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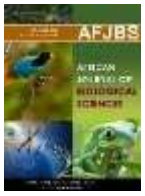

Phytochemistry And Gas Chromatography-Mass Spectroscopy (Gc-Ms) Analysis Of Methanolic Extracts Of *Aegle Marmelos* And *Euphorbia Hitra* Leaves

M Sabitha[1&3], P Sreevani[2], M Sridevi[4], P Tirupathi Rao[6], S Joseph[5], G Dinesh Ratna[5], Pangi Vijaya Nirmala[5]*

[1]TDR-HUB, Andhra University, Visakhapatnam -530003, AP
[2*]Department of Botany, Government Degree College, Vizianagaram Town, AP
[3]Department of Botany, S K V T Government Degree College, Rajamahendravaram -533103, AP
[4]Department of Botany, S K R Government Degree College, Rajamahendravaram -533103, AP
[5]School of Life and Health Sciences, Adikavi Nannaya University, Rajamahendravaram, AP
[6]Crystal growth & Nanoscience Research Center, Department of Physics, Government College (A), Rajamahendravaram, AP.

***Corresponding Author:** Prof. P. Sreevani
*Department of Botany, Government Degree College, Vizianagaram Town, Andhra Pradesh. Email: srvani6@gmail.com

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Abstract:
This study investigates the phytochemicals of two medicinal plants, *Aegle marmelos* and *Euphorbia hitra*, using qualitative, quantitative, and GC-MS analysis. The quantitative phytochemical investigation of the leaf methanolic extracts revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids, coumarins, diterpenes, and atropine equivalent (AE)/g of dried extract. The total polyphenol content (TPC) of *Aegle marmelos* was 73.7, while *Euphorbia hitra* had 133.53. Total flavonoid content (TFC) was 73.2 and Euphorbia hitra had 85.5. Total alkaloid content (TAC) was 79.43. The GC-MS analysis of methanolic extracts of *Aegle marmelos* and *Euphorbia hitra* leaves revealed a rich diversity of compounds, predominantly fatty acids and their derivatives. *Aegle marmelos* exhibited 54 compounds, while *Euphorbia hitra* demonstrated 41 distinct compounds. This comprehensive profiling highlights the phytochemical richness of both plants, underscoring their potential medicinal and therapeutic applications. The results will help in understanding the potential of these plants as natural medicines for treating various diseases and ailments in humans and non-human animals.

Keywords: Phytochemicals, *Aegle marmelos, Euphorbia hitra*, GC-MS.

1. INTRODUCTION:

Indian subcontinent has abundant wealth of medicinal and aromatic plants. Since ancient time, several drugs or medicines have been formulated using plants and their extracts (Krishnamoorthy and Subramaniam, 2014). Plants produce tremendous diversity of secondary metabolites called phytochemicals or bioactive compounds which are found in different parts of the plants such as stem, bark, leaves, flower, fruits, seeds and root etc. (Kumar and Fathima, 2017). Phytochemical constituents such as alkaloids, anthraquinone glycosides, carbohydrates, cardic glycosides, coumarins, flavonoids, phenols, saponins, steroids, tannins and terpenoids etc. have been isolated from plants and investigated their pharmacological activities thoroughly (Batiha et al., 2020) (Izzany et al., 2020). The quantity and types of phytochemicals are different from one species to other species of plants. In recent decades, different phytochemicals bioactive compounds with pharmacological activities have been investigated (Ahmad et al., 2018). A several reports stated that naturally derived compounds or drugs have fewer side effects than other synthetic medicines (Batiha et al., 2020). Many synthetic, non-natural drugs exhibit adverse side effects and some are unable to treat the terminal stages of diseases such as cancer. Several phyto active compounds found in plants showed effective pharmacological activities and devoid of side effects (Gavamukulya et al., 2015). Natural medicines are widely used in many regions of the world to treat many diseases and ailments in humans and non-human animals (Chattopadhyay et al., 2016). Still there are several substances with medicinal or pharmacological activities yet to be revealed in plant kingdom (De et al., 2013).

Two medicinal plants *Aegle marmelos* and *Euphorbia hitra* were selected for this study to investigate the phytochemicals using qualitative, quantitative and GC-MS analysis.

Aegle marmeols (*Rutaceae*) is a perennial tree indigenous to India and commonly known as Bilva in Sanskrit. Every part of bilva has medicinal activity and fruit of Bilva has great medicinal value which is used in folk medicine since Charaka (1500 B.C.), and is important environmental protective plant (Perumal and Krishna, 2018).The phytochemicals which are present in bilva possess pharmacological activities against cancer, gastrointestinal and cardio vascular diseases. Animal models treated with the active extracts of various bilva plant parts reported anti-ulcer, anti-hyperlipidaemic, anti-oxidant, anti-diabetic, anti-inflammatory and anti-microbial properties (Mujeeb et al., 2014) (Kumar et al., 2019).

Euphorbia hitra (*Euphorbiaceae*), a small annual hairy plant, commonly found in tropical countries and an ascending herb (Karki et al., 2021). This plant used in folk medicine against various skin diseases, gonorrhoea, warts, intestinal parasites, conjunctivitis, asthma and respiratory problems. It also exhibit analgesic, anti-microbial, anti-oxidant, ant-helminthic, anti-cancer, anti-allergic and anti-diuretic activities. Recent studies found that this plant contains the enzyme inhibitory activity against tyrosinase, α-glucosidase, acetylcholinesterase, α-amylase and butyrylcholinesterase enzymes (Rautela et al., 2020) (Izzany et al., 2020) (Chitra et al., 2014).

Thus, the objectives of the present study are to evaluate the following:

(i) Extraction of phytochemicals using decoction method from *Aegle marmelos* and *Euphorbia hitra* leaves.
(ii) Evaluation of phytochemicals using qualitative and quantitative investigations.
(iii) GC-MS analysis of phytocompounds.

2. Methodology:

2.1. Collection of plant leaves:

The leaves of two medicinal plants *Aegle marmelos* (Maredu- *Rutaceae)* and *Euphorbia hitra (*Nanapaala – *Euphorbiaceae)* were collected within the premises of Rajahmundry, Andhra Pradesh, India. All the leaves were washed with tap water 2-3 times and subjected to shade drying under sunlight for 3-4 days. The dried leaves were finely powdered and packed in sealed polythene bags separately.

2.2. Decoction:

Decoction is more convenient method for water soluble and heat stable compounds, which is prepared by dissolving and boiling of crude material in water for 30 minutes followed by cooling and filtering to yield the required volume. 5grams of each leaf powder was added to the 100mL of methanol separately and kept for boiling up to 45 minutes in water bath. The boiled solution was subjected to double filtration with Whatman number 1 filter paper and resultant solution was transferred to a screw cap bottle and stored at 25^{o}C temperature. Polar solvents are not compatible with non-polar column of GC-MS analysis, hence the methanol was selected as solvent. Depending upon the operating temperature, water can oxidize both the column and parts of the MS in GC-MS equipment. Oxidation in the MS leads to poor tuning and dramatic loss in sensitivity (Al-Manhel and Kareem Niamah 2012).

