Phytochemical profiling using GC-MS, antioxidant and antidepressant properties of methanolic leaf extract of *Clerodendrum polycephalum* Baker in Swiss mice

ABSTRACT

Background:
Clerodendrum polycephalum Baker is used by the traditional people in southwest Nigeria for arresting bleeding from cuts and treating bacterial infections especially wound infection without scientific proof of its efficacy. The plant was investigated in animal models for its antidepressant activity in Swiss mice.

Methods:
The GC-MS, phytochemical analyses, antioxidant activities, tail suspension test, forced swimming test and oxidative stress parameters were determined using standard procedures.

Results:
A total of 27 compounds were identified consisting of five prominent compounds and 22 minor compounds. The five prominent compounds constitute 63.99% of the *Clerodendrum polycephalum* Baker plant. The five major compounds and their percentage abundance are: Bicyclo[3.1.1]heptane, 2,6,6-trime thyl- (21.36%), Squalene (18.69%), Neophytadiene (10.71%), 2-Tridecanol (6.66%) and 2-Dodecanol (6.57%). The phytochemicals present in the methanolic leaf extract of *C polycephalum* are: flavonoids, steroid, tannins, saponins, anthraquinones, alkaloids, terpenoids, anthocyanin, phenolic compounds and carbohydrate The extract has the ability to scavenge DPPH activity and it contains other components like: total proanthocyanidine (1.369±0.184), flavonoids (2.4%), β-Carotene (0.1336± 0.45 µg) and lycopene (0.0340±0.053µg/g). The antidepressant result showed robust and dose-dependent antidepressant-like activity of *Clerodendrum polycephalum* Baker. There are statistically significant ($P<0.05$) reductions in the duration of immobility time both in the tail suspension and forced swimming test. *Clerodendrum polycephalum* extract produced significant ($P<0.05$) increase in total protein of the plasma, liver and kidney homogenate of the treated groups (Group C, D and E) compared to the untreated mice in group B. The level of antioxidant parameter such as catalase, and superoxide dismutase were significant increased ($P<0.05$) and MDA values significantly reduce ($P<0.05$) in the treated groups administered with the extract and imipramine compared to the untreated mice in group B.

Conclusion: The results show that methanolic leaf extract of the *Clerodendrum polycephalum* Baker has potential antioxidant and antidepressant activities and further studies should be conducted to identify, isolate and evaluate its potential active compound responsible for such effect.

Keywords: Antidepressant, *Clerodendrum polycephalum* Baker, Depression, GC-MS, FST, TST

1.0 INTRODUCTION

"*Clerodendrum polycephalum* Baker is a shrub that belongs to the family Lamiaceae. They usually grow up to 1–12 m tall, with opposite or whorled leaves and its common names include glory bower, bag flower and bleeding-heart" [1]. "In Nigeria, the Yorubas commonly refer to it as 'aporo' which said to mean, 'it kills pain', and it is used as antidotes for venomous stings and bites" [2]. "Studies have shown that *Clerodendrum polycephalum* have been used as an antidote for venomous bites, as painkiller and medicine for the treatment of convulsion, epilepsy, spams and paralysis. The leaf sap was known to cure epilepsy, paralysis and also in the treatment of convulsion and spasm. In Eastern Cameroun and Southern Nigeria, the leaf sap is used to wash the face of persons subject to fainting, giddiness and attacks of epilepsy" [3]. "The powder or paste form and the various extracts of the leaves, stem and root of the plant are reported to be used as medicine for the treatment of cataract, asthma, malaria, pyreticosis and diseases of blood, skin and lung. It possess anti-diabetic, antitumor, anti-inflammatory, antidiarrheal and antimicrobial activities have been reported in literature in order to prove their ethnomedical uses" [4, 5].

"GC-MS is an effective method for secondary metabolites profiling in plant and non-plant species, and it is also a useful way to extract polar solvents and produce volatile oils with bioactive compounds. Some of our previous studies have shown different secondary metabolites profiling for numerous medicinal plants. Some of these plant include: methanolic root, stem and leaf extracts of *Vernonia amygdalina* [6], *Carica papaya* seed oil [7], *Carica papaya* leaf [8], *Hunteria umbellata* seed extract [9], hexane leaf, stem and root extracts of *Azadirachta indica A. Juss* [10], GC-MS of *Azadirachta indica* (neem) root [11], methanolic leaf extract of *Morinda lucida* also called Ewe Oruwo [12], Phytochemical screening, GC-MS and antidiabetic properties of aqueous extract of ginger (*Zingiber officinale*) in alloxan: induced diabetic Wistar rats [13], phytochemical Screening, AAS, GC-MS and Antibacterial activities of Turmeric [14] and evaluation of secondary metabolites profiling of ginger (*Zingiber officinale* Roscoe) rhizome using GC-MS" [15].

"Depression is becoming a rising problem in the world currently as almost 450 million people around the world suffer from it according to WHO. Depression along with anxiety is now the most prevalent mental

disorders all over the world; together they are responsible for 50% of psychiatric and substance use disorders" [16]. "Epidemiological studies have shown that more than 20% of the general populations have at least one of these disorders during their lifetime" [17]. "Nearly one in four women and one in six men experience depression in their life [18], and up to 65% of individuals have recurrent episodes of the disorder" [19-21]. "Currently, Depression has been found to be the fourth leading cause of overall disease burden and the leading cause of nonfatal disease burden worldwide" [22]. "Drugs such as Monoamine Oxidase Inhibitors, Tricyclic antidepressants, Reuptake Inhibitors, Selective Serotonin are used for the treatment of depression which possesses some serious drawbacks like weight gain, anxiety, insomnia, sedation, etc. Studies have shown that natural products have less side effects and complications compared to orthodox drugs" [23]. The study examines the **p**hytochemical profiling using GC-MS, antioxidant and antidepressant properties of methanolic leaf extract of *Clerodendrum polycephalum* in Swiss mice.

2.0 METHODOLOGY

2.1 Collection and identification of plant extract

The leaves of *Clerodendrum polycephalum* Baker were obtained from Lagos State University of Science and Technology, Ikorodu in Lagos State, Nigeria, and were authenticated in University of Lagos, in Lagos State, Nigeria.

2.2 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of *Clerodendrum polycephalum* Baker

GC-MS analysis of the *Clerodendrum polycephalum* Baker was carried out on an Agilent technology 7890 GC system equipped with a mass spectrometric detector (MSD) as described by Momoh et al. [12].

Detection of Components

Analysis of mass spectrum GC-MS was conducted by the database of the National Institute Standard and Technique (NIST) which contained more than 62,000 patterns. The spectrum of the unidentified compound was compared with the spectrum of the identified compounds stored in the National Institute Standard and Technique library. The names, molecular weight, structure of the compounds in the test material were ascertained.

2.3 Preparation of methanolic leaf extract of *Clerodendrum polycephalum*

The leaf of *Clerodendrum polycephalum* was washed properly with distilled water to remove dirt's, it was air dried under shade in the Chemistry Laboratory, pulverized into coarse power using industrial machine. The extraction was carried out by dispersing 200g of the grounded *C. polycephalum* plant material into one

litre of 75% methanol at room temperature. Shaking and maceration was done for 72 hours. The solution was filtered by passing it through cotton wool followed by Whatman filter paper and the resultant filtrate was concentrated using rotary evaporator at a temperature not exceeding 40^{o}C. The concentrated leaf extract of *C. polycephalum* was dried to complete dryness in an aerated oven at 40^{o}C for 48 hours. The extract of *C. polycephalum* was later stored in a refrigerator at 4^{o}C.

2.4 Preliminary phytochemical analysis

The presence of reducing sugars, tannins, saponin, alkaloids, anthraquinones, flavonoids and glycosides were determined by qualitative methods using procedures described by Momoh et al. [24] and Momoh et al. [25]. The simple qualitative analyses of the extract were based on the intensity of the colour change.

2.5 Flavonoid content determination

"One hundred millilitres of 80% aqueous methanol was used to repeatedly extract 1 g of the defatted sample at room temperature. The solution was then filtered through Whatman filter paper. The filtrate was evaporated to dryness in a crucible over a water bath and weighed to a constant weight using method" described by Longe et al. [26].

