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Assessment of Volatile Chemical Composition of Essential Oil of *Schinus molle* (L.), *Myrtus communis* (L.), and *Eucalyptus camaldulensis* (Dehnh.) Grown in Erbil, Iraq

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Abstract

Myrtus communis (L.) and Eucalyptus camaldulensis (Dehnh.), both members of the Myrtaceae family, are extensively prevalent throughout the Middle Eastern region. *Schinus molle* (L.), a member of the Anacardiaceae family, is native to South America and has recently been introduced to Iraq to determine its ability to adapt and grow under the climatic conditions of Iraq. This study focused on the chemical comparison of essential oils (EOs) extracted from the leaves of this species grown under the climatic conditions of Erbil by hydrodistillation, followed by analysis using GC-FID-MS. The results showed that a total of fifty-two compounds were identified, revealing that the EO was rich in monoterpenes, with percentages of 71%, 83%, and 91% for SMEO, MCEO, and ECEO respectively. 1,8-Cineole (46.4%), α-Phellandrene (10.68%), Isobicyclogermacrenal (5.85), β-Atlantol (5.73%), α-Vetispirene (5.09%), and Bicyclogermacrene (4.5%) were the dominant components of SMEO. The most prevalent components of MCEO were *α*-pinene (26.84%), 1,8-Cineole (16.79 %), 3-methylthio-Propanal (13.76%), Limonene (12.57%), and Linalool (11.3 %). Moreover, the major components of ECEO were Limonene (41.52%), α-Phellandrene (34.99%), α-Pinene (5.49%), Myrcene (2.8%), and Elemol (2.59%). It has been concluded that geographical region and climatic conditions play a major role in the variation of EOs chemical composition.

Keywords: GC-MS; Essential Oils; Chemical Comparison; *Schinus molle*; *Myrtus communis*; *Eucalyptus camaldulensis*; Erbil; 1,8-Cineole.

تقييم المحتوى الكيميائي المتطاير للزيوت الأساسية لكل من *Schinus molle* (L.)، *Myrtus communis* (L.) و *Eucalyptus camaldulensis* (Dehnh.) النامية في أربيل, العراق

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الخلاصة

ينتمي كل من نبات الآس (L.) *Myrtus communis* و الأوكالبتوس *Eucalyptus camaldulensis* (Dehnh.) الى الفصيلة الآسيّة Myrtaceae اللذين ينتشران على نطاق واسع في منطقة الشرق الأوسط. من جانب آخر, فأن نبات (L.) *Schinus molle* من الفصيلة البطمية Anacardiaceae، التي تعتبر أمريكا الجنوبية موطنه الأصلي, تم أدخاله مؤخراً إلى العراق لتحديد قدرته على التكيف والنمو تحت الظروف المناخية للعراق. تركّز هذه الدراسة على المقارنة الكيميائية بين الزيوت الأساسية (EOs) التي تم استخراجها من أوراق هذه الأنواع الثلاثة المزروعة في ظل الظروف المناخية لمدينة أربيل عن طريق التقطير المائي, ومن ثم تحليلها بواسطة GC–FID–MS. في النتيجة، تم تشخيص اثنين وخمسين مركبًا مع الكشف عن وجود زيوت أساسية غنية بالتربينات الأحادية بنسب 71%، 83%، و91% لكل من SMEO، MCEO، وECEO على التوالي. كانت 1,8–Cineole (46.4%), α–Phellandrene (10.68%), Isobicyclogermacrenal (5.85%), β–Atlantol (5.73%), α–Vetispirene (5.09%), و Bicyclogermacrene (4.5%) هي المكونات السائدة لـ SMEO. في حين المكونات الرئيسية لـ MCEO كانت α–pinene (26.84%), 1,8–Cineole (16.79 %), 3–methylthio–Propanal (13.76%), Limonene (12.57%), و Linalool (11.3 %). علاوة على ذلك، كانت المكونات الرئيسية لـ ECEO هي Limonene (41.52%), α–Phellandrene (34.99%), α–Pinene (5.49%), Myrcene (2.8%), و Elemol (2.59%). استنتجت من هذه الدراسة أن المنطقة الجغرافية والظروف المناخية تلعبان دورًا رئيساً في تباين التركيب الكيميائي للزيوت الأساسية والعطرية كماً و نوعاً.

