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Comparative Study of Peels Essential Oil Constituents of some *Citrus* Species

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Abstract

Peels of *Citrus* as byproducts are considered a valuable source of various bioactive compounds such as essential oils and flavonoids with vital properties. So, the study goal is to determine which are the most suitable methods of extraction of the essential oils of three *Citrus* species peels *C.aurantifolia (CA), C.limon (CL)* and *C.sinensis (CS).* A comparative study of conventional techniques; hydrodistillation (HD), solvent extraction (SE), and innovative techniques; microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), were used for extraction of the essential oils from the three *Citrus* species peels. The chemical composition with percentages of all constituents was determined using gas chromatography/mass spectroscopic analyses (GC/MS) of different oil peels. The GC/MS analyses results indicated that both HD and SE techniques were more desirable for the extraction, and they gave a higher percentage of monoterpene and sesquiterpene hydrocarbons (85.87 % and 46.69 %, respectively). These ingredients are considered the basic ingredients in many cosmetic and pharmaceutical industries. In contrast, MAE and UAE techniques were more efficient for extracting hydrocarbons, oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, and fatty acids from different essential oils peels. The results proved that the use of different extraction methods lead to different yields of essential oils with the highest yield with microwave-assisted extraction (MAE) techniques for the three *Citrus* species peels (*CA*), (*CL*) and (*CS*) in percentages of 1.52 %, 2 %, 2.8 %, respectively.

Keywords: *Citrus* peels, Conventional and Innovative extraction methods, GC/MS, Essential oils.

1. Introduction

Citrus belonging to the Rutaceae family included 140 genera and 1300 species [1,2]. It has vastly grown in tropical and subtropical regions and is considered the most abundant crop globally. Most *Citrus* species are found in Africa and Australia, often in semiarid woodlands [3]. Egypt is one of the largest countries producing *Citrus* fruits with wide varieties (i.e., orange, lemon, lime, tangerines, and grapefruit). These fruits have important biological activities such as antibacterial, antidiabetic, antioxidant, anticancer, cardiovascular protection, analgesic, anti-inflammatory, and anti-anxiety properties [4]. A huge amount of *Citrus* wastes from lemon, orange, mandarin, and tangerine is produced every year [5]. In Egypt, the data of cultivated crops area indicated that *Citrus* fruits cultivated approximately 541723 Fadden with production in about 4.1 million tons and the waste about 2.5 % of the total fruit production [6].

Phytochemical screening of the *Citrus* peels waste verified that they are a rich source of essential oils, flavonoids, phenols, coumarins, fibers, and vitamin C [7,8]. The essential oils of the *Citrus*, mainly fixed in the peel, considers 40 % - 50 % of the fruit portion. It

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mainly consists of monoterpenes hydrocarbons, sesquiterpenes hydrocarbons, and their oxygenated derivatives [9,10]. Aboudaou et al., in 2019 [11] extracted essential oil from *C. sinensis* peels using three different methods, conventional techniques as hydrodistillation (HD), cold-pressing (CP), and other innovative techniques as solvent-free microwave assisted extraction (SFME). The monoterpene hydrocarbons were the major component extracted by three techniques, mainly limonene.

Conventional extraction techniques as solid-liquid extraction, soxhlet, steam distillation, and cold press are still used in Egypt. These extraction methods lack selectivity, give lower yields, and use more organic solvents. Innovative techniques such as microwave-assisted extraction, supercritical fluid extraction, accelerated solvent extraction, and ultrasonic-assisted extraction is used as new extraction techniques for shortening the extraction time, reducing solvent composition, and obtaining high yields [12]. The main objective of the current work deals with the extraction of essential oils from three varieties of *Citrus* waste peels (*C.aurantifolia*, *C.limon*, and *C.sinensis*) using the innovative microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) techniques, in comparison with the conventional hydrodistillation (HD) and solvent extraction (SE) methods.

2. Experimental

2.1. Plant material

The fresh lemon fruits banzaher (*Citrus aurantifolia*; *CA*), lemon addalia (*Citrus limon*; *CL*) and orange (*Citrus sinensis*; *CS*) were collected from Nubaria farm (National Research Center, Cairo, Egypt) during the study period from January 2019 to May 2020.The fresh plant material were authenticated kindly by Mrs. Treza Labib, a taxonomist at Al-Orman Garden-Giza.

The peels were manually removed and dried in the air for 14 days under laboratory conditions at 28±2 °C. The dried materialswere ground using the mill, stored in cotton bags, and kept dry.

2.2. Extraction methods

The extraction of essential oils was achieved by using hydrodistillation (HD), microwave-assisted extraction (MAE), solvent extraction method (SE), and ultrasound-assisted extraction (UAE) as follow:

2.2.1. Hydrodistillation method (HD)

The essential oils were obtained by steam distillation from the powdered peels (50 g) of three *Citrus* species (*CA*, *CL*, and *CS)* using a Clevenger apparatus for 4 hrs. The volatile oils were collected and taken with dry diethyl ether. The solvent was evaporated in a water bath maintained at 40 °C, and the remaining oils were weighed and stored at -4°C for analysis.

2.2.2. Microwave-assisted extraction method (MAE)

The essential oil was obtained from (50 g) each of the peels (*CA*, *CL*, and *CS*) by hydrodistillation for 60 min. using a Clevenger-type apparatus placed in a modified microwave oven (MARS 240v/ 50 Hz). During distillation, time, temperature, pressure, and power were monitored and controlled with the "easy-control" software package. Microwave power applied to the plant material was controlled by a shielded thermocouple inserted directly into the flask. The oven was operated for 55 minutes at 800 Watts up to 90ºC, followed by 5 minutes of ventilation. The obtained essential oils were collected in amber-coloured vials, dehydrated with anhydrous sodium sulfate, capped under nitrogen, and kept at -4ºC for analysis [13].

