European Journal of Medicinal Plants

23(1): 1-16, 2018; Article no.EJMP.38739
ISSN: 2231-0894, NLM ID: 101583475

Identification of Phytocomponents and Acute Toxicity Evaluation of *Corchorus olitorius* Leaf Extract

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Authors' contributions

This work was carried out in collaboration between all authors. Author DO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OCO and III managed the analyses of the study. Author SNI managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2018/38739
Editor(s):
(1) Shanfa Lu, Professor, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, China.
(2) Marcello Iriti, Professor, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Kadiri Oseni, Obafemi Awolowo University, Nigeria.
(2) Juan Carlos Troiano, University of Buenos Aires, Argentina.
(3) Arun Kumar, Hindu Post Graduate College, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/24276

Original Research Article

Received 14th November 2017
Accepted 23rd January 2018
Published 23rd April 2018

ABSTRACT

In this study, the identification of secondary metabolites was carried out alongside acute toxicity evaluation on *Corchorus olitorius* leaf extract (COLE). Results of phytochemical analysis of the extract revealed the presence of flavonoids (4.00±0.035 mg/100 g), steroids (0.89±0.031 mg/100 g), terpenes (1.27±0.016 mg/100 g), phenolic compounds (2.05±0.514 mg/100 g), alkaloids (3.10±0.026 mg/100 g), saponins (4.00±0.054 mg/100 g), tannins (0.32±0.044 mg/100 g) and cardiac glycoside (1.61±0.068 mg/100 g) while GCMS analysis of the extract showed the presence of 46 compounds with 2-Dodecenal having the highest concentration. 3-Methyl-1-penten-4-yn-3-ol, 2,4-Decadienal and Ethanone were also found in higher amounts. No toxicity behaviour and mortality were observed during the acute toxicity study period, even at a dose of 5000 mg/kg body.

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From results obtained, we conclude that *Corchorus olitorius* leaf extract may be a potential source of antioxidant, anti-inflammatory, anticancer and cardiovascular system modulatory agent and may be safe for use as food and medicine.

Keywords: Corchorus olitorius; GC-MS; phytochemical; toxicity.

1. INTRODUCTION

Of late, there appears to be an upsurge interest in plant-based medicines. This keen interest, particularly in African countries and other developing nations of the world is not surprising due to the extensive availability of plants of assorted species in their flora and fauna. Africa is indeed heavily endowed with abundant natural resources and is home to millions of medicinal plants [1,2]. Currently, the use of plants for medicinal purposes is not only limited to African and other developing nations of the world but have achieved global status, hence the current assertion that over 80% of the world's population relies on herbal medicines [3,4].The high cost, inadequate efficacy and numerous side effects of currently available orthodox medicines appear to heighten interests in plants medicine, precipitating numerous scientific researchers whose aim is to identify and isolate the active compounds from plants and harness such into more useful forms. This may be why orthodox medicine appears to be strongly anchored on traditional medicine [5].

The evaluation of phytochemical constituents of plants have been of value in investigating the healing potential of such plants and assessing possible toxicity effects. The healing and toxicity effects of plants have greatly been associated with their phytochemical compositions [6]. Gas Chromatography-Mass Spectrometry (GC-MS) has of late proven to be of greater advantage in the study of plant phytochemical studies since it does not just identify phytocomponents as groups of compounds but reveals in many details the individual compounds present and enables researchers to pin down observed pharmacological effects to individual constituents [1]. *Corchorus olitorius* is only one of the numerous plants that are currently being studied. *Corchorus olitorius* is a green leafy vegetable popularly consumed among the Yorubas of southwestern Nigeria where it is commonly called Ewedu. Among the Igbos of southeastern, Nigeria, it is called Ahihara while in English, the plant is known as jute mallow or bush okra. *C. olitorius* plant is not found in Nigeria only but also in other countries such as Egypt, Sudan, Malaysia, South America, and the Caribbean [7,8,9]. Nutritional substances; including calcium, potassium, phosphate, iron, ascorbic acid, carotene and large amount of mucilaginous polysaccharides have all been identified in the plant [10]. Medicinally, *C. olitorius* are used as a demulcent, diuretic, purgative, bitter tonic, laxative, refrigerant, carminative and lactagogue [11]. The leaves extract has given positive results in the management of chronic cystitis, dysuria and hyperglycaemia. Its reported high antibacterial activity gives credence to its use traditionally for the treatment of dysentery, fever and gonorrhea [10,11].

In this study, we evaluated the phytochemical composition and acute toxicity effect of *C. olitorius* in rats.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Fresh leaves of *C. olitorius* were collected from a local settlement in Umudike, Ikwuano Local Government Area of Abia State, Nigeria and was identified at the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike. A voucher number MOUAU/VPP/17/009 was assigned and a sample was deposited at the herbarium of the Department of Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike.

2.2 Preparation of Plant Extract

Fresh leaves of *C. olitorius* were air dried at room temperature in an open laboratory space for 14 days and ground into powder using a locally fabricated milling machine powered by a petrol motor (Honda Company, Japan). Fifty (50) grams of the powdered material was introduced into the extraction chamber of the Soxhlet extractor and extraction was done using methanol as solvent. Extraction temperature was maintained at 60^0C for 48 hours. At the end of the period, the solvent was evaporated at a temperature of 40ºC in a hot

air oven to obtain a crude solid extract which weighed 4.09 g and represented a percentage yield of 8.18%. The extract was preserved in the refrigerator until needed. The extract is hereafter referred to as *Corchorus olitorius* Leaf Extract (COLE).

2.3 Preliminary Phytochemical Investigation

Preliminary Phytochemical studies were carried out according to the methods of Trease and Evans [12] and used by Ijioma et al. [1].

2.4 Gas Chromatography-mass Spectrometry (GC-MS) Analysis of APE

The characterization of the Phytochemicals in COLE was done using GC-MS QP2010 Plus (Shimadzu, Japan) while 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 set at 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 1 min and was later increased linearly at 70ºC min-1 to 220ºC, held for 3 min followed by linear increased temperature 10ºC min-1 to 290ºC for 10 minutes. 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. Identification of compounds was accomplished by comparison of retention time and fragmentation pattern, as well as with mass spectra of the GC-MS. The identity of the active components in the extract was by comparison of their retention indices, peak area percentage and mass spectra fragmentation pattern with those stored on the National Institute of Standards and Technology (NIST) digital library data and also with published Literature. NIST08. LIB [13], WILEY 8 LIB, [14] library sources were used for matching the identified components from the plant material to ascertain the name, molecular weight, formula, structure and bioactivities of the compounds were then ascertained.

2.5 Acute Toxicity Evaluation of *C. olitorius*

Acute toxicity value of COLE was determined following new Lorke's method as was used by Chinedu et al. [15]. Twelve rats were divided and spread into 4 stages of the study in this order: 4 for the first, 3 for the second and another 3 for the third stage. A final 2 was used for the confirmatory test. At the 1st stage, each of the 4 animals was administered specific dose levels of COLE in the order 100, 200, 400 and 800 mg/kg, while in the 2nd stage 500, 1000, and 2000 mg/kg were administered and at the 3rd stage, the doses administered were 1250, 2500 and 5000 mg/kg. The highest dose used (5000mg/kg) was administered to the last 2 rats during the confirmatory test period. All administrations were done via the oral route. The animals were observed for 7 days and a number of deaths recorded was used to determine the LD_{50} value of the extract by Lorke's formula in compliance with OECD, (2001) guidelines for acute toxicity study.

3. RESULTS

3.1 Preliminary Phytochemical Composition of *C. olitorius*

Results of quantitative phytochemical analysis of COLE revealed the presence of flavonoids (4.00±0.035 mg/100 g), steroids (0.89±0.031 mg/100 g), terpenes (1.27±0.016 mg/100 g), phenolic compounds (2.05±0.514 mg/100g), alkaloids (3.10±0.026 mg/100 g), saponins (4.00±0.054 mg/100 g), tannins (0.32±0.044 mg/100 g) and cardiac glycoside (1.61±0.068 mg/100 g). This is presented in Table 1.