2.3. Qualitative Phytochemical Screening:

Using standard protocols, a preliminary phytochemical screening of the ASE and ATE extract was conducted to determine whether secondary metabolites, including flavonoids, phenols, tannins, alkaloids (Mayer's and Wagner's), terpenoids, anthraquinones, saponins, quinones, coumarins, glycosides, steroids and reducing sugars were present or absent (Dubale et al., 2023).

2.4. Quantitative phytochemical investigation

The leaf methanolic extracts of two plants were subjected for quantitative phytochemical determination separately for Phenols, Flavonoids and Alkaloids and all were done in triplicates, according to the *Senguttuvan et al. 2014*.

2.4.1. Total Phenol estimation:

Preparation of standard: 10 mg Gallic acid was dissolved in 10 mL methanol and labelled as standard stock solution. Various aliquots of 50- 250 µg/mL were prepared in methanol from standard stock solution.

Preparation of reagents: The FC (Folin-ciocaltaeau) reagent was prepared by dissolving the 1mL of FC reagent solution in 10mL of distilled water (1:10 v/v). 7.5% of sodium carbonate was prepared by dissolving the 0.75gms of sodium carbonate in 10mL of distilled water.

Preparation of plant extract solution: 10 mg of dried plant extract was dissolved in 10mL of methanol and filtered the solution using Whatmann number 1 filter paper.

Procedure:

2 mL of extract or standard solution was taken into test tubes and added 1 mL of FC reagent (1:10 v/v). 1 mL of 7.5% sodium carbonate was added to this solution and kept for incubation up to 2 hours. The absorbance was read at 765 nm wavelength using UV-Vis Spectrophotometer against blank.

2.4.2. Total flavonoids estimation:

Preparation of standard: 10 mg Quercetin was dissolved in 10 mL methanol and labelled as standard stock solution. Various aliquots of 50- 250 µg/ml were prepared in methanol from standard stock solution.

Preparation of reagent: 5% sodium nitrate solution was prepared by dissolving the 0.5grams of sodium nitrate in 10mL of distilled water. 10% aluminum chloride solution was prepared dissolving 1gram of aluminum chloride powder in 10mL of distilled water. 1M NaOH solution was prepared by dissolving 0.04grams of sodium hydroxide in 10mL of distilled water.

Preparation of reagents: 5% sodium nitrate was prepared by dissolving 0.25 grams of sodium nitrate in 5mL of distilled water. 10% aluminum chloride was prepared by dissolving 0.5grams of $AlCl_3$ in 5mL of distilled water. 1M NaOH was prepared by dissolving 2grams of sodium hydroxide in 50mL of distilled water.

Preparation of plant extract solution: 10 mg of dried plant extract was dissolved in 10mL of methanol and filtered the solution using Whatman No 1 filter paper.

Procedure: 2 mL of plant extract or standard was added to test tube and 0.3mL of 5% sodium nitrate was added to this solution and kept for incubation for 5 minutes at room temperature and followed by addition of 0.3 mL of 10% aluminum chloride solution. 2 mL of 1M NaOH was added to this solution and make up the solution to 2.5mL with distilled water and kept for 15 minutes incubation at room temperature and absorbance was read at 510 nm wavelength using UV-Vis Spectrophotometer against blank.

2.4.3. Total alkaloids estimation:

Preparation of standard: 10 mg Atropine was dissolved in 10 mL methanol and labelled as standard stock solution. Various aliquots of 50- 250 µg/ml were prepared in methanol from standard stock solution.

Preparation of reagent: Sodium phosphate buffer was prepared by dissolving 0.99 grams of Na2HPO4 and 1.98 grams of citric acid in 200mL of distilled water. 2N sodium hydroxide was prepared by dissolving 8 grams of NaOH in 100mL of distilled water. 10mg bromo cresol green was dissolved in 100mL of 2N NaOH.

Preparation of plant extract solution: 10 mg of dried plant extract was dissolved in 10mL of methanol and filtered the solution using Whatman No 1 filter paper.

Procedure: 500 µl of plant extract solution or standard was added to 2.5 mL of Bromo cresol green solution dissolved 2N NaOH and 2.5mL of phosphate buffer solution. 1 mL of chloroform added to this solution and mixed well and separated the chloroform layer using separating funnel. This was repeated by adding 2mL, 3mL and 4mL of chloroform. Separated chloroform subjected for absorbance reading at 470nm using UV-Vis Spectrophotometer against blank.

2.5. GC-MS analysis:

For GC-MS analysis 5% of leaf extract powders were dissolved in methanol and boiled for 60 minutes on water bath. After boiling the solution was heat filtered using whatmann no 1 filter paper and solution stored in screw capped bottle.

The Gas chromatography of methanolic extracts was carried out using Shimdzu GC-2010 plus gas chromatograph (IIT Madras, India) which was equipped with a 2mm direct inject liner and a column (Alltech EC-5) with 15mm. The experiment was done as follows: The sample was introduced with split injection and split ratio of 10:1. The oven temperature ramp at 20°C per minute to 450°C and held for 5 minutes. The helium carrier gas was set to 2 ml/minute flow rate (constant flow mode).

The mass spectrum operated in electron ionization (EI) mode with GCMS solution ver.2.6 software. The Low-resolution mass spectra were attained at resolving power of 1000 and scanning from 25-1000 m/z at 0.3sec/scan with a 0.2 second inter scan delay. At 5000 resolving power high-resolution spectra were attained and scanning from 65-1000 m/z at 1 sec/scan and both low and high resolution power were done at 20% height definition.

Identification of the different components of the compounds was matching their recorded spectra with the data bank mass spectra of NIST library V 11 provided by the instruments software. GC/MS metabolomics Database was used for the similarity search with retention index (Karki et al., 2021).

3. RESULT AND DISCUSSION

Two plant extracts were subjected to determine the phytochemical constitutions such as Steroids, anthraquinones, carbohydrates, coumarins, alkaloids, flavonoids, tannins, terpenoids, saponins, glycosides and phenols. The result of qualitative phytochemical investigation given the table 1.

Table 1: Qualitative phytochemical investigation of methanolic extracts.

S.No	Constituents	*Aegle marmelos*	*Euphorbia hitra*	Observation
1	Steroids	+	+	Formation of steroidal ring was observed.
2	Anthraquinones	-	-	No appearance of far bright pink colour and the solutions were found in green colour.
3	Carbohydrates	+	+	Formation of dull violet colour was observed.
4	Coumarin	+	-	Appearance of yellow colour solution was observed in *Aegle marmelos* and *Euphorbia hitra* was appeared in yellow colour.
5	Alkaloids	+	+	Appearance of turbidity was observed.
6	Flavonoids	+	+	Appearance of dark greenish colour was observed.
7	Tannins	+	+	Appearance of light to dark greenish colour was observed
8	Terpenoids	+	+	Orange to greyish colour was observed
9	Saponins	+	+	Formation of turbidity was observed
10	Glycosides	+	+	*Aegle marmelos* and *Euphorbia hitra*
11	Phenols	+	+	Dark green colour solutions were observed.

