

## Phytochemical and Gas Chromatography-Mass Spectrophotometric (GC-MS) Analyses of *Vitex doniana* Leaf from Abakaliki, Ebonyi State.

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**Abstract:** The phytochemical and GC-MS analyses of *Vitex doniana* leaf were carried out by standard methods. The results of the phytochemical constituents showed that alkaloids had the highest concentration of (491.13±5.09%) in the sample while other phytochemical constituents determined were tannins, total phenol, glycoside, flavonoids and saponins (4.81±0.047%) being the least in leaf of *Vitex doniana*. Ten (10) GC-MS constituents were also identified in methanol leaf extract of *Vitex doniana* with 6-Octadecenoic acid (24.19%) as the major and 1-tridecyne (2.4%) as the least constituents respectively. These relatively diverse phytochemicals and GC-MS constituents may be responsible for the medicinal properties of *Vitex doniana*.

**Key words:** *Vitex doniana* leaf, Phytochemicals, screening, GC/MS constituents

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### I. Introduction

The use of plants for medicinal purposes started with life. Plants have been used in the treatment of variety of diseases and the introduction of the orthodox medicine did not affect their use (Chan et al., 2006). Plants are known to have sustained mankind not only as source of food but also as medicines (Abdu-Aguye, 1997). Herbal medicine is the study and use of plants for medicinal purposes (Abdu-Aguye, 1997). Modern medicine recognizes herbal medicine as a form of alternative medicine. Phytochemical chemistry is the study of variety of organic compounds present in plant and the chemical structure of the compound (Johns, 1996). It is a non nutritive compound that contributes to the characteristics of plant like flavour, texture and colour (Johns, 1996; Craig, 1991). Majority of secondary metabolites are antioxidant thereby reducing the risk of many diseases.

*Vitex doniana* belongs to the family of Verbenaceae. The plant is commonly called black plum while is locally known as 'Uchakiri' in Igbo, 'Dinya' in Hausa, 'Oori-nla' in Yoruba, 'Tinya' in Fulani, 'Vabga' in Ghana, 'Mfuru' in Tanzanian, 'Muhonozi' in Uganda (Atawodi et al., 2003). This plant is a shrub which grows into a tree in open wood land and Savannah regions of tropical Africa. Various parts of the plant are used by traditional medicinal practitioners in Nigeria in the management and treatment of various disorders. They can be used in treatment of inflammation, dysentery, diarrhoea, improve fertility and eye problem.

With recent drop in the price of crude oil in the international market and its attendant effect on purchasing power of less developed nations, it has become vivid that medicinal plants will play increasing role in the food, nutrition and health security of the rural populace and the increasing urban poor. As popular as this medicinal plant is in Nigeria, there is a paucity of information on GC-MS analysis of *Vitex doniana* leaf. This study therefore evaluates the phytochemical and GC-MS analyses of the methanol leaf extracts of the plant leaf.

### II. Materials and Methods

This study was conducted in March, 2014 in Biochemistry Department, Ebonyi State University, Abakaliki. The leaf of *Vitex doniana* were collected from Outskirt Abakaliki in Ebonyi State and were identified and authenticated by a taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki.

**Preparation of plant leaf:** Fresh leaves of *Vitex donian* were collected, washed and dried at ambient temperature (25°C). After drying, the leaves materials were pulverized using electric blender. Leaves powder were stored in refrigerator in well labelled, air tight container prior for analysis.

#### Quantitative Phytochemical Analysis of *Vitex donian*

The phytochemical constituents of the samples were carried out by the methods modified by the following

#### **Determination of Flavonoids:**

This was determined by the method of Harborne, 1973.

**Principle:** Flavonoids reacts with dilute ammonia (NH<sub>3</sub>) to produce a coloured complex which can be measured spectrophotometrically at 470nm.

**Procedure:** 1g of the samples each were macerated with 20mls each of ethylacetate for 5mins, 5mls each were transferred into a triplicate tubes, and 5mls of dilute ammonia (NH<sub>3</sub>) each were added and stirred for 5mins and allowed to stand for some time. The lower layers were collected and the absorbance was read at 470nm against dilute ammonia.