2.2.3. Solvent extraction method (SE)

About 50 g of different peels (*CA*, *CL*, and *CS*) were extracted by maceration with hexane for 24 hrs. (3 × 1L). The combined extracts were evaporated *in vacuo* at 40˚C till free from solvent. The combined solvent was dried over anhydrous sodium sulfate and evaporated, and then the extracts were collected and kept at -4 ºC for analysis.

2.2.4. Ultrasound-assisted extraction method (UAE)

About 50 g of different peels (*CA*, *CL*, and *CS*) were extracted 3 times with hexane (solid/liquid 1: 8, w/v) under the action of ultrasonic-assisted extraction for 20 min. (i.e., temperature 25 ˚C, and power 100 W). After that, the extracts were combined, filtrated, and removed the solvent *in vacuo* at 40˚C till free from solvent. The extracts obtained were stored in a refrigerator at -4 ˚C until use [14].

2.3. GC/MS analysis

A 10 mg of each essential oil from the above extraction methods were dissolved in 5 ml diethyl ether and subjected to GC/MS analysis. The samples were carried out using gas chromatography-mass spectrometry instrument stands at Central Agricultural Pesticides Laboratory of Agricultural Research Center, Egypt, with the following specifications. Instrument: Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric detector, direct capillary interference, and fused silica capillary column PAS-5ms (30 m x 0.32 mm i.d., 0.25 μm film thickness). Samples were injected under the following conditions: helium was used as carrier gas at approximately 1.0 ml/min, pulsed spitless mode. The solvent delay was 3 min., and the injection size was 1.0 μl. The mass spectrometric detector was operated in electron impact ionization mode with ionizing energy of 70 e.v. The ion source temperature was 230 ˚C, the electron multiplier voltage was maintained at 1250 v above auto-tune. The instrument was manually tuned using perfluorotributyl amine (PFTBA). The GC temperature program was started at 40 ˚C (2 min) and then elevated to 300 ˚C at a rate of 5 ˚C /min. The injector temperature was set at 280 ˚C. Wiley and Wiley Nist mass spectral database was used in the identification of the separated peaks.

3. Results and discussion

The essential oils of three *Citrus* species (*CA*, *CL*, and *CS*) were characterized by a specific colour and odour. As shown in Table 3, the yields of *CA* obtained by the four extraction techniques HD, MAE, SE, and UAE were 0.8 %, 1.52 %, 1.38 %, and 1.06 %, respectively. For *CL*, the obtained yields were 1.2 %, 2.0 %, 1.44 %, and 1.6 % through HD, MAE, SE, and UAE. On the other hand, the yield of the species *CS* was 2.4 %, 2.8 %, 1.9 %, and 0.8 % using HD, MAE, SE, and UAE, respectively. The results indicated that the MAE technique produced the highest essential oils (EOs) yield from the three *Citrus* peels compared to other techniques. The highest yield obtained from *CS* may be due to the too compact EOs glands having many oil sacks ruptured by steam, microwaves, soaking with organic solvents, and ultrasonic waves [13]. Golmakani and Moayyedi2015., [15] stated that the yield difference could be related to the extraction time, heat transfer within the samples where in MAE, heat transfer through the sample by (irradiation, conduction, and convection but within HD by conduction and convection only). Allaf et al., 2013., [16] indicated that the obtained EOs yield from HD technique did not obtain a good yield due to the resistance to thermal treatment of the too compact EOs glands.

Tables 1 and 2 showed 38, 27, 30, and 39 compounds identified from *CA* essential oils using four different extraction techniques (HD, MAE, SE, and UAE). Bousbia et al., 2008 [17] identified 22 and 23 compounds from *CA* essential oils using hydrodistillation and microwave hydrodiffusion and gravity (MHG), respectively, while Saad M 2013., [18] identified 28 compounds from the peel of *CA* using HD technique. A 29, 40, 41, and 37 compounds were identified from *CL* essential oils and 23, 20, 37, and 17 compounds from *CS*EOs using the HD, MAE, SE, and UAE techniques, respectively.

The data in Table 3 presented the identified and percentages of different compound classes found in EOs of the three *Citrus* species. For *CA* EOs, the highest monoterpene hydrocarbons obtained using HD technique were (43.41 %), mainly d-limonene (18 %) and β-pinene (14.37 %). Our results were agreed with Lota et al., 2002 [19] where they found that limonene is the main abundant component in the lime peel essential oil extracted using hydrodistillation (HD). It was reported that, limonene was the major volatile composition of lime peel extracted using hydrodistillation (HD), microwave hydrodiffusion and gravity (MHG), and cold pressing (CP) techniques (63.44 %. 60.56 %, 68.81 %, respectively), followed by β-pinene (13.09 %, 11.6 %, 14%, respectively), and γ-terpinene (11.17 %, 11.91 %, 8.9 %, respectively) [17]. El-Gengaihi et al., 2020 [6] reported that the percentage of limonene from fresh lemon peel was (6.06%), while the obtained results indicate that limonene was the major component in *CA* (18%) by hydrodistillation technique which may be due to seasonal variation and different locality. The highest monoterpene hydrocarbons of *CL* obtained by the HD extraction technique were (61.09%), followed by the SE (22.24%) and UAE (2.84 %) techniques (Table 1 and 2).

