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Comparative study of volatile secondary metabolite of Cistus libanotis during different process

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ABSTRACT

Chemical composition of the essential oils of Cistus libanotis from eastern Morocco (Tafoughalt and Jerrada) extracted from different season was analyzed by GC-MS and GC-FID. The hydro-distilled essential oil content ranged from 0, 27 % to 0, 46%, the maximum amounts were observed in summer while minimum in winter for Tafoughalt, whereas the oil obtained from Jerrada had the highest yield (0, 61 %). The essential oils consisted of terpineol-4 as the most abundant component (18, 70 % - 24, 91 %), followed by γ-terpinene (9, 82 % - 12, 25 %), camphene (5, 86 % - 13, 58 %), sabinene (7, 86 % - 9, 89 %) and α-thujene (6, 92 % - 10, 12 %). Samples collected in all seasons were found to be richer in monoterpenes hydrocarbons (52, 68 % - 57, 59 %). None sesquiterpene hydrocarbons found in the oils from summer and autumn. Drying period of leaves at normal air laboratory and distillation time also affect the oil content and composition. There was variation in terpineol-4 content from 22, 23 % (4th day of drying) to 19, 27 % (29th day of drying), and from 35, 60 % (1 hour of distillation) to 24, 17 % (4 hours of distillation). It can be concluded that the harvesting season, period of drying and the time of distillation had an effect on the yield of essential oil and chemical composition of various compounds.

Key words: Cistus libanotis, Essential oil, GC-MS, Kinetics, Yeilds

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INTRODUCTION

Essential oils are defined as complex mixtures of volatile substance [1]. They are produced by special cells within the plant and protect the plant from: disease, parasite, predators, attracting certain insects for reproductive pollination and more others [2].

Essential oils can be occurring by different methods such as: hydro-distillation, steam distillation, simultaneous distillation–extraction, Soxhlet extraction, micro-distillation, direct sampling from secretory structures, Solvent-free microwave extraction, headspace techniques, static HS methods, solid-phase micro-extraction, etc [3].

The interactions between constituents of essential oil are known to exhibit antagonistic, additive or synergistic effects, for example, synergistic interactions found in the mixture of 1, 8-cineole / α-pinene and 1, 8-cineole / caryophyllene oxide, whereas in combination of 1, 8 cineole / camphor, they found antagonism effect for inhibitor of acetylcholinesterase [4].

Although reports on the extract composition and their biological activities (antioxidant, antimicrobial …) of different Cistus species (Cistus ladanifer, Cistus monspeliensis…) in Morocco and other countries were achieved [5, 6].

For the chemical composition of Cistus ladanifer and Cistus libanotis essential oils growing in eastern Morocco was reported for the first time by Zidane H. et al 2013 [7].

The present study aims to survey and to compare the variability of the chemical composition of Cistus libanotis of a hydro-distilled essential oil from the leaves of these plants growing in eastern Morocco (Tafoughalt and Jerrada). The variability was evaluated as a function of different parameters: seasonal variation, days of drying of plants, distillation time and geographical variation. Furthermore the kinetics and yields of the extraction have been determined.

EXPERIMENTAL SECTION

1.1. Material collection

Cistus libanotis was collected from eastern Morocco (Tafoughalt (latitude 34°48'26" (N) and 2°24'48" (W), humid climate) and Jerrada (latitude 34°18'42" (N) and longitude 2°09'49" (W), arid climate)). The samples were identified by Dr Haloui of the department of biology (University Mohamed Premier Oujda). Aerial parts were carefully deposed on several groups to dray in normal air laboratory.

1.2. Extraction of essential oil

The essential oil was obtained by using Clevenger apparatus (150 g of leaves in 170 ml of water). The distillation time was approximately 4 h. The essential oil was dried over anhydrous sodium sulfate and kept at 4°C.

1.3. Gas chromatography-mass spectrometry (GC-MS)

The oils were analyzed by GC/MS using a HP HEWLETT PACKARD (HP 6890 series GC system), VF-WAXms capillary column (30 m × 0.25 mm, film thickness 0.25 µm), Injector and detector temperatures were set at 260°C. Helium was the carrier gas, at a flow rate of 1, 3 mL/min.