#### **III. Determination of Total Phenols:**

This was determined by the method of Malick and Singh, 1980.

**Principle:** Phenols react with phosphomolybdic acid in folin-ciocalteu reagent in alkaline medium to produce a blue coloured complex (molybdenum blue) which can be estimated spectrophotometrically at 650nm.

**Procedure:** 1g of the sample was macerated with 20mls of 80% methanol for 10mins and centrifuged for 5mins. Then, 1ml of the supernatant was transferred into triplicate tubes. In the tubes 4mls of distilled water and 0.5ml of folin-ciocalteu were added and mixed properly. After 5mins 2mls of 20% Na<sub>2</sub>CO<sub>3</sub> (sodium carbonate) was also added and stirred and allowed to stand for 30mins. The absorbance was taken at 650nm against the blank. The same procedure was repeated with the second sample.

#### **IV. Determination of Alkaloids:**

This was determined using the method of Harborne, 1973.

**Principle:** H<sub>2</sub>SO<sub>4</sub> reacts with alkaloids in the presence of formaldehyde to form a coloured complex which is read spectrophotometrically at 565nm.

**Procedure:** 1g of the sample was macerated with 20mls of methanol and 20% sulfuric acid at the ratio of 1:1 (i.e. 10ml of each) for 5mins and centrifuged for 5mins. Then, 0.5ml of the supernatant was transferred into triplicate tubes. In the tubes 2.5mls of 60% sulfuric acid was added and stirred. After 5mins, 2.5 ml of 0.5% formaldehyde in 60% sulfuric acid was added and allowed to stand for 3hrs. The absorbance was taken at 565nm against the blank. The same procedure was repeated with the second sample.

#### **V. Determination of Tannins:**

This was determined by the method of Harborne, 1973.

**Principle:** Tannins reduce phosphotungstomolybdic acid in alkaline solution to produce highly coloured blue solution, the intensity of which is proportional to amount of tannins. The intensity is measured in spectrophotometer at 720nm.

**Procedure:** 1g of the sample was macerated with 20mls of methanol for 10mins and centrifuged for 5mins. Then, 5mls of the supernatant was transferred into triplicate tubes. In the tubes, 0.3ml of 0.1molar ferric chloride in 0.1molar HCl was added and stirred. Then 0.3ml of 8/10000 molar potassium ferricyanide was added and mixed, and stood for 5mins. The absorbance was taken at 720 nm against the blank. The same procedure was repeated with the second sample.

#### **Determination of Saponins:**

This was determined by the method of Harborne, 1973.

**Principle:** Saponins react with anisaldehyde and ethylacetate to give a coloured complex which is read spectrophotometrically at 430nm.

**Procedure:** 0.5g of the sample was macerated with 10mls of methanol for 10mins and centrifuged for 5mins; 2mls of the supernatant was transferred into triplicate tubes. The tubes were placed in water bath to evaporate the methanol and allowed to cool. Then, 2mls of ethylacetate and 1ml of 0.5% anisaldehyde in ethylacetate and 1ml of 5% H<sub>2</sub>SO<sub>4</sub> in ethyl acetate were added and placed in a hot water bath at 60°C for 20mins and allowed cool in cold water for 10mins. The absorbance was taken at 430nm. The same procedure was repeated with the second sample.

#### **VI. Determination of Glycosides**

About 5 g of the sample was macerated with 100 ml of distilled water for 2 hours, it was filtered. 1 ml of the filtrate was pippered into three test tubes and 2 ml of 3,5 DNS was added and boiled for 15 minutes and allowed to cool. The absorbance was read at 540 nm (Oloyede, 2005)

## VII. GC-MS Analysis

### Procedure:

C-MS analysis of the methanol extract of *Vitex donia* leaves and seeds were performed using Shimadzu Japan gas chromatography QP2010PLUS with a fused GC column (2010) coated with polymethyl silicon (0.25mm x 50m) and the conditions were as follows: Temperature programming from 80–200<sup>o</sup>C held at 80<sup>o</sup>C for 1 min, rate 5<sup>o</sup>C/min and at 200oc for 20 min. Field ionization detector (FID) temperature 300<sup>o</sup>C, injection temperature 220oc, carrier gas nitrogen at a flow rate of 1 ml/min, split ratio 1:75. Gas chromatography mass spectrum was conducted using GCMS –QP 2010 Plus Shimadzu Japan with injector temperature of 220<sup>o</sup>C and carrier gas pressure of 116.9 kpa.