+ Positive,

- Negative,

The results obtained from the qualitative phytochemical investigation confirmed the presence of steroids, carbohydrates, alkaloids, flavonoids, tannins, terpenoids, saponins, glycosides and phenols in the two plant extracts. Anthraquinones were not detected in both plants, coumarins were found only in plant *Aegle marmelos* and not in *Euphorbia hitra*.

3.2. Quantitative phytochemical investigation of methanolic extracts:

The Phenols, Flavonoids and Alkaloids of two plants leaf methanolic extracts were quantitatively determined and the result were represented in Table 2.

Table 2: Quantitative phytochemical investigation.

S.No	Plant extract	Quantity of phytochemical constituents
Total Polyphenol Content (TPC)		
1	*Aegle marmelos*	73.7*
2	*Euphorbia hitra*	133.53*
Total Flavonoid Content (TFC)		
1	*Aegle marmelos*	73.2**
2	*Euphorbia hitra*	85.5**
Total Alkaloid Content (TAC)		
1	*Aegle marmelos*	84.71***
2	*Euphorbia hitra*	79.43***

*Total polyphenol content (TPC) mg Gallic acid equivalent (GAE)/g of dried extract.

**Total flavonoid content (TFC) mg Quercetin equivalent (QE)/g of dried extract.

***Total alkaloid content (TAC) mg Atropine equivalent (AE)/g of dried extract.

Aqueous leaf extract of *Euphorbia hitra* does not shown the presence of anthraquinone glycosides and coumarin in this study. Methanol extract of *Euphorbia hitra* leaves does not shown coumarin, and cardiac glycosides and n-hexane does not shown any phytochemical constituents except the tannins and quinones (Karki et al., 2021). The root of *Euphorbia hitra* shown carbohydrates, proteins, tannins, saponins, flavonoids, alkaloids, coumarins, diterpenes in at least one of the extracts of methanol, ethanol, ethyl acetate, acetone and aqueous extracts (Kumar and Choudhary, 2020). In a study of leaf extracts, alkaloids terpenoids and carbohydrates appeared only in ethanol and chloroform but not appeared in hexane. Flavonoids appeared in all three extracts and tannins and saponins only found in chloroform and ethanol respectively (Ahmad et al., 2018). Whole plant methanolic extract shown the presence of alkaloids, flavonoids, saponins, tannins and phenols but not steroids (Nkafamiya and Shagal, 2019). Anthraquinone glycosides were not appeared in the qualitative phytochemical investigation of leaf aqueous extracts of *Aegle marmelos* in our study. Leaf aqueous extracts of *Aegle marmelos* shown the phytochemical constituents such as alkaloids, terpenoids, tannins, saponins, steroids, coumarin, anthocyanin and carbohydrates but does not shown the phenols, reducing sugar, proteins, anthocyanin, and glycosides (Batiha et al., 2020). Aqueous leaf extracts does not shown terpenoids, phenols, amino acids and proteins (Rajeswari et.,al 2011). Chloroform leaf extract shown all the phytochemical constituents but leucoanthocyanin, steroids and phytosterols does not found (Ariharan and Prasad, 2014). In a study, alkaloids, phenols and flavonoids found in aqueous, acetone and chloroform extracts, saponins and phytosterols not found in chloroform and aqueous extracts respectively (Kumar and S, 2013).

In a study, the fruit pulp aqueous extract of *Aegle marmelos* showed 76.28mg/TAE (tannic acid equivalent) and 15.20mg/RE (Rutin equivalent) of phenols and flavonoids respectively, which is less than the present study. Whereas methanolic and ethanolic extracts showed slightly more quantity of phenols and flavonoids (Tagad et al., 2018). Aqueous fruit extract of *Aegle marmelos* showed 129mg/QE (Quercetin equivalent) and 147.66mg/GAE (gallic acid equivalent) of flavonoids and phenols respectively, which are higher in quantity of aqueous leaf extracts of *Aegle marmelos* in the present study (Rajan et al., 2011). Combined leaf and bark aqueous extracts of *Aegle marmelos* showed 11.37/QE (Quercetin equivalent) and 65.20mg/GAE (gallic acid equivalent) of flavonoids and phenols respectively which is less than in quantity when compared with the present

study (Paradisiaca, 2021). Leaf aqueous extract of *Aegle marmelos* showed 8.62mg/g of alkaloids, 26.2mg/g of flavonoids and 22.8mg/g of phenols which are lesser in quantity than the present study (Mujeeb et al., 2014). In the present study of aqueous leaf extracts of *Aegle marmelos* showed 73.7mg/100mg GAE of phenols, 73.2mg/100mg QE of flavonoids, and 84.71mg/100mg Atropine equivalent of alkaloids.

A study on methanolic leaf extract of *Euphorbia hitra* showed 6.68mg/100mg QE of flavonoids, 0.0023mg/100mg GAE of phenols, 0.3502mg/100mg Atropin equivalent of alkaloids (Nwofor Chioma N et al., 2021), which are in less quantity than the present study of aqueous leaf extract of *Euphorbia hitra*. Whole plant methanolic extract of *Euphorbia hitra* showed 109.86mg/100mg GAE of phenols and 18.92mg/100mg QE of flavonoids (Tran et al., 2020) which are less than the present study. Aqueous plant extract of *Euphorbia hitra* excluding root showed 64.31mg/g RE of flavonoids and 152.95mg/g GAE of phenolic compounds (Izzany et al., 2020) which are similar to the present study. In the present study of aqueous leaf extracts of *Euphorbia hitra* showed 133.53mg/100mg GAE of phenols, 85.5mg/100mg QE of flavonoids, and 79.43mg/100mg Atropine equivalent of alkaloids.

3.3. GC-MS analysis:

The GC-MS method is a valuable instrument for quantifying the concentration of active compounds in herbal extracts. This technique is particularly useful in the cosmetic, pharmaceutical, food, environmental, and forensic industries. This method integrates two analytical techniques to analyse mixtures of chemical substances. Gas chromatography is a technique used to separate the different components of a mixture, whereas mass spectroscopy is used to analyse each of these components individually. Chemical analyses have revealed that it mostly consists of cardenolides, pregnane glycosides, and volatile constituents. The majority of the volatile components consist of long-chain unsaturated fatty acids, which serve crucial functions in biological systems. These fatty acids act as structural components for various useful molecules and also serve as significant sources of energy (Gomathi et al., 2015).

