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Identification of The Phyto Components in *Telfairia occidentalis* Methanolic Extract By Gas Chromatography-Mass Spectrometry

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ABSTRACT: The identification of the phytocomponents in the methanolic extract of *Telfairia occide.ntalis* was done using GCMS analysis. Ten phytocompounds were identified. The most abundant compounds were n-Hexadecanoic acid with Peak area%: 39.47, RT:19.92,Mol. Formula: $C_{16}H_{32}O_2$; Linoleic acid with Peak area%: 34.15, RT:22.691, Mol. Formula: $C_{18}H_{32}O_2$ and Ethyl hexadecanoate with Peak area%: 7.26, RT:23.084, Mol. Formula: $C_{18}H_{32}O_2$ which showed similar antioxidant and antherosclerotic activity. *Telfairia occidentalis* phytocompounds could therefore be effective in the control and prevention of cancer, free radical oxygen or free radical nitrogen species. Angiotensin converting enzyme present in 2-Butyl propionate with Peak area%: 1.08, 14.823, Mol. Formula: $C_7H_{12}O_2$ which may be antihypertension.

KEYWORDS: Angiotensin converting enzyme, Androgen/Estrogen receptor antagonist, GCMS, n-Hexadecanoic acid, *Telfairia occidentalis.*

I.INTRODUCTION

Fluted pumpkin (*Telfairia occidentalis)* is a plant with dark green three bladed leaves that also bears an inedible pod containing edible seeds belonging to the family of cucurbitaceae. It is widely cultivated and eaten in the Eastern part of Nigeria, West Africa having a high commercial importance even in neighbouring countries like Ghana and Sierra Leone who are also major producers [1]. *Telfairia occidentalis* is called *ugu* in Igbo, *umueke* in Edo, *krobonko* in Ghana *gonugbe* in Sierra Leone and *cestillada* in Spain. *Telfairia occidentalis* (fluted pumpkin) is a common tropical green leafy vegetable native to many African countries especially Eastern Nigeria [2]. It is used primarily in soups and herbal medicines to produce blood tonic used when a person is weak or ill. The seeds are planted directly in the soil in groups of 3 to increase output in case of a failure of some seeds to germination [3] and as the leaves appear the plant will be staked to a frame work of long sticks or bamboo for it to climb when twining. The fruit pod although inedible produces edible seeds that have different colours. *Telfairia occidentalis* is high in protein and fat and therefore contribute to a well balanced diet. The leaves have some mineral composition like the macro nutrients which are calcium 6.8%, Phosphorous 8.1% Magnesium 4.0%, potassium 29.8% and the micro nutrients which are Iron 51.9%, Manganese 45.4%, Copper 17.4% and Zinc 9.4% [4] due to high Iron content found in the leaves, it is used to control anaemia [5].The young shoot and leaves of the plants are also used as soup because of their pleasant taste [6].The plant is also used in herbal preparation for the treatment of sudden attack of convulsion, malaria and anaemia [7]. All these properties and observations of *Telfairia occidentalis* stimulated research to find out scientifically the phytochemicals responsible for the bioactivities.

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II. MATERIAL AND METHODS

A. Plant Materials

leaves of *Telfairia occidentalis* were harvested at Ohafia town in Abia State, Nigeria. The plant leaves were identified by Prof M C Dike at the Taxonomy section of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.

B. Preparation of Plant Extract

The plant material of *Telfairia occidentalis* was collected from home garden, shade dried for 10 days and pulverized to powder using mechanical grinder. The plant extract was prepared using Soxhlet method described by [8]. Thirty five grams (35 g) of powdered sample was introduced into the extraction chamber of the Soxhlet extractor using methanol as solvent. Temperature was maintained at 70^{o} C throughout the extraction period of 48 hrs. At the end of the extraction period, the extract was concentrated using oven at 35^{o} C to obtain dried extract which was sent for GCMS analysis.

C. GCMS analysis of *Telfairia occidentalis*

The characterization of the Phytochemicals in *Telfairia occidentalis* was done using GC-MS QP2010 Plus (Shimadzu, Japan). The identification of the phytochemicals in the sample was carried out using a QP2010 gas chromatography with Thermal Desorption System, TD 20 coupled with Mass Spectroscopy (Shimadzu). The ionization voltage was 70eV. Gas Chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60 m, XTI-5).The initial column temperature was 80^{o}C for 1min, and then increased linearly at 70^{o}C min^{-1} to 220^{o}C, held for 3 min followed by linear increased temperature 10^{o}C min^{-1} to 290^{o}C for 10 min. The temperature of the injection port was 290^{o}C and the GC-MS interface was maintained at 290^{o}C .The sample was introduced via an all-glass injector working in the split mode, with helium carrier gas low rate of 1.2 ml min^{-1}. The identification of compounds was accomplished by comparison of retention time and fragmentation pattern, as well as with mass spectra of the GC-MS.

