



## Regular Articles

# Chemical investigation of decanted and hydrophilic fractions of *Salvia sclarea* essential oil

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### Abstract

The decanted and recovered (hydrophilic fraction) essential oils of *Salvia sclarea* obtained from a field distillation unit and Clevenger apparatus distilled essential oil were analyzed by GC and GC-MS. A total of 20 components representing 96.45 %–99.53 % of the oils were identified. The major components of the oils were linalool (27.08 %–62.51 %), linalyl acetate (nil–43.01 %),  $\alpha$ -terpineol (2.12 %–20.58 %),  $\beta$ -myrcene (2.03 %–7.29 %), geraniol (0.74 %–4.84 %), (*E*)- $\beta$ -ocimene (1.19 %–4.83 %) and geranyl acetate (0.36 %–3.11 %). The oxygenated monoterpenes were found to be higher in the recovered oil (90.28 %) followed by Clevenger distilled oil (87.13 %) and decanted oil (80.39 %). Monoterpene and sesquiterpene hydrocarbons were found to be higher in the decanted oil (17.38 % and 1.45 %, respectively) compared with Clevenger oil (11.38 % and 0.64 %, respectively) and recovered oil (6.06 % and nil, respectively). One of the major components of the decanted oil, linalyl acetate, was not present in the recovered oil obtained from the field distillation unit.

**Key words:** *Salvia sclarea*; field distillation; decanted oil; recovered oil; GC-MS; linalool; linalyl acetate

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### Introduction

*Salvia sclarea* L., belonging to the family Lamiaceae is also popularly known as 'Clary Sage'. The plants are 60-100 cm high with large hairy leaves and small blue, white or purple flowers <sup>[1, 2]</sup>. The plant is native to Mediterranean countries, southern France, Italy and Morocco, and is one of the most important plants cultivated worldwide as a source of essential

oils and other perfumery products <sup>[1, 3]</sup>. In traditional herbal medicine the plant is used as an antispasmodic, carminative and oestrogenic agent <sup>[4]</sup>. In aromatherapy, it is reported to be used as an effective relaxant for the treatment of stress, asthma, digestive and menstrual problems and, in addition, it is also reported to have anti-inflammatory, antitumoral, antituberculosis larvicidal, and genotoxic activities <sup>[1, 5, 6]</sup>. The analysis of the essential oils of *S. sclarea* inflorescence and leaves has been reported by a number of researchers <sup>[7-14]</sup>.

In order to meet domestic needs, efforts have now been made to cultivate Clary Sage in the hilly regions of northern India since it was not commercially cultivated earlier in any part of India. It was introduced in the hilly parts of Uttarakhand a few years ago with the objective of making Clary

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Sage a commercially viable crop to meet the domestic and international demands. A number of experiments that are mandatory requirements for agro technology development are currently under progress.

The extraction of essential oils of aromatic crops is generally carried out by hydro/hydro-steam/steam distillation processes and, during the distillation process, the hydrophilic part of the essential oils dissolves in the aqueous distillate or condensed water which is rich in organoleptically important oxygenated components [15, 16]. Therefore, different methods have been developed to recover the dissolved essential oils from aqueous distillates [17-19]. Since *S. sclarea* is a new crop for this region and there has been no report describing the chemical composition of decanted and recovered essential oils derived from a commercial distillation unit, a comparison of these two types of oil with Clevenger apparatus distilled oil has been conducted and the results are described here.

## Materials and methods

### *Plant material and isolation of essential oils*

The fresh spikes of Clary Sage (*S. sclarea*) were collected from the experimental field of the Central Institute of Medicinal and Aromatic Plants, Research Centre Purara, Uttarakhand during first week of July and used for isolation of the essential oils. The site is located at an altitude of 1250 m where the climate is mild. A 32 kg sample of spikes of the Clary Sage was hydro-cum-steam distilled in small stainless-steel field distillation unit (30-35 kg capacity/batch) for 4 hr. A total 43.5 ml essential oil decanted from the receiver was designated as 'decanted oil' or 'direct oil'. The aqueous distillate (21 liters) was collected in a separate container. The pooled 3 liters of the aqueous distillate (hydrosol) was extracted with 600 ml diethyl ether. The organic layer was dried over anhydrous sodium sulphate, filtered and the solvent was evaporated under reduced pressure to obtain 'recovered essential oil'. A sample of *S. sclarea* spikes was also distilled

in the Clevenger apparatus to compare the yield and composition with that of the field distillation unit. Essential oils obtained from the Clevenger apparatus and direct oil from the field distillation unit were dried over anhydrous sodium sulphate, filtered and used for subsequent analyses.

