Chemical composition of essential oils of *Citrus limon* peel from three Moroccan regions and their antioxidant, anti-inflammatory, antidiabetic and dermatoprotective properties

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**A B S T R A C T**

**Introduction:** The current study aimed to explore the *in vitro* antioxidant, anti-inflammatory, antidiabetic, and dermatoprotective properties of lemon peel essential oil (EO).

**Methods:** The chemical composition of lemon EOs extracted from the lemon of three cities in Morocco was investigated using gas chromatography-mass spectrometry (GC-MS) analysis. The antioxidant property was estimated by two complementary tests: Ferric ion reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH). The *in vitro* anti-inflammatory activity was assessed by the inhibition of albumin denaturation and proteinase. Inhibitory properties of α-glucosidase and α-amylase were used to reveal the antidiabetic activity of lemon peel EOs. Dermatoprotective property was evaluated by the tyrosinase inhibition method.

**Results:** In addition to high amounts of polyphenols and flavonoids, GC-MS analysis of lemon peel EOs demonstrated the presence of D-limonene, β–pinene, and γ-terpinene as the main compounds in the three samples studied. Lemon peel EOs exhibited significant antioxidant activities by IC50 values ranging from 40.57 µg/mL to 100.22 µg/mL and 113.63 µg/mL to 180.90 µg/mL obtained by DPPH and FRAP tests, respectively. *in vitro* inhibition of enzymes involved in inflammatory response revealed that lemon peel EOs presented remarkable inhibitory activities against albumin denaturation (230.48 µg/mL > IC50 < 341.13 µg/mL) and proteinase (199.70 µg/mL > IC50 < 307.05 µg/mL). Moreover, lemon peel EOs demonstrated powerful inhibition of α-amylase and α-glucosidase with various IC50 values (1689.06 µg/mL > IC50 > 4000 µg/mL and 1021.58 µg/mL > IC50 < 2467.62 µg/mL), respectively. These EOs also revealed significant inhibition of tyrosinase with IC50 values ranging from 248.42 µg/mL to 378.02 µg/mL.

**Conclusion:** These results revealed that lemon peel EOs might constitute a new product with beneficial biological abilities against the mentioned complications.
(2–4). Recently, interest has been revived and many studies have focused on the beneficial activities of EOs and their major contents; terpenoids and terpenes, which are mostly sesquiterpenes and monoterpenes, and their biological abilities (5).

Citrus fruits such as grapefruits, oranges, lemons, tangerines, limes, and mandarins, whose productions are rising each year with the increase in consumer demand, are among the most popular fruits cultivated around the world (6). Native to the Himalayan foothills of Northern India, Southern China, Northern Myanmar, and Southeast Asia (7), citrus fruits are also grown in Mexico, Turkey, the United States, Iran, Argentina, Italy, Brazil, the People’s Republic of China, and Spain (8). They are from the Rutaceae family and originated from the hybridization of Lemon with primitive papeda (9). After oranges, they are the 3rd most important species of citrus fruit, grown with more than 4.4 million tons each year (10).

In addition to the flesh usually inside of the lemon, the peel is also very important; the extracted EOs from this part have been widely used for centuries by humankind (11). They are used either in pharmaceuticals, foods, or as preservatives and are generally regarded as safe, wherein the extract can be obtained through extraction by different techniques such as steam and hydro-distillation (12). In fact, citrus peel EOs have been studied extensively for their compositions (13) and biological properties by many researchers, including lemon peel EOs (14,15), among others. Lemon peel is considered a major source of monoterpenes compounds with biological activities. Therefore, in order to determine the healthier variety of lemon from three cities in Morocco (Fes, Beni Mellal, and Agadir), this paper aims to 1) explore the biologically active compounds, mainly those belonging to the monoterpenes and sesquiterpenes groups and to 2) study, in vitro, their anti-diabetic, antioxidant, anti-inflammatory, and dermatoprotective properties.

**Materials and Methods**

**Plant material**

Freshly harvested lemons were collected from the following three regions: Fez, Beni-Mellal, and Agadir, corresponding to the following coordinates (34° 2’ 11.278” N 5° 1’ 2.41” W), (32° 20’ 21.998” N 6° 21’ 38.999” W), and (30° 25’ 39.918” N 9° 35’ 53.185” W), respectively. The species were identified by Professor El Mahjoub Aouane and the specimens were deposited in the herbarium of Ibn Tofail University, Faculty of Science, Kenitra, Morocco, under number: CIT-307/2022. The harvesting of lemons was carried out between January and March 2022. The fruits were peeled, and the peels were kept at a temperature of -4°C in the dark.

