



Chemical Composition and Biological Activities of Essential Oils and Organic Extracts from Fresh and Sun-Dried *Citrus limon* Peels

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Abstract

The present study deals with the characterization of essential oils extracted from both fresh and sun-dried lemon (*Citrus limon* (L.) Burm.f) peels by hydrodistillation, followed by extraction with ether or ethyl acetate, yielding four essential oil samples. The extraction yield varied between 0.1 and 1.65%. The essential oils constituents were identified using GC/MS analysis; limonene was the major compound ranging between 3.75 and 76.78%. In addition, ethyl acetate and methanol extracts were prepared from sun-dried lemon peels, and characterized by HPLC–PDA–ESI–MS. Four flavanone-*O*-diglycosides (neoreiocitrin, neohesperidin, melitidin and naringin), three flavone di-*C*-glycosides (diosmetin-6,8-di-*C*-glycoside, apigenin-6,8-di-*C*-glycoside, di-*C*-glycosideflavone), two flavone *O*-diglycosides (vicenin 2, neodiosmin), three polymethoxyflavones (tangeretin, nobiletin, 5,6,7,4'-tetramethoxyflavone), and two coumarins (scoparin, isomeranzin) were identified. Lemon peels ethyl acetate extract exhibits a powerful antioxidant activity ($IC_{50} = 0.09 \mu\text{g}/\text{mL}$) compared to those of vitamin E ($IC_{50} = 0.017 \mu\text{g}/\text{mL}$) and BHT ($IC_{50} = 0.026 \mu\text{g}/\text{mL}$). On the other hand, essential oils from fresh and sun-dried lemon peels as well as organic extracts exhibited interesting antimicrobial activities against *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus* (Gram⁺) and *Salmonella enterica*, *Escherichia coli*, *Klebsiella pneumoniae* (Gram⁻). Lemon peels would be used in the agro-food industry as alternative to the undesirable chemical additives.

Keywords *Citrus limon* · Chemical composition · Phenolic contents · Antioxidant activity · Antibacterial activity

1 Introduction

Citrus is a flowering plant which belongs to the Rutaceae family comprising about 140 genera and 1300 species. *Citrus* fruit is considered among the most important fruit in the world which is produced in several countries with tropical or subtropical climates. The main *Citrus* producers are USA, Mexico, Brazil, China, Pakistan, Japan and Mediterranean countries basin especially Tunisia. In general, *Citrus* peels are widely used as a flavoring additive for cakes, ice cream drinks, perfumes and household products [1]. The fresh exocarp of *Citrus sinensis* is traditionally used in Tunisia to

prepare several traditional dishes and to flavor tea, and the powdered air dried peels are used to prepare cake. *Citrus* fruit and its by-products have multiple uses in folk medicine and cosmetics with a high economic value [2]. *Citrus* flavonoids show a wide spectrum of biological activities including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities [3]. The most important product obtained from the *Citrus* by-products is the peel essential oil, which is endowed with many biological properties such as antioxidant [4], anti-inflammatory, anxiolytic [5], antimicrobial [6], and antifungal [7]. *Citrus* essential oils contain high amounts (70–95%) of monoterpene hydrocarbons and low amounts of sesquiterpene hydrocarbons, which are accountable for a characteristic flavor. *Citrus* peels contain alcohols, aldehydes and aliphatic esters [8, 9]. *Citrus limon* (L.) Osbeck is a significant medical plant known as a typical healthy food for a long time. *Citrus limon* (L.) Burm.f is extensively cultivated in Tunisia, especially in the Cap Bon (Nabeul) and other Mediterranean countries like Italy, Spain, Turkey, and Egypt [10]. This specie gives an important quantity of waste or by-products such as seeds, peels and pulps [11].

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Ascorbic acid (vitamin C, an antioxidant with important biological activities *in vivo*) [12] is present abundantly in the lemon juice. In addition, it has been reported that lemon juice and *Citrus* flavonoids have desmutagenic [13] and anti-mutagenic effects [14]. Lemon fruit contains a number of nutrients such as flavonoids, ascorbic acid, citric acid, and minerals. It is mostly cultivated for its flavonoids, which are endowed with antibacterial and anticancer activities. Organic extracts from different lemon organs (peels, leaves and flowers) have shown interesting antimicrobial activity against various clinically pathogenic bacterial strains [15]. To our knowledge, in the previous studies performed on Tunisian *Citrus limon*, researchers have extracted essential oil from fresh peels and there was no further investigations dealing with dry plant material. Indeed, the absence of water in the dried peels would increase the extraction yield and give more concentrated components in the essential oil. In the present work, we were interested in the extraction of essential oils from both fresh and sun-dried lemon peels in order to select the best plant material in term of its essential oil chemical composition. In addition, the nature of the solvent used for the essential oil recovery from the hydro-distillation-aqueous phase was optimized in order to obtain the best extraction yield.

The objective of the present study was to compare the chemical composition of four essential oils extracted from sun-dried (temperature average about 20 °C and during for 1 week) and fresh lemon peels using diethyl ether and ethyl acetate, after characterization by GC/MS analysis, and to evaluate their antioxidant and antibacterial activities. In addition, the intention of this work was also to determine the chemical composition of organic extracts from sun-dried lemon peels using HPLC-PAD ESIMS.

2 Material and Methods

2.1 Collection of Plant Material

Lemon peels (*Citrus limon*(L.) Burm.f) (Weregathered from Sfax (Tunisia) during January 2015). All the fruit was an eating quality, without harm or blemishes. A voucher specimen of the plant material (LCSN129) was deposited at the Laboratory of Organic Chemistry in the Faculty of Sciences of Sfax (Tunisia). The fruits were peeled off carefully using a sharp razor blade to avoid any ravage of oil glands. The lemon peels to be analyzed were processed in two different ways: one part was used fresh; the other part was sun-dried for one week at a temperature average about 20 °C.