### *Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS)*

The GC analysis of the oil samples was carried out on a Nucon gas chromatograph model 5765 and a Perkin Elmer Auto XL GC equipped with FID and fused silica capillary columns with two different stationary phases, BP-20 (coated with Carbowax 20 M, 30 m × 0.25 mm × 0.25 µm film thickness) and PE-5 (60 m × 0.32 mm; 0.25 µm film coating). Hydrogen was the carrier gas at 1.0 ml/min. Temperature programming was carried out from 70 °C–230 °C at 4 °C /min with an initial and final hold time of 2 min (for BP-20) and from 70 °C–250 °C at 3 °C /min (for PE-5). The split ratio was 1: 30. The injector and detector temperatures were 200°C and 230°C for the BP-20 column and 220°C and 300°C for the PE-5 column. GC-MS data were recorded on a Perkin Elmer Auto System XL GC and a Turbo Mass Spectrometer fitted with a fused silica capillary column, PE-5 (50 m × 0.32 mm, film thickness 0.25 µm). The column temperature was programmed from 100 °C–280 °C at 3 °C/min, using helium as carrier gas at constant pressure of 10 psi. MS conditions were: EI mode 70 eV, ion source temperature 250 °C. Compound identification was based on the retention time, Kovats Index, MS Library search (NIST & WILEY), *n*-alkane (C<sub>9</sub>-C<sub>22</sub>) hydrocarbons pattern (Nile, Italy) and by comparing the mass spectra with the MS literature data [20 - 22]. The relative amounts of individual components were calculated based on the GC peak areas without using correction factors.

## Results and discussion

The recovery of Clevenger distilled, decanted



(direct oil) and recovered essential oils was 0.30 %, 0.136 % and 0.011 %, respectively. The recovery of essential oils in the Clevenger apparatus was higher compared with the field distillation unit due to continuous re-distillation of the condensate water and more controlled distillation of the biomass in the former and incomplete recovery of essential oils from the crop biomass in the latter [23]. Furthermore, the decanted oil accounted for 92.52 % while the recovered oil accounted for 7.48 % from the total oil recovery of the field distillation unit (0.147 %). Similar observations have also been reported for other aromatic crops [15, 23, 24].

GC and GC-MS analyses of the Clevenger

distilled essential oil, decanted and recovered essential oils resulted in the identification of twenty components which are listed in Table 1. The gas chromatograms of these three essential oils are given in Fig. 1, Fig. 2 & Fig. 3. The essential oil obtained from the Clevenger apparatus was found to be rich in linalool (40.24 %), linalyl acetate (34.51 %),  $\beta$ -myrcene (5.47 %),  $\alpha$ -terpineol (5.17 %), geranyl acetate (3.03 %), (*E*)- $\beta$ -ocimene (3.15 %), geraniol (1.76 %), limonene (1.76 %) neryl acetate (1.26 %) and (*Z*)- $\beta$ -ocimene (1.0 %), whereas the decanted oil contained linalyl acetate (43.01 %), linalool (27.08 %),  $\beta$ -myrcene (7.29 %), limonene (3.09 %), (*E*)- $\beta$ -ocimene (4.83 %), (*Z*)- $\beta$ -ocimene (1.99 %)