Our results agree with Aguilar-Hernández et al., 2020 [20] and Paw et al., 2020 [21] who stated that limonene was the most abundant compound in *C. limon* peel extracted by HD and our obtained data disagreed with Golmakani and Moayyedi (2015)[15] who extracted the EOs of *C. limon* by microwave-assisted hydrodistillation (MAHD), solvent-free microwave extraction (SFME) and HD techniques.

As shown in Table 3, the EOs of *CS* obtained by HD and SE were richer with monoterpene hydrocarbon compounds (85.87 %, 70.42 %, respectively). The d-limonene was the main detected compound in HD, SE, and UAE techniques with a ratio (80.09 %, 67.37 %, 4.76 %, respectively). Taktak et al., 2021[22] identified twenty-eight components in *C. sinensis* peel using conventional hydrodistillation (HD), and limonene was the principal identified component (86.7 %). The HD technique obtained the highest monoterpene hydrocarbons from *CS*, more than from *CA* and *CL*. The colourless hydrocarbon limonene was the major component found in all obtained oil. These results agreed with those reported by Bousbia et al., (2008) [17] and Saad et al., (2016) [18] who revealed that the EOs were more concentrated by monoterpenes that obtained from *Citrus* waste (*C. limon*, *C. aurantifolia* and *C. sinensis*) and *C. sinensis* having the highest limonene incomparable to other species.

In *CA* essential oils, the yield of oxygenated monoterpenes obtained using MAE were the highest (22.24 %) with α-terpineol (8.35 %), linalool (7.08 %), and geraniol (4.34%) as major compounds, followed by the HD technique (17.8 %) where citral and α-terpineol were the main components (7.34 %, 3.49 %, respectively).

The obtained results are matched with Bousbia, et al., 2008 [17] who revealed that the highest yield of oxygenated monoterpenes was obtained from *Citrus limon* (lime) peel using microwave hydrodiffusion and gravity (MHG).

For *CL*, the EOs extracted by MAE (Table 3) showed higher amounts of oxygenated monoterpenes (17.17 %), compared to those extracted by HD (7.03 %), SE (3.88 %), and UAE (5.98 %), where linalool (5.62 %) was the main component by using MAE extraction technique and citral is the major one by using HD (2.57 %), and UAE (3.41%) extraction techniques. In *CS* EOs, the highest percentage of oxygenated monoterpene components were obtained by HD (4.17 %), followed by (MAE 2.65 %), SE (0.87 %), and absence in UAE technique. Linalool was the main oxygenated monoterpene in HD (1.39 %), SE (0.87%), and α- terpineol (2.65 %) was the main in the MAE technique.

Ferhat et al., 2016 [23] indicated that the oxygenated monoterpenes (linalool, geranial, and nerol) were present in a low percentage from EOs of 4 varieties of Algerian *C. sinensis* fresh peel by using hydrodistillation (HD), cold pressing (CP), and microwave accelerated distillation (MAD).

For *CA* EOs, sesquiterpene hydrocarbons (Table 3) have mainly consisted of caryophyllene (13.93 %) and α-bisabolene (9.1 %) by (SE); γ-elemen (8.56 %) and β-bisabolene(7.12 %) by (HD) technique; and alloaromadendrene (8.56 %) by (MAE). In *CL* EOs, the four techniques (HD, MAE, SE, and UAE) contain major sesquiterpene hydrocarbons (15.9 %, 16.38 %, 22.54 %, and 31.77 %, respectively), and the UAE method showed the highest percentage. β-bisabolene (10.93 %) and bergamotene (8.48 %) were the major components by using the UAE technique. The sesquiterpene hydrocarbons of *CS* EOs, were found in slightly larger amounts in the MAE technique (14.33 %) than in the UAE (13.88 %), SE (6.71 %), and the lowest amount in HD (4.5 %). In *CS* EOs caryophyllene was present in four extraction techniques HD, MAE, SE and UAE(0.55% ,1.87%, 1.85% and 1.28%, respectively).

In *CA* EOs, the oxygenated sesquiterpenes obtained by HD, MAE, SE, and UAE are 2.27 %, 22.03 %, 12.56 %, 2.56 %, respectively. Our results indicate that the EOs obtained by MAE were rich with oxygenated sesquiterpene hydrocarbons. α-bisabolol (10.18 %) and farnesol (12.56 %) represent the main constituents. For *CL* EOs, the highest oxygenated sesquiterpene hydrocarbons extracted by MAE technique (15.85 %), mainly α-bisabolol (11 %) followed by SE (9.42 %) then by

TABLE 1: GC/MS of EOs of *Citrus* peels (*C. aurantifolia*, *C. limon*and *C. sinensis*) using HD and MAE extraction techniques**.**

