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IDENTIFICATION OF THE PHYTOCOMPONENTS IN LORANTHUS MICRANTHUS METHANOLIC EXTRACT BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Igwe K. K.[1*], Nwankwo P. O.[2],Otuokere I. E.[3], Ijioma S .N.[1], Amaku F.J.[3] Chika Ikenga [4]
[1*]Departmemt of Veterinary Physiology, Pharmacology and Biochemistry, Michael Okpara University of Agriculture, Umudike, Nigeria.
[2]National Root Crops Research Institute, Umudike,Abia State,Nigeria
[3]Departmemt of Chemistry, Michael Okpara University of Agriculture, Umudike, Nigeria.
[4]Recare Natural Products, Lagos, Nigeria
Correspondence Author: kkigwe191@gmail.com

Keywords: GCMS analysis, Loranthus micranthus ,Antimetabolic syndrome, Anticardiovascular risk factor, arachidonate 5-lipoxygenase inhibitor, epigenetic enzymes inhibitor

Abstract
Phytocomponents in methanolic extract of Loranthus micranthus leaf was studied using GC MS analysis. 12 compounds were identified from the extract. The major chemical constituents were cis-Octadecenoic acid (Peak area 44.82% ; RT 22.743), Octadecanoic acid (18.34% ; RT 18.34),Ethylhexanoate(Peak area 8.7% ; RT 32.142), Curcumin(1E,4Z,6E)-5-hydroxy-1-7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one (Peak area 7.25% ; RT 27.456),1,3-Hexyloxacyclotridec-10-en-2-one (Peak area 5.34% ; RT 21.382). The bioactive compounds in the methanolic leaf extract of Loranthus micranthus exhibited phytopharmacological significance and hence could be of therapeutic use against some diseases.

Introduction
Animals in the wild have been surviving their health challenges by depending on the medicine in the plants they eat. Plants have therefore been used in treating human diseases for many years now. Bioactive compounds from plants continue to play a major role in health care benefits [1]. *Loranthus micranthus* is a semi parasitic shrub belonging to family *Loranthaceae*. The plant in Nigeria grows by obtaining nutrients and support from a host of trees including *Kola acuminata, Kola nitida, Azadirachta indica, Jatropha curcas and Persia spp*. The leaves extracts of *Loranthus micranthus* has been used traditionally to treat diabetes mellitus, hypertension and schizophrenia[2]. The plant has also been reported to have antidiarrheal, antioxidant, hypoglycaemic, antiarthritic/analgesic and immunological activities[3] Phytochemical composition of the plant reveal the presence of terpenoids, steroids, oils, proteins, resins, flavonoids, tannins, saponins and glycosides[3].The aim of this research is to identify phytochemicals in the plants by GC-MS analysis as preliminary study. Then structural-functional relationship to bioactivity will be established thereby charting a new road map for drug discovery by molecular docking of the medicinal compounds. The compounds with the highest peak area percentage in the extract could be further characterized and could be adopted for molecular modeling because of the presence of particular bioactive compound.Furthermore our focus is to chart a new course for plant based drug which can be docked and modelled for the control of diseases like Ebola, Avian flu, Cancer,HIV, Leukamia, Lassa fever, Swine fever, Gumboro or New Castle disease. GCMS analysis for bioactive components in plants remain the most appropriate technique for identification of new phytochemicals with medicinal potentials against many diseases [4, 5, 6]. There is increase awareness in phytomedicine which in future will help to standardize the plant constituents for health benefits.

Material and methods
Plant materials
Fresh leaves of *Loranthus micranthus* was 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 and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.
Preparation of plant extract
The plant material of *Loranthus micranthus* was collected from wild, shade dried for 10 days and pulverized to powder using mechanical grinder. The plant extract was prepared using Soxhlet method described by [7]. 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.
GCMS analysis of Loranthus micranthus
The characterization of the Phytochemicals in *Loranthus micranthus* 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

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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 80ºC for 1min, and then increased linearly at 70ºC min^{-1} to 220ºC, held for 3 min followed by linear increased temperature 10ºC min^{-1} to 290ºC for 10 min. The temperature of the injection port was 290ºC and the GC-MS interface was maintained at 290ºC .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.

Identification of phytocompoments in loranthus micranthus

GC-MS Chromatogram of *Loranthus micranthus* revealed twelve peaks showing that twelve 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 [8], WILEY8.LIB [9], 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.

Results & discussion

Results

GCMS chromatogram of the methanolic extract of *Loranthus micranthus* (Figure 1) showed twelve peaks which indicated the presence of twelve phytochemicals constituents.