1. Introduction

Aromatic plants have been utilized for centuries in food, food additives, cosmetics, and medicines since time immemorial. Essential oils (EOs) are complex mixture of volatile compounds extracted by steam distillation, hydrodistillation or solvent extraction. These oils are a part of various plant secondary metabolites that responsible for plant aroma characteristic. The primary constituents of EOs are terpenoids and phenylpropanoids, along a few aromatic and aliphatic constituents are also present.

Myrtle (*Myrtus communis*) L. and River Red Gum (*Eucalyptus camaldulensis* Dehnh.) are two significant members of the Myrtaceae family, primarily found in Central Asia, the Middle East, and the Mediterranean Basin [1-3]. These plants are abundant in EOs, which have led to their extensive use in traditional medicine since ancient times [4-6]. The main constituents of EO obtained from *M. communis* (MCEO) were discovered to be Z-geraniol, E-caryophyllene, eugenol, estragole, α-terpineol, 1,8-cineol, α-terpinyl acetate, 1,8-cineole, linalool, linalool acetate, α-pinene, and limonene in the majority of locations [1, 2, 4, 7-9]. Additionally, 1,8-cineole, terpinen-4-ol, spathulenol, sabinene, linalyl acetate, linalool, p-cymene, cryptone, cuminaldehyde, α-pinene, α-phellandrene, γ-terpinene, β-pinene, E-pinocarveol were found in *E. camaldulensis* EO (ECEO) from different countries [5, 10-14].

Furthermore, *Schinus molle* L., commonly known as the Pepper tree, is a cosmopolitan plant belonging to the Anacardiaceae family and is native to South America widely used in folk medicine [15]. EO of *S. molle* L. (SMEO) have been studied in many researches for its promising pharmaceutical applications [16, 17]. The most abundant constituents were β-myrcene (58.7 %) from Morocco [18], O-Cymene (29.04%) from Mexico [17], epi-α-cadinol (22.8%) from Brazil, in addition to some other ingredients viz.; α-Pinene, camphene, α-Phellandrene, Limonene, β-Phellandrene, γ-cadinene and, β- pinene [15-21].

Previous studies point out that the proportions of EOs components considerably vary even within the same species, depending on many factors, including geographic location and surrounding climate. In addition, plant part used and the extraction method affect the yield and constituents of the EOs [10, 22]. Nevertheless, *S. molle* L. is recently cultivated in Erbil Governorate, northern Iraq, as one of the new plants imported by the Research Center-Ministry of Agriculture in the Kurdistan Region, Iraq. Therefore, this study aims to evaluate

the impact of geographical and climatic effect on volatile chemical constituents of EO obtained from the leaves of SMEO grown in Erbil. Additionally, it seeks to compare it with both MCEO and ECEO under Erbil climate condition.

2. Experimental

Plant materials and Isolation of EOs

The leaves of the studied plants were collected in June 2023 in Erbil (Altitude 418 m). A voucher specimen has been deposited by Assist. Prof. Dr. Abdullah Shukur Sardar from the college of education, Salahaddin University- Erbil. EO samples were extracted from the leaves using a Clevenger-type apparatus and hydrodistillation for 3 hours. The oils were then stored in airtight vials at 4 ºC until analysis [23].

Chromatographic analyses:

Gas Chromatography- Flame Ionization Detector (GC–FID) and Gas Chromatography-Mass Spectrometer (GC–MS) analysis

GC analysis was carried out on a ThermoQuest–Finnigan Trace gas chromatograph with a flame ionization detector (FID). The analysis was performed by using fused silica capillary DB-5 column (60 m × 0.25 mm; film thickness 0.25 μm). Nitrogen was used as carrier gas at a flow rate of 1.0 mL/min. The oven temperature was programed from 60 to 250 °C at the rate of 5 °C/min, and finally held isothermally for 10 min. GC–MS analysis was carried out on a ThermoQuest–Finnigan gas chromatograph equipped with above mentioned column, used under the same conditions coupled to a TRACE mass spectrometer. Helium was used as the carrier gas at a flow rate of 1.1 mL/min with a split ratio of 1:50. The quadrupole mass spectrometer was scanned over 45–465 amu (atomic mass unit) with an ionizing voltage of 70 eV. Ion source and interface temperatures were kept 200 and 250 °C, respectively. The injector and detector temperatures were kept at 250 and 300 °C, respectively [23].

