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A Comparison between Three Organic Solvents in Extracting Essential Oils from Fresh and Dry Leaves Of *Salvia Officinalis* L

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Abstract

Salvia officinalis L. is a Mediterranean species, naturalized in many countries. The composition of essential oil isolated from *Salvia officinalis* L, wild growing in Libya, was analyzed. In this study, essential oils from fresh and dry leaves of *Salvia officinalis* L, Libya, were extracted with ethanol, hexane or chloroform as solvents using Soxhlet apparatus and the extracts were analyzed using gas chromatography/mass spectrometry method. Each solvent showed differences in the extraction yields between fresh and dry leaves. Physical constants of the extracts were shown variation in color, viscosity, density, PH and percentage of production between fresh and dry leaves for each solvent. GC-MS results showed that ethanol could extract the most compounds compared to hexane and chloroform from both fresh and dry leaves. In total 41, 28 and 31 compounds were identified in the essential oils of fresh and dry leaves of *Salvia officinalis* L using ethanol, hexane or chloroform respectively. For each solvent the number of compounds extracted from dry leaves was bigger than the number extracted from fresh leaves.

Keywords: *Salvia Officinalis*.L Essential oils, ethanol, hexane, chloroform, soxhlet, and gas chromatography/mass spectrometric (GC-MS).

1. Introduction

Essential oils are aromatic oily liquids extracted from plants. The most usual purpose of Essential oils is the use in food, perfumes and pharmaceuticals [1]. Salvia is one of the most widespread member of the Lamiaceae family. It features prominently in the pharmacopoeias of many rural areas throughout the world from the Far East, through Europe and other different places and several of the almost thousand Salvia species have been utilized in many ways [2]. The chemical composition of Salvia officinal L varies widely [3]-[4]. The first dominant constituents in many sage essential oils are cis-thujone, 1,8-cineole, camphor, trans-thujone, α-humulene and linalool. Germacrene D as the first major constituent was found entirely in one sage oil sample from Cuba [5]. Viridiflorol dominated in the wild plant essential oils [6]-[7]. The latter compound and manool were the major constituents in one sample of the essential oil of *Salvia. officinalis* L growing in Cuba [5]. The sage essential oils rich in viridiflorol and the manool were found alone in the last decade [3]-[7]-[8] and the information about the healing power of these oils was not set up. Some sage oils were rich in α-pinene, limonene and borneol [3]-[4].

Leaf senescence is a highly regulated physiological process that leads to nutrient remobilization during stress, therefore leaving the rest of the plant to benefit from the nutrients accumulated during the lifetime span of the leaf [9]. Leaf in *Salvia officinalis* L grown under Mediterranean field conditions, with an emphasis on the potential participation of the phytohormones, salicylic acid and jasmonic acid in the process. The initial points of leaf senescence (0–27 days of water deficit) were characterized by salicylic acid accumulation by 80% and decrease of jasmonic acid levels by 40%, which occurred in parallel with a severe loss of photosynthetic pigments up to 65%. The later levels of leaf senescence (until 42 days of water deficit) were instead characterized by maintenance of the levels of jasmonic acid and salicylic acid [9]. The aims of this study were to extract the essential oils from dry and fresh leaves of Salvia officinal L using Soxhlet apparatus with three different organic solvents and to learn the different pieces of the extracted oils.

2. Material and Methods

Preparation of plant extracts

Fresh leaves of *Salvia officinalis L* were collected in March during the flowering stage from Airport Road area, Tripoli, Libya. The collected plant leaves were divided into two halves, one was used fresh as collected and the other was washed gently with water, drained as much as possible and finally fine spread over a wide filter paper and left in the shade at room temperature for a week.

Soxhlet Extraction

The extraction experiments were carried out in Oil Research Center (Tripoli, Libya). Twenty grams of the fresh/dry leaves of *Salvia officinalis L* were extracted using three different organic solvents (ethanol 99.8%, hexane 99.5% and chloroform 99%: 250 mL from each one) in a Soxhlet apparatus for two hours. The solvents were then evaporated in a rotary evaporator and the extracts were dried until a constant weight using a mild flow of nitrogen gas.

