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Original Article

Hydrodistillation and Microwave-assisted hydrodistillation Extraction of Essential Oil from *Ageratum conyzoides* L. Aerial Parts: Comparative Study

Manar A. Mohamed[1], Mostafa M. Abazied[1,*], Abou El-Hamd H. Mohamed[1], Hussein A. El-Damarany[1]

[1] **Chemistry Department, Faculty of Science, Aswan University, Aswan 81528, Egypt.**

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Abstract

Limited essential oil (EO) investigation has been conducted on the genus *Ageratum*. In particular, the annual herb *Ageratum conyzoides* L., which grows throughout equatorial regions of South America, Asia, and West Africa, is well-known for its many chemical components as well as its traditional medicinal applications. The EO yields form two extraction methods one is conventional, and the other is modern method which is referred to as a green method. Both extraction techniques were successful in obtaining phytochemicals from this species, as evidenced by the discovery of 79 and 42 components in the two methods by GC/MS analysis. Results highlight *Ageratum conyzoides* L. conventional medicine's use and its EO potentiality as a source of bioactive compounds.

Hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD) were the two primary techniques used to extract the EO of Egyptian *Ageratum conyzoides* L. aerial parts. GC/MS was used for the investigation, so as to compare the outcomes of the HD and MAHD methods, the chemical composition of the EO was assessed.

Keywords: *Ageratum conyzoides* L.; GC/MS; Hydrodistillation; Microwave-assisted hydrodistillation.

Corresponding author*: E-mail address: mostafa.abazied@aswu.edu.eg

1. Introduction

The *Ageratum* genus includes approximately 30 species, with only a handful of them having been subject to phytochemical studies (Gbotto et al., 2015). *A. Conyzoides,* a tropical plant native to South America, Asia, and West Africa, is an annual herb that has been traditionally used for medicinal purposes across various countries (Kissmann & Groth, 1991), particularly in equatorial regions (Babushok et al., 2011; Dubey et al., 1989; Jaya et al., 2014). This particular species has been found to contain a diverse array of chemical compounds (Cragg & Newman, 2013). Research indicates that the EO obtained through steam distillation from this plant is rich in bioactive compounds (Okunade, 2002). Furthermore, *A. Conyzoides* has been identified for its efficacy as a weedicide and nematicide (Cruz et al., 2013; Moura et al., 2005; Shirwaikar et al., 2003).

2. Methods and Materials

2.1. Materials of Plant

In 2023, the aerial components of *A. conyzoides* were gathered from the arid area surrounding Sahari campus of Aswan University. A voucher specimen (Mohamed 18), identified by Dr. M. Gaber and is deposited in the Herbarium of the Botany Department, Aswan-Faculty of Science, Aswan University, Aswan, Egypt.

2.2. Hydrodistillation (HD)

300 g of *A. conyzoides* was used in HD to extract the EO. Utilizing a Clevenger-style device in compliance with the methods, for three hours (Ekundayo et al., 1988; Kováts, 1958; Tyagi et al., 2010).

2.3. Microwave-assisted hydrodistillation (MAHD)

A Clevenger-type apparatus situated outside a microwave oven was utilized to conduct HD of 300 grams of dried aerial parts in a 1 L round-bottom flask. The extraction process lasted for 60 minutes at 800 W of power (Xu et al., 2023). The temperature maintained during the process was set at 100°C. Following the collection of the EO, its volume was measured using a micropipette (Hammouda et al., 2014; Kasturi & Manithomas, 1967; Vasantha-Srinivasan et al., 2024). Subsequently, the EO was dehydrated using anhydrous sodium sulfate and then stored in a refrigerator for later analysis (Vigil De Mello et al., 2016). The percentage yield of the extraction was then determined (Babushok et al., 2011).

3. Results and Discussion

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The percentage yields of the EO obtained by both methods, HD and MAHD, were (0.4 %) (v/w) and (0.45 %) (v/w), respectively. 42 components were found in the microwave extracted oil, which made up 100.002 percent of its composition alone, and 79 components were found in the HD oil, which made up 99.814 percent of the total oil composition (Fig. 1, 2) display chromatograms of HD and MAHD were used to extract the EO of *A. conyzoides*. (Hammouda et al., 2014).

Table (1): Results comparative study between both HD and MAHD techniques

No.	Name compounds	HD	MV
1	(1*R*,2*S*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl acetate	0.95%	0.64%
2	Caryophyllene	2.49%	0.35%
3	Eicosane	0.20%	0.26%
4	Diisooctyl Phthalate	0.27%	1.09%

In terms of yield and distillation times, MAHD is quicker (60 min) than the other method. However, HD takes three hours as water having a high dielectric constant absorbs microwave radiation, which raises the temperature faster than in MAHD (Xu et al., 2023). Microwave irradiation did not significantly alter the volatile oil composition, but it did speed up the extraction process (Arya et al., 2011).

It is intriguing to observe the distillation process time and efficacy (Eissa & Abdelmoneim, 2023). The oil yield obtained with MAHD was higher than that obtained with HD after 180 minutes (0.4 % and 0.45 % v/w), respectively. Therefore, MAHD is suggested as an eco-friendly extraction method (Eissa et al., 2024; Kosar et al., 2005).

Fig. (1): Photo shape of a chromatogram of *A. conyzoides* EO extracted by MAHD.

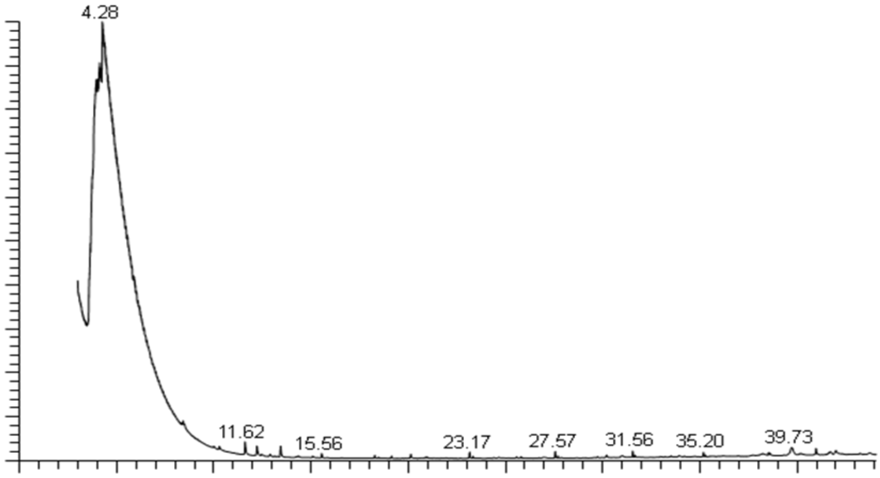

Table (2): Major Identified components in *A. conyzoides* EO, extracted using MAHD and their relative percentages.