2.2 Preparation of the Extracts

The dried lemon peels (500 g) were extracted sequentially by maceration using three different solvents: hexane, ethyl acetate and methanol (3 × 500 mL of each). After filtration and evaporation under reduced pressure, samples were stored in darkness at 4 °C before analysis.

2.3 Isolation of Essential Oils

The samples of fresh and dried lemon peels were subjected to hydrodistillation for 2 h using a Clevenger-type apparatus. Each lemon peels type was extracted twice to give two separated aqueous phases. To improve their recovery, essential oils from each two aqueous phases related to the same peels type were taken up separately with two solvents: one aqueous phase was extracted with diethyl ether and the other with ethyl acetate. The obtained four extracts were dried over anhydrous sodium sulphate until the last traces of water were removed and then concentrated under reduced pressure using a rotary evaporator. Finally, the obtained essential oils were transferred into brown bottles and stored in the refrigerator at 4 °C until tested and analyzed. The four types of essential oils obtained were designated as follows:

- Lemon fresh peels essential oil extracted with ether (LFPEO-E).
- Lemon dried peels essential oil extracted with ether (LDPEO-E).
- Lemon fresh peels essential oil extracted with ethyl acetate (LFPEO-A).
- Lemon dried peels essential oil extracted with ethyl acetate (LDPEO-A).

2.4 GC/MS Analysis

Essential oils were analyzed using an Agilent-Technologies 19091S-433 instrument, equipped with a capillary column HP-5MS (5% phenyl, 95% methyl siloxane), with a length of 30 m, a nominal diameter of 0.25 mm and a film thickness of 0.25 µm. The injection of 0.4 µL of each sample is carried out with a split mode with a ratio of 1: 100, the carrier gas is H₂. Analysis was performed in programmed temperature: 50 °C for 5 min, then (50–250 °C) over 25 min. The injection temperature was 250 °C, the equilibration time was 0.5 min and the flow rate was equal to 1 mL/min. Mass spectrometric detection was used operating in electron impact (EI) mode (70 eV) in a scan range 50–550 m/z. Most component were tentatively identified by comparison of their GC retention indices (RI), determined with reference to an homologous series of C₅–C₂₈ n-alkanes and with those of

authentic standards disposable in the authors' laboratory. Identification was confirmed by comparison of their mass spectral fragmentation patterns with those stored in the MS data base (National Institute of Standards and Technology and Wiley libraries) and with mass spectra literature data [16, 17].

2.5 HPLC–PDA–ESI–MS Analysis

HPLC–MS files were converted to netcdf file format using the File Converter tool in X-Calibur software. Detected diode array was performed simultaneously at three different wavelengths: 245, 280 and 360 nm. The mass analysis was performed with an ESI interface in the positive ionization mode. The data were acquired in the full scan (range of mass was 200–1200 *m/z*) and MS tandem modes. Two mobile phases were used for the chromatographic separation elution: mobile phase A was water containing 0.1% of formic acid and mobile phase B was 100% acetonitrile (ACN). The gradient elution program was conducted as follows: 0 (90% A); 5.0 min (90% A); 47.0 min (100% B); 52.0 min (100% B); 55.0 min (90% A); finishing at 60.0 min (90% A). The flow rate was 4 $\mu\text{L}/\text{min}$, and column temperature was 50 °C. Analytes were detected as protonated molecules. Compounds identification was based on comparison of their λ_{max} values, retention times, and MS data with previous studies as indicated in the results and discussions section.

2.6 Determination of Phenolics Content (TPC)

In order to determine the total phenolics content, organic extracts were analyzed by the Folin–Ciocalteu method using gallic acid as a standard [18]. 1 mg of extract was dissolved in 1 mL of methanol, 0.5 mL of Folin–Ciocalteu reagent and 3 mL of distilled water were added successively. After 5 min stirring, 0.5 mL of 2% sodium carbonate solution was added. Absorbance was measured at 760 nm after incubation in the dark for 90 min, and the results are expressed in mg gallic acid equivalents per gram extract (mg GAE/g extract).

2.7 Antioxidant Activity

2.7.1 DPPH Radical Scavenging Assay

The tested antioxidant activity of lemon's extracts and its essential oils was based on the assay of free 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity following the procedure described in a previous study [19]. 50 μL of the sample's of various concentrations were dissolved in methanol and added to 5 mL of a 0.004% methanol solution of DPPH. After being incubated in darkness for 30 min and at room's temperature, the antioxidant activity was read at 517 nm. Methanol was used as the blank.

Scavenging of the free radical (DPPH) presented in percent (I%) was calculated by the following expression:

$$I\% = (A_0 - A_1/A_0) \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of sample tested. Sample (extract or essential oil) concentration providing 50% DPPH scavenging (IC_{50}) was graphically calculated. The antioxidants butylate-hydroxyl-toluene (BHT) and vitamin E were used as a positive controls. All tests were carried out in triplicate.

2.7.2 Reducing Activity of Potassium Ferricyanide (FRAP)

Total reducing capacity (TRC) was tested using the previous reported method by Ferreira et al. [20]. 2 mL of sample were diluted into 10 mL distilled water. 1 mL of diluted sample was taken, 2.5 mL of sodium phosphate buffer (0.2 mol/L, pH 6.6) and potassium ferrocyanide ($\text{K}_3\text{Fe}(\text{CN})_6$) solution (1%) were combined, then kept for 20 min in a water bath at 50 °C.

Thereafter, an extra trichloro-acetic acid solution (10%, 2.5 mL) was mixed. Before being kept still for 10 min 2.5 mL of the reaction solution was mixed with a solution of 2.5 mL of distilled water and 1 mL of a ferric trichloride (0.1%) solution, were integrated and vortexed. The mixture absorbance was determined at 700 nm and as a blank the distilled water was used. The distilled water was used as a blank however Gallic acid and ascorbic acid were employed as references.