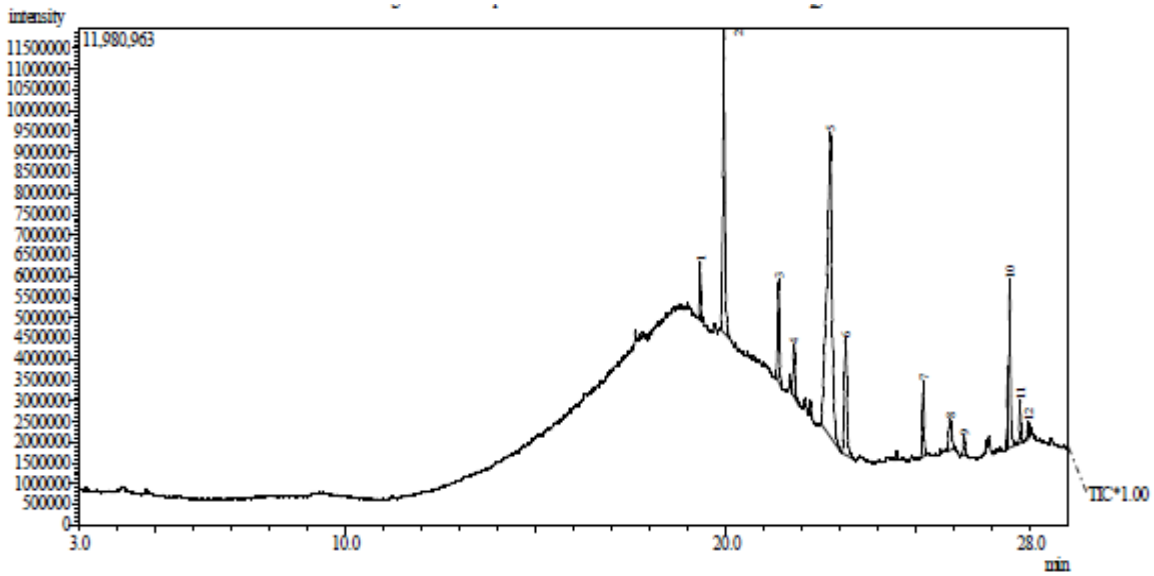

Figure 1 shows GCMS chromatogram of extract of Loranthus micranthus

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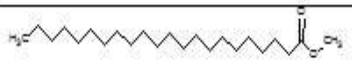
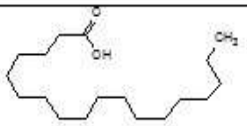
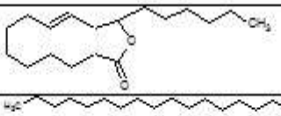
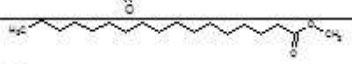
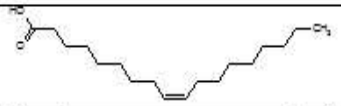
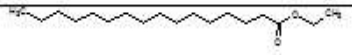
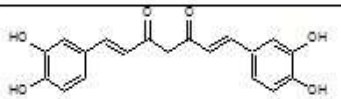
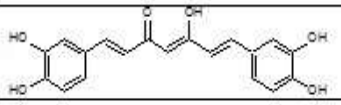
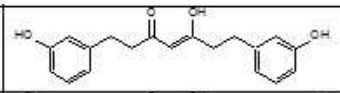
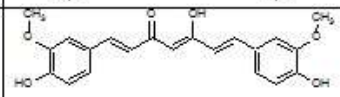
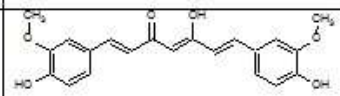
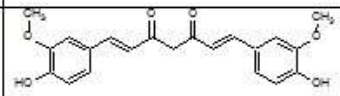
Table 1shows the name, retention time,molecular weight,formular,structure and bioactivities of Loranthus micranthus