Front detector (FID): Temperature: 260°C, H_2 flow: 40.0 mL/min, air flow: 450.0 mL/min, mode: Constant makeup flow, makeup flow: 20.0 mL/min, makeup Gas Type: Nitrogen

Temperature program: Initial temperature: 55 'C; equilibration time: 1.00 min; initial time: 0.10 min; rate: 10.00 'C/s; end temp: 260 'C; hold time: 1.00 min

For MS parameters, an electron ionization system with an ionization energy of 70 eV was used (low mass: 31.0; high Mass: 400.0; threshold: 150; MS Source: 230°C maximum 250°C; MS Quad: 150°C maximum 200°C

Diluted samples (a drop of essential oil in 5 ml of hexane) were injected (0, 5 µl) in the splitless mode. Quantitative data were obtained electronically from FID area data without using correction factors. The components were identified using Wiley library 275 data of the GC/MS system, Kovats index and literature data.

RESULTS AND DISCUSSION

1. Kinetic of extraction of essential oil

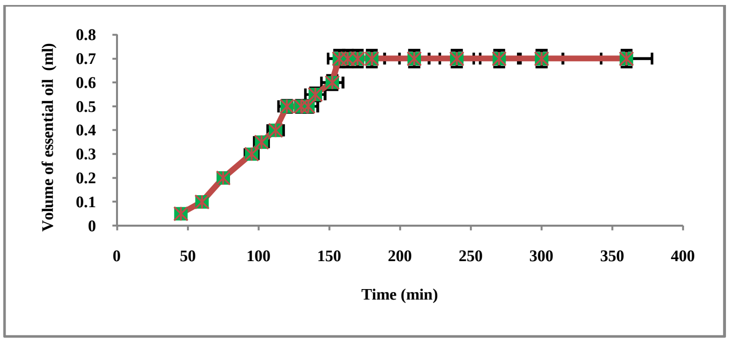

Figure 1: Kinetics of extraction of essential oil

The results shows that the maximum essential oil percentage was obtained at (2, 6) hours duration of extraction (Figure 1). The increasing duration of hydro-distillation not affect the volume of essential oil extracted from Cistus libanotis.

2. Seasonal and regional variation of essential oil of Cistus libanotis

All the oil samples have been analyzed by GC-MS and GC-FID. The seasonal and regional variation of the chemical composition of the oil samples of Cistus libanotis are reported in the table 1. α-thujene, camphene, sabinene, α-terpinene, γ-terpinene, ρ-cymene and terpineol-4 were the major constituents, accompanied by α-terpinolene, α-terpineol and bornyl L found at appreciable contents in all samples. The oil samples contained high amounts of hydrocarbons and oxygenated monoterpenes compounds.

The oils from all seasons varied greatly in composition, some components were found just in one oil. For example, in winter we had 1, 8-Epoxy-p-menth-2-ene (0, 43 %), limonene (0, 29 %) and thymol (0, 09 %). Others were present only in summer (cyclofenchene (0, 12 %) and a phellandrene epoxide (0, 16 %)). For α-terpinyl acetate was present in all seasons except winter. Whereas other constituents were common to all oils analysed, but with different amounts in oils. The high amounts of terpineol-4 was obtained in autumn season (24, 91%) followed by summer (20, 19%) and comparative content in spring and winter (18, 75 % and 18, 70 %, respectively).

In the essential oil of Cistus libanotis from Jerrada 44 compounds were identified, representing 94, 98 % of the total oil. The major constituents being: terpineol-4 (22, 98 %), γ- terpinene (12, 14 %), sabinene (9, 28 %), camphene (6, 13 %), ρ-cymene (6, 83 %), α-terpinene (5, 80 %) and borneol (5, 04 %). The amount of these components indicates quantitative and qualitative variations of the oil collected in the same season (winter) and from different regions (Tafoughalt and Jerrada). The oil of Jerrada region was characterized by the domination of terpinol-4 (22, 98 %) while is less abundant in Tafoughalt oil (18, 70 %). Futhermore, the oil of Tafoughalt was found to have higher content of α-thujene (6, 92 %), camphene (10, 46 %), sabinene (9, 89 %) and α-terpineol (1, 63 %) in comparaison with Jerrada. In the other hand, α-terpinene (5, 80 %), γ-terpinene (12, 14 %), ρ-cymene (6, 83 %) and borneol (5, 04 %) were more abundant in Cistus libanotis essential oil from Jerrada than Tafoughalt oil.