2.7.3 Total Antioxidant Capacity

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto, Pineda and Aguilar [21]. The trial is based on the reduction of Mo(VI) to Mo(V) by the samples and subsequent formation of a green phosphate/Mo(V) complex at acid pH. The solution of 1 mg/mL of sample in 1 methanol was added to 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was incubated at 95 °C for a period of 90 min. After cooling it to the room's temperature the solution absorbance was measured at 695 nm using a spectrophotometer UV–Vis against blank after cooling to room temperature. Methanol (0.3 mL) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of vitamin E per grams of extract (mg/g of extract).

2.8 Determination of Antibacterial Activity

The Bacterial cultures were first grown on Muller Hinton agar (MH) plates at 37 °C for 18–24 h before being planted onto the nutrient agar. Colonies of the respective bacteria were transferred into API suspension medium (Bio-Merieux)

and adjusted to 0.5 McFarland turbidity standards with a Densimat (Bio-Merieux) [22, 23]. The obtained inocula were lean into MH agar plates using a sterile swab prior to drying at 37 °C during 15 min. Discs with 6 mm of diameter were prepared from Whatman paper and sterilized. The obtained sterile discs were putted at the surface of MH agar and then 5 µL of each ethyl acetate extract solution (2 mg/mL) was dropped onto the corresponding paper disc [24]. The incubation of the prepared Petri-dishes should be at 37 °C for 18 and 24 h. By measuring the diameter of the clear zone surrounding the Whatman paper disc the antibacterial activity was evaluated and as a positive antibacterial control Ampicillin was used and ethyl acetate without extract was used as negative control.

3 Results and Discussion

3.1 Organic Extraction Yields

Extraction is a significant step for the retrieval and segregation of bioactive phytochemicals from plant materials before testing. The amount and the type of compounds in the extract are determined by the selection of the extraction solvents [25]. After maceration of dry lemon peels in solvents with increasing polarity (hexane, ethyl acetate and methanol), the corresponding extraction yields were calculated. The extraction yield obtained with methanol was the highest (4.55%) followed by those hexane (0.43%) and ethyl acetate (0.42%). Consequently, lemon peels are rich in polar compounds such as polyphenols.

3.2 Essential Oil Yields

The investigated lemon peels were obtained from plants belonging to the same *Citrus* variety and cultivated under the same climatic and agricultural conditions. After hydro-distillation of the (dry and fresh) lemon peels, ether and ethyl acetate were used as extraction solvents of essential oils from the aqueous phases. This method afforded four different essential oils, which were produced and analyzed under the same conditions.

3.3 Chemical Composition of Lemon Peel Essential Oils

The composition of essential oils from fresh and sun-dried lemon peels is shown in Table 1. The identified compounds appertain to hydrocarbon and oxygenated classes such as monoterpenes, sesquiterpenes, monoterpeneols, aldehydes, alcohols and esters. Quantitatively, limonene was the major monoterpene identified in the four essential oils, representing 76.78%, 48.52%, 28.25% and 4.75% of the total

identified compounds in LFPEO-E, LFPEO-A, LDPEO-E and LDPEO-A, respectively. Other monoterpenes were identified such as sabinene, β -pinene, myrcene, and *p*-cymene, in different concentrations. α -Humulene, 3-carene and phellandrene were detected only in essential oils from fresh peels: 9.37–8.2%, 1.02–0.59% and 6.47–3.68%, in LFPEO-E and LFPEO-A, respectively. Moreover, another level of sesquiterpenes was identified in the LDPEO in function of the extraction solvent. γ -Terpinene (3.9%) in LDPEO-A and α -terpinene (2.55%) in LDPEO-E. The alcohols were abundant only in LDPEO, especially in LDPEO-A such as 1-octanol, linalool, *Z*-carveol and *cis*-geraniol with different concentrations varying from 0.17% to 1.19%. Hexanol, bicyclo[2.2.1]heptan-2-ol, farnesol and thymol were present only in LDPEO-E representing 4.02%, 1.29%, 1.07%, 0.43% of the total identified compounds, respectively. Among the aldehydes, *Z*-citral and *E*-citral were present either in LFPEO-E (1.45% and 1.2%, respectively) or in LFPEO-A (0.54% and 0.55%, respectively). One ester, α -terpinyl acetate (10.55%) was identified in LDPEO-E. Regarding the chemical composition of the essential oils, there were significant difference in terms of profiles and yields when the extraction was performed either by diethyl ether or by ethyl acetate, related to their different polarity. Previous studies showed that monoterpene hydrocarbons were the main components in *Citrus* peel essential oil; they represented 80.03% of Pakistan *Citrus* peel essential oil [26] and ranged between 97.6 and 99.3% in a Tunisian sample [10]. According to Di Vaio et al. [27], limonene was the major constituent (72.52–76.40%) in 18 lemon cultivars from Campania and Sicily. Some other reports revealed high levels of limonene in various *Citrus* fruits: *Citrus sinensis* (80.9%), *Citrus paradisi* (50.8%) and *Citrus reticulata* (69.9%) from Pakistan [28]; between 89.1 and 95.5% in *Citrus reticulata* from China [29]. In our work, the percentage of limonene ranged from 74.97 to 95.17% in the essential oil LFPEO-E. This result is higher than that of Mushtaq et al. [26] who stated that the limonene level ranged between 53–86% in four citrus fruits (*Citrus sinensis*(L.)*Osbeck*, *Citrus limetta*, *Citrus paradisi*, *Citrus lemon* 'Eureka). On the other hand, myrcene was present at a high concentration in LDPEO-E (2.09–10.41%), which was in agreement with the result of Adebisi [30]. This percentage is higher than others, ranging between 0.90 and 6.20% for Pakistan and Turkey *Citrus limon*(L.)*Osbeck*, respectively [26, 31]. The α -pinene concentration ranged between 0.65 and 3.33% in essential oil of *Citrus* lemon peels from Nigeria [30] and between 0.42 and 1.26% in Florida and Iran [31, 32]. These values were not in agreement with our results where α -pinene is not available in the Tunisian lemon peel essential oils. These different concentrations can be explained by plant organ, stage, geographic origin, season, environmental factors and genetic differences [33]. The occurrence of some volatile substances