2.6 Determination of total proanthocyanidins

Determination of proanthocyanidin was based on the procedure reported by Longe et al. [26]. "A volume of 0.5 ml of 0.1 mg/ml of extract solution was mixed with 3 ml of 4% vanillin methanol solution and 1.5 ml hydrochloric acid; the mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total proanthocyanidin content were expressed as catechin equivalents (mg/g) using the following equation based on the calibration curve: y = 0.5825x, R2 = 0.9277, where x was the absorbance and y is the catechin equivalent (mg/g)". [26]

2.7 Determination of β-carotene and lycopene

β-Carotene and lycopene were determined by the method of Barros et al. [27]. The dried extract of *Clerodendrum polycephalum* 100 mg was vigorously shaken with acetone-hexane mixture (4:6, 10 mL) for 5 minutes and filtered through a disposable filter. The absorbance of the filtrate was measured at 453, 505, and 663 nm. Contents of β-carotene and lycopene were calculated according to the following equations:

β-carotene (mg/100 mL) = 0.216 (A663) − 0.304 (A505) + 0.452 (A453);

lycopene (mg/100 mL) = −0.0458 (A663) + 0.372 (A505) − 0.0806 (A453).

The assays were carried out in triplicates, the results were mean ± SD and expressed as µg of carotenoid/g of extract.

2.8 DPPH radical-scavenging activity

The antioxidant activity of the methanol extracts of *Clerodendrum polycephalum* was assessed by using 1,1-diphenyl 1-2-picryl-hydrazyl (DPPH) assay [28]. The DPPH (2.4 mg) was dissolved in 100 ml of methanol and diluted with methanol to obtain an absorbance of about 0.98 (± 0.02) at 517 nm. 0.01 ml of the methanolic extract of the different concentrations of 25-250 µg/ml was added in 3 ml of the DPPH solution. After incubation for 15 minutes in dark; absorbance of the mixture was determined at 517 nm. The DPPH radical scavenging activity was determined using the formula shown below;

Percentage inhibition = [(control absorbance−sample absorbance)/(control absorbance)] x100:

IC_{50} is the concentration value which scavenged 50% of the DPPH radicals. Ascorbic acid and rutin were used as reference compounds [29, 30].

2.9 Experimental animals

Male Swiss mice of 20 to 30g were obtained from University of Lagos Idi-Araba, Lagos- Nigeria.The rat were housed in clean polypropylene cages and kept in a well-ventilated room and allowed them to acclimatize for six weeks, feed and water were given all through. Animals were maintained in 12 hours light: 12 hours dark at a controlled temperature (20-25°C) and kept in animal house.

2.10 Experimental design

The animals were divided into five groups (five animals per group)

Group A -Normal control (They did not undergo the experiment)

Group B-Negative control (No drug and they undergo FST and TST)

Group C-Positive control (30 mg/kg B.WT of Imipramine)

Group D-100 mg/kg.B.WT of methanolic extract of *Clerodendrum polycephalum*

Group E-300 mg/kg.B.WT of methanolic extract of *Clerodendrum polycephalum*

Drugs and treatment schedule

Imipramine IMP (30 mg/kg) was used as the standard drug for depression. The methanolic extract of *Clerodendrum polycephalum* and standard drug were administered per orally (p.o).

2.11 Depression models

2.11.1 Tail suspension test

The tail suspension test (TST) was performed according to the method described by Steru et al. [31] with slight modification. The principle of this test is suspending mice suspended upside down leads to

characteristic behavior immobility which resembles human depression. After the administration of respective extract and standard drugs, mice were suspended on the edge of the table 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility duration was recorded during 6 minutes period. Mice were considered immobile when they hanged passively and completely motionless. Single oral administrations of methanol leaf extract of *Clerodendrum polycephalum* (100 and 300 mg/kg B.W) and *Imipramine,* (IMP) 60 mg/kg were given one hour prior to the test.

Figure 1 shows Tail suspension test (TST) assay

2.11.2 Forced swim test (FST)

Antidepressant activity of methanolic extract of *Clerodendrum polycephalum* was assessed using modified Porsolt test. The forced swim test (FST) is the most widely used pharmacological *in vivo* model for assessing antidepressant activity and was performed according to the method of Porsolt et al. [32]. The apparatus consists of a transparent plastic cylinder filled to 18 cm depth with water. Animals were divided into groups of five animals each per group. One of the groups received only distilled water treatment. In the pre-test session, every animal was placed individually into the cylinder for 15 minutes, 24 hours prior to the 6 minutes swimming test, in which the duration of immobility was recorded for the last 5 minutes.

Oral administration of the methanol extract *of Clerodendrum polycephalum* (100 and 300mg/kg) and IMP (30 mg/kg.) was administered one hour prior to final swimming test session. The period between when the mouse was immersed and when no further attempts to escape were made (apart from the movements necessary to keep its head above the water) was recorded as the immobility time.

The following behaviors were recorded during the experiment

1. **Immobility**: Floating in water without swimming.
2. **Swimming**: Active movements of mice and circling in the container.
3. **Climbing**: Active movements of forelimbs on the container wall.

Figure 2a. Shows the forced swimming test assay.

Figure 2b. Shows the forced swimming test assay.

2.12. Collection of Blood Samples

The Swiss mice in the tail suspension test were sacrificed. Blood were collected from the mice by ocular puncture into EDTA bottles for hematological analysis and the remaining blood were collected in an heparinized bottle and centrifuge at 3000 rpm for 20 minutes using a centrifuge and the plasma stored at 4°C.

2.12.1 Determination of total protein, and uric acid

Plasma total protein and liver and kidney homogenate uric acid (UA**)** values were determined using Randox diagnostic kits.

2.13. Estimation of Oxidative Stress Parameters

2.13.1. Preparation of Liver Homogenate

The Liver tissues of some of the sacrificed albino Swiss mice were excised, the kidney and liver samples were cut into small pieces and homogenized in phosphate buffer saline (PBS) to give a 10% (w/v) liver homogenate. The homogenates were then centrifuged at 12,000 rpm for 50 minutes. The supernatant obtained was later used for assay of total protein, uric acid, thiobarbituric acid reactive substances (TBARS) content, superoxide dismutase and catalase.

2.13.2. Estimation of Lipid Peroxidative (LPO) Indices

Lipid peroxidation as evidenced by the formation of TBARS was measured in the homogenate by the method of Momoh and Oshin [33].

2.13.3 Estimation of Catalase (CAT)

The liver homogenate was assayed for catalase colorimetrically at 620mm and expressed as µmoles of H_2O_2 consumed/min/mg protein as described by Momoh and Oshin [33].

2.13.4 Estimation of superoxide dismutase (SOD)

The SOD activity was estimated by its capacity of inhibiting pyrogallol autooxidation in alkaline medium. The liver and kidney homogenate were assayed for the presence of SOD% by utilizing the technique described by Zou et al. [34] and Momoh et al. [35].

2.14. Histopathological Studies

"The histopathological analyses were assayed in the Department of Anatomy, college of Medicine, University of Lagos, Idi-Araba, Surulere, Lagos, Nigeria. Abdomens were opened to remove the liver. Some of the livers were fixed in Boucin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12 hours, and then embedded in paraffin using conventional methods" [33]. They were cut into 5µm thick sections and stained using haematoxylin-eosin dye and finally mounted in di-phenyl xylene. The sections were then examined under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

2.15 Statistical Analysis

The data were expressed as mean ± SD. Comparisons across each of the rows were done using one way analysis of variance (ANOVA) complemented with Bonferroni`s to compare all pair of columns. For all the analyses, a $P<0.05$ was considered statistically significant.

