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GC-MS analysis for Structural Identification and Bioactive Compounds in Methanolic Leaf Extract of *Mallotus oppositifolius*.

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ABSTRACT

The aim of the present study is to investigate the bioactive compounds from the leaf extract of *Mallotus oppositifolius* using GCMS analysis. The chromatogram showed nine peaks indicating the presence of nine compounds in the extract. The major phytocompounds in the leaf were Glutaconic anhydride with the highest concentration in the extract, 40.19 peak area %, RT 22.686 and molecular formula $C_5H_4O_3$; 2-Mercaptophenol with 18.23 peak area %, RT 22.068 and molecular formula C_6H_6OS. Iso-Valreic and Valeric acids with 12.39,2.53 peak area %, RT 3.676,7.037 and the same molecular formula $C_5H_{10}O_2$ which had been proposed to have anticonvulsant effect in valerian and act as neurotransmitter. Oleamide with the least concentration of 2.15 peak area %, RT 27.959 and molecular formula $C_{18}H_{35}NO$ which could induce sleep in animals, being studied as a potential medical remedy for mood and sleep disorder and cannabinoid–regulated disorder. The phytochemicals in *Mallotus oppositifolius* could be of therapeutic importance.

Keywords: GCMS analysis, *Mallotus oppositifolius* , anticonvulsant, neurotransmitter, sleep depressant.

INTRODUCTION

The use of plants in the treatment of ailments has been long time immemorial [1].*Mallotus oppositifolius*(Geisel) is one the plant used by Nigerians for the treatment of skin diseases [2]. *Mallotus oppositifolius*is an erect branching perennial shrub up to 3.6 m high when fully matured. The plant is commonly found in drier types of forest and grow throughout the West Africa region [3]. Ethnobotanically, *Mallotus oppositifolius* is used as chewing sticks for cleaning the teeth and the stem for yam stakes. The Ohafia people in Nigeria use the cold infusion to expel placenta blood clot after delivery, while the decoction is a vermifuge in Ivory Coast. In Ghana, the crushed leaves are applied to inflammation of the eye during an attack of small pox [3]. Rottlerin has also been found in its bark and leaves [4]. The aqueous and ethanol extracts of the plant show antifungal properties [5] and anti parasitic activity against blastocystishominis [6] The bioassay-guided fractionation of an ethanol extract of the leaves and inflorescence of *Mallotu oppositifolius* collected in Madagascar led to the isolation of the two new bioactive dimeric phloroglucinols mallotojaponins B and C, together with mallotophenone. These compounds show antiproliferative and antiplasmodial activities [7]. The crude extracts of *Mallotus oppositiformis* possess antifungal activity on most of the fungi and inhibits the growth of *Aspergillus flavus, Candida albicans, Microsporium audouinii, Penicillium spp, Trichophyton mentagrophytes, Trichoderma spp* and *Trichosporon cutaneum* [5]. The leaves are ingredients of common anti-malaria and anti-inflammatory remedies [8].Phytochemical screening of *Mallotus oppositiformis* revealed the presence of secondary metabolites such as alkaloids, phenols, flavonoids, anthroquinones and cardenolides. A higher concentration of these resides in the leaves than in the root [9]. Hydroalcoholic extract of leaves of *Mallotus oppositifolius* plant is used for CNS conditions in Ghana, which exhibits antidepressant effects mediated by enhancement of serotoninergic neurotransmission and inhibition of glycine receptor activation [10].There is increase in fungal related cases for the last decade. Fungal related diseases may not be as common as

other microbial infections but, when present, they are difficult to treat especially if patients immunity is low [11]. Therefore traditional doctor who tries to cure an ailment using plant may use the whole plant or extract from leave, stem, root, and seed or mix all together. This type of treatment is wrong so there is need to scientifically analyze the medicinal plant. GCMS analysis has been employed in this research to identify the phytochemicals responsible for bioactivities associated with *Mallotus oppositifolius*.

2. MATERIAL AND METHODS

2.1 Plant Materials

Fresh leaves of *Mallotus oppositifolius* 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.

2.2 Preparation of Plant Extract

The plant material of *Mallotus oppositifolius* 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 [12]. 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 70o C throughout the extraction period of 48 hrs. At the end of the extraction period, the extract was concentrated using oven at 35o C to obtain dried extract which was sent for GCMS analysis.

2.3 GCMS analysis of *Mallotus oppositifolius*

The characterization of the Phytochemicals in *Mallotus oppositifolius* 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 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.

2.4 Identification of Phytocompoments in *Mallotus oppositifolius*

GC-MS Chromatogram of *Mallotus oppositifolius* revealed nine peaks showing that nine 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 [13], WILEY8.LIB [14], 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.