Table 1 Chemical composition of essential oils from dry and fresh peels of *Citrus limon*

Compound name	RT (min)	RI	LFPEO-E (%)	LFPEO-A (%)	LDPEO-E (%)	LDPEO-A (%)
1-Octanol	3.075	859	–	–	0.73	0.82
Hexanol	3.076	859	–	0.11	4.02	–
Ocimene	9.076	1010	–	–	0.59	–
3-Carene	9.089	1011	1.02	0.59	–	–
Sabinene	10.302	1078	4.89	0.04	–	–
β-Pinene	10.381	1081	–	3.13	–	–
<i>Cis</i> -Geraniol	10.391	1089	–	–	0.51	0.64
Linalool	11.087	1098	–	–	1.19	0.54
α-Terpinene	11.862	1102	–	–	2.55	–
Limonene	11.983	1120	76.78	48.52	28.25	4.75
γ-Terpinene	12.916	1168	–	–	–	3.9
α-Gumulene	12.924	1170	9.37	8.2	–	–
Phellandrene	12.927	1171	6.47	3.68	–	0.68
<i>p</i> -Cymene	16.693	1312	–	1.07	0.55	–
α-Terpinyol acetate	16.864	1327	–	–	10.55	–
Safranal	17.138	1421	–	–	0.73	–
<i>Z</i> -Carveol	17.679	1489	–	–	0.17	–
Perilla alcohol	17.924	1502	–	–	2.57	–
<i>Z</i> -Citral	18.325	1561	1.45	0.54	–	–
Verbenol	18.337	1562	–	0.88	–	–
<i>Cis</i> -Carvone	18.398	1571	–	–	0.56	–
<i>E</i> -Citral	19.184	1592	1.2	0.55	–	–
Thymol	19.963	1612	–	–	0.43	–
Myrcene	21.596	1684	–	–	3.46	–
Farnesol	25.274	1731	–	–	1.07	–
Bicyclo[2.2.1]heptan-2-ol	41.529	1983	–	–	1.29	–
% Monoterpenes	–	–	99.9	80	35	2.5
% Sesquiterpenes	–	–	–	7.6	38	19
Other compounds	–	–	–	–	–	17.14

RI retention indices on DB-5MS column in reference to n-alkanes, RT retention time

in low quantities could be explained by differences environmental factors, weather and genetic conditions [34].

3.4 HPLC–PDA–ESI–MS

The ethyl acetate and methanol extracts of lemon dried peels were analyzed by HPLC–PDA–ESI–MS. Fig. 1 shows the HPLC–ESI–MS profiles of the two extracts. The obtained chromatograms in positive ion mode show pseudomolecular ions at m/z 597, 595, 611, 683, 723, 565, 581, 261, 373, 403, 343, 595, 625, 463 and 609 as well as other ions depending on the voltage applied to the source.

In ethyl acetate and methanol extracts fifteen major compounds were determined based on their λ_{\max} values, retention times and MS data (Table 2). Analyses of MS and UV spectra allowed the identification of fourteen compounds: three methoxyflavones (13, 14, and 15), four flavanone-*O*-diglycosides (3, 6, 9, and 11), three flavone di-*C*-glycosides

(1, 2, and 10), two flavone-*O*-diglycosides (4 and 7), and two coumarins (5 and 12). Compound 8 remained unidentified.

Flavanones were identified in the two extracts. It has been reported that the major phenolic compounds of *Citrus* generally are flavanones [35].

The molecular ions related to the protonated molecules of the *O*-diglycosyl flavonoids 3, 4, 6, 7, 9 and 11 ($[M+H]^+$ at m/z 597, 595, 611, 609, 723 and 581, respectively) showed fragments at m/z (451, 289); (449, 287); (456, 303); (463, 301); (577, 415) and (435, 273), respectively. These fragments are related to the cleavage of two glycoside linkages leading to the loss of 146 and 308 amu. Consequently, the disaccharide sequence is determined as deoxyhexose–hexose–flavonoid. This data indicated that products 3, 4, 6, 7, 9 and 11 are rutinose derivatives which were reported as the main flavonoid diglycosides in *Citrus* juice [35]. UV spectra of compounds 3, 6, 9, 11 presented two maxima at 285 nm and 330–335 nm, which is indicative for flavanones [36].

