

Original Research Article

Antioxidant potential of Crude Methanol Leaf extract and fraction of *Mallotus oppositifolius*.

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Abstract

Mallotus oppositifolius (Geiseler) Mull. Arg. (Euphorbiaceae) is a predominant edible shrub in Nigeria. It is locally used for treatment of common infections and wounds. This study evaluated the toxicity and antioxidant potential of the crude extract and fractions of *M. oppositifolius* using invitro 1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging assay. The result showed abundance of flavonoids (28.21), tannins (17.72), alkaloids (7.79) and Saponins (0.23) in the crude extract of *M. oppositifolius* leaf extract. Also, the result showed that the LD₅₀ is above 5000 mg/kg, therefore the crude extract is safe for consumption. The result further showed that the ethyl-acetate and butanol had significant ($p < 0.05$) antioxidant activity when compared with the control at 125, 62.5 and 31.125 µg/ml respectively while at 250 and 500 µg/ml there was no significant different in the level of reduction of oxidation between the butanol and ethyl acetate when compared with the control. The result further showed that the butanol and ethyl-acetate fraction of *M. oppositifolius* had the best antioxidant potential among the fractions studied.

The result of GCMS studies of ethyl-acetate fraction showed the presence of 7,10,13-eicosatrienoic acid methyl ester, korormicin, agosterol, phyllanthin, ricinoic acid, 2,4,6-decatrienoic acid 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9, limonin, compounds that

have shown to possess strong antioxidant activities. Also, the GCMS result of butanol fraction showed the presence of seven compounds which include: ferulylhistamine, hippastrine, 10,13-eicosadienoic acid methyl ester, ethyl 5,8,11,14-eicosatetraenoate, 6-hydroxy-powelline-N-nitroso-7-demethoxy-aldehyde, 6,7-epoxypregn-4-ene-9,11,18-triol-3,20-dione 11,18-diacetate and 5 α -androstane-3,17-dione 17-monooxime, compounds which may serve as a new lead for the treatment of diseases associated with the oxidative stress.

Background

Oxidative stress occurs as a result of the activities of free radicals, leading to public health issues with a huge economic burden. Oxidation and lipid peroxidation has a significant negative impact on living cells and have caused numerous disease conditions and sometimes apoptosis. Free radicals are atoms, molecules or ions derived from oxygen, nitrogen, and sulphur molecules with unpaired electrons, which are highly reactive capable of causing cell damage or death [1]. Free radicals such as reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulphur species (RSS) are implicated in numerous degenerative disorders such as diabetes, rheumatoid arthritis, cancer, neurodegenerative diseases, atherosclerosis and ischemic heart disease. ($O_2^{\cdot-}$), perhydroxyl radical (HO_2^{\cdot}), hydroxyl radical ($\cdot OH$), nitric oxide, hydrogen peroxide (H_2O_2), singlet oxygen (1O_2), hypochlorous acid (HOCl) and peroxynitrite ($ONOO^-$) [2]. When the human endogenous antioxidant defence system is overwhelmed by exogenous or endogenous free radical secretion mechanisms, disease conditions such as cancer, heart disease and diabetes set in due to the imbalance between the exogenous or endogenous pro-oxidants and endogenous antioxidants. Cellular damage, ulcer, inflammatory diseases, auto-immune diseases, microbial infections and numerous neurodegenerative disorders, are all linked to the activities of free radicals in the human body [3, 4]. Numerous studies have proven that since these disease conditions are mediated by oxidative stress and imbalance between pro-oxidant and antioxidant factors, antioxidants may play a pivotal role in preventing or slowing the progression of these disease conditions. Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole also diminish oxidation, but they have been proved to be dangerous to humankind; therefore, need for investigation for non-toxic antioxidants which will serve as an alternative [5]. Plant and plant-based resources remain the potential sources of antioxidants active compounds with great potential for the treatment of diverse

disease conditions caused by free radicals globally. It is believed that natural products from plants have always been of great resources for antioxidant therapy since time immemorial. Among the phytochemicals, phenolic compounds have a broad spectrum of biochemical activities and represent the largest group of these compounds [6, 7, 8, 9, 10]. Recent scientific studies globally revealed that medicinal plants and other natural resources have proven to be a source of antioxidants because of their effective pharmacological activities, economic viability, acceptability, availability, and low toxicity. Natural antioxidants can be obtained through diet, dietary supplements and herbs in the form of fruits, spices, vegetables [5]. Plant-based antioxidants such as flavonoids, phenolic acids and tannins have diverse effects like anti-inflammatory, anti-carcinogenic and antiantherosclerotic agents as a result of their antioxidant activity [11]. It is also reported that medicinal plants possessing anthraquinones, flavonoids, aromatic acids, and tannins have shown ROS scavenging and lipid peroxidation prevention effects [12]. The commercial development of plants as sources of antioxidants to enhance health is of current interest [13]. It is believed there is a positive association between the consumption of phenolic-rich foods or beverages and the prevention of diseases associated with oxidative stress, and this has been attributed to antioxidant components such as plant phenolic, including flavonoids and phenylpropanoids among others [13]. Researchers have reported the following plant species as great sources of anti-oxidants: *Diospyros abyssinica* [14], *Geranium sanguineum* [15], *Polyalthia cerasoides* [16], *Uncaria tomentosa* (Willd) [17,18, 19], *Momordica charantia* L [20], *Ficus microcarpa* L. [21], *Diospyros abyssinica* [22, 23]. There is therefore need for continuous search for effective antioxidant agents from natural origin that is effective, available, and acceptable with little or no adverse effects considering the increasing global public health burdens and costs associated with oxidative stress. *Mallotus oppositifolius* (Geiseler) Mull. Arg. (*M. oppositifolius*) (Euphorbiaceae) is a predominant edible shrub in South-Eastern Nigeria where it is commonly identified as 'Ukpo' [24]. It is used in Nigerian folk medicine for the treatment of common infections caused by bacteria and fungi pathogens. It has been reported that *M. oppositifolius* showed antifungal properties [25, 26], antibacterial activity against a wide range of bacteria including *E. coli*, *S. aureus*, among others [26]. This study evaluated the toxicity and antioxidant potential of the crude extract and fractions of *M. oppositifolius* using invitro 1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging assay.