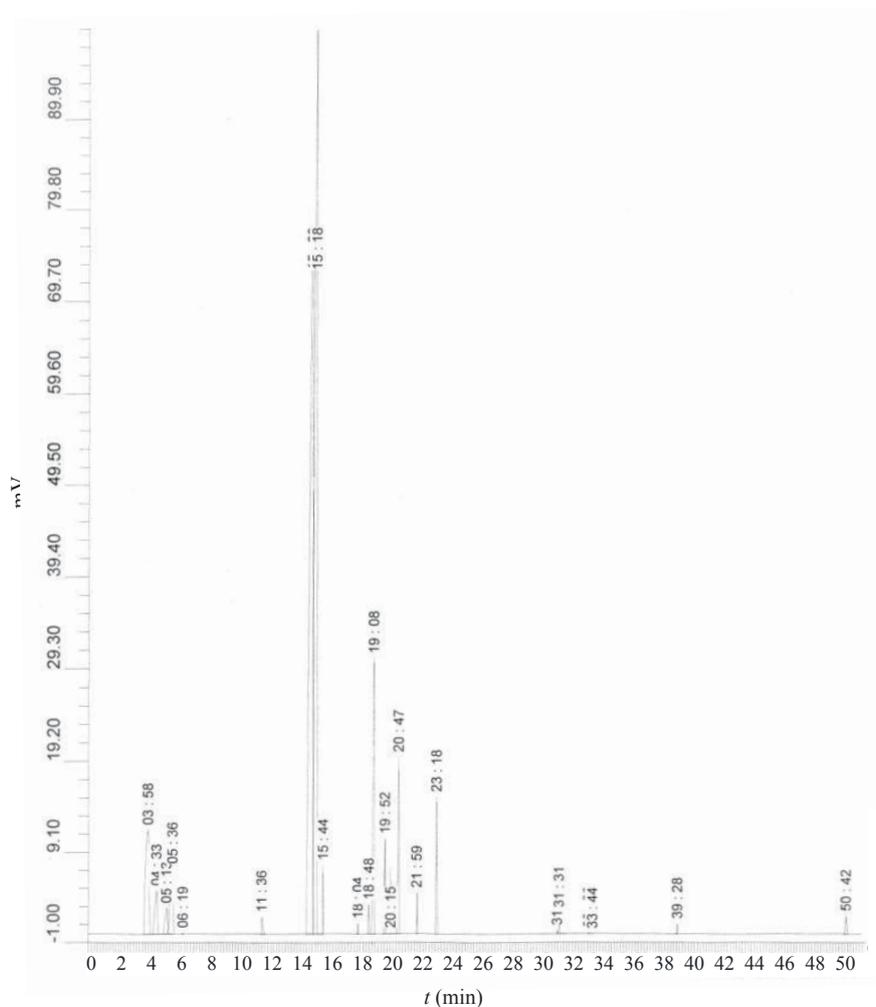


Fig. 1. Gas chromatogram of Clevenger apparatus distilled essential oil of *Salvia sclarea*



Table 1. Chemical composition of Clevenger distilled, decanted and recovered essential oils of *Salvia sclarea*

S. No.	Compound	RI <sup>a</sup>	RI <sup>b</sup>	Peak Area (%)			Detection
				CA	FDU		
					A	B	
1	$\beta$ -Myrcene	1158	989	5.47	7.29	2.03	RI, MS
2	Limonene	1194	1030	1.76	3.09	2.16	RI, MS
3	(Z)- $\beta$ -Ocimene	1234	1038	1.00	1.99	0.47	RI, MS
4	(E)- $\beta$ -Ocimene	1251	1054	3.15	4.83	1.19	RI, MS
5	<i>p</i> -Cymene	1271	1025	t	0.18	0.21	RI, MS
6	(Z)-Linalool oxide	1435	1072	0.35	0.77	t	RI, MS
7	Camphor	1507	1143	-	t	-	RI, MS
8.	Linalool	1550	1098	40.24	27.08	62.51	RI, MS, Co-In
9.	Linalyl acetate	1561	1257	34.51	43.01	-	RI, MS, Co-In
10.	$\beta$ -Caryophyllene	1594	1419	0.64	1.37	t	RI, MS
11	Neral	1685	1238	0.41	2.13	-	RI, MS
12	$\alpha$ -Terpineol	1685	1189	5.17	2.12	20.58	RI, MS
13	$\alpha$ -Terpinyl acetate	1689	1351	-	t	-	RI, MS
14	Germacrene-D	1708	1480	-	0.10	-	RI, MS
15	Neryl acetate	1720	1356	1.26	1.27	0.21	RI, MS
16	Geranyl acetate	1760	1373	3.03	3.11	0.36	RI, MS
17	Nerol	1801	1225	0.40	0.16	1.78	RI, MS
18	Geraniol	1859	1237	1.76	0.74	4.84	RI, MS
19	Caryophyllene oxide	1995	1584	-	-	0.11	RI, MS
20	Sclareol	2387	2224	0.38	-	t	RI, MS
<b>Class composition</b>							
Monoterpene hydrocarbons				11.38	17.38	6.06	
Oxygenated monoterpenes				87.13	80.39	90.28	
Sesquiterpene hydrocarbons				0.64	1.45	-	
Oxygenated sesquiterpenes				t	-	0.11	
<i>Total identified</i>				99.53	99.24	96.45	
<b>Yield</b>							
Essential oil yield *				0.30	0.136	0.011	
Total oil recovery (FDU)				-	0.147 %		
Decanted oil					92.52 %		
Recovered oil (dissolved oil)					7.48 %		

CA: Clevenger Apparatus; FDU: Field Distillation Unit; t: trace (<0.1%)

A: Decanted essential oil from FDU; B: Recovered essential oil from hydrosol of FDU; -: not found;

\* Fresh weight basis; RI<sup>a</sup>: Retention Indices on the BP-20 column; RI<sup>b</sup>: Retention Indices on the PE-5 column;