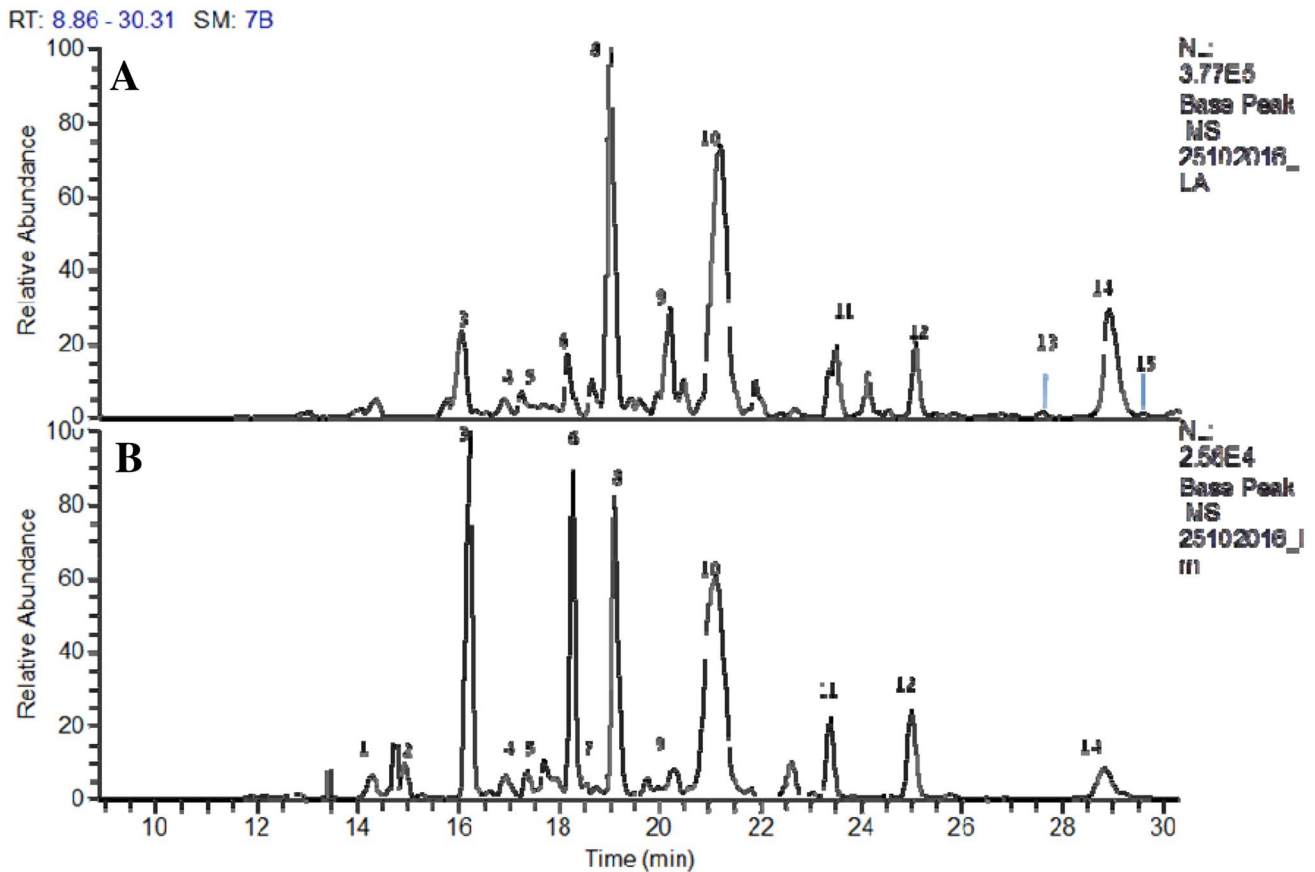


Fig. 1 HPLC–PDA–ESI–MS of ethyl acetate (a) and methanol (b) extracts from lemon peels. Nh (Neohesperidose). HMG (6-(3-hydroxy-3-methylglutaryl))

Table 2 Retention times, UV and ESI–MS Data of compounds detected in Ethyl acetate (EtOAc) and methanol (MeOH) extracts

Peaks	Compounds	RT (min)	[M+H] ⁺ (m/z)	[M+Na] ⁺ (m/z)	Other ions (m/z)	UV λ _{max} (nm)	EtOAc	MeOH
1	Apigenin-6,8-di-C-glucoside	14.26	595	–	577, 559, 499, 475, 457, 439, 355, 325, 295	280, 345	a	b
2	Diosmetin-6,8-di-C-glucoside	14.93	625	–	607, 589, 529, 505, 487, 439	285, 346	a	b
3	Neohesperidin	16.20	597	–	579, 435, 451, 418, 355, 289	285, 330	b	b
4	Vicenin 2	16.80	595	–	449, 287	280, 345	b	b
5	Scoparin	17.29	463	–	391, 283, 301	330, 340	b	b
6	Neohesperidine	18.15	611	633	593, 539, 445, 456, 303	285, 333	b	b
7	Neodiosmin	18.34	609	631	463, 301	280, 340	a	b
8	Nd	19.05	683	–	653	274, 353	b	b
9	Melitidin	20.01	723	–	675, 617, 577, 559, 415, 347, 303, 271	285, 331	b	b
10	Di-C-glucosylflavone	21.14	565	587	305, 283	286, 340	a	b
11	Naringin	23.47	581	–	563, 545, 509, 435, 273	285, 330	b	b
12	Isomeranzin	25.07	261	–	243, 189, 177	330, 334	b	b
13	Tangeretin	27.69	373	–	358, 343, 312, 328	285, 340	b	b
14	Nobiletin	28.90	403	–	388, 373, 342	285, 343	b	b
15	5,6,7,4'-tetramethoxyflavone	29.44	343	–	343, 328, 282, 312	280, 345	b	b

a absent, b present

However compounds **4** and **7** possessed UV spectra with two maxima at 330–350 nm and 275–290 nm, characteristic of flavones. All these data allowed to identify compounds **3**, **4**, **6**, **7**, **9** and **11** as neoeriocitrin, vicenin-2, neohesperidine, neiodiosmin, melitidin, and naringin, respectively (Fig. 2) [37, 38].

Compounds **1**, **2** and **10** showed UV spectra suggesting a flavone structure, and in positive ion mode product ions (Table 2) typical of di-*C*-hexosyl flavones were observed [39]. These glycosylated flavones, which were detected only in methanolic extracts were identified as apigenin-6,8-di-*C*-glycoside, diosmetin-6,8-di-*C*-glycoside and di-*C*-glycosylflavone (**1**, **2** and **10**, respectively). These compounds were previously identified in *Citrus aurantifolia* leaves [40] and in hydroalcoholic extract of *Citrus aurantium* L. var. *amara* peels [38].

