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Exploitation of sweet lemon residues in the production of essential oils

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Abstract

Background and objective: The constituents of sweet lemon essential oil (EO) have different biological and medical properties. The exploitation of sweet lemon residues in the production of EO is an important means of increasing natural products and disposing of those residues. The aim of this study was to evaluate the EO extracted from various sweet lemon residues such as leaves, flowers, and peels of fruits to find out their content of active substances.

Materials and methods: The EO of different residues of sweet lemon was isolated by hydrodistillation (HD) method, then they were analyzed by GC/MS. Data were statistically analyzed using ANOVA-1.

Results: The content of EO (%) was higher in peels than in flowers or leaves. Citronellal, nerol, and limonene were the major constituents of EO extracted from leaves, flowers, and peels, respectively. All detected components of various oils belonged to four chemical fractions (monoterpene hydrocarbons (MH), oxygenated monoterpenes (OM), sesquiterpene hydrocarbons (SH), and oxygenated sesquiterpenes (OS)). The MH was the major faction of peel EO while the OM was the major fraction of leaf and flower EOs. The SH and OS were formed as the minor fractions in all EOs.

Conclusion: Different variations were observed in sweet lemon EO extracted from various residues which lead to diversity in natural sources of EO production.

Keywords: Sweet lemon, Essential oil, Leaves, Flowers, Peels, Citronellal, Nerol and limonene

Background

Essential oils are secondary metabolites formed in aromatic plants. They are volatile, terpenoids, and usually isolated by distillation methods. They have odor and several pharmaceutical, medical, and biological properties such as antiseptic, bactericidal, virucidal, and fungicidal, as well as against liver and lung carcinogenesis, colon tumors, and gastric cancer (Davis 1982; Yeung 1999; Khursheed et al. 2016). The Administration of Food and Drug (FDA) indicated that EO of citrus is safe natural product, so it can be added to canned food and cosmetics to prevent the propagation of pathogens and spoiling microorganisms (Fisher and Phillips 2006; Nannapaneni et al. 2009; Velázquez-Nuñez et al. 2013). The residues of different organs of citrus tree such as peels, leaves, and flowers that are produced after juice extraction, pruning of branches and change to precipitation flowers are very important sources of EO (Viuda-Martos et al. 2009), as well as citrus residues which can serve as raw material for the extraction of EOs needed for various domestic and industrial uses (Giwa et al. 2018). Sweet lemon (*Citrus limettioides* Tan.), one of the citrus species, belongs to family Rutaceae. Just one literature was carried out in México to isolate and characterize the constituents of sweet lemon EO that was isolated from peels and leaves (Pino et al. 2010); this study reported that different variations were observed in the chemical constituents due to the differences in plant organs, and the major component of peel EO was limonene while the main constituents of leaf EO were limonene, citronellal, and linalool.

The yield and chemical constituents of EO can be changed by various conditions such as fertilizers, irrigation, climate, location, plant organ, and others (Krayni et al. 2015). The highest yield of EO and major constituents (thymol and carvacrol) of thyme were detected in the EO isolated from the aerial parts during the flowering stage (Jordan et al. 2006; Nejad-Ebrahimi et al. 2008; Omidbaigi et al. 2010). Different changes were recorded in the composition of oregano (*Origanum onites*) EO due to various plant organs (Kizil et al. 2008). The EO extracted from

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Eucalyptus cinerea fruits produced higher amount of 1,8-cineole (81%) than that isolated from leaves (75%) and flowers (78.8%) (Silva et al. 2011). The EO of Sodom apple extracted from the leaf, stem, flower, and fruit was analyzed by GC/MS (Wahba and Khalid 2018); leaf EO resulted in the highest values of *E*-phytol, myristicin, myristic acid, oxygenated sesquiterpenes (OS), and oxygenated diterpenes (OD); fruit EO recorded the greatest amounts of *E,E*-farnesyl acetone, monoterpene hydrocarbons (MH), oxygenated monoterpenes (OM), and sesquiterpenes hydrocarbons (SH). The EO components of *Sarcopoterium* plants were evaluated by Sipahi et al. (2017); the major components of EO isolated from the stem were aldehydes (40%), aromatic constituents (34.0%), and OM (21%); aldehyde component (43%), OS (26%), and aliphatic hydrocarbons (21%) were detected as the main components in leaf EO, while aldehydes (52%) and aromatic components (48.4%) were identified as the main constituents of root EO.