3.0 RESULT

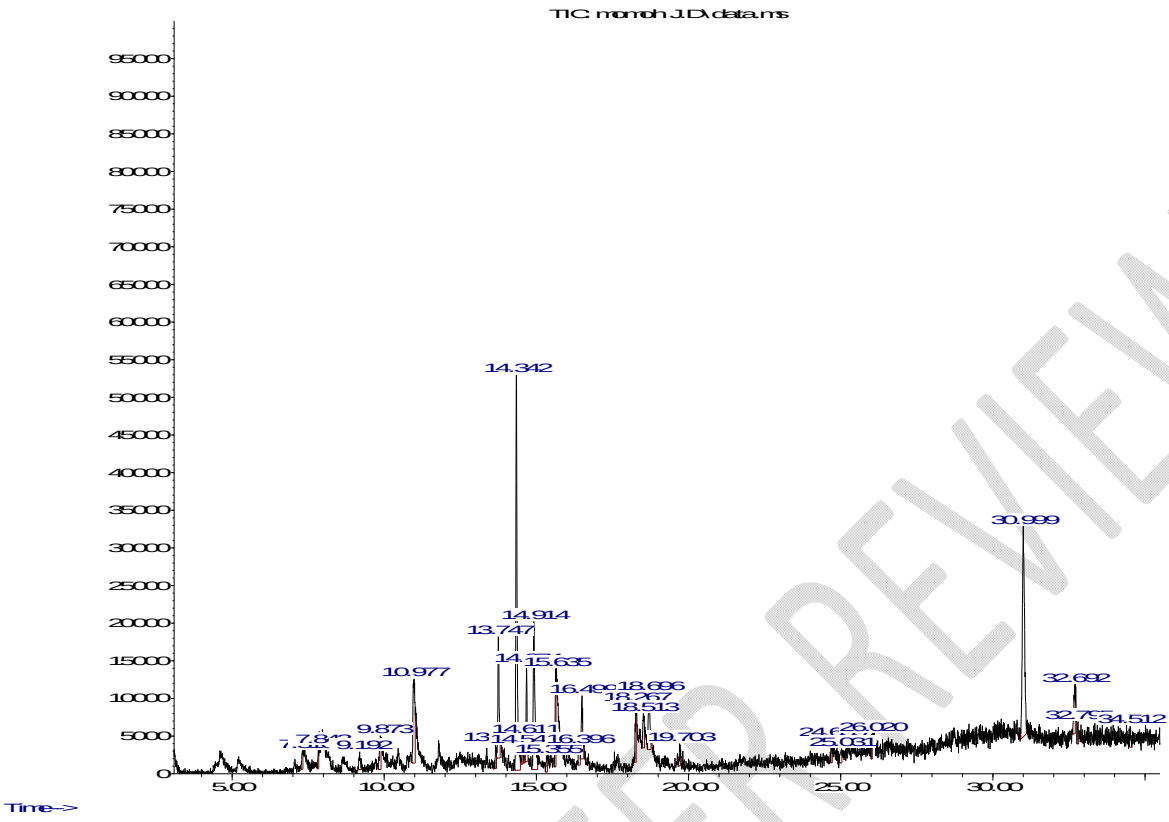

Figure 3. Gas Chromatography-Mass Spectrometry chromatogram for the leaf of *Clerodendrum polycephalum* Baker

Table. 1. Chemical composition for leaf of *Clerodendrum polycephalum* Baker identified by Gas Chromatography-Mass Spectrometry

Pk#	Retention Time	Name of compound	Molecular Formulae	Molecular Weight (g/mol)	Peak Area (%)	Ref#	CAS#	Quality
1	7.310	Chloromethyl 9-chloroundecanoate	$C_{12}H_{22}Cl_2O_2$	269.20	0.87	127944	080418-95-7	64
2	7.842	Oxirane, decyl-	$C_{12}H_{24}O$	184.3184	0.72	51292	002855-19-8	38
3	9.192	Doconexent	$C_{22}H_{32}O_2$	328.4883	0.93	186706	006217-54-5	4
4	9.873	Naphthalene, 1,2,3,5,6,8a-hexahydro -4,7-dimethyl-1-(1-methylethyl)- ,(1S-cis)-	$C_{15}H_{24}$	204.3511	2.19	68800	000483-76-1	35
5	10.977	2-Dodecanol	$C_{12}H_{26}O$	186.3342	6.57	53004	010203-28-8	47
6	13.661	1-Octyn-3-ol	$C_8H_{14}O$	126.1962	0.98	11540	000818-72-4	36
7	13.747	2-Tridecanol	$C_{13}H_{28}O$	200.3608	6.66	65223	001653-31-2	80
8	14.342	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R-(1.alpha.,2.beta.,5.alpha.)]-	$C_{10}H_{18}$	138.2499	21.36	17379	000473-55-2	64
9	14.542	4-Cyclopentene-1,3-diol, trans-	$C_5H_8O_2$	100.12	0.68	3785	000694-47-3	10
10	14.611	Cyclohexane, (1-methylpropyl)-	$C_{10}H_{20}$	140.2658	0.66	18478	007058-01-7	50
11	14.674	(Z)-11-Tetradecen-1-ol acetate	$C_{16}H_{30}O_2$	254.4082	4.37	113491	033925-72-3	47
12	14.914	Neophytadiene	$C_{20}H_{38}$	278.5157	10.71	138502	000504-96-1	43
13	15.355	Crotyl methacrylate	$C_8H_{12}O_2$	140.18	0.85	18870	007376-45-6	28
14	15.635	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507	3.14	130813	000112-39-0	95
15	16.396	Cyclohexane, 1,2,3-trimethyl-, (1.	C_9H_{18}	126.2392	1.13	11877	001839-88-9	12

		alpha.,2.alpha.,3.alpha.)-						
16	16.499	Behenic alcohol	$C_{22}H_{46}O$	326.600	3.64	184686	000661-19-8	70
17	18.267	trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.4879	2.19	155762	1000333-61-3	49
18	18.513	Phytol	$C_{20}H_{40}O$	296.539	2.06	155852	000150-86-7	47
19	18.696	Methyl stearate	$C_{19}H_{38}O_2$	298.5038	4.11	157880	000112-61-8	83
20	19.703	1-Octadecene	$C_{18}H_{36}$	252.4784	0.87	113634	000112-88-9	50
21	24.699	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5- dimethyl-3-(prop-1-en-2-yl) octahydronaphthalen-1(2H)-one	$C_{15}H_{24}O_2$	236.35	0.75	98580	066884-74-0	27
22	25.031	Cyclohexane-1,3-dione, 2-allylamin omethylene-5,5-dimethyl-	$C_{12}H_{17}NO_2$	207.27	0.84	71553	104926-37-6	9
23	26.020	Cyclohexane, 1,2,4,5-tetraethyl	$C_{10}H_{20}$	140.2658	0.72	61887	061142-00-5	14
24	30.999	Squalene	$C_{30}H_{50}$	410.730	18.69	243225	000111-02-4	74
25	32.692	2-Methyltetracosane	$C_{25}H_{52}$	352.6804	2.54	207500	001560-78-7	38
26	32.795	3,5-Dimethylbenzaldehyde thiocarbamoylhydrazone	$C_{10}H_{13}N_3S$	207.30	0.95	71186	1000195-15-1	38
27	34.512	2-Hydroxychalcone	$C_{15}H_{12}O_2$	224.255	0.82	87703	000644-78-0	43

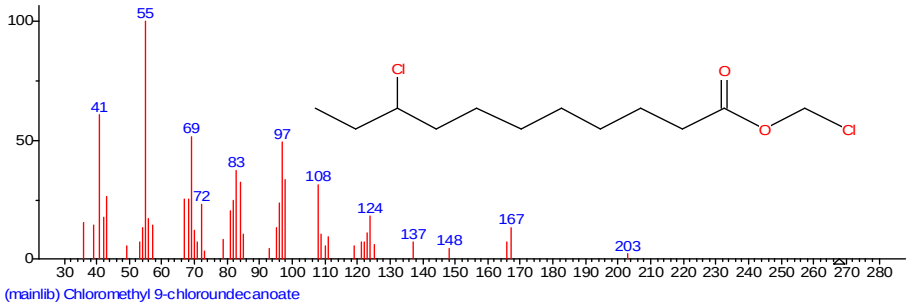

Fig. 4a. Mass spectrum of Chloromethyl 9-chloroundecanoate structure (0.87%, RT 7.310)