**Extraction of lemon peel essential oil**

Extraction of EOs from lemon peel was performed by hydro-distillation method using a Clevenger-type machine (16). Peels were ground with an electric grinder. Then, 200 g of the zest of each sample were placed in a flask with water and boiled for 3 hours.

**Total phenolic and flavonoid contents**

The total phenolic content (TPC) of the EO was evaluated using Folin-Ciocalteu reagent (17). Gallic acid (GA) was used as a standard. The TPC was expressed in milligrams of GA equivalent per gram of the EO (mg GAE/g EO).

Total flavonoid content (TFC) was quantified according to the aluminum chloride colorimetric assay (17). Different concentrations of the EO were prepared for this assay. The absorbance was assessed at 430 nm. The TFC was presented as milligrams of quercetin (QE) equivalent per gram of the EO (mg QE/g EO). QE was used as a standard.

**Chemical composition analysis**

The phytochemical compositions of lemon peel EOs were characterized using gas chromatography-mass spectrometry (GC-MS) (TQ8040 NX type), fitted with an apolar capillary RTx- 5 Sil MS column (30 m x 0.25 mm id.; 0.25 μm of film thickness) and coupled with triple quadrupole detector using acquisition mode of full scan with NIST version 2019 Library. EOs were diluted with hexane as a solvent with a split opening of 4 minutes. Furthermore, Helium was used as the carrier gas with a pressure of 37.1 kPa with an injection volume of 1 µL at a source ion temperature of 200°C. Additionally, the temperature programming was 50°C for 2 minutes at the rate of 5°C/min up to 160°C and finally 280°C at 5°C/min for 2 minutes, respectively. Furthermore, 1 µL of each EO was injected by using a split mode with an injection temperature of 250°C for an analysis time of 50 minutes.

**Antioxidant activity**

**Radical scavenging activity by DPPH assay**

Lemon peel EOs were evaluated for their radical scavenging property by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method (18). Briefly, a mixture of 0.25 mM DPPH radical in ethanol and EOs was prepared to test various concentrations. The reaction mixtures constituted by DPPH radical and EOs were prepared by using Folin-Ciocalteu reagent at 517 nm. Ascorbic acid was used as a standard reference (positive control) with a concentration ranging from 1.5 to 0.1 µg/mL. All tests were carried out in triplicates. The following formula was used:

$$DPPH \text{ scavenging percentage } \% = 100 \times \left(\frac{(As - Ac)}{Ac}\right)$$

where, As and Ac are the absorbance of the tested samples and control, respectively.

**Ferric ion reducing antioxidant power (FRAP) assay**

Essential oils of lemon’s reducing powers were assessed
The protocol consisted of 500 µL of reaction mixture

In vitro inhibition assay of α-glucosidase and α-amylase activities
The inhibitory ability of the EOs of lemon peel against α-glucosidase propriety was evaluated spectrophotometrically by monitoring the D-glucose release according to the method previously described by Ouassou et al (20) with slight modifications. The mixture contained 1000 µL of phosphate buffer (50 mM), 100 µL of sucrose (50 Mm), and 100 µL of α-glucosidase enzyme solution (10 IU). Then, EOs solutions at various concentrations (250, 500, 750, 1000, 1500, 2000, 2500, and 4000 µg/mL) solubilized in dimethyl sulfoxide were added. In a water bath, the mixture was then incubated for 25 minutes at 37°C. To stop the enzymatic reaction, the mixture was heated for 5 minutes at 100°C, and the released D-glucose was assessed by the D-glucose oxidase method using a specific commercial kit. The absorbance was evaluated at 500 nm. In addition, the α-amylase inhibition ability by the EOs was assessed according to the protocol described by Daoudi et al (21). The mixture contained 200 µL of phosphate buffer (0.02M), 200 µL of α-amylase enzyme solution (13 IU), and 200 µL of EO (250, 500, 750, 1000, 1500, 2000, and 4000 µg/mL) solubilized in dimethyl sulfoxide (1%; to solubilize the oil in the perfusion solution). Two hundred microliters of starch (1%) dissolved in phosphate buffer (0.02M), 200 µL of α-glucosidase enzyme solution (13 IU), and 200 µL of EO (250, 500, 750, 1000, 1500, 2000, and 4000 µg/mL) solubilized in dimethyl sulfoxide (1%; to solubilize the oil in the perfusion solution). Two hundred microliters of starch (1%) dissolved in phosphate buffer was added and incubated for 20 minutes at 37°C after pre-incubating the mixtures at 37°C for 10 minutes. Then, 600 µL of 3,5-dinitrosalicylic acid reagent (2.5%) was added to stop the enzymatic reaction. Then, the mixtures were incubated at 100°C for 8 minutes. These mixtures were then placed in ice-cold water for a few minutes. The absorbance was measured at 540 nm after adding 1 mL of distillate water to the mixture. Acarbose in both assays was used as a positive control.