Materials and methods

2.1. Materials and Chemicals

Ascorbic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), methanol, ethyl acetate, n-hexane, and butanol of analytical grade were purchased from Sigma Aldrich. Distilled water was secured from the Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

2.2. Collection and identification of plant leaves

Fresh leaves of *M. oppositifolius* were collected from its natural habitat at Amawbia in Awka South Local Government Area, Anambra State, during the rainy season (June). The plant was identified by a plant taxonomist Mr. Felix Nwafor of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka. A voucher number PCG/UNN/0337/Euphorbiaceae was obtained. The plant leaves were air dried at room temperature for 14 days. The dried leaves were pulverized using a pestle and mortar, sieved, and stored in a dry clean, sterile container.

2.3. Extraction and Fractionation

A kilogram of the coarsely ground leaves of *M. oppositifolius* was weighed and extracted with 1.5 liter of absolute methanol by a cold maceration method as described by [27]. The mixture was covered in order to prevent solvent evaporation and was allowed to stand for 74 hours. Thereafter, it was filtered using Whatman filter paper No. 1. The filtrate was concentrated to dryness using a rotary evaporator at 40 °C. The concentrated extract was scrapped into a sterile bottle, covered properly with foil paper, and kept in refrigerator. The methanol extract was fractionated using the liquid-liquid partition modified method described by [27] using n-hexane, ethyl-acetate, butanol, and distilled water. The extract was suspended in 300 ml of distilled water and was transferred into a separating funnel. Furthermore, 250mL of n-hexane, the least polar solvent was added and the mixture was shaken. The content was allowed to settle, and the bottom of the separating funnel opened to remove the aqueous layer. The remaining content in the separating funnel was poured into a clean container to get the n-hexane fraction. An equal volume of n-hexane was added again, shaken, and separated; this was repeated until no reasonable quantity of extract appeared to move into the n-hexane portion. A similar cycle was performed for ethyl-acetate, butanol, and distilled water to n-hexane, get ethyl-acetate, butanol, and distilled water fractions.

2.4. Phytochemical Screening

The Phytochemical analysis was conducted using the method of Trease and Evans [28], 29, 30] as described by [31] to determine the presence of secondary metabolites in the leaves of *M.*

oppositifolius. Estimation of the amount of phytochemicals present was assayed by using standard procedures described by [29, 32, 33,34].

2.5. Acute Toxicity Studies

The acute toxicity test to determine the LD₅₀ as an index of the safety of the extract was done in 2 phases according to the method of [35] with modifications for *M. oppositifolius* as described by [31]. Briefly, nine animals (rats) were randomly allocated into 3 groups of 3 rats each. Animals in groups 1, 2, and 3 were given 10, 100, and 1000 mg/kg of the crude extract respectively orally. Animals were therefore monitored for signs of toxicity and mortality for 48 hours. All the animals survived, so the crude extract was further subjected to acute toxicity test with higher doses in the second trial. In the second trial, 4 animals were randomly allocated to 4 groups of one animal each. Animals in groups 1, 2, 3, and 4 were given 1200, 1600, 2900, and 5000 mg/kg of the crude extract respectively.

2.6. *Invitro* Antioxidant Assays

DPPH Radical Scavenging Activity Assay

The DPPH free radical scavenging activities of the extract and fractions were evaluated with the method of [36] modified as described by [37]. Freshly prepared DPPH solution (25 µl, 0.6 mmol) was added to 25 µl of different concentrations of the extract and fractions (3.125, 6.25, 12.5, 25, 50, 100 and 200 µg/mL). The volume of the solution was adjusted with methanol to a final volume of 200 µl. The control tube contains 175 µl methanol and 25 µl of DPPH. After incubation in the dark for 30 minutes at room temperature, the absorbance of the mixtures was obtained at 490 nm using a microplate reader. All the tests were performed in triplicate, and ascorbic acid was used as standard. The DPPH radical percentage scavenging potentials of the extracts, fractions, and standard (ascorbic acid) were calculated from the equation below.

$$\% \text{ Inhibition of free radical} = [(A_0 - A_1) / A_0] \times 100 \dots (1)$$

Where A₀ is the absorbance of the control, and A₁ is the absorbance of the test/standard. The IC₅₀ was determined from a plot of percentage scavenging potentials against concentration.