The GC-MS analysis of methanolic extracts of *Aegle marmelos* and *Euphorbia hitra* leaves, prepared using the decoction method, revealed a rich diversity of compounds, predominantly fatty acids and their derivatives. *Aegle marmelos* exhibited a total of 54 compounds, while *Euphorbia hitra* demonstrated 41 distinct compounds, as detailed in Tables 3 and 4. This comprehensive profiling highlights the significant phytochemical richness of both plants, underscoring their potential medicinal and therapeutic applications. The identification of these compounds provides a valuable foundation for further pharmacological studies and validates the traditional uses of these plants in herbal medicine. The GC-MS chromatogram of both the samples were represented in Fig 1&2.

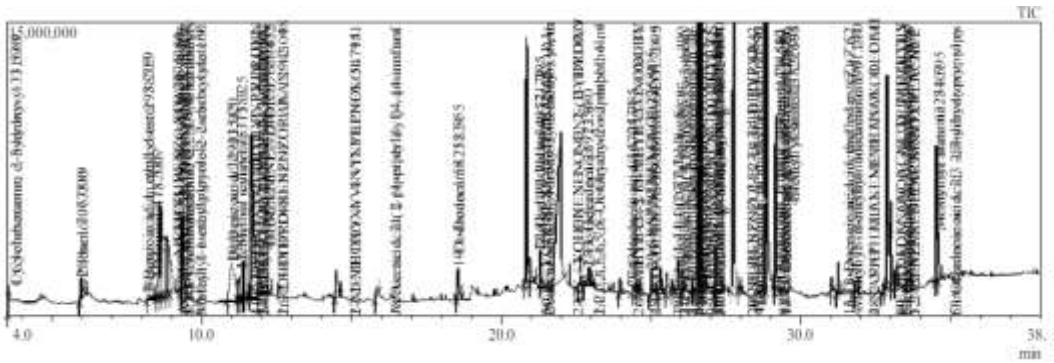


Figure 1: GC-MS chromatogram of leaf methanolic extract of *Aegle marmelos*

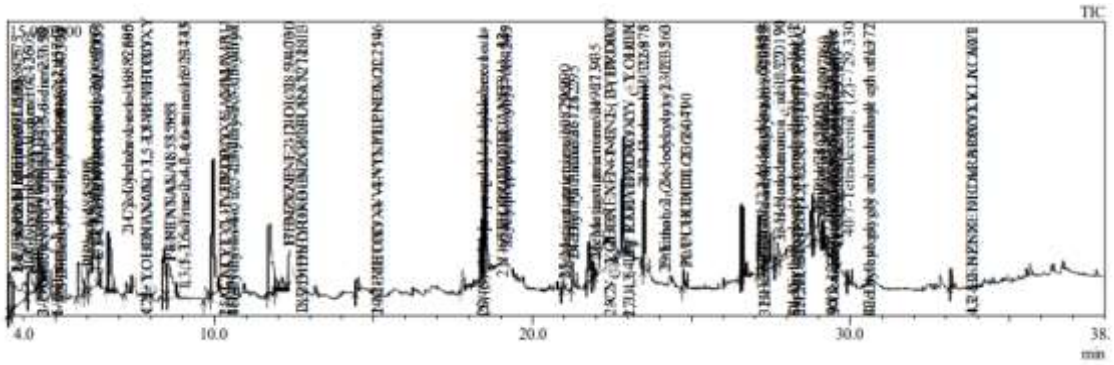
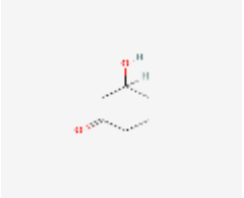
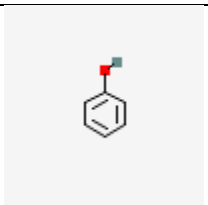
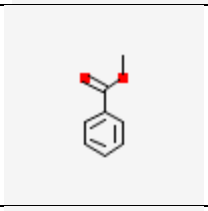
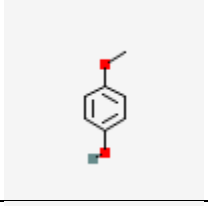
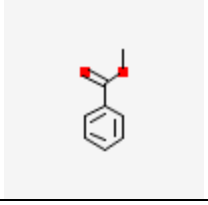
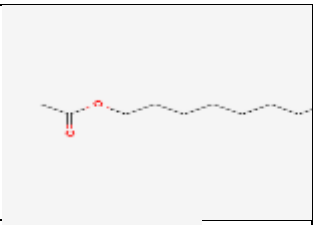
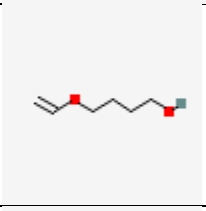
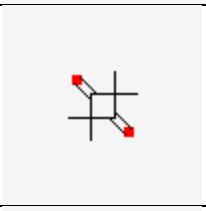
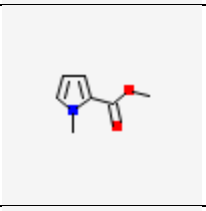
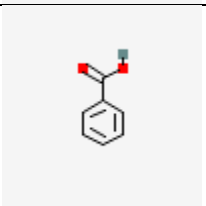
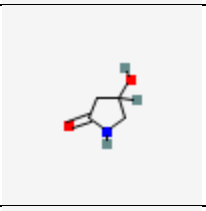
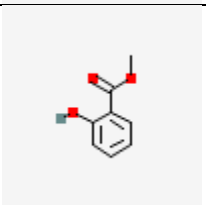
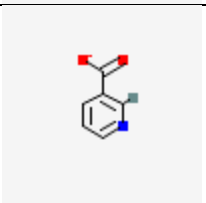
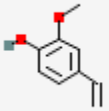
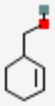
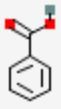


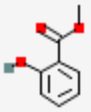
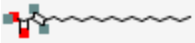
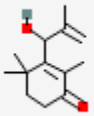
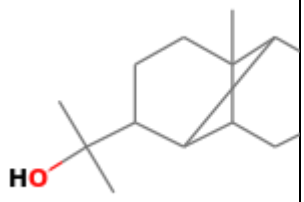
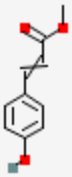
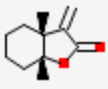
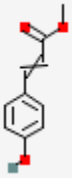
Figure 2: GC-MS chromatogram of leaf methanolic extract of *Euphorbia hitra.*

