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GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC (GC-MS) ANALYSIS OF ETHANOL LEAF-EXTRACT OF *VIGNA UNGUICULATA* (COWPEA)

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ABSTRACT

*Vignaunguiculata*leafis a common vegetable in Nigeria. The GC-MS analysis was carried out using standard method. The result revealed the presence of 15 chemical constituents which includes oct-2-ene (24.68%), nona-3,5-diene (18.86%), non-4-ene (14.02%), octadecanoic acid (12.93%) and hexadecanoic acid (11.45%) as the major chemical constituents. The presence of these various bioactive compounds may be responsible for the use of *Vignaunguiculata* leaf in ethno-medicine.

Key Words: GC-MS analysis, Chemical constituents, *Vignaunguiculata*leaf, ethanol extract

INTRODUCTION:

Vignaunguiculata(Cowpea) is a proteinous food which serves as staple in Nigeria, other African countries and all over the world. It is recognized as a good source of plant protein. Different cultivars are available and differ in seed, shape, size, colour and tastes [1]. It is readily available and relatively cheap. The three ethnic groups in Nigeria call it different names, “Iwake” in Hausa, “Ewa” in Yoruba and “Agwa” in Igbo. *Vignaunguiculata* is one of the ancient crops for man and animal consumption [2]. *Vignaunguiculata* originated in Africa, America and southern United States. The crop is trace to ancient West Africa cereal farmers 5 to 6 thousand years ago where it was closely associated with the cultivation of sorghum and millets. In some countries they cultivate the crop primarily for seed and also as a vegetable [3].

Vignaunguiculata serves as a nutrient component of the human diet and as a good component of livestock feed. The leaves equally serve as good sources of vegetable, while several snacks and man meal dishes; especially African dishes are prepared from the grain of cowpea leaf. Vitamin is the one of the major components from cowpea especially vitamin B 6 and minerals [4].

It is widely cultivated throughout the world, particularly in the developing countries, like Asia and Africa where plant protein comprise of 83% of recommended diet [5]. Cowpea constitutes a major important food legume in the world [6].

Despite numerous researches work done in *Vignaunguiculata*seedand fruitno documented data/information has been done on the GC-MS constituents of the leaf. Though, with recent drop in the price of crude oil in the international market and its attendant effect on economy of less developed nations like Nigeria, it has become vivid that medicinal plants/ staple crops will play increasing role in the food, nutrition and health security of the rural populace and the increasing urban poor. As popular

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as*Vignaunguiculata is* in Nigeria, there is a paucity of information on GC-MS analysis of the leaf .This study therefore evaluates GC-MS analysis of the ethanol leaf extract of*Vignaunguiculata* leaf.

Figure 1: *Vignaunguiculata*Leaf

Materials and Methods
Materials

Plant Collection:

The fresh leaves of *Vignaunguiculata*werecollected by hand picking in the month of October, 2014 from Agubia village in Ikwo Local Government of Ebonyi State, Nigeria. The collected fresh leaves of*Vignaunguiculata*were identified and authenticated byDr.Mrs. K. Nnamani of Applied Biology Department,Ebonyi State University, Abakakliki, Nigeria. Some parts of the plant were also deposited in the herbarium for reference purpose.

Preparation of Plant Sample

The leaves were destalked, washed and shade dried at ambient temperature with constant turning to averts fungal growth. The dried leaves were later milled to obtained the vegetable leaf meals (VLMs) using an electric blender and was stored in 4°C temperature in refrigerator in well labeled air-tight containers for analysis.

Preparation of *Vignaunguiculata*Ethanol Leaf-Extract

Exactly 40grams of dried powdered leaves of *Vignaunguiculata*were extracted successively with 300ml of ethanol in an orbital shaker for 24hours at room temperature. The extract was filtered using what-man No.1 filter paper to remove extractable substances at every 3hrs interval. The combined extracts were then evaporated with rotary evaporator and the dried extracts were stored at 4°C in air-tight sterile container in refrigerator.

