International Journal of Secondary Metabolite
2023, Vol. 10, No. 1, 38–47

https://doi.org/10.21448/ijsm.1093888

Published at https://dergipark.org.tr/en/pub/ijsm

Research Article

Optimization of supercritical fluid CO2 extraction of Opoponax essential oil (C. erythraea)

Fatih Durak[1], **Aydin Alemdar**[1], **Munevver Sokmen**[2,*]

[1]MG International Fragrance Company Research and Development Laboratory, Department of Synthesis and Naturals, Kocaeli, Türkiye
[2]Department of Bioengineering, Faculty of Engineering and Architecture, Konya Food and Agriculture University, Konya, Türkiye

Abstract: Essential oil of Opoponax (*Commiphora erythraea*) was extracted by means of steam distillation and supercritical CO_2 (SFE-CO_2) extraction. Experiments were performed using a Clevenger-type apparatus. On the other hand, SFE-CO_2 runs were carried out using OCOLABS extraction unit at the operating conditions of 150, 200, and 250 bars and within temperature range from 50 to 65 °C. Other extraction parameters such as particle size and extraction period were also investigated in order to validate their effect on the processes and the oil yield. The extraction yields were almost similar (2.46%) for the optimized SFE-CO_2 at 250 bar, 65 °C, and 2 hr, and 2.45% for the steam distillation. Our findings confirmed that the grinding of the material (particle size), pressure, and temperature had an important effect on the SFE-CO_2 extraction process. GC/MS analyses revealed that major compounds, namely α-santalene, α and β-bisabolone, and trans-alpha-bergamotene were of similar quantities except for ocimene. α-Santalene concentration was 7.15% for steam distillation and 15.32% for SFE-CO_2 at the abovementioned conditions.

ARTICLE HISTORY

Received: Mar. 26, 2022
Accepted: Jan. 26, 2023

KEYWORDS

Opoponax,
Essential Oil,
SFE,
Steam distillation,
GC-MS.

1. INTRODUCTION

Supercritical fluid extraction (SFE) is an environmental friendly, a fast, and an efficient method for the extraction of many compounds especially in non-polar nature from plant matrices (Tao *et al*., 2014). Carbon dioxide (CO_2) is used under supercritical conditions to extract the principle aromatics from plant materials without using organic solvents. High pressure and temperature transform CO_2 into supercritical carbon dioxide which is neither a gas nor a liquid. In this phase CO_2 supercritical fluid uses both properties being in gas and liquid forms. Therefore, it is able to act as a good and effective solvent. Moreover, after the extraction period, CO_2 quickly and completely evaporates from the extract which is free from solvent residue. It means the process does not require additional steps to remove the solvent itself. Since it is extracted at mild conditions, the resulting oil has an aroma closer to that of the natural plant. As oxidation or degradation during the extraction is mostly prevented, an essential oil mixture obtained from the nature represents the aroma of each oil component and the combination of the aromas gives

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*CONTACT: Munevver Sokmen ✉ munevver.sokmen@gidatarim.edu.tr MG International Fragrance Company Research and Development Laboratory, Department of Synthesis and Naturals, Kocaeli, Türkiye

ISSN-e: 2148-6905 /

the distinct fragrance. Trace components can be very important. Although their concentration is low in the natural source, they may give the specific scent to the oil. Thus, the natural proportion of the components should be maintained during the extraction of the essential oil from any resources. The supercritical fluid extraction (SFE) technique has remarkable advantages when compared to those of known traditional procedures such as liquid-liquid solvent extraction. Due to the low temperature process, the natural characteristics of the oils and their original components are preserved during the extraction process. As a result of organic solvent free low temperature, extraction and transformation of the components from thermal degradation or hydrolysis can be prevented while solvent contamination is not observed. If controlled extraction conditions are applied, the SFE provides rapid extraction of oils from complex matrices and provides high selectivity for certain compounds (Anitescu *et al*., 1997)**.** There are some important reports in the literature about the selectivity of SFE (Ensieh *et al*., 2007; Khajeh *et al*., 2010; Ouzzar *et al*., 2015).

