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PHYTOCHEMICAL EVALUATION BY GC-MS ANALYSIS OF THE SEEDS OF *MUCUNA FLAGELLIPES* EXTRACT.

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Abstract

Mucuna flagellipes seeds were analyzed for essential oil composition. The ethanol extract of the seeds was subjected for GC-MS studies. Ten phyto-constituents were identified from *M. flagellipes* comprising 60 % of esters and 30 % of carboxylic acids and 10 % hydrocarbon. 9-octadecenoic acid, ethyl ester constitutes the bulk of the oil (18.66 %), followed by linoleic acid, ethyl ester (16.99 %) and a carboxylic acid, 9-octadecanoic acid (16.71%). Other Compounds identified in the seeds were hexadecanoic acid (11.14 %), ethyl hexadecanoate (14.76 %), ethyl octadecanoate (4.18 %), Eicosanoic acid (1.39 %), ethyl icosanoate (0.84%), 3-hydroxypropyl-9-octadecenoate (1.11 %) and 9-methylbicyclo (3,3,1) nonane (14.21%). The presence of many esters in the seeds may be the reason the seeds enhance the aroma of soup. These compounds present in the plant made it possible for the use of *M. flagellipes* seed extract in phytomedicine in Nigeria for the treatment of different ailments.

Key words : Mucuna, ester, phytomedicine, ailment.

Introduction

Mucuna flagellipes is popularly known and called “Ukpo” by the Igbo tribe of Nigeria. It occurs naturally in Sierra Leone to Nigeria, Zaire and Uganda (Keay et al, 1989).

M. flagellipes is a tropical forest climbing perennial herb. The plant is up to 12m long by 3cm diameter at base of riverine and swamp forest (Keay et al, 1989). It produces long, fairly longitudinal ribbed pods which are covered with brownish yellow hairs. The pods are green when immature but black when dry and mature. Each pod contains one to four seeds (Okwu and Okoro, 2007; Enwere 1998). The seeds can be used either when they are fresh and tender or when dry. The seeds of *Mucuna flagellipes* when mature are broken to remove testa and processed into flour which can then be used as soup thickener, flavor and stabilizer. Besides, being used as soup thickners, they can be used as additives in other foods to impart desirable textural and functional properties such as enabling fast coagulation in food preparation of the different finished products particularly the “convenience foods” which contain one or more gums (Uzomah and Ahiligwo,1999, Okorie et al, 2013). It is among the food thickeners consumed in South Eastern South Nigeria (Ene- obong 2001; Okwu and Okoro, 2007).

In herbal medicine, a decoction of this plant is taken to arrest diarrhea and headache. It is used in Nigerian Medicine to expel tapeworm (Keay et al, 1989).

In spite of the various uses of this plant in food and as medicine, much work has not been done on the plant. The bioactive constitutes of the seeds have not been fully documented. Thus this present research will examine the essential oil present in the seeds to assess their potential usefulness as food supplement and pharmaceutical raw material for drug production.

MATERIALS AND METHOD:

Plant Materials: The seeds of *M. flagellipes* were bought from Ndoro market in Ikwuano L.G.A, both in Abia state, Nigeria.

Sample Preparation / Extraction: The seeds were cracked, the testa were removed and the seeds were milled into fine powder with Thomas Willey milling machine. The powdered plant sample (200g) were percolated in ethanol for 24 hrs and filtered. The filtrate was concentrated under reduced pressure and subjected to systematic GC and GC-MS analysis.

General Experimental Procedure:

The GC analysis were carried out in SHIMADZU JAPAN gas chromatography 5890-11 with a fused GC column (OV- 101) coated with polymethyl silicon (0.25nm x 50m) and the conditions were as follows: Temperature programming from 80- 200^{o}C held at 80^{o}Cfor 1 minute, rate 5^{o}C/min and at 200^{o}C for 20 min. FID temperature 300^{o}C, injection temperature of 250^{o}C and carrier gas nitrogen at a flow of 1ml /min, split ratio 1:75. GC- MS analysis was conducted using GCMS- QP 2010 PLUS SHIMADZU JAPAN with injector temperature of 230oC and carrier gas pressure of 100kpa. The column length was 30m with a diameter of 0.25mm and the flow rate of 50ml/min. the elutes were automatically passed into a mass spectrometer with a dictator voltage set at 1.5kv and sampling rate of 0.2 sec. The mass spectrum was also equipped with a computer fed mass spectra data bank. HERMLE Z 233 M-Z centrifuge Germany was used. Reagents and solvents like ethanol, chloroform, diethyl ether, hexane were all analytical grade and were procured from MERCK, GERMANY.