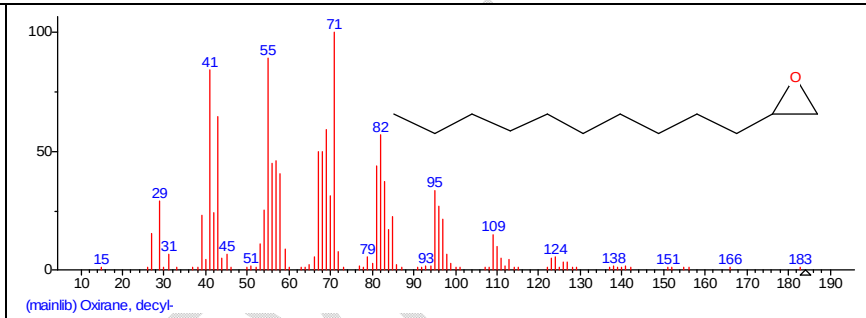

Fig. 4b. Mass spectrum of Oxirane, decyl- structure (0.72%, RT 7.842)

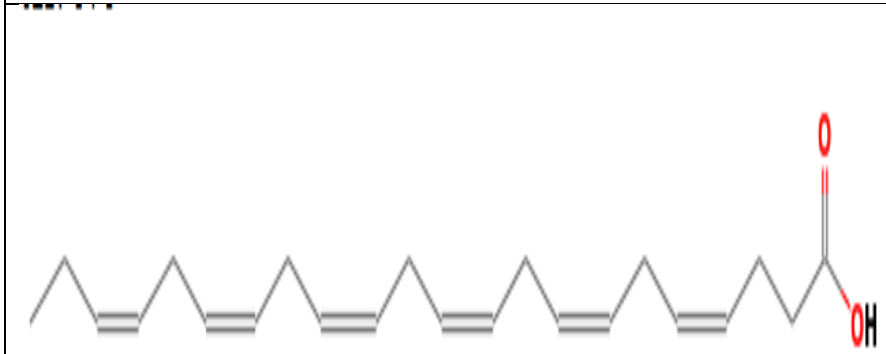

Fig. 4c. Structure of Doconexent (0.93 %, RT 9.192)

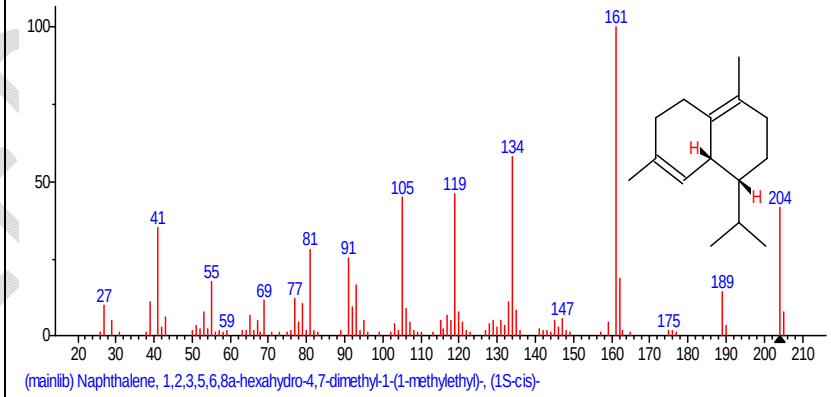

Fig. 4d. Mass spectrum of Naphthalene, 1,2,3,5,6,8a-hexahydr o-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)- structure (2.19%, RT 9.873)

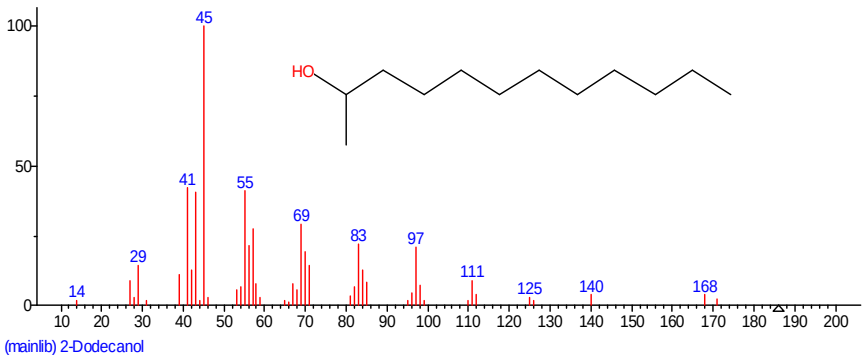

Fig. 4e. Mass spectrum of 2-Dodecanol structure (6.57%, RT 10.977)

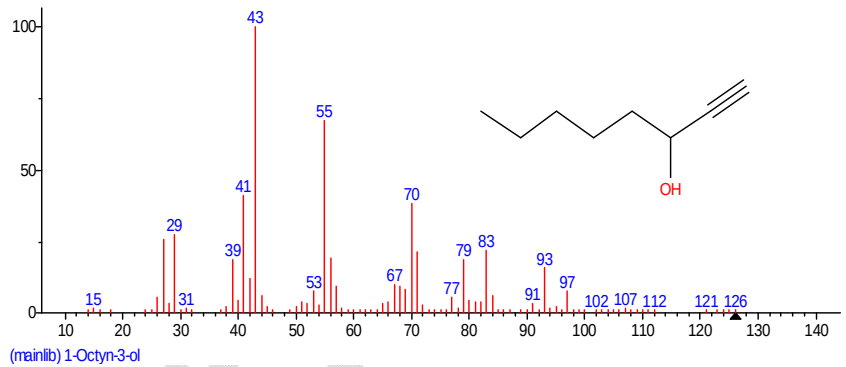

Fig. 4f. Mass spectrum of 1-Octyn-3-ol structure (0.98%, RT 13.661)

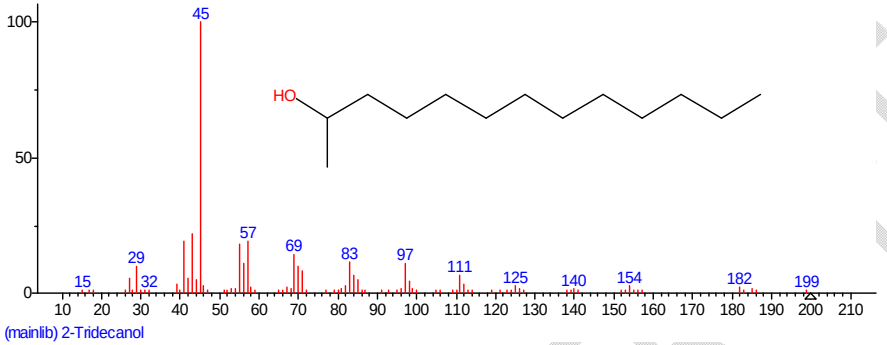

Fig. 4g. Mass spectrum of 2-Tridecanol structure (6.66%, RT 13.747)

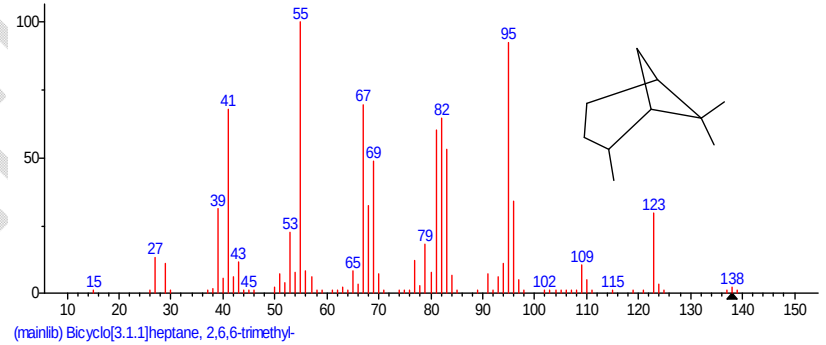

Fig. 4h. Structure of Bicyclo [3.1.1]heptane, 2,6,6-trime thyl-structure (21.36%, RT 14.342)