Table 1. Results of preliminary phytochemical components in *C.olitorius* leaf extract

Phytochemical agent	Amount in mg/100 g
Saponins	4.00±0.054
Flavonoids	4.00±0.035
Tannins	0.32±0.044
Steroids	0.89±0.031
Terpenes	1.27±0.016
Alkaloids	3.10±0.026
Glycoside	1.61±0.068
Phenolic compounds	2.05±0.514

3.2 Results of Gas Chromatography-mass Spectrometry (GC-MS) Analysis of COLE

Forty-six peaks were observed following GC-MS analysis of COLE indicating the presence of 46 compounds with 2-Dodecenal as the compound

that is most present. Also present in higher amounts are 3-Methyl-1-penten-4-yn-3-ol, 2,4-Decadienal and Ethanone, 1-(2,2-dimethylcyclo pentyl).

The identified peaks of compounds in *C. olitorius* leaf extract are presented in Fig. 1, their structures in Fig. 2 and composition in Table 2 as shown.

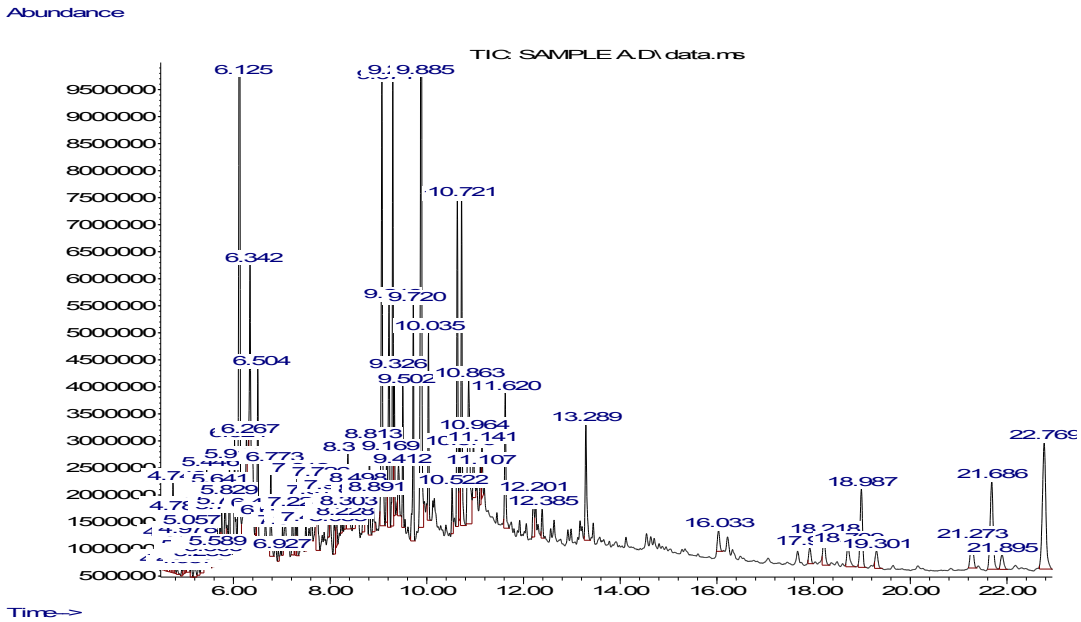
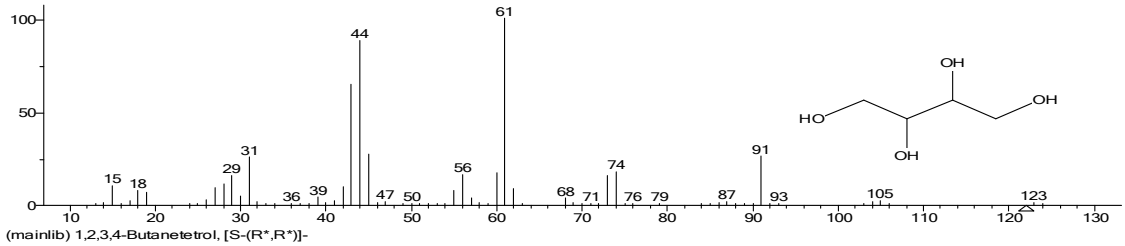
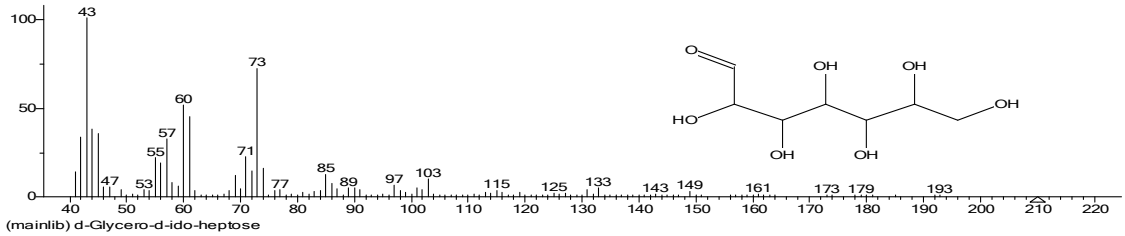
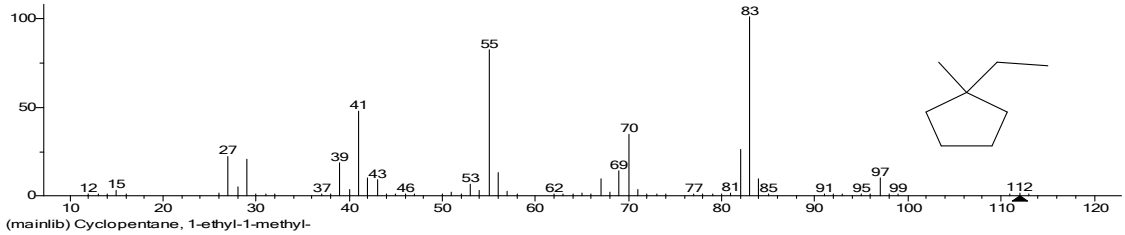
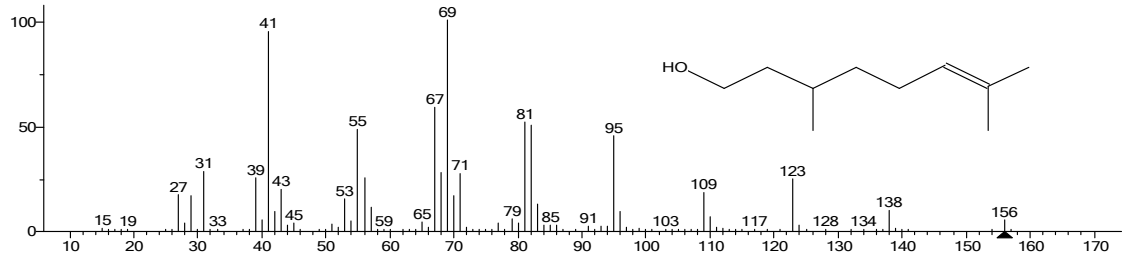


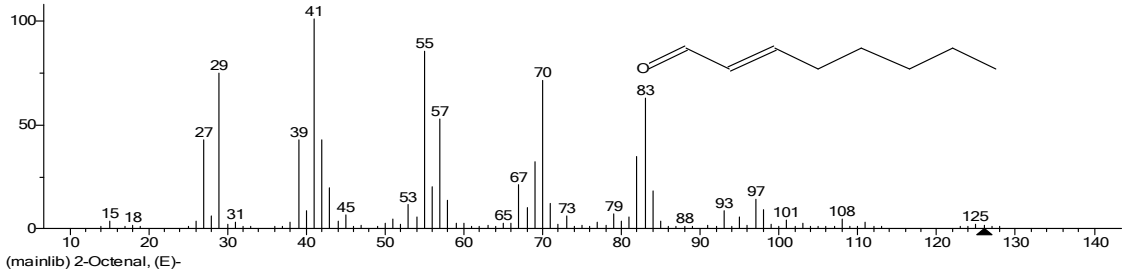
Fig. 1. Chromatogram showing 46 peaks following GC-MS analysis of COLE



