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Research Paper

Analysis of the essential oils of *Salvia Libanotica* and *Origanum Syriacum*

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ABSTRACT

Essential oils (EO's) of *Salvia libanotica* and *Origanum syriacum* grown in Lebanon were extracted by two different techniques; hydrodistillation and cold solvents extraction using different solvents systems according to their polarity. The essential oil was analyzed using GC/MS. The results identified 35 constituents in each of *Salvia Libanotica* and *Origanum syriacum* extracts.

Keywords: *Salvia Libanotica*; *Origanum Syriacum*; Essential oil; GC/MS.

INTRODUCTION

Spices are actually not only evaluated for their seasoning properties, but are also appreciated for their bioactive efficacy as antioxidants, and nutriments. In recent years, there has been growing interest in finding natural antioxidants, including volatile chemicals, in plants because they inhibit oxidative damage and may consequently prevent inflammatory conditions (Silva, et al., 2003; Santos, et al., 2000). Most of the chemical constituents of essential oils are terpenoids, characterized by a low molecular weight which allows easy transport across cell membranes to induce different biological activities, including antioxidant, anti-inflammatory and anticholinesterase effects (Bakkali, et al., 2008; Hammer, et al., 1999).

Lebanon, located on the Mediterranean littoral, presents a climatic and ecological diversity that is unique to the eastern Mediterranean region and the whole country is recognized as a centre of plant diversity. An estimated 2600 plant species are recorded in Lebanon, of which 221 are broad endemics and 90 are narrow endemics (Loizza, et al., 2009). This study includes that of two medicinal plants of the "Lamiaceae" family, the *Salvia libanotica* and the *Origanum syriacum.*

The essential oils of Origanum species vary in respect of the total amount produced by plants as well as in their qualitative composition. Origanum essential oils are characterized by a number of main components which are implicated in the various plant odors (Chalchat, et al., 1998; Longaray Delamare, et al., 2007; Kowalski, et al., 2009).

The aim of this study was to identify the essential oil's composition of *Salvia* and *Origanum* using GC/MS and the best way of extraction. By comparing the results obtained in hydrodistillation and solvent extraction, we can affirm that hydrodistillation presents the most effective way to identify the EO's composition. Identification of the components by GC/MS was based on their retention time.

MATERIALS AND METHODS

***Sample collection*:** *Origanum Syriacum* and S*alvia Libanotica* were collected from Dennieh, North Lebanon, in July 2009 and authenticated by Dr. Ali CHAKAS, Botanist, Lebanese University, faculty of science III.

Chemicals: All reagents were of analytical grade. The following reagents were obtained from Sigma-Aldrich: ethanol (99.8%), acetone (99.8%), ethyl acetate (99%), ether (99%), hexane (97%) and dichloromethane (99.5%).

Oil extraction:

(i) ***Cold extraction:*** 20gm powder of dry plants were stirred each in 100 ml of different solvents at room temperature, agitated at 125 rpm for 24 h.

(ii) Hydrodistillation: Essential oil from crushed plants was isolated by hydrodistillation for 4 h using a Clevenger-type apparatus. The oil obtained was collected in dark glass vessels and stored at below 4°C until chromatographic determination. Percent of oils calculated on a moisture free basis are 2.5% from salvia libanotica and 3% from origanum syriacum.

Gas chromatography/mass spectrometry: A Shimadzu QP 2010 plus gas chromatography system interfaced to a 2010 mass spectrometer was used for analysis of the samples. The separation was performed on a 30 m x 0.25 mm internal diameter fused silica capillary column coated with 0.25 μm film Rtx-5MS.

The injector and the detector temperatures were respectively 250 and 280°C. The oven temperature was held at 40°C for 5 min, and programmed from 40 to 100°C at 4°C/min then to 280°C at 19°C/min and finally maintained at 280°C for 5 min. Split injection was conducted with a split ratio of 5:10. Helium was used as carrier gas, and flow-rate was 1.62 ml/min. The mass spectra were recorded over a range of 30-1000 atomic mass unit at 0.5s/scan. Solvent cut time was 3 min. Ionization energy was 70 eV. The inlet and ionization source temperature were 280°C.

RESULTS AND DISCUSSION

The main constituents of the essential oils of *Salvia Libanotica* and *Origanum Syriacum* cultivated in north Lebanon and used in present study are shown in Table-1; 2 respectively.

S. Libanotica essential oil was characterized by the presence of 34 components. Eucalyptol (47.55%), Camphor (5.57%), α-pinene (4.75%), β-pinene (5.8%), camphene (3.2%), β-myrcene (2.9%) and α-Terpineol (1.7%) were the major constituents (Table 1).