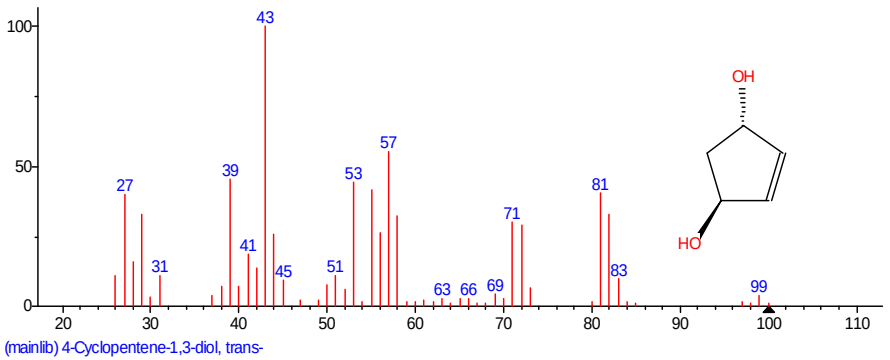

Fig. 4i. Mass spectrum of 4-Cyclopentene-1,3-diol, trans- structure (0.68 %, RT 14.542)

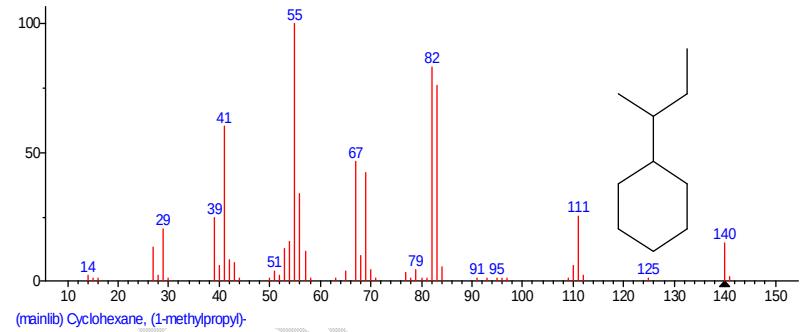

Fig. 4j. Mass spectrum of Cyclohexane, (1-methylpropyl)- structure (0.66 %, RT 14.611)

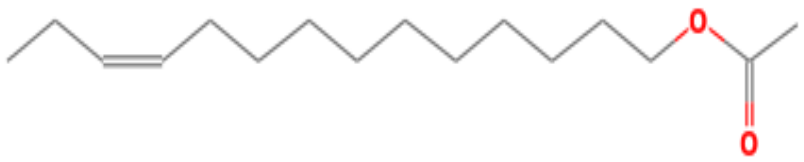

Fig. 4k. structure of (Z)-11-Tetradecen-1-ol acetate (4.37%, RT 14.674)

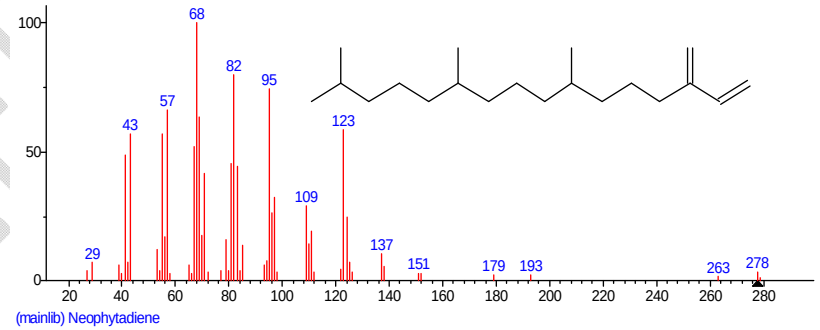

Fig. 4l. Mass spectrum of Neophytadiene structure (10.71 %, RT 14.914)

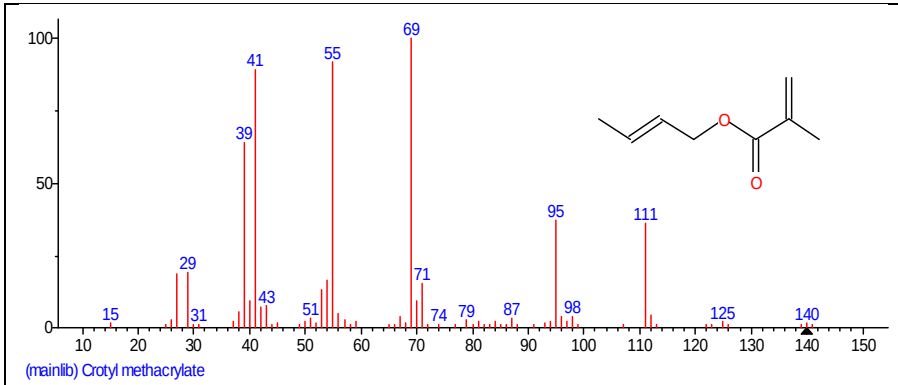

Fig. 4m. Mass spectrum of Crotyl methacrylate structure (0.85 %, RT 15.355).

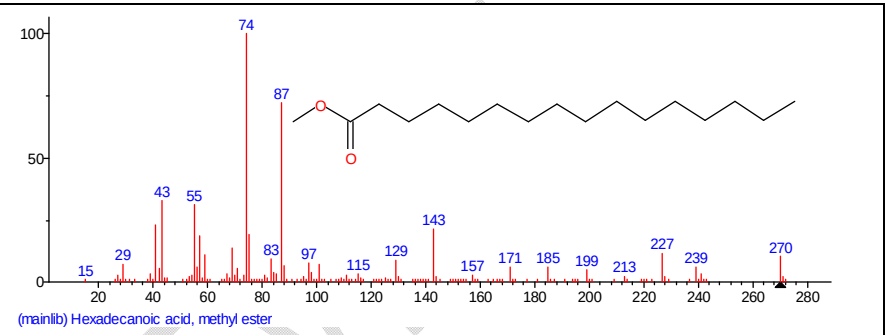

Fig. 4n. Mass spectrum of Hexadecanoic acid, methyl ester (3.14 %, RT 15.635).

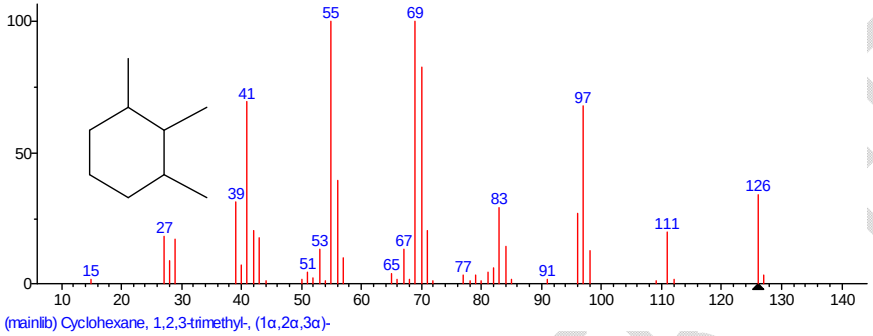

Fig. 4o. Mass spectrum of Cyclohexane, 1,2,3-trimethyl-, (1. alpha.,2.alpha.,3.alpha.)- structure (1.13%, RT 16.396).

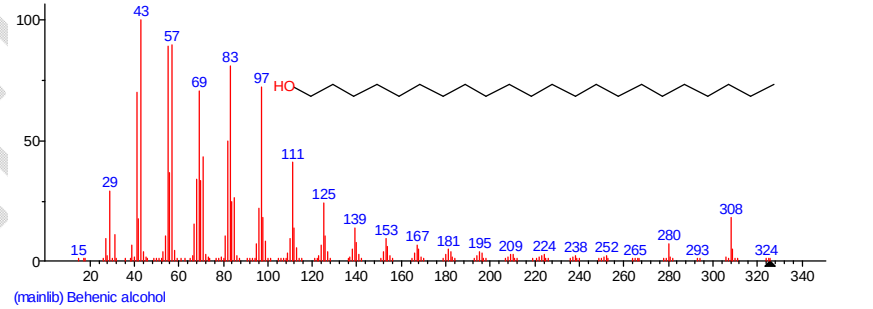

Fig. 4p. Mass spectrum of Behenic alcohol structure (3.64 %, RT 16.499).