Peak No.	Compounds	Mol.Wt	B.P	*C. aurantifolia*		*C. limon*		*C. sinensis*	
				HD %	**MAE%**	**HD%**	**MAE%**	**HD%**	**MAE%**
	Monoterpene Hydrocarbons			**43.41**	**0**	**61.09**	**0**	**85.87**	**0**
1	α-Pinene ($C_{10}H_{16}$)	136	93	4.39	-	4.27	-	-	-
2	β-Pinene($C_{10}H_{16}$)	136	93	14.37	-	12.47	-	5.25	-
3	D-Limonene ($C_{10}H_{16}$)	136	68	18	-	36.78	-	80.09	-
4	γ-Terpinene ($C_{10}H_{16}$)	136	93	2.16	-	6.34	-	-	-
5	α-Terpinolene ($C_{10}H_{16}$)	136	93	1.01	-	0.99	-	0.53	-
6	Allo-Ocimene ($C_{10}H_{16}$)	136	121	1.07	-	0.24	-	-	-
7	α-Terpinene ($C_{10}H_{16}$)	136	121	2.41	-	-	-	-	-
	Oxygenated Monoterpenes			**17.80**	**22.24**	**7.03**	**17.17**	**4.17**	**2.65**
8	Linalool Oxide($C_{10}H_{18}O_2$)	170	59	-	-	-	0.72	-	-
9	Linalool ($C_{10}H_{18}O$)	154	71	2.06	7.08	1.14	5.62	1.39	-
10	Borneol($C_{10}H_{18}O$)	154	95	-	1.05	-	0.22	-	-
11	α- Terpineol($C_{10}H_{18}O$)	154	59	3.49	8.35	-	2.59	0.65	2.65
12	Carveol($C_{10}H_{16}O$)	152	109	-	-	-	0.45	0.22	-
13	Geraniol($C_{10}H_{18}O$)	154	69	-	4.34	1.5	3.33	-	-
14	Citral($C_{10}H_{16}O$)	152	69	7.34	-	2.57	1.22	0.7	-
15	Citronella($C_{10}H_{18}O$)	154	41	-	-	0.38	1.4	0.78	-
16	Verbenol ($C_{10}H_{16}O$)	152	41	1.6	-	-	-	-	-
17	4-Terpineol ($C_{10}H_{18}O$)	154	71	3.31	1.42	1.44	1.62	0.43	-
	Sesquiterpene Hydrocarbons			**31.53**	**18.76**	**15.90**	**16.38**	**4.50**	**14.33**
18	Bergamotene($C_{15}H_{24}$)	204	93	-	0.92	4.01	0.58	-	-
19	β-Patchoulene($C_{15}H_{24}$)	204	161	-	0.58	-	-	-	-
20	Ledene($C_{15}H_{24}$)	204	107	-	-	-	0.87	-	-
21	Valencene($C_{15}H_{24}$)	204	161	-	-	2.46	0.73	2.01	6.45
22	β-Copaene($C_{15}H_{24}$)	204	119	-	-	-	-	0.21	-
23	β-Elemene ($C_{15}H_{24}$)	204	93	2.77	-	-	-	-	-
24	α-Farnesene ($C_{15}H_{24}$)	204	41	0.74		1.2	0.45	-	-
25	Caryophyllene($C_{15}H_{24}$)	204	93	1.41	0.65	2.24		0.55	1.87

26	β-Cubebene($C_{15}H_{24}$)	204	161	-	-	-	-	0.46	
27	γ-Elemen ($C_{15}H_{24}$)	204	121	8.56		-	-	-	-
28	Alloaromadendrene($C_{15}H_{24}$)	204	91	-	6.39	-	-	-	-
29	Humulene ($C_{15}H_{24}$)	204	93	1.16	-	0.72	-	-	-
30	γ-Curcumene($C_{15}H_{24}$)	204	119	-	-	0.24	-	-	-
31	Cadinene ($C_{15}H_{24}$)	204	161	1.57	-	-	0.25	0.59	2.29
32	α-Santalene($C_{15}H_{24}$)	204	94	-	-	-	5.46	-	-
33	Germacrene ($C_{15}H_{24}$)	204	161	2.34	-	-	-	-	-
34	β-Bisabolene ($C_{15}H_{24}$)	204	69	7.12	4.58	4.66	4.68	0.68	2.16
35	α-Bisabolene ($C_{15}H_{24}$)	204	69	0.48	-	0.37	3.36	-	-
36	Selina-3,7(11)-Diene ($C_{15}H_{24}$)	204	161	1.08	5.64	-	-	-	-
37	Longicyclene ($C_{15}H_{24}$)	204	94	2.61	-	-	-	-	-
38	γ-Selinene ($C_{15}H_{24}$)	204	161	1.46	-	-	-	-	-
39	Isolongifolene ($C_{15}H_{22}$)	202	161	0.23	-	-	-	-	1.56
	Oxygenated Sesquiterpenes			**2.27**	**22.03**	**7.48**	**15.85**	**2.49**	**22.26**
40	Nerolidol($C_{15}H_{26}O$)	222	69	-	0.62	0.38	0.52	-	3.06
41	Caryophyllene oxide($C_{15}H_{24}O$)	220	43	0.21	-	0.2	0.75	0.36	10.63
42	Eudesmol($C_{15}H_{26}O$)	222	41	-	2.07	-	1.45	-	3.01
43	T-Muurolol($C_{15}H_{26}O$)	222	95	-	5.32	-	-	-	-
44	α-Bisabolol($C_{15}H_{26}O$)	222	109	0.85	10.18	1.66	11	-	-
45	Juniper camphor($C_{15}H_{26}O$)	222	189	0.54	3.84	-	-	-	-
46	β-Spathulenol($C_{15}H_{24}O$)	220	43	-	-	0.79	-	-	-
47	Nootkatone($C_{15}H_{22}O$)	218	147	-	-	1.98	1.86	0.76	3.57
48	Farnesol ($C_{15}H_{26}O$)	222	69	0.67	-	2.47	-	-	-
49	α-Sinensal($C_{15}H_{22}O$)	218	93	-	-	-	-	1.37	1.99
50	Campherenone($C_{15}H_{24}O$)	220	41	-	-	-	0.27	-	-
	Oxygenated Diterpenes			**0**	**0**	**0**	**0.21**	**0**	**3.54**
51	Geranyl-geraniol($C_{20}H_{34}O$)	290	69	-	-	-	-	-	3.54
52	Geranyllinalool($C_{20}H_{34}O$)	290	69	-	-	-	0.21	-	-
	Triterpenes			**0**	**0**	**0**	**3.13**	**0**	**0**
53	Squalene($C_{30}H_{50}$)	410	69	-	-	-	3.13	-	-
	Hydrocarbon Compounds			**0.14**	**15.52**	**0**	**15.49**	**0**	**2.85**