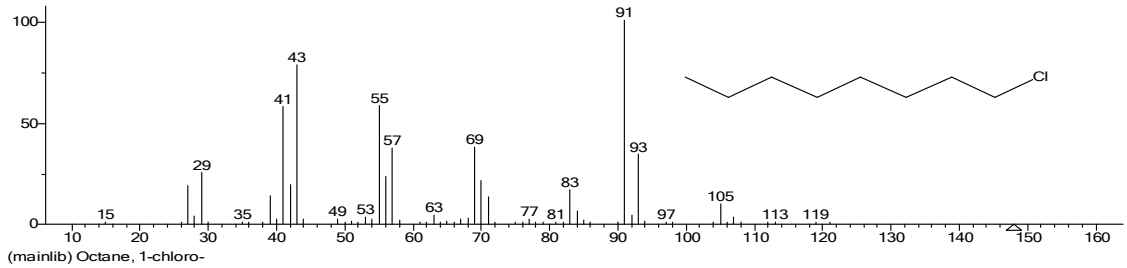




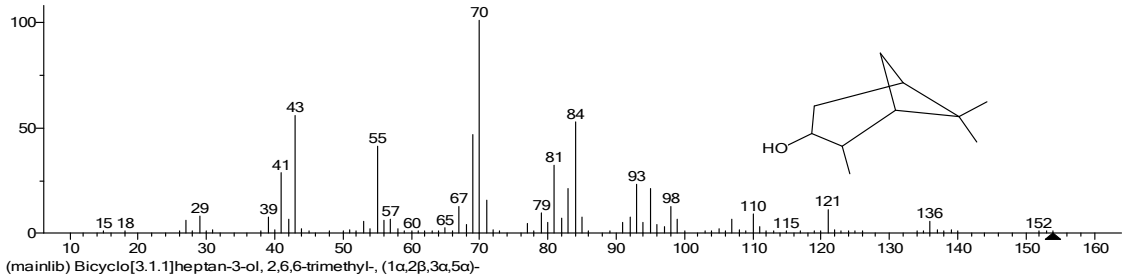


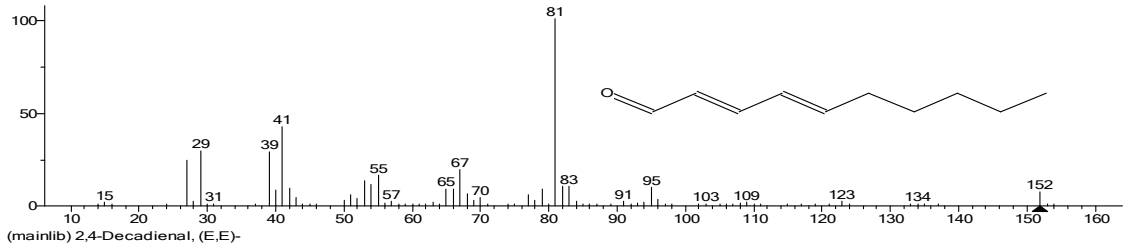


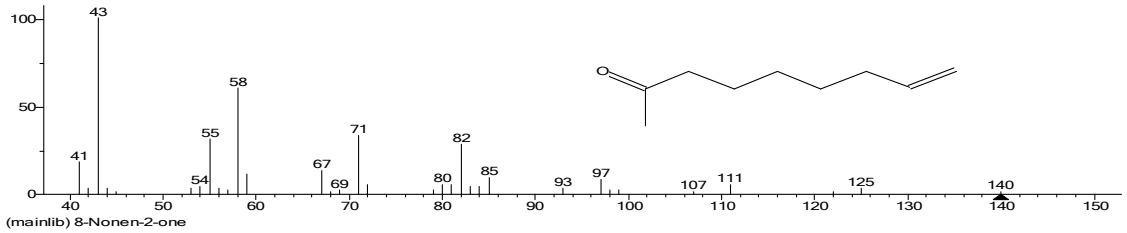
a flavoring ingredient for improving the aroma and flavor of cherries, dairy products, nuts, and meat. It is a colorless to slightly yellow liquid with fresh cucumber, fragrant herbs, banana leaf-like flavor



Liquid fractions are central nervous depressants when absorbed from alimentary tract, or severe pulmonary irritants when aspirated



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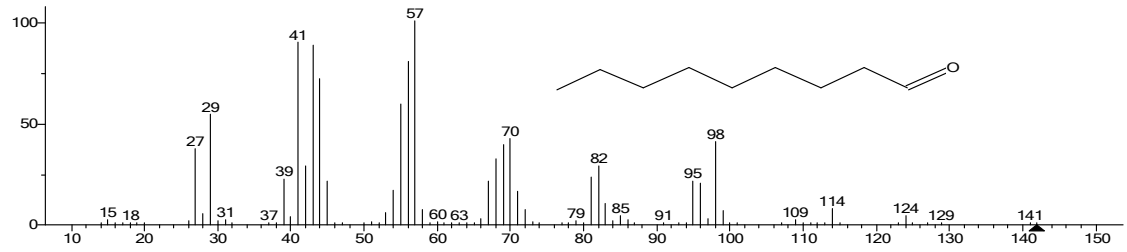
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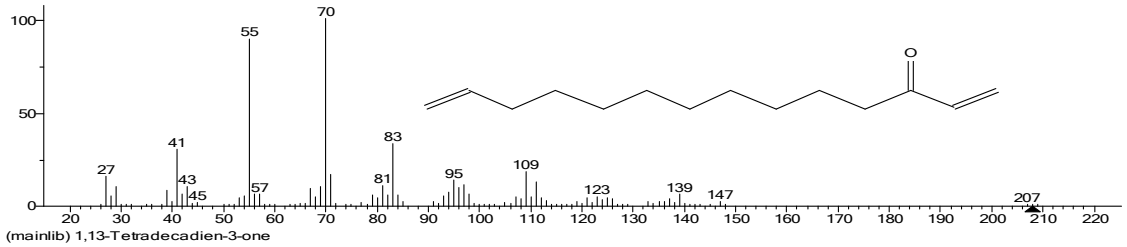
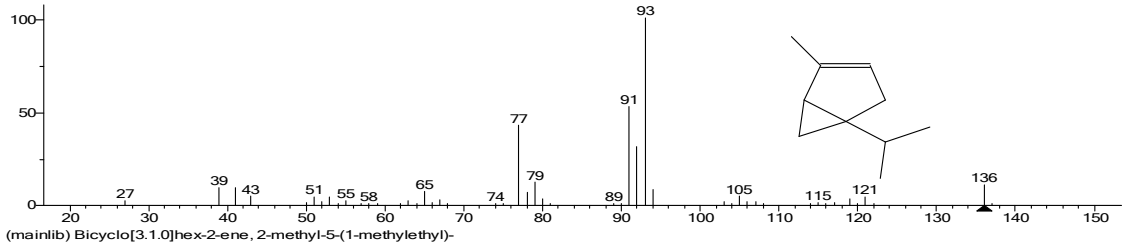
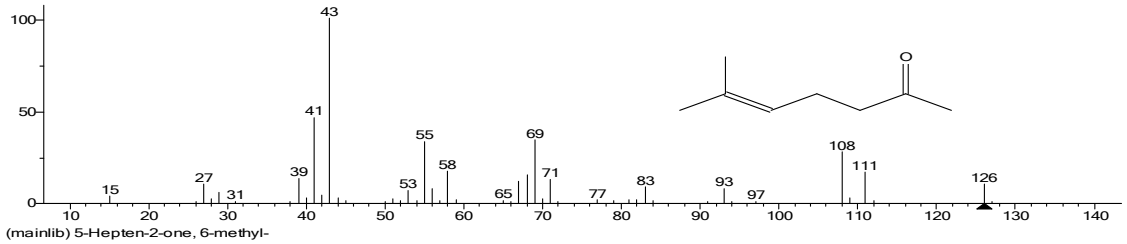