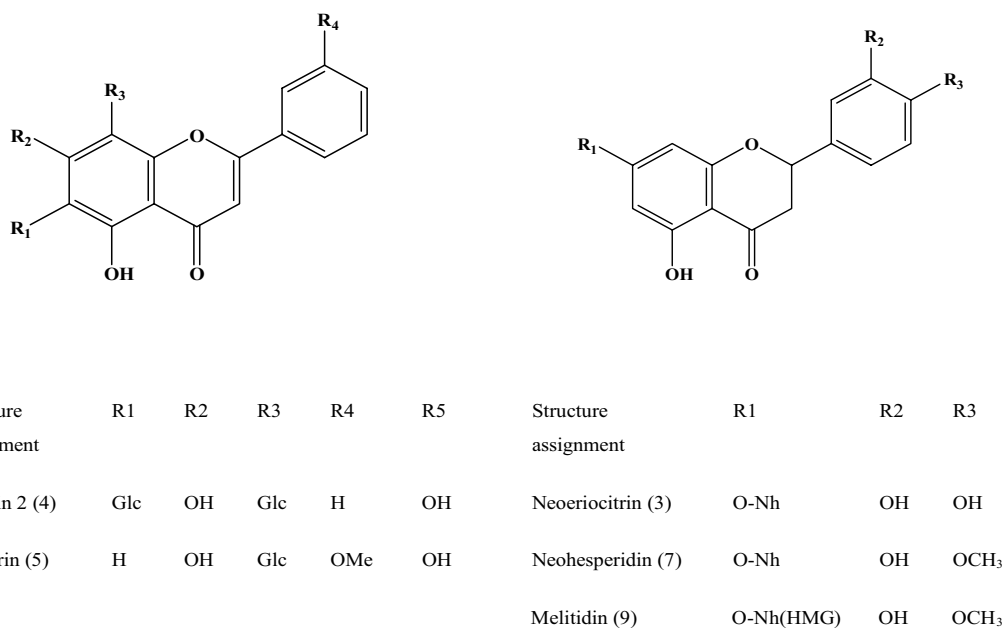
The less polar products **13**, **14** and **15** were identified as polymethoxylated flavones based on their protonated molecular ions $[M+H]^+$ at m/z 373, 403, and 343, respectively, and by their characteristic UV spectra. The MS spectra of products **13**, **14** and **15** presented base peaks for the $[M+H-Me]^+$ ion (m/z 358, 388 and 328 for **13**, **14** and **15**, respectively). On the other hand, their fragmentations were in agreement with the loss of 30 amu ($[M+H-2Me]^+$ at m/z 343, 373, 312 for **13**, **14**, **15**, respectively; 61 ($[M+H-Me-CO-H_2O]^+$ at m/z 312, 342 and 282 for **13**, **14** and **15**, respectively; and 46 ($[M+H-2Me-H_2O]^+$ at m/z 328 for **13**). These data allowed us to identify compounds **13**, **14** and **15** as tangeretin, nobletin and

5,6,7,4'-tetramethoxyflavone (Fig. 3). These components were previously identified in *Citrus* juice and *Citrus aurantium* L. [38, 41].

A typical UV spectrum of coumarins characterized by a λ_{max} at 330 nm [42] was observed for compounds **5** and **12**. Compound **3** (m/z 463 $[M+H]^+$) showed two fragment ions at m/z 283 and 301, consistent with the elimination of an hexose moiety and water from the coumarin structure. This compound was identified as scoparin, which was in agreement with the literature [38, 43, 44]. The presence of a tropylium ion at m/z 189 in compound **12** suggested the structure of isomerazin [45].

3.5 Antioxidant Activity Evaluation

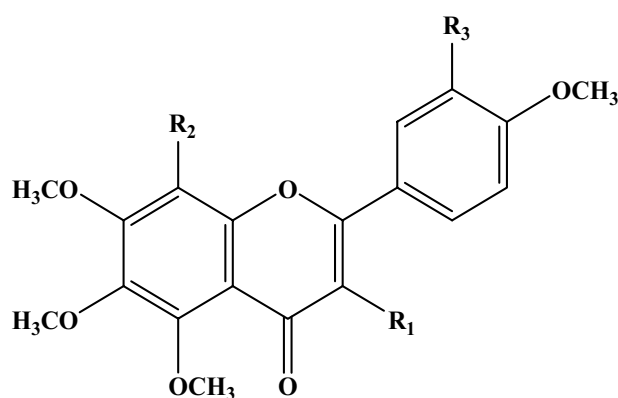
The free radical scavenging activity (FRSA) of *Citrus limon* peels extracts and essential oils was determined using the DPPH scavenging test (Table 3). The ethyl acetate extract displayed an important antioxidant activity with $IC_{50}=0.09$ mg/ mL followed by the methanol ($IC_{50}=0.11$ mg/ mL) and hexane ($IC_{50}=0.13$ mg/ mL) extracts (Table 3). Other studies have reported before that *Citrus limon* peels have a significant antioxidant activity. Mbeng and Jide [46] showed IC_{50} values ranging from 0.29 to 0.31 mg/mL for acetone and ethanol extracts, respectively. The FRSA is related to the ability of molecules to donate hydrogen to another molecule as known for phenolic compounds [47]. Therefore, the study of total phenol contents (TPC) of *Citrus limon* peels essential oils and extracts is



Nh (Neohesperidose). HMG (6-(3-hydroxy-3-methylglutaryl))

Fig. 2 Chemical structures of flavonones glycoside

Fig. 3 Chemical structures of Polymethoxyflavones. LFPEO-E Lemon fresh peels essential oil extracted with ether, LDPEO-E Lemon dried peels essential oil extracted with ether, LFPEO-A Lemon fresh peels essential oil extracted with ethyl acetate, LDPEO-A Lemon dried peels essential oil extracted with ethyl acetate, BHA butylated hydroxyanisole



Polymethoxyflavones	R1	R2	R3
Tangeretin (13)	H	OCH ₃	H
Nobiletin (14)	H	OCH ₃	OCH ₃
5,6,7,4'-tetramethoxyflavone (15)	OCH ₃	OCH ₃	OCH ₃

Table 3 Antiradical activity of extracts and essential oils from *Citrus limon*

Extracts	IC ₅₀ (mg/mL)
Hexane extract	0.13 ± 0.01
Ethyl acetate extract	0.09 ± 0.01
Methanol extract	0.11 ± 0.01
LFPEO-E	0.15 ± 0.02
LFPEO-A	0.17 ± 0.03
LDPEO-E,	0.21 ± 0.04
LDPEO-A	0.25 ± 0.03
Vitamin E	0.017 ± 0.001
BHT	0.026 ± 0.002

important. The ethyl acetate extract had the highest TPC (143.11 mg GA/g) followed by methanol (114 mg GA/g) and hexane (109 mg GA/g) extracts.