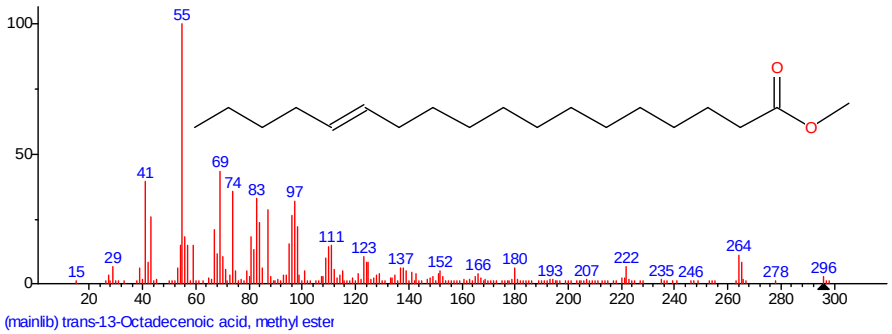

Fig. 4q. Mass spectrum of trans-13-Octadecenoic acid, methyl ester structure (2.19%, RT 18.267).

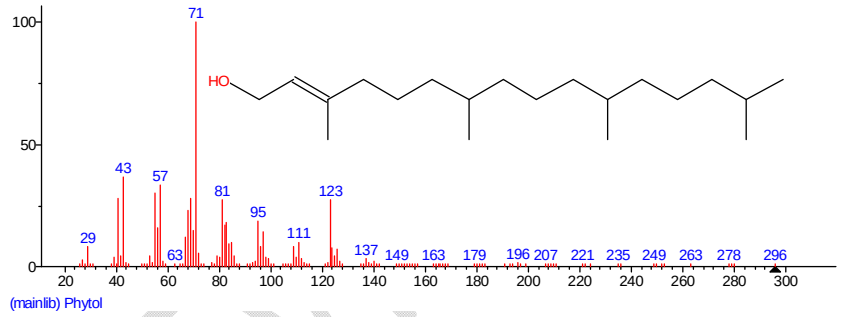

Fig. 4r. Mass spectrum of Phytol structure (2.06%, RT 18.513).

Fig. 4s. Structure of Methyl stearate (4.11%, RT 18.696).

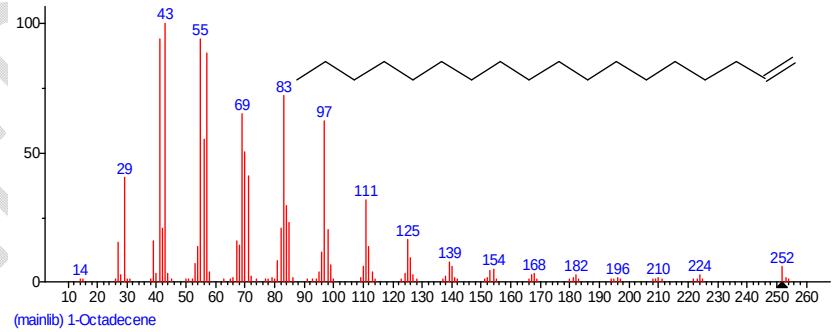

Fig. 4t. Mass spectrum of 1-Octadecene structure (0.87%, RT 19.703).

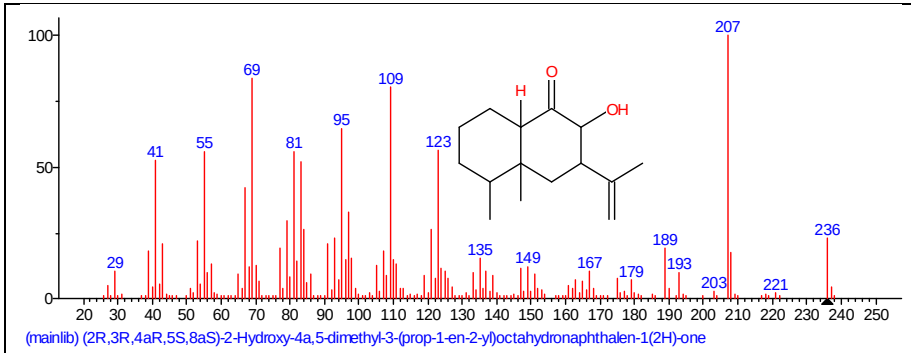

Fig. 4u. Mass spectrum of (2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahyd ronaphthalen-1(2H)-one structure (0.75 %, RT 24.699).

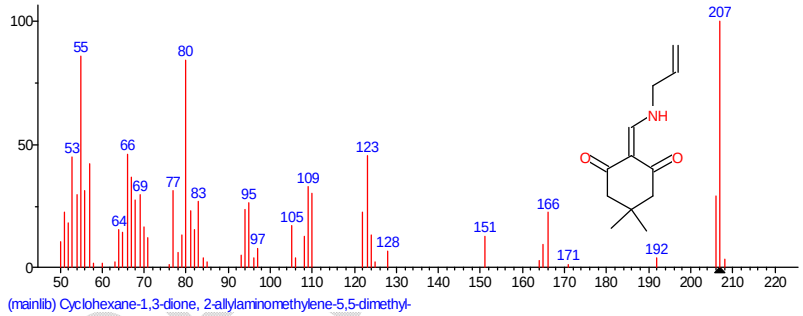

Fig. 4v. Mass spectrum of Cyclohexane-1,3-dione, 2-allylamin omethylene-5,5-dimethyl- structure (0.84%, RT 25.031).

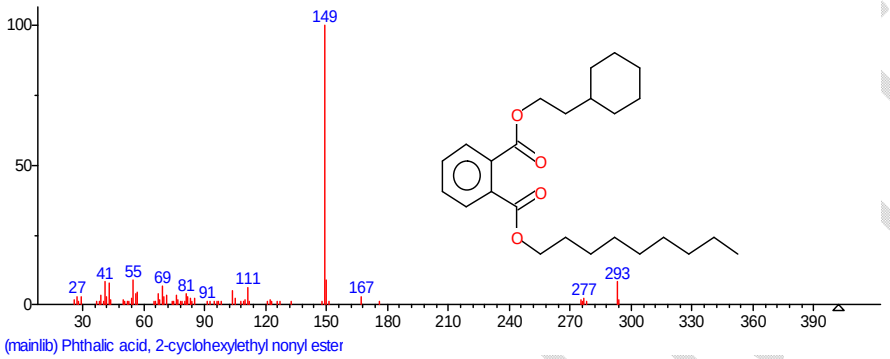

Fig. 4w. Mass spectrum of Phthalic acid, 2-cyclohexylethyl nonyl ester structure (0.72 %, RT 26.020).

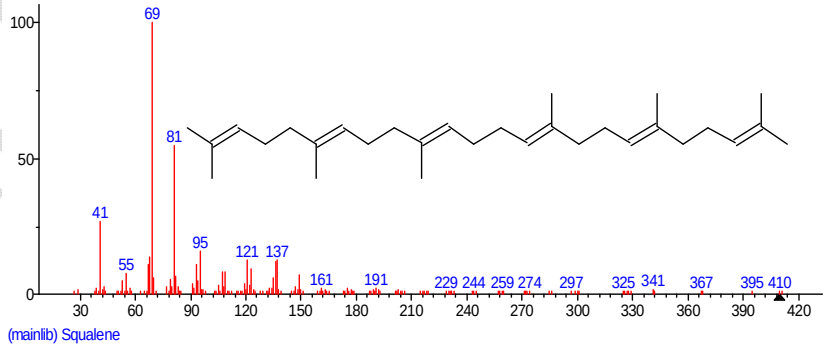

Fig. 4x. Mass spectrum of Squalene structure (18.69%, RT 30.999).

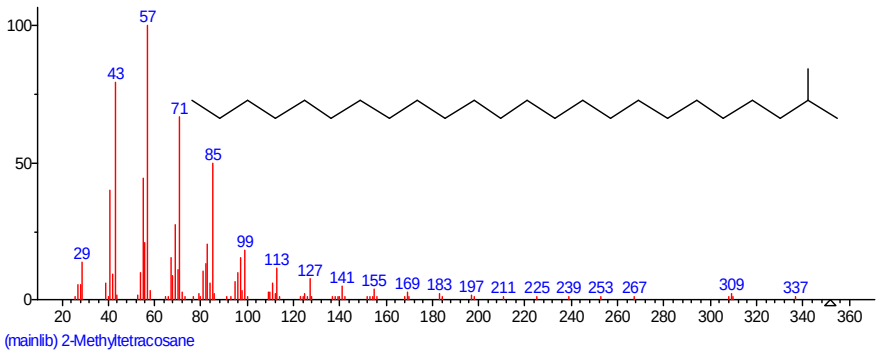

Fig. 4y. Mass spectrum of 2-Methyltetracosane structure (2.54%, RT 32.69).