There are no investigations on the EO composition of sweet lemon extracted from various part residues (leaves, flowers, peels) in Egypt, so the aim of this study was to describe the composition of sweet lemon EO extracted from different residues. This study may increase the sources of natural products (EO) used in food and drug industries.

Materials and methods

Plant materials

Plant materials (leaves, flowers, and peels) were collected from the citrus farm of National Research Centre (NRC) during 2 years (2017 and 2018). The citrus farm is located at the reclaimed zone and is characterized by sandy soil. The leaves were obtained from pruning trees which were collected in February. Flowers produced from the precipitation were collected in June while fruit peels were collected in November at both seasons.

EO isolation

The fresh plant materials were collected, and then 250 g from each replicate (three replicates) were subjected to hydrodistillation (HD) for 3 h using a Clevenger-type apparatus (Clevenger 1928).

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC–MS) conditions

Gas chromatography analyses were performed using a Shimadzu GC-9 gas chromatograph equipped with a DB-5 (dimethylsiloxane, 5% phenyl) fused silica column (J&W Scientific Corporation) (30 m x 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was held at 50 °C for 5 min and then programmed to rise to 240 °C at a rate of 3 °C/min. The flame ionization detector (FID) temperature was 265 °C, and the injector temperature was 250 °C. Helium was used as the carrier gas with a linear velocity of 32 cm/s. The percentages of compounds were calculated by the area normalization method, without considering response factors. GC–MS analyses were carried out in a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 lm); the oven temperature was 50–240 °C at a rate of 4 °C/min, transfer line temperature 260 °C, carrier gas, helium, with a linear velocity of 31.5 cm/s, split ratio 1:60, ionization energy 70 eV, scan time 1 s, and mass range 40–300 amu.

Identification of volatile components

The components of EOs were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices (RI), either with those of authentic compounds or with data published in the literature (Adams 1995). Mass spectra from the literature were also compared (Adams 1995). Further identification was made by comparison of their mass spectra on both columns with those stored in NIST-98 and Wiley-5 Libraries.

Statistical analysis

In this experiment, one factor was considered: plant part residues (leaves, flowers, and peels). For each residue, there were three replicates; the experimental design followed a complete random block design. The average data of both seasons were statistically analyzed using one-way analysis of variance, ANOVA-1 (Snedecor and Cochran 1990). Significant values were determined according to P values ($P < 0.05$ = significant and $P < 0.01$ = highly significant). The applications of that technique were according to the STAT-ITCF program version 7 (Foucart 1982).

Results

EO content

Different variations are detected in sweet lemon EO (%) with various plant part residues (leaves, flowers, and peels) (Table 1). The greatest EO percentage is found in

Table 1 Changes in EO contents and chemical fractions of various plant residues

EO composition	Leaves		Flowers		Peels		*F* values
	Mean	SD	Mean	SD	Mean	SD	
Total EO (%)	0.2	± 0.1	0.1	± 0.0	0.3	± 0.1	4.5*
Total chemical fractions							
MH	24.9	± 0.0	24.7	± 0.3	79.6	± 0.4	34,650.8**
OM	71.1	± 0.1	61.4	± 0.4	12.2	± 0.2	42,742.4**
SH	1.6	± 0.3	1.8	± 0.2	4.3	± 0.3	70.2**
OS	1.7	± 0.4	11.4	± 0.4	3.3	± 0.3	715.9**
Total identified	99.3		99.3		99.4		

Note: *$P < 0.05$ = significant; **$P < 0.01$ = highly significant. Values are given as Mean ±SD