Proteinase inhibition assay
The proteinase inhibitory test of EOs of lemon peel was performed according to the protocol described by Oyedapo and Famurewa (22). The absorbance was read at 210 nm against the buffer as blank. The percentage of inhibition of proteinase propriety was calculated. In this test, Diclofenac was prepared in the same condition as the sample and was used as a reference. The experiment was performed in triplicate.

In vitro tyrosinase inhibition
The dermatoprotective propriety of lemon peel EOs was determined spectrophotometrically by evaluating the tyrosinase inhibitory activity according to the protocol conducted by Batubar et al (23), with slight modifications. In brief, 25 µL of the sample were added to 100 µL of tyrosinase solution (333 units/mL in phosphate buffer 5 ×10⁻⁴ mol L⁻¹). The reaction mixture was incubated for 10 minutes at 37°C. Then, 300 µL of L-DOPA (5 × 10⁻³ mol L⁻¹) as a specific substrate were added to the reaction mixture and then incubated for 30 minutes at 37°C. The absorbance of the mixture was determined at 510 nm in three replicates for each sample concentration (the concentrations were selected according to their percent inhibition of the tyrosinase activity). Kojic acid was used as a positive control.

Data analysis
All results were presented as the mean ± standard deviation (SD), using GraphPad Prism 8.0 software for Windows. One-way analysis of variance (ANOVA) followed by a post-hoc (LSD test) was used to determine the differences between IC50 of EOs versus standard reference molecules used. The difference was considered statistically significant at $P<0.05$.

Results
Chemical composition
The yield and phenolic contents of EOs obtained by the hydro-distillation procedure are presented in Table 1. As shown in this table, the yields of lemon EOs were 1.35 % for EO-2, 1.22% for EO-3, and 1.03% for EO-1. Regarding the phenolic contents, EO-1 exhibited the highest TPC consisting of 50 µL of EO sample and 450 µL of bovine serum albumin (5%). The mixture was incubated in a water bath for 20 minutes at 37°C, and then the mixture was heated for 30 minutes at 57°C. Two and a half microliters of phosphate buffered saline was added to the mixture. The control contained phosphate buffer solution without EOs. Diclofenac was prepared in the same condition as the sample and was used as a reference. The absorbance was evaluated at 416 nm using a UV/VIS spectrometer.

%Inhibition=100 x ((absorbance of test / absorbance of control)−1).

Inhibitory activity (%) = ((DOcontrol − DO Test)/DOcontrol) × 100

Anti-inflammatory activity evaluation
Albumin denaturation inhibition assay
The protocol consisted of 500 µL of reaction mixture

proteinase inhibition assay
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<table>
<thead>
<tr>
<th>EOs</th>
<th>Yield (%)</th>
<th>TPC (mg GAE/g EO)</th>
<th>TFC (mg QE/g EO)</th>
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<tr>
<td>EO-1</td>
<td>1.03</td>
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<td>15.48 ± 2.03</td>
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<tr>
<td>EO-2</td>
<td>1.35</td>
<td>75.76 ± 6.83</td>
<td>12.77 ± 1.96</td>
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<tr>
<td>EO-3</td>
<td>1.22</td>
<td>80.25 ± 9.27</td>
<td>19.07 ± 1.46</td>
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</table>

Values are represented as mean (n=3) ± SD. EO-1: Essential oil (Beni Mellal region), EO-2: Essential oil (Fez region), EO-3: Essential oil (Agadir region). GAE: Gallic acid equivalent; QE: Quercetin; TPC: Total phenolic content; TFC: Total flavonoid content.