MS: Mass fragmentation pattern

and  $\beta$ -caryophyllene (1.37 %) as major constituents. On the other hand, the recovered oil was rich in

linalool (62.51 %),  $\alpha$ -terpineol (20.58 %), geraniol (4.84 %), nerol (1.78 %) and caryophyllene oxide

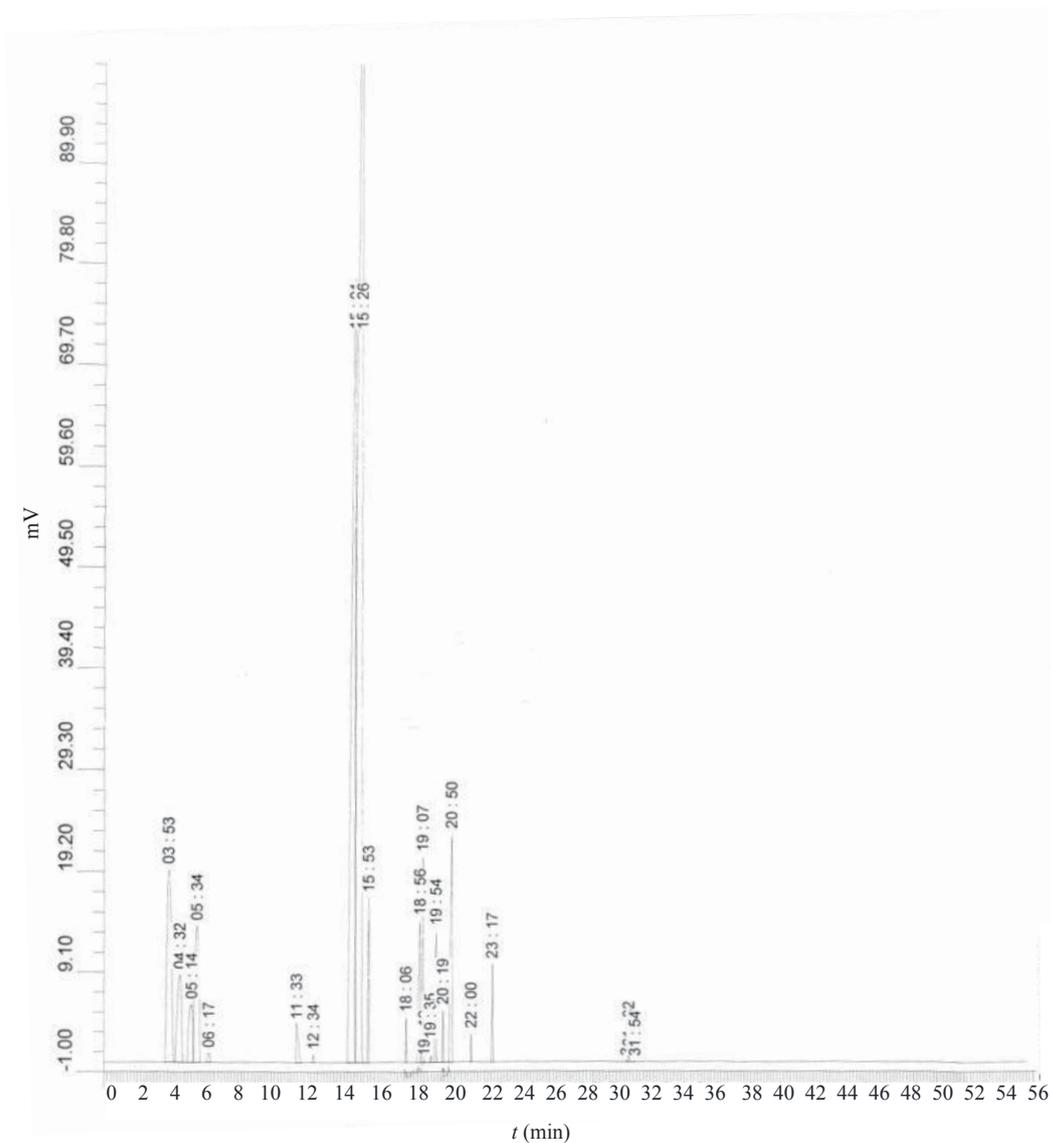


Fig. 2. Gas chromatogram of decanted essential oil of *Salvia sclarea*

(0.11 %). The higher concentration of linalool,  $\alpha$ -terpineol and geraniol in the recovered oil make it an alternate source of these components for industrial applications. The presence of a high concentration of alcohols (linalool,  $\alpha$ -terpineol and geraniol) in the recovered oil and monoterpene and sesquiterpene hydrocarbons in the decanted oil has also been reported in case of rose, lavender and palmarosa [15, 16, 23, 25]. This is due to the relatively higher aqueous solubility of oxygenated

compounds compared with terpene hydrocarbons [19, 26].