pk	RT	Area %	Compound Name	Molecular Weight	Molecular Formula
1	3.64	4.97	*trans*- 2-ethyl-3-methyl-Oxirane	154	$C_5H_8Cl_2O$
2	3.73	14.36	3-methylpentane	86	C_6H_{14}
3	3.86	30.92	3-methyldihydrofuran-2(3H)-one	100	$C_5H_8O_2$
4	4.29	13.39	*n*-Hexane	86	C_6H_{14}
5	5.91	1.51	4-Methyl-1,3-oxazine-2,6[3H]-Dione	127	$C_5H_5NO_3$
6	6.18	1.10	3-Pentanone	86	$C_5H_{10}O$
7	8.43	1.51	Eucalyptol (1,8-Cineole)	154	$C_{10}H_{18}O$
8	11.62	2.06	1,7,7-Trimethylbicyclo [2.2.1] Heptan-2-One	152	$C_{10}H_{16}O$
9	12.22	1.67	(*E*)-2,4,4,7-tetramethylocta-5,7-dien-3-Ol	182	$C_{12}H_{22}O$
10	13.44	1.73	2,6,6-trimethylcyclohepta-2,4-dien-1-One	150	$C_{10}H_{14}O$

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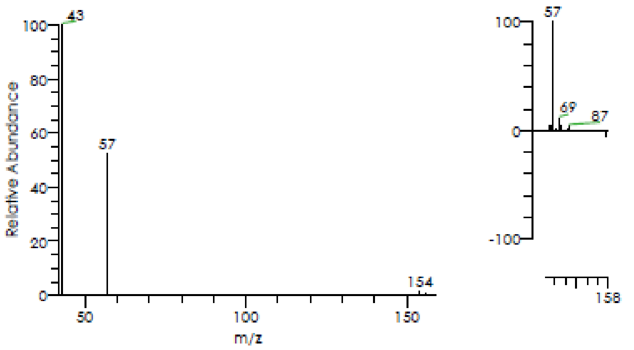
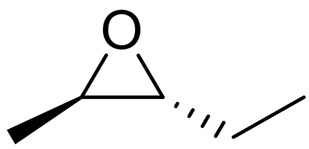
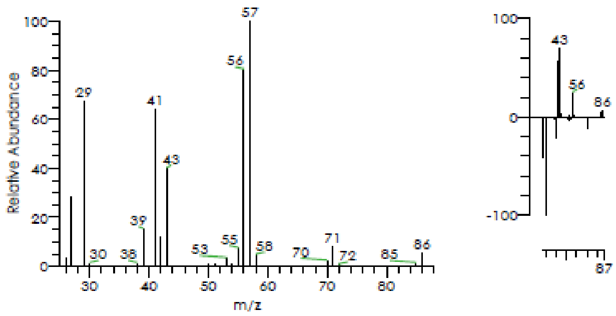
MASS SPECTRUM OF PEAKS FOR DOMINANT COMPONENTS IN A. CONYZOIDES ESSENTIAL OIL, EXTRACTED USING MICROWAVE-ASSISTED HYDRODISTILLATION (MAHD).

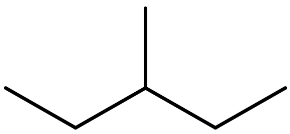
1 *trans*- 2-ethyl-3-methyl-Oxirane.



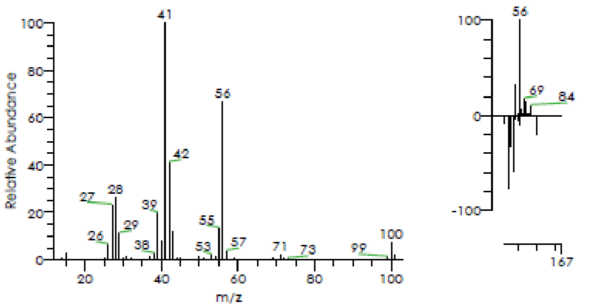


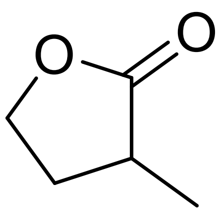
2 3-methylpentane.