In vitro antioxidant activity

The antioxidant activity findings are presented in Figure 2 with the IC50. As depicted in this figure, EOs extracted from lemon peel exhibited remarkable antioxidant propriety. The CG-MS of the EOs demonstrated several compounds able to significantly decrease the DPPH radical. EO-1 revealed the highest potential for radical scavenging, with an IC50 value of 40.57 µg/mL, followed by EO-2, with an IC50 value of 52.16 µg/mL, whereas EO-3 displayed the lowest DPPH radical scavenging capacity, with an IC50 value of 100.22 µg/mL. In addition, in the FRAP test, EO-1 showed the highest antioxidant power, with an IC50 value of 113.63 µg/mL, followed by EO-3, with an IC50 value of 148.51 µg/mL. EO-2 in this test exhibited the lowest antioxidant power activity, with an IC50 value of 180.90 µg/mL. Moreover, all IC50 values of the EOs studied were significantly higher than that of ascorbic acid in both tests (P<0.001), with IC50 values of 0.29 ± 0.02 µg/mL and 0.54 ± 0.03 µg/mL for DPPH and FRAP tests, respectively.

In vitro α-amylase and α-glucosidase inhibition

In the present study, the inhibitory propriety of α-glucosidase and α-amylase of lemon peel EOs was assessed (Figure 3). These EOs showed a dose-dependent inhibitory property on intestinal α-glucosidase as well as pancreatic α-amylase. The IC50 values for α-amylase inhibition were 1689.06 µg/mL and 2500.09 µg/mL for EO-1 and EO-2, respectively, while the IC50 for α-amylase inhibition was higher than 4000 µg/mL for EO-3. As shown in this figure, the results confirmed the significant α-glucosidase inhibitory abilities of these EOs. The IC50 for pancreatic α-glucosidase inhibition were 1021.58 µg/
mL, 1982.01 µg/mL, and 2467.62 µg/mL for EO-1, EO-2, and EO-3, respectively. In the present study, all IC50 values of lemon EOs were significantly higher than that of acarbose in both tests (P<0.001), with IC50 values of 619.11 ± 9.85 µg/mL and 374.98 ± 5.72 µg/mL for α-amylase and α-glucosidase tests, respectively.

### In vitro anti-inflammatory activity

In this study, proteinase inhibition and protein denaturation assays were used as markers of the anti-inflammatory activity of lemon peel EOs over a range of concentrations, and the findings are presented in Figure 4. As depicted in this figure, EOs were significantly able to

<table>
<thead>
<tr>
<th>Compound</th>
<th>Family</th>
<th>EO-1 RT</th>
<th>Content %</th>
<th>EO-1 RT</th>
<th>Content %</th>
<th>EO-1 RT</th>
<th>Content %</th>
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<td>11.681</td>
<td>12.33</td>
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<td>2.80</td>
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<td>0.99</td>
<td>15.273</td>
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<td>1.19</td>
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<td>10.615</td>
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 EO-1: Essential oil (Beni Mellal region), EO-2: Essential oil (Fez region), EO-3: Essential oil (Agadir region). ND: not determined. RT: retention time.

Figure 2. Antioxidant activities of lemon peel essential oils (EOs) and ascorbic acid at various concentrations: (A) FRAP assay (B) DPPH trapping activity. Results are presented as mean±SD (n=3). EO-1: EO-1: Essential oil (Beni Mellal region), EO-2: Essential oil (Fez region), EO-3: Essential oil extracted (Agadir region).
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inhibit Albumin denaturation. EO-1 revealed the highest potential of protein denaturation inhibition, with an IC50 value of 230.48 µg/mL, followed by EO-3, with an IC50 value of 325.49 µg/mL. However, EO-2 presented the lowest protein denaturation inhibition ability, with an IC50 value of 341.13 µg/mL. Likewise, these EOs were significantly able to inhibit proteinase in a concentration-dependent manner. EO-3 showed the highest potential of proteinase inhibition (75.99 ± 2.87 % at 350 µg/mL), with IC50 value of 199.70 µg/mL followed by EO-1 (67.89 ± 3.01% at 350 µg/mL) with IC50 value of 238.09 µg/mL. Nevertheless, EO-2 exhibited the lowest proteinase inhibition activity (56.49 ± 3.22% at 350 µg/mL), with IC50 value of 307.05 µg/mL. These IC50 values were significantly higher than that of diclofenac in both tests (*P* < 0.001).