The column length is 30 m with a diameter of 0.25 mm and flow rate of 50 ml/min. The elutes were automatically passed into a mass spectrometer with a dictator voltage set at 1.5 kv and sampling rate of 0.2 sec. The mass spectrum was also equipped with a computer fed mass spectra data bank. Hermlez 233 M-Z centrifuge (Germany) was used.

## VIII. Component Identification:

Chemical constituent components of the extracts were identified by matching the peaks with Computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature.

This involves two analytical techniques used to expose more of the bioactive compound present in the extracts.

**Statistical Analysis:** Statistical analysis was done using mean standard deviation.

## IX. Results and Discussion

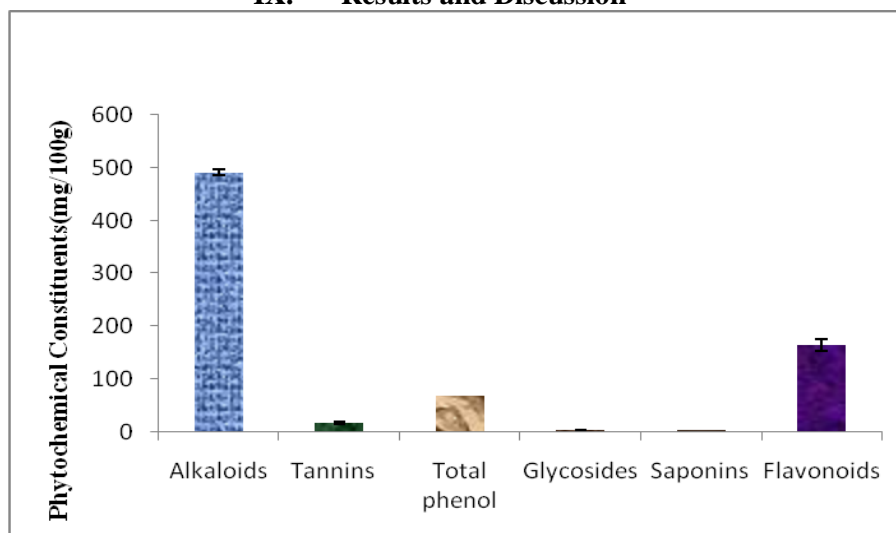


Figure 1: Phytochemical Constituents of *Vitex doniana*

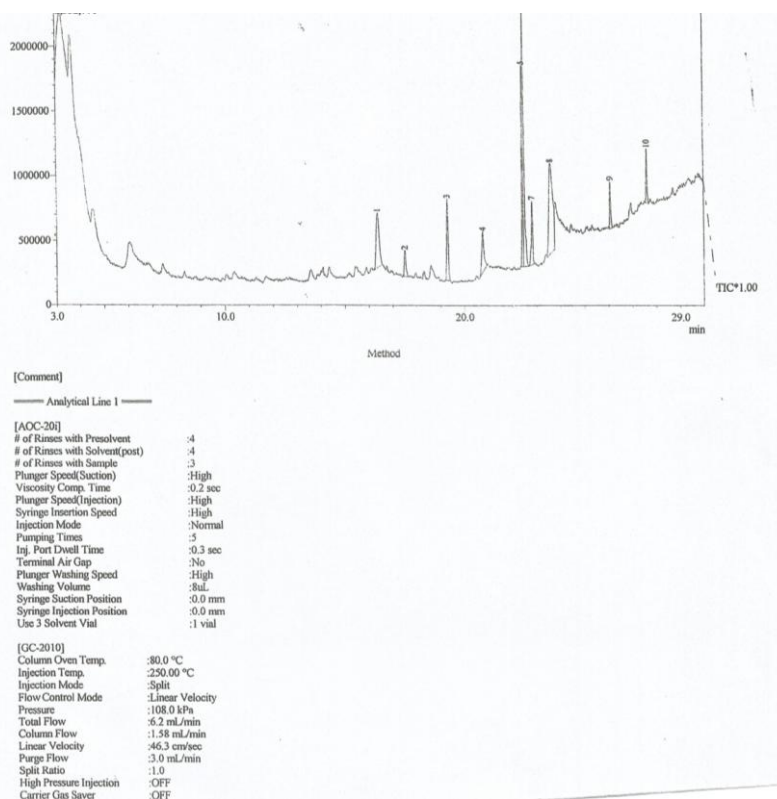


Figure 1: GC-MS Chromatogram of *Vitex doniana* Methanol Leaf extract

**GC-MS Spectral Analysis of Methanol Extract in *Vitex doniana* leaf indicating peaks and retention time and Percentage content.**

S/N	Peak compound	Molecular formula	Molecular weight	Retention time	Percentage content	Base peak
1	1-chloro-3-methyl-butane	C <sub>5</sub> H <sub>11</sub> Cl	106	16.370	15.73	43.05
2	1-Tridecyne	C <sub>13</sub> H <sub>24</sub>	180	17.520	2.4	43.05
3	14 methylpentadecanoic acid	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	10.304	7.66	74.05
4	Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	20.783	8.06	43.05
5	9,12-Octadecadienoic acid	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	22.453	9.27	41.05
6	6-Octadecenoic acid	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296	22.505	24.19	41.05
7	Octadecanoic acid	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298	22.835	6.05	74.05
8	Oleic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	23.595	16.94	41.05
9	Octylether	C <sub>16</sub> H <sub>34</sub> O	242	26.090	9.44	57.05
10	2,2,4-Trimethylpentylvinyl ether	C <sub>10</sub> H <sub>20</sub> O	156	27.631	6.05	57.05

The result of quantitative phytochemical analysis of *Vitex doniana* leaf revealed that *Vitex doniana* leaf is rich in phytochemicals (bioactive compounds) with alkaloids (491.13±5.095mg/100g) as the highest constituent, which followed by flavonoids (164.58± 11.815mg/100g) and the least was saponins (4.81±0.047 mg/100g) as shown in Figure 1. This result is correlation with the findings of Aja et al. (2010 and 2015).