A good antioxidant activity of *Citrus limon* peels extracts was shown by (FRSA and TPC) as indicators. The total antioxidant activity and the total phenol contents (TPC) of lemon peels extracts were strongly correlated. In the same way, Anagnostopoulou et al. [48] showed that the ethyl acetate extract of sweet orange dry peels has the highest TPC (105 ± 10 mg GA/g). The ethyl acetate extract has the higher TPC and antiradical activity due to its polarity. The four essential oils exhibited antioxidant activity as DPPH free radical scavenger. The IC₅₀ values for radical scavenging efficacy of LFPEO-E, LFPEO-A, LDPEO-E, and LDPEO-A were 0.15 mg/ mL, 0.17 mg/ mL, 0.21 mg/ mL, and

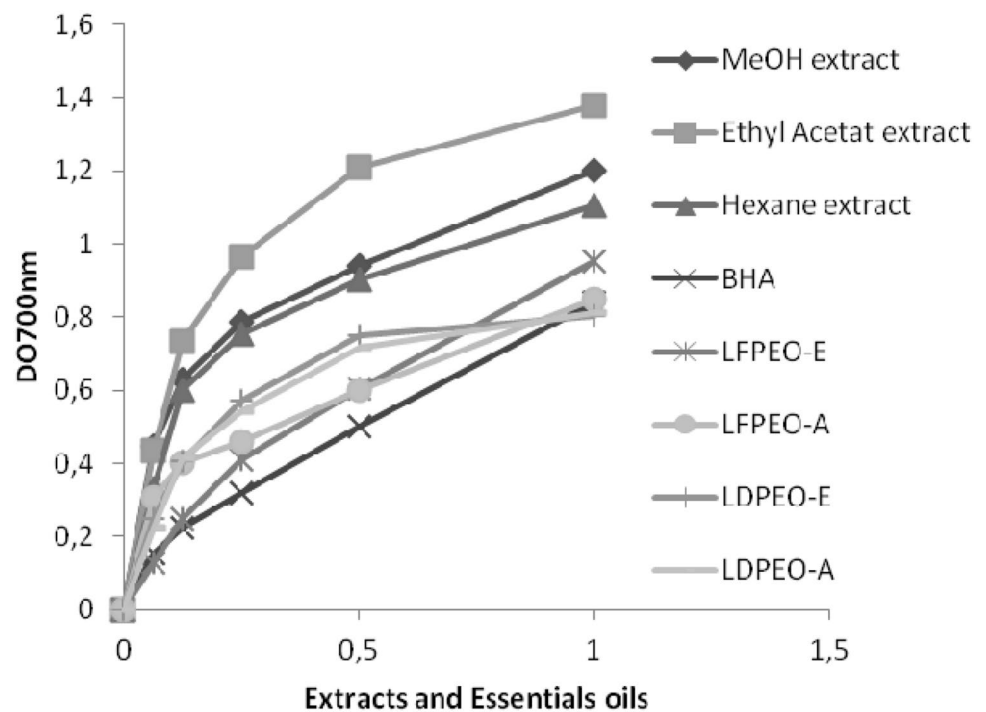
0.25 mg/ mL, respectively. These results are in agreement with those reported in the literature [41, 49] who obtained a high antiradical activity of essential oil from lemon peels cultivated in Portugal and Calabria, respectively. To confirm this result, we tested the antioxidant activity by the reducing power method, which is based on the capacity of an extract to reduce a Fe³⁺ ferricyanide complex to a Fe²⁺ ferrous complex. As observed in Fig. 4, the ethyl acetate extract from dried peels of *Citrus limon* has a higher reducing power activity than the other extracts in this order: Ethyl acetate > Methanol > Hexane extracts (Fig. 4). The four essential oils of *Citrus limon* peels exhibited the highest antioxidant activity as determined in this assay. This result is in agreement with those of Loizzo et al. [41], who found values in the range of 2.55–157.40 μM Fe (II)/g of C. × limon extracts. In order to confirm our results, the total antioxidant capacity test was used by the phosphor-molybdenum method based on the reduction of Mo(VI) to Mo(V).

The antioxidant capacity value of the ethyl acetate extract (1.5 mg of vitamin E/g of extract) was also more important than those of methanol (1.3 mg of vitamin E/g of extract) and hexane (1.1 mg of vitamin E/g of extract) extracts (Fig. 5). This method confirms that lemon peel essential oil is endowed with an interesting antioxidant activity.