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Methods
GC-MS Analysis:

Procedures:

GC-Ms analysis of the ethanol extract of *Vignaunguiculata*leaf was performed using Shimadzu Japan gas chromatography QP2010 plus with a fused gas chromatography (GC) column (2010) coated with poly-methyl silicon (0.25nm x 50m) and the conditions were as follows: Temperature programming from 80-200°C held at 80°C for 1minute, rate 5°C/min and at 200°C for 20min. Field ionization detector (FID) Temperature of 300°C, injection temperature of 220°C, carrier gas nitrogen at a flow rate of 1ml/min, split ratio of 1:75. Gas chromatography mass spectrum was conducted using GCMS-QP 2010 plus Shimadzu Japan with injector temperature of 220°C and carrier gas presence of 116.9kpa. The column length is 30m with a diameter of 0.25mm and flow rate of 50ml/min. 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 bank. German Hermlez 233M-Z centrifuge was used.

Component Identification

Chemical constituent of the extract was identified by matching the peak with computer Wiley Ms libraries and confirmed by those comparing mass spectra of the peaks and those from literature **Results**

Result of GC-MS Analysis of *Vignaunguiculata* Leaf.

The result of GC-MS analysis revealed 15 peaks from the Chromatogram (Figure 2). These peaks (1-15) indicate the presence of 15 compounds (1-15) in the extract (Table 1). The molecular formula, percentage content and molecular mass of the compounds are shown in Table 1. These compounds comprise mainly hydrocarbons, fatty acids, alcohols, esters and phenols. The composition of the extract comprises; oct-2-ene (24.68%), nona-3,5-diene (18.86%), non-4-ene (14.02%), octadecanoic acid (12.93%) and hexadecanoic acid (11.45%) as the major chemical constituents (Table 2).

Table 2:GC-MS Analysis And Mass Spectral Data Of Extracts From The Leaf of *VignaUnguiculata*Showing Molecular Formula, Molecular Weight, Percentage Content, Retention Time And Base Peak.

PEAK	COMPOUNDS	MOLCULAR FORMUAR	MOLECULAR WEIGHT	RETENTION TIME	PERCENTAGE CONTENT	BASE PEAK
1	1-ethyl-3-methylcyclopentane	C_8H_{16}	112	3.092	0.3%	55.00
2	Non-4-ene	C_9H_{18}	126	3.242	0.35%	43.05

3	Ethylbenzene	**C_8H_{10}**	**106**	**3.508**	**0.84%**	**91.05**
4	Propan-2-ylcyclohexane	**C_9H_{17}**	**125**	**3.617**	**0.71%**	**43.00**
5	Ethylbenzene	**C_8H_{10}**	**100**	**3.800**	**0.64%**	**91.05**
6	(1-Methylethyl)benzene (cumene)	**C_9H_{12}**	**120**	**5.142**	**0.59%**	**105.10**
7	Oct-2-ene	**C_8H_{15}**	**111**	**13.067**	**2.4%**	**41.00**
8	Oct-2-ene	**C_8H_{15}**	**111**	**13.217**	**1.71%**	**41.00**
9	Oct-2-ene	**C_8H_{15}**	**111**	**15.667**	**3.92%**	**41.00**
10	Non-4-ene	**C_9H_{18}**	**126**	**19.317**	**6.64%**	**43.05**
11	Hexadecanoic acid	**$C_{16}H_{32}O_2$**	**256**	**19.633**	**11.45%**	**43.05**
12	Oct-2-ene	**C_8H_{15}**	**111**	**22.317**	**24.68%**	**41.00**
13	Octadecanoic acid	**$C_{18}H_{36}O_2$**	**284**	**22.550**	**12.92%**	**43.05**
14	Non-4-ene	**C_9H_{18}**	**126**	**24.567**	**14.02%**	**43.05**
15	Nona-3,5-diene	**C_9H_{16}**	**124**	**26.408**	**18.86%**	**43.05**