Clary sage oil is an important commercial oil and is characterized in the European Pharmacopoeia (EP 5) as containing large amounts of linalool (6.5 %–24 %) and linalyl acetate (56 %–78 %) [14]. However, in the present study, linalyl acetate was lower and linalool was higher than that in EP 5. This could be due to the method of extraction used in this study. Nevertheless, the decanted oil is better than Clevenger

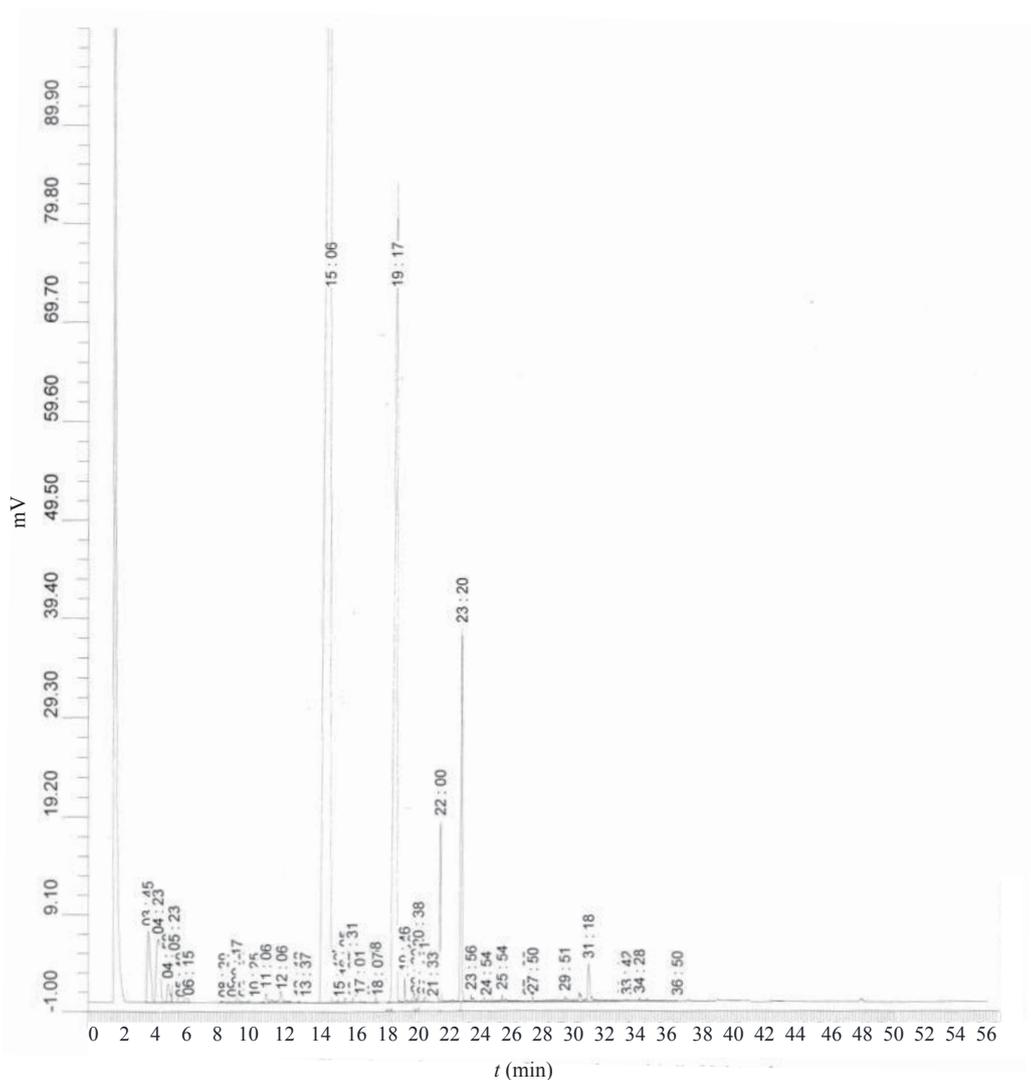


Fig. 3. Gas chromatogram of recovered essential oil of *Salvia sclarea*

and recovered oils in term of the amount of these two major components. Therefore, on the basis of EP 5 specifications, it is concluded that the recovered oil may be used for other purposes and should not be added to the decanted oil to avoid any deterioration in the quality of Clary Sage oil.

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