35 compounds characterized *Origanum syriacum* essential oil. Carvacrol (43.6%), p-cymene (16.36%), γ-terpinene (3.95%), naphtalonene (1.52%), caryophyllene (1.3%) and thymol (6.88%) were the major constituents (Table 2).

Table 1 shows that the dominant component is eucalyptol (figure 1) (47.55%), it is called also 1, 8-cineol. 1, 8-cineol, the major constituent of eucalyptus oil (eucalyptol), is known chemically as terpenoids oxide (Juergens, et al., 2004). Reactive oxygen species (ROS) are the causative factor involved in many human degenerative diseases, including cancer, and antioxidants have been found to have some degree of preventive effect on these disorders.

Table 2 shows the carvacrol (figure 2) (43.6%) as the dominant component in origanum. Carvacrol, a monoterpenolic phenol, possesses strong antioxidant properties equivalent to those of ascorbic acid, butyl hydroxyl toluene (BHT) and vitamin E. It is an antibacterial, antifungal, antispasmodic, white blood cell macrophage stimulate and a cardiac depressant activity compound (Arunasree, et al., 2010; Durling, et al., 2006).

Table 1, 2 show that hydrodistillation was the most effective way to extract the highest number of essential oils contents, but not always the highest percentage of each one. The percentage of each component differs from solvent to another due to their different solubility in each one. For instance, in salvia's essential oil the highest percentage of eucalyptol was obtained in hexane (54.01%) and dichloromethane (56.86%) (Non polar), whereas in hydrodistillation the percentage obtained was 45.87% only. In origanum's essential oil, the highest percentage of carvacrol was obtained in dichloromethane (56.06%) and acetone (54.63%), whereas it decreases in hydrodistillation to 15.06% only. Acyclique terpene (C_{10}) and sesquiterpene (C_{15}) (myrcene, ocimene and β-farnesene) hydrocarbons find little use in flavor and fragrance compositions. They are relatively unstable and have a slightly aggressive odor due to their highly unsaturated structure.

The most important families to which salvia and origanum essential oils belong are monoterpenic hydrocarbons (such as p-cymene, o-cymene, β-myrcene, α-pinene, β-pinene, camphene), monoterpenic aldehydes and alcohols (α-therpineol, thymol and carvacrol), monoterpenic epoxides (eucalyptol, thujone), and sesquiterpenic hydrocarbons (caryophyllene).

CONCLUSION

The present study has elucidated the chemical composition of the essential oil of *Salvia Libanotica* and *Origanum Syriacum* obtained by hydrodistillation and solvent extraction. The result indicates that the hydrodistillation must be the effective way to identify the EO's composition. The essential oil of *S. libanotica* contained the largest amount of eucalyptol, in comparison with *O. syriacum* that contained the largest amount of carvacrol, that grown in north Lebanon.

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Table-1: Chemical compositions of *Salvia libanotica* essential oil.

Name	Tr	% Area Hydro-distillation	% Area Hexane	% Area Dichloromethane	% Area Ethanol	% Area Acetone	%Area Ether
α-Thujene	10.525	0.16	0	0	0	0	0
α-pinene	10.792	4.36	5.36	6.78	3.66	3.94	4.43
camphene	11.4	3.78	3.23	4.25	2.45	2.56	2.85
β-pinene	12.633	6.2	6.59	7.75	4.48	5.1	4.95
β-myrcene	13.388	3.66	3.03	3.55	2.28	2.52	2.35
α-phellandrene	13.817	0.07	0	0	0	0	0
α-terpinene	14.358	0.2	0	0	0	0	0
P-cymene	14.683	0.15	0.62	0.62	0.52	0.55	0.44
1.5-isopropenyl-1-methyl-1-cyclohexene	14.84	0	0	0	0	0	0
Eucalyptol	15.15	45.87	54.01	56.86	43.79	48.44	36.33
γ-terpinene	16.125	0.44	0	0	0	0	0
Thujanol	16.467	0.24	0	0	0.23	0.28	0
α-terpinolene	17.3	0.18	0	0	0	0	0
Linalool	17.842	0.49	0	0	0.18	0.17	0
α-thujone	18	1.78	0.91	0.58	1.13	0.76	0.37
β-thujone	18.4	0	0	0	0	0	0
Camphor	19.542	7.46	5.17	5.34	6.29	6.25	2.94
Pinocamphone	20.092	0.29	0	0	0	0	0
Borneol	20.325	1.2	0	0	0.68	0.75	0
Myrcenol	20.39	1.02	0	0.57	0.78	0.89	0
4-terpineol	20.783	0.93	0	0	0.25	0.3	0
α-terpineol	21.417	2.91	1.1	1.35	2.06	2.15	0.53
Myrtenol	21.65	0.27	0	0	0	0	0
Bergamol	24.8	0.47	0	0	0.54	0	0
Thymol	26.103	0	0.6	0	0	0	1.95
Bornyl acetate	26.12	0.2	0	0	0	0	0
Carvacrol	26.384	0	1.13	0	0	0	3.09
α-farnesene	27.428	0	0	0	0.21	0	0
D-sylvestrene	27.609	2.32	0.85	1.13	2.07	1.63	0.29
caryophyllene	28.667	1.61	1.35	3.14	3.75	4.24	0.85
Aromadendrene	28.9	0	0	0	0.84	0.92	0
α-humulene	29.075	0.65	0	1.07	1.08	1.52	0
Viridiflorene	30.35	0	0	0	0	0	0
sclareol	33.29	0	0	0	0	0	0
	TIC	86.91	83.95	92.99	77.27	82.97	61.37