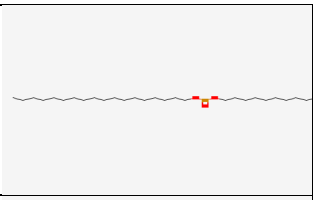
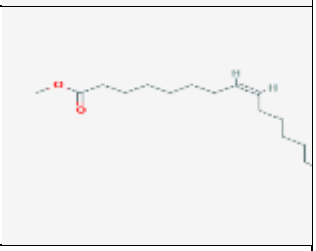
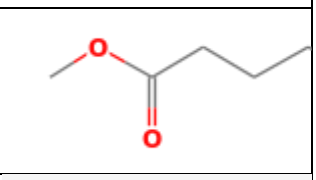
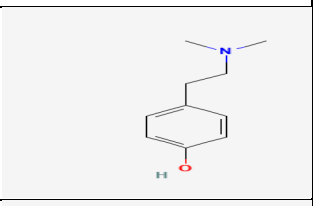
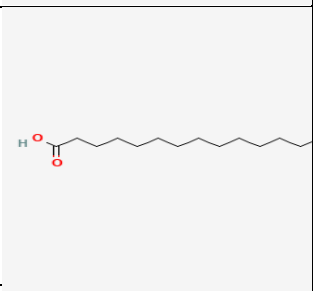
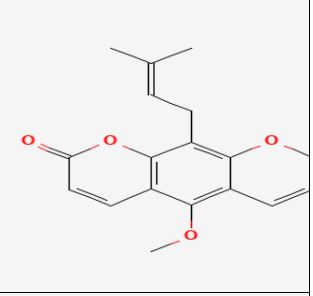
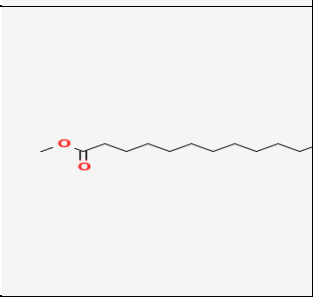
Table 3: GC-MS analysis of leaf methanolic extract of *Aegle marmelos*

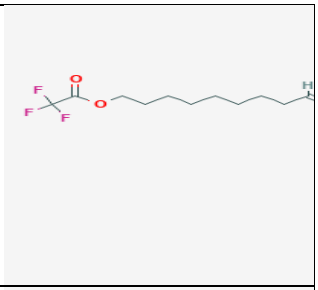
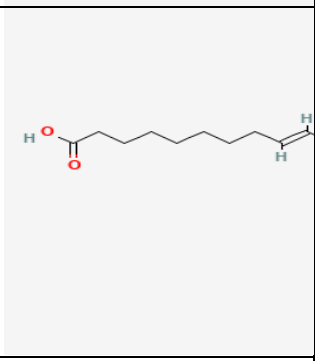
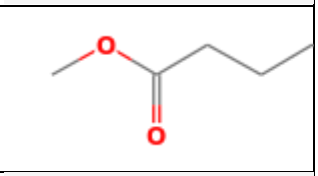
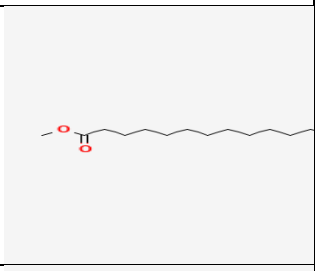
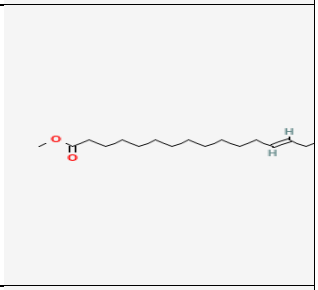
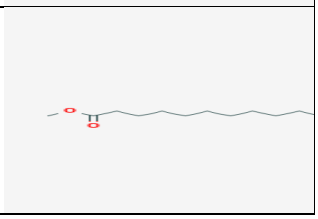
S.NO	Retention time	Name of the Compound	Molecular formula	Molecular weight *(m/z)*	Chemical structure
1.	3.529	Cyclohexanone, 3-hydroxy (Synonyms: 3-Hydroxycyclohexanone 3-hydroxycyclohexan-1-one)	$C_6H_{10}O_2$	114.14	
2.	5.998	Phenol	C_6H_5OH	94.11	
3.	8.205	Benzoic acid, methyl ester	$C_6H_5COOCH_3$	136.14	
4.	8.535	Mequinol	C_7H_7OH	124.13	
5.	8.613	METHYL BENZOATE	$C_6H_5COOCH_3$	136.14	

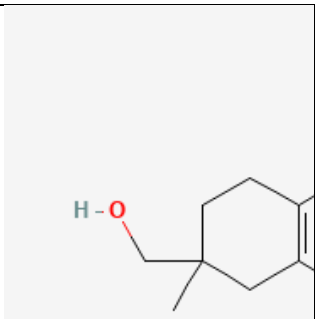
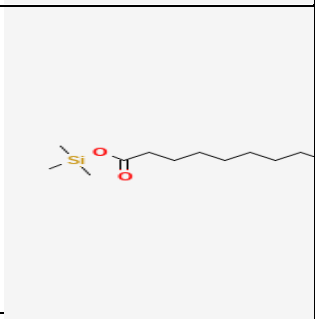
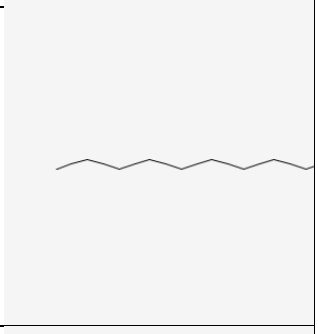
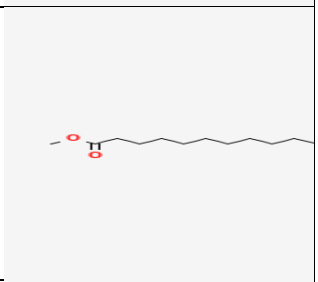
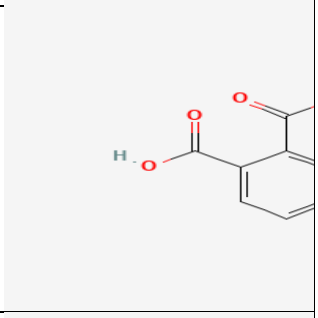
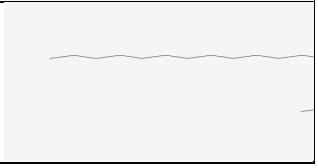
6.	8.776	ACETIC ACID, OCTYL ESTER	$C_{10}H_{20}O_2$	172.26	
7.	8.850	1-BUTANOL, 4-(ETHENYLOXY)-	$C_6H_{12}O_2$	116.16	
8.	8.961	1,3-Cyclobutanedione, 2,2,4,4-tetramet	$C_8H_{12}O_2$	144.21	
9.	9.342	Methyl 1-methylpyrrole-2-carboxylate	$C_7H_9NO_2$	139.15	
10.	11.018	Benzoic acid	C_6H_5COOH	122.12	
11.	11.210	4-HYDROXY-2-METHYL-PYRROLID	$C_4H_7NO_2$	101.10	
12.	11.325	Methyl salicylate	$C_8H_8O_3$	152.15	
13.	11.395	2-DEUTERIO PYRIDINE-3-CARBOX	C_5H_4DN-COOH	123.11	
14.	11.448	N-Methylpyrrole-2-carboxylic acid BENZENE-1,2-DIOL			