Opoponax gum resin (*Commiphora erythraea*) is produced in Somalia and known as sweet myrrh and bisabol myrrh because of its earthy-sweet, balsam-like, and lavender-like odour. It is traditionally said to be the noblest of all incense gums. It was used to guard people from negative influences, to strengthen the senses, and to increase awareness and intuition (www.scents-of-earth.com)**.**

The essential oil of *C. erythraea* is used in perfume industry as the class of the Oriental type. The term “opoponax” is confused with other species, *Commiphorae* oil, and with the gum latex oil of some *Umbelliferae*. Most of the published studies related to the analysis of opopanax oil were performed on commercial oils. The first study was reported by Ikeda et al. (1962) on the composition of the essential oil confirming mostly presence of ocimene. Presence of isomeric bisabolenes, and of α-santalene were reported by Nigam and Neville (1968). Following these studies Wenniger and Yates (1969) reported the presence of sesquiterpene hydrocarbons of commercial opopanax oil and along with bisabolenes, they identified other components such as α-cubebene, α-copaene, caryophyllene, β-elemene, *epi*-β-santalene, δ- and γ-cadinene, α- and β-santalene, δ- and γ-elemene, *cis*-, and *trans*-α-bergamotene. Unlike Tucker (1986) some researchers believe that opopanax also belongs to other species such as *C. kataf* (Forsk.) Engl. and *C. guidottii* (Chiov). Therefore, some close morphological similarities of Yemeni shrubs of *C. kataf* to *C. erythraea* are reported (Wood, 1997; Marcotullio *et al*. 2009).

C. erythraea is an industrial plant containing specific essential oil constituents and adds great value to the perfumery industry. Trace components can add distinctive quality to final product if they are present in the preparation. The oil obtained mostly by steam distillation is usually exported to perfume producing companies. The aim of the present work is to compare two different techniques, steam distillation, and supercritical CO_2 extraction of *C. erythraea* essential oil with respect to the extract yields and process time, pressure, and temperature for supercritical CO_2 extraction. The study was also structured to investigate the effects of the particle size of the gum material (ground and unground) on its extraction performance.

2. MATERIAL and METHODS

2.1. Gum Material

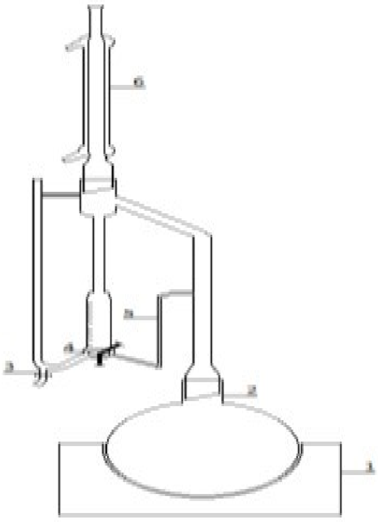
Opoponax gum (*Commiphora erythraea*) was purchased from Neo Gr Limited (England), origin of the product Somalia at Grade 1 and is an amorphous non uniform solid in its natural form (Figure 1). Natural gum is used either directly or ground in a mechanical grinder (15 s) to get a smaller but uniform particle size distribution. 100g of gum is used for the extraction either in the natural form or ground to an approximate powder size (0.2-1mm).

Figure 1. Opoponax (*Commiphora erythraea*) gum in natural form.

2.2. Steam Distillation

The steam distillation experiment was carried out in a Clevenger-type apparatus following the procedure given in the 10th edition of the French Pharmacopeia (Pharmacopée Française). As shown in Figure 2 the system was operated at atmospheric pressure and the mass ratio (1:10) was kept constant by adding water during the process. 100 g of opoponax gum and 1L water were placed in a ball and the mixture was heated to the boiling temperature. The oil was collected over water column concentrated in a flow tube after 4 hours extraction time.

Figure 2. Steam distillation equipment.