Component Identification :

Oil components were identified by matching the peaks with Computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks with those from literature (Brillo and Selvakymari, 2006; Adams 2001; Okwu and Ighodaro, 2010).

Results and Discussion:

The dark brown oil (3.00 g) obtained from ethanol extract of the seeds of *Mucuna flagellipes* on GC-MS spectrum showed ten peaks (fig 2). These peaks indicate the presence of ten compounds (1-10) in the oil (fig 1).

The molecular formula, the molecular weight, the retention time and the percentage constituents of the compounds are shown in Table 1.

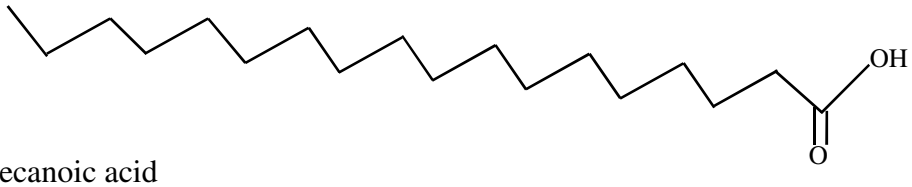
The first compound is hexadecanoic acid with molecular formula $C_{16}H_{32}O_2$ (m/z 256). The composition of compound [1] in the oil is 11.14 %. Detachment of propyl fragment (C_3H_7) from the compound produced the base peak m/z 43. Compound [2] is an ester, ethyl hexadecanoate and constitutes 14.76 % of the oil. It has the molecular formula $C_{18}H_{36}O_2$ (m/z 284). The base peak occurred at m/z 88 due to the detachment of $C_4H_7O_2$ (m/z 87 calculated). Compound [3] is a hydrocarbon, 9-methylbicyclo (3,3,1) nonane with the molecular formula $C_{10}H_{18}$ (m/z 138). The percentage constituent is 14.21 % and the base peak is at m/z 81 which resulted due to the cleavage of C_6H_{10} (m/z 82 calculated). Compound [4] is a carboxylic acid, 9-octadecanoic acid. The molecular formula is $C_{18}H_{32}O_2$ (m/z 282) with base peak of m/z 55. It comprises 16.71 % of the oil. Compound [5] is an ester, ethyl linoleate with molecular formula $C_{20}H_{36}O_2$ (m/z 308). It constitutes 16.99 % of the oil. Compound [6] is also an ester, 9-octadecenoic acid, ethyl ester with molecular formula of $C_{20}H_{38}O_2$ (m/z 310), the base peak occurs at m/z 55 (C_4H_7). It constitutes the highest percentage of the oil, 18.66 %. Compound [7] is ethyl octadecanoate, an ester. It has the molecular formula $C_{20}H_{40}O_2$ (m/z 312) and comprises 4.18 % of the oil. The base peak occurred at m/z 88 ($C_4H_8O_2$). This peak occurred due to Mclafferty re-arrangement. Compound [8] is a carboxylic acid, Eicosanoic acid with the molecular formula $C_{20}H_{40}O_2$ (m/z 312). It constitutes 1.39 % of the oil. Compound [9] is an ester, ethyl icosanoate. The molecular formula is $C_{22}H_{44}O_2$ (m/z 340). It constitutes 0.84 % of the oil. Compound [10] is also an ester, oleic acid, 3-hydroxypropyl ester or 3-hydroxypropyl-9-octadecenoate with molecular formula $C_{21}H_{40}O_3$ (m/z 340). It comprises 1.11 % of the oil.