3 3-methyldihydrofuran-2(3H)-one





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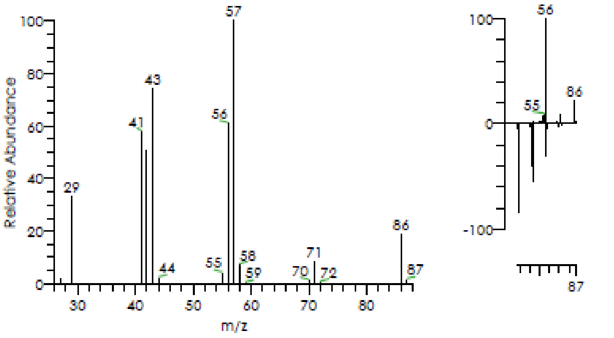
<tr><td></td><td>
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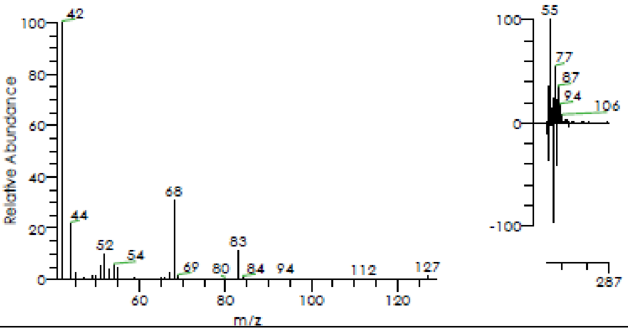
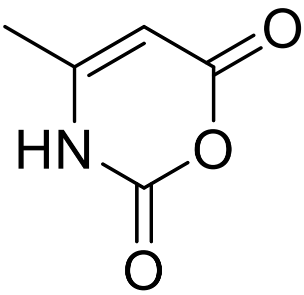
<tr><td>5</td><td colspan="2">Methyl-1,3-oxazine-2,6[3H]-Dione.</td></tr>
<tr><td></td><td>
</td><td>
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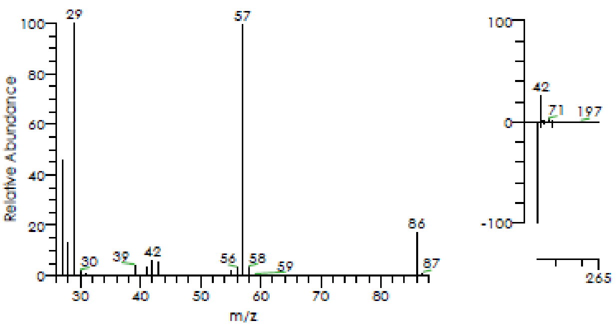
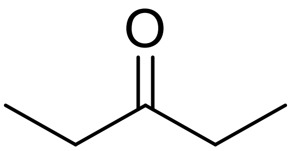
<tr><td></td><td>
</td><td>
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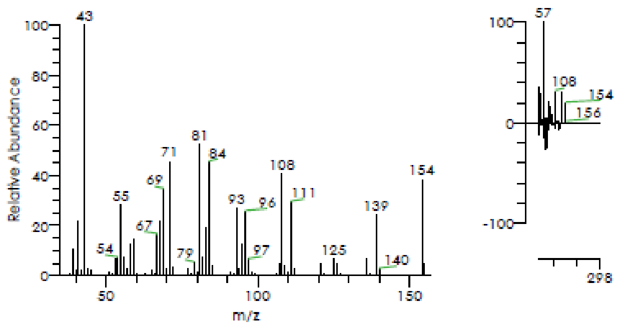
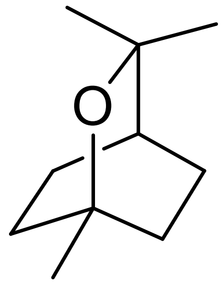
<tr><td></td><td>
</td><td>
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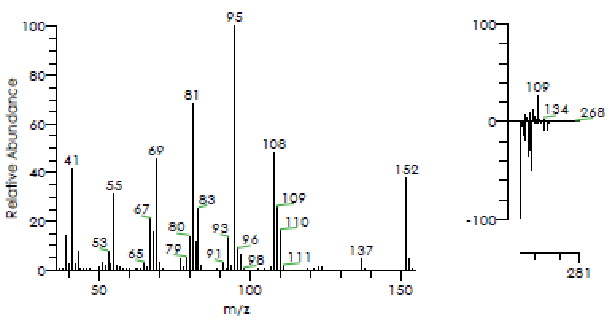
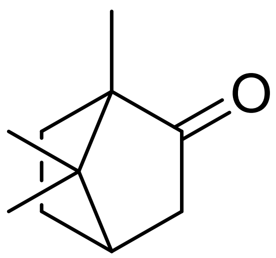
<tr><td>8</td><td colspan="2">1,7,7-Trimethylbicyclo [2.2.1] Heptan-2-one.</td></tr>
<tr><td></td><td>
</td><td>
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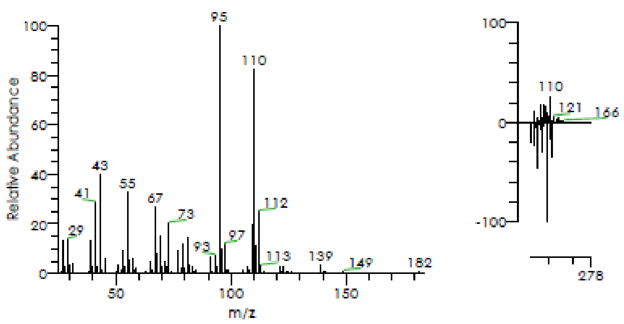
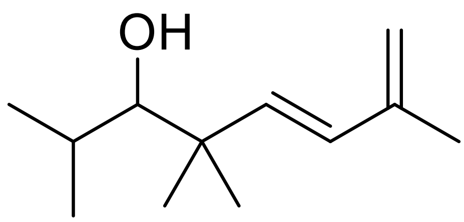
<tr><td>9</td><td colspan="2">(E)-2,4,4,7-tetramethylocta-5,7-dien-3-Ol</td></tr>
<tr><td></td><td>
</td><td>
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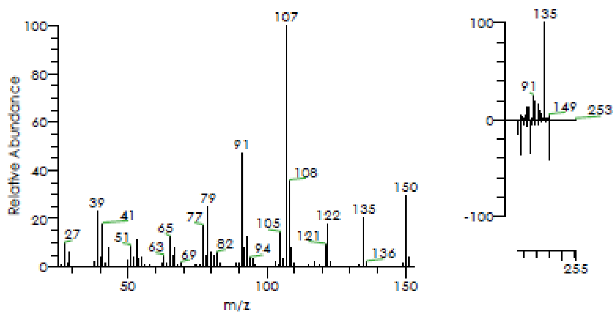
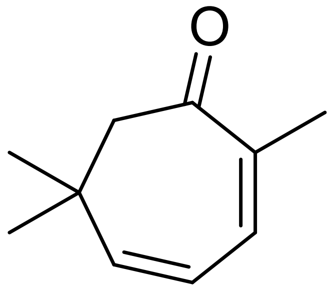
10	2,6,6-trimethylcycloheptа-2,4-dien-1-One	
		

Fig. (2): Photo shape of a chromatogram of *A. conyzoides* essential oil extracted by Hydro-Distillation.

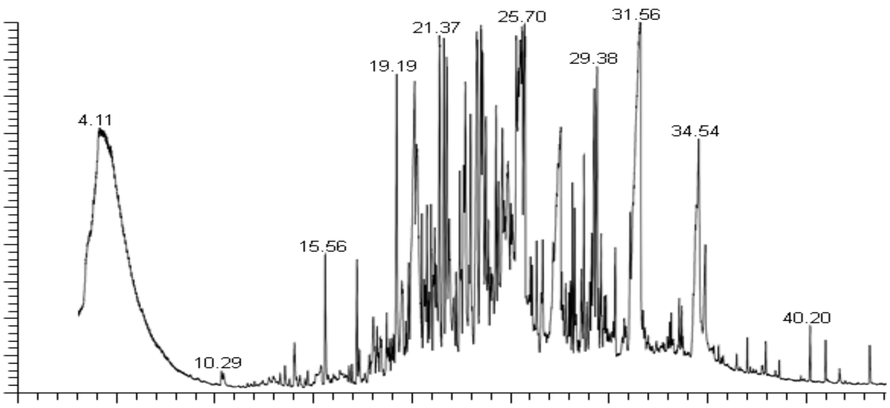


Table (3): Major Identified components in *A. conyzoides* EO, extracted using HD.