2.7. GC-MS Analysis

Samples of the Fractions of *M. oppositifollous* leaf extract obtained using liquid-liquid chromatographic techniques were characterized using a gas chromatography-mass spectrum (GC-MS)

2.7.1. GC-MS Procedure for Structural Elucidation

From the sample of butanol and ethyl acetate fractions 2ul of the sample extract was injected into the GC column for analysis. The GC (Agilent 6890N) and MS (5975B MSD) are equipped with DB-5ms capillary column (30 m×0.25 mm; film thickness 0.25 µm). The initial temperature was set at 40°C which increased to 150°C at the rate of 10°C/minute. The temperature was again increased to 230°C at the rate of 5°C/minute. The process continued till the temperature reached 280°C at the rate of 20°C/minutes which was held for 8 minutes. The injector port temperature remained constant at 280°C and detector temperature was 250°C then. Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector and transfer line temperatures were set at 250°C and 285°C, respectively. The column temperature was initially kept at 10°C for 1 min, then gradually increased to 250 °C at the rate of 5 °C /min, and finally to 285 °C at the rate of 1 °C /min. The GC (Agilent 6890N) and MS (5975B MSD) is also interfaced with an Agilent mass selective detector 5973N and operated by HP Enhanced ChemStation software (Hewlett-Packard, Palo Alto, CA, USA). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used.

To identify the unknown compounds, present in the extract, their individual mass spectral peak value was compared with the database of the National Institute of Science and Technology 2014 and PubChem database. Then the compounds were identified after comparing the unknown peak values and chromatogram from GCMS against known chromatogram and peak values from the NIST Library database and PubChem database. Subsequently the details about their molecular formula, molecular weight, retention time, and percent content were also obtained. Quantification of the components was performed based on their peak area on the HP-5MS column.

2.8. Statistics

Statistical analyses were carried out using one-way ANOVA using SPSS (IBM, USA), with the level of significance set at $p < 0.05$. Differences among the means were evaluated using Duncan multiple range test and the turkey post hoc tests. All experiments were carried out in triplicate and repeated twice.

3. Result and Discussion

3.1. Phytochemical

The result of the phytochemical screening is presented in Table 1. The result showed an abundance of flavonoids (28.21), tannins (17.72), alkaloids (7.79), saponins (0.23), and steroids (0.08) in the *M. oppositifolius* leaf extract while terpenoids were absent. Also, the plant contained reducing sugar (2.0) and carbohydrates (33.79) respectively (Table 1).

Table 1: The phytochemical constituents of *M. oppositifolius* leaf

SN	Bioactive compound		<i>M oppositifolius</i>
1	Alkaloids	Present ⁺⁺	7.797 ± 0.023
2	Flavonoids	Present ⁺⁺⁺	28.213 ± 0.029
3	Tannins	Present ⁺⁺⁺	17.723 ± 0.029
4	Saponins	Present ⁺⁺	0.239 ± 0.003
5	Steroid	Present ⁺	0.077 ± 0.000
6	Terpenoids	Absent	-
7	Carbohydrates	Present ⁺	33.793 ± 0.046
8	Reducing sugar	Present ⁺	2.013 ± 0.029

Heavily present: +++; slightly present: ++; present: +; absent: -

3.2. Acute toxicity test of methanol leaf extract of *M. oppositifolius*

The result of the acute toxicity studies is presented in Table 2. It showed that the LD₅₀ of *M. oppositifolius* was above 5000 mg/kg of crude extract because no death was recorded above 5000 mg/kg in the rats and this implies that the crude extract is safe for consumption.

Table 2: Acute toxicity test of methanolic leave extract of *M. oppositifolius*

Phase	Dose (Mg/kg)	No of animals	Death ratio
Phase 1	10	3	0/3
	100	3	0/3
	1000	3	0/3
Phase 2	1200	1	0/1
	1600	1	0/1
	2900	1	0/1
	5000	1	0/1

3.3. Antioxidant Activity of *M. oppositifolius*

The result of the anti-oxidant activity of crude methanol extract of *M. oppositifolius* is presented in Table 3. The result showed a statistical difference ($p < 0.05$) in the antioxidant potential of *M. oppositifolius* crude extract when compared with the standard (ascorbic acid) at 125, 62.5 and 31.125 $\mu\text{g/ml}$ respectively. At 125 $\mu\text{g/ml}$ the crude extract of *M. oppositifolius* had a greater significant ($p < 0.05$) (78.51 %) reduction of oxidation when compared to the standard drug (25.07 %). Also, at 62.5 and 31.125 $\mu\text{g/ml}$, the crude extract had 56.53 and 39.76 % inhibition of oxidation when compared with the standard 11.75 and 4.3 respectively. The result further showed that at 500 and 250 $\mu\text{g/ml}$ there was no significant difference between the crude extract and the standard antioxidant drug in reduction of oxidation.