(mainlib) 1,13-Tetradecadien-3-one


(mainlib) Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-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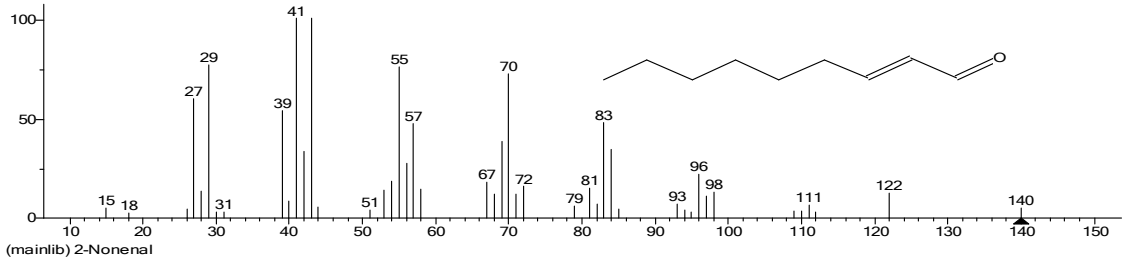
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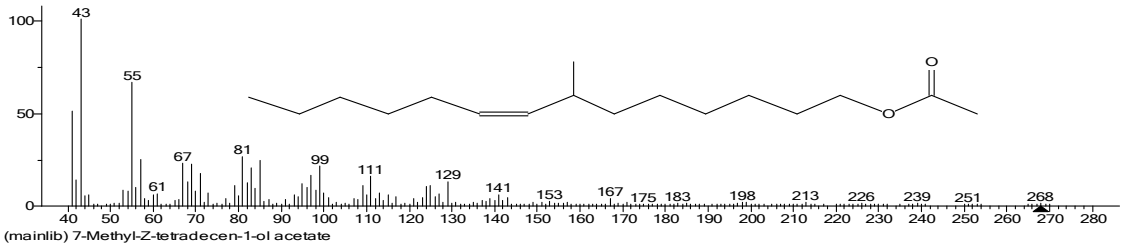
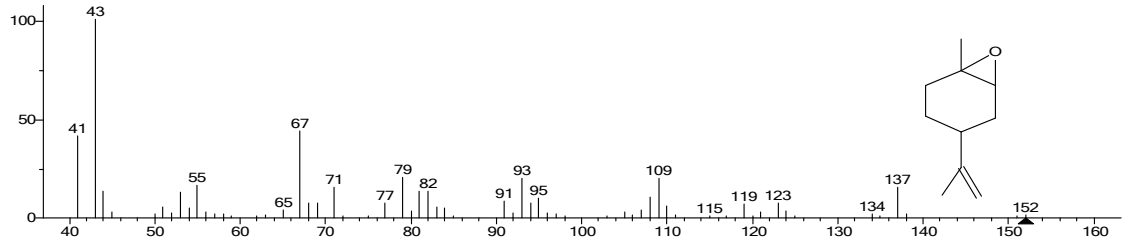
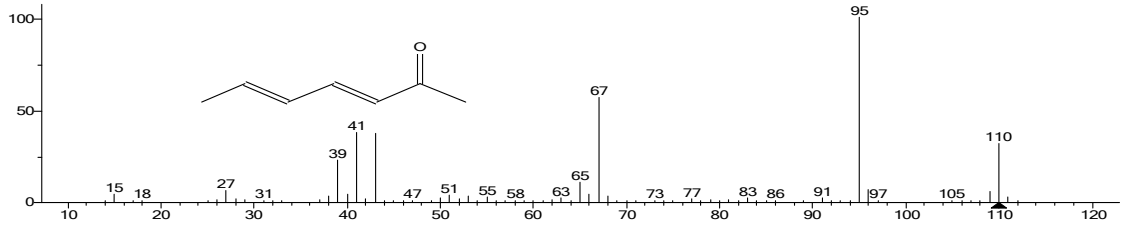


(mainlib) 7-Methyl-Z-tetradecen-1-ol acetate



(mainlib) Limonene oxide, cis-



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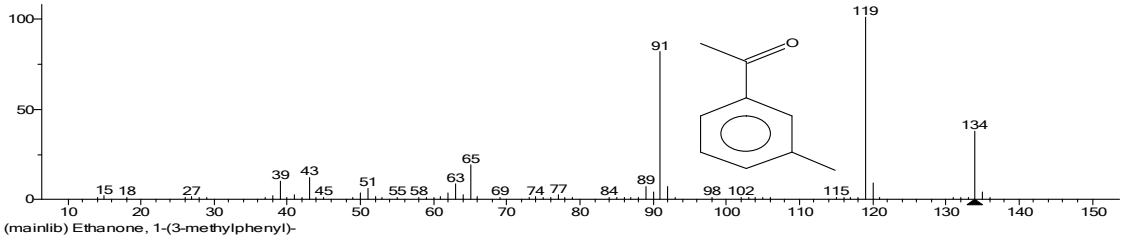
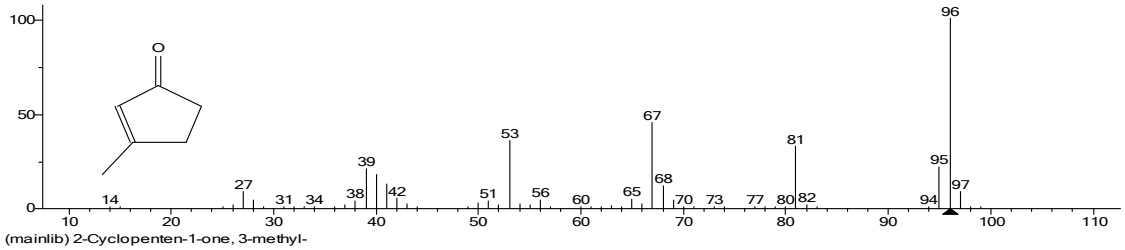


(mainlib) Ethanone, 1-(3-methylphenyl)-



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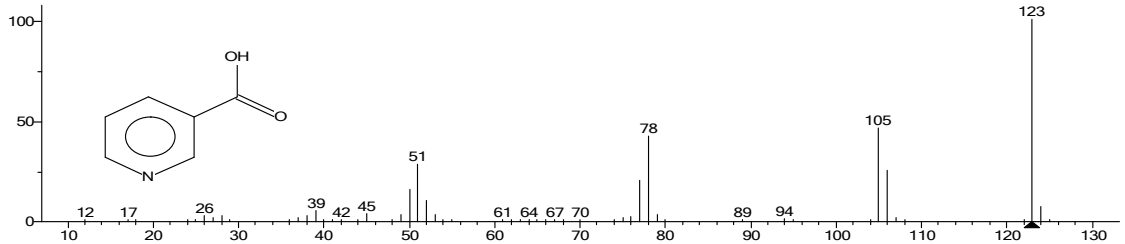

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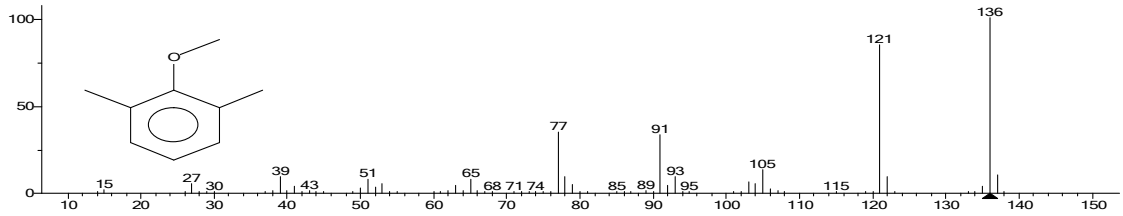
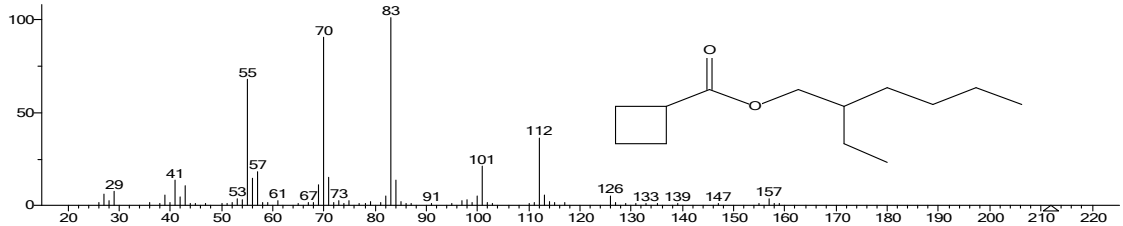
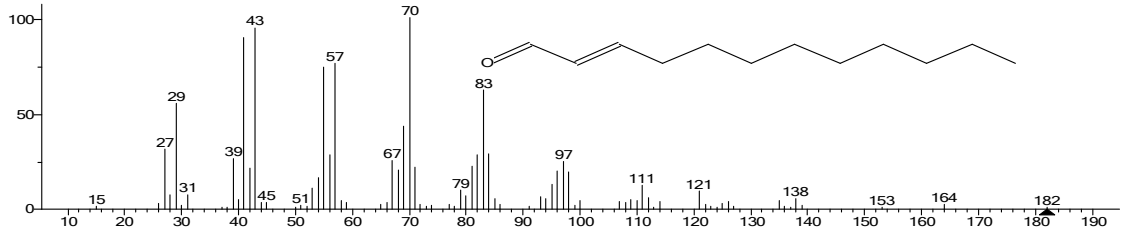