D. Identification of Phytocompoments in *Telfairia occidentalis*

GC-MS Chromatogram of *Telfairia occidentalis* revealed ten peaks showing that ten different compounds were present. Identity of the active components in the extract was done by comparison of their retention indices, peak area percentage and mass spectra fragmentation pattern with those stored in the database of National Institute of Standards and Technology (NIST) and also with published literature, NIST08.LIB [9], WILEY8.LIB [10], PESTEI-3.LIB and FA-ME.LIB library sources were used for matching the identified components from the plant material. The name, molecular weight, formula, structure and bioactivities of the compounds were ascertained.

III. RESULTS

GCMS chromatogram of the ethanolic extract of *Telfairia occidentalis* (Figure 1) showed ten peaks which indicated the presence of ten phytochemical constituents. The mass spectra data of *Telfairia occidentalis* is show in figure 2. The retention time (RT), peak area percentage, molecular weight, molecular formula and bioactivities of *Telfairia occidentalis* are shown in table 1.

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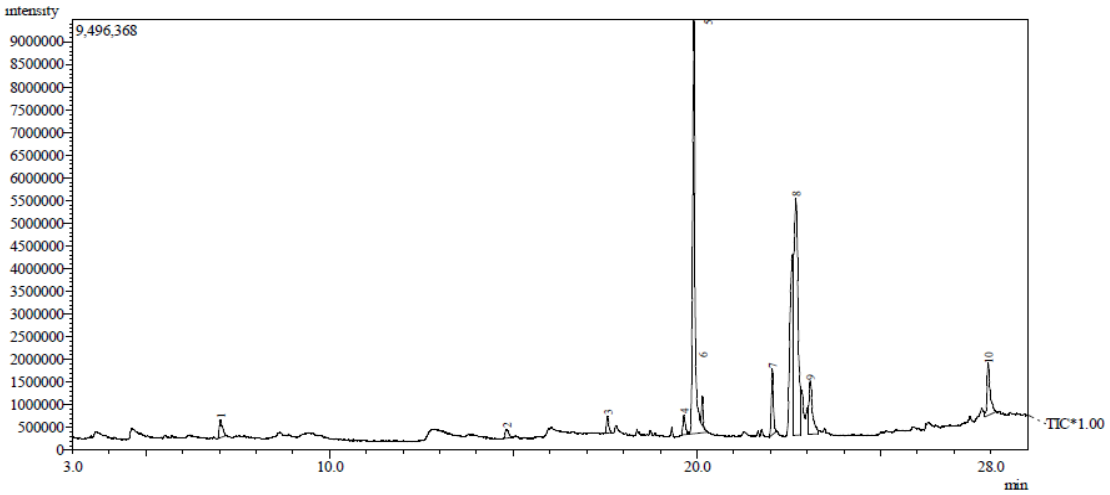
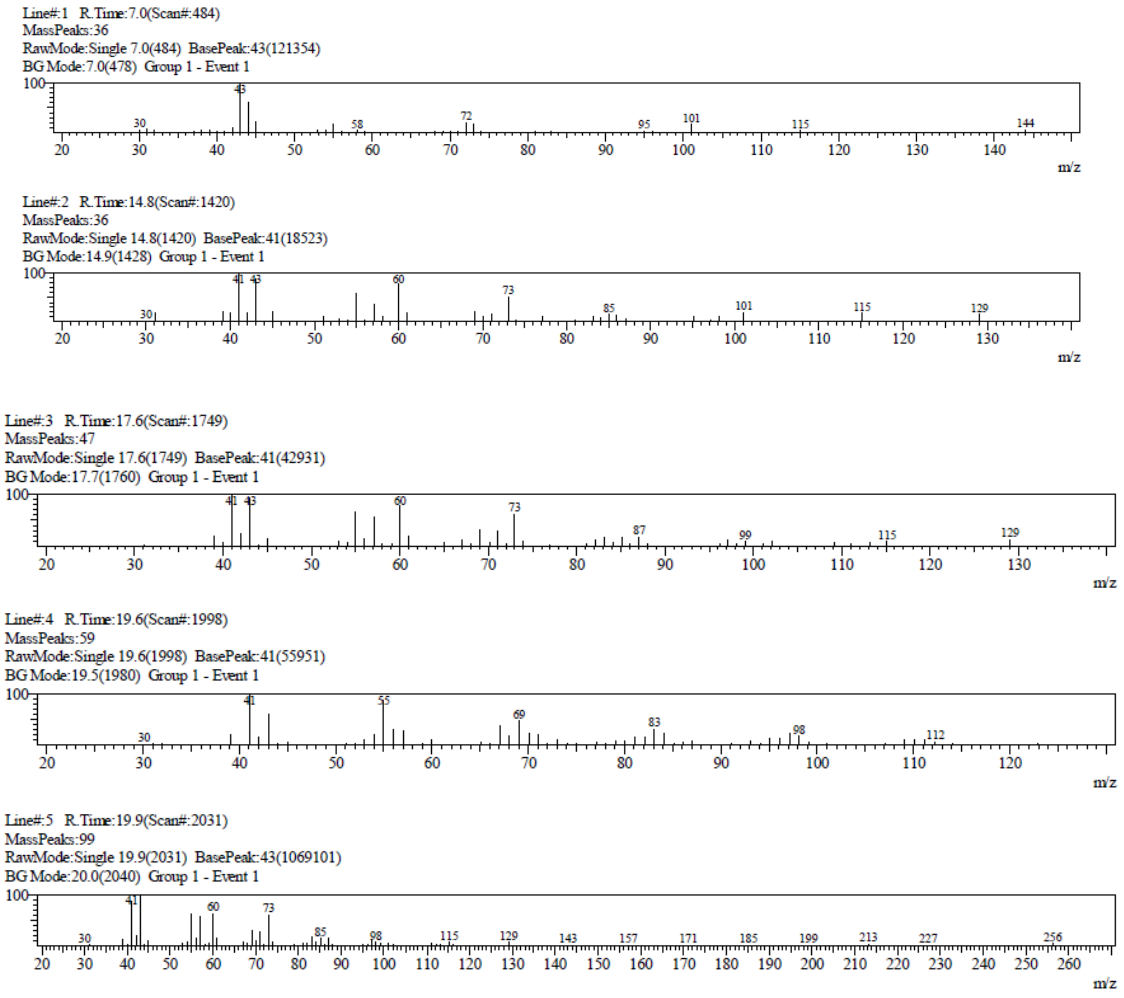