Identification of compounds

The constituents of the EO were identified by calculation of their retention indices under temperature programmed conditions for homologous series of *n*-alkanes (C_6–C_{24}) and the EO on a DB-5 column under the same chromatographic conditions. individual Compounds were identified by comparing their mass spectra with internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature [24]. Each sample was analyzed three times using GC-FID to determine the percentage concentration of each constituent.

3. Results and Discussion

The main components in the EOs of three different species *S molle*, *M. communis*, and *E. camaldulensis* grown under the same conditions were determined using GC-MS. The hydrodistillation of the leaves of all studied plants gave light yellowish oil with yields GC-MS. The hydrodistillation of the leaves of all studied plants produced a light yellowish oil with yields of 0.73 %, 0.45%, and 0.88% (v/w) for *S. molle*, *M. communis*, and *E. camaldulensis*, respectively. The yield results are in slight contrast to those found by [4, 5, 7, 10, 15, 17].

According to chemical categories, it has been demonstrated that EOs of all studied species exhibit quantitative differences while maintaining qualitative similarities. As shown in Figure 1, the subclass chemical groups were mainly monoterpenes, with a relative percentage of 71% for SMEO, including oxygenated monoterpenes (50.0%) and monoterpene hydrocarbons (21.0%). For MCEO, the percentages of both groups were (54.0%) and (29.0%), respectively, while for ECEO they were (43.0%) and (48.0%). For oxygenated sesquiterpene, both SMEO and MCEO possessed the same percentage (16%), but

it was relatively low (3%) in ECEO. The compositions of SMEO varied significantly with both MCEO and ECEO. Furthermore, as shown in Figure 1, the content of sesquiterpene hydrocarbons detected in SMEO is notably higher at 13%, compared to MCEO and ECEO representing a small portion of the EO with percentage (1%) and (3%), respectively.