Table 3. antioxidant potential of crude methanol extract of *M. oppositifolius*

S/N	Dosage ($\mu\text{g/ml}$)	Standard /Ascorbic Acid	<i>M. oppositifolius</i>
1	31.25	4.37 ± 0.22^a	39.76 ± 1.65^b
2	62.5	11.75 ± 2.72^a	56.53 ± 1.50^b
3	125	25.07 ± 0.72^a	78.51 ± 1.41^b
4	250	79.66 ± 0.00^a	80.16 ± 1.22^a
5	500	82.09 ± 0.57^a	82.31 ± 0.50^a

(NB: Any two or more means having a common alphabet are not statistically different at the 5% level of significance)

3.4. Antioxidant Activity of *M. oppositifolius* Fractions

The result of the antioxidant potential of the n-hexane, ethyl-acetate, butanol, and water fraction of *Mallotus oppositifolius* is presented in Table 4. The result showed that the butanol and ethyl-acetate had significant ($p < 0.05$) antioxidant activity when compared with the control at 125, 62.5, and 31.125 $\mu\text{g/ml}$ respectively while at 250 and 500 $\mu\text{g/ml}$ there was no statistical difference in the antioxidant activity when compared with the control.

At 500 and 250 $\mu\text{g/ml}$ butanol fraction had 78.94 % and 82.09 % inhibition of oxidation respectively, and they were not statistically different when compared with the standard (ascorbic acid) that had 82.09 and 79.66 % respectively. Also, at 500 and 250 $\mu\text{g/ml}$, water fraction had the least inhibition of oxidation of 55.44 and 35.44 % respectively but was statistically lower when compared with the standard drug. The result further showed that at 500 and 250 $\mu\text{g/ml}$, ethyl acetate fraction had 69.77 and 76.65 % inhibition of oxidation, while n-hexane had 61.53 % and 48.21 % inhibition of oxidation and these were statistically lower when compared with the standard and butanol fraction respectively. At 125, 62.5, and 32.5 $\mu\text{g/ml}$, ethyl acetate had highest inhibition of oxidation of 76.22, 54.73, and 50.07 %, which were significantly higher when

compared with the standard that had 25.07, 11.75 and 4.37 % respectively. Also, at 125 and 62.5 µg/ml, butanol had 65.12 and 40.90% inhibition of oxidation whereas n-hexane had 30.59 and 25.93 % inhibition of oxidation. Water at 125 and 62.5 µg/ml had 22.06 and 18.05% inhibition of oxidation. Also, at 32.25 µg/ml, Butanol and n-hexane had 26.36 and 29.59 % inhibition of oxidation respectively.

Table 4: The antioxidant potential of *Mallotus oppositifolius* fractions

SN	Dosage (µg/ml)	Standard /Ascorbic Acid	Butanol	Ethyl-acetate	N-Hexane	Water
1	31.25	4.37 ± 0.22 ^a	26.36 ± 3.87 ^c	50.727 ± 1.93 ^e	29.59 ± 8.24 ^{c^d}	15.62 ± 2.57 ^b
2	62.5	11.75 ± 2.72 ^a	40.90 ± 2.51 ^d	54.73 ± 0.57 ^e	25.93 ± 3.59 ^c	18.05 ± 2.57 ^b
3	125	25.07 ± 0.72 ^a	65.12 ± 1.08 ^c	76.22 ± 0.43 ^d	30.59 ± 1.65 ^b	22.06 ± 4.72 ^a
4	250	79.66 ± 0.00 ^d	80.23 ± 0.86 ^d	76.65 ± 0.00 ^c	48.21 ± 4.08 ^{a^b}	35.10 ± 3.44 ^a
5	500	82.09 ± 0.57 ^c	78.94 ± 0.57 ^d	69.77 ± 1.15 ^c	61.53 ± 0.65 ^b	55.44 ± 0.00 ^a

. (NB: Any two or more means having a common alphabet are not statistically different at the 5% level of significance)

3.5 Result of the IC₅₀ of *M. oppositifolius* Crude Extract and Fractions

The result of the concentration of the crude extract and fractions needed to decrease the initial DPPH concentration by 50 % (IC₅₀) is presented in Table 5.0. The result showed that the *M. oppositifolius* crude extract had the best IC₅₀ value of 219.7 µg/ml, followed by butanol fraction (234.41 µg/ml), ethyl-acetate (242.83 µg/ml), ascorbic acid (256.27 µg/ml), and n-hexane fraction (338.5 µg/ml) while water had the least (407.07µg/ml) activity.

Table 5: IC₅₀ of the Crude extract and factions of *M. oppositifolius*

S/N	Treatment	IC50
1	Standard /Ascorbic acid	256.27
2	<i>M.oppositifolius</i> crude extract	219.68
3	Butanol fraction	234.41
4	Ethyl-acetate fraction	242.83
5	N-Hexane fraction	338.524
6	Water fraction	407.068

3.6. GCMS Result

3.6.1. GCMS Result of Ethyl-acetate Fraction.

The GCMS result of the ethyl acetate fraction is presented in Table6 and Figure 6. The result showed the presence of seven compounds which includes: 7,10,13-eicosatrienoic acid methyl ester, korormicin, agosterol, phyllanthin, ricinoic acid, 2,4,6-decatrienoic

acid1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9, limonin. (Table 10). The result showed that ricinoic acid (41%) is the most abundant compound in ethyl acetate fraction, followed by limonin (33%) while agosterol (2.1%) and korormicin are the least compounds.