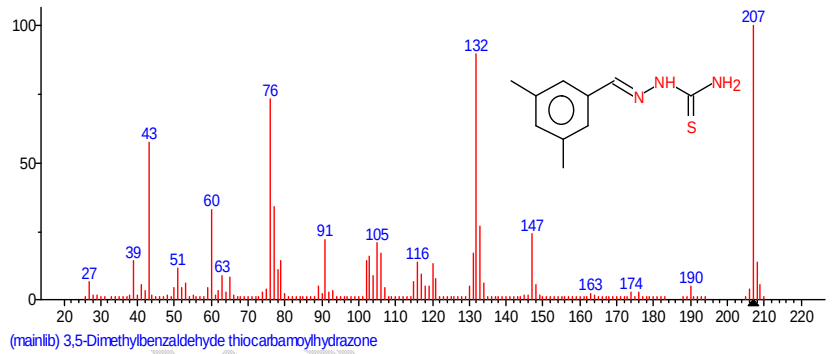

Fig. 4z. Mass spectrum of 3,5-Dimethylbenzaldehyde thiocarba moylhydrazone structure (0.95%, RT 32.795).

Fig. 4za. Structure of 2-Hydroxychalcone (0.82%, RT 34.512).

Figure 4. Mass spectrum and structure of 27 different compounds obtained during GC-MS analysis for leaf of *Clerodendrum polycephalum* Baker with their peak area and retention time.

Phytochemical screening of methanolic leaf extract of *Clerodendrum polycephalum*

Phytochemical analysis of the methanolic leaf extract of *Clerodendrum polycephalum* shows the presence of secondary metabolites like flavonoids, steroid, tannins, saponins, anthraquinones, alkaloids, terpenoids, anthocyanin, phenolic compounds and carbohydrate (Table 2).

Table 2: Phytochemical screening of methanolic leaf extract of *Clerodendrum polycephalum*

Phytoconstituent	**Qualitative abundance**
Tannins	+
Flavonoids	+
Alkaloids	+
Carbohydrate	+
Anthocyanin	+
Steroids	+
Anthraquinones	+
Terpenoids	+
Saponin	+
Phenolic compound	+

(+) indicate present

Antioxidant assays

The quantitative phytochemical constituents of the methanolic leaf extract of *Clerodendrum polycephalum* are shown in Table 3 below. The DPPH scavenging activity of the extract was less ($P < 0.05$) than those of standard compounds such as rutin. and ascorbic acid.

Table 3. Quantitative analysis of the phytochemical constituents of the methanolic leaf extract of *Clerodendrum polycephalum*

Phytochemical constituents	**Result**
Total proanthocyanidins	1.369±0.184
Flavonoids	2.4%
β-Carotene	0.1336± 0.45 µg
Lycopene	0.0340±0.053µg/g
Rutin	49.90 ± 1.20^{b}
Ascorbic acid	38.40 ± 1.30^{a}
C. polycephalum	87.70 ± 2.10^{c}

Each value is represented as mean ± SD (n = 3). Values with different letters indicate significantly different ($p < 0.05$).

Antidepressant of activity of *Clerodendrum polycephalum* and imipramine

The effects of the methanolic leaf extract of *Clerodendrum polycephalum* and imipramine (IMP) on active behaviors in the tail suspension test (TST) of mice are shown in Table 3. Oral administration of various doses of the extract (100 and 300 mg/kg) were used to assess the extent of immobility in mice exposed to TST.

Table 4. Effect of methanolic leaf extract of *Clerodendrum polycephalum* and imipramine on tail suspension test model in **Swiss mice.**

Groups	Dose (mg/kg)	Duration of mobility (sec)	Duration of immobility (sec)
Group A	-	-	-
Group B	-	182.80±3.80^{d}	177.20±1.75^{a}
Group C	30	265.31±3.63^{a}	94.69±2.06^{d}
Group D	100	225.49±2.32^{c}	134.51±1.21^{b}
Group E	300	248.21±1.80^{b}	111.79±1.71^{c}

Data represent means ± SD for five Swiss mice per group during tail suspension test.

Effect of *C. polycephalum* on forced swim test (FST)

Effect of various treatments on immobility behavior in FST experiments is shown in Table 5. The methanolic leaf extract of *C. polycephalum* markedly ($p<0.001$) decreased the immobility time in comparison to control group B mice while significant difference in activity was noticed in contrast to imipramine.

Table 5. Effect of methanolic leaf extract of *Clerodendrum polycephalum* on Swiss mice that undergo forced swim test.

Group	Dose(mg/kg B.WT)	Swimming test (sec)	Immobility (sec)
Group A	-	-	-
Group B	-	176.55±2.45^{d}	123.45±1.58^{a}
Group C	30	228.55±1.40^{a}	71.45±1.88^{d}
Group D	100	200.54±2.62^{c}	99.46±1.82^{b}
Group E	300	221.65±3.05^{b}	78.35±1.93^{c}

Data represent means ± SD for five Swiss mice per group during forced swim test.

Table 6. Effect of methanolic leaf extract of *Clerodendrum polycephalum* on oxidative stress parameters in Swiss albino mice.

Parameters	Group A	Group B	Group C	Group D	Group E
Plasma					
Total protein plasma (g/l)	105.16±2.76	88.63±1.38	92.38±1.23	113.44±2.34	116.08±3.58
Liver homogenate					
Total protein in liver homogenate (g/l)	18.27±1.36	11.52 ±1.83	15.41±1.18	21.95 ±1.15	20.65 ±1.23
Malanoaldehyde (x 10^3 mM MDA/mg protein)	5.88 ±0.25	10.76±1.36	8.91±1.75	8.84 ±2.43	8.34 ±0.78
Catalase (µmol/min/mg protein)	19.63±6.41	5.87±2.43	9.48 ±2.43	14.63 ±2.98	18.75±4.82
Uric acid (mg/dl)	1.062±0.186	2.14±0.671	1.735 ±0.174	1.131 ±0.184	1.332±0.295
SOD % Inhibition	95.88±3.36	85.39±2.6	93.42±3.16	92.15±2.32	95.81±2.76
SOD Unit	23.27±1.41	5.84±0.92	14.20±1.54	11.74±0.93	22.87±1.25
Kidney homogenate					
Total protein in kidney homogenate (g/l)	6.24 ±0.78	4.29 ±0.94	5.57 ±0.79	7.33 ±1.13	7.06±1.92
Malanoaldehyde (x 10^3 mM MDA/mg protein)	4.04 ±0.93	9.38 ±0.71	8.61 ±0.89	7.88 ±0.86	6.87±0.72
Catalase (µmol/min/mg protein)	15.44 ±0.91	9.71±0.85	14.41±0.76	15.28±1.02	15.77±1.19
Uric acid (mg/dl)	0.079±0.0031	0.132±0.0024	0.281±0.0024	0.182±0.0013	0.0782±0.0018
SOD % Inhibition	93.63±2.50	71.31±1.89	82.44±3.41	80.17±2.13	86.12±2.15
SOD Unit	14.70±0.93	2.49±0.27	4.69±0.95	4.04±0.57	6.20±0.47

4.0 DISCUSSION

Depression is a life-threatening psychiatric disorder and a major public health problem worldwide. Numerous antidepressant compounds are available which act through different mechanics involving Selective serotonin reuptake inhibitors (SSRIs), Serotonin-norepinephrine reuptake inhibitors SNRIs and Norepinephrine reuptake inhibitors (NRIs) system. Although a number of synthetic drugs are being used as treatment for clinically depressed patients, they have adverse effects that can compromise the therapeutic treatments and also provide an opportunity for alternative remedies based on natural products. But new drugs are still needed for the control of depression related disorders. "Tail Suspension Test (TST) and Forced Swim Test (FST) are employed as an exemplary systems to probe depressing condition in rodents [36], Immobility or despair behavior produced in both TST and FST were hypothesized to display animal's hopelessness and low mood (behavioral despair), and are taken as paradigm of depression. This simple behavioral procedure is widely used test for screening novel antidepressants" [37]. *Clerodendrum polycephalum* Baker plant was used to study its potential antidepressant activity.