The essential oil from Jerrada had the highest hydro-distillation yield (0, 61 %), whereas the hydro-distilled oil from Tafoughalt had the lowest yield (0, 27 %).

A recent study of the chemical composition of hexane extract from the leaves of Cistus libanotis collected on March from Tunisia has been described by Ben Mariem et al 2013 [8], they identified 47 constituents of the extract such as α-pinene (2, 1 %), sabinene (1, 2 %), β-pinene (3, 0 %), camphene (3, 6 %) and borneol (1, 2 %).

This variability can be explained by genetic and environmental factors (climate, soil) [9]. In addition, some variables can affect the chemical composition, for example, the part of the plant used for extraction (leaf, flower, stem, etc), fertilizer, method of extraction, harvest season and altitude [10], geographical region [11]. The effect of stage development of plant and temperature of drying were studied by Jens Rehloff et al 2005 [12]. They obtained high essential oil of Mentha x Piperita L. yield at full bloom with a higher amount of menthol and menthone (43 – 54 % and 12 – 30 %, respectively) at 30 °C (drying temperature).

3. Effect of drying and distillation time on the chemical composition of essential oil

The analyses of the chemical composition of Cistus libanotis essential oil extracted at different time of distillation (1, 2, 3 and 4 hours) and at different period of drying period are given in the table below (table 2).

A comparative study of volatile components of Cistus libanotis leaves oil extracted at different time of distillation indicates qualitative and quantitative variations.

The majority of compounds existed in the samples, but some of them increased considerably by prolonging the time of distillation, such as α-thujene varied from 3, 36 % after one hour of distillation to 9, 37 % after four hours, α-terpinene reached the maximum after four hours of distillation (6, 59 %), γ-terpinene (3, 51 % - 13, 07 %), ρ-cymene (2, 34 % - 2, 82 %), α-terpinolene (0, 70 % - 2, 53 %) and sabinaketone (0, 38 % - 0, 68 %). Whereas others decreased: linalool L (0,48 % - 0,33 %), cis sabinene hydrate (2,43 % - 0,81 %), p-menth-2-en-1-ol, stereoisomer (1,61 % - 0,88 %), bornyl acetate (0,70 % - 0,34 %), terpinen-4-ol (35, 60 % - 24, 17 %), 1-terpineol (1, 79 % - 0, 54 %), myrtenal (1, 12 % - 0, 61 %), α-terpineol (1, 38 % - 0, 61 %), α-terpinyl acetate (4, 53 % - 2, 77 %), borneol (4, 69 % - 1, 48 %) and cuminic aldehyde (1, 36 % - 0, 53 %). Or even appeared and disappeared in the same time, for example: cistonellol (0, 11 %), cis sabinol (0, 16 %), (+) spathulenol (0, 07 %), p-cumenol (0, 09 %), p-menth-1(7), 2-dien-8-ol (0, 38 %) and β-thujene (0, 65 %) after three hours of hydro-distillation.

Table 1: Chemical composition of Cistus libanotis essential oil from different harvest season and region