pk	RT	Area %	Compound Name	Molecular Weight	Molecular Formula
1	19.19	2.49	Caryophyllene	204	$C_{15}H_{24}$
2	19.45	1.04	4a,8-dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene	204	$C_{15}H_{24}$

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3	20.10	1.52	6-(Tert-Butyl)-2(1H)-Pyridone	151	$C_9H_{13}NO$
4	20.23	1.28	2,3-Dihydro-2,2-Dimethyl-4-Methoxy-1H-Inden-1-One	190	$C_{12}H_{14}O_2$
5	20.93	1.18	4-Methyl-2-(2-Methyl-2-Propenyl)-4-(2-Propynyl)- 2,5-Cyclohexadien-1-One	200	$C_{14}H_{16}O$
6	21.11	1.04	(-)-3,7,7-Trimethyl-11-methylenespiro [5.5] undec-2-ene	204	$C_{15}H_{24}$
7	21.38	2.61	(*R*)-1-Methyl-4-(1,2,2-Trimethylcyclopentyl) Benzene	202	$C_{15}H_{22}$
8	21.59	2.64	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulen-4-ol	222	$C_{15}H_{26}O$
9	21.73	2.45	2,3,4,6,7,8-hexahydro-2,2-dimethyl-5H-1-Benzopyran-5-one	180	$C_{11}H_{16}O_2$
10	22.40	1.68	Epoxy-α-terpenyl acetate	212	$C_{12}H_{20}O_3$
11	22.68	1.98	3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol	222	$C_{15}H_{26}O$
12	22.94	2.40	(3e)-3-Hexenyl Phenylacetate	218	$C_{14}H_{18}O_2$
13	23.27	4.00	Caryophyllene oxide	220	$C_{15}H_{24}O$
14	23.57	2.42	2-Methyl-4-Isobutylpyrimidine	150	$C_9H_{14}N_2$
15	23.72	1.17	*α*-Methyl-Cyclo-Hexanemethanol	142	$C_9H_{18}O$
16	24.24	1.44	1-(cyclohexylmethyl)-4-(1-methylethyl) Cyclohexane	222	$C_{16}H_{30}$
17	24.55	1.41	(1a*R*,3a*S*,7b*S*)-1,1,3a,7-Tetramethyl-1a,2,3,3a,4,5,6,7b-octahydro-1H-cyclopropa[*a*]naphthalene	204	$C_{15}H_{24}$
18	24.85	1.22	10-Methyl-E-11-tridece-1-ol acetate	254	$C_{16}H_{30}O_2$

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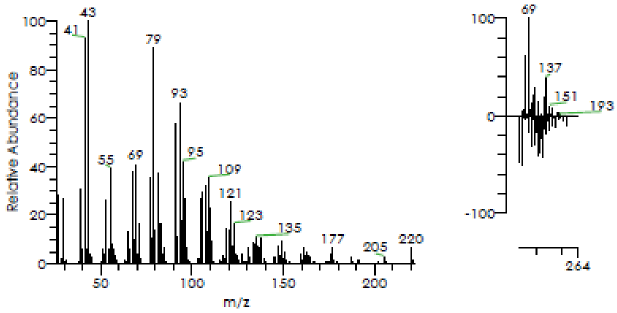
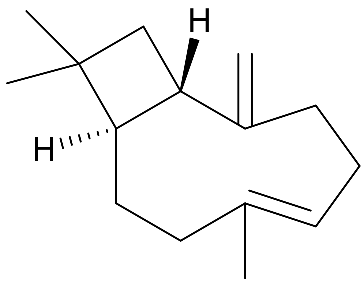
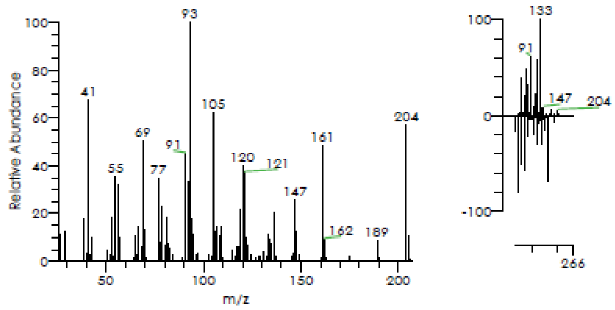
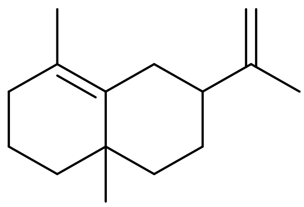
19	25.27	1.84	2-Methyl-4'-Methoxybiphenyl	198	$C_{14}H_{14}O$
20	25.38	1.56	1-(4-Phenylcyclohexyl)-1-Ethanone	202	$C_{14}H_{18}O$
21	25.46	2.22	1,2,3,3a,4,5,6,7-octahydro-*α*, *α*,3,8-tetramethyl- [3S-(3*α*,3a*α*,5*α*)]-5-Azulenemethanol	222	$C_{15}H_{26}O$
22	25.56	1.57	*Trans* -3,5-Dimethyl-1,6-octadiene	138	$C_{10}H_{18}$
23	25.70	2.26	2-Tridecanone	198	$C_{13}H_{26}O$
24	26.61	1.02	3-Isopropyl-6,7dimethyltricyclo [4.4.0.0(2,8)] decane-9,10-diol	238	$C_{15}H_{26}O_2$
25	27.52	3.87	Tetradecanoic acid	228	$C_{14}H_{28}O_2$
26	28.24	1.11	*α*-humulene	204	$C_{15}H_{24}$
27	28.71	1.20	6,10,14-trimethyl-2-Pentadecanone	268	$C_{18}H_{36}O$
28	29.23	2.62	Pentadecanoic Acid	242	$C_{15}H_{30}O_2$
29	31.57	9.88	*n*-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
30	34.54	3.34	(8)-carbonic acid-(1) Heptadecene	282	$C_{18}H_{34}O_2$

MASS SPECTRUM OF PEAKS FOR COMPONENTS IN *A. CONYZOIDES* ESSENTIAL OIL, EXTRACTED USING HYDRO-DISTILLATION (HD)