3. RESULTS AND DISCUSSION

3.1 Results

GCMS chromatogram of the methanolic extract of *Mallotus oppositifolius* (Figure 1) showed nine peaks which indicated the presence of nine phytochemicals constituents.

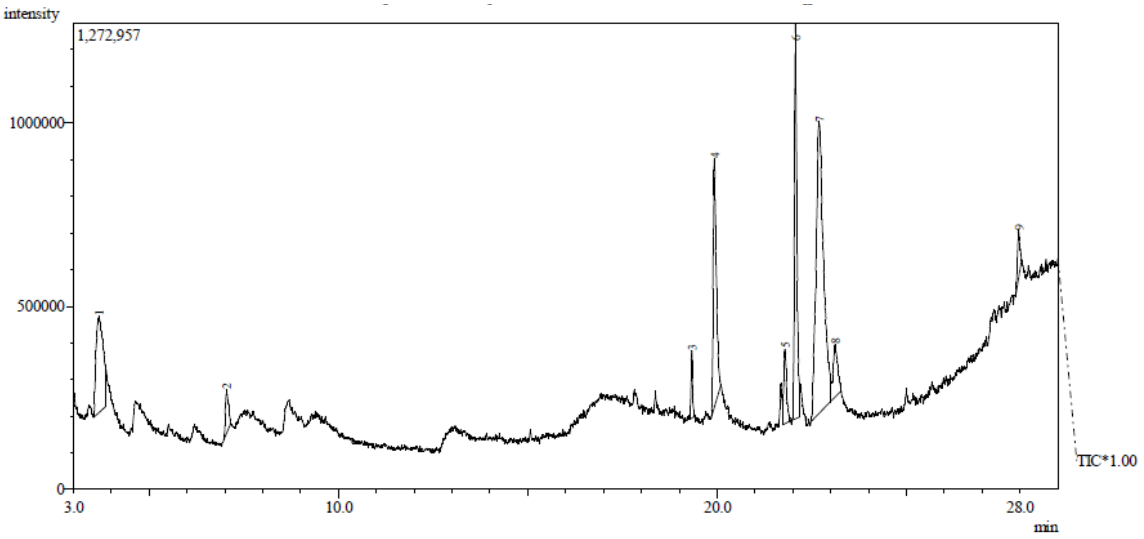
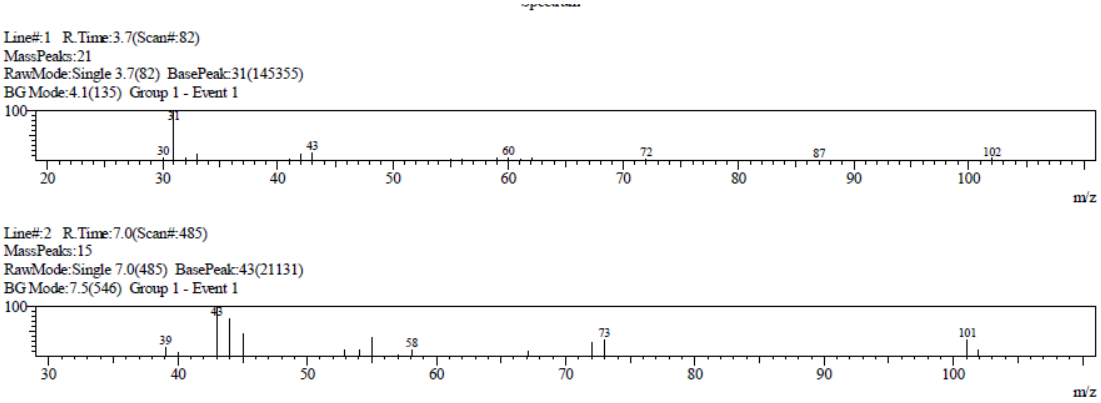