Dermatoprotective activity: Tyrosinase inhibition

Figure 5 presents the tyrosinase inhibition percentage versus the EOs concentrations. As shown in this figure, the inhibitory property increased gradually with increasing concentration of these EOs, i.e., from 50 µg/mL to 600 µg/mL. To compare the obtained findings, the IC50 values of the tyrosinase inhibition were measured, and the obtained results are presented in the same figure. EO-1 revealed higher inhibition than EO-2 and EO-3; the IC50 were 248.42 µg/mL, 378.02 µg/mL, and 307.01 µg/mL, respectively. All EOs revealed lower efficacy compared to kojic acid, which exhibited an IC50 of 3.43 µg/mL.

**Discussion**

This study aimed to explore the biologically active compounds, mainly those belonging to the monoterpenes, and to study their antioxidant, antidiabetic, dermatoprotective, and anti-inflammatory abilities. In this study, the findings revealed that lemon peel contained a significant amount of EOs. These results follow the work carried out by Moosavy et al (17), who reported a yield of 1.33%. Moreover, another study conducted by Bourgou et al (24), revealed a yield of 1.30% of lemon peel EO obtained from the Tunisia region.

We optimized the extraction of the EOs in an attempt to get the highest monoterpenes levels as well as TPC and TFC. The presented findings go in line with previous works (24,25), which have revealed that lemon peel EO has significant contents of these compounds. In addition, our findings were approximately similar to those obtained
by Moosavy et al (17). In contrast, in a previous study, high content of phenolic components ranging from 104.2 to 223 mg GAE/g of EO was found in lemon peel (26). This difference is certainly due to the plant’s geographical origins.

D-limonene was found to be the main monoterpene constituent in all lemon peel EOs in this study. These findings are approximately in accordance with the results found in a previous study (26). Moreover, another study found that limonene and γ-terpinene were the most abundant components in lemon peel (25,27). The results of a previous study revealed that limonene and β-myrcene were the major monoterpenes in lemon peel (28). However, EOs had high variability of their contents, both in quantitative and qualitative terms (29). Many parameters are responsible for this variability and might be divided into 2 categories (30,31): 1) intrinsic factors associated with the soil type and composition, the plant’s age, the seasons, geographical origin, and time of collection, 2) extrinsic factors linked to the environment and the extraction method.

Phenolic compounds are known as powerful antioxidants; therefore, any changes in their amounts may have a significant influence on their antioxidant activities. In this context, the EOs of lemon peel collected from different regions were assessed for their antioxidant activities over a range of concentrations using two methods viz. DPPH and FRAP tests. These assays are widely used tests to assess the free radical scavenging activity and the ferric reducing ability from various studied samples (32).

The study results revealed a powerful and specific antioxidant activity for each sample studied. The variation observed between the different samples might be explained by the difference in the chemical compositions, the climate, and soil type and the composition of each region. Previous works also investigated the antioxidant property of the lemon peel EOs (17,33). The antioxidant property might be explained by the presence of important amounts of monoterpenes (34,35). In the present study, the major compounds, for instance, D-limonene, β-pinene, and γ-terpinene, seem to be highly implicated in this activity. These compounds have been revealed previously as potent antioxidants (30,36). These molecules act as reducing agents, hydrogen donors, and metal chelating potentials (37).

Moreover, all IC50 values of the EOs studied were significantly higher than that of ascorbic acid in both tests. It is well known that ascorbic acid is a potent antioxidant, as well as a powerful free radical scavenger and a substance needed for many enzyme reactions (38). Ascorbic acid has been shown to attenuate oxidative damage significantly by suppressing free radical species generation (38). All these findings confirm the role of EOs as natural antioxidants and also in human health protection against several diseases, such as diabetes.