(mainlib) Benzene, 2-methoxy-1,3-dimethyl-


(mainlib) Cyclobutanecarboxylic acid, 2-ethylhexyl ester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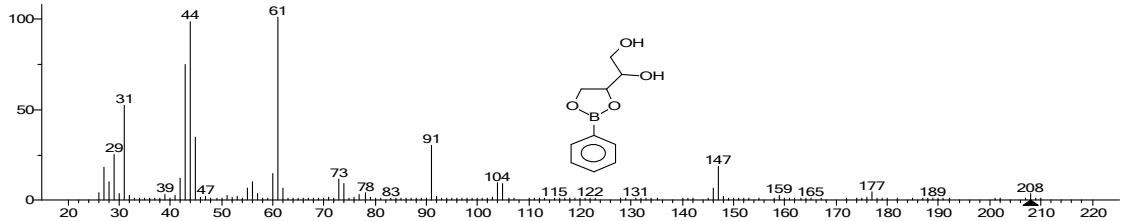
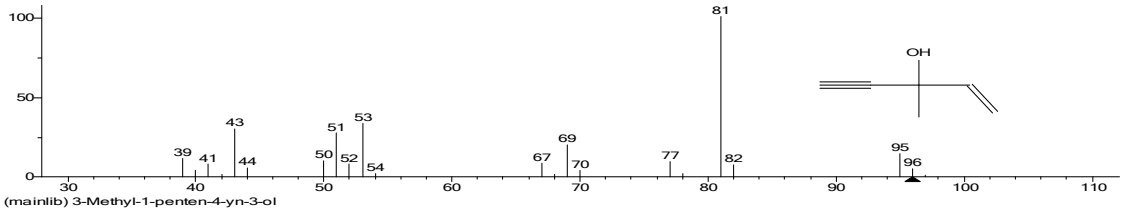

(mainlib) 1,2-Ethanediol, 1-(2-phenyl-1,3,2-dioxaborolan-4-yl)-, [S-(R*,R*)]-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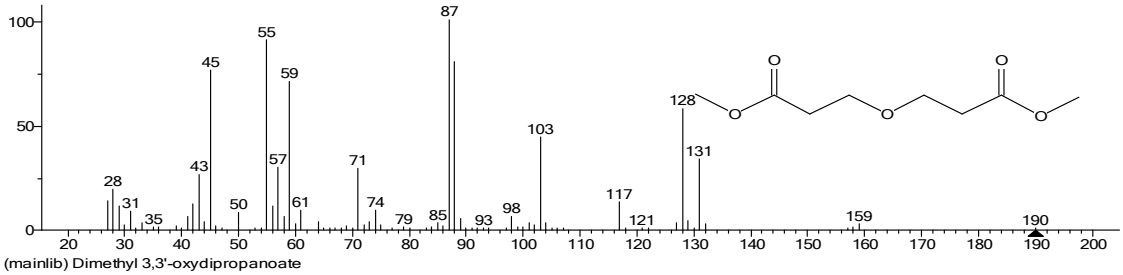
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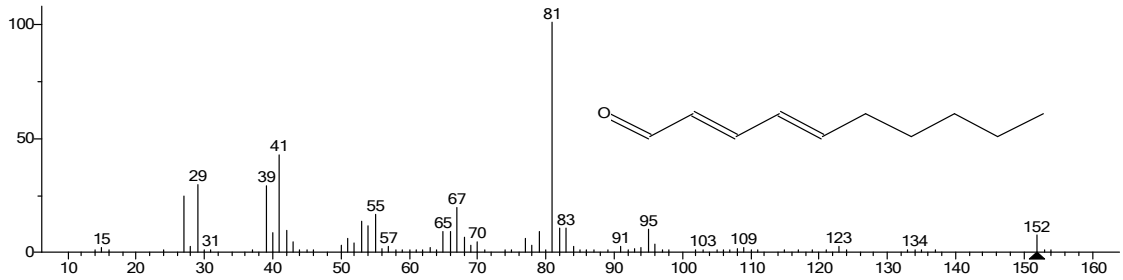
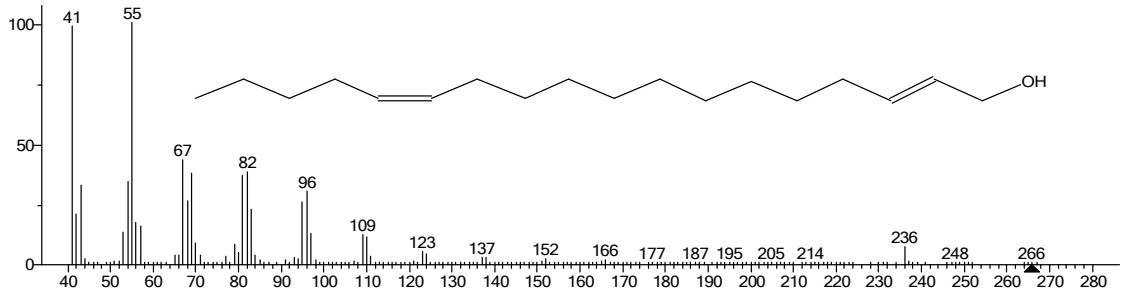


(mainlib) 2,4-Decadienal, (E,E)-



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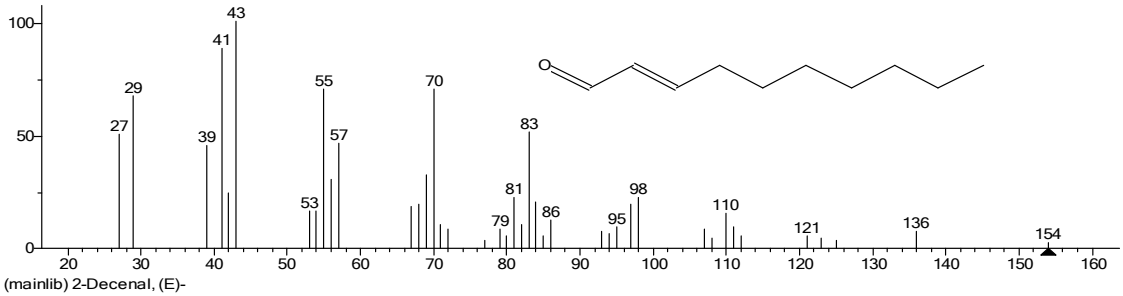

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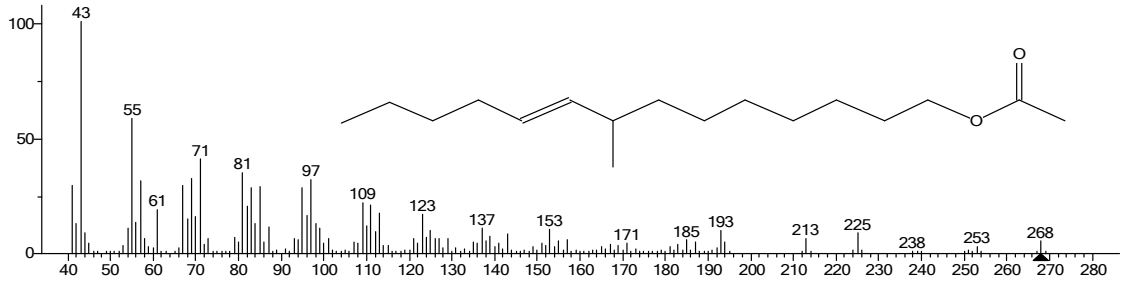
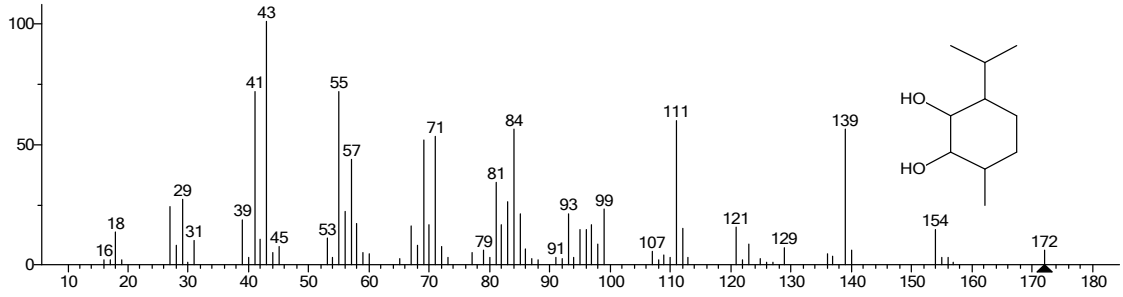