GC-MS is the best technique for examining biologically active compounds or constituents in plants. GC-MS is an ideal technique for the analysis of volatile components; hence they are frequently used for the resolution of plant samples in which the molecular formula, chemical structure and functional group prediction are possible from the GC-MS data [38]. Analysis of leaf of *Clerodendrum polycephalum* Baker by GC-MS revealed the presence of four major components as shown in Figures 1. From the chromatogram and mass spectra, the compounds were identified as shown in Table 1, and Figure 1 and 2 respectively. The phytochemical compositions of the leaf of *C. polycephalum* with compound name, molecular formula, molecular structure, retention time are shown in Table 1. A total of 27 compounds were identified consisting of five prominent compounds and 22 minor compounds (Table 1). The five prominent compounds constitute 63.99% of the *Clerodendrum polycephalum Baker* plant. The five major compounds and their percentage abundance are: Bicyclo[3.1.1]heptane, 2,6,6-trime thyl- (21.36%), Squalene (18.69%), Neophytadiene (10.71%), 2-Tridecanol (6.66%) and 2-Dodecanol (6.57%). These prominent compounds were represented by peaks 8, 24, 12, 7 and 5 with retention times of 14.342, 30.999, 14.914, 13.747 and 10.977 respectively. The major component found in the *Clerodendrum polycephalum Baker* plant was Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R-(1.alpha.,2.beta.,5.alpha.)]-

Toha et al. [39] study shows that "the three major compounds in the air dried plants of *Clinacanthus nutans* leaf extract samples (constituting 18.236% of the peak area) were Bicyclo[3.1.1]heptane, 2,6,6-trimethyl(1.alpha.,2. beta., 5.alpha.), Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, and Bicyclo[3.1.1] heptane,

2,6,6-trimethyl-,[1R (1.alpha.,2.beta., 5 alpha.)]. The study also shows that the plant exhibited free radical scavenging activity and selective antiproliferative activity against MCF-7 (breast cancer) but not MCF-10A (normal breast) cell lines. The properties of this plant may be as result of Bicyclo[3.1.1] heptane, 2,6,6-trimethyl-,[1R-(1.alpha.,2.beta.,5 alpha.)] compound with synergy with other compounds found in the plant".

Squalene is a triterpenoid that possess hepatoprotective, hypolipidemic cardioprotective, and antioxidant activity [38, 40]. "Squalene is a linear triterpene having a hydrocarbon chain of six isoprene units which is chemically 2,6,10,15,19,23-hexamethyl-6,6,10,14,18,20-tetracosa-hexane (Fig. 2m), with a $C_{30}H_{50}$ molecular formula showing solubility only in organic solvents" [38, 41, 42]. Squalene is synthesized by many bacteria, fungi, animals, and plants that act as an intermediate for the biosynthesis of secondary metabolites like: vitamins (E, D and K), sterols and hormones [43]. "Studies have shown that squalene possess useful bioactivities which including antioxidant, hypolipidemic, hepatoprotective, cytoprotective effects and antitumor activities. The compound was suggested for supplementation which may be responsible for tumor growth inhibition and the prevention of normal cells from mutating into tumor cells when exposed to oxidative stress" [38, 44-46]. "Squalene is found in the human body, where it is produced by the sebaceous glands to protect the skin and makes up 10–15% of lipids on the skin surface in concentrations of 300–500 g/g, and less than 75 g/g in internal organs including the liver and small intestine" [38, 47, 48]. Study has shown that squalene possess anti-inflammatory, anti-atherosclerotic and anti-neoplastic role in skin aging and pathology and Adjuvant activities [49]. A study has shown that squalene from *Aurantiochytrium sp* modulate neurotransmitter systems through the anti-inflammatory and antidepressant-like properties [50]. Venkata Raman et al. [49] study shows that neophytadiene possess antioxidant, anti-inflammatory, antimicrobial, antipyretic and analgesic activities. Other compound found in the plant like: Hexadecanoic acid, methyl ester possess Anti-oxidant, antimicrobial, decrease blood cholesterol, anti-inflammatory [10]. Phytol has been shown to possess immunostimulatory, antimicrobial, anticancer, anti-inflammatory, anti-diabetic and anti-diuretic activities [49].

From the analysis done, It was revealed that methanolic leaf extract of *Clerodendrum polycephalum* contains phytochemicals such as tannins, saponnins, anthraquinones , alkaloids, carbohydrate etc. (Table 2). Egharevba et al. [2] study shows that the phytochemical screening of *Clerodendrum polycephalum* indicated the presence of saponins, flavonoids, reducing sugar, carbohydrate, tannins, resins, balsams, phlobatannins and alkaloid. The extract contains total proanthocyanidin (1.369±0.184mg quercetin/gdry

plant material), flavonoids (2.4%), β-Carotene (0.1336± 0.45 µg) and lycopene (0.0340±0.053µg/g)., these parameters shows that the plant possesses antioxidant activity and protect human cells against oxidative damage. The scavenging effect of the methanolic leaf extract of *C. polycephalum* and standards compounds against DPPH radicals varied a great deal indicating that scavenging activities are related to the electron transfer or donating ability. The extract exhibited inferior IC_{50} values for DPPH radical scavenging activity as compared to the rutin and ascorbic acid. These models (tail suspension and forced swimming test) were used in this study because they are the two most widely used animal models for screening new antidepressant drugs [31]. "The efficacies of these two models of depression have been reviewed". Petit-Demouliere et al. [51] reviewed "FST with mice and concluded that the assay has good reliability and predictive validity". Likewise, Steru et al. [31] described "TST as another test as primary screening procedure which induces a state of despair in animals like that as in FST". The main advantages of these procedures are the use of a simple objective test situation, the concordance of the results with the validated "behavioral despair" test. [32] and the sensitivity to a wide range of drug doses [31, 52]. After oral administration of methanolic leaf extract of *C. polycephalum*, the test doses of 100 and 300 mg/kg body weight of the extract shows a statistical significant antidepressant-like activity in the tail suspension test ($P < 0.05$). The methanolic extract of the plant exhibited a reduction in the time of immobility at the dose of 100 and 300 mg/kg body weight respectively when compared to controlled group B mice. The use of Imipramine produces a better result when compared to the methanolic leaf extract of *C. polycephalum* used in the study during TST. FST is mostly used as a behavioral model to evaluate rodent depression in the screening of antidepressant drugs [53]. Specifically, the physical immobility of rodents in FST is thought to be an indication of behavioral despair and is inferred as depressive-like behavior. In the forced swim test assay**,** the methanolic leaf extract of *C. polycephalum* displayed a notable reduction in the duration of immobility in comparison to the control animals (group B), which is suggestive of a considerable antidepressant-like activity. The significant reduction in immobility was seen at all doses of the methanolic extract of *C. polycephalum* used. This indicates an increase in the concentration of the active principles or in the ability to neutralize the effect of inhibitors with increasing dose of the extract. The Imipramine used in the study produces a better result when compared to the extract. The antidepressant activity of the methanolic leaf extract of *C. polycephalum* may be due to the presence of squalene working in synergy with other compounds found in the plant since squalene have been shown to have antidepressant activity [50].

There were significant reduction (P<0.05) in plasma total protein and activities of CAT and SOD in group B liver and kidney homogenate compared to Group A, C, D and E mice respectively. The reduced level of antioxidant enzymes (SOD and CAT) in group B clearly indicates oxidative stress due to the production of reactive oxygen species. MDA values significantly increase (P<0.05) in the liver and kidney homogenate of group B mice compared to group A, C, D and E animals respectively. Increase in MDA value indicates increased in lipid peroxidation which indicates oxidative stress in group B animals. There was no significant difference in uric acid values of all the animals studied. (Table 6).

CONCLUSION

The findings obtained from this study indicate that the methanolic leaf extract of the *Clerodendrum polycephalum* possesses a significant antidepressant-like activity. This is indicated by the decrease in the duration of immobility in the treated groups. The antidepressant-like effect of the extract as observed in the TST and FST were found statistically significant.

Ethical Approval:

Animal Ethic committee approval has been collected and preserved by the author(s)

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