Diabetes mellitus (DM) is associated with an alteration of insulin release by pancreatic islet beta cells (β-cells), which induce an abnormal glucose metabolism (39). α-Glucosidase and α-amylase are enzymes responsible for intestinal absorption and degradation of hydrocarbons. Consequently, the inhibition of these enzymes is an effective means in the treatment of type 2 diabetes mellitus (T2DM). Actually, the pharmaceutical sector provides different anti-diabetic drugs to treat patients with T2DM, but their undesirable effects are exceedingly dangerous. To address this problem, we have turned to natural herbal therapies that metabolize glucose without inducing significant side effects (32). In this regard, we assessed the inhibitory propriety of α-glucosidase and α-amylase of lemon peel EOs.

The antidiabetic property of these EOs might be due to the volatile molecules known by their antihyperglycemic capacities such as limonene (40), β-pinene, and p-cymene (41). To the best of our knowledge, the antidiabetic activities of EOs extracted from Moroccan lemons have not been evaluated. Nevertheless, a recent work was interested in Moroccan *C. aurantium* (D-limonene represents the main compound of this sample with a percent of 35.17%), which has potential α-glucosidase and α-amylase inhibitory ability (42). Moreover, a study revealed that the EO of lemon peel (collected from Southwest Nigeria) possessed a significant α-amylase and α-glucosidase inhibitory ability (43).

In the present study, all IC50 values of lemon EOs were higher than that of acarbose (used as positive control) in both tests. Acarbose, as a drug used by people with T2DM, is a powerful inhibitor of α-glucosidase and α-amylase. Nevertheless, several undesirable effects are associated with acarbose use (44). For example, it provokes hepatic injury and hepatotoxicity with an increase in liver enzyme concentrations (45). Other studies have reported diarrhea by excessive α-amylase inhibition in the gastrointestinal tract (46). Excessive pancreatic amylase inhibition may
Several enzymes play a significant role in the skin aging resistance loss in the dermis. This resistance loss causes inflammatory activity in obese mice induced by a high-fat diet (53).

A peel, which revealed significant anti-inflammatory activity experimental study was interested in fermented lemon of lemon peel EOs have not been reported; however, to our knowledge, the in vitro anti-inflammatory activities α-terpinene are also responsible for this activity, probably by synergism with D-limonene (52). To the best of our knowledge, the in vitro anti-inflammatory activities of lemon peel EOs have not been reported; however, an experimental study was interested in fermented lemon peel, which revealed significant anti-inflammatory activity in obese mice induced by a high-fat diet (53).

Skin aging is the principal cause that induces resistance loss in the dermis. This resistance loss causes pigment loss, inflammation irritation, roughness and dryness. Several enzymes play a significant role in the skin aging process such as elastase and tyrosinase. This latter is a key enzyme implicated in the melanogenesis process; therefore, its inhibition is a significant target therapeutic strategy in hyperpigmentation treatment. The tyrosinase enzyme known as polyphenol oxidase is a metal-oxidase implicated in the first 2 steps of the biosynthesis of melanin in mammals (54). Consequently, the functional compounds that could inhibit tyrosinase properties would be promising dermatoprotective products. In the present study, the capacity of EO of lemon peel to protect the skin was evaluated by its tyrosinase inhibitory activity. These EOs exhibited a significant tyrosinase inhibition. Due to the complexity and variability of lemon peel EO content, this inhibitory ability is possibly attributable to a synergistic interaction of their constituents with the tyrosinase. A previous study revealed that the EO of lemon peel possessed a significant tyrosinase inhibitory activity (55).

Conclusion
The present study revealed that EOs of lemons rich in monoterpene compounds such as D-limonene, γ-terpinene, α- and β-pinene demonstrated remarkable antioxidant, antiadipic, anti-inflammatory, and dermatoprotective activities. Therefore, these EOs could be used as antioxidant and antiadipic agents and as supplement products. In pharmaceutical applications, lemon peel EO could be used as an antiadipic drug by its significant ability to inhibit α-glucosidase and α-amylase. Indeed, due to its important inhibition of tyrosinase and proteinase, the EO of lemon peel could also be used as a dermatoprotective and anti-inflammatory agent. Nevertheless, further in vivo studies of lemon peel EOs and their bioactive compounds are needed to validate these activities.

Authors’ contributions
ME, BD, and SB invented the work’s conception, carried out the in vitro experiments, and performed statistical analysis; BA helped in manuscript preparation; AE performed the GC-MS analysis; YA participated in the study design and critical revision of the manuscript; EA performed the manuscript revision. All authors read and approved the final manuscript.

Conflict of interests
All authors of this study certify that they have no conflict of interest.

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References