(mainlib) E-8-Methyl-9-tetradecen-1-ol acetate



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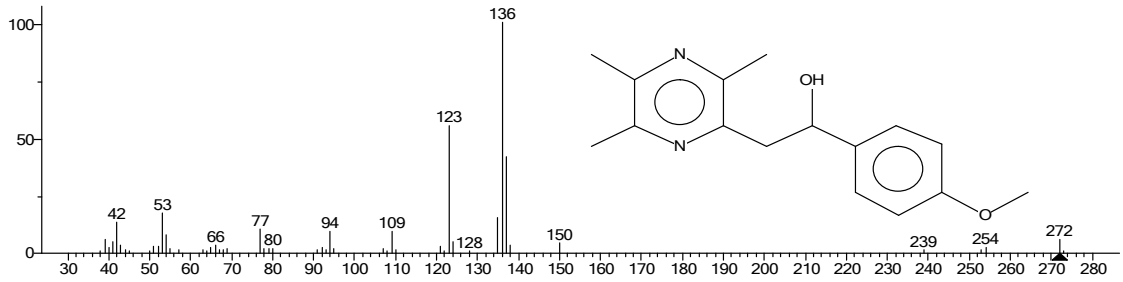
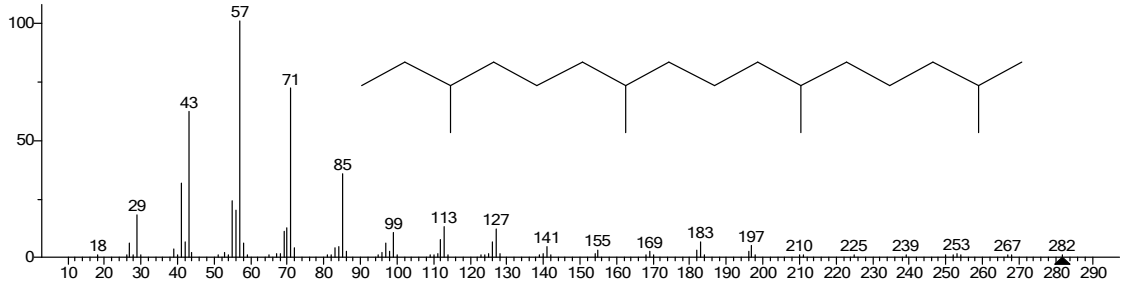
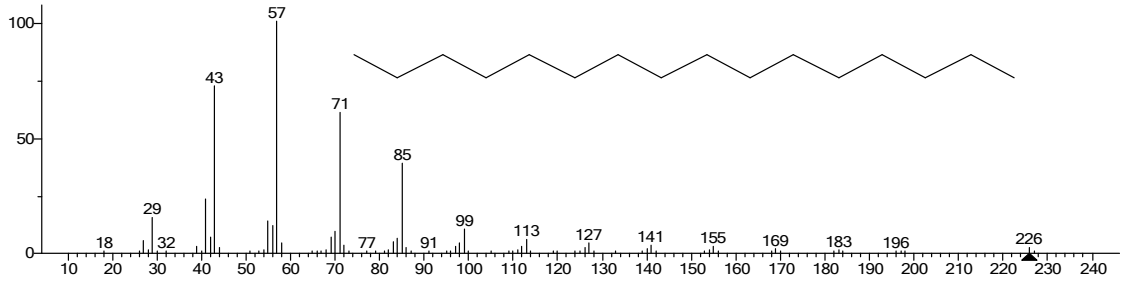


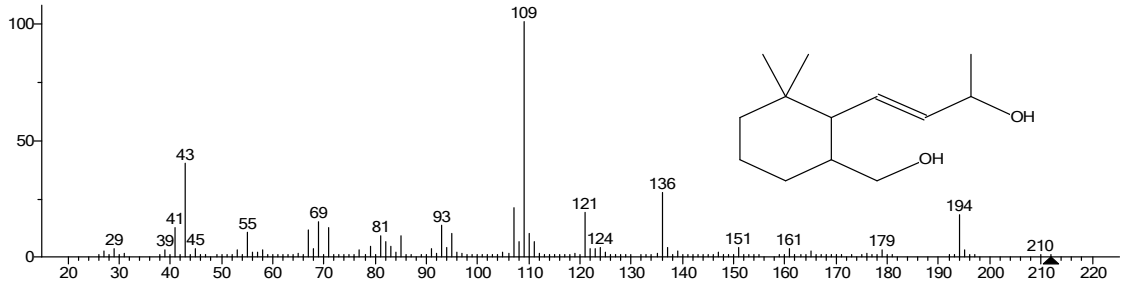
(mainlib) 2-(2-Hydroxy-2-p-methoxyphenylethyl)-3,5,6-trimethylpyrazine



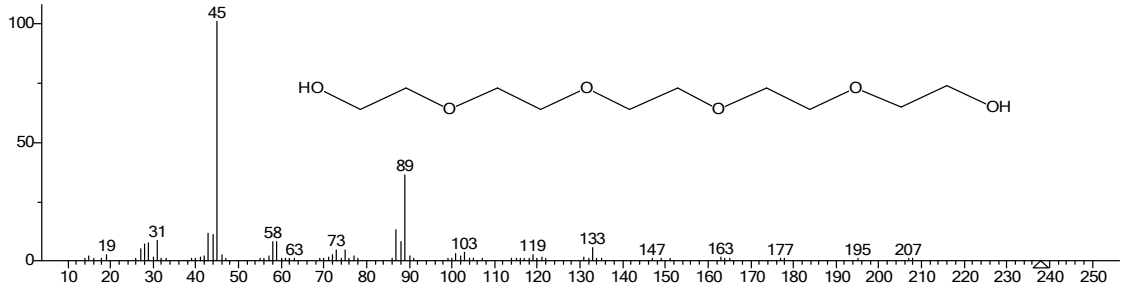
(mainlib) Hexadecane, 2,6,10,14-tetramethyl-



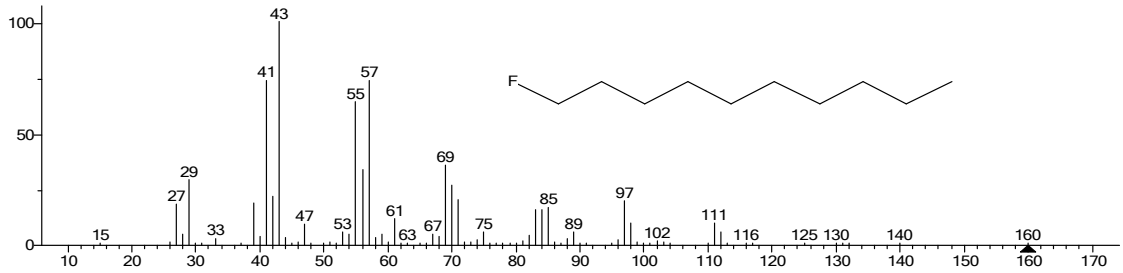
(mainlib) Hexadecane



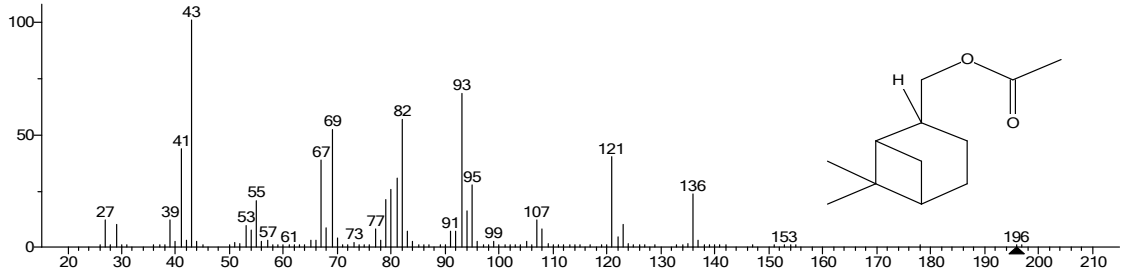
(mainlib) Cyclohexanemethanol, 3,3-dimethyl-2-(3-methyl-1-butenyl)-