1	Caryophyllene	
		
2	4a,8-dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene	
		
3	6-(Tert-Butyl)-2(1H)-Pyridone	

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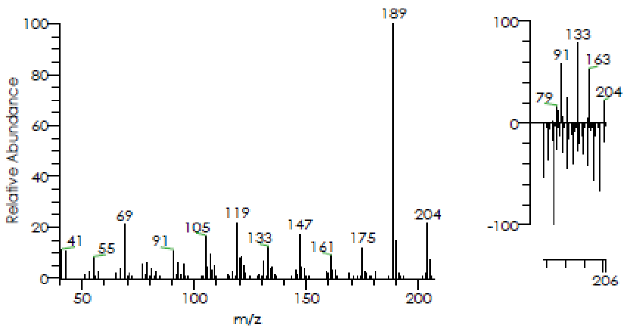
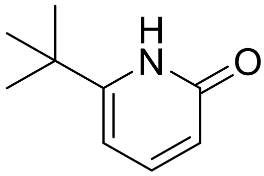
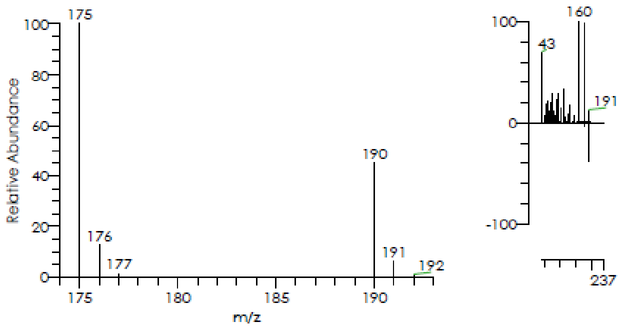
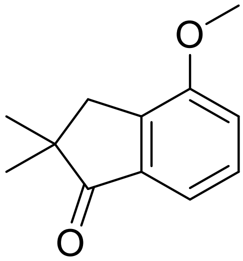
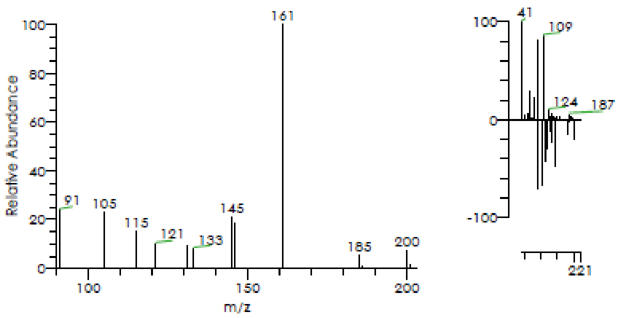
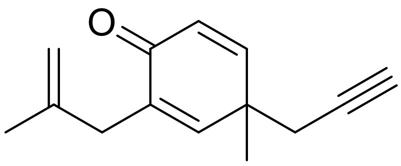
		
4	2,3-Dihydro-2,2-Dimethyl-4-Methoxy-1*H*-Inden-1-One	
		
5	4-Methyl-2-(2-Methyl-2-Propenyl)-4-(2-Propynyl)- 2,5-Cyclohexadien-1-One	
		
6	(-)-3,7,7-Trimethyl-11-methylenespiro [5.5] undec-2-ene	

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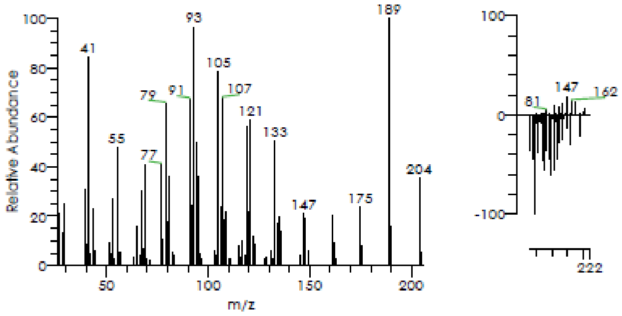
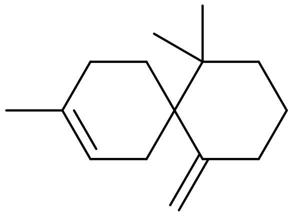
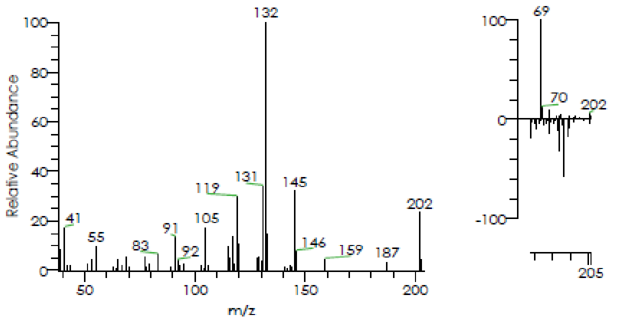
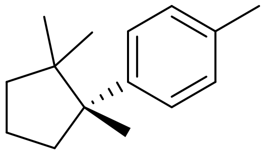
		
7	(*R*)-1-Methyl-4-(1,2,2-Trimethylcyclopentyl) Benzene	
		
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	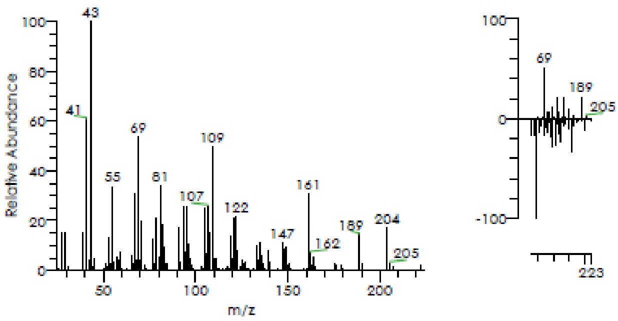	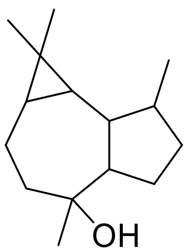
9	2,3,4,6,7,8-hexahydro-2,2-dimethyl-5H-1-Benzopyran-5-One	