54	α-Ionene($C_{13}H_{18}$)	174	159	-	-	-	0.24	-	-
55	1-Butylcyclohexene($C_{10}H_{18}$)	138	81	-	1.31	-	-	-	-
56	Docosane($C_{22}H_{46}$)	310	57	-	2.66	-	2.13	-	-
57	Tricosane($C_{23}H_{48}$)	324	57	-	-	-	3.36	-	-
58	Heneicosane ($C_{21}H_{44}$)	296	57	0.14	4.29	-	7.65	-	1.39
59	Tetracosane($C_{24}H_{50}$)	338	57		2.14		0.27		
60	Pentacosane($C_{25}H_{52}$)	352	57	-	4.48	-	1.11	-	0.9
61	Hexacosane($C_{26}H_{54}$)	366	57	-	0.64	-	-	-	
62	Heptacosane($C_{27}H_{56}$)	380	57	-	-	-	0.5	-	0.56
63	Nonacosane($C_{29}H_{60}$)	408	57	-	-	-	0.23	-	-
	Fatty Acids			**0.85**	**17.48**	**0**	**29.19**	**0.02**	**51.68**
64	Lauricacid($C_{12}H_{24}O_2$)	200	73	-	2.47	-	1.98	-	2.25
65	Myristicacid($C_{14}H_{28}O_2$)	228	73	-	-	-	4.02	-	3.44
66	Linoleic acid($C_{18}H_{32}O_2$)	280	67	0.11	4.82	-	4.19	0.02	19.08
67	Palmiticacid ($C_{16}H_{32}O_2$)	256	43	0.74	10.19	-	19	-	26.91
	Other Oxygenated Compounds			**3.86**	**3.57**	**8.42**	**2.18**	**2.46**	**2.23**
68	Perialldhyde ($C_{10}H_{14}O$)	150	68	0.09	-	-	-	-	-
69	Decanal($C_{10}H_{20}O$)	156	57	-	-	0.86	-	0.42	-
70	Neryl acetate($C_{12}H_{20}O_2$)	196	69	0.88	-	3.7	0.57	0.62	
71	Bornylacetate ($C_{12}H_{20}O_2$)	196	95	0.52	-	-	-	-	-
72	Dodecanal ($C_{12}H_{24}O$)	184	57	0.16	-	0.46	-	-	-
73	Geranylacetate ($C_{12}H_{20}O_2$)	196	69	2	-	3.4	0.55	0.48	-
74	Tetradecanal($C_{14}H_{28}O$)	212	57	-	2.79	-	-	-	-
75	Dibutylphthalate($C_{16}H_{22}O_4$)	278	149	0.21	0.78	-	1.06	0.94	2.23

GC/MS: Gas chromatography/ Mass SpectrometryMol.Wt: Molecular weight

B.P:Base peak

TABLE 2: GC/MS of EOs of *Citrus* peels (*C.aurantifolia*, *C.limon*and *C.sinensis*) using SE and UAE extraction techniques.

Peak No.	Compounds	Mol.Wt	B.P	*C.aurantifolia*		*C. limon*		*C. sinensis*	
				SE%	UAE%	SE%	UAE%	SE%	UAE%
	Monoterpene Hydrocarbons			**0**	**5.95**	**22.24**	**2.84**	**70.42**	**4.76**
1	α-Pinene($C_{10}H_{16}$)	136	93	-	-	-	0.65	-	-
2	β-Myrcene($C_{10}H_{16}$)	136	93	-	2.82	0.83	-	1.22	-
3	D-Limonene($C_{10}H_{16}$)	136	68	-	-	16.52	-	67.37	4.76
4	4-Carene($C_{10}H_{16}$)	136	93	-	-	0.63	-	0.46	-
5	Ocimene($C_{10}H_{16}$)	136	93	-	2.08	0.63	-	0.67	-
6	γ-Terpinene($C_{10}H_{16}$)	136	93	-	-	1.06	0.22	0.7	-
7	α-Terpinolene($C_{10}H_{16}$)	136	93	-	-	2.57	1.97	-	-
8	IsoCamphane($C_{10}H_{18}$)	138	95	-	1.05	-	-	-	-
	Oxygenated Monoterpenes			**0**	**0.32**	**3.88**	**5.98**	**0.87**	**0**
9	Citronella($C_{10}H_{18}O$)	154	41	-	-	-	0.02	-	-
10	4-Terpineol($C_{10}H_{18}O$)	154	71	-	0.32	-	0.17	-	-
11	Geraniol ($C_{10}H_{18}O$)	154	69	-	-	1.82	2.38	-	-
12	Citral ($C_{10}H_{16}O$)	152	69	-	-	0.92	3.41	-	-
13	Linalool ($C_{10}H_{18}O$)	154	71	-	-	0.48	-	0.87	-
14	α-Terpineol($C_{10}H_{18}O$)	154	43	-	-	0.66	-	-	-
	Sesquiterpene Hydrocarbons			**46.69**	**5.7**	**22.54**	**31.77**	**6.71**	**13.88**
15	β-Elemene($C_{15}H_{24}$)	204	81	5.46	-	-	0.32	-	2.75
16	Bergamotene($C_{15}H_{24}$)	204	93	-	0.32	0.66	8.48	-	-
17	β-Farnesene($C_{15}H_{24}$)	204	41	-	-	-	2.16	-	-
18	Germacrene ($C_{15}H_{24}$)	204	161	4.42		1	-	-	2.36
19	β-Bisabolene($C_{15}H_{24}$)	204	69	-	1.62	-	10.93	-	-
20	δ-Cadinene($C_{15}H_{24}$)	204	161	-	0.14	-	-	-	-
21	γ-Selinene($C_{15}H_{24}$)	204	161	-	0.59	-	-	-	-
22	Valencene($C_{15}H_{24}$)	204	161	-	-	-	3.96	-	7.49
23	Humulene ($C_{15}H_{24}$)	204	93	3.24	-	-	-	0.8	
24	Caryophyllene ($C_{15}H_{24}$)	204	93	13.93	0.13	4.1	5.12	1.85	1.28
25	α-Himachalene ($C_{15}H_{24}$)	204	93	-	-	6.1	-	-	-