Table 2 Changes in EO constituents of various plant residues

No	EO constituents (%)	RI	Fractions	Leaves		Flowers		Peels		*F* values
				Mean	SD	Mean	SD	Mean	SD	
1	α-Thujene	931	MH	0.3	± 0.1	0.1	± 0.0	1.1	± 0.1	126.1**
2	α-Pinene	939	MH	0.4	± 0.1	1.6	± 0.2	0.2	± 0.1	86.1**
3	Camphene	953	MH	0.4	± 0.1	0.1	± 0.0	0.1	± 0.0	27.0**
4	Sabinene	976	MH	0.5	± 0.1	0.6	± 0.1	1.8	± 0.2	78.5**
5	β-Pinene	980	MH	0.3	± 0.1	1.6	± 0.2	0.3	± 0.1	112.5**
6	Myrcene	991	MH	3.8	± 0.2	2.8	± 0.2	0.7	± 0.2	187.8**
7	α-Phellandrene	1005	MH	0.3	± 0.2	0.1	± 0.0	0.7	± 0.2	9.9**
8	Δ-3-carene	1011	MH	0.2	± 0.1	1.4	± 0.4	0.4	± 0.1	20.7**
9	α-Terpinene	1018	MH	0.2	± 0.1	1.4	± 0.4	0.1	± 0.0	27.7**
10	p-Cymene	1026	MH	0.3	± 0.1	0.4	± 0.1	0.1	± 0.0	22.8**
11	Limonene	1031	MH	13.8	± 0.2	12.5	± 0.5	72.5	± 0.5	19,576.1**
12	Cis-β-Ocimene	1040	MH	3.7	± 0.3	0.6	± 0.1	0.3	± 0.1	289.9**
13	γ-Terpinene	1062	MH	0.4	± 0.1	0.9	± 0.1	0.7	± 0.2	14.1**
14	α-Terpinolene	1088	MH	0.3	± 0.1	0.6	± 0.1	0.6	± 0.1	9.1*
15	Cis-Sabinene hydrate	1097	OM	0.5	± 0.1	0.9	± 0.1	0.1	± 0.0	72.0**
16	Linalool	1098	OM	11.1	± 0.1	0.8	± 0.2	1.6	± 0.1	4924.5**
17	Isopulegol	1145	OM	0.1	± 0.0	0.1	± 0.0	0.2	± 0.1	3.0^{ns}
18	Citronellal	1153	OM	51.1	± 0.1	0.2	± 0.1	1.9	± 0.1	250,717.0**
19	Terpinen-4-ol	1177	OM	0.3	± 0.1	0.1	± 0.0	0.3	± 0.1	6.1^{ns}
20	α-Terpineol	1189	OM	0.4	± 0.1	0.4	± 0.1	1.1	± 0.1	49.1**
21	Decanal	1204	OM	0.1	± 0.0	0.1	± 0.0	0.1	± 0.0	5.2^{ns}
22	Citronellol	1228	OM	4.9	± 0.1	0.4	± 0.1	2.1	± 0.1	1549.0**
23	Nerol	1228	OM	0.4	± 0.1	53.9	± 0.1	0.1	± 0.0	431,758.5**
24	Cis-Carveol	1229	OM	0.2	± 0.1	0.1	± 0.0	0.1	± 0.0	3.0^{ns}
25	Citral	1240	OM	0.1	± 0.0	1.1	± 0.1	1.2	± 0.2	66.6**
26	Geranial	1270	OM	0.3	± 0.1	1.6	± 0.4	0.6	± 0.2	19.9**
27	Thymol	1290	OM	0.4	± 0.1	0.5	± 0.1	0.8	± 0.2	6.5*
28	Cis-Limonene oxide	1294	OM	0.4	± 0.1	0.1	± 0.0	0.9	± 0.1	73.5**
29	Citronellyl acetate	1354	OM	0.1	± 0.0	0.1	± 0.0	0.1	± 0.0	5.2^{ns}
30	Neryl acetate	1365	OM	0.2	± 0.1	0.1	± 0.0	0.1	± 0.0	3.0^{ns}
31	Geranyl acetate	1383	OM	0.5	± 0.1	0.9	± 0.1	0.9	± 0.1	16.0**
32	β-Humulene	1440	SH	0.3	± 0.1	0.1	± 0.1	0.3	± 0.1	36.0**
33	α-Caryophyllene	1454	SH	0.3	± 0.1	0.6	± 0.1	0.8	± 0.2	9.5**
34	β-Selinene	1485	SH	0.2	± 0.1	0.4	± 0.1	1.1	± 0.1	67.0**
35	Germacrene D	1490	SH	0.5	± 0.1	0.4	± 0.1	0.4	± 0.1	1.0^{ns}
36	β-Bisabolene	1509	SH	0.3	± 0.1	0.3	± 0.1	1.7	± 0.3	53.5**
37	β-Nerolidol	1546	OS	0.3	± 0.1	10.3	± 0.3	0.1	± 0.0	3061.2**
38	Spathulenol	1576	OS	0.4	± 0.1	0.1	± 0.0	0.1	± 0.0	27.0**
39	Caryophyllene oxide	1581	OS	0.5	± 0.1	0.2	± 0.1	1.8	± 0.4	20.4**
40	α-Eudesmol	1652	OS	0.2	± 0.1	0.3	± 0.1	0.6	± 0.1	13.0**
41	α-Bisabolol	1683	OS	0.2	± 0.1	0.4	± 0.1	0.5	± 0.1	7.0*
42	È,È-Farnesol	1722	OS	0.1	± 0.0	0.1	± 0.0	0.2	± 0.1	3.0^{ns}