Gas Chromatography/Mass Spectrometric (GC/MS) analysis

The analyses were carried out in the Centre for Chemical Protection in Tajoura (Tripoli, Libya). Analyses of the oils were performed using GC-MS. The analytical conditions were employed for GC-MS analysis are a HP G 1800C Series II GCD system equipped with HP-5MS column (30 m x 0.25 mm, 0.25μm film thickness). The transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV) in an m/z range of 40-400. Identification of the individual oil components was accomplished by comparison of the retention times with standard substances and by matching mass spectral data with those held in the Wiley 275 library of mass spectra. For quantitative analysis, area percent obtained by FID was used as a base. The qualitative analysis was based on the comparison of retention indexes on both columns and mass spectra with corresponding data in literature [10].

Determination of physical constants of the essential oil samples

The color of the prepared essential oils was described. Density and PH were measured in the laboratories of the faculty of education Janzour (Tripoli/Libya).

The percentage (v/w) of the prepared essential oils relative to the dry weight of the plants or Production percentage of the essential oil for different samples were calculated by following equation

Production percentage = weight of leaves (mg) / volume of essential oil (cm^3)*100.

3. Results and Discussion

Physical constants of the extraction yield

The yields of the extracted oils from dry and fresh leaves including physical constants for each sample with different solvents was described in Table 1 and Picture 1. Essential oils were extracted by different solvents (ethanol, hexane or chloroform) using soxhlet device. Thus, a modest quantity of solvent was allowed with the extracted oil, which touched on the density and hydrogen number. The samples extracted with ethanol were darker in color than the samples extracted by the other two solvents. The density values of both fresh and dry samples of each solvent are nearly equal but these values differentiate for the three solvents. The hexane samples showed the lowest density followed by ethanol and chloroform which showed the highest density. Viscosity of the samples was found high with chloroform compared to ethanol and hexane which was the lightest. The PH- values of the samples differ slightly for the three solvents. However, the percentage (v/w) of the prepared essential oils with chloroform was higher than hexane, and ethanol which was the lowest. Our results were in accordance with another publisher, who found that the essential oil obtained by hydrodistillation from dry sage leaves was transparent liquid of light yellow color, low-viscosity and specific odor [11].

Figure 1: Picture shown fresh and dry leaves Soxhlet extracts (DE: Dryethanol, FE: Fresh ethanol, DC: drychloroform, FC: freshchloroform, DH: Dry hexane and FH: Fresh hexane).

In total 100 compounds were identified, 41 from ethanol extracts (13 from fresh and 28 from dry leaves), 28 from hexane extracts (10 from fresh and 18 from dry leaves) and 31 from chloroform extracts (12 from fresh leaves and 19 from dry leaves) (Tables 2-7).

Differences were observed between the components of fresh and dry leaves of *Salvia officinalis* L when extracted with ethanol (Table 2 and 3). Five compounds (number 4, 5, 6, 7, 8, in Table 2) were common between ethanol extracts of fresh and dry leaves. A pair of them, thujone and Borneol were determined as a main compound. In a previous study, it was reported that the major constituents in Salvia officinalis. L oil are 1,8-cineole (33.27%), β-thujone (18.40%), α-thujone (13.45%), Borneol (7.39%) [12]. In another study, it was found that α-thujone was the major compound, representing about 55, 30 and 18% of the essential oils from stems, leaves, and flowers respectively from plants harvested in northern Portugal [6]. In this study, 28 compounds were detected in ethanol extract of dry leaves (Table 3). The most important active compounds in the *Salvia officinalis* L (1,8-cineole, borneol, camphor, and thujone) were extracted with ethanol from dry leaves as well as from fresh leaves and these compounds are well known as anti-inflammatory and antioxidants [13].

When hexane was used as an extraction solvent ten compounds were detected from fresh leaves extract (Table 4) and eighteen from dry leaves extract (Table 5). Three compounds (number 1, 7, 9 in Table 4) were common between dry and fresh leaves extracts. The most important compounds were borneol and sabinol which is applied as a disinfectant for its anti-inflammatory effect [14]. The results revealed from dry leaves extract were shown in Table 5. Eighteen compounds were detected, such as borneol, bornyl ester and camphor. Borneol was the only compound extracted with ethanol and hexane from fresh leaves and six compounds (number 2, 3, 8, 9, 10, 15 in Table 5) were common between ethanol and hexane from the dry leaves.