1 - Heating mantle, 2 - Two liters flask, 3 - Essential oil output 4 - Three ways valve, 5 - Return tube, 6 – Condenser.

2.3. Supercritical Fluid Extraction (SFE-CO_2) of Opoponax Gum

SFE-CO_2 extraction experiments were carried out at different conditions (temperature, pressure, and time) in the OCOLABS Experimental Supercritical CO_2 Extraction system (USA). The bench scale apparatus consists of CO_2 dip-tube, supercritical fluid extractor; five cylinder separators were assembled in series first with inner volume of 100 mL and the others with 400 mL. The desired pressure and temperature values in the tubes were maintained by feed valve by adequate regulation of CO_2 flow rates, measured by a barometer. When the pressure came to adequate amount in the system, the feed valve was closed.

The filling of the extraction vessel with CO_2 was made directly from a moderately heated cylinder (30-90°C) fitted with a dip tube. 100 g of opoponax was filled to the four cylinder separators for each run. One experiment was also carried out with unground material in order to see the effect of particle size on oil yield.

SFE-CO_2 runs were carried out using OCOLABS extraction unit at the operating conditions of 150, 200, and 250 bars range pressure and at 50-65°C range temperature. Particle size was 0.2-1mm for ground samples and non-uniform 1-2 cm larger particles were also used for extraction. SFE-CO_2 was carried out for 2 or 4 hours at different pressure and temperature to investigate the effect of process periods on oil yield.

2.4. Gas Chromatography-Mass Spectrometry Analysis (GC-MS)

GC-MS data were obtained using the Agilent Technologies: 7890B GC System Agilent Technologies 5977A MSD equipped with a split-splitless injector (250 °C) and with a fused silica DB-1 column (60 m; 0:25 mm i.d; 0.25 mm thickness, Agilent J&W). Helium was used as carrier gas, with a rate through the column of 1.0 mL/ min and septum purge of 4 mL/min. The split ratio was 1:100, and the volume of injected samples was 0.1 mL. The GC oven was programmed as follows: first, hold 5 min at 50 °C to 220 °C with a heating rate 4 °C/min and then hold 27.5 min at 220 °C. The interface temperature was 280 °C. Data acquisition was performed with Enhanced Chem Station for the mass range 40± 400 a.m.u. with a scan speed of 1 scan/sec. The ionization energy of electrons was 70 eV. The identification of compounds was based on a comparison of their mass spectra with NIST (2017) mass spectral library and by relative retention time of compounds identified previously. The percentage composition of the essential oil was computed in each case from GC peak areas without using correction factors.

2.5. AT-FTIR, Color and Refractive Index Analysis

Attenuated total reflectance Fourier transform infrared (ATR-FT-IR) results provided the information about the structural characteristic of the essential oils. ATR-FT-IR spectra of samples were characterized by Agilent Cary 630 ATR- FTIR Spectrometer equipped with the ATR accessory system in 650-4500 cm^{-1} ranges.

Color analysis data were obtained by Konica Minolta CR-5 at room temperature. L, a, and b parameters express the color analysis. The three coordinates were measured by CIELAB in terms of 1) the lightness of the color (*L*= 0 yields black and *L*= 100 indicates diffuse white), 2) its position between red and green (*a*, where negative values indicate green and positive values indicate red), and 3) its position between yellow and blue (*b*, where negative values indicate blue and positive values indicate yellow). Refractive Index data were obtained by Anton Pear DMS 4500M. Operation condition was at room temperature.

