Clary Sage Oils

Variation in composition and enantiomeric analyses of commercial vs. new Kashmir clary sage oils

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Commercial clary sage oils were analyzed along with a newly available Kashmir oil (Himalayan Foothills Oils, Srinagar, India). All of the oils were high in linalool (14.7–25.1%) and linalyl acetate (55.0–72.4%). Several of the commercial clary sage oils failed to meet one or more of the European Pharmacopoeia 5.0 (EP 5) specifications for linalool, linalyl acetate, germacrene D or sclareol. The new Himalayan Foothills Oils (Inc.) clary sage oil met all the EP 5 specifications as did the oil from Liberty Natural Products, Oregon. Chiral analyses of linalool and linalyl acetate revealed that the ratio of (-)-R-linalool to total linalool was not very variable, ranging from 73.4% to 83.1%. However, the ratio of (-)-(R)-linalyl acetate to total linalyl acetate was variable with both Russian (82.4%) and Ukrainian (86.5%) oils being clearly different from the other oils that were 99% or more (-)-(R)-linalyl acetate.

Introduction

Clary sage oil is an important commercial oil obtained from the flowering stems of *Salvia sclarea* L. It is characterized in the EP 5 as having large amounts of linalool (6.5–24%), linalyl acetate (56–78%) and moderate amounts of germacrene D (1.0–12.0%) and sclareol (0.4–2.6%). Brian Lawrence has reviewed the literature through 2000 and he reported considerable variation in these components.[1] More recently, Carrubba et al. analyzed inflorescences and leaves of an Italian biotype.[2] The oils of the inflorescences and leaves were found to contain large amounts of linalool (28.9% and 25.65%), linalyl acetate (34.9% and 52.7%) and germacrene D (10.6% and 3.9%), with very small quantities of sclareol (0.1% and 0.06%). Analysis of the oil of a Uruguayan biotype of clary sage showed considerable variation based on flowering stages and years: linalool (7.9–22.5%), linalyl acetate (38.6–48.1%), germacrene D (8.2–19.8%) and sclareol (1.1–2.7%).[3] Farkas et al. reported that the clary sage oil from flowers (full bloom) of plants cultivated in Slovak Republic were moderate in linalool (18.9%) and germacrene D (5.0%), but low in linalyl acetate (13.7%) and very high in sclareol (15.7%).[4]

Commercial Kashmir clary sage oil has been analyzed by Shawl et al. who reported it to contain 30% linalool, 52% linalyl acetate and 7.2% α-terpineol as major components.[5] The same lab (presumably using the same oil sample) reported that both sclareol and 13-epi-sclareol were present.[6]

Chiral analysis of the major components (linalool and linalyl acetate) of clary sage has been the subject of only a few papers. Casabianca and Graff analyzed French clary sage oil and reported (R)-(-)-linalool (80.6–95.0%):(S)-(+)-linalool (5.0–19.4%) and (R)-(-)-linalyl acetate (93.0–98.1%):(S)-(+)-linalyl acetate (1.9–7.0%).[7] Demirci et al. found 76.3% (-)-(R)- and 23.7% (+)-(S)-linalool for locally grown (Turkey) clary sage flower oil.[8] They did not report on +/- linalyl acetate. Lorenzo et al. performed chiral analyses on Uruguayan clary sage oil and found (R)-(-)-linalool (72.9%):(S)-(+)-linalool (27.1%) and (R)-(-)-linalyl acetate (99.1%):(S)-(+)-linalyl acetate (0.9%). A review of the literature did not reveal any reports on the chiral analyses of common commercial clary sage oils.

The purpose of this study is to compare both the oil composition and chiral analysis of linalool and linalyl acetate of a new commercial source of Kashmir clary sage oil with other commercially available clary sage oils from various geographic regions.

Experimental

Clary sage oils (with lab accession numbers) from Bulgaria (11047), France (11049), Hungary (11034), Oregon (USA) (11033), Russia (11050) and Ukraine (11051) were obtained from Liberty Natural Products (LNP), Portland, Oregon, USA. Kashmir clary sage oil (10979) was furnished by Himalayan Foothills Oils, Srinagar, India.

The oils were analyzed on a HP5971 MSD mass spectrometer, directly coupled to a HP 5890 gas chromatograph, using a J&W DB-5, 0.26 mm x 30 m, 0.25 µ coating thickness, fused silica capillary column, using the following conditions: carrier gas He, 30.5 cm/s (ca. 1 mL/min), 0.2 µL of 10% solution injected, split 1:15; injector 220°C, oven temperature linear programmed 60°–246°C at 3°C/min., transfer line 240°C. Identifications were made by library searches of our volatile oil library, using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds.[9] Quantitation was by FID on an HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 µ coating thickness, fused silica capillary column, using