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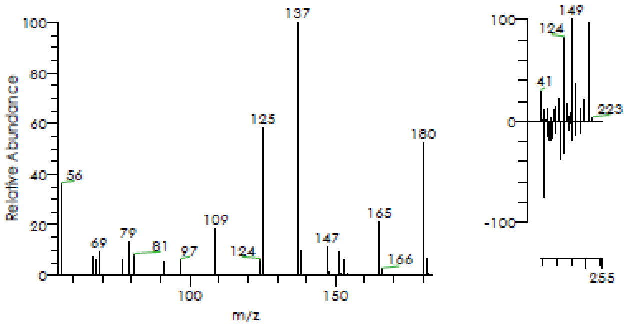
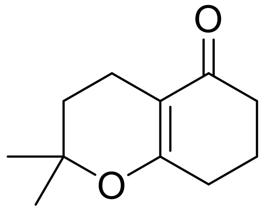
<tr><td></td><td>
</td><td>
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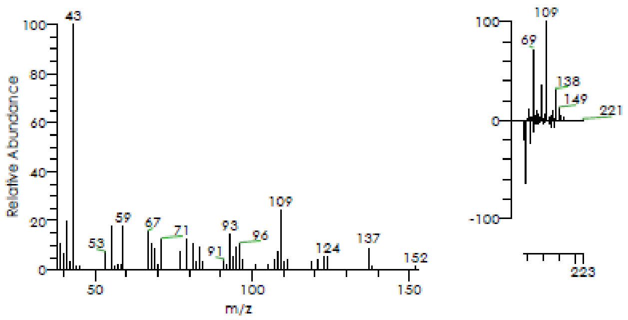
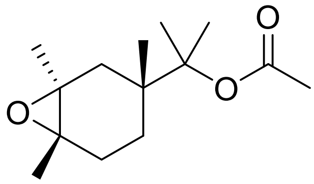
<tr><td></td><td>
</td><td>
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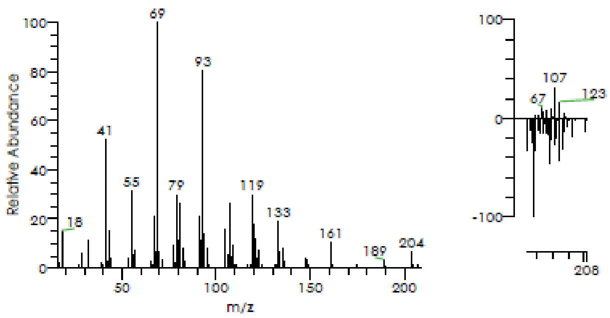
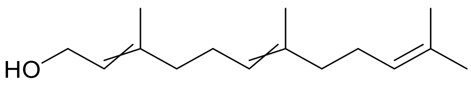
<tr><td></td><td>
</td><td>
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	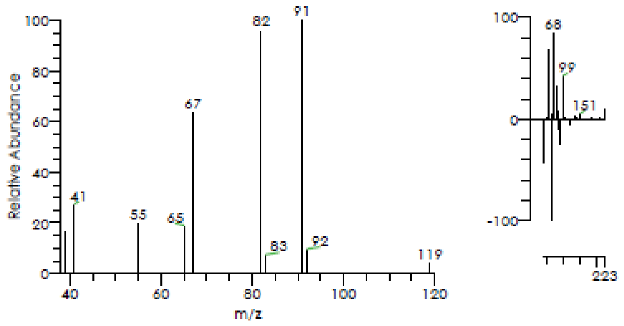	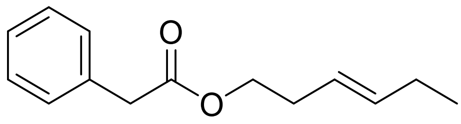
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	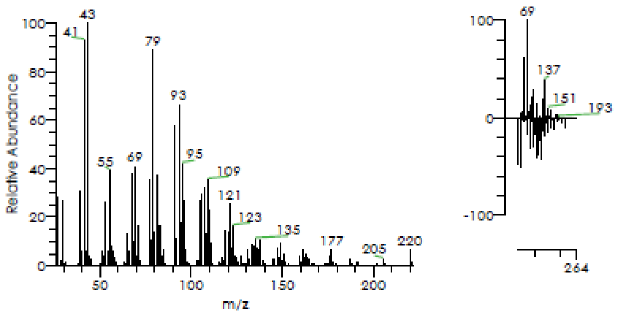	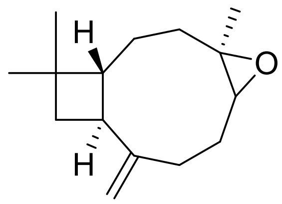
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	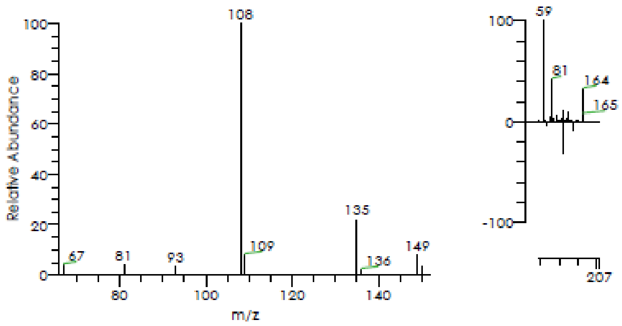	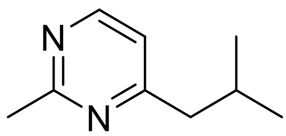
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	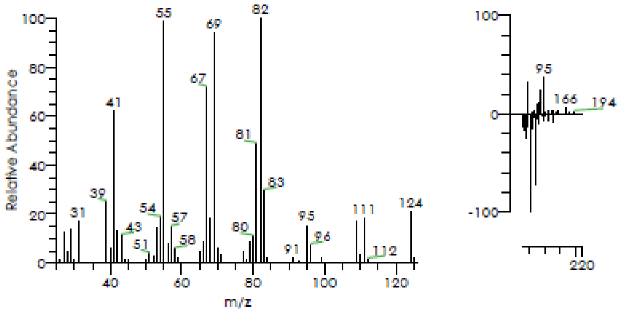	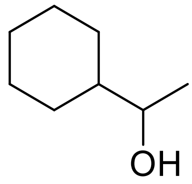
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	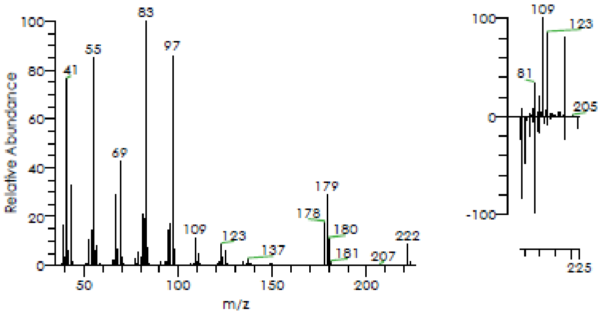	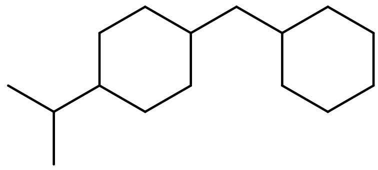
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	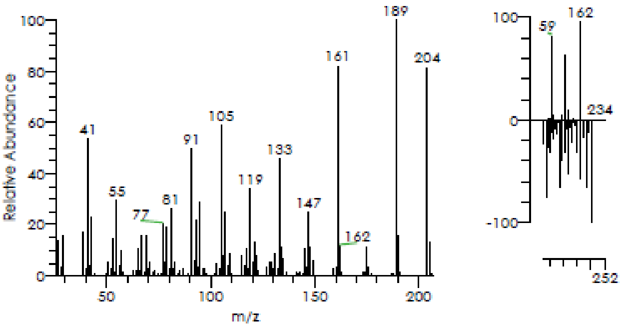	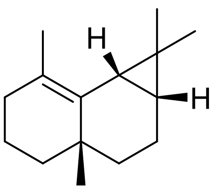