26	Curcumene($C_{15}H_{24}$)	204	119	-	1.25		-	-	-
27	Farnesene ($C_{15}H_{24}$)	204	41	3.12		0.51	-	-	-
28	β-santalene($C_{15}H_{24}$)	204	94		1.65	0.78	-	-	-
29	β-Cubebene($C_{15}H_{24}$)	204	161	3.12	-	-	-	0.85	-
30	Ledene($C_{15}H_{24}$)	204	107	0.99	-	-	-	-	-
31	Valencene ($C_{15}H_{24}$)	204	161	-	-	0.98	-	0.85	-
32	α-Bisabolene ($C_{15}H_{24}$)	204	69	9.1	-	0.74	0.8	0.76	-
33	α-Copaene($C_{15}H_{24}$)	204	161	-	-	7.67	-	1.22	-
34	Cadinene ($C_{15}H_{24}$)	204	161	-	-	-	-	0.38	-
35	Patchoulene($C_{15}H_{24}$)	204	161	3.31	-	-	-	-	-
	Oxygenated Sesquiterpenes			**12.56**	**2.56**	**9.42**	**2.71**	**3.20**	**11.21**
36	Nerolidol($C_{15}H_{26}O$)	222	69	-	0.19	0.38	-	0.26	-
37	Elemol($C_{15}H_{26}O$)	222	59	-	-	-	-	0.38	1.54
38	Caryophyllene Oxide($C_{15}H_{24}O$)	220	43	-	-	0.83	-	0.9	3.88
39	α-Bisabolol($C_{15}H_{26}O$)	222	109	-	1.57	2.37	1.54	-	-
40	Juniper Camphor($C_{15}H_{26}O$)	222	189	-	0.44	-	-	0.36	-
41	Nootkatone($C_{15}H_{22}O$)	218	147	-		0.71	0.64	0.62	2.8
42	α-Sinensal($C_{15}H_{22}O$)	218	93	-	0.36	-	-	0.28	2.99
43	Isolongifolol($C_{15}H_{26}O$)	222	109	-	-	1.26	-	-	-
44	Farnesol ($C_{15}H_{26}O$)	222	69	12.56	-	3.87	0.53	0.4	-
	Diterpenes			**0**	**0**	**0.87**	**0**	**0.52**	**0**
45	Geranyl -α-Terpinene($C_{20}H_{32}$)	272	69	-	-	0.87	-	0.52	-
	Triterpenes			**0**	**0.52**	**6.24**	**1.5**	**2.73**	**8.14**
46	Squalene($C_{30}H_{50}$)	410	69	-	0.52	6.24	1.5	2.73	8.14
	Oxygenated Triterpenes Hydrocarbon			**0**	**0.67**	**0**	**0**	**0**	**0**
47	α-Amyrine($C_{30}H_{50}O$)	426	218	-	0.67	-	-	-	-
	Hydrocarbon Compounds			**5.79**	**19.12**	**7.92**	**22.46**	**5.06**	**11.20**
48	2,3,3-Trimethylcyclohexan(C_9H_{16})	124	68	-	0.52	-	-	-	-
49	Bicyclo[4.1.0]Heptane,7-(1-Methylethylidene)($C_{10}H_{16}$)	136	93	2.5	-	-	-	-	
50	Undecane($C_{11}H_{24}$)	156	57	-	-	0.76	4.24	-	-
51	Dodecane($C_{12}H_{26}$)	170	57	-	-	1.01	5.12	1.2	-