The various phytochemical compounds detected are known to have medicinal values. According to Aja et al. (2010) and Aja et al. (2015) phytochemical components are responsible for both pharmacological and toxic activities in plants. Some of these metabolites are said to be useful to both animal and plant itself. But some could be toxic to animals, including man at higher concentration (Aja et al., 2015). Alkaloids are good in treatment of malaria, hypertension, tumors and mental disorders and used as analgesics, It can also be use as pain relievers (morphine) but its additive properties limit its usefulness. These compounds have antimicrobial properties due to their ability to intercalate with DNA of microorganisms (Kasolo et al., 2010).

Tannins have anti-bacterial and astringent properties which have action upon mucous secreting tissues such as tongue and inside mouth. The astringency from the tannins is that which causes the dry feeling in the mouth following the consumption of red wine, strong tea, or an unripe fruit (Mann et al., 2011). Tannins may be employed medicinally as anti-diarrheal and anti-haemorrhoidal compound. They bind to compound such as

fibre, protein and prevent their digestibility and subsequently reduce iron growth rate of the animals (Huang et al., 2010).

Saponins bind to bile salt and cholesterol in the intestinal tract. Bile salts from small micelles with cholesterol facilitate its absorption. Saponin causes a reduction of blood cholesterol by preventing its re-absorption (Anwar et al., 2003). Heffman (1997) reported that saponins inhibit sodium ion ( $\text{Na}^+$ ) efflux by the blockage of the entrance of  $\text{Na}^+$  -  $\text{Ca}^{2+}$  anti-porter in cardiac muscle, which strengthens the contraction of heart muscle. Considering diosgenin a type of saponin which is found to resemble cholesterol in structure and thus interfere with both dietary and endogenous cholesterol absorption, thus leading to increased rate of hepatic and intestinal cholesterol synthesis (Cayen and Dvornick, 1979). Diosgenin also markedly enhance cholesterol secretion into bile which in conjunction with unabsorbed cholesterol, resulted in increased fecal excretion of cholesterol without excretion of bile acid (Cayen and Dvornick, 1979). It can be used as anti-cancer, anti-inflammatory, antioxidant, hypoglycaemia and weight loss.