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	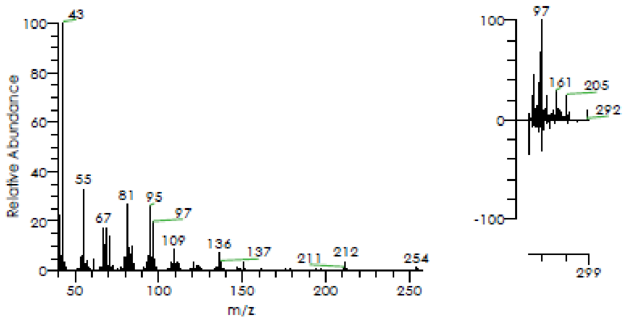	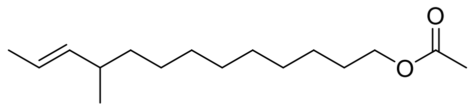
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	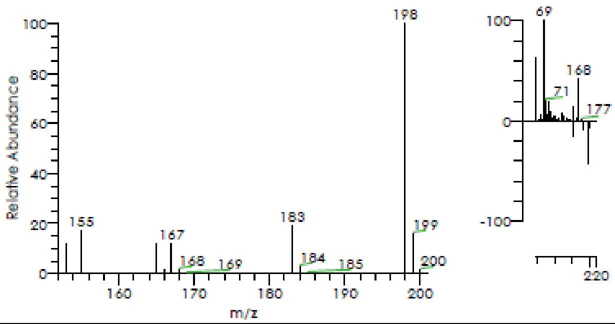	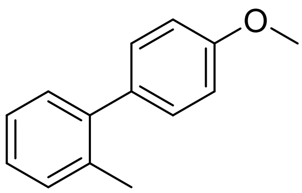
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	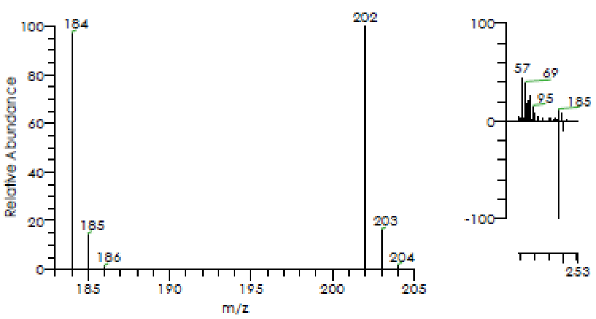	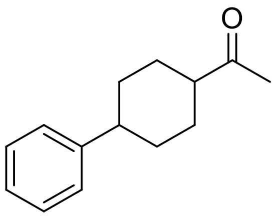

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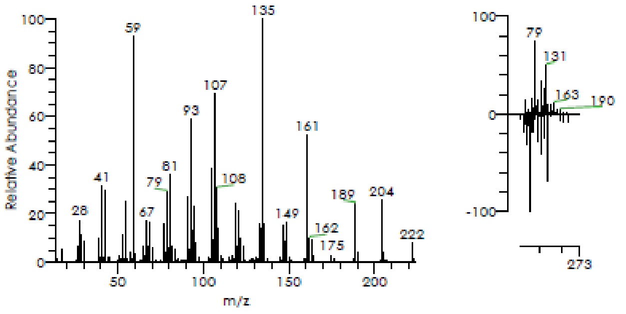

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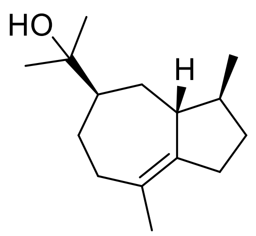

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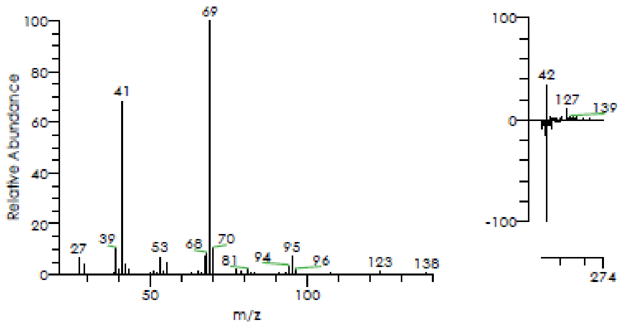

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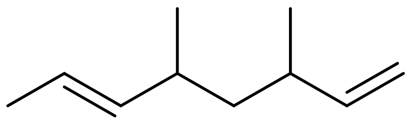
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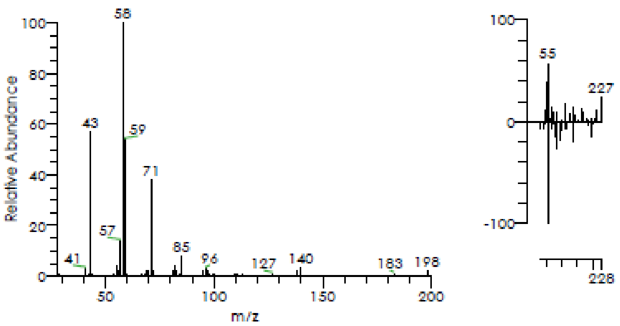

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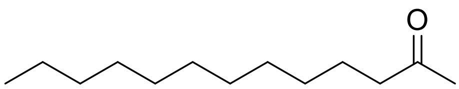