3.6 Antibacterial Activity

Antibacterial activity of *Citrus limon* peel essential oils and extracts was evaluated against a set of human pathogenic bacterial strains, including both Gram-positive and

Fig. 4 Reducing power of *citrus* lemon peels essential oil and extracts. *LFPEO-E* Lemon fresh peels essential oil extracted with ether. *LDPEO-E* Lemon dried peels essential oil extracted with ether, *LFPEO-A* Lemon fresh peels essential oil extracted with ethyl acetate. *LDPEO-A* Lemon dried peels essential oil extracted with ethyl acetate



Gram-negative ones. The inhibitory effect on bacterial growth was determined using the agar disc diffusion assay (Table 4). LFPEO-E showed a strong antibacterial activity against *B. subtilis* (Gram+) (18 mm), which was higher than that of ampicillin (14 mm). In addition LFPEO-E and LFPEO-A presented an interesting activity comparable to that of ampicillin against *B. cereus* (Gram+) (13 mm and 10 mm, respectively) and *E. coli* (Gram-) (12 mm and 11 mm, respectively). On the other hand, for dry lemon peels, LDPEO-A revealed an antibacterial activity against

most tested microorganisms with moderate inhibition zone diameters: *B. cereus* (13 mm) and *B. subtilis* (13 mm) (Gram+) as well as *E. coli* (14 mm) and *K. pneumonia* (14 mm) (Gram-). However, LDPEO-E was active only against *E. coli* with an inhibition zone of 8 mm. These results reveal that the antibacterial activity was influenced by the extraction solvents and hence thus the extracts composition. It has been reported in the literature [50, 51] that fresh *Citrus* essential oil has an antibacterial activity against *B. cereus*, *E. coli* and *S. aureus*.

Fig. 5 Total antioxidant capacity of *citrus* lemon extracts and essential oils

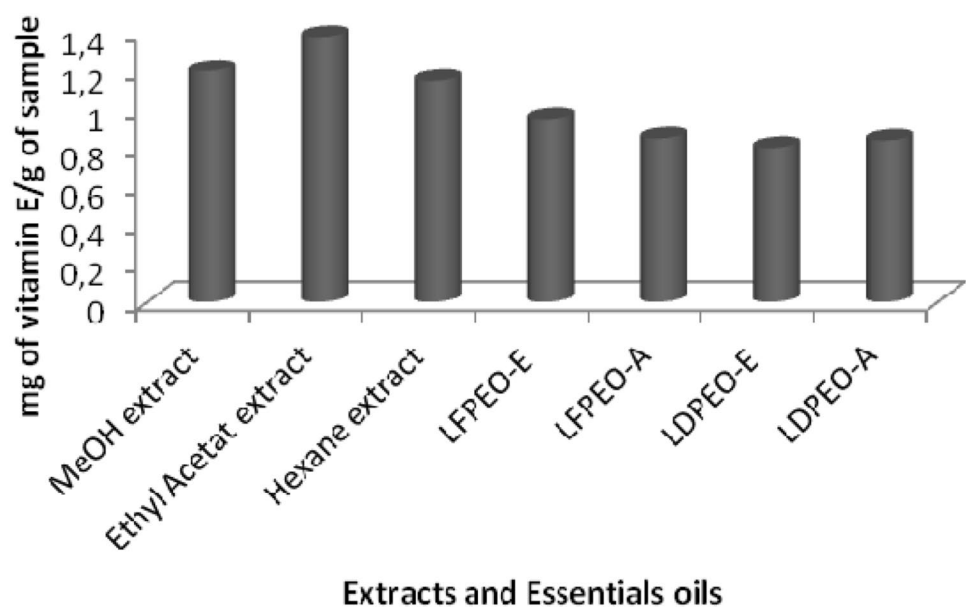


Table 4 Antibacterial activity of *citrus limon* peels essential oils and extracts using agar disc method

Strains	Diameters of inhibition (mm) (10 µg/disc)				Hexane extract	AcOEt extract	MeOH extract	Ampicillin
	LFPEO-E	LFPEO-A	LDPEO-E	LDPEO-A				
Gram-positive bacteria								
<i>Bacillus cereus</i>	13.0±0.1	10.0±0.1	na	13.0±0.2	8.0±0.1	9.0±0.1	na	10.0±0.1
<i>Bacillus subtilis</i>	18.0±0.3	na	na	13.0±0.2	10.0±0.1	na	14.0±0.2	14.0±0.3
<i>Staphylococcus aureus</i>	na	na	na	na	na	8.0±0.1	10.0±0.1	9.0±0.1
Gram-negative bacteria								
<i>Salmonella enteric</i>	10.0±0.1	na	na	na	na	8.0±0.1	12.0±	11.0±0.1
<i>Escherichia coli</i>	12.0±0.2	11.0±0.2	8.0±0.1	14.0±0.2	na	8.0±0.1	13.0±	12.0±0.1
<i>Klebsiella pneumonia</i>	na	na	na	14.0±0.3	14.0±0.2	10.0±0.1	na	13.0±0.2

na not active

The hexane extract was effective in inhibiting the growth of *K. pneumonia* (14 mm) (Gram -) followed by *B. subtilis* (10 mm) and *B. cereus* (8 mm) (Gram +). In addition, the ethyl acetate extract presented a medium antibacterial activity against *B. cereus* (9 mm) and *S. aureus* (8 mm) of (Gram +) as well as *S. enteric* (8 mm), *E. coli* (8 mm) and *K. pneumonia* (10 mm) (Gram-). This result is in agreement with the findings of Chanthaphon et al. [52] who found that ethyl acetate extracts from fresh and dried Lime Kaffir showed a moderate antibacterial activity against *S. aureus* and *E. coli*. Furthermore, the extract of methanol is granted by an interesting antibacterial activity against all tested bacteria except for *K. pneumonia* and *B. cereus* which weren't affected. On the other hand, both Mbeng and Jide [46] clarified that ethanol and acetone extracts of *Citrus limon* L. peel present a highest activity against *B. subtilis*, *S. aureus*, and *E. coli*.

4 Conclusion

The aim of this study was to compare the chemical composition and yields of essential oils from fresh and dried *Citrus limon* peels and their non-volatile organic extracts. Results obtained with GS/MS and HPLC–PDA–ESI–MS analysis showed a noticeable difference in chemical composition of the studied samples. Our study demonstrated that monoterpenes are the most abundant compounds in the essential oils, limonene being the major volatile component. In addition, *Citrus limon* peels are rich in phenolic and flavonoids compounds. For these reasons the *Citrus limon* peel essential oils and extracts exhibited powerful antioxidant and antimicrobial activities. Further studies are needed for the isolation of the natural compounds responsible for those activities.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

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