15.	11.654	2,3-DIHYDRO-BENZOFURAN	C_8H_8O	120.15	
16.	12.089	2- METHOXY-4-VINYLPHENOL	$C_9H_{10}O_2$	150.7	
17.	14.493	Acetic acid, 2-(1-piperidyl)-, [4-aminofu 1-Dodecanol	n/a	n/a	n/a
18.	15.850	3- CYCLOHEXENE-1-METHANOL, .	$C_7H_{11}OH$	112.17	
19.	18.553	Benzoic acid, 4-hydroxy-3-methoxy-, m Diethyl Phthalate	n/a	n/a	n/a
20.	20.845	2-CYCLOHEXEN-1-ONE, 2-(HYDRO	$C_5H_6O_2$	112.18	
21.	20.960	1,2,3,4,5,6,7,8-Octahydro-2-naphthol, 4 1-Hexadecanol	n/a	n/a	n/a
22.	21.296	2-METHYL-2-(3-METHYL-2-OXOBU	n/a	n/a	n/a
23.	21.979	Benzoic acid	C_6H_5COOH	122.12	
24.	22.631	4-HYDROXY-2-METHYL-PYRROLID	$C_4H_7NO_2$	101.10	

25.	22.882	Methyl salicylate	$C_8H_8O_3$	152.15	
26.	23.959	2-DEUTERIO PYRIDINE-3-CARBOX	C_5H_4DN-COOH	123.11	
27.	24.385	Octadecanoic acid	$C_{17}H_{35}COOH$	284.5	
28.	24.517	4-OXO-.BETA.-ISODAMASCOL	$C_{13}H_{20}O$	208.29	
29.	25.007	(-)-SPATHULENOL	$C_{15}H_{24}O$	220.35	
30.	25.317	Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-meth	$C_{15}H_{24}O$	220.35	
31.	25.527	2-Propenoic acid, 3-(4-hydroxy-3-meth	$C_{10}H_{10}O_3$	208.21	
32.	25.720	2,3a-Dimethylhexahydro benzofuran-7a-	$C_{10}H_{18}O_2$	170.25	
33.	25.906	2-Propenoic acid, 3-(4-hydroxy-3-meth	$C_{10}H_{10}O_3$	208.21	

34.	26.060	PHOSPHONIC ACID, DIOCTADECY	$C_{36}H_{75}O_3P$	587	
35.	26.386	9-HEXADECENOIC ACID, METHYL	$C_{16}H_{30}O_2$	254.41	
36.	26.600	Tricyclo[7.2.1.0(3,8)]dodeca-3(8),4,6-tr	n/a	n/a	n/a
37.	26.670	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.54	
38.	26.795	Hordenine	$C_{10}H_{15}NO$	165.23	
39.	27.181	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	
40.	27.752	2H,8H-BENZO[1,2-B:3,4-B']DIPYRA	$C_{21}H_{20}O_6$	368.35	
41.	27.970	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	284.5	

42.	28.680	Oleyl alcohol, trifluoroacetate	$C_{20}H_{35}F_3O_2$	364.5	
43.	28.791	9,12-Octadecadienoic acid (Z,Z)-, meth	$C_{18}H_{32}O_2$	280.4	
44.	28.866	11-Octadecenoic acid, methyl ester, (Z)	$C_{19}H_{36}O_2$	296.5	
45.	29.163	Methyl stearate	$C_{19}H_{38}O_2$	298.5	
46.	31.006	13-Docosenoic acid, methyl ester, (Z)-	$C_{23}H_{44}O_2$	352.6	
47.	31.251	Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$	326.6	

48.	31.816	2-NAPHTHALENEMETHANOL, DEC	$C_{15}H_{26}O$	222.36	
49.	32.755	HEXADECANOIC ACID, TRIMETHY	$C_{19}H_{40}O_2Si$	328.6	
50.	32.881	Hexadecanoic acid, 2-hydroxy-1-(hydro	$C_{19}H_{38}O$	330.50	
51.	33.026	Docosanoic acid, methyl ester	$C_{23}H_{44}O_2$	352.6	
52.	33.205	1,2-BENZENEDICARBOXYLIC ACID	$C_8H_6O_4$	166.14	
53.	34.527	Octadecanoic acid, 2,3-dihydroxypropy	$C_{31}H_{62}O_4$	498.8	