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	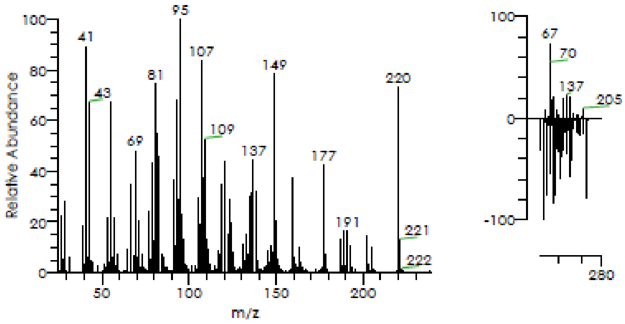	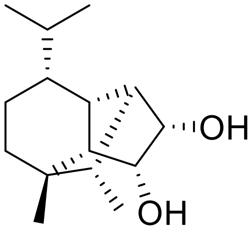
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	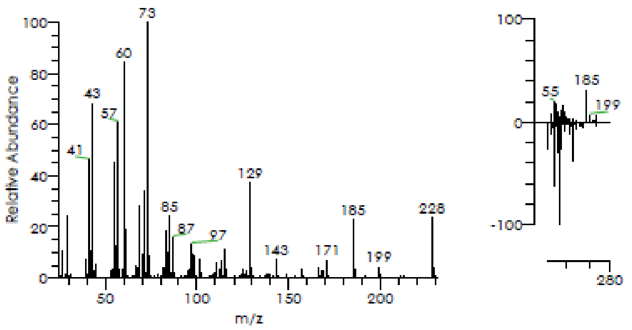	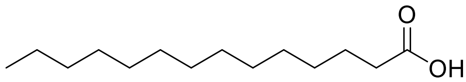
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	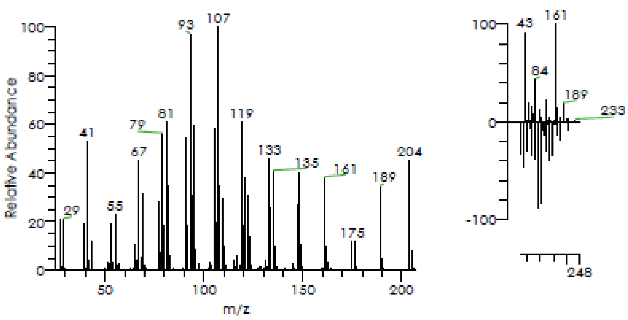	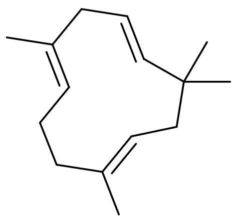

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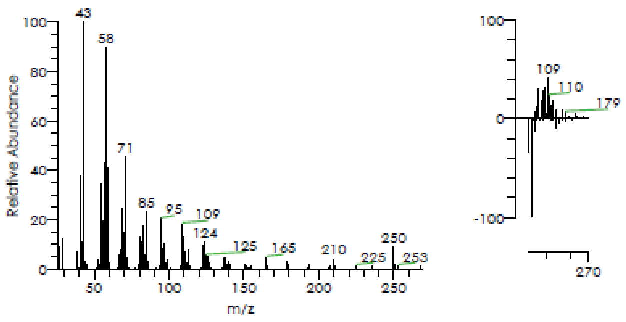
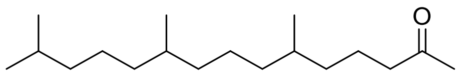
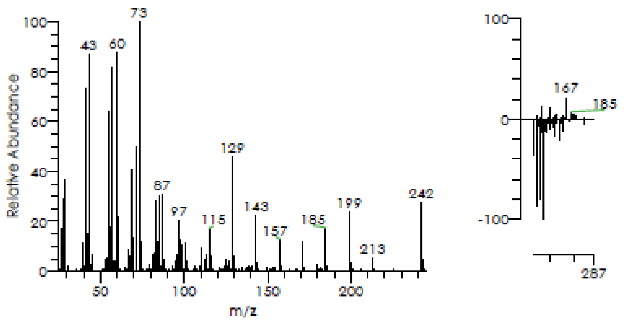
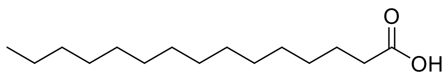
27 6,10,14-trimethyl-2-Pentadecanone



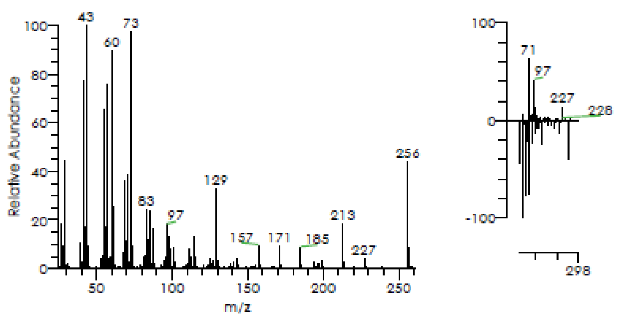


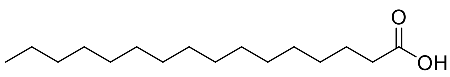
28 *Pentadecanoic Acid*





29 *n*-Hexadecanoic acid





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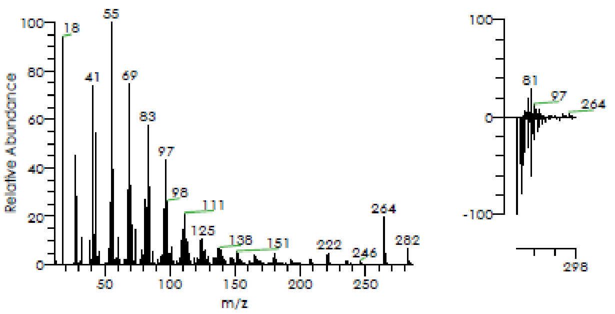
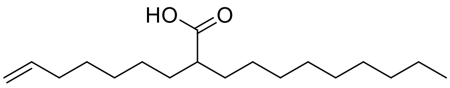
30	(8)-carbonic acid-(1) Heptadecene	
		

4. *Conclusion*

In conclusion, research on the chemical makeup of the EO from *A. conyzoides* offers valuable information on the potential medicinal benefits of the plant and the effectiveness of various extraction methods. The comparison between HD and MAHD revealed distinct advantages of each method. MAHD exhibited a faster extraction process and higher oil yield, indicating its efficiency in extracting EO. Notably, MAHD demonstrated a swifter extraction process without significant alteration in EO composition, indicating its potential as a green extraction method for EO. These findings not only contribute to the scientific understanding of *A. conyzoides* essential oil but also enhance the importance of exploring indigenous approaches in conjunction with scientific methods. Furthermore, more research is to be conducted to fully elucidate the therapeutic values of *A. conyzoides* and to optimize the extraction techniques for its essential oil. This research direction holds promise for the advancement of natural product-based pharmaceutical applications and underscores the significance of integrating traditional wisdom with contemporary scientific exploration.

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Ethics/bioethics: This research did not cause harm to any human or animal.

Declaration of Competing Interest: The authors declare no conflict of interest.

Author Contributions: The final manuscript was read, approved, and each author made an equal contribution to this work.

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