Figure 1 shows ten peaks which indicated the presence of ten phytochemicals constituents in GCMS chromatogram of the ethanolic extract of *Telfairia occidentalis*.



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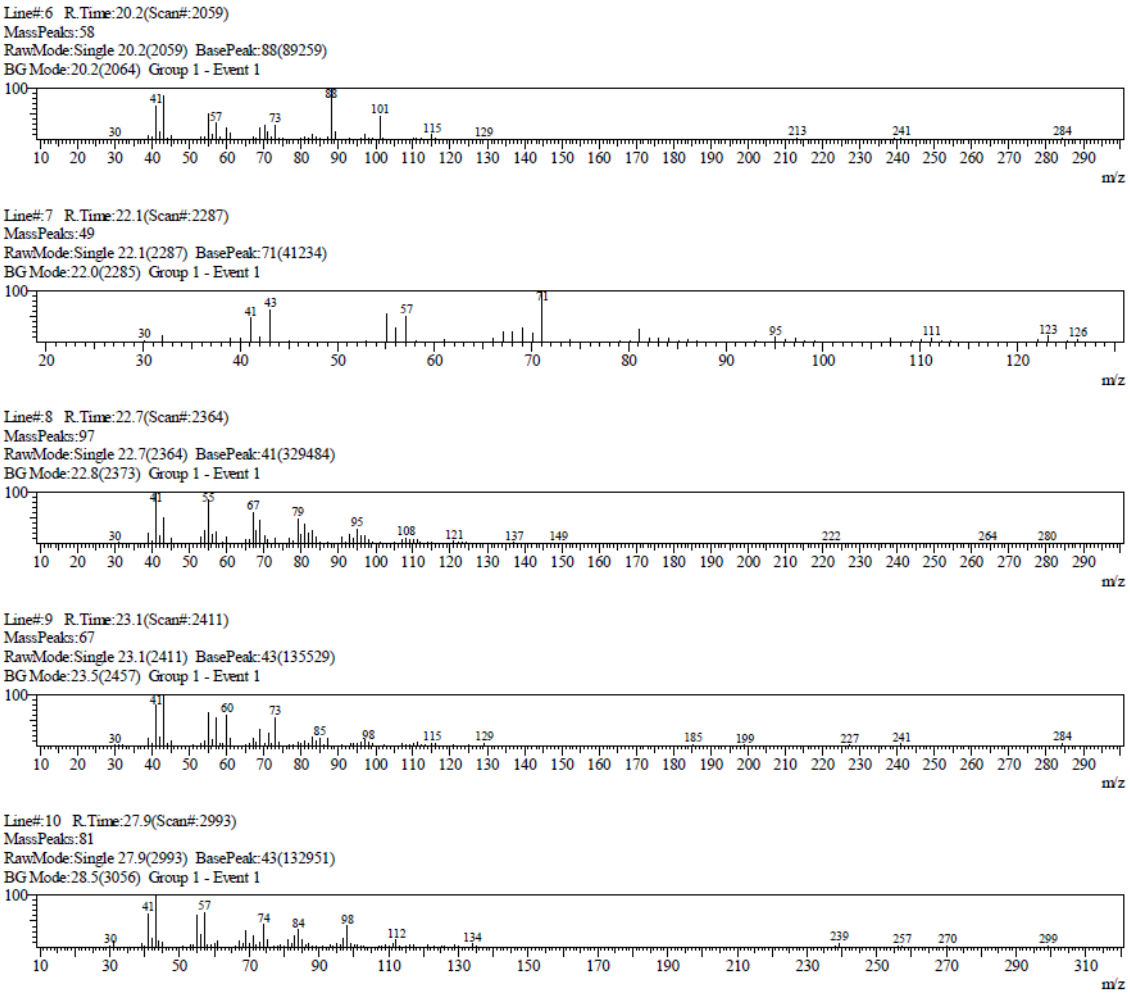
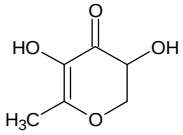
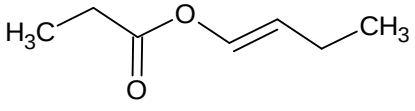


Figure 2: Shows the mass spectra of the ten phytocompounds in *Telfairia occidentalis* identified by GCMS analysis

Table1: Shows the names, retention time, peak area percentage, molecular weight, molecular formula and bioactivity of compounds identified in *Telfairia occidentalis* by GCMS analysis.

S.No	Name of Compound	Retention time	Peak area %	Molecular weight	Molecular formular	Molecular structure	Bioactivity
1	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	7.024	2.13	144.12	$C_6H_8O_4$		S-adenosyl-homocysteine hydrolase
2	2-Butenyl propionate	14.823	1.08	128.16	$C_7H_{12}O_2$		ACE, angiotensin-converting enzyme,