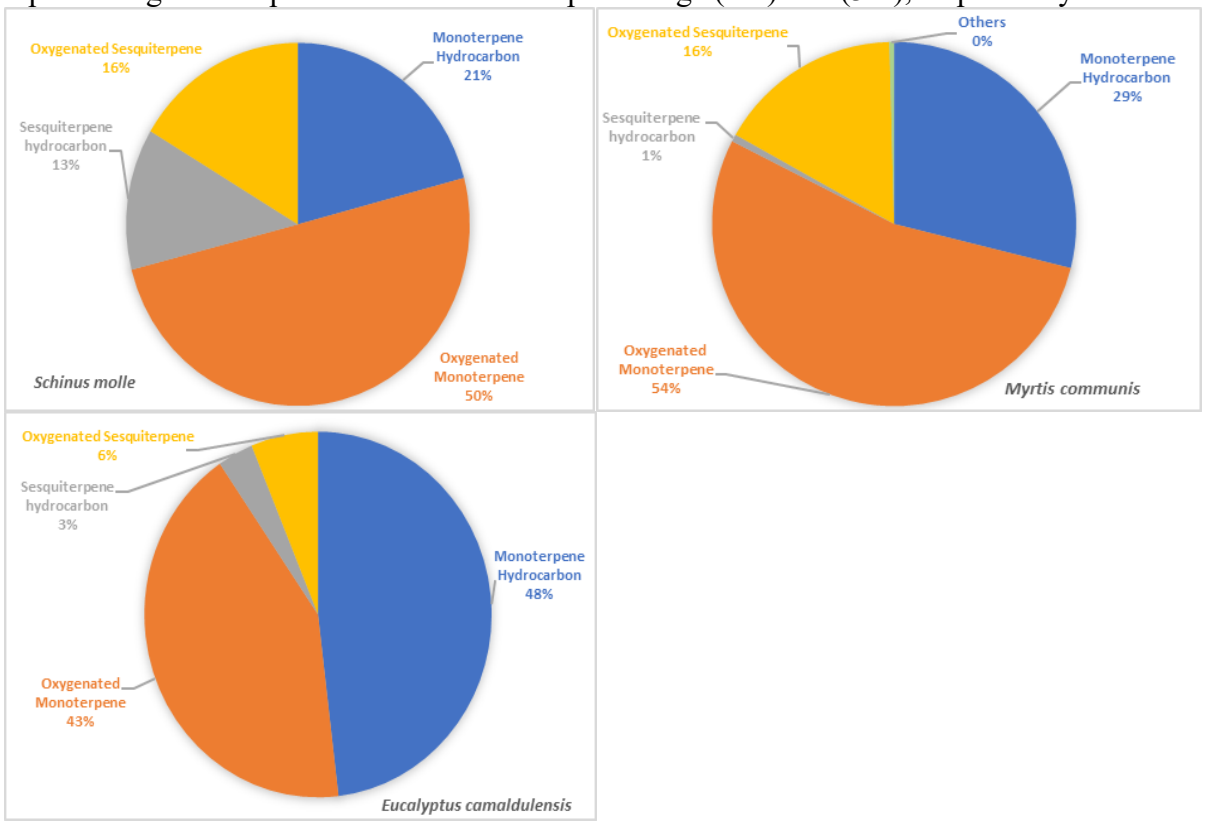

Figure 1: Chemical categories of the EOs from leaves of *S. molle*, *M. communis*, and *E. camaldulens*

The results in Table 1 demonstrate the chemical variability of all EOs although sharing a few common components at relatively high concentrations (>4%). To gain deeper insights into the distinctions among the three EOs, a comparative analysis of their primary chemical constituents was conducted. The total relative contents of SMEO, MCEO, and ECEO were 98.28, 98.94, and 99.2%, respectively. The dominant components of SMEO were 1,8-Cineole (46.4%), α-Phellandrene (10.68%), Isobicyclogermacrenal (5.85), β-Atlantol (5.73%), α-Vetispirene (5.09%), and Bicyclogermacrene (4.5%) (Figure 2). The principal components of MCEO were *α*-pinene (26.84%), 1,8-Cineole (16.79 %), 3-methylthio-Propanal (13.76%), Limonene (12.57%), and Linalool (11.3 %) (Figure 3). Moreover, the key components of ECEO were Limonene (41.52%), α-Phellandrene (34.99%), α-Pinene (5.49%), Myrcene (2.8%), and Elemol (2.59%) (Figure 4). As shown in the (Table 1), 1,8-Cineole (also known as eucalyptol) was only found in SMEO and MCEO, exhibiting the highest relative content in SMEO (46.4%), followed by MCEO with ratio (16.79 %). Moreover, α-Phellandrene with a percentage of (34.99%) constituted the highest component of ECEO, followed by SMEO with a ratio (10.68%), where it was almost undetectable in MCEO. In contrast, Limonene, one of the most predominant components of MCEO and ECEO, was not detected in SMEO, while Linalool was only detected in MCEO as a principal agent. Furthermore, Isobicyclogermacrenal, β-Atlantol, and α-Vetispirene were the major components of SMEO, showing high percentages (5.85%, 5.73% and, 5.09% respectively), whereas they were undetectable in MCEO and ECEO. In contrast, Elemol with a ratio (2.59%) was only detected in ECEO. Likewise, 3-methylthio-Propanal was only found in MCEO with a high percentage (13.76%), with Elemol, β-Atlantol, and Isobicyclogermacrenal absent.

Table 1: Chemical composition of the EOs from leaves of *S. molle*, *M. communis*, and *E. camaldulensis*