The total compounds obtained when chloroform is used as an extraction solvent are 31 compounds, 12 of them were extracted from fresh leaves (Table 6) and 19 from dry leaves (Table 7). Six compounds (number 4, 5, 6, 7, 8, 12 in Table 6) were common between chloroform extracts of fresh and dry leaves. Nine compounds (number 10 in Table 2 and 6, 7, 12, 13, 14, 19, 20, 22 in Table 3) were extracted from both ethanol and chloroform and only three compounds (number 3, 4, 8 in Table 7) were common between hexane and chloroform. Some compounds were unique for each solvent and only two compounds (number 7 and 12 in Table 3) were common between the three solvents.

Our results with the three solvents were in agreement with studies reported the presence of 1,8-cineole, camphor, β-pinene, myrcene and α-pinene in their samples of *S. triloba* growing in eastern of Libya [15] and in Egypt [16]. Another study confirmed 1,8-cineole and camphor as predominant constituents in the Turkish sage samples [17] and the same applies for S. fruticosa from Greece [18]-[19]. On the other hand, other researchers found α-thujone as a major compound in their analyses [19] -[20], which is not in contrast with our results and other available information.

The results of this study show clearly that some compounds were extracted from the dry leaves which could not be extracted from the fresh leaves. A decrease in the number of extracted compounds was observed for fresh samples for the three solvents. This may be referred to the presence of water in fresh leaves which represents close to 10% and the bonding of this water with some compounds can impede the extraction with the organic solvent. Another explanation may refer to an increase in concentration of the components and therefore the accumulation of essential oil inside dry leaves after losing water during the drying process. The results also shown the presence of some compounds only in fresh samples. Absence of these compounds in dry samples may refer to lost them with water during the drying process.

Ethanol was the most capable of extracting compounds from the leaves. This is because of the oxygen present in most compounds in the leaves and its ability of forming hydrogen bonds with ethanol and thus solubility.

4. Conclusion

In total, 100 compounds were identified in the essential oils of fresh and dry leaves of *Salvia officinalis* L using ethanol, hexane or chloroform as extraction solvents. The three solvents were different in extracting compounds from fresh and dry leaves. Ethanol extracted the highest number of compounds.

5. Acknowledgements

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Table1: Physical constants of the extraction yield ((DE: Dry leaves/ethanol, FE: Fresh leaves/ ethanol, DH: Dry leaves/ hexane, FH: Fresh leaves/ hexane, DC: Dry leaves/ chloroform and FC: Fresh leaves/chloroform).

	Ethanol		**Hexane**		**Chloroform**	
Sample	**FE**	**DE**	**FH**	**DH**	**FH**	**DH**
Color	Dark green	Light green	Light yellow	Yellowish green	Yellowish orang	Yellowish brown
Viscosity	Light	Light	Vary light	Vary light	Heavy	Heavy
Density	0.831	0.804	0.661	0.665	1.474	1.474
PH	5.25	4.50	5.85	7.13	5.40	7.20
Percentage	32.78%	23.25%	45.45%	21.27%	55.5%	27.4%

Table 2: List of compounds from GC/MS analysis present in essential oil extracted from fresh leaves using ethanol. (Highlighted compounds were detected only with ethanol)