Cistus libanotis							
			Region				
N°	Compounds	IK	Tafoughalt				Jerrada
			Area (%)				
			Spring	Summer	Autumn	Winter	Winter
1	Tricyclene	1017	1,38	1,32	0,66	1,00	0,48
2	Alpha thujene	1032	7,13	10,12	8,60	6,92	6,13
3	Camphene	1074	13,58	12,35	5,86	10,46	9,28
4	Beta pinene	1114	2,52	1,29	0,54	1,59	1,00
5	Sabinene	1127	9,82	7,86	8,84	9,89	4,96
6	2,4(10) thujadien	1133	0,95	-	-	1,21	-
7	Alpha phellandrene	1169	-	0,24	1,13	0,21	0,36
8	Alpha terpipene	1184	4,62	5,44	5,93	4,67	5,80
9	1,8-Epoxy-p-menth-2-ene	1192	-	-	-	0,43	-
10	Limonene	1202	-	-	-	0,29	-
11	Beta phellandrene	1213	-	-	-	-	0,86
12	Beta thujene	1213	-	0,84	0,51	0,56	-
13	Gamma terpinene	1253	9,82	11,36	12,25	10,41	12,14
14	para cymene	1275	3,63	4,35	5,56	4,70	6,83
15	Alpha terpinolene	1287	1,90	2,24	2,61	2,29	2,73
16	Beta thujone	1448	-	-	0,24	0,17	0,24
17	Cymenene	1441	-	0,07	0,17	0,14	0,22
18	Trans-sabinene hydrate	1461	0,67	0,96	1,80	2,01	1,06
19	L-Camphor	1525	0,56	1,06	0,45	0,29	0,74
20	Cis-sabinene hydrate	1546		0,69	1,29	1,35	
21	Alpha campholene aldehyde	1494	0,16	0,16		0,09	
22	ALPHA CUBEBENE	1498	0,18			0,11	
23	Cis p-Menth-2- en-1-ol	1546					0,49
24	Iso-pinocamphone	1556					0,22
25	p-Menth-2-en-1-ol, stereoisomer	1561	0,75	0,84	1,17	0,91	0,85
26	Pinocarvone	1577	0,86	0,77	0,45		0,31
27	(-)-Bornyl acetate	1585	0,41	0,48	0,33	0,42	1,45
28	Terpinen-4-ol	1608	18,75	20,19	24,91	18,70	22,98
29	Cyclofenchene	1616		0,12			
30	1-Terpineol	1625	0,48	0,51	0,41	0,53	0,34
31	Myrtenal	1639	0,95	0,97	0,71	0,64	0,50
32	Sabinaketone	1643	0,68	0,79	0,65	0,75	0,50
33	Sabinyl acetate	1651			0,16	0,11	
34	Trans-Pinocarveol	1658		0,11	0,95	0,76	0,67
35	Alpha terpineol	1694	1,06	1,14	0,77	1,63	0,63
36	alpha terpinyl acetate	1698	1,78	1,38	1,59		2,05
37	Borneol L	1702	1,79	1,82	1,79	2,06	5,04
38	Verbenone	1715	0,59	0,60	0,55	0,46	0,37
39	Trans-p-menth-2-ene-1,8-diol	1723				0,39	0,05
40	p-mentha-1,5-dien-8-ol	1719	0,56	0,46	0,39		0,06
41	cis-p-Menth-1-en-3-ol	1743	0,35		0,40	0,13	0,16
42	p-Menth-1-en-3-ol, trans-	1744		0,36			0,05
43	(2-Methylprop-1-enyl) cyclohexa-1,5-diene	1751				0,21	
44	A-phellandrene epoxide	1783		0,16			
45	Sabinol	1783					0,50
46	Benzaldehyde, 4-(1-methylethyl)-	1787	0,61	0,69	0,72	0,61	0,64
47	Myrtenol	1792	0,85	0,79	0,56	0,65	0,28
48	Trans-(+)-carveol	1832	0,58	0,39	0,29	0,45	0,68
49	1S, Cis-calamenene	1838	0,48	0,49	0,40	0,52	0,83
50	Para-cymen-8-ol	1845	0,09	0,54	0,72	0,63	0,06
51	Caryophyllene oxide	1994	0,49	0,76	0,61	0,59	1,46
52	p-Mentha-1,4-dien-7-ol	2053		0,40	0,49	0,06	0,40
53	p-Cymen-7-ol	2100	0,32	0,28	0,31	0,64	0,25
54	Thymol	2178				0,09	
55	Carvacrol	2209		0,12	0,22	0,18	0,26
56	P-cumenol	2211		0,13	0,11	0,11	
57	(+) Spathulenol	2124			0,14		
58	1,4-Dimethyl-7-(1-methylethyl)azulene	2232					0,06
Total monoterpenes hydrocarbons			55,34	57,59	52,68	54,56	50,81
Total oxygenated monoterpenes			30,65	34,80	40,23	34,63	38,31
Total sesquiterpenes hydrocarbons			0,18	-		0,11	0,06
Total oxygenated sesquiterpenes			0,97	1,25	1,15	1,10	2,29
Total others			2,19	1,99	2,19	0,64	3,51
% Total			89,34	95,63	96,25	91,03	94,98
% Yields			0,30	0,46	0,41	0,27	0,61

IK: Kovats index calculated on polar column (Vf-waxms)

Table 2: Variation in the chemical composition of Cistus libanotis essential oil with distillation time and drying days of plant material