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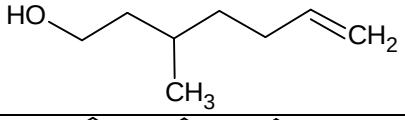
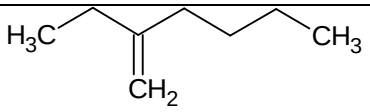
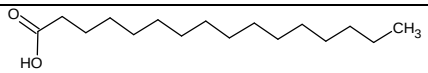
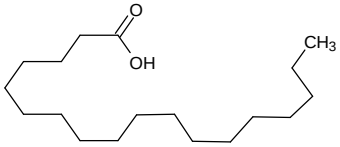
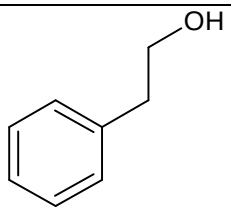
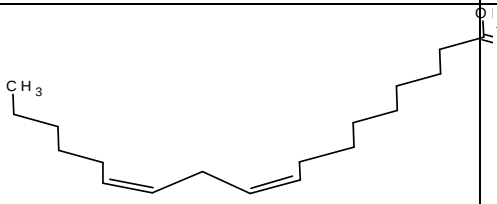
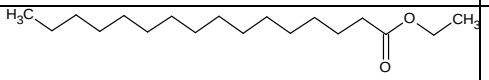
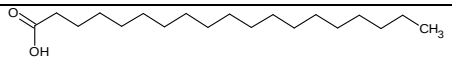
3	3-Methyl-6-hepten-1-ol	17.566	1.43	128.21	$C_8H_{16}O$		Glucocorticoid receptor
4	2-Ethyl-1-hexene	19.644	1.83	112.21	C_8H_{16}		Androgen receptor, estrogen receptor agonist
5	n-Hexadecanoic acid or Palmitic acid	19.920	39.47	256.42	$C_{16}H_{32}O_2$		Mild antioxidant and anti-atherosclerotic activity [11]
6.	Octadecanoic acid or Stearic acid	20.144	1.48	284.47	$C_{18}H_{36}O_2$		Antiinflammatory, Antiandrogenic Cancer preventive, Dermatitigenic Hypocholesterolemic, 5-Alpha reductase inhibitor, anemiagenic insectifuge, flavor [12]
7	Phenylethyl alcohol	22.049	5.10	122.16	$C_8H_{10}O$		Antiinfective agent and disinfectant
8	Linoleic acid	22.691	34.15	280.44	$C_{18}H_{32}O_2$		Anti-carcinogenic, lipid metabolism regulation, anti-inflammatory, anti-obese and antioxidant activities [13]
9	Ethyl hexadecanoate or Ethyl palmitate	23.084	7.26	284.47	$C_{18}H_{36}O_2$		Mild antioxidant and anti-atherosclerotic activity [11]
10	n-Nonadecanoic acid (nonadecylic acid)	27.928	6.07	298.50	$C_{19}H_{38}O_2$		Antiproliferative agent, cell signaling, enzyme cofactor, fuel and energy storage and membrane stability [14].

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IV .DISCUSSION

The chromatogram of *Telfairia occidentalis* showed ten peaks indicating the presence of ten different phytocompounds. The compound 3, 5-Dihydroxy-6-methyl-2, 3 – dihydro-4H – pyran-4-one with the retention time of 7.024 and a peak area percentage of 2.13% had S-adenosyl-homocysteine hydrolase activity. S-adenosyl homocysteine hydrolase is an enzyme that hydrolyzes S-adenosyl homocysteine into adenosine and homocysteine [15].The compound 2-Butenyl propionate that had a retention time of 14.823 with a peak area percentage of 1.08% had angiotensin-converting enzyme (ACE) action. Angiotensin-converting enzyme (ACE) is a dipeptidyl-carboxypeptidase that splits off histidyl-leucine from the physiolofically inactive angiotensin I ,forming the octapeptide angiotensin II [16] various nonapeptides analogs of angiotensin I and other compounds act as competitive inhibitors of converting enzyme and are used to treat renin-dependent hypertension [17].

The compound n-Hexadecanoic acid or Palmitic acid which had a retention time of 19.920 and a peak area percentage of 39.47% and Ethyl hexadecanoate or ethyl palmitate which had a retention time of 23.084 and a peak area percentage of 7.26% had a mild antioxidant and anti-atherosclerotic activity.Antioxidants are substance that prevents or reduces damage caused by reactive oxygen species or reactive nitrogen species. They are used as dietary supplements in animals for aiding in the treatment of cancer, enhancing immune function and reducing treatment toxicity [18]. Atherosclerosis is a risk factor of hypertension which can predispose to coronary heart failure, stroke, peripheral artery disease and death [19]. The risk for coronary artery disease and stroke depends to a great extent on other risk factors, such as obesity, smoking and elevated cholesterol levels [19], therefore, the compound linoleic acid which has a retention time of 22.691 and had a high concentration of 34,15 peak area had also anti-obesity and anti-oxidant activity [13].

V. CONCLUSION

Therefore, *Telfairia occidentialis* could be beneficial for the control of atherosderotic related cases which could lead to hypertension, stroke and eventual death. This is because the leaf extract contains n-Hexadecanoic acid in abundance and linoleic acid which is an anti-obese factor ,Table 1.It could also be an effective tool against cancer which is number one killer disease in the world, because the GCMS analysis shows the presence of the phytocompound, linoliec acid. The plant extract is indicated to prevent the free oxygen species and nitrogen species that ravage the cell because of the presence of the compound n-Hexadecanoic acid and Ethyl hexadeanoate identified by the GCMS analysis. Structures of these compounds identified by GCMS analysis could also be docked for molecular modelling and charting a new avenue for drug discovery.

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