#	Compounds	Schinus		Myrtis		Eucalyptus		KI[b]
		RT[a]	%Area	RT	%Area	RT	%Area	
1	3-methylthio-Propanal	ND[C]	ND	5	13.76	ND	ND	901
2	Tricyclene	ND	ND	ND	ND	5.12	0.29	921
3	α-Thujene	5.24	0.2	ND	ND	ND	ND	924
4	α-Pinene	5.52	4.41	5.58	26.84	5.57	5.49	932
5	Camphene	5.95	0.73	ND	ND	5.98	2.11	946
6	Sabinene	6.68	0.33	ND	ND	6.7	0.32	969
7	β-Pinene	6.82	1.01	6.75	0.29	6.84	1.3	974
8	Myrcene	7.23	1.57	7.16	0.22	7.28	2.8	988
9	α-Phellandrene	7.8	10.68	7.66	0.2	8.09	34.99	1002
10	δ-3-Carene	ND	ND	7.82	0.15	ND	ND	1008
11	Limonene	ND	ND	8.62	12.57	8.95	41.52	1024
12	1,8-Cineole	8.86	46.4	8.78	16.79	ND	ND	1026
13	E-β-Ocimene	ND	ND	9.11	0.37	ND	ND	1044
14	γ-Terpinene	9.5	0.32	9.47	0.24	9.55	0.12	1054
15	Terpinolene	10.48	1.27	10.43	0.28	10.5	0.31	1086
16	Linalool	ND	ND	11.1	11.3	ND	ND	1095
17	trans-Sabinol	ND	ND	ND	ND	14.17	0.15	1137
18	Terpinen-4-ol	13.38	0.79	13.38	0.29	ND	ND	1174
19	α-Terpineol	13.81	0.38	13.9	2.91	ND	ND	1186
20	Methyl chavicol	ND	ND	14	0.44	ND	ND	1195
21	Nerol	ND	ND	14.95	0.17	ND	ND	1227
22	Geraniol	ND	ND	15.2	0.32	ND	ND	1249
23	Linalool acetate	ND	ND	15.72	5.78	ND	ND	1254
24	Methyl nerolate	ND	ND	17.35	0.09	ND	ND	1280
25	Bornyl acetate	ND	ND	ND	ND	16.59	0.34	1284
26	Carvacrol	ND	ND	ND	ND	17.2	0.12	1298
27	8-hydroxy-neo-Menthol	ND	ND	17.62	0.55	ND	ND	1328
28	α-Terpinyl acetate	ND	ND	18.38	1.93	ND	ND	1346
29	Geranyl acetate	ND	ND	19.26	1.2	ND	ND	1379
30	β-Bourbonene	19.27	0.13	ND	ND	ND	ND	1387
31	β-Elemene	19.48	0.59	ND	ND	ND	ND	1389
32	E-Caryophyllene	ND	ND	20.27	0.93	20.25	0.16	1417
33	Aromadendrene	20.78	1.2	ND	ND	ND	ND	1439
34	α-Humulene	ND	ND	21.15	0.63	ND	ND	1452
35	Germacrene D	ND	ND	ND	ND	21.94	2.22	1484
36	α-Vetispirene	21.39	5.09	ND	ND	ND	ND	1489
37	β-Selinene	21.7	0.58	ND	ND	ND	ND	1489
38	Bicyclogermacrene	22.31	4.5	ND	ND	22.26	0.5	1500
39	trans-Cycloisolongifol-5-ol	22.55	1.22	ND	ND	ND	ND	1513
40	γ-Cadinene	22.86	0.29	ND	ND	ND	ND	1513
41	δ-Cadinene	ND	ND	ND	ND	22.88	0.34	1522
42	Elemol	ND	ND	ND	ND	23.67	2.59	1548
43	Germacrene B	ND	ND	ND	ND	23.77	0.23	1559
44	Spathulenol	24.36	3.49	ND	ND	ND	ND	1577
45	Caryophyllene oxide	ND	ND	24.43	0.36	ND	ND	1582
46	β-Atlantol	24.56	5.73	ND	ND	ND	ND	1608
47	β-Biotol	24.82	1.07	ND	ND	ND	ND	1612
48	γ-Eudesmol	ND	ND	ND	ND	25.59	0.87	1630
49	α-Eudesmol	ND	ND	ND	ND	26.14	2.43	1652
50	Deodarone	ND	ND	27.41	0.33	ND	ND	1698
51	Isobicyclogermacrenal	28.05	5.85	ND	ND	ND	ND	1733
52	β-Costol	28.43	0.45	ND	ND	ND	ND	1766

[a] Retention Time
[b] Kovats Index relative to C6 – C24 n-alkanes on the DB-5 column.
[C] not detected.

Out of a total of fifty-two chemotypes identified, all studied genera shared some minor components. For instance, the chemotypes of Spathulenol (3.49%), Cycloisolongifol-5-ol (1.22%), Aromadendrene (1.2%), β-Biotol (1.07%), β-Elemene (0.59%), γ-Cadinene (0.29%), α-Thujene (0.2%), and trans- β-Bourbonene (0.13%) were detected only in SMEO. Likewise, the minor compounds were also quite different in both MCEO and ECEO, i.e.; Linalool acetate, Geranyl acetate, and α-Humulene were only identified in MCEO, whereas α-Eudesmol, Germacrene D, γ-Eudesmol, δ-Cadinene, Bornyl acetate were of ECEO constituents.