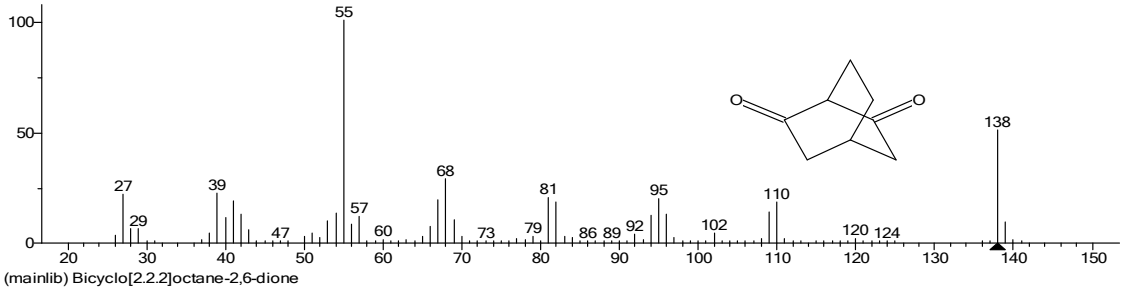
(mainlib) Pentaethylene glycol



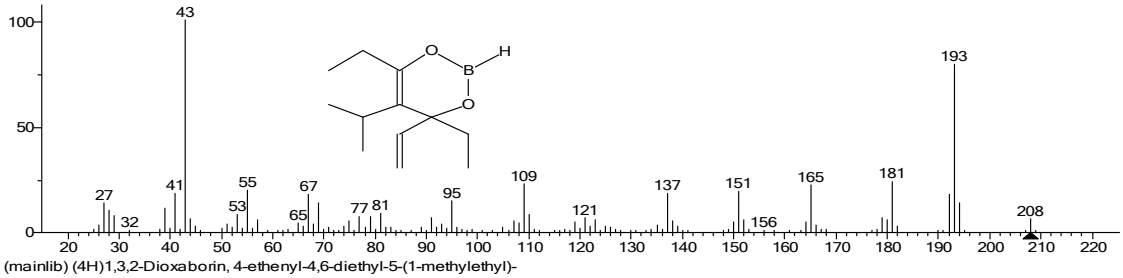
(mainlib) Decane, 1-fluoro-



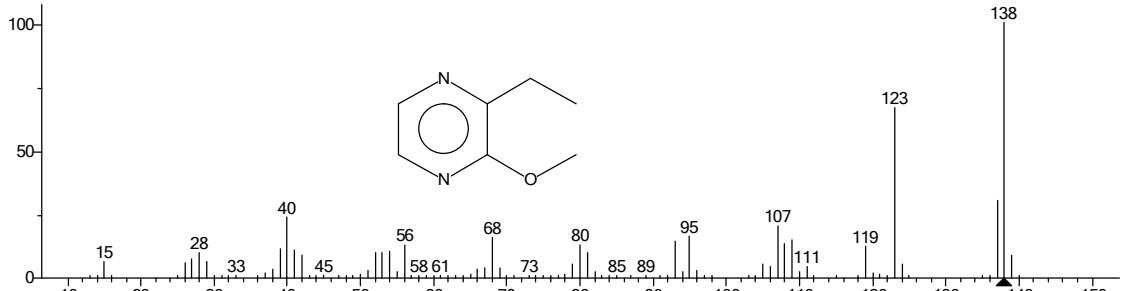
(mainlib) (-)-trans-Myrtanyl acatate



(mainlib) Bicyclo[2.2.2]octane-2,6-dione



(mainlib) (4H)1,3,2-Dioxaborin, 4-ethenyl-4,6-diethyl-5-(1-methylethyl)-



(mainlib) 2-Ethyl-3-methoxypyrazine

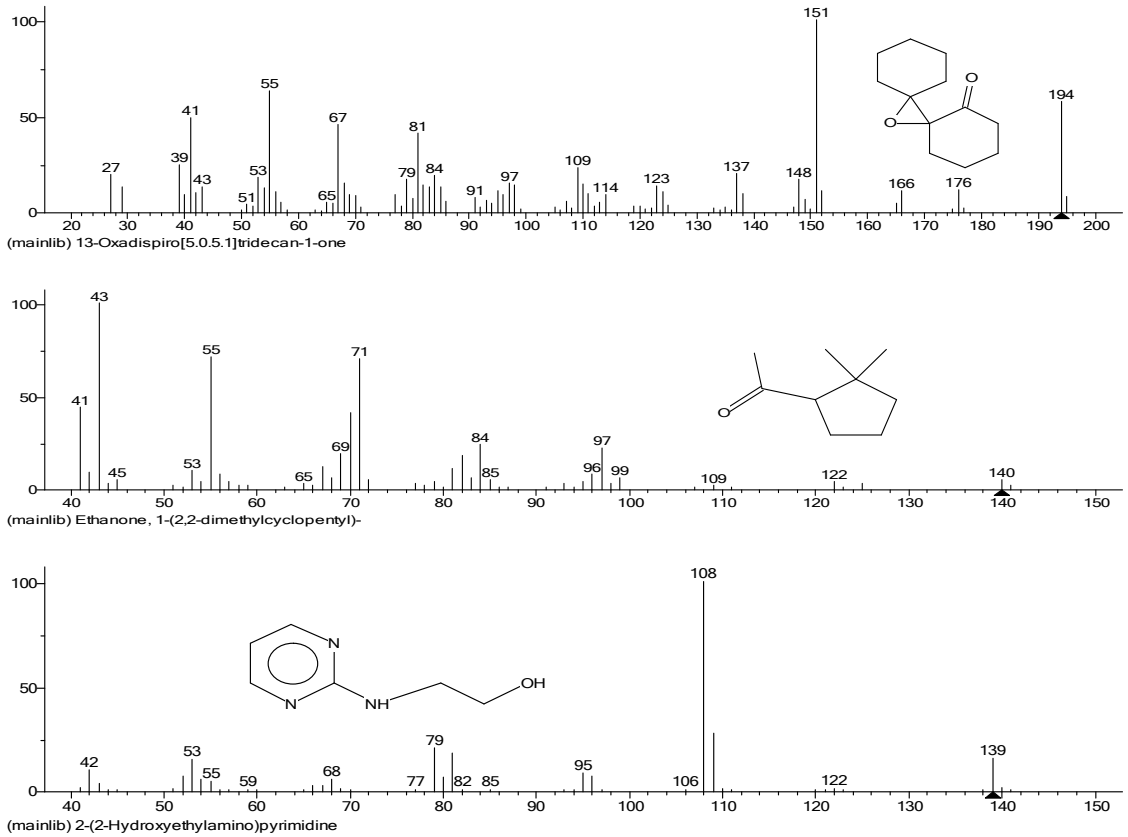


Fig. 2. Structures of compounds in COLE

3.3 Acute Toxicity Evaluation of *Corchorus olitorius* Leaf Extract

No toxicity behaviours and mortality were observed after all stages of single-dose administration of *C. olitorius* leaf extract during the acute toxicity study period which lasted for 1 to 7 days, even at a dose of 5000 mg/kg body. A repeat of this highest dose on 2 animals at the confirmatory stage of the test still produces no mortality. Though animals were calm immediately after the administration, they, however, regained their agility and physical activities within 2 hours. With this result, it was concluded that the LD_{50} value of *C. olitorius* leaf extract is above 5000 mg/kg and that the extract has a high margin of safety.

4. DISCUSSION

The presence of some phytochemical agents including flavonoids, steroids, terpenes, phenolic compounds, alkaloids, saponins, tannins and cardiac glycoside in *C. olitorius* leaf extract suggest that the plant is enriched with bioactive substances and may be a potential source of medication, just like several other green leafy vegetables. Results of a number of scientific investigations carried out on several green leafy plants had shown that these plants are rich in bioactive substances which confer on them several healing properties [1,16,17].