3. RESULTS

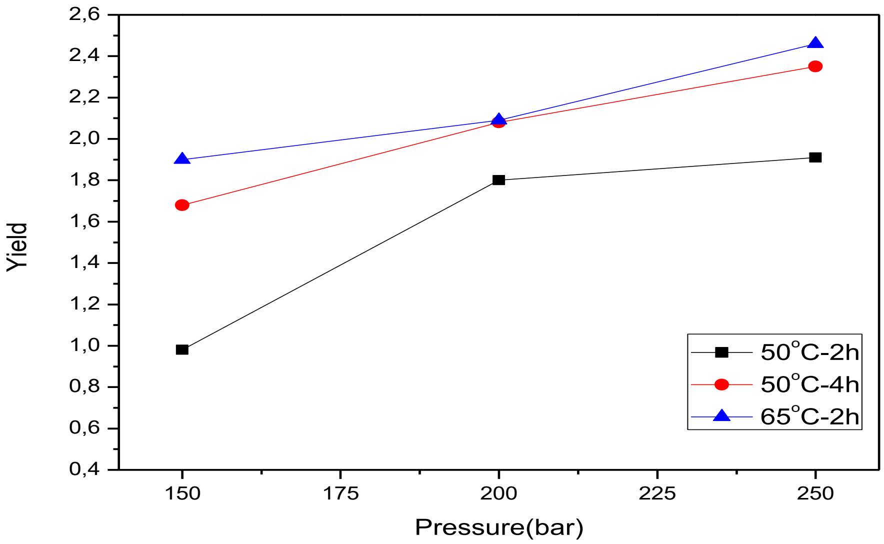
3.1. Extraction Yields: Effect of the Extraction Pressure, Temperature and Extraction Period in SFE-CO_2

Effect of the studied extraction parameters on oil yields are given in Table 1. These results are shown in Figure 3 to make better evaluation. The extraction process is affected from various parameters; therefore, the optimization of the experimental conditions is critical in the development of a SFE method. Generally, applied pressure, temperature, and extraction period are considered as the most important factors during SFE. It is clear from Table 1 that particle size, increased extraction time, and pressure led to a greater amount of the accumulated essential oil. Additionally, the oil yield increased by increasing the temperature at the same extraction period and pressure, which is due to the enhancement of the mass transfer area and provision of shorter diffusion paths.

Table 1. Oil yields (%w/w) of SFE-CO_2 extraction for the parameters studied.

Amount (g)	Pressure (bar)	Temperature (°C)	Time (hr)	Yield (%)	Sample Type
100	150	50	2	0.98	Ground
100	200	50	2	1.80	Ground
100	250	50	2	1.91	Ground
100	150	50	4	1.68	Ground
100	200	50	4	2.08	Ground
100	250	50	4	2.35	Ground
100	150	65	2	1.90	Ground
100	200	65	2	2.09	Ground
100	250	65	2	2.46	Ground
100	250	65	2	0.80	Unground
100				2.45	Steam Distillation (Ground)

Figure 3. Essential oil extraction yield (%) as a function of the pressure, time, and temperature.