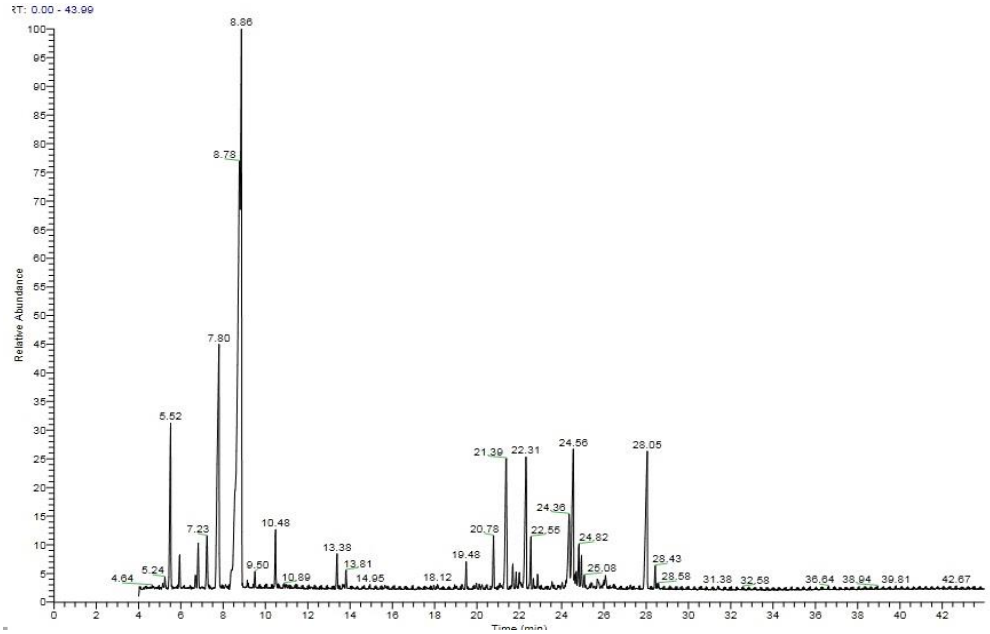

Figure: 2 Chromatogram obtained by GC-FID analysis from SMEO.

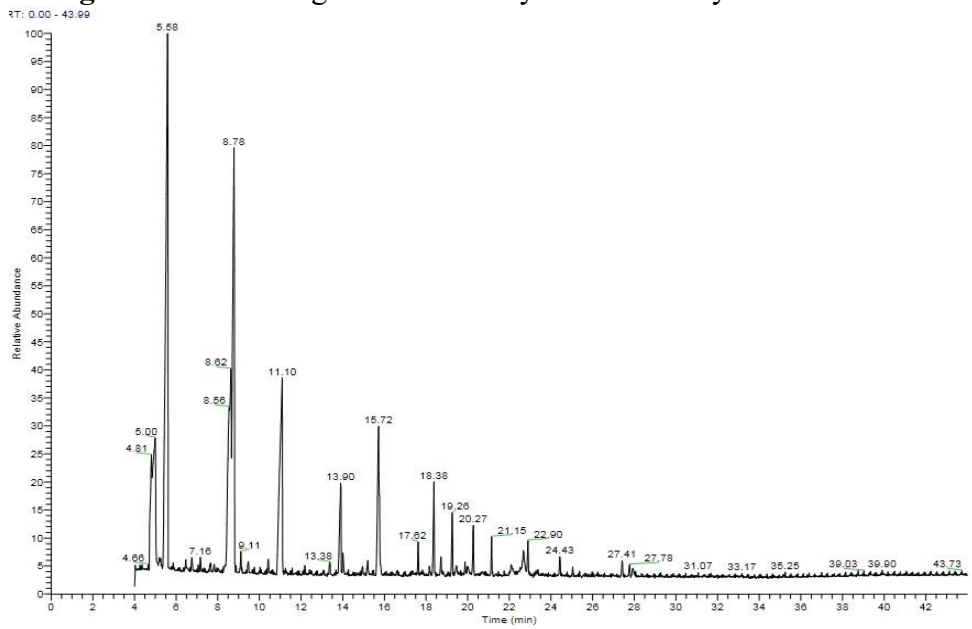

Figure 3: Chromatogram obtained by GC-FID analysis from MCEO.