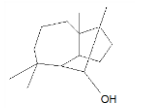
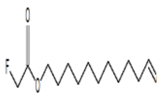
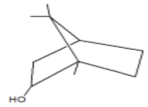
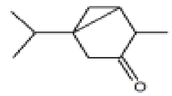
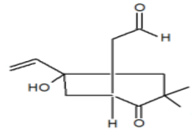
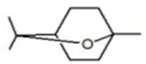
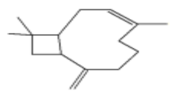
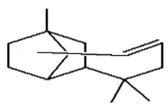
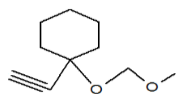
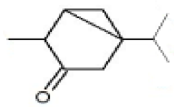
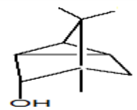
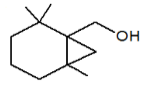
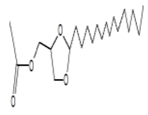
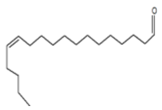
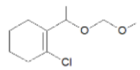
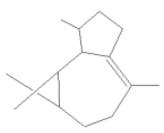
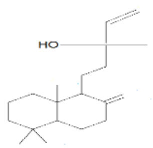
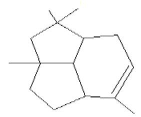
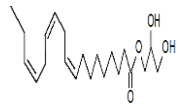
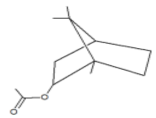
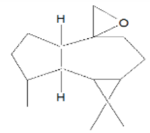
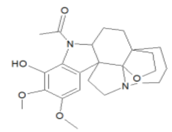
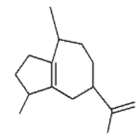
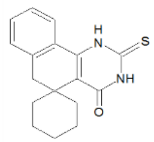
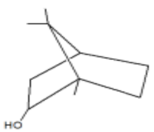
	Compound	**Chemical formula**	**M. WT**	**Chemical structure**
1	1,4-Methanozulen-9-ol,decahydro-1,5,5,8a-tetramethyl-,(1R-(1.alpha. ..	$C_{15}H_{26}O$	222	
2	11-Dodecen-1-ol monofluoroacetate	$C_{14}H_{25}FO_2$	244	
3	1-Heptadecanol	$C_{17}H_{36}O$	256	
4	Bicyclo(2.2.1)heptan-2-ol, 1,7,7-trimethyl-,(1S-endo)-& Borneol	$C_{10}H_{18}O$	154	
5	Bicyclo(2.2.1)heptan-2-one, 1,7,7-trimethyl-,(1R)-	$C_{10}H_{16}O$	152	
6	Bicyclo(3.1.0)hexan-3-one, 4-methyl-1-(1-methylethyl)-	$C_{10}H_{16}O$	152	
7	Bicyclo(3.2.0)heptan-2-one, 5-formylmthyl-6-hydroxyl-3,3-dimethyl-6-vinyl-	$C_{13}H_{18}O_3$	222	
8	Eucalyptol	$C_{10}H_{18}O$	154	
9	Humulen-(v1)	$C_{15}H_{24}$	204	
10	Longifolene-(V4)	$C_{15}H_{24}$	204	
11	Methane, ((1-ethylcyclohexyl)oxy) methoxy-	$C_{10}H_{16}O_2$	204	
12	Thujone	$C_{10}H_{16}O$	168	
13	Tricyclo(2.2.1.0(2,6))heptan-3-ol, 4,5,5-trimethyl-	$C_{10}H_{16}O$	152	

Table 3: List of compounds from **GC/MS analysis** present in essential oil extracted from dry leaves using ethanol. (Highlighted compounds were detected only with ethanol)

	Compound	**Chemical formula**	**M. WT**	**Chemical structure**
1	(2,2,6-Trimethyl-bicyclo(4.1.0)hept-1-yl)-methanol& Myrtinol	$C_{11}H_{20}O$	168	
2	1,3-Dioxolane-4-methanol, 2-pentadecyl-,acetate	$C_{21}H_{40}O_4$	365	
3	13-Octadecenal, (Z)-	$C_{18}H_{34}O$	266	
4	1-Chloro-2-(1-methoxy-ethyl)-cyclohexene	$C_{10}H_{17}ClO_2$	204	
5	1H-Cycloprop(e)azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-,(1aR-(1a.alpha.,7.alpha.,7a.beta.,7b.alpha.))-	$C_{15}H_{24}$	204	
6	1-Naphthalenepropanol, .alpha.-ethenyldecahydro-.alpha.,5,8a-tetramethyl-2-methylene-, (1S-(1.alpha.(S*),4a.beta.,8a.alpha.))-	$C_{20}H_{34}O$	290	
7	1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo(5.3.1.0(4,11))undec-8-ene	$C_{15}H_{24}$	204	
8	7-Tetradecene	$C_{14}H_{28}$	196	
9	8-Hexadecyne	$C_{16}H_{30}$	222	
10	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester,(Z,Z,Z)-	$C_{21}H_{36}O_4$	352	
11	Acetic acid,1,7,7 –trimethyl –bicyclo (2.2.1) hept-2-yl ester & Bornyl acetate	$C_{12}H_{20}O_2$	196	
12	Alloaromadendrene oxide-(1)	$C_{15}H_{24}O$	220	
13	Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy-	$C_{23}H_{30}N_2O_5$	414	
14	Azulene,1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethyl)-,(1S-(1.alpha.,4.alpha.,7.alpha.))-	$C_{15}H_{24}$	204	
15	Benzo(h)quinazolin-4(1H)-one,2,3,5,6-tetrahydro-5-spirocyclohexane-2-thioxo-	$C_{17}H_{18}N_2OS$	298	
16	Bicyclo(2.2.1)heptan-2-ol, 1,7,7-trimethyl-,(1S-endo)- & Borneol	$C_{10}H_{18}O$	154	
17	Bicyclo(2.2.1)heptan-2-one, 1,7,7-trimethyl-,(1R)-	$C_{10}H_{16}O$	152	