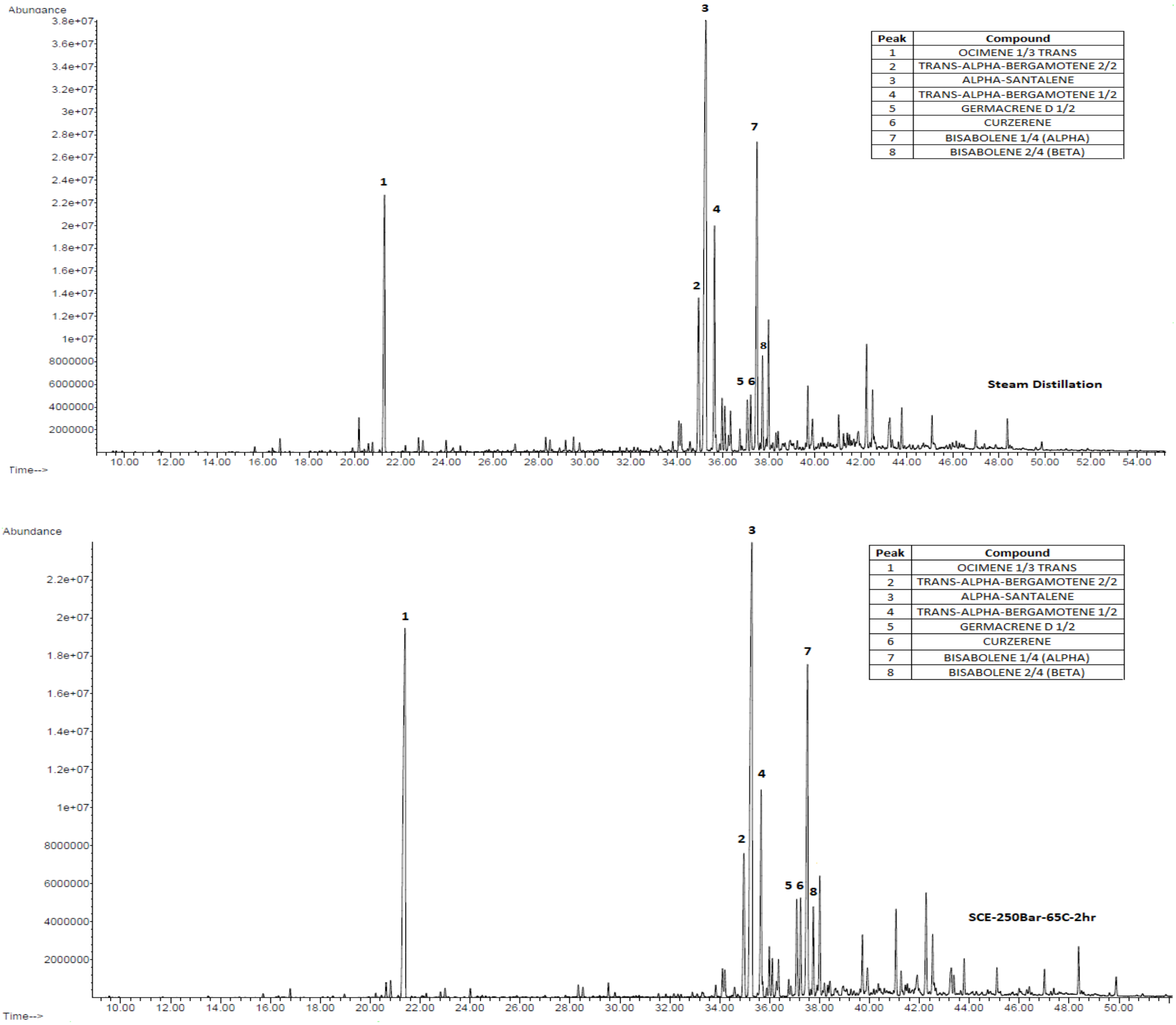


As a result, the decrease of the diameter enhanced mass transfer leading to a better extraction yield. The increased yield of the initial parts of the extraction curves with increasing pressure indicated the accelerated solubility of the oil in supercritical fluid. The yield of opoponax oil increased from 0.98% to 2.46% as pressure increased from 150 to 250 bar. The solubility of the oil in supercritical fluid at high pressure increases the driving force and consequently the mass transfer rate is improved significantly (Özkal, 2004). Similarly, the solubility of the oil in SFE-CO_2 increased by the increasing temperature. The oil yield increased from 1.91% to 2.46% at 250 bar with increase in temperature from 50 to 65 °C after 2 h extraction. Results showed that increasing extraction time to 4 h produced higher extraction yield as recommended in the literature (Anitescu *et al.*, 1997). The optimum yield (2.46%) was obtained at 250 bar, 65 °C temperature and 2 hours' extraction period. It should be noted that oil yield was only 0.8% when unground gum is extracted at the same conditions. It shows how grinding has a positive effect providing large surface area that allows the better mass transfer. The oil yield was 2.45% in Clevenger extraction after 4 h steam distillation (Table 1) using the ground gum. This oil was used to make comparisons related to oil components between SFE-CO_2 and steam distillation processes.