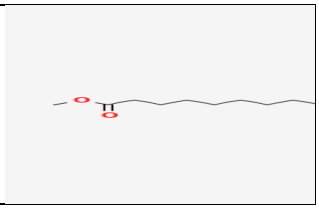
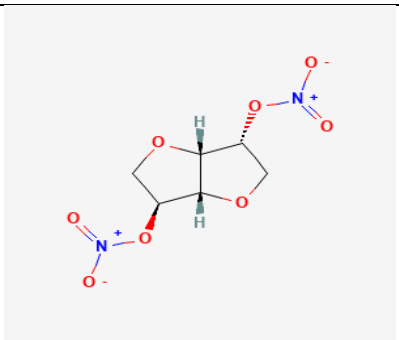
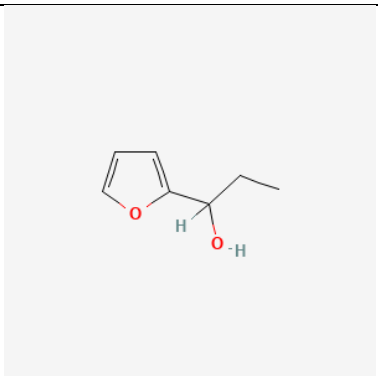
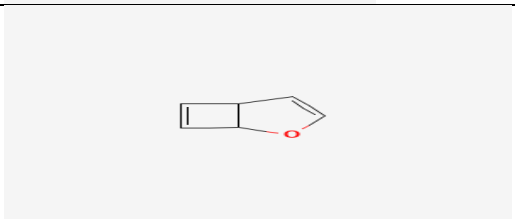
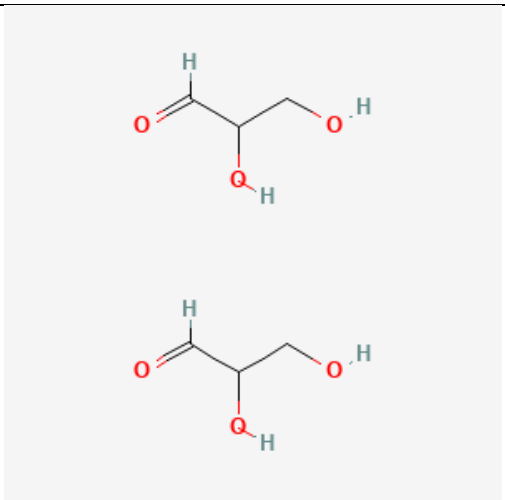
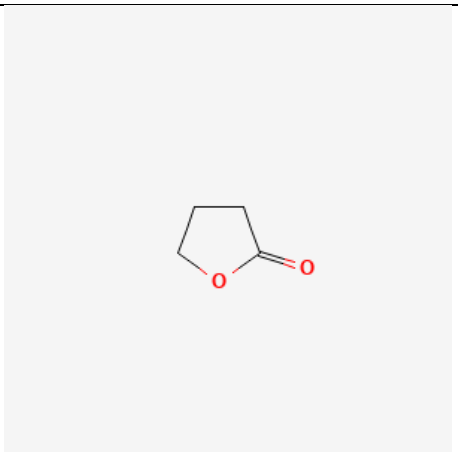
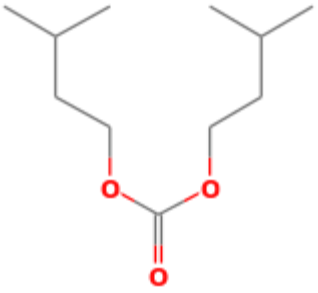
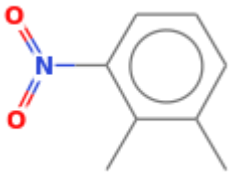
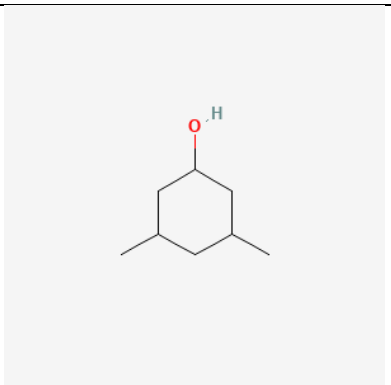
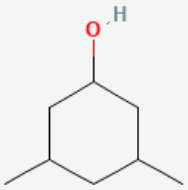
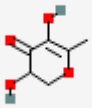
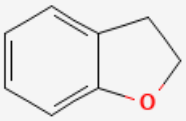
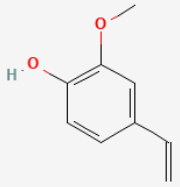
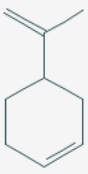
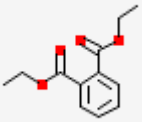
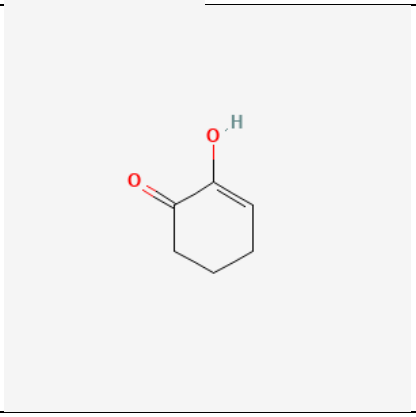
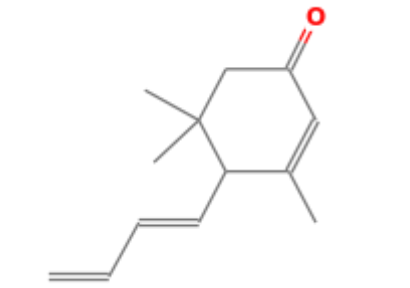
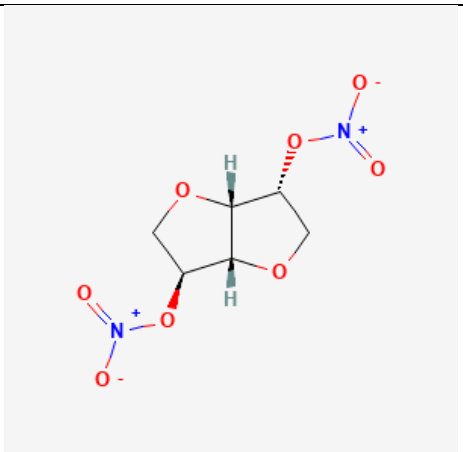
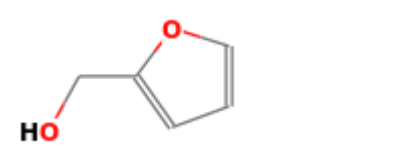
54.	34.597	Methyl stearate	$C_{19}H_{38}O_2$	298.5	

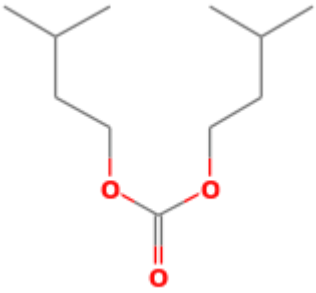
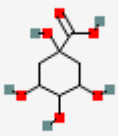
Table 4: GC–MS analysis of leaf methanolic extract of *Euphorbia hitra*

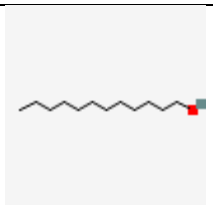
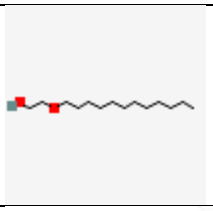
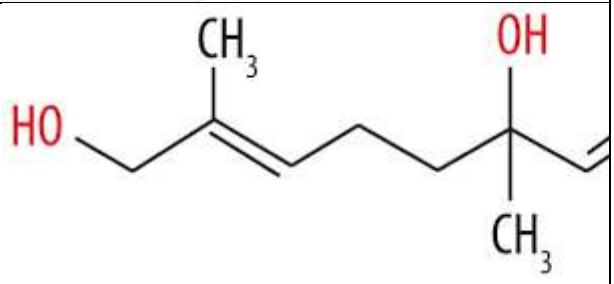
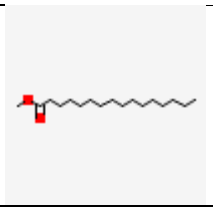
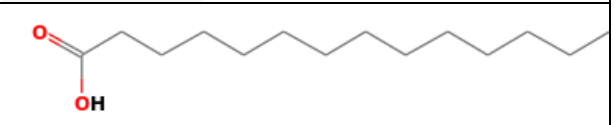
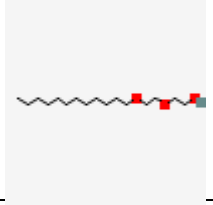
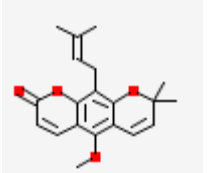
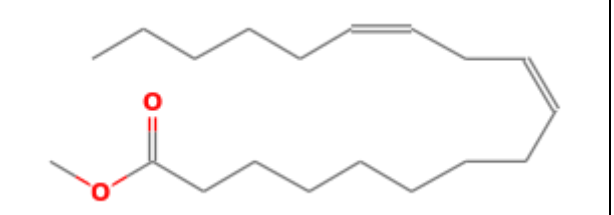
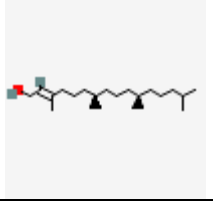
S.NO	Retention time	Name of the Compound	Molecular formula	Molecular weight *(m/z)*	Chemical structure
1.	3.529	Isosorbide Dinitrate	$C_6H_8N_2O_8$	236.14	
2.	3.576	2-FURANMETHANOL	$C_7H_{10}O_2$	126.15	
3.	3.990	2-Oxabicyclo[3.2.0]hepta-3,6-diene	C_6H_6O	94.11	
4.	4.135	dl-Glyceraldehyde dimer	$C_6H_{12}O_6$	180.16	