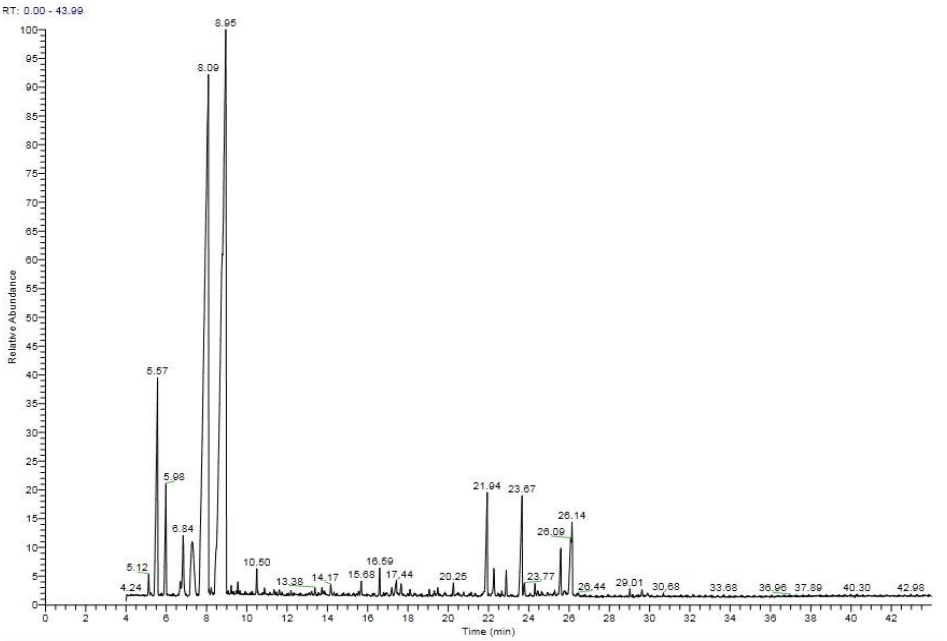

Figure 4: Chromatogram obtained by GC-FID analysis from ECEO.

The variation in the abundances of several components observed in our study aligns with the findings of [1, 4, 12-14, 18, 19]. Some of these components have been previously reported in the EOs of studied species as major compounds. For example, SMEO is proposed to mainly contain O-Cymene, α-Pinene, camphene, β-myrcene, α-Phellandrene, Limonene, β-Phellandrene, γ-cadinene and epi-α-cadinol, β- pinene, sabinene, elemol, p-cymene, spathulenol, bicyclogermacrene, δ-cadinene, guaiol, and γ-eudesmol [15-21]. Our main components Isobicyclogermacrenal, β-Atlantol, and α-Vetispirene did not identified in the literature. In addition, MCEO is composed of 1,8-cineole, linalool, linalool acetate, α-pinene, myrtenol, cis-4-thujanol, myrtenal, limonene, (E)-β-ocimene, linalyl acetate, α-terpineol, myrtenyl acetate, geranyl acetate, β-fenchyl alcohol, p-menth-1-enol, cis-geraniol, trans-caryophyllene, eugenol, estragole, 1,8-cineol, α-terpinyl acetate [1, 2, 4, 7-9]. Interestingly, 3-methylthio-Propanal, a major component in our study, was not detected in these studies. ECEO mainly contains 1,8-cineole, terpinen-4-ol, spathulenol, sabinene, linalyl acetate, linalool, p-cymene, cryptone, cuminaldehyde, α-pinene, α-phellandrene, γ-terpinene, β-pinene, Trans-pinocarveol [5, 10-14]. 1,8-Cineole was not detected in our study. Therefore, the variations in the abundance of numerous components of the EOs could be attributed to many factors viz.; environment, geographic conditions, climate, and other factors [10, 22].

Conclusions

EOs of leaves from *S. molle* (SMEO) collected in Erbil, Iraq contain high percentage monoterpene-rich volatile fractions representing in 1,8-Cineole, α-Phellandrene, and Isobicyclogermacrenal. These compounds have been reported to demonstrate promising biological activities. In contrast, the concentration of sesquiterpene hydrocarbons in SMEO was substantially greater compared to the levels observed in MCEO and ECEO. Moreover, several main components like Isobicyclogermacrenal, β-Atlantol, and α-Vetispirene did not identified in the literature. Interestingly, 3-methylthio-Propanal, a major component of MCEO, was not detected in previous studies. Likewise, 1,8-Cineole, the most abundant compound in ECEO from literature, was absent in our study. Future studies should confirm the reproducibility of these results under a wider range of climatic conditions and determine the molecular mechanisms of the biological activities of these EOs.

Conflict of Interest

The author declares that he has no conflicts of interest.

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