (1997). "Antioxidants and free radical scavengers of natural origin", current organic chemistry, 1(4): 415-440.
9. Sies, H. (2011). "Oxidative stress: oxidants and antioxidants" experimental physiology 82 (2): 291-295.
10. Soetain, K.O (2008) "pharmacological and other beneficial effects of anti-nutritional factors in plants- a review, African Journal of Biotechnology, 7(25) 47 13-4721.
11. Sofowora. A.E. (2013). The state of medical plants in Nigeria, University of Ibadan, Ibadan Ni.geria.
12. Vardarajan, P. Rathinas G. wamy, D (2011). Asirtochemical constutents of Rheodiscolor", Ethanobotanical leaflet, 12,841-845.
13. Vnchi, M.A., Umar, A.M., king, M.A; Liman, A.A. Jeremiah, G. And Aigbe, C. O (2011). Proximate vitamins and minerals composition of vitex doninna (black plum) fruit PUPP Nig. *Journal. Of basic and Applied Science*. 19 (1): 97-101.
14. Yakubu, O.E., Ojogbane, E., Nwodo Ofe, Nwanevi- Chidozie V,0., Dasofim, J.O.K. (2013) Comparative antioxidant and hypoglycaemic effects of aqueous, ethanol and n-hexane extracts of leaf of uced dia betes in abino rats. *African Journal of Biotechnology*.: 12 (40): 5933-5940.