The seeds of *M. flagellipes* plant contain a lot of esters and this may be the reason the seeds enhance the aroma of the soup they are used as thickeners. Fatty acids and alcohols present in the plant undergo esterification reaction to form the esters. One or both of the oxygen atoms of carboxylic acid can be replaced by sulphur forming a thio acid or dithio acid respectively. Thio acids react readily with alcohols to form thio esters. Thio esters play an important role in the breakdown and synthesis of lipids and steroids in living tissues (Okwu and Ighodara, 2010). However, the presence of fatty acids and aromatics of *M. flagellipes* shows that the plant has pharmacological properties.

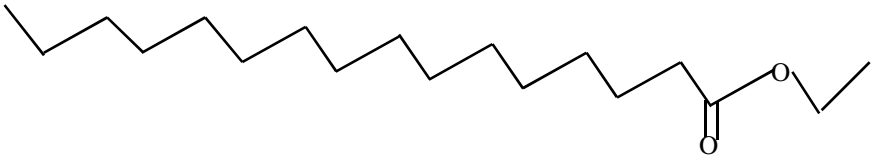
From this investigation, *M. flagellipes* plant can be seen as quality food with good medicinal properties, thus the pharmaceutical industries should research into the potential use of this plant for drug formulation.

Table 1: GC-MS Analysis of Ethanol Extract of the Seeds of *M. flagellipes*

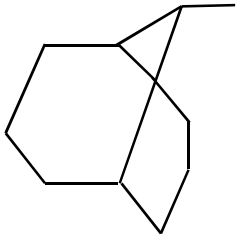
Peak	Compound	Molecular formula	Molecular weight	Retention time	% content	Fragment peaks m/z
1	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	27.975	11.14	27(20%),41(80%),43(100%),60(90%), 73(99%),85(30%),98(20%) 129(35%),213(20%), 256(50%).
2	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	284	28.150	14.76	27(20%), 41(30%), 55(30%), 57(2 0%), 73(15%),88(100%), 101(50%), 157(10%).
3	9–Methylbicyclo [3,3,1] nonane	$C_{10}H_{18}$	138	29.125	14.21	39(30%), 41(50%),55(40%), 67(80%),81(100%), 95(40%), 109(10%), 123(20%),138(10%).
4	9-Octadecanoic acid	$C_{18}H_{34}O_2$	282	29.158	16.71	27(10%), 41(70%),55(100%), 69(80%),83(70%), 97(50%), 98(30%), 114(20%),264(50%) 20%).282(20%).
5	Linoleic acid, ethyl ester	$C_{20}H_{36}O_2$	308	29.233	16.99	41(70%), 55(80%), 67(100%), 81(90%), 95(70%), 109(40%), 123(20%).
6	9-octadecenoic acid, ethyl ester	$C_{20}H_{38}O_2$	310	29.275	18.66	(40%)88(50%)101(40%),123(15%),222(10%),57(264(20%), 310(10%))
7	Octadecanoic acid, ethyl ester.	$C_{2o}H_{40}O_2$	312	29.435	4.18	27(10%),41(35%),43(50%),57(30%),73(20%),88(100%),101(70%), 157(10%).
8	Eicosanoic acid	$C_{20}H_{40}O_2$	312	30.400	1.39	27(15%),41(60%), 43(100%), 57(80%), 73(80%), 85(30%), 98(30%), 129(40%), 269(10%), 298(10%), 312(50%).
9	Eicosanoic acid, ethylester.	$C_{22}H_{44}0_2$	340	30.575	0.84	27(10%), 41(30%), 43(40%), 57(30%), 73(20%), 88(100%), 101(70% 157(10%).
10	0leic acid, 3 – hydroxypropyl ester.	$C_{21}H_{40}O_3$	340	32.442	1.11	27(10%), 41(95%), 55(100%), 69(60%), 81(40%), 95(30%), 98(60%), 112(20%), 137(20%), 264(70%).