Compounds	Time distillation				Days distillation		
	1 hour	2 hour	3 hour	4 hour	4^{th} day	26^{th} day	29^{th} day
	Area (%)				Area (%)		
Tricyclene		0,58	0,45	0,43	0,94	1,16	1,00
Alpha thujene	3,36	7,11	9,17	9,37	6,31	6,61	6,99
Camphene	3,68	8,14	6,42	5,67	9,78	10,95	10,48
Beta pinene	0,82	1,68	1,35	1,23	1,89	1,86	1,61
Sabinene	9,35	16,86	12,57	10,60	9,81	11,27	9,98
Myrcene		0,29					
Alpha phellandrene		0,26	0,23				
Alpha terpipene	1,51	5,62	6,08	6,59	4,21	4,27	4,69
Limonene		0,34	0,33				
Beta phelandrene		0,51		0,73		0,46	0,55
Beta thujene			0,65				
Gamma terpinene	3,51	11,02	12,03	13,07	9,63	9,16	10,29
para cymene	2,34	2,65	2,82	2,82	4,79	4,12	4,67
Alpha terpinolene	0,70	2,27	2,49	2,53	1,94	1,84	2,12
Cymenene							0,21
Beta thujone	0,50	0,30	0,24				
Trans-sabinene hydrate	1,86	2,17	1,66	1,29	0,84	3,51	2,03
Alpha cubebene							0,12
L-Camphor	0,95	0,27	0,26		3,32	0,20	0,30
Linalool l	0,48	0,29	0,27	0,33			
Cis-sabinene hydrate	2,43	1,42	1,09	0,81			1,36
Linalyl acetate	0,72	0,21	0,17				
Isopinocamphone		0,89					
p-Menth-2-en -1-ol,cis					0,57	2,59	
p-Menth-2- en-1-ol, stereoisomer	1,61	0,09	0,94	0,88	0,88	0,94	0,93
Pinocarvone		0,46	0,38	0,38	0,60	0,40	0,52
(-)-Bornyl acetate	0,70	0,38	0,36	0,34	0,31	0,56	0,42
Terpinen-4-ol	35,60	18,75	22,97	24,17	22,23	18,20	19,27
Beta caryophyllene	2,66	0,36					
1-Terpineol	1,79	0,51	0,57	0,54		0,19	0,17
Myrtenal	1,12	0,69	0,65	0,61	0,90	0,52	0,53
Sabinaketone	0,38	0,59	0,66	0,68	0,75	0,65	0,64
(+)-2-Carene					2,22		
Sabinyl acetate	2,61	0,16	0,15				
Pinocarveol						0,69	
Trans Pinocarveol	0,60	0,19		0,14	0,93		0,76
Alpha terpineol	1,38	0,75	0,69	0,61	0,25	0,58	0,46
Alpha terpinyl acetate	4,53	2,09	2,52	2,77		0,16	1,65
Borneol L	4,69	1,35	1,43	1,48	1,90	1,55	2,12
Verbenone	0,96	0,21	0,28	0,35	0,64	0,49	0,47
p-mentha-1,5-dien-8-ol		0,03		0,49			
P-mentha-1(7), 2dien 8 ol			0,38		0,50		0,35
cis-p-Menth-1-en-3-ol		0,08	0,26	0,40		0,24	0,29
Sabinyl acetate							0,16
Citronellol			0,11				
Cis sabinol			0,16				
A-Phellandrene epoxide	1,00	0,10					
Cuminic aldehyde	1,36	0,45	0,51	0,53	0,37	0,24	0,63
Myrtenol	0,97	0,49	0,66	0,78	0,72	0,11	0,67
p-Mentha-1,5-dien-7-ol		0,32					
Trans-(+)-carveol	0,72	0,39	0,44	0,52			0,46
1S, Cis-calamenene		0,26	0,47	0,56	0,47	0,50	0,53
Para-cymen-8-ol	0,86	0,09	0,37	0,45	0,63	0,55	0,65
4,5--epoxy-1-isopropyl-4-methyl-1- Cyclohexene							0,15
Caryophyllene oxide	1,74	0,74	1,00	1,20	0,56	0,55	0,64
p-Mentha-1,4-dien-7-ol	0,54	0,20	0,06		0,49	0,36	0,63
p-Cymen-7-ol	0,42	0,11	0,43	0,33	0,43	0,14	0,45
(+)spathulenol			0,07				
Thymol							0,09
Carvacrol			0,10	0,15		0,08	
P-cumenol			0,09				0,12
Total monoterpenes hydrocarbons	25,27	57,33	54,59	53,05	51,51	51,70	52,59
Total oxygenated monoterpenes	60,23	31,20	35,58	35,93	36,95	32,24	33,93
Total sesquiterpenes hydrocarbons	2,66	0,36	-	-	-	0	0,12
Total oxygenated sesquiterpenes	1,74	1,01	1,54	1,76	1,04	1,05	1,17
Total others	8,56	2,84	3,29	3,11	0,31	0,72	2,35
% Total	98,45	92,73	95,00	93,85	89,81	85,71	90,16