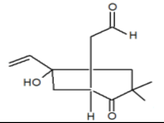
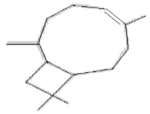
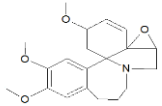
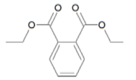
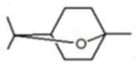
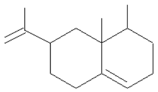
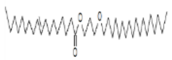
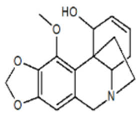
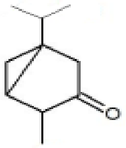
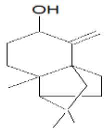
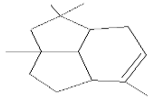
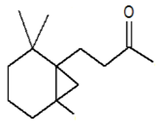
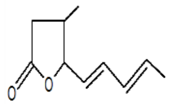
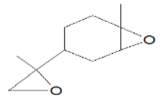
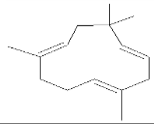
18	Bicyclo(3.2.0)heptan-2-one,5-formylme thy l-6-hydroxyl-3,3-dimethyl-6-vinyl-	$C_{13}H_{18}O_3$	222	
19	Caryophyllene	$C_{15}H_{24}$	204	
20	Caryophyllene oxaide	$C_{15}H_{24}O$	220	
21	C-Homoerythrinan,1,2-didehydro-6,7-epoxy-3,15,16-trimethoxy-,(3.beta.,6.xi.)	$C_{20}H_{25}NO_4$	343	
22	Diethyl phthalate	$C_{12}H_{14}O_4$	222	
23	Eucalyptol	$C_{10}H_{18}O$	154	
24	Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-,(1R-(1.alpha.,7.beta.,8a.alpha.))	$C_{15}H_{24}$	204	
25	Oleic acid, 3-(octadecycloxy)propyl ester	$C_{39}H_{76}O_3$	592	
26	Puphanamin	$C_{17}H_{19}NO_4$	301	
27	Thujone	$C_{10}H_{16}O$	352	
28	Tricyclo(5.2.2.0(1,6))undecane-3-ol, 2-methylene-6,8,8-trimethyl-	$C_{15}H_{24}O$	220	

Table 4: List of compounds from **GC/MS analysis** present in essential oil extracted from fresh leaves using hexane. (Highlighted compounds were detected only with hexane)

	Compound	**Chimical formula**	**M.W T**	**Chemical structure**
1	1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo(5.3.1.0(4,11))undec-8-ene	$C_{15}H_{24}$	204	
2	4-(2,2,6-Trimethyl-bicyclo(4.1.0)hept-1-yl)-butan-2-one& 4-(2,2,6-Trimethylbicyclo(4.1.0)hept-1-yl)-2-butanone	$C_{14}H_{24}O$	208	
3	4-Methyl-5-penta-1,3-dienyltetrahydrofuran-2-one	$C_{10}H_{14}O_2$	166	
4	7-Oxabicyclo(4.1.0)heptane,1-methyl-4-(2-methyloxiranyl)-	$C_{10}H_{16}O_2$	168	
5	Alpha.-Caryophyllene	$C_{15}H_{24}$	204	