Note: Under table, *$P < 0.05$ = significant; **$P < 0.01$ = highly significant; ^{ns}insignificant. Values are given as Mean ±SD

peel EO (0.3%) followed by leaf EO (0.2%) and flower EO (0.1%). The changes in EO oil contents are significant ($P < 0.05$) for different plant residues (Table 1).

EO constituents

In this study, 42 components are observed by GC/MS analysis (range from 99.3 to 99.4%) in leaf, flower, and peel EOs of sweet lemon trees (Table 2). Different changes are found in various constituents due to the differences in plant part residues. The major constituents of leaf EO are citronellal (51.1%), limonene (13.8), and linalool (11.1%). Nerol (53.9%), limonene (12.5%), and β-nerolidol (10.3%) are identified as the major components in flower EO while limonene (72.5%) is the major component in peel EO. All detected components are grouped into four chemical fractions (MH, OM, SH, and OS) (Table 1 and Figs. 1, 2, 3, and 4). The MH is the major fraction of peel EO while the OM is the major fraction of leaf and flower EOs. The SH and OS are formed as the minor fractions in all EOs (Table 1). The

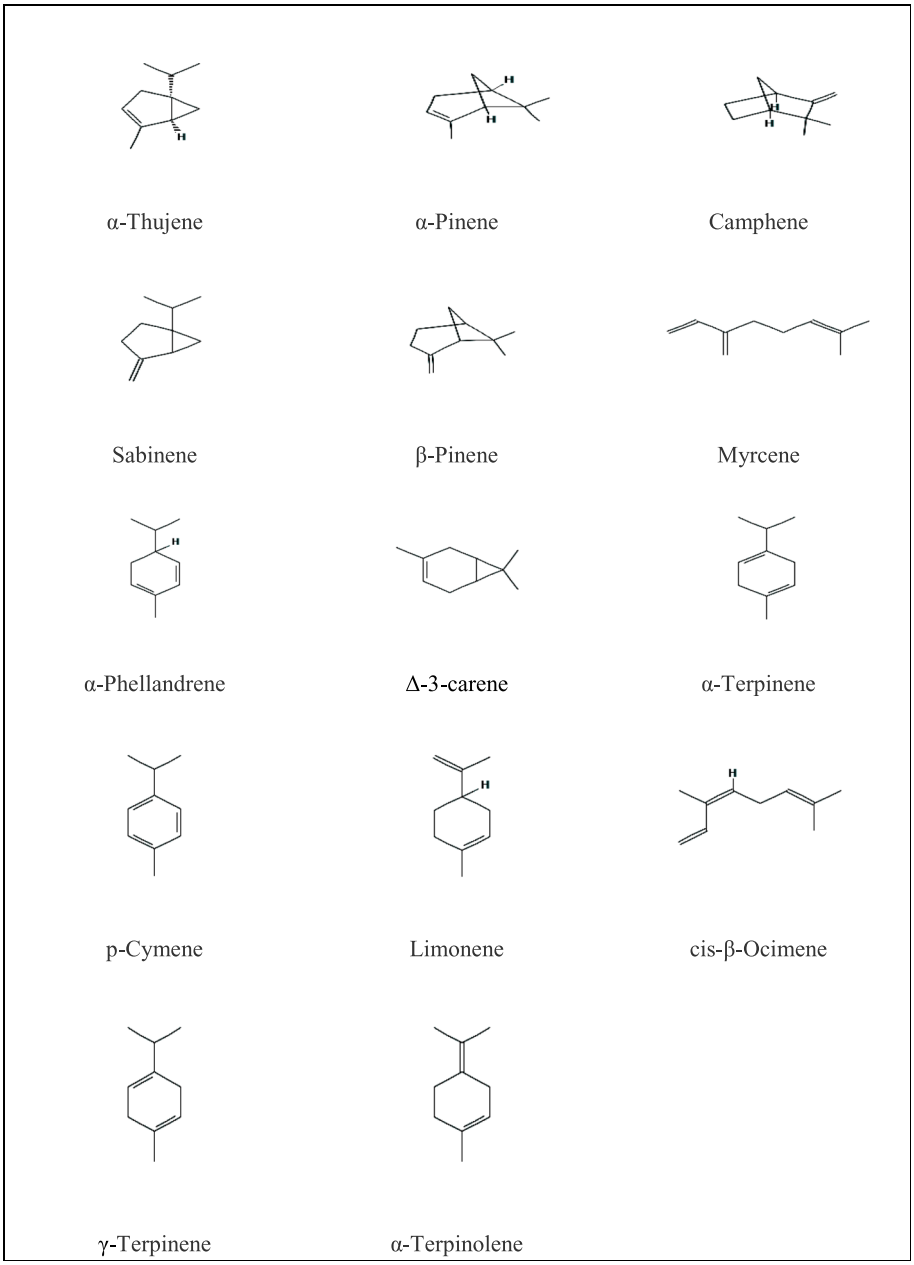

Fig. 1 MH constituents

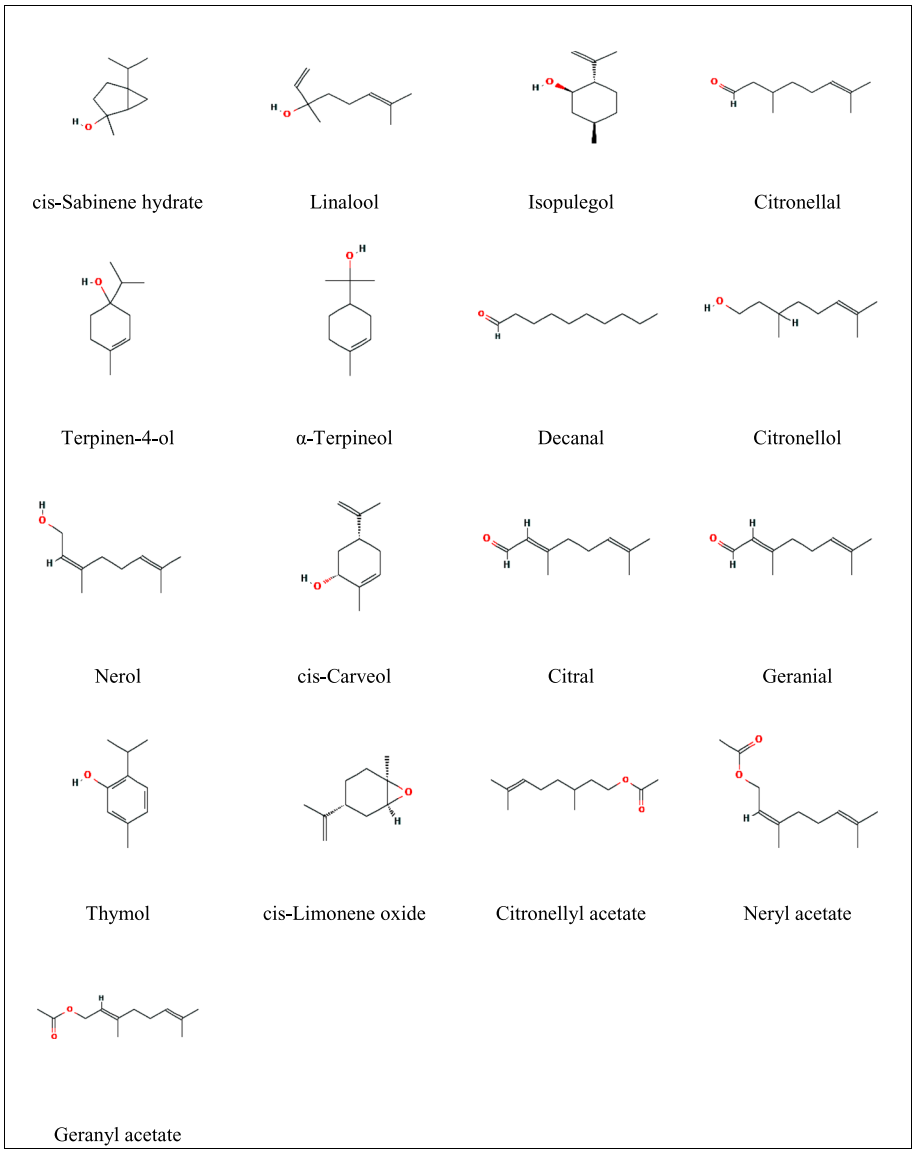

Fig. 2 OM constituents

changes in the most detected components and chemical fractions are highly significant ($P < 0.01$) while α-terpinolene, thymol, and α-bisabolol are only significant ($P < 0.05$). Insignificant variations are observed in isopulegol, terpinen-4-ol, decanal, *cis*-carveol, citronellyl acetate, neryl acetate, germacrene D, and È,È-farnesol.

Discussion

In this study, various plant residues resulted in different variations of quantity and quality of sweet lemon EO. These changes might be due to the different variations in the metabolism and enzyme activities of EO formations in various plant residues (Burbott and Loomis 1969). The changes in EO composition with different plant residues were confirmed by previous investigators on some medicinal plants. Significant variations were reported in EO and its major constituents of oregano, thyme, and *Eucalyptus* sp. as a result of the difference of the plant part (Jordan et al. 2006; Kizil et al. 2008; Nejad-Ebrahimi et al. 2008; Omidbaigi et al. 2010; Ghasemi-Pirbalouti et al. 2011; Silva et al. 2011; Golparvar et al. 2015). The various fractions (aldehydes, aromatic components, MH, OM, SH OS, OD, and aliphatic hydrocarbons) of EO extracted from *Sarcopoterium spinosum* and Sodom apple plants were significantly changed with different plant organs (Sipahi et al. 2017; Wahba and Khalid 2018). On the other hand, the present study indicated