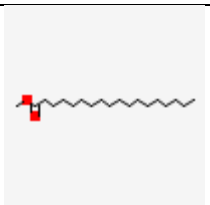
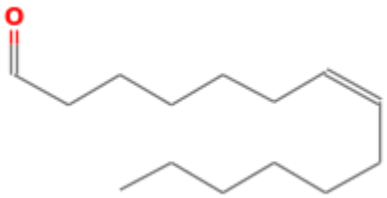
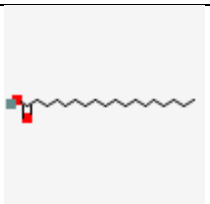
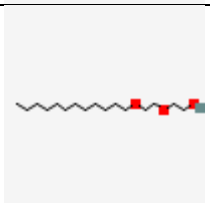
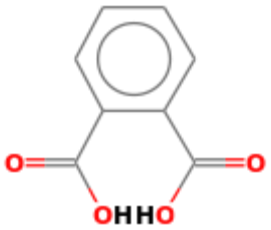
5.	4.415	Butyrolactone	$C_4H_6O_2$	86.09	
6.	4.472	1-Butanol, 3-methyl-, carbonate (2:1)	$C_{11}H_{22}O_3$	202.2906	
7.	4.573	1,2-Dimethyl-3-ethyldiaziridine	$C_8H_9NO_2$	151.1626	
8.	5.733	CYCLOHEXANOL, 3,5-DIMETHOXY	$C_8H_{16}O$	128.21	
9.	5.997	1,3,5-Triazine-2,4,6-triamine PENTANAL	n/a	n/a	n/a

10.	6.218	2-ACETYL-2-HYDROXY-.GAMMA.-	$C_6H_8O_3$	128.13	
11.	6.685	4H-Pyran-4-one, 2,3-dihydro-3,5-dihyd BENZENE-1,2-DIOL	$C_6H_8O_4$		
12.	7.220	2,3-DIHYDRO-BENZOFURAN	C_8H_8O	120.15	
13.	8.435	2-METHOXY-4-VINYLPHENOL	$C_9H_{10}O_2$	150.17	
14.	8.563	(S)-(-)-4-Isopropenyl-1-cyclohexene-1-c 1-CHLORODODECANE	C_9H_{14} $C_{12}H_{25}Cl$	122.21 204.78	

					
15.	9.710	Cyclopropane, nonyl- Megastigmatrienone Diethyl Phthalate	$C_{12}H_{14}O_4$		
16.	9.969	2-CYCLOHEXEN-1-ONE, 2-(HYDRO	$C_6H_8O_2$	112.13	
17.	11.730	Megastigmatrienone	$C_{13}H_{18}O$	190.28	
18.	12.115	Isosorbide Dinitrate	$C_6H_8N_2O_8$	236.14	
19.	14.493	2-FURANMETHANOL	$C_7H_{10}O_2$	126.15	

20.	17.838	2-Oxabicyclo[3.2.0]hepta-3,6-diene dl-Glyceraldehyde dimer Butyrolactone	C_6H_6O $C_6H_{12}O_6$	94.11 180	
21.	18.427	1-Butanol, 3-methyl-, carbonate (2:1) 1,2-Dimethyl-3-ethyldiaziridine	$C_{11}H_{22}O_3$	202.29	
22.	18.543	3-CYCLOPENTEN-1-OL	C_5H_8O	84.12	
23.	20.989	Phenol	C_6H_6O	94.11	
24.	21.295	1,2-Cyclohexanedione 2-Cyclohexen-1-one	$C_6H_8O_2$ C_6H_8O	112.13 96.13	
25.	21.821	CYCLOHEXANOL, 3,5-DIMETHOXY	$C_8H_{16}O_3$	160.12	
26.	21.933	1,3,5-Triazine-2,4,6-triamine PENTANAL	n/a	n/a	n/a
27.	22.408	1,3,4,5-TETRAHYDROXY-CYCLOHE	$C_7H_{12}O_6$	192.12	

28.	22.875	1-Dodecanol	$C_{12}H_{26}O$	186.33	
29.	23.561	Ethanol, 2-(dodecyloxy)-	$C_{14}H_{30}O_2$	230.39	
30.	24.791	PLUCHIDIOL	$C_{13}H_{20}O_2$	208	
31.	26.666	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	
32.	27.171	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	
33.	27.561	Diethylene glycol monododecyl ether	$C_{16}H_{34}O_3$	274.44	
34.	27.753	2H,8H-BENZO[1,2-B:3,4-B']DIPYRA	$C_{21}H_{20}O_6$	368.38	
35.	28.789	9,12-Octadecadienoic acid (Z,Z)-, meth	$C_{18}H_{32}O_2$	280	
36.	29.011	Phytol	$C_{20}H_{40}O$	296	

37.	29.159	Methyl stearate	$C_{19}H_{38}O_2$	298.5	
38.	29.330	7-Tetradecenal, (Z)-	$C_{14}H_{26}O$	210.35	
39.	29.579	Octadecanoic acid	$C_{18}H_{35}O_2$	284.48	
40.	29.958	Diethylene glycol monododecyl ether	$C_{16}H_{34}O_3$	274.44	
41.	33.202	1,2-BENZENEDICARBOXYLIC ACID	$C_8H_6O_4$	166.14	

Conclusion:

The study highlights the significant abundance of phytochemicals in *Aegle marmelos* and *Euphorbia hitra*, as demonstrated by the wide range of compounds found using qualitative, quantitative, and GC-MS studies. Both plants displayed substantial quantities of alkaloids, flavonoids, saponins, tannins, steroids, coumarins, and diterpenes, along with noteworthy overall polyphenol and flavonoid levels. *Aegle marmelos* and *Euphorbia hitra* exhibited polyphenol concentrations of 73.7 and 133.53, respectively, and flavonoid values of 73.2 and 85.5. The GC-MS study identified 54 different compounds in *Aegle marmelos* and 41 in *Euphorbia hitra*, mainly consisting of fatty acids and their derivatives. This finding emphasises the potential therapeutic and pharmacological uses of these substances.

Although these findings show promise, GC-MS technology has limitations when it comes to phytochemical profiling. Its main purpose is to detect and analyse substances that easily evaporate or partially evaporate, possibly disregarding those that do not evaporate easily. Moreover, the intricate combination of plant extracts might result in co-elution, which makes compound identification more challenging. Hence, it is imperative to employ complementary analytical techniques in order to obtain a thorough phytochemical profile.

In order to gain a deeper understanding of the pharmacological potential and therapeutic

effectiveness of these plants, future study should combine modern chromatographic and spectroscopic techniques with in vitro and in vivo studies. These investigations have the potential to facilitate the development of organic remedies for a wide range of human and animal illnesses.

Declaration

The authors declare no conflict of interest

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