Comparison of the percentage composition of commercial clary sage oils

T-1

KI	Compound	Kash	Hung	Russ	Bulg	Ukrn	Fren	OR	EU Pharm
932	α-pinene	0.1	t	t	t	t	0.1	t	
969	sabinene	t	t	t	t	t	t	t	
974	β-pinene	0.1	0.2	t	0.1	t	t	0.1	
988	myrcene	0.9	1.2	0.6	2.3	1.0	0.8	1.0	
1006	dehydroxy-*cis*-linalool oxide	t	0.1	t	0.1	t	0.1	0.1	
1020	p-cymene	t	0.1	t	t	t	t	0.1	
1024	limonene	0.3	2.2	0.4	0.7	0.3	0.3	0.3	
1026	1,8 cineole	0.1	t	t	t	0.1	t	–	
1032	(Z)-β-ocimene	0.3	0.3	0.1	0.8	0.4	0.2	0.4	
1044	(E)-β-ocimene	0.4	0.5	0.2	1.4	0.5	0.3	0.6	
1067	*cis*-linalool oxide (furanoid)	t	t	0.1	t	t	t	t	
1084	*trans*-linalool oxide (furanoid)	0.1	t	t	t	t	t	–	
1086	terpinolene	0.1	0.2	0.2	0.3	0.1	t	0.2	
1095	**linalool**	**14.7**	**17.8**	**18.1**	**23.8**	**25.1EP**	**19.7**	**19.9**	**6.5–24.0**
1101	***cis*-thujone (α-thujone)**	–	–	–	–	–	–	–	**< 0.1**
1112	***trans*-thujone(β-thujone)**	–	–	–	–	–	–	–	**< 0.1**
1141	camphor	0.1	t	t	t	t	t	–	
1154	nerol oxide	t	t	t	t	t	t	t	
1165	borneol	t	t	t	t	t	0.1	t	
1174	terpinen-4-ol	0.4	t	t	0.1	0.2	0.1	t	
1179	p-cymen-8-ol	0.1	t	t	t	t	0.1	t	
1186	**α-terpineol**	**2.5**	**1.5**	**3.1**	**4.5**	**3.9**	**2.3**	**4.0**	**< 5.0**
1214	linalyl formate	0.4	0.2	0.2	0.2	t	0.1	0.2	
1227	nerol	0.4	–	0.4	0.8	0.6	0.4	0.8	
1254	linalyl acetate	**72.4**	**71.0**	**68.2**	**55.0EP**	**58.8**	**67.1**	**61.5**	**56.0–78.0**
1264	geranial	t	t	0.4	0.2	0.3	0.2	0.2	
1280	neryl formate	t	t	t	0.1	t	t	t	
1288	lavandulyl acetate	t	0.4	0.3	t	0.1	t	t	
1298	geranyl formate	t	t	0.2	0.2	0.1	t	t	
1346	α-terpinyl acetate	–	t	0.1	t	t	t	–	
1359	neryl acetate	0.6	0.4	0.9	1.3	1.1	0.8	1.3	
1374	α-copaene	0.4	0.2	0.2	0.4	0.4	0.7	0.3	
1379	geranyl acetate	1.3	0.9	2.0	2.6	2.1	1.5	2.6	
1387	β-bourbonene	0.1	0.1	0.1	t	0.2	0.3	t	
1387	β-cubebene	0.3	t	t	0.2	0.1	0.2	0.1	
1387	β-elemene	t	t	t	t	0.1	0.1	0.1	
1417	β-caryophyllene	1.3	0.7	0.9	1.8	1.6	1.3	1.4	
1430	β-copaene	t	t	–	t	t	t	t	
1452	α-humulene	t	t	t	0.1	0.1	t	0.1	
1454	(E)-β-farnesene	t	t	t	t	0.1	t	t	
1484	**germacrene D**	**1.4**	**0.3EP**	**0.3EP**	**1.8**	**0.7EP**	**1.0**	**1.5**	**1.0–12.0**
1489	β-selinene	t	t	0.1	t	0.1	0.2	0.2	
1500	bicyclogermacrene	0.2	t	0.3	0.4	0.2	0.2	0.3	
1505	(E,E)-α-farnesene	t	t	t	0.1	t	t	0.1	
1519	cis-dihydroagarofuran	t	t	t	t	t	t	0.1	
1522	δ-cadinene	t	t	t	0.1	0.1	0.1	0.1	
1577	spathulenol	0.2	0.1	0.1	0.1	0.2	0.2	0.1	
1582	caryophyllene oxide	–	0.2	0.6	0.1	0.3	0.4	0.3	
1649	β-eudesmol	t	t	0.1	t	t	t	0.2	
1652	α-eudesmol	t	t	t	t	t	t	0.1	
2222	**sclareol**	**0.6**	**0.1EP**	**0.5**	**0.2EP**	**tEP**	**0.3EP**	**0.9**	**0.4–2.6**

KI = Kovat's Index on DB-5(= SE54) column; t = trace (< 0.1%); unidentified components less than 0.5% are not reported; Kash = Kashmir oil; Hung = Hungarian oil; Russ = Russian oil; Bulg = Bulgarian oil; Ukrn = Ukrainian oil; Fren = French oil; OR = USA oil (Oregon); EU Pharm = European Pharmacopoeia compounds specified in EP 5.0 are in bold