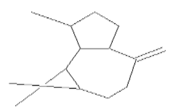
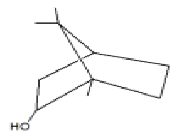
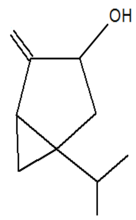
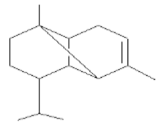
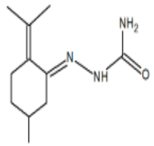
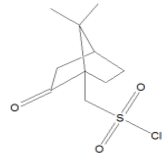
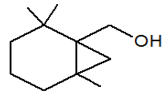
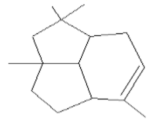
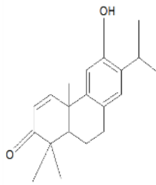
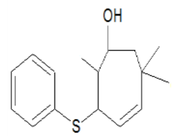
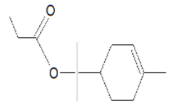
6	Aromadendrene	$C_{15}H_{24}$	204	
7	Bicyclo(2.2.1)heptan-2-ol, 1,7,7-trimethyl-,(1S-endo)-& Borneol	$C_{10}H_{18}O$	154	
8	Bicyclo(3.1.0)hexan-3-ol, 4-methylene-1-(1-methylethyl)-,(1S-(1.alpha.,3.beta.,5.alpha.))-& 4(10)-Thujen-3-ol,(1S,3R,5S)-(+)-&(+)-Sabinol &cis-Sabinol &1-Isopropyl-4-methylenebicyclo(3.1 .0)hexan-3-ol	$C_{10}H_{16}O$	152	
9	Copaene	$C_{15}H_{24}$	204	
10	Pulegone semicarbazone &Hydrazincarboxamide,2-(5-methylt hyldene)cyclohexylidene)-,(R)-&p-Menth-4(8)-en-3-one,semicarbazone,(R)- & (1E)- 5-Methyl- 2-(1-methylethylidene)cyclohexanone semicarbazone	$C_{11}H_{19}N_3O$	209	

Table 5: List of compounds from **GC/MS analysis** present in essential oil extracted from dry leaves using hexane. (Highlighted compounds were detected only with hexane)

	Compound	**Chemical formula**	**M.WT**	**Chemical structure**
1	(+)-Camphor-10-sulfonyl chloride &D-(+)-10-Camphorsulfonyl chloride & Bicyclo (2.2.1) heptane-1-methanesulfonyl chloride,7,7-dimethyl-oxo-,(1S)	$C_{10}H_{15}ClO_3S$	250	
2	(2,2,6-Trimethyl-bicyclo(4.1.0)hept -1-yl)-methanol &Myrtinol	$C_{11}H_{20}O$	168	
3	1R,4S,7S,11R-2,2,4,8-Tetramethyltr icyclo(5.3.1.0(4,11))undec-8-ene	$C_{15}H_{24}$	204	
4	2(1H)-Phenanthrenone,4a,9,10,10a-tetrahydro-6-hydroxy-1,1,4a-trimet hylethyl)-,(4aS-trans)-	$C_{20}H_{26}O_2$	298	
5	2,6,6-Trimethyl-3-(phenylthio)cycl ohept-4-enol	$C_{16}H_{22}OS$	262	
6	3-Cyclohexene-1-methanol,.alpha.,. alpha.,4-trimethyl-,propanoate &.alpha.-Terpinyl propionate &Terpinyl n-propionate	$C_{13}H_{22}O_2$	210	