52	Tetradecane($C_{14}H_{30}$)	198	43	-	-	0.79	-	0.58	-
53	(Diphenylmethylene)Cyclopropane($C_{16}H_{14}$)	206	205	-	2.48	-	-	-	-
54	7-Hexadecene($C_{16}H_{32}$)	224	55	-	-	-	2.77	-	-
55	Heptadecene($C_{17}H_{34}$)	238	83	-	-	3	-	-	-
56	Eicosane($C_{20}H_{42}$)	282	57	-	2.75	-	1.48	0.9	7.56
57	Docosane($C_{22}H_{46}$)	310	57	1.43	-	1.25	2.13	1.59	-
58	Tricosane($C_{23}H_{48}$)	324	57	-	-	1.11	-	-	1.27
59	Tetracosane($C_{24}H_{50}$)	338	57	-	5.41	-	3.16	-	-
60	Benzene,[3-(2-Cyclohexylethyl)-6-Cyclopentylhexyl]($C_{25}H_{40}$)	340	92	0.91	-	-	-	-	-
61	Pentacosane($C_{25}H_{52}$)	352	57	-	4.14	-	1.04	-	2.37
62	Hexacosane($C_{26}H_{54}$)	366	57	-	0.49	-	0.52	-	-
63	Cyclohexane,1,4-Dimethyl-2-Octadecyl($C_{26}H_{52}$)	364	111	0.95	-	-	-	-	-
64	3-Methylpentacosane($C_{26}H_{54}$)	366	365	-	1.38	-	-	-	-
65	Octacosane ($C_{28}H_{58}$)	394	57	-	0.71	-	-	-	-
66	Nonacosane($C_{29}H_{60}$)	408	43	-	1.24	-	2	0.79	
	Fatty acids			**4.02**	**31.75**	**12.88**	**8.02**	**6.6**	**40.31**
67	Lauric Acid($C_{12}H_{24}O_2$)	200	73	-	0.74	-	-	-	-
68	Myristic Acid($C_{14}H_{28}O_2$)	228	73	-	1.92	1.03	-	-	-
69	Palmitic Acid($C_{16}H_{32}O_2$)	256	73	-	2.59	1.28	2	1.5	20.1
70	Linoleic Acid($C_{18}H_{32}O_2$)	280	67	-	21.98	5.29	6.02	3.9	20.21
71	Stearic Acid ($C_{18}H_{36}O_2$)	284	117	-	2.15	-	-	1.2	-
72	Gibberellic Acid($C_{19}H_{22}O_6$)	346	136	-	-	1.23	-	-	-
73	Octadecanoic Acid,2-(Hexadecyloxy)Ethyl Ester($C_{36}H_{72}O_3$)	552	57	1.31	-	-	-	-	-
74	Nonanoic Acid,9-(O-Propylphenyl),Methyl ester($C_{19}H_{30}O_2$)	290	105	2.71	-	-	-	-	-
75	Lignoceric acid ($C_{24}H_{48}O_2$)	368	74	-	0.97	-	-	-	-
76	Ethyl Lignocerate ($C_{26}H_{52}O_2$)	396	88	-	1.4	-	-	-	-
77	PhthalicAcid,di(2-Propylpentyl)Ester($C_{24}H_{38}O_4$)	390	149	-	-	4.05	-	-	-
	Sterols			**22.94**	**1.69**	**0**	**1.37**	**0**	**2.68**
78	Carda-4,20(22)-Dienolide,3-[(6-Deoxy-3-O-Methyl-Allopyranosyl)Oxy]-1,14-Dihydroxy($C_{30}H_{44}O_9$)	548	43	1.32	-	-	-	-	-
79	Ethyl Iso-Allocholate($C_{26}H_{44}O_5$)	436	43	7.82	-	-	-	-	-

80	14- Methylcholesta-7,9(11)-Dien-3-Yl Acetate($C_{30}H_{48}O_2$)	440	43	2.09	-	-	-	-	-
81	3-Hydroxyspirost-8-En-11-One($C_{27}H_{40}O_4$)	428	43	1.13	-	-	-	-	-
82	Ergosta-5,22-Dien-3-Ol,Acetate, (3á,22E)($C_{30}H_{48}O_2$)	440	43	1.31	-	-	-	-	-
83	Stigmasterol($C_{29}H_{48}O$)	412	55	-	0.35	-	0.47	-	-
84	Campesterol ($C_{28}H_{48}O$)	400	43	-	0.18	-	-	-	-
85	Clionasterol($C_{29}H_{50}O$)	414	57	0.95	-	-	-	-	-
86	7,8-Epoxylanostan-11-ol,3-Acetoxy($C_{32}H_{54}O_4$)	502	44	1.82	-	-	-	-	-
87	Cholan-24-Oicacid,3-(Acetyloxy)-7,12-Dioxo-,MethylEster,(3à,5á)($C_{27}H_{40}O_6$)	460	245	6.5	-	-	-	-	-
88	Stigmast-5-En-3-Ol($C_{29}H_{50}O$)	414	414	-	1.16	-	0.9	-	2.68
	Carotenoids			**4.11**	**0**	**0**	**0**	**0**	**0**
89	Lycoxanthin($C_{40}H_{56}O$)	552	91	1.19	-	-	-	-	-
90	Lutein($C_{40}H_{56}O_2$)	568	91	2.03	-	-	-	-	-
91	Carotene,1,1',2,2'-Tetrahydro-1,1'-Dimethoxy($C_{42}H_{64}O_2$)	600	73	0.89	-	-	-	-	-
	Coumarins			**0**	**28.06**	**0**	**0**	**0**	**0**
92	Limetin($C_{11}H_{10}O_4$)	206	206		28.06	-	-	-	-
	Other Oxygenated Compounds			**3.43**	**1.47**	**13.33**	**23.29**	**3.32**	**7.31**
93	4-Methyl-2-Phenyl-1,3-Dioxolane($c_{10}H_{12}O_2$)	164	44	1.59			-	-	-
94	1-Decanol,2-Methyl($C_{11}H_{24}O$)	172	57	-	-	-	-	0.91	-
95	Formic Acid, Ethenyl Ester($C_3H_4O_2$)	72	43	0.93	-	-	-	-	-
96	Citronellyl Acetate ($C_{12}H_{22}O_2$)	198	43	-	-	-	1.04	0.56	-
97	Neryl Acetate($C_{12}H_{20}O_2$)	196	69	-	-	-	8.87	-	-
98	Geranyl Acetate ($C_{12}H_{20}O_2$)	196	69	-	-	7.03	7.27	0.81	-
99	Di Butyl Phthalate($C_{16}H_{22}O_4$)	278	149	-	0.46	-	-	0.45	-
100	1-Eicosanol($C_{20}H_{24}O$)	298	41	-	-	-	-	0.59	-
101	Heptanal($C_7H_{14}O$)	114		0.91	-	-	-	-	-
102	1-Docosanol($C_{22}H_{46}O$)	326	43	-	-	3.4	-	-	-
103	Bis(2-Ethylhexyl)Phthalate($C_{24}H_{38}O_4$)	390	149	-	-	-	1.5	-	-
104	2-Hexadecyloxirane($C_{18}H_{36}O$)	268	82	-	-	-	3.1	-	-
105	Linalyl Propionate($C_{13}H_{22}O_2$)	210	93	-	-	2.9	-	-	-
106	Vitamin E($C_{29}H_{50}O_2$)	430	430	-	1.01	-	1.51	-	7.31