Table- 2: Chemical compositions of *Origanum Syriacum* essential oil.

Name	Tr	%Area hydro-distillation	%Area Hexane	%Area Dichloro methane	%Area Acetone	%Area Ether	%Area Ethanol
α-thujene	10.5	3.05	0	0	0	0	1.86
α-pinene	10.776	1.64	0	0	0	0	0.95
Bicyclo[3,1,0] hex-2-ene	11.3	0.06	0	0	0	0	0
Camphene	11.384	0.25	0	0	0	0	0
Sabinene	12.526	0.05	0	0	0	0	0
β-pinene	12.6	0.43	0	0	0	0	0
1 Octen-3-ol	12.95	3.19	2.27	1.03	0.62	1.3	0
β-Myrcene	13.375	3.25	2.34	1.3	1.39	1.33	1.78
3 octanol	13.62	2.18	1.62	0.68	0.61	0.86	0
α-phellandrene	13.825	0.82	0	0	0	0	0
3 carene	14.067	0.43	0	0	0	0	0
1 methyl 4[1 methyl diene] cyclohexene	14.367	4.79	0	0	0	0	0
2 carene	14.4	0	2.82	1.59	1.77	1.72	2.11
p-cymene	14.733	11.35	25.52	15.01	13.59	14.68	18.03
o-cymene (E)	15.342	0.2	0	0	0	0	0
Hyacinthin	15.55	0.13	0	0	0	0	0
o-cymene (Z)	15.75	0.26	0.26	0.62	0.63	0.56	0
γ-terpinene	16.175	14.4	0	0	0	0	9.33
4 carene	16.467	2.59	1.92	0.99	0.66	1.32	0.79
α-Terpinolen	17.209	0.39	0	0	0	0	0
3,7 dimethyl 1,6 octadien-3-ol	17.3	0.39	0	0	0	0	0
Borneol	20.315	0.39	0	0	0	0	0
4 terpineol	20.784	1.98	0	0	0	0	0
2 isopropyl 5 methyl Anisole	24.275	0.28	0	0	0	0	0
1,2(H) Naphtalonene	24.684	2.57	0	0	0	0	6.59
Thymol	26.441	12.87	4.62	5.2	6.66	5.93	6.04
Carvacrol	26.709	15.06	35.86	56.06	54.63	53.13	46.84
Caryophyllene	28.674	1.44	0.99	1.27	1.15	1.37	1.49
α-humulene	29.02	0.06	0	0	0	0	0
β-Bisabolen	29.61	0.25	0	0	0	0	0
6 methyl 5[1 methyl ethyl] 5 hepten-3-yn-2-ol	30.034	0.64	0	0	0	0	0
Caryophyllene oxyde	30.36	0.51	0	0	0	0	0
Cholesta 5, 2,4 dien-3-ol	33.633	0.39	0	0	0	0	0
9-10 dihydro cycloisolongiclene	33.726	0.09	0	0	0	0	0
	TIC	85.6	78.22	83.75	81.71	82.2	95.81

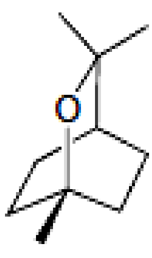

Figure-1: Eucalyptol

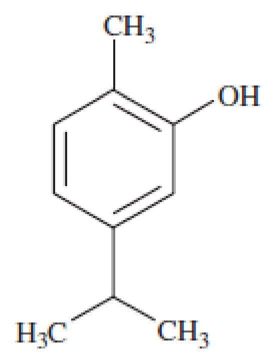

Figure-2: Carvacrol