



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

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### ABSTRACT

*Vigna unguiculata* leaf is 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 *Vigna unguiculata* leaf in ethno-medicine.

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

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### INTRODUCTION:

*Vigna unguiculata* (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. *Vigna unguiculata* is one of the ancient crops for man and animal consumption [2]. *Vigna unguiculata* 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].

*Vigna unguiculata* 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 B6 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 *Vigna unguiculata* seed and fruit no 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

as *Vigna unguiculata* 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 *Vigna unguiculata* leaf.



Figure 1: *Vigna unguiculata* Leaf

## Materials and Methods

### Materials

#### Plant Collection:

The fresh leaves of *Vigna unguiculata* were collected 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 *Vigna unguiculata* were identified and authenticated by Dr. 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 avert fungal growth. The dried leaves were later milled to obtain 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 *Vigna unguiculata* Ethanol Leaf-Extract

Exactly 40 grams of dried powdered leaves of *Vigna unguiculata* were extracted successively with 300 ml of ethanol in an orbital shaker for 24 hours at room temperature. The extract was filtered using Whatman No. 1 filter paper to remove extractable substances at every 3 hours 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.



## Methods

### GC-MS Analysis:

#### Procedures:

GC-MS analysis of the ethanol extract of *Vigna unguiculata* leaf was performed using Shimadzu Japan gas chromatography QP2010 plus with a fused gas chromatography (GC) column (2010) coated with poly-methyl silicon (0.25mm x 50m) and the conditions were as follows: Temperature programming from 80-200°C held at 80°C for 1 minute, rate 5°C/min and at 200°C for 20 min. 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 pressure 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 detector 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 Hermle Z 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 *Vigna unguiculata* Leaf.

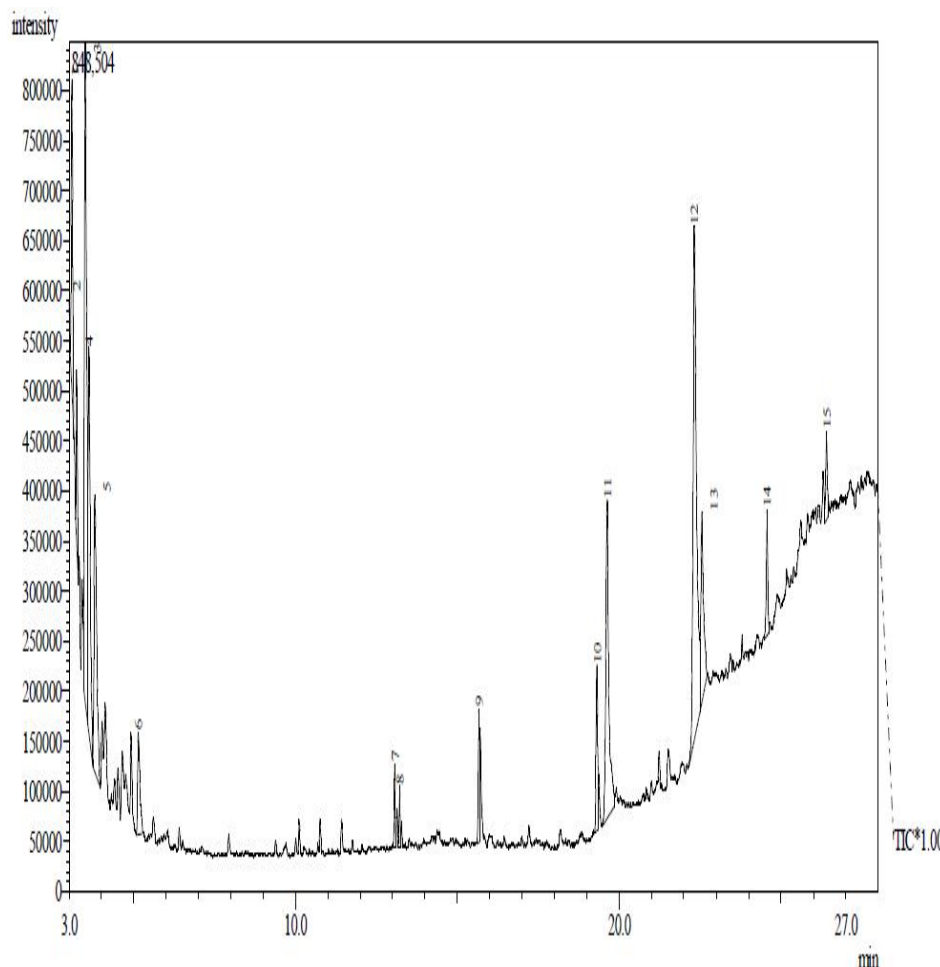
The result of GC-MS analysis revealed 15 peaks from the Chromatogram (Figure 2). These peaks (1-15) indicate the presence of 15 compounds (1-15) in the extract (Table 1). The molecular formula, percentage content and molecular mass of the compounds are shown in Table 1. These compounds comprise mainly 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 *Vigna unguiculata* Showing Molecular Formula, Molecular Weight, Percentage Content, Retention Time And Base Peak.**

PEAK	COMPOUNDS	MOLECULAR FORMULAR	MOLECULAR WEIGHT	RETENTION TIME	PERCENTAGE CONTENT	BASE PEAK
1	1-ethyl-3-methylcyclopentane	C <sub>8</sub> H <sub>16</sub>	112	3.092	0.3%	55.00
2	Non-4-ene	C <sub>9</sub> H <sub>18</sub>	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



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

### Discussion:

In this study the chemical profiles of *Vigna unguiculata* were 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 Ajaet *al.* (2014) [5] and Nweke *et al.* (2015) [6] on *Moringa oleifera* and *Vitex doniana* leaves respectively. The study also conformed to the report of Urakuet *al.* (2015) [7] on the G.C-MS constituents of essential oil from *Hyptis spiciger* leaf

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

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