GC/MS: Gas chromatography/ Mass Spectrometry Mol.Wt.: Molecular weight

B.P: Base peak

TABLE 3: Summary of all the identified classes of compounds in all extraction methods of *Citrus* peels (*Citrus aurantifolia*, *Citrus limon*, and *Citrus sinensis*)

Compounds	*C. aurantifolia*				*C. limon*				*C. sinensis*			
	HD %	**MAE %**	**SE %**	**UAE %**	**HD %**	**MAE %**	**SE %**	**UAE %**	**HD %**	**MAE %**	**SE %**	**UAE %**
Yields %	0.8	1.52	1.38	1.06	1.2	2	1.44	1.6	2.4	2.8	1.9	1.8
No. of components	38	27	30	39	29	40	41	37	23	20	37	17
Hydrocarbons	0.14	15.52	5.79	19.12	0	15.49	7.92	22.46	-	2.85	5.06	11.2
Monoterpene Hydrocarbons	43.41	-	-	5.95	61.09	-	22.24	2.84	85.87	-	70.42	4.76
Oxygenated Monoterpenes	17.8	22.24	-	0.32	7.03	17.17	3.88	5.98	4.17	2.65	0.87	-
Sesquiterpene Hydrocarbons	31.53	18.76	46.69	5.7	15.9	16.38	22.54	31.77	4.5	14.33	6.71	13.88
Oxygenated Sesquiterpenes	2.27	22.03	12.56	2.56	7.48	15.85	9.42	2.71	2.49	22.26	3.2	11.21
Diterpene Hydrocarbons	-	-	-	-	-	-	0.87	-	-	-	0.52	-
Oxygenated Diterpene Hydrocarbons	-	-	-	-	-	0.21	-	-	-	3.54	-	-
Triterpene Hydrocarbons	-	-	-	0.52	-	3.13	6.24	1.5	-	-	2.73	8.14
Oxygenated Triterpene Hydrocarbons	-	-	-	0.67	-	-	-	-	-	-	-	-
Fatty acids	0.85	17.48	4.02	31.75	-	29.19	12.88	8.02	0.02	51.68	6.6	40.31
Sterols	-	-	22.94	1.69	-	-	-	1.37	-	-	-	2.68
Carotenoids	-	-	4.11	-	-	-	-	-	-	-	-	-
Coumarins	-	-	-	28.06	-	-	-	-	-	-	-	-
Other Oxygenated Compounds	3.86	3.57	3.43	1.47	8.42	2.18	13.33	23.29	2.46	2.23	3.32	7.31
Total %	**99.86**	**99.6**	**99.54**	**97.81**	**99.92**	**99.6**	**99.32**	**99.94**	**99.51**	**99.54**	**99.43**	**99.49**

HD (7.48 %) and UAE (2.71 %). In *CS*, the EOs obtained by MAE and UAE were richer with oxygenated sesquiterpenes (22.26 %, 11.21 %, respectively) than by the HD, and SE (2.49 %, and 3.2 %, respectively). Caryophyllene oxide, nootkatone, and α-sinensal were the major oxygenated sesquiterpene components found in four extraction techniques. Squalene was the principal

triterpene component in the three *Citrus* species extracted mainly by SE and UAE techniques.

Fatty acids from EOs of *C.A, C.L* and *C.S* were more concentrated and found in large amounts by using innovative techniques, MAE (17.48 %, 29.19 %, 51.68 %, respectively) and UAE technique (31.75 %, 8.02 %, 40.31 %, respectively), in comparison with fatty acids obtained using conventional techniques (HD and SE). Palmitic acid and linoleic acid were the main fatty acids identified. Table 3 showed the highest percentage of sterols found in *CA* EOs using the SE technique (22.94 %), and a small amount was found in *CL*EOs and *CS* EOs. Yilmaz and Güneser in 2017 [24] proved the presence of fatty acids, sterols, and α-tocopherol in the lemon seed oil where linoleic, oleic, and palmitic acids were the major fatty acids components. Coumarin compounds, mainly limetin (28.06 %) present in *CA* EOs by using UAE technique and absent in the other techniques. Limetin considered a defense compound and present in high concentration in *C.aurantifolia* [25,26,27].

Conclusion

Essential oils were extracted from the peels of *C.aurantifolia*, *C.limon* and *C.sinensis* by two innovative techniques (microwave-assisted extraction and ultrasound-assisted extraction) and compared to that extracted by conventional techniques (hydrodistillation and solvent extraction), then analyzed the extracts using GC/MS. The obtained results confirmed the effectiveness of innovative techniques by saving extraction time and without causing any changes in the volatile oil composition. The MAE and UAE techniques were more efficient for extraction of hydrocarbons, oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, and fatty acids from different essential oils *Citrus* peels, which were considered the basic ingredients in many cosmetic and pharmaceutical industries (antioxidant, antibacterial, antifungal, antispasmodic, hypotensive, anticancer and vasorelaxant).

Conflicts of interest

The authors declare no conflicts of interests.

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