Table 6: GCMS result of ethyl-acetate fraction of *Mallotus oppositifolius*.

Rt	Name of components	Formula	Mw	% Abundance
11.56	7,10,13-Eicosatrienoic acid, methyl ester	$C_{21}H_{36}O_2$	320	4.022
11.648	Korormicin	$C_{25}H_{39}NO_5$	433	2.231
12.224	Agosterol	$C_{31}H_{50}O_6$	518	2.113
12.707	Phyllanthin	$C_{24}H_{34}O_6$	418	13.614
13.592	Ricinoic acid	$C_{18}H_{34}O_3$	298	41.365
13.906	2,4,6-Decatrienoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9	$C_{30}H_{40}O_6$	496	3.78
17.835	Limonin	$C_{26}H_{30}O_8$	470	32.874

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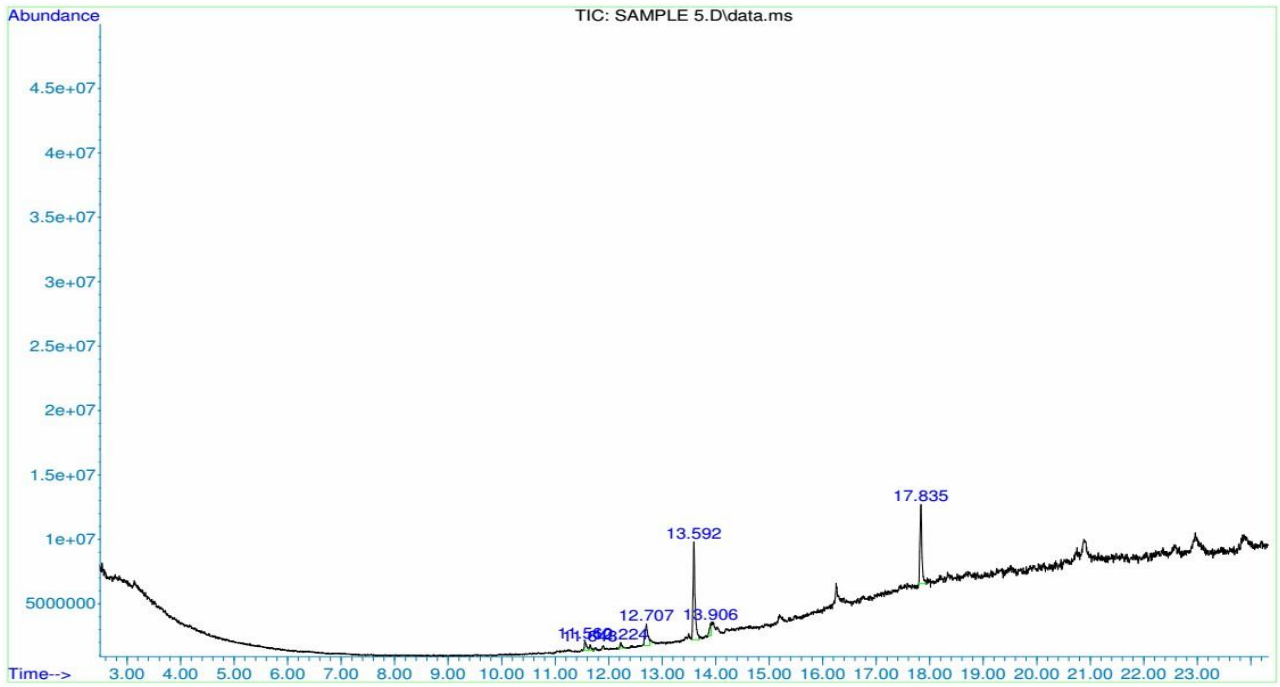