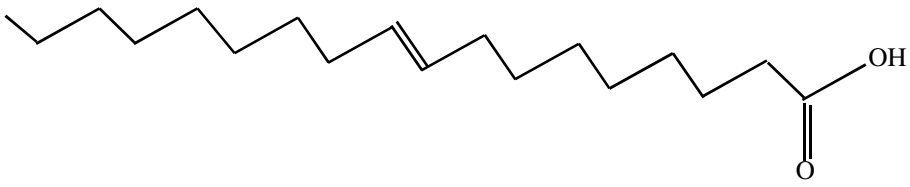
[1] Hexadecanoic acid



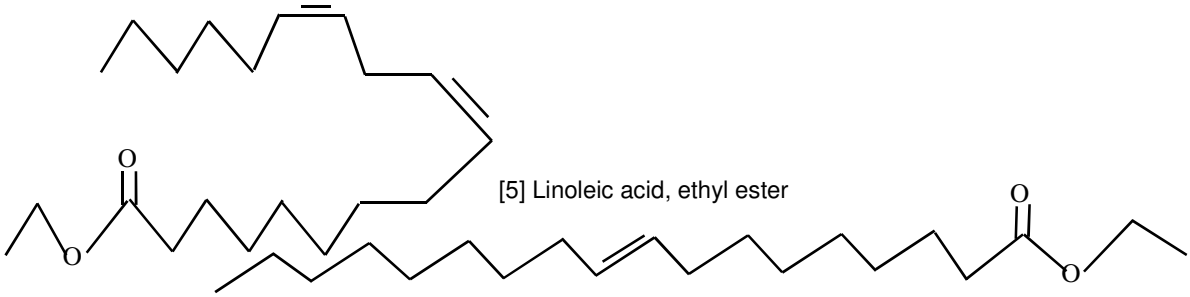
[2] Ethyl hexadecanoate



[3] 9–Methylbicyclo [3,3,1] nonane

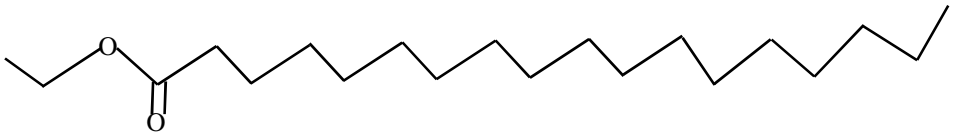


[4] 9-Octadecanoic acid

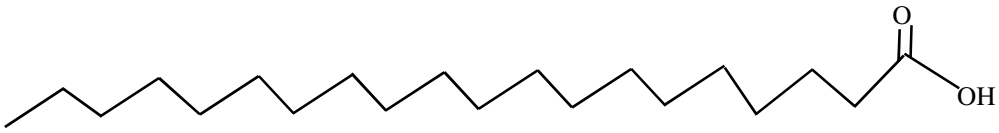


[5] Linoleic acid, ethyl ester

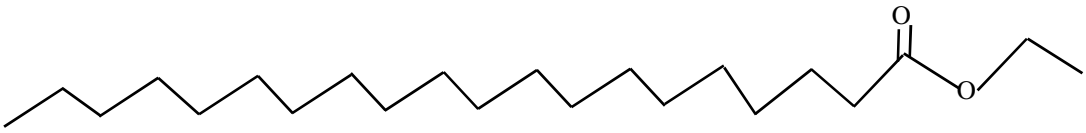
[6] 9-octadecenoic acid, ethyl ester



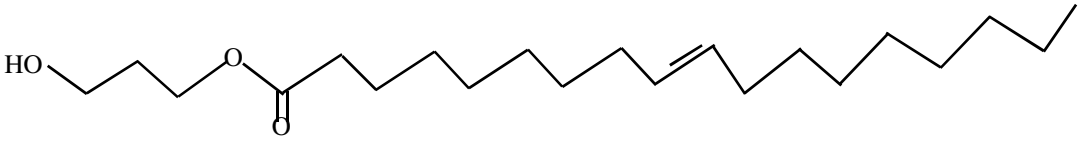
[7] Octadecanoic acid, ethyl ester



[8] **Eicosanoic acid**



[9] Eicosanoic acid, ethylester



[10] 0leic acid, 3 – hydroxypropyl ester

Fig 1: Structures of Compounds from GC-MS Analysis of *M. flagellipes* seeds.

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