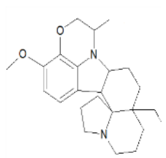
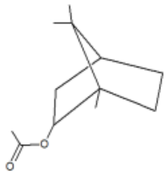
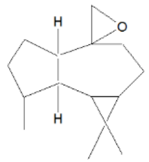
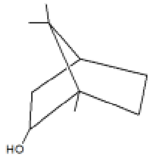
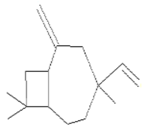
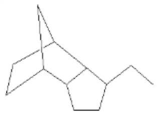
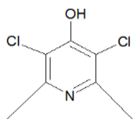
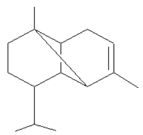
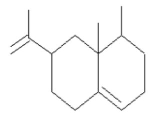
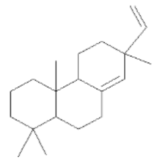
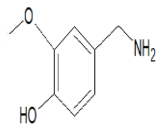
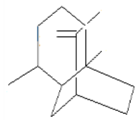
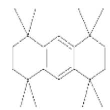
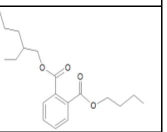
7	4,25-Secoobscurinervan,21-deoxy-16-methoxy-22-methyl-,(22.alpha.)-	$C_{23}H_{32}N_2O_2$	368	
8	Aceticacid,1,7,7-trimethyl-bicyclo(2.2.1)hept-2-yl ester& Bornyl acetate & 2-Camphanol acetate &Bornyl acetic ether	$C_{12}H_{20}O_2$	196	
9	Alloaromadendrene oxide-(1)	$C_{15}H_{24}O$	220	
10	Bicyclo(2.2.1)heptan-2-ol, 1,7,7-trimethyl-,(1S-endo)	$C_{10}H_{18}O$	154	
11	Bicyclo(5.2.0)nonane,2-methylene-4,8,8-trimethyl-4-vinyl-	$C_{15}H_{24}$	204	
12	Cis-3-etyl-endo-tricyclo (5.2.1.0 (2.6))decane	$C_{12}H_{20}$	194	
13	Clopidol	$C_7H_7Cl_2NO$	191	
14	Copaene	$C_{15}H_{24}$	204	
15	Naphthalene, 1,2,3,5,6,7,8,8a -octahydro-1,8a-dimethyl-7-(1-methylethenyl)-,(1R- (1.alpha.,7.beta.,8a.alpha.))	$C_{15}H_{24}$	204	
16	Phenanthrene,7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,1,4a,7-tetramethyl-,(4aS-(4a.alpha.,4b.beta.,7beta.,10a.beta))	$C_{20}H_{32}$	272	
17	Phenol, 4-(aminomethyl) -2-methoxy-&p-Cresol, .alpha.-amino-2-methoxy-&vanillylamine &3-Methoxy-4-hydroxybenzylamine&4-hydroxy-3-methoxybenzylamine& 4-(Aminomethyl)-2-methoxyphenol	$C_8H_{11}NO_2$	153	
18	Seychellene	$C_{15}H_{24}$	204	

Table 6: List of compounds from **GC/MS analysis** present in essential oil extracted from fresh leaves using chloroform. (Highlighted compounds were detected only with chloroform)

	Comupound	**Chemical formula**	**M.W T**	**Chemical structure**
1	1,2,3,4,5,6,7,8-Octahydro-1,1,4,4,5,5,8,8-octamethyl-	$C_{22}H_{34}$	298	
2	1,2-Benzene dicarboxylic, butyl2-ethyl2-ethylhexyl ester	$C_{20}H_{30}O_4$	334	

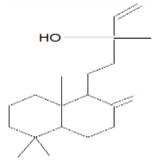
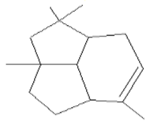
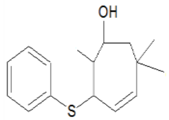
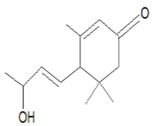
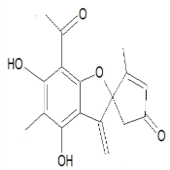
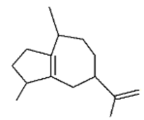
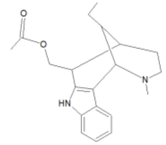
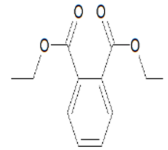
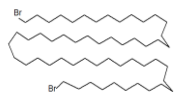
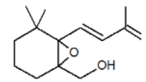
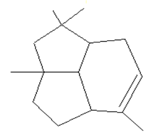
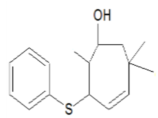
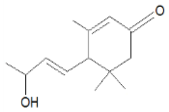
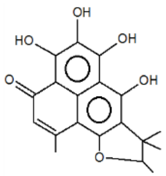
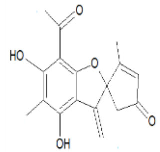
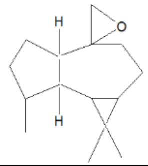
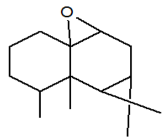
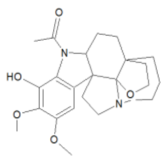
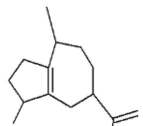
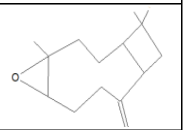
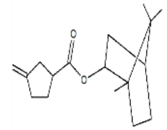
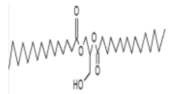
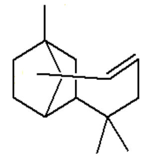
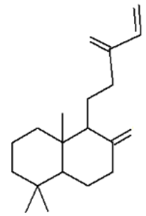
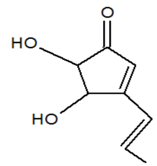
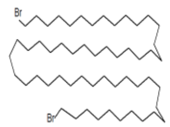
3	1-Naphthalenepropanol ,.alpha.-ethenyldecahydro-.alpha.,5,5,8a-tetramethyl-2-methylene-,(1S-(1.alpha .(S*),4a.beta.,8a.alpha.))	$C_{20}H_{34}O$	290	
4	1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo(5.3.1.0(4,11))undec-8-ene	$C_{15}H_{24}$	204	
6	2,6,6-Trimethyl-3-(phenylthio)cyclohept-4-enol	$C_{16}H_{22}OS$	262	
7	2-Cyclohexene-1-one,4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-	$C_{13}H_{20}O_2$	208	
8	7-Acetyl-4,6-dihydroxy-2,5-dimethyl-3-methyl-3-methylenespiro(benzofuran-2(3H),1-(2)cyclopenten)4-one	$C_{17}H_{16}O_5$	300	
9	Azulene,1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethyl)-,(1S-(1.alpha.,4.alpha.,7.alpha.))-	$C_{15}H_{24}$	204	
10	Caryophyllene	$C_{15}H_{24}$	204	
11	Dasycarpidan-1-methanol,acetate (ester)	$C_{20}H_{26}N_2O_2$	326	
12	Diethyl phthalate	$C_{12}H_{14}O_4$	222	
13	Tetrapentacontane, 1,54-dibromo-	$C_{45}H_{108}Br_2$	914	