Chiral analysis of linalool and linalyl acetate for the clary sage oils in this study **T-2**

	linalool			linalyl acetate		
Oil	**(-)-(R)-**	**(+)-(S)-**	**% (-)-(R)-**	**(-)-(R)-**	**(+)-(S)-**	**% (-)-(R)-**
Kashmir	11.3	3.4	76.9	72.4	t	99.9
Hungary	13.5	4.3	76.0	61.4	9.6	86.5
Russia	14.3	6.0	78.9	56.2	12.0	82.4
Bulgaria	17.5	6.3	73.4	54.6	0.4	99.3
Ukraine	19.5	5.6	77.6	58.8	t	99.9
France	16.4	3.3	83.1	66.5	0.5	99.3
Oregon, USA	14.7	5.2	73.8	60.8	0.7	98.7

the following conditions: carrier gas He, 30.5 cm/s (ca. 1 mL/ min), 0.2 µL of 10% solution injected, split 1:15; injector 220°C, oven temperature linear programmed 60°–246°C at 3°C/min., FID detector 240°C, H_2 66 mL/ min, make-up He 30 mL/min, air 300 mL/min. The FID signal (uncorrected) was analyzed using the HP Chemstation software to obtain percent of total oil for individual components.

Chiral analyses were performed on a Restek Rt-DEXse 2,3-di-O-ethyl-6-O-tert-butyl dimethylsilyl β-cyclodextrin infused into 14% cyanopropylphenyl/ 86% dimethyl polysiloxane, 0.25 µ coating thickness, 0.25 mm x 30 m, fused silica capillary column, using the following conditions: carrier gas He, 30.5 cm/s (ca. 1 mL/min), 0.2 µL of 10% solution injected, split 1:15; injector 220°C, oven temperature linear programmed 70°–230°C at 2°C/min, FID detector 240°C, H_2 66 mL/min, make-up He 30 mL/min, air 300 mL/min. Standards [(-)-R-linalool, (+/-) racemic linalool, (+/-) racemic linalyl acetate] were obtained from Aldrich-Sigma Chem. Co.

Results and Discussion

The compositions of the clary sage oils are given in **T-1** along with the EP 5 specifications. All of the oils were high in linalool (14.7–25.1%) and linalyl acetate (55.0–72.4%). The Ukrainian oil was slightly above the EP 5 standard for linalool (24.0%) and the Bulgarian oil was just below the EP 5 lower limit for linalyl acetate (56.0%). All of the oils met the α-terpineol EP 5 limit (< 5.0%). Three of the oils (Hungarian, Russian and Ukrainian) were below the lower limit for germacrene D (EP 5, 1.0–12.0%). Four of the oils were below the EP 5 limit (0.4–2.6%) for sclareol (T-1).

The ketones α- and β-thujones were not detected in any oils thus fulfilling the EP 5 standard of < 0.2% (combined).

The previous report that the commercial Kashmir clary sage oil contained 30% linalool, 52% linalyl acetate and 7.2% α-terpineol differed considerably from the Himalayan Foothills clary sage oil studied in this report.[5] The differences between the Kashmir oil and the Himalayan Foothills oil may reflect different plant germplasm, environmental growth conditions and/or different extraction methodology.[5] Additional field work is in progress to determine the source(s) of these differences.

Chiral analyses (**T-2**) gave a different picture of the variability among the clary sage oils. EP 5 does not set a standard for chiral purity. Bulgarian, Russian and Ukrainian oils had the largest amounts of (+)-(S)-linalool, ranging from 6.3% to 5.6% (T-2). The smallest amounts of (+)-(S)-linalool were in the French and Kashmir oils (3.3% and 3.4%, respectively).

The amount of (+)-(S)-linalyl acetate was quite variable among oils (T-2). The largest amounts of (+)-(S)-lin-

alyl acetate was in Russian (12.0%) and Hungarian oils (9.6%). The other oils had nearly pure (-)-(R)-linalyl acetate, with Kashmiri and Ukrainian oils having only trace amounts of (+)-(S)-linalyl acetate (T-2).

The ratio of (-)-(R)-linalool to total +/- linalool was not very variable, ranging from 73.4% to 83.1%. However, the ratio (-)-(R)-linalyl acetate to total +/- linalyl acetate was variable with both Russian (82.4%) and Ukrainian (86.5%) being clearly different from the other oils that had 99% or more (-)-(R)-linalyl acetate (T-2).

These analyses agree with previous reports for French oil of 80.6–95.0% (-)-(R)-linalool and 93.0–98.1% (-)-(R)-linalyl acetate and Uruguayan clary sage oil 72.9% (-)-(R)-linalool and 99.1% (-)-(R)-linalyl acetate.[3,4] Clary sage oil from Turkey (garden plot) was reported to contain 76.3% (-)-(R)-linalool in good agreement with our results.[6]

In summary, the Himalayan Foothills clary sage oil met all the specifications of the EP 5. Most of the other clary sage oils examined were very close to the EP 5 standards.

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