Figure 1: GCMS chromatogram of ethyl-acetate fraction showing 7 peaks

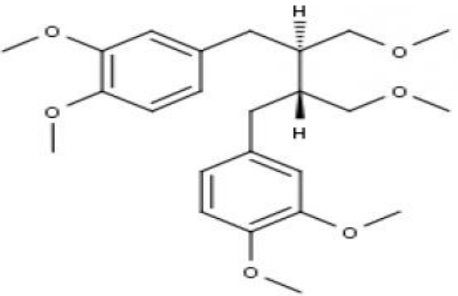
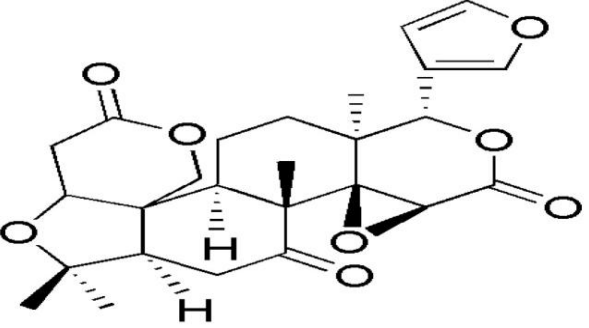
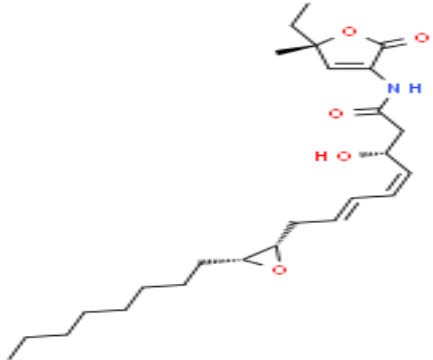
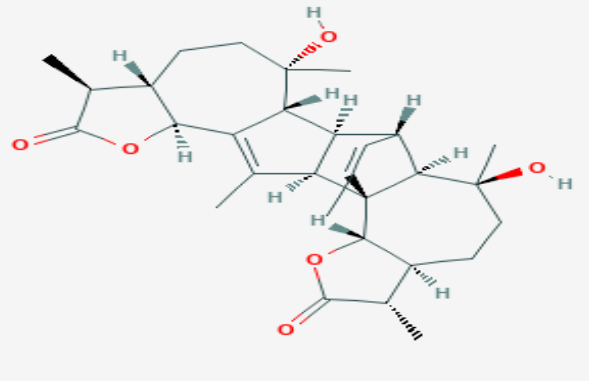
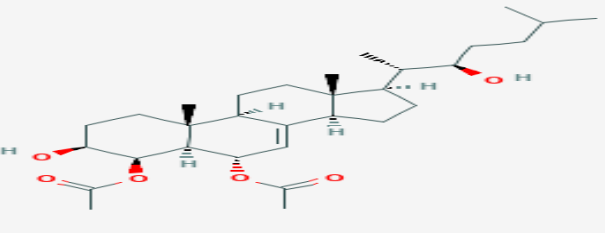
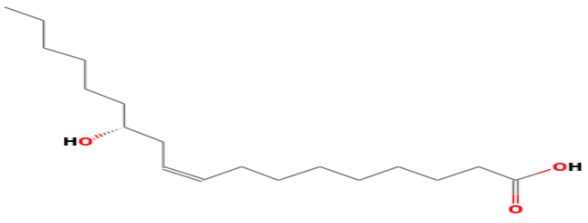
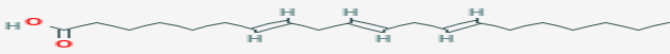
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 <p>Name Korormicin Molecular Formula $C_{25}H_{39}NO_5$</p>	 <p>Name: Absinthin Molecular formula $C_{30}H_{40}O_6$</p>
 <p>Name: Agosterol A Chemical Formula $C_{31}H_{50}O_6$</p>	 <p>Name: Octadecanoic acid, 9,10-epoxy-, cis- Chemical Formula: $C_{18}H_{34}O_3$</p>
 <p>Name: 7,10,13-Eicosatrienoic acid, methyl ester Formula: $C_{21}H_{36}O_2$</p>	

Figure 2: Compounds Isolated from Ethyl-acetate Fraction

3.6.1. GCMS Result of Butanol Fraction.

The result of the GCMS result of the butanol fraction is presented in Table 7 and Figure 3. The chromatogram of the GCMS result showed 7 peaks as shown in figure 3.0. The GCMS result of the butanol fraction showed the presence of seven compounds which include: ferulylhistamine, hippeastrine, 10,13-eicosadienoic acid methyl ester, ethyl 5,8,11,14-eicosatetraenoate, 6-hydroxy-powelline-N-nitroso-7-demethoxy-aldehyde, 6,7-epoxypregn-4-ene-9,11,18-triol-3,20-dione 11,18-diacetate and 5Alpha-androstane-3,17-dione 17-monooxime. The result showed that 10,13-eicosadienoic acid (28.95 %), methyl ester and hippeastrine (28.0 %) were the most abundant compounds in butanol fraction of *Mallotus oppostifollous* followed by 6-Hydroxy-powelline-N-nitroso-7-demethoxy-, aldehyde (15.04 %) while Ferulylhistamine (4.9 %) was the least.

Table 7: GCMS Result of butanol fraction of *Mallotus oppostifollous*.

SN	RT	NAME OF COMPONENTS	FORMULAR	MW	% Abundance
1	12.224	Ferulylhistamine	C ₁₅ H ₁₉ N ₃ O ₂	273	4.906
2	12.818	Hippeastrine (=Trispherine)	C ₁₇ H ₁₇ NO ₅	315	28
3	14.022	10,13-Eicosadienoic acid, methyl ester	C ₂₁ H ₃₈ O ₂	322	28.948
4	15.477	Ethyl 5,8,11,14-eicosatetraenoate	C ₂₂ H ₃₆ O ₂	332	6.66
5	16.03	6-Hydroxy-powelline-N-nitroso-7-demethoxy-, aldehyde	C ₁₆ H ₁₆ N ₂ O ₅	316	15.035
6	16.257	6,7-Epoxypregn-4-ene-9,11,18-triol-3,20-dione, 11,18-diacetate	C ₂₅ H ₃₂ O ₈	460	9.774
7	16.647	5Alpha-androstane-3,17-dione 17-monooxime	C ₁₉ H ₂₉ NO ₂	303	6.677

File :E:\Ifeanyi Onyeka\SAMPLE 3.D
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Vial Number: 1