Table 7: List of compounds from **GC/MS analysis** present in essential oil extracted from dry leaves using chloroform. (Highlighted compounds were detected only with chloroform)

	Compound	**Chemical formula**	**M. WT**	**Chemical structure**
1	(5,5-Dimethyl-buta-1,3-dienyl)-7-oxa-bicyclo(4.1.0)hept-1-yl)-methanol	$C_{14}H_{22}O_2$	222	
2	1-Heptatriacotanol	$C_{37}H_{76}O$	536	
3	1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo(5.3.1.0(4,11))undec-8-ene	$C_{15}H_{24}$	204	
4	2,6,6-Trimethyl-3-(phenylthio)cyclohept-4-enol	$C_{16}H_{22}OS$	262	

5	2-Cyclohexene-1-one,4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-	$C_{13}H_{20}O_2$	208	
6	4,5,6,7-Tetrahydroxy-1,8,8,9-tetramethyl-8,9-dihydrophenaleno(1,2-b)furan-3-one	$C_{19}H_{18}O_6$	342	
7	7-Acetyl-4,6-dihydroxy-2,5-dimethyl-3-methyl-3-methylenespiro(benzofuran-2(3H),1-(2)cyclopenten)4-one	$C_{17}H_{16}O_5$	300	
8	Alloaromadendrene oxide-(1)	$C_{15}H_{24}O$	220	
9	Aristolene epoxide	$C_{15}H_{24}O$	220	
10	Aspidospermidin-17-ol, -acetyl-19,21-epoxy-15,16-dimethoxy-	$C_{23}H_{30}N_2O_5$	414	
11	Azulene,1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethyl)-,(1S-(1.alpha.,4.alpha.,7.alpha.))-	$C_{15}H_{24}$	204	
12	Caryophyllene oxide	$C_{15}H_{24}O$	220	

13	Cyclopentanecarboxylic acid,3-methylene-,1,7,7-trimethylbicyclo(2.2.1)hept-2-yl ester	$C_{17}H_{26}O_2$	262	
14	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568	
15	Longifolene-(V4)	$C_{15}H_{24}$	204	
16	Naphthalene,decahydro-1,1,4a-trimethylene-5-(3-methylene-4-pentenyl)-,(4aS-(4a.alpha.,5.alpha.,8a.beta.))-	$C_{20}H_{32}$	272	
17	Terrein	$C_8H_{10}O_3$	154	
18	Tetrapentacontane, 1,54-dibromo-	$C_{45}H_{108}Br_2$	914	

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