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Exploring the Potential of Lemon Peel Extracts in Cosmetics: Chemical Composition and Bioactive Properties

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Research Article

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ABSTRACT

The study aimed to explore the potential of lemon peel extracts as a cosmetic raw material, with a focus on the composition, aroma, antimicrobial and bioactivities. Lemon Peel Essential Oil (LPEO), Lemon Peel Extract (LPE) and Lemon Peel Absolute Oil (LPAO) were prepared by hydrodistillation and organic solvent extraction, respectively. The GC/MS analysis revealed that LPEO, LPE and LPAO contained 22, 39 and 9 components, respectively, with terpenoids being the predominant component. LPE had the highest total flavonoid content and surpassing that of total phenolic content. LPEO exhibited the strongest aroma intensity and persistence as measured by electronic nose (e-nose). All three lemon peel extracts showed strong antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* as well as antioxidant and anti-tyrosinase properties with inhibition rates exceeding 90% in a dose-dependent manner. Among them, the antioxidant capacity of LPE and LPAO was stronger than that of LPEO. LPEO demonstrated superior anti-inflammatory effects compared to LPAO and LPE, with inhibitory rates of 87.79% ± 3.86% and 80.75% ± 2.33% on Tumor Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6) at a concentration of 1×10^{-2} mg/mL. Lemon peel extract was also found to promote HaCaT cell migration, with LPEO exhibiting greater effectiveness than LPE and LPAO. The healing rate of scratched HaCat cells treated with LPEO at a concentration of 1×10^{-2} mg/mL for 12 h was 95.29% ± 3.41%. In addition to antioxidant properties, LPEO has demonstrated superior overall performance compared to LPE and LPAO. These three extracts can also be combined to expand their application as cosmetic additives, providing benefits such as enhancing aroma, antioxidant, whitening, antibacterial, anti-inflammatory and skin wound healing.

Keywords: Lemon peel extracts; Ingredient; Aroma; Antimicrobial; Bioactivity; Cosmetic raw material

INTRODUCTION

In the formulation of cosmetics, there are usually flavors, preservatives and active ingredients. The existing cosmetic formulas are developing towards a natural and chemical free direction [1,2]. Cosmetics often add artificial flavors to enhance their fragrance and use preservatives to extend the shelf life of products. However, synthetic spices generally lack biological activity in addition to imparting fragrance and some can also cause skin allergies. Some preservatives, like formaldehyde-releasing agents and methylchloroisothiazolinone/methylisothiazlinone, may also lead to contact dermatitis [3]. Overuse of preservatives in cosmetics can lead to the emergence of drug-resistant strains and pose a serious risk to consumers [4].

The essential oil extracted from natural plants has a series of biological activities, including aroma, antioxidant, anti-aging, antibacterial, anti-acne and anti-inflammatory properties [5]. Lemon, as the third largest variety of citrus in *Rutaceae* is famous for its dual use in medicine and food. Lemon peel accounts for about 20% of the whole lemon, which is rich in terpenes, alkaloids, phenolic acids and other active compounds and has a pleasant aroma and unique biological activities [6]. Scientific research has verified the benefits of lemon essential oil in promoting microvascular circulation, promoting subcutaneous fat combustion, stimulating collagen synthesis, restoring skin oil-water balance and inhibiting melanin production [7].

Furthermore, lemon essential oil can modulate cell permeability and exhibit potent inhibitory effects on *Aeromonas aeruginosa*. The terpenoids in lemon extract have strong antioxidant activity and have obvious scavenging effect on 2,2-diphenyl-1-picrylhydrazyl and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid free radicals [8]. (Z)-Citral in lemon extract has a strong inhibitory effect on tyrosinase, which can effectively whiten the skin [9]. Lemon peel is rich in antifungal compounds such as limonene, *β*-pinene and *γ*-terpene, which can destroy the balance of organelles and inhibit the metabolism of *Candida albicans* [10].

Additionally, (Z)-Citral has been shown to reduce cell injury induced by LPS by inhibiting inflammation, oxidative stress and epithelial-mesenchymal transition in THLE-2 cells. It can also affect the brain through the olfactory system and regulate anxiety and depression [11]. Nerolide acetate and limonene in lemon essential oil are the key components of advanced aromatics. The flavonoids and polyphenols contained in lemon peel extract have effectively scavenge free radicals, inhibit tyrosinase activity and inhibit the production of Nitric Oxide (NO) by macrophage RAW 264.7 [12]. It is worth noting that the polyphenols in lemon peel extract have strong antibacterial effect on *Staphylococcus aureus* and *Escherichia coli* [13].

The current research on lemon peel mainly focuses on the extraction and application of lemon essential oil. However, the potential of flavonoids and polyphenols in lemon peel has not been explored to a great extent. Although the research on lemon essential oil emphasizes its antioxidant and antibacterial properties, it is mainly used in the field of food. With the growing preference for natural ingredients in the cosmetics industry, there is a high demand for plant extracts with natural aroma, antibacterial properties and biological activities. In this paper, the lemon peel extracts obtained by three different extraction methods were compared from the aspects of composition, aroma, antioxidant, anti-inflammatory, antibacterial, whitening and cell repair ability. The aim is to develop a lemon peel extract with enhanced flavor, antibacterial, antiseptic properties and a variety of biological activities, so as to provide the basis for its use as cosmetic raw materials.

MATERIALS AND METHODS

Reagents and materials

Rutin, gallic acid, arbutin, ascorbic acid, folin phenol, polyphenol oxidase, tyrosine, aluminum chloride hexahydrate, potassium persulfate, sodium nitrite, Phosphate-Buffered Saline (PBS, pH6.8), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazide (DPPH), as well as solvents (ethanol, dimethylsulfoxide (DMSO), petroleum ether, propylene glycol, sodium chloride) were analytical grade and purchased from Sinopharm (China). HaCaT cell lines (No.KMCC-001-0256), Luria-Bertani medium (LB medium), recombinant broth, Fetal Bovine Serum (FBS), Cell Counting Kit-8 (CCK-8), Tumor Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6) ELISA Kits were purchased from Beina Biological company (China). *Escherichia coli* (No.CMCC(B)44102), *Staphylococcus aureus* (No.CMCC(B)26003) and *Candida albicans* (No.CMCC(B)98001).

Instruments and equipments

Electronic balance (BSA124S, Sartorius); rotary evaporator (RV10, Ika); capillary column (HP-5MS), Gas Chromatography-Mass Spectrometry (GC-MS, 7693A-5977B, Agilent); electronic nose (PEN3.5, Airsense); spectrophotometer (TU-1810, Beijing general instruments); high-speed centrifuge (5417R, Sigma); microplate reader (FC, Thermofisher); constant temperature incubator (THZ-D, Ningbo southeast instruments); carbon dioxide incubator (4111FO, Thermofisher).

Preparation of lemon peel extracts

The lemon peel was first washed and then roasted at 40°C for 2 h~4 h. Subsequently, it was ground in a 10 mesh mill and stored in a sealed container.

Lemon Peel Essential Oil (LPEO) was obtained using the steam distillation method. Lemon peel and distilled water (with 2% w/v NaCl) in a solid-liquid ratio of 1:10 were placed in a distillation flask for a micro boiling reflux extraction process until the distillate was free of oil droplets. The resulting oily liquid was separated using an oil-water separator, dried