Figure 1: Shows the chromatogram of *Mallotus oppositifolius*



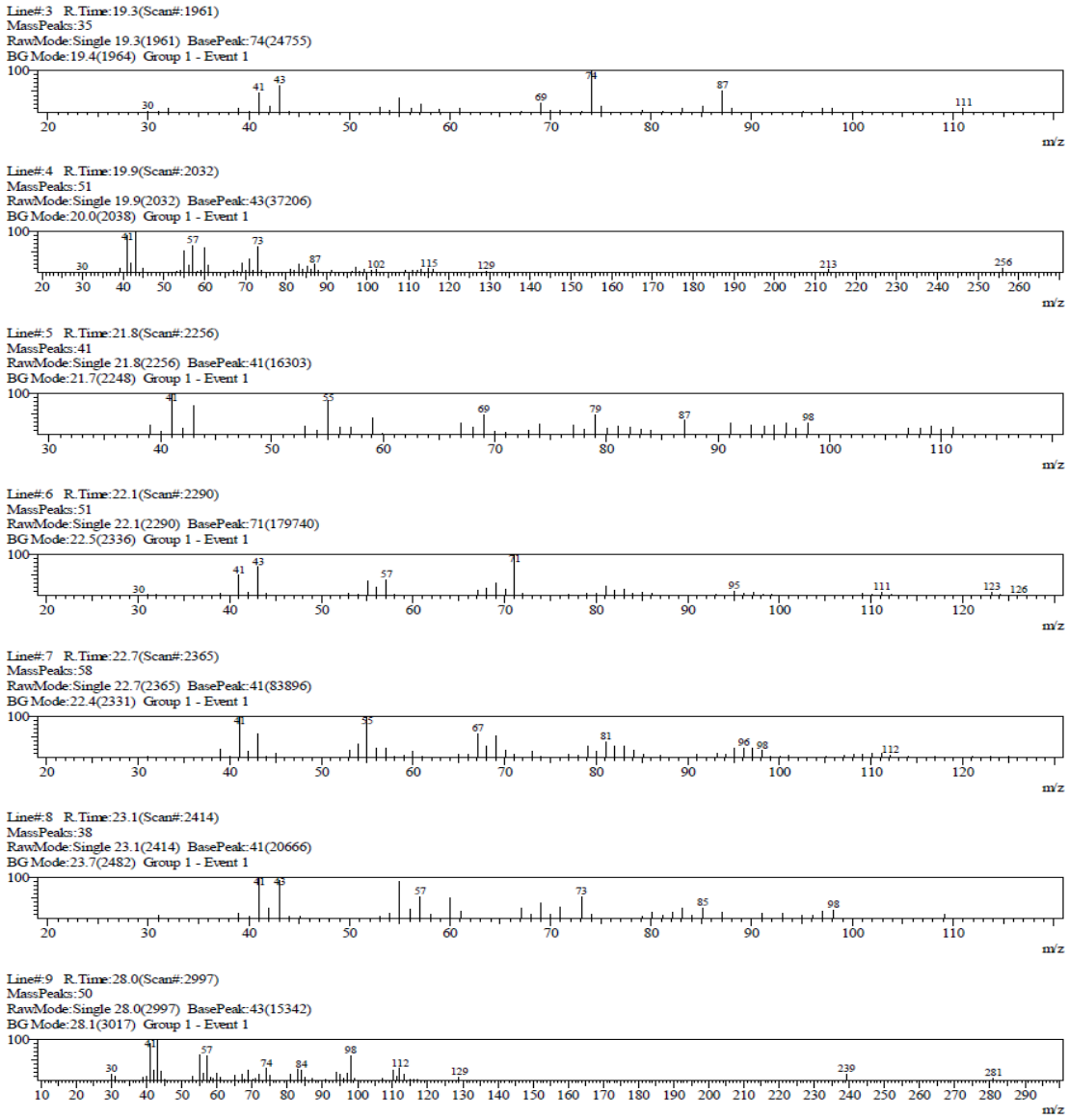
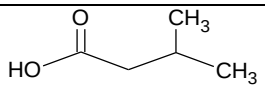
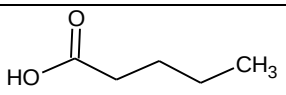
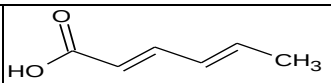
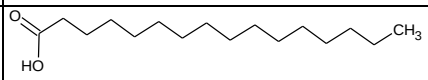
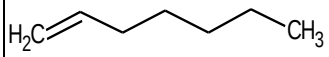
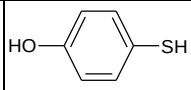
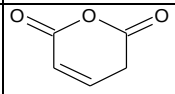
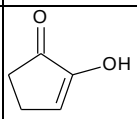
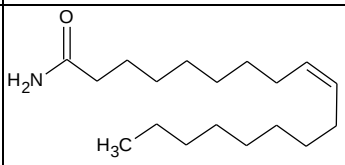


Figure 2: Shows the mass spectra of the nine phytocompounds in *Mallotus oppositifolius* identified by GCMS analysis

Table 1 shows the phytocompounds in *Mallotus oppositifolius* methanol extract.