S.No	Name of Compound	Retention time	Peak area %	Molecular weight	Molecular formular	Molecular structure	Bioactivity
S.No	Name of Compound	Retention time	Peak area %	Molecular weight	Molecular formular	Molecular structure	Bioactivity
1	Methyl docosanoate or n-Docosanoic acid methyl ester	19.324	2.01	354.61	$C_{23}H_{46}O_2$		Humectants skin conditioning [10]
2	Octadecanoic acid or Stearic acid	19.952	18.34	284.47	$C_{18}H_{36}O_2$		Antiinflammatory, Antiandrogenic Cancer preventive, Dermatitigenic Hypocholesterolemic, 5-Alpha reductase inhibitor, anemiagenic insectifuge, flavor [4]
3	13-Hexyloxacyclotridec-10-en-2-one	21.382	5.34	280.44	$C_{18}H_{32}O_2$		Protein kinase c gamma inhibitor [11]
4	Methylheptanoate	21.787	3.05	284.47	$C_{18}H_{36}O_2$		Food and flavoring ingredient
5	cis-,Octadecenoic acid or cis-Oleic Acid	22.743	44.82	282.46	$C_{18}H_{34}O_2$		Protective against metabolic syndrome and cardiovascular disease risk factors [12].
6	Ethylhexanoate	23.142	8.78	284.47	$C_{18}H_{36}O_2$		Food-Grade Flavor Ingredient
7	(1E,6E)-1,7-bis(3,4-dihydroxyphenyl)hepta-1,6-diene-3,5-dione	25.180	3.75	340.32	$C_{19}H_{16}O_6$		neuroprotective activity against glutamate-induced oxidative stress [13]
8	(1E,4Z,6E)-1,7-bis(3,4-dihydroxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one	25.883	2.76	340.32	$C_{19}H_{16}O_6$		neuroprotective activity against glutamate-induced oxidative stress [13]
9	(4Z)-5-hydroxy-1,7-bis(3-hydroxyphenyl)hept-4-en-3-one	26.256	1.02	312.35	$C_{19}H_{20}O_4$		Trypanocidal activity [14]
10	(1E,4Z,6E)-5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,4,6-trien-3-one commonly known as Curcumin (enol form)	27.456	7.25	368.37	$C_{21}H_{20}O_6$		inhibit certain epigenetic enzymes and arachidonate 5-lipoxygenase enzyme in vitro [15,16]
11	(1E,4Z,6E)-5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,4,6-trien-3-one commonly known as Curcumin (enol form)	27.726	1.91	368.37	$C_{21}H_{20}O_6$		inhibit certain epigenetic enzymes and arachidonate 5-lipoxygenase enzyme in vitro [15,16]
12	(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione commonly known as Curcumin (Keto form)	27.944	0.97	368.37	$C_{21}H_{20}O_6$		inhibit certain epigenetic enzymes and arachidonate 5-lipoxygenase enzyme in vitro [15,16]

Discussion

Phytocomponents in methanolic extract of *Loranthus micranthus* leaf was studied using GC MS analysis and twelve compounds were identified from the extract figure 1. Table 1, shows the name of the compounds, retention time, peak area percentage, molecular weight, molecular formular, molecular structure and bioactivity of the twelve compounds identified. The major chemical constituents were cis-Octadecenoic acid $C_{18}H_{34}O_2$ (Peak area 44.82% ; RT 22.743),Octadecanoic acid $C_{18}H_{36}O_2$ (Peak area 18.34%; RT 19.95), Ethylhexanoate $C_{18}H_{36}O_2$ (Peak area 8.7%; 23.142), Cucrcuumin $C_{21}H_{20}O_6$ (Peak area 7.25%; RT 27.456),1,3Hexyloxacyclotridec-10-en-2-one $C_{18}H_{32}O_2$ (Peak area 5.34%; RT 21.382).

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The plant exhibited antiinflamatory,antiandrogenic,cancer preventiveand 5-alpha reductase inhibitor activity because of the compound,octadecanoic acid present in the extract of *Loranthus micranthus* leaf [4].The extract showed protective compound against metabolic syndrome and cardiovascular disease risk factors [12].

DNA and chromosomal structural changes that are molecular and cellular mechanisms termed epigenetic mechanisms involves changes in the pattern of gene expression without a change in the DNA [17].Epigenetic mechanisms may silence gene such as tumor suppressor gene, so that even though the gene is present, it is not expressed and a cancer-suppressing protein is not made [17] ,leading to higher chances of cancer occurrence. Tumor suppressor proteins prevent cancer [18].The compound (1E,4Z,6E)-5-hydroxy-1-7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one,commonly known as curcumin (enol form), identified by GCMS analysis inhibits certain epigenetic enzymes and arachidonate 5-lipoxygenase enzyme *in vitro* [15]. Compounds that block activation or suppress activity of the HIV-1 LTR could be useful for extending the viral latency period or inhibition of the persistence progressive infection. Curcumin, the major active component of the spice turmeric has been demonstrated by Harvard researchers to block HIV- LTR activity [19].Therefore the curcumin identified in our GCMS analysis in the leaf extract of *Loranthus micranthu* could also be effective against HIV LTR activity. Curcumin is effective in both acute and chronically infected cells [19].This compound could be beneficial for cancer and HIV- LTR control. This plant extract could also be useful as humectants skin conditioner or as a skin moisturizer because of the compound, methyl decosanoate present in the extract.

The bioactive compounds in the methanolic leaf extract of *Loranthus micranthus* exhibited phytopharmacological significance and hence could be of therapeutic use for disease control.

Conclusion

Plants are important source of drug for both man and animals. No treatment is given to those animals in the wild so they depend on phytocomponents in plants as their phyto - remedy in the case of disease attack. In the present research, twelve compounds were identified from the methanolic leaf extract of *Loranthus micranthus* using GC-MS analysis. These bioactive compounds identified in the extracts of *Loranthus micranthus* justifies the use of the plant for various ailments by traditional medicinal practitioners. We recomend further investigation into isolation of pure compounds for pharmacological drug modelling and molecular docking. From the above results, *Loranthus micranthus* could be regarded as a plant of phytopharmaceutical significance.

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