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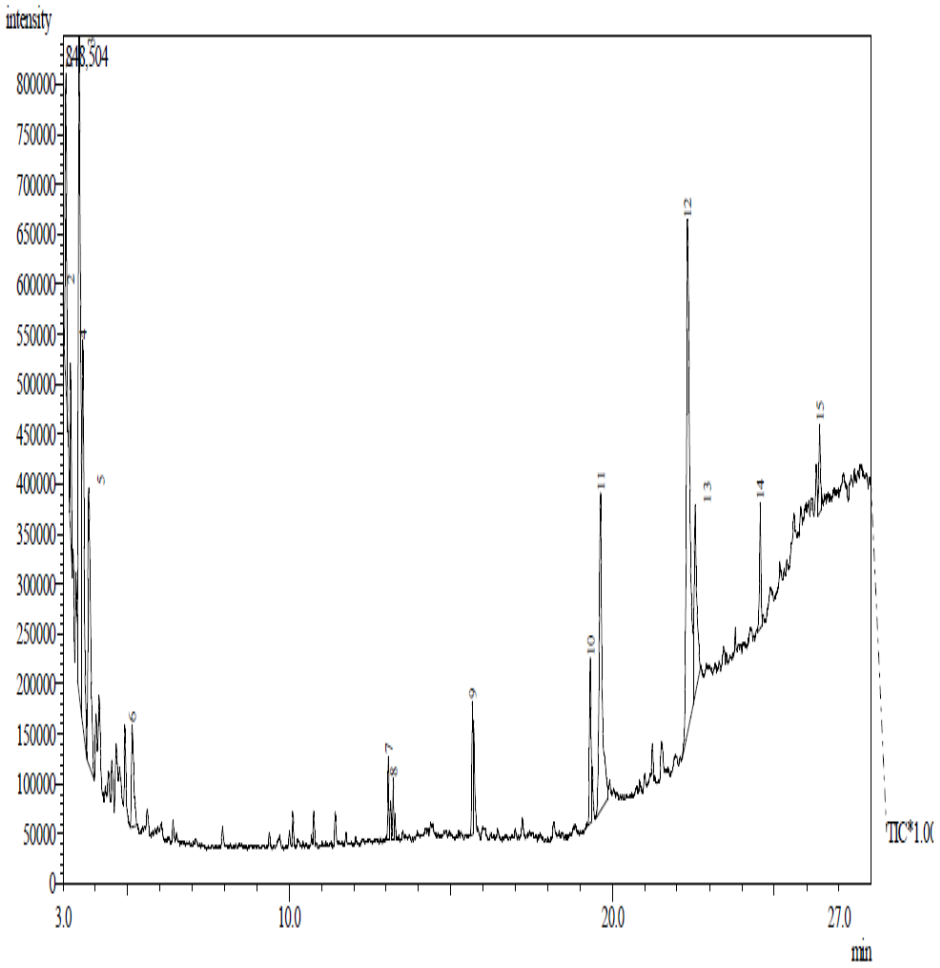

Figure 2: GC/MS Chromatogram of *V.unguiculata*EthanolLeaf-Extract

Discussion:

In this study the chemical profiles of *Vignaunguiculata*werewe characterized using GC-MS analysis. The chromatogram showed the relative concentration of various compounds getting eluted as a function of retention time. The heights of the peaks indicate there are high concentrations of the components present in the plant. In addition to this the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant. The result of the present study supported the previous reports of Aja*et al.* (2014) [5] and Nweke*et al.* (2015) [6] on *Moringaoleifera* and *Vitexdoniana leaves* respectively. The study also conformed to the report of Uraku*et al.* (2015) [7] on the G.C-MS constituents of essential oil from*Hyptisspicigera*leaf

GC-MS chromatogram of the *Vignaunguiculata* leaf showed 15 peaks indicating the presence of 15 chemical constituents which includes oct-2-ene, non-4-ene, nona-3,5-diene, octadecanoic acid, 1-ethyl-3-methylcyclopentane and hexadecanoic acid. 1-ethyl-3-methylcyclopentane is a phenolic compound and it acts as an antioxidant, antimicrobial, antifungal and anti-inflammatory agents. Non-4-ene is a fatty acid

and it an active anti-microbial and anti-diarrhea agents. Ethylbenzene is an aliphatic alcoholic compound and it used as an antimicrobial.

Conclusion

GC-MS analysis *Vignaunguiculata* leaf showed the existence of various compounds with different chemical structures. The presence of various these bioactive compounds confirms application of *Vignaunguiculata*for various ailments by traditional practitioners.

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