S.No	Name of Compound	Retention time	Peak area %	Molecular weight	Molecular formular	Molecular structure	Bioactivity
1	3-Methylbutanoic acid or more commonly isovaleric acid	3.676	12.39	102.13	$C_5H_{10}O_2$		It has been proposed that it is the anticonvulsant agent in valerian.
2	Valeric acid or pentanoic acid	7.037	2.53	102.13	$C_5H_{10}O_2$		neurotransmitter

3	Sorbic acid	19.329	2.08	112.12	$C_6H_8O_2$		antibacterial drug fungicide
4	n-Hexadecanoic acid or Palmitic acid	19.926	14.22	256.42	$C_{16}H_{32}O_2$		Mild antioxidant and anti-atherosclerotic activity [15]
5	Hept-1-ene	21.791	3.87	98.18	C_7H_{14}		Surfactant
6.	2-Mercaptophenol	22.068	18.23	126.17	C_6H_6OS		
7	Glutaconic anhydride	22.686	40.19	112.08	$C_5H_4O_3$		peroxisome proliferator activated receptor
8	2-Hydroxy-2-cyclopenten-1-one	23.115	4.33	98.09	$C_5H_6O_2$		estrogen receptor; agonist
9	Oleamide	27.959	2.15	281.47	$C_{18}H_{35}NO$		It accumulates in the cerebrospinal fluid during sleep deprivation and induces sleep in animals. It is being studied as a potential medical treatment for mood and sleep disorders, and cannabinoid-regulated depression [16].

3.2 Discussion

The chromatogram of *Mallotus oppositifolius* indicated the presence of nine phytocompounds. The compound 3-Methylbutanoic acid demonstrated anticonvulsion activity. Convulsion is produced by a number of metabolic disorder such as hypoglycemia, hypocalcaemia and hormonal imbalances [17].This compound identified by GCMS analysis could counter the effect of convulsion and therefore could be used as a therapeutic remedy for idiopathic seizures. A seizure represents the abnormal behaviour caused by an electrical discharge from neurons in the cerebral cortex presents clinical signs and symptoms that vary according to the site of neuronal discharge in the brain[18]. Manifestations of seizure generally include sensory, motor, autonomic or psychic phenomena. A convulsion refers to the specific seizure type of a motor seizure involving the entire body [18]. The neurotransmitter effect was demonstrated by phytocompound, Valeric acid or Pentanoic acid as was identified by GCMS analysis. Neurotransmitters carry nerve impulses across synapse and are small molecules that incorporate a positively charged nitrogen atom. They include several amino acids, peptides and monoamines. The amines acid, glutamine, glycine and gamma amino butyric acid (GABA) serve as neurotransmitters at most CNS synapse [19].

The compound Glutaconic anhydride with a retention time of 22.686 and a peak area percentage of 40.19% had a peroxisome proliferator activated receptor activity. Peroxisomes play important role in B-oxidation leading to the formation of acetyl Co A and hydrogen peroxide which is broken down by catalase. [20]. The peroxisome system facilates the oxidation of very long fatty acids example C_{20} and C_{22}. Peroxisomes shorten the side chain of cholesterol in the bile acid formation and also takes part in the synthesis of etherglycerolipids [21]. Therefore this compound Glutaconic anhydride could play a biochemical role of facilitating B-oxidation in the cell. Oleamide was identified also with GCMS at retention time of 27.959 with 2.15% peak area percentage.

The compound exhibits a bioactivity of influencing mood and sleep disorder especially if it accumulates in the cerebrospinal fluid during sleep deprivation. The compound also induces sleep in animals [16].

CONCLUSION

The result of this analysis showed the presence of various phytocompounds in methanolic extract of *Mallotus oppositifolius.* Glutaconic anhydride which had the highest concentration in the extract (40.19%; $C_5H_4O_3$) and n-Hexadecanoic acid (14.22%; $C_{16}H_{32}O_2$) showed peroxisome proliferetor receptor activation and antioxidantion, anti-atherosclerotic activity respectively. The compound 3-Methylbutanoic acid ($C_5H_{10}O_2$) commonly known as isovaleric acid and its isomer Valeric acid ($C_5H_{10}O_2$) which is also known as pantanoic acid were found to be anticovulsants and nuerotrasmitters respectively. Sorbic acid $C_6H_8O_2$ and Oleamide $C_{18}H_{35}NO$ with close range concentration of (2.08%; 2.15%) in the *Mallotus oppositifolius* extract exhibited activity of antibacterial,fungicidal, and sleep inducer respectively. The phytocompound,oleamide could therefore be pharmacologically useful as pre-anaesthetic agent. The plant has a wide array of medicinal usage and those compound identified by Gas Chromatography-Mass Spectrometry could undergo molecular docking to creat new roadmap for drug modelling.

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