3.2. GC-MS Analysis

GC/MS chromatograms of the oils obtained by both methods are given in Figure 4. The 46 compounds were identified and are presented in Table 2. According to GC/MS analyses, similar results were obtained from the steam distillation and supercritical fluid extraction. It is clear that the oil composition was quite similar for major compounds in both of the oil samples; however, some minor compounds were observed in only one process.

Figure 4. GC-MS chromatogram for opoponax oil obtained by steam distillation and SFE-CO_2.



These compounds are α-thujene, sabinene, β-pinene, Δ-careven, carvomenthene, 2-methoxyphenol, cis-verbenol, alcohol C12 dodecylique, furanoeudesma-1,3-diene, and lindestrene for steam distillation and limonene, linalool, methyl acetophenone, 8-hydroxy p-cmene, trans-carveol, α-cubenene, α-longipene, α-copaene, α-humulene, allaoro mandedrene and tau cadinol for SFE-CO_2 extraction. GC-MS analyses revealed that major compounds (α-santalene, α and β-bisabolone, trans-alpha-bergamotene) were of similar quantities except for ocimene. Its concentration was 7.15% for steam distillation and 15.32% for SFE-CO_2, while several important differences were observed between these oils. The percentage of the most desired oxygenated compounds is higher in the oil obtained by SFE-CO_2. Some fatty acids and trace amount of esters were also extracted by this process, but these do not have a negative influence on the oil quality. Organoleptic comparison of the oils showed that the aroma of the oil obtained from SFE-CO_2 was more intense and more pleasant.

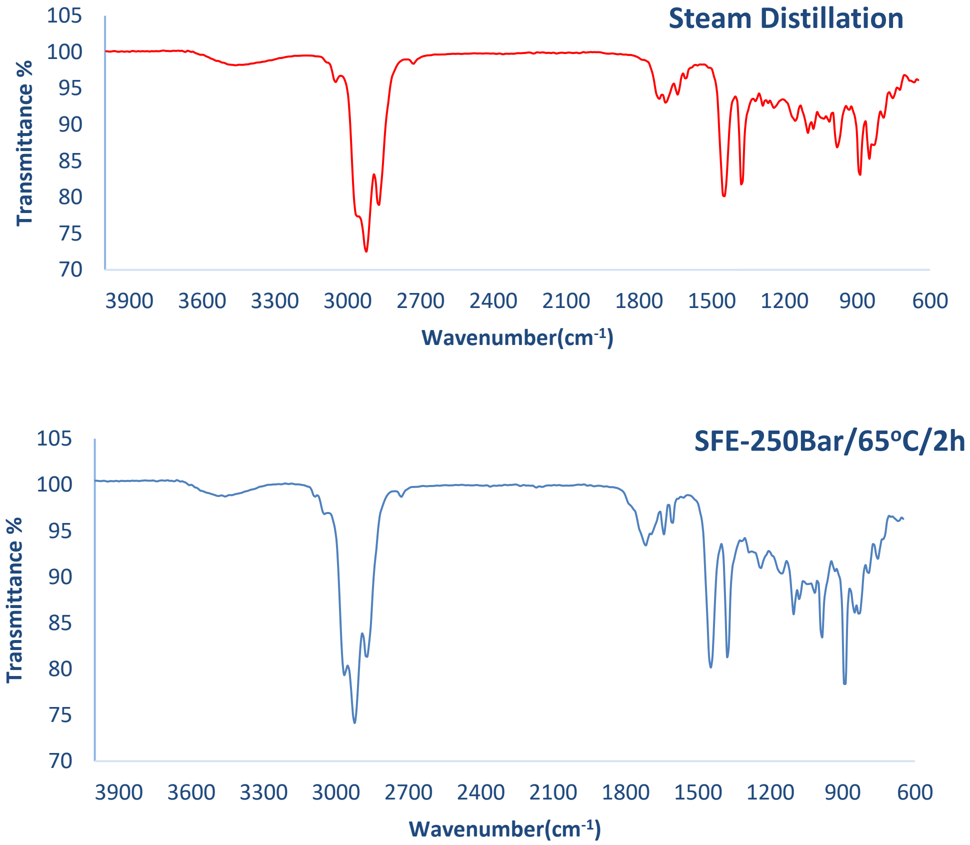
Table 2. Comparative percentages of the identified compounds in opoponax oils.