Flavonoids and other phenolic compounds have been isolated from several plants and trials on animal models revealed strong antioxidant activity due to the free radical scavenging capacity of these compounds [1]. The consumption of flavonoids in food has also been reported to be of value in the prevention of coronary heart diseases and management of cancer [18]. Flavonoids also have been implicated in wound healing, cellular regeneration and cytoprotection [19,20] and as such may be an agent of interest in the management of ulcer. Only recently, antimalarial properties have been linked to flavonoids. The observed anti-malarial effect of flavonoids may be due to the ability of flavonoids to inhibit fatty acid biosynthesis of the parasite and the influx of

Table 2. GC-MS composition of COLE

SN	RT	Component	Formula	MW	%
1	4.543	1,2,3,4-Butanetetrol, [S-(R*,R*)]-	$C_4H_{10}O_4$	122	0.07
2	4.592	d-Glycero-d-ido-heptose	$C_7H_{14}O_7$	210	0.06
3	4.659	Cyclopentane, 1-ethyl-1-methyl-	C_8H_{16}	112	0.41
4	4.712	6-Octen-1-ol, 3,7-dimethyl-	$C_{10}H_{20}O$	156	0.69
5	4.712	2-Octenal, (E)-	$C_8H_{14}O$	126	0.98
6	4.787	Octane, 1-chloro-	$C_8H_{17}Cl$	148	0.48
7	4.843	2-Acetoxy-1,1,10-trimethyl-6,9-epidioxydecalin	$C15H_{24}O_4$	268	0.07
8	4.937	Bicyclo [3.1.1]heptan-3-ol, 2,6,6-trimethyl-, (1α,2β,3α,5α)-	$C_{10}H1_8O$	154	0.11
9	4.978	2,4-Decadienal, (E,E)-	$C_{10}H_{16}O$	152	0.36
10	5.008	8-Nonen-2-one	$C_9H_{16}O$	140	0.19
11	5.057	Nonanal	$C_9H_{18}O$	142	0.61
12	5.132	1,13-Tetradecadien-3-one	$C_{14}H_{24}O$	208	0.22
13	5.199	Bicyclo [3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	$C_{10}H_{16}$	136	0.24
14	5.285	5-Hepten-2-one, 6-methyl-	$C_8H_{14}O$	126	0.19
15	5.368	7-Decen-1-ol acetate	$C_{12}H_{22}O_2$	198	0.35
16	5.446	2-Nonenal	$C_9H_{16}O$	140	1.10
17	5.506	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	0.21
18	5.589	Limonene oxide, cis-	$C_{10}H_{16}O$	152	0.21
19	5.641	Trans,trans-3,5-Heptadien-2-one	$C_7H_{10}O$	110	1.00
20	5.724	Ethanone, 1-(3-methylphenyl)-	$C_9H_{10}O$	134	0.41
21	5.758	2-Cyclopenten-1-one, 3-methyl-	C_6H_8O	96	0.35
22	5.829	Niacin	$C_6H_5NO_2$	123	0.67
23	5.919	2,6-Dimethylanisole	$C_9H_{12}O$	136	0.84
24	6.024	Cyclobutanecarboxylic acid, 2-ethylhexyl ester	$C_{13}H_{24}O_2$	212	1.45
25	6.125	2-Dodecenal	$C_{12}H_{22}O$	182	10.95
26	6.267	1,2-Ethanediol, 1-(2-phenyl-1,3,2-dioxaborolan-4-yl)-, [S-(R*,R*)]-	$C_{10}H_{13}BO_4$	208	0.43
27	6.342	3-Methyl-1-penten-4-yn-3-ol	C_6H_8O	96	1.99
28	6.462	Dimethyl 3,3'-oxydipropanoate	$C_8H_{14}O_5$	190	0.23
29	6.504	2,4-Decadienal, (E,E)-	$C_{10}H_{16}O$	152	1.83
30	6.661	E,Z-2,13-Octadecadien-1-ol	$C_{18}H_{34}O$	266	
31	6.773	2-Decenal, (E)-	$C_{10}H_{18}O$	154	1.06
32	6.927	E-8-Methyl-9-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	0.11
33	7.017	1,2-Cyclohexanediol, 3-methyl-6-(1-methylethyl)-, (1α,2β,3β,6α)-	$C_{10}H_{20}O_2$	172	0.26
34	7.220	2-(2-Hydroxy-2-p-methoxyphenylethyl)-3,5,6-trimethylpyrazine	$C_{16}H_{20}N_2O_2$	272	0.55
35	7.283	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	282	0.34
36	7.313	Hexadecane	$C_{16}H_{34}$	226	0.67
37	7.497	Cyclohexanemethanol, 3,3-dimethyl-2-(3-methyl-1-butenyl)-	$C_{13}H_{24}O_2$	212	0.35
38	7.617	Pentaethylene glycol	$C_{10}H_{22}O_6$	238	0.44
39	7.726	Decane, 1-fluoro-	$C_{10}H_{21}F$	160	1.27
40	7.973	(-)-trans-Myrtanyl acetate	$C_{12}H_{20}O_2$	196	0.42
41	8.085	Bicyclo [2.2.2]octane-2,6-dione	$C_8H_{10}O_2$	138	0.46
42	8.134	(4H)1,3,2-Dioxaborin, 4-ethenyl-4,6-diethyl-5-(1-methylethyl)-	$C_{12}H_{21}BO_2$	208	0.33
43	8.228	2-Ethyl-3-methoxypyrazine	$C_7H_{10}N_{2O}$	138	0.13
44	8.303	13-Oxadispiro [5.0.5.1]tridecan-1-one	$C_{12}H_{18}O_2$	194	0.23
45	8.367	Ethanone, 1-(2,2-dimethylcyclopentyl)-	$C_9H_{16}O$	140	1.37
46	8.498	2-(2-Hydroxyethylamino)pyrimidine	$C_6H_9N_{3O}$	139	0.62

L-glutamine and myoinositol into infected erythrocytes [21]. Both alkaloids and tannins are reported to be widely used as cancer chemotherapeutic agents [22,23] possibly due to their free radical scavenging effect and ability to prevent cellular oxidative damage and lipid peroxidation [24,25]. Most plants that contain tannin as their main component have been used for treating intestinal disorders such as diarrhea and dysentery [26]. Steroids and terpenes increase protein synthesis, promote the growth of muscles and bones and show some level of antiviral activities [25]. Saponins and glycosides alleviate cardiac problems associated with hypertension [27]. Saponins, in particular, have been used to treat hypercholesterolemia in humans. This is because it binds to cholesterol in the body to inhibit the reabsorption of the later thereby facilitating its excretion from the body.

4.1 GC-MS analysis of *C. olitorius*

Gas chromatography-mass spectrometry is an established technique for evaluating in greater details the chemical compounds present in any plant-based product, particularly volatile components [28,1,29,30]. In this study, GC-MS analysis of *C. olitorius* leaf extract showed the presence of 46 different compounds out of which some are known to possess some pharmacological activities and some are used as flavoring agents and in the production of cosmetics.

Niacin is reported to have an anticancer effect and lowers cardiovascular risks by boosting the levels of high-density lipoprotein (HDL). There is also evidence that niacin helps to lower atherosclerosis and the risk of Alzheimer's disease, cataract, osteoarthritis and type-1 diabetes. Other reported medicinal values of niacin are; improving liver and digestive functions including carbohydrate and protein breakdown [31]. Nonanal is a perfuming and flavouring agent [32] while 2-Nonenal which is found in alcoholic beverages, coffee, water melon, cucumbers, red currants, palm oil and potatoes known for its flavouring potential [33]. 2,4-Decadienal may be toxic and is reported to be possibly a carcinogenic substance [34]. 2-Decenal induced malformation of somatic muscles during trials in nematodes [35].Hexadecane found in most spices is known to possess anti-inflammatory, beta-oxidant and thermogenic functions [36]. A large number of other compounds identified in *C. olitorius* by GC-MS are flavour agents and as such are widely used as cosmetic agents.

The fact that an LD_{50} value greater than 5000 mg/kg was obtained in the course of evaluating the acute toxicity potentials of *C. olitorius* leaf extract suggest that the extract may not be toxic to living systems and gives credence to the use of the plant as food and medicine over the years without any reported case of acute toxicity. In Nigeria, *C. olitorius* is a major vegetable consumed by the Yorubas, where it is popularly called Ewedu [37]. The conclusion that *C. olitorius* leaf extract may be safe is indeed in line with the OECD guideline for acute toxicity studies. The guideline stipulates that mortality is the expected endpoint of acute toxicity and that were no mortality occurred within the acute toxicity study period in a population treated with a dose range at which mortality is expected, then the administered agent may be adjudged as being well tolerated and free of acute toxicity [38]. Similar conclusions were made in other acute toxicity investigations involving plant materials [3]. This Acute Toxicity result no doubt suggests that *C. olitorius* has no any form of acute toxicity effects and may not be different from other edible vegetables.

5. CONCLUSION

The present study on phytochemical, GC-MS analysis and acute toxicity evaluation of *C. olitorius* leaf extract reveals the presence of various phytochemical constituents and suggest that the plant is a potential source of antioxidant, anti-inflammatory, anticancer and cardiovascular system modulatory agent. The extract may also be safe with no form of acute toxicity effect.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24276