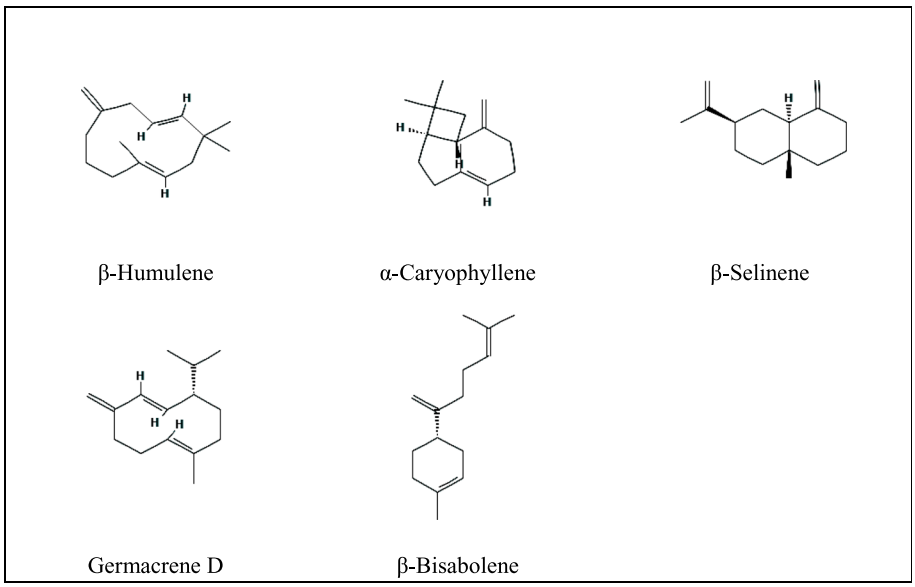

Fig. 3 SH constituents

that the EO of sweet lemon consists of various kinds of terpenes (MH, OM, SH, and OS). Theses terpenes included natural flavor additives for food or fragrances in perfumery and in traditional and alternate medicines as aroma therapy (Brahmkshatriya and Brahmkshatriya 2013). Other important therapeutic uses of terpenoids include antimicrobial, antifungal, antiviral, antihyperglycemic, antiinflammatory, antioxidant, anticancer, antiparasitic, and immunomodulatory, and as a skin permeation enhancer (Brahmkshatriya and Brahmkshatriya 2013).

Conclusion

The compositions of EO isolated from sweet lemon residues were evaluated. The yields of peel EO were higher than those isolated from flowers and leaves. The leaf EO is rich with citronellal, limonene, and linalool. Flower EO is distinguished by high values of nerol, limonene, and β-nerolidol while peel EO is characterized by its high quantity of limonene. Various types of terpenes such as MH, OM, SH, and OS were found in different EOs extracted from sweet lemon residues. This investigation indicated that sweet lemon residues contain

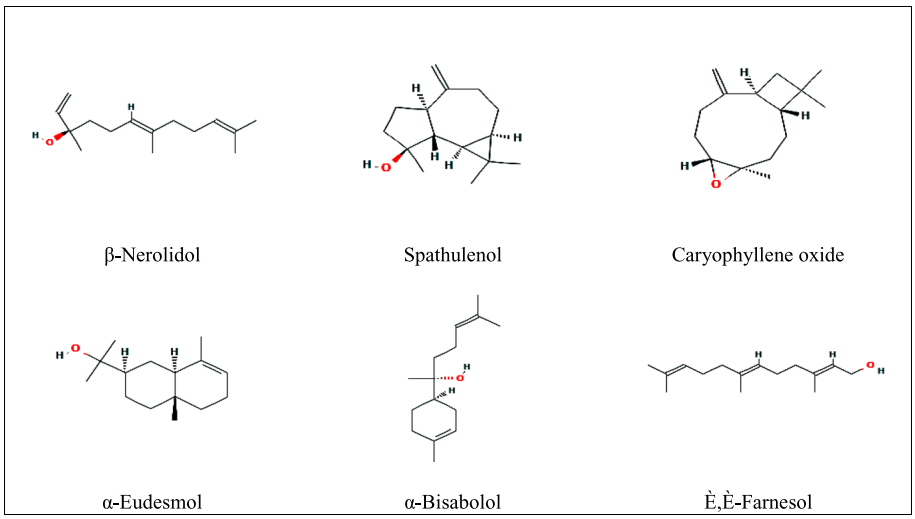

Fig. 4 OS constituents

various effective substances that have different activities. Therefore, this study will lead to increase the natural sources of active substances, especially EOs.

Abbreviations

ANOVA: Analysis of variance; EO: Essential oil; GC: Gas chromatography; GC/MS: Gas chromatography–mass spectrometry; HD: Hydrodistillation; MH: Monoterpene hydrocarbons; OM: Oxygenated monoterpenes; OS: Oxygenated sesquiterpenes; RI: Retention index according to DB5 column; SH: Sesquiterpenes hydrocarbons

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Authors' contributions

All authors have contributed significantly to the conception and design of the study, the interpretation of data, and the drafting and revision of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets supporting the results are included within the article.

Ethics approval and consent to participate

The manuscript does not contain studies involving human participants, human data, or human tissue.

Consent for publication

The authors declare that the work has consent for publication.

Competing interests

The authors declare that they have no competing interests.

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