The oil obtained after one hour of distillation was characterized by oxygenated monoterpenes (60, 23 %), followed by monoterpenes hydrocarbons (25, 27 %). For the other oils, monoterpenes hydrocarbons were the chief (57, 33 %, 54, 59 %, 53, 05 %) afterward we found oxygenated monoterpenes (31, 20 %, 35, 58 %, 35, 93 %) of 2, 3 and 4 hours, respectively. No sesquiterpenes hydrocarbons found in the oils obtained after three and four hours of distillation.

The essential oil content of Cistus libanotis extracted at different day of drying ranged from 0, 11 % to 0, 20 % (Figure 2). The high oil content was found when the leaves were left between 25 and 27th days of drying, after that there was a reduction of the yield.

In addition, results showed that drying period in the normal air laboratory had an effect on the chemical composition of Cistus libanotis essential oil. The higher amount of camphene (10, 95 %) and sabinene (11, 27 %) was obtained on the 26th day (table 2).

On the 4th day of drying, the major components (terpineol-4 (22, 23 % - 19, 27 %), L-camphor (3, 32 % -0, 30 %) in the essential oil were decreased.

In the other hand, some components were increased: Tricyclene (0, 94 % - 1, 16 %), α-thujene (6, 31 % - 6, 99 %), γ-terpinene (9, 63 % - 10, 29 %), α-terpinolene (1, 94 % - 2, 12 %) and trans sabinene hydrate (0, 84 % - 2, 03 %). Cis sabinene hydrate was appeared in the oil extracted on the 29th day of drying.

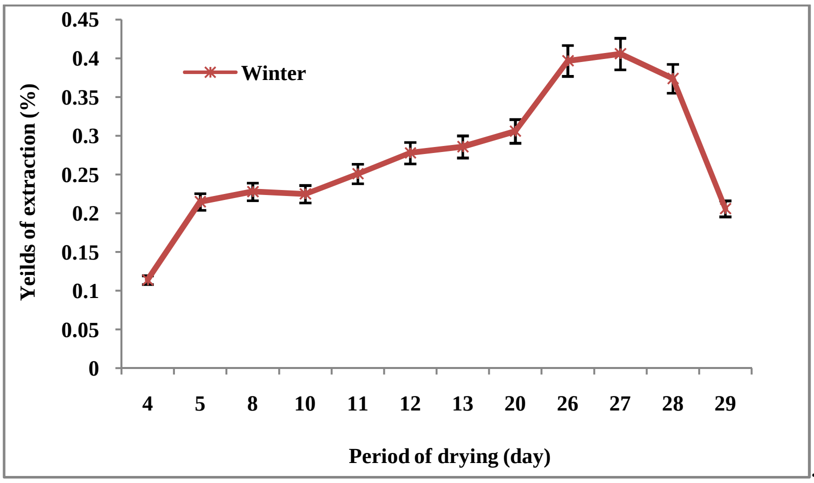

Figure 2: Effect of drying period on yields of Cistus libanotis essential oil

CONCLUSION

It can be concluded that the harvesting season, period of drying and the time of distillation had an effect on the yield of essential oil and chemical composition of various compounds (some compounds decreased, increased and disappeared or appeared). The distillation time may left for 3 hours in order to increase the yield of extraction, also the period of during of leaves should be allowed in order to increase the yield of extraction. In general, growing season affected the chemical composition of Cistus libanotis essential oils. These differences can be attributed to the seasonal changes, harvest region and stage development of plant.

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