Compound	Retention Time (min)	Steam Distillation (%)	SFE 250 Bar 65 ºC 2 h (%)	Kovats RI
4,4-DIMETHYL BUTENOLIDE	15.65	0.108	0.075	-
TRICYCLENE	16.28	0.030	0.033	921
ALPHA-THUJENE	16.42	0.080	-	924
ALPHA-PINENE	16.75	0.260	0.164	939
SABINENE	18.25	0.016	-	969
BETA-PINENE	18.46	0.028	-	979
MYRCENE	18.93	0.046	0.062	990
DELTA-CARENE	19.90	0.100	-	1002
PARA-CYMENE	20.18	0.638	0.082	1024
CARVOMENTHENE	20.41	0.064	-	1026
LIMONENE	20.63	-	0.287	-
OCIMENE 2/3 (CIS)	20.77	0.182	0.333	1037
OCIMENE 1/3 (TRANS)	21.29	7.147	15.321	1050
LINALOOL	23.13	-	0.013	-
2-METHOXY PHENOL 1/2	23.77	0.277	-	-
4-ACETYL-1-METHYL CYCLOHEXENE	23.97	0.245	0.172	1130
MYROXIDE 1/2	24.58	0.121	0.031	1132
CIS-VERBENOL	24.88	0.033	-	1141
METHYL ACETOPHENONE 1/3	25.87	-	0.045	1182
8-HYDROXY P-CYMENE	26.13	-	0.015	-
TRANS-CARVEOL	27.52	-	0.020	-
ALCOOL C-10	29.51	0.303	0.276	1269
ALPHA-CUBEBENE	32.91	-	0.117	-
ALPHA-LONGIPINENE	33.1	-	0.077	-
ALPHA-COPAENE	33.84	-	0.268	1376
SESQUITHUJENE (7-EPI) 2/2	34.08	0.695	0.613	1389
BETA-ELEMENE 1/2	34.18	0.617	0.568	1390
SESQUITHUJENE (7-EPI) 1/2	34.57	0.270	0.255	1400
TRANS-ALPHA-BERGAMOTENE 2/2	34.94	4.389	4.047	1412
ALPHA-SANTALENE	35.26	19.167	18.464	1417
TRANS-ALPHA-BERGAMOTENE 1/2	35.63	5.457	4.725	1434
EPI-BETA-SANTALENE	35.96	1.034	0.964	1447
BETA-SESQUIPHELLANDRENE 2/2	36.08	0.891	-	-
ALCOOL C-12 DODECYLIQUE	36.24	0.359	-	1470
ALPHA-HUMULENE	36.27	-	0.350	
BETA-SANTALENE	36.33	0.833	0.756	1459
ALLOAROMADENDRENE	36.54	-	0.039	
AR-CURCUMENE	36.73	0.446	0.336	1480
GERMACRENE D 1/2	37.06	1.388	2.196	1481
CURZERENE	37.20	1.263	2.027	1499
BISABOLENE 1/4 (ALPHA)	37.48	8.588	9.848	1505
BISABOLENE 2/4 (BETA)	37.72	1.932	1.776	1507
DELTA-CADINENE	38.16	0.204	0.294	1523
TAU-CADINOL	40.43	-	0.230	1640
FURANOEUDESMA-1,3-DİENE	41.24	0.900	-	-
LNDESTRENE	41.31	0.448	-	-

3.3. Spectral Analyses

AT-FT-IR spectrums of oils are given in Figure 5. Results show that only minor differences were observed between the extraction methods. Specific absorption bands were identified at: 2929-2914 cm^{-1} and wavenumber region showed C-H band from CH_3 and CH_2, 1647-1640 cm^{-1} (C=C) band/exocyclic methylene groups, 1453-1438 cm^{-1} CH_2 and CH_3 bending bands, 1379-1364 cm^{-1} CH_3 bending bands ,1244-1237 cm^{-1} (C-O) stretching bands,1021-1013 cm^{-1} C-O bending band (Carrión-Prietoa *et al.*, 2017).