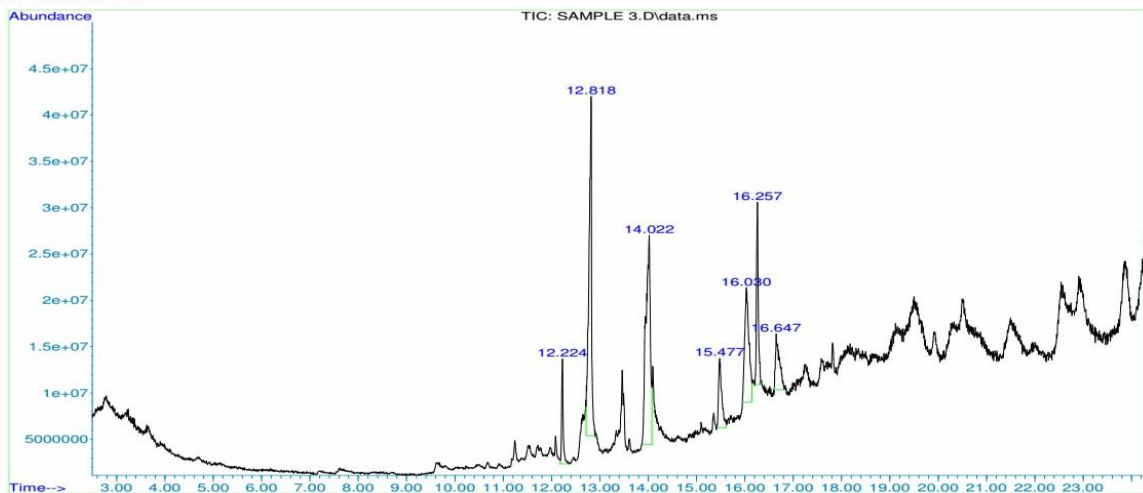


Figure 3: GCMS chromatogram of butanol fraction showing 7 peaks

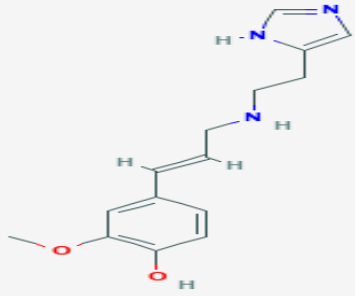
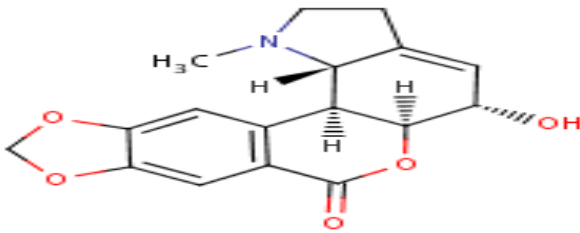

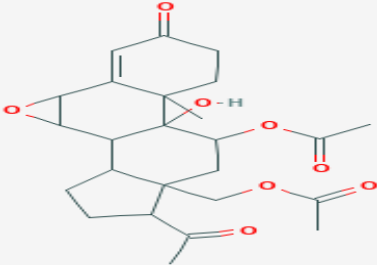

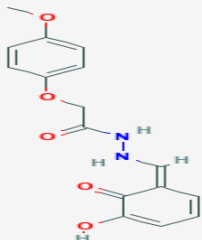
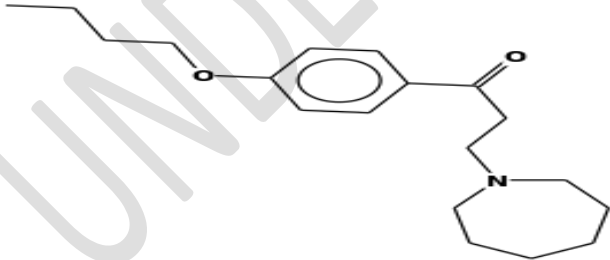
 <p>Name of Compound: Ferulylhistamine Chemical Formula: $C_{15}H_{19}N_3O_2$</p>	 <p>Name of Compound: hippastrine Chemical Formula: $C_{17}H_{17}NO_5$</p>
 <p>Name of Compound: 11,13-Eicosadienoic acid, methyl ester Chemical Formula: $C_{21}H_{38}O_2$</p>	 <p>Name of Compound: 6,7-Epoxypregn-4-ene-9,11,18-triol-3,20-dione, 11,18-diacetate Chemical Formula: $C_{25}H_{32}O_8$</p>
 <p>Name of Compound: Benzoic acid, pentadecyl Chemical Formula: $C_{22}H_{36}O_2$</p>	 <p>Name of Compound: 5-hydroxy-6-oxocyclohexa-2,4-dien-1-ylidene) methyl]-2-(4-methoxyphenoxy) acetohydrazide Chemical Formula: $C_{16}H_{16}N_2O_5$</p>
 <p>Name of Compound: 5Alpha-androstane-3,17-dione 17-monooxime Chemical Formula: $C_{19}H_{29}NO_2$</p>	

Figure 4: Compounds Isolated from Butanol Fraction Discussion

Free radicals cause detrimental alterations in a variety of ways in biomolecules (such as proteins, lipids, and DNA) of the living cells resulting in disease conditions like cancer, diabetes, etc., with a huge economic burden. The present studies showed that *M. oppositifolius* is rich in phenolic compounds such as flavonoids, tannins, and alkaloids which could be associated with its high antioxidant activity when compared with the standard.