Flavonoids have antioxidant activity and can reduce the risk of many diseases. Reactive oxygen – free radicals (ROS), have been implicated in many diseases and in ageing process, tissue damage are generated by aerobic respiration, inflammation, lipid per-oxidation. Flavonoid which is antioxidant minimizes or prevents deleterious effects of the ROS (Valko et al., 2007). Oxidation of LDL-cholesterol has been recognized to play an important role in atherosclerosis. Immune systems cells recognize and engulf oxidized LDL- cholesterol a process that lead to the formation of atherosclerosis plaques in the arterial wall. Flavonoids can directly react with superoxide anions and lipid peroxy radical and consequently inhibit or break the chain of lipid per-oxidation (Aja et al., 2010).

Phenol and glycoside have antioxidant properties. The antioxidant activity of phenol could be attributed to their redox properties, presence of carboxylic group which have reported to inhibit lipid per-oxidation.

Results of GC-MS analysis of *Vitex doniana* leaf showed ten different peaks which corresponded to ten compounds as shown in the chromatogram in Figure 2. The result showed that 14- methyl-pentadecanoic acid (74.05%) had the highest percentage composition and oleic acid (41.05) had the least as shown in Table 1. The findings were in correlation with Aja et al. (2014) which reported sixteen different GC-MS constituents in

*Moringa oleifera* leaf. The constituent compounds in the extract are long chain aliphatic carboxylic acids, (saturated and unsaturated) and their derivatives including alcohols, aldehydes as well as benzene carboxylic acid esters and a steroidal compounds. The presence of fatty acids and their derivatives in *Vitex doniana* leaf extract dictates the pharmacological properties of the plant. Fatty acids and alcohols in the plant undergo esterification reaction to form esters. One or both of the oxygen atoms of carboxylic acid can be replaced by sulphur giving a thio acid or dithio acid respectively. Thio acids react readily with alcohols to form thio-esters. Thio-esters play an important part in the break down and synthesis of lipids and steroids in living tissues. Carboxylic acids are transferred from one enzyme reaction to another as thioesters of the complex thiol, Co enzyme A (CoA-SH). The thio-ester of benzoic acid with Co-enzyme A is the form in which acetate enters the sequence of enzyme catalyzed reactions which results in the synthesis of fatty acids and glycerides (Okwu and Ighodaro, 2010).

Oleic acid (18:0) is the most abundant monounsaturated fatty acid (MUFA) in the human body (Aja et al., 2014). The health benefits of oleic acid are broad and profound. Numerous studies have shown that consumption of MUFAs is important to maintain low levels of LDL in the blood and is also likely to be associated with the potential for elevated high density Lipoprotein (HDL-C) (Ogunleshi et al., 2010). Oleic acids are among the fatty acid known to have potential antibacterial and antifungal properties (Ogunleshi et al., 2010). Free fatty acids include long chain  $\text{C}_{16}$ - $\text{C}_{20}$  unsaturated fatty acids were suggested to be responsible for the anti-inflammatory activity (Ogunleshi et al., 2010). Some of other compound like octal ether- 2,2,4-Trimethylpentyl vinyl ether are groups of ether group which act as anaesthetics, it has also demonstrated that they protect mammalian cells against the damaging effect of reactive oxygen species (Nagan and Zoeller, 2001) Also dodecanoic acid, are among the fatty acid known to have potential antibacterial and antifungal properties (Ogunleshi et al., 2010).

## X. Conclusion

The presence of the identified bioactive compounds makes this plant pharmacologically active. These bioactive compounds may be responsible for their usefulness in the management and treatment of various diseases. We believe that these compounds in *Vitex doniana* leaves could be harnessed for industrial and medicinal sciences utilization.

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