Figure 5. FT-IR analysis of opoponax oil obtained by both methods.



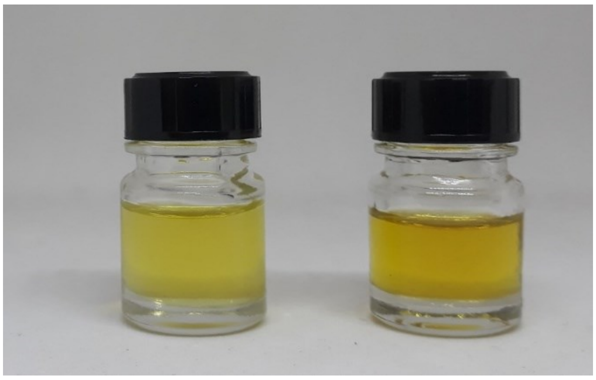
Detailed vibrational frequencies are given in Table 3.

Table 3. Vibrational spectra of opoponax oil.

Frequency (cm^{-1})	Vibration Type
2929	asymmetric *v*(CH) from CH_3 asymmetric *v*(CH) from CH_2 asymmetric *v*(CH) from CH_3
1640	ν(C=C) disubstituted olefins; olefinic terpenoids, *v*(C=C) aromatic ring
1446	*δ*(C-H) from CH_2 or CH_3 groups; CH_2
1379	*δ*$(CH_2)_2$ bending deformation
1238	*δ*(C-H), ν(C-O-H).
	methyl ester, ν(CO), *v*(C-C)
1021	stretching vibration of C–O ester groups

Color values of the oil obtained from steam distillation are L= 67.82, a=-8.48, b=44.98 and from SFE-CO_2 they are L=87.28, a=-6.4, b=61.98. Apparently, oil color was darker in the latter (Figure 6).

Figure 6. Colors of the essential oils; steam distillation (left), SFE-CO_2 (right).



The refractive index (n) of a material has no universal units but expresses how fast light travels through the material. A higher refractive index corresponds to decreasing the speed of light in the material. The n values are 1.4737 and 1.4896 for steam distillation and SFE-CO_2 opoponax oil, respectively.

4. DISCUSSION and CONCLUSION

The opoponax essential oils were extracted by supercritical fluid extraction and steam distillation method and the results (either as yield% or oil composition) obtained from these two methods have been compared. The SFE-CO_2 was carried out for four parameters, namely temperature, pressure, time, and particle size and the effect of these parameters on the extraction yield was studied. The experiments were designed to obtain a high extraction yield with better organoleptic and spectral properties. The pressure and temperature were the most important factors in the SFE-CO_2. The extraction yields significantly increased as the pressure/temperature increased. The best extraction yield was 2.46% and obtained at 250 bar pressure and 65°C with a mean particle size of 0.2-1 mm. This result was almost the same as the one obtained from the steam distillation. The essential oil obtained from supercritical fluid extraction was deeper in color and odor if compared to the one obtained from steam distillation. The main components of opoponax essential oil were α-santalene and bisabolene 1,4 alpha, however ocimene concentration was two times higher than that in SFE.

Acknowledgments

The authors would like to MG International Fragrance Company for providing financial support throughout this research.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Fatih Durak: Investigation, Resources, Experimental, Data Analysis. **Aydin Alemdar:** Investigation, Resources, Experimental, Data Analysis, Writing. **Munevver Sokmen:** Methodology, Supervision, Validation, Experimental, Data Analysis and Writing.

Orcid

Fatih Durak https://orcid.org/0000-0002-6199-9925
Aydin Alemdar https://orcid.org/0000-0002-8069-1248
Munevver Sokmen https://orcid.org/0000-0002-6769-118X

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