It is believed that herbal medicine has great potential as antioxidants due to the presence of phytochemicals which have been scientifically proven to contain polyphenolic compounds that have shown to have antioxidant activities. It is reported that polyphenols have ROS scavenging and lipid peroxidation prevention effects [38]. The past epidemiological study opines positive associations between the consumption of phenolic-rich foods, and the prevention of diseases and this has been attributed to antioxidant components particularly the plant phenolics[13].The GCMS result of the Ethyl-acetate fraction showed the presence of 7,10,13-eicosatrienoic acid methyl ester, korormicin, agosterol, phyllanthin, ricinoic acid, 2,4,6-decatrienoic acid 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9, limonin, these compounds have all been reported for their antioxidant activities in previous studies.

Phyllanthin is an active lignan present in various *Phyllanthus* species and number of studies revealed that it exhibits various biological activities such as antioxidant [39]; anticancer [40, 41] and anti-inflammatory activities [42,43].

Limonin also known as obaculactone and evodin, belongs to the tetracyclic triterpenoids derived from the plants of Rutaceae and Meliaceae. It is a secondary metabolite with a high biological activity in plants and has been reported for anti-cancer [44,45, 46, 47] and anti-inflammatory activities [48].

Korormicin is a novel antibiotic isolated from pseudoalteromonads reported as an antibiotic that selectively kills gram negative bacteria that express the Na⁺-pumping NADH quinone oxidoreductase (Na⁺-NQR)[49]. Absinthin is a triterpene, first isolated from a Chinese herb, *Artemisia absinthium* (Wormwood) with high potential for anti-inflammatory properties [50].

Agosterol-A was reported to have enhanced the susceptibility to anti-tumor agents such as vincristine, colchicine, doxorubicin, and etoposide in P-gp- and MRP1- overexpressing MDR cells by inhibiting ATP-dependent drug efflux by P-gp and MRP1 [51].

Oxiraneoctanoic acid, 3-octyl-, cis- isolated from h-hexane fraction of *Acacia modesta* has been reported for antibacterial and insecticidal activity [52]. Also, the GCMS result of the butanol fraction showed the presence of seven compounds which include: ferulylhistamine, hippeastrine, 10,13-eicosadienoic acid methyl ester, ethyl 5,8,11,14-eicosatetraenoate, 6-hydroxy-powelline-N-nitroso-7-demethoxy-aldehyde, 6,7-epoxypregn-4-ene-9,11,18-triol-3,20-dione 11,18-diacetate and 5 α -androstane-3,17-dione 17-monooxime. Ferulylhistamine an imidazole alkaloid [53] was isolated from the root of *Ephedra* and was shown to have histidine decarboxylase inhibitory, antiulcer and antihepatotoxic activities [54]. Hippeastrine is an alkaloid isolated from Amaryllidaceae family which has been reported for biological activity such as antitumor, antiviral, antibacterial, antifungal, antimalarial, and analgesic [55]. It has been reported that 11,13-eicosadienoic acid, methyl ester is a fatty acid derivative which was isolated from chloroform extract of *Hugonia mystax*, a plant that was shown to have antioxidant property [56]. The compound 6,7-Epoxypregn-4-ene-9,11,18-triol-3,20-dione, 11,18-diacetate has been isolated in *Phyllanthus amarus*; a plant that was shown to possess great antimicrobial potential [57]. The compound benzoic acid, pentadecyl ester is a fatty acid which was isolated from *Sanbai* melon seed by [58], the essential oil was reported for antidiabetic activity.

Conclusion

This study has shown that LD₅₀ of *M. oppositifolius* crude extract is above 5,000 mg/kg and therefore relatively safe at dosage less than or equal to 5,000 mg/kg at acute administration orally. The findings also showed that both the crude extract and fractions have great potential for antioxidant activity which could be associated with high content of the flavonoids and alkaloids. Furthermore, the GCMS result of ethyl acetate fraction showed the presence of 7,10,13-eicosatrienoic acid methyl ester, korormicin, agosterol, phyllanthin, ricinoic acid, 2,4,6-decatrienoic acid 1 α ,2,5,5 α ,6,9,10,10 α -octahydro-5,5 α -dihydroxy-4-(hydroxymethyl)-1,1,7,9, limonin, compounds reported to possess strong antioxidant activities. Also, the GCMS result of the butanol fraction showed the presence of seven compounds which include: ferulylhistamine, hippeastrine, 10,13-eicosadienoic acid methyl ester, ethyl 5,8,11,14-eicosatetraenoate, 6-hydroxy-powelline-N-nitroso-7-demethoxy-aldehyde, 6,7-epoxypregn-4-ene-9,11,18-triol-3,20-dione 11,18-diacetate and 5 α -androstane-3,17-dione 17-monooxime, compounds that may have great potential for antioxidants and anti-inflammation.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication and etc.) have been completely observed by the authors. The ethical approval for the research studies was obtained from Nnamdi Azikiwe University Awka animal and research ethical committee (NAU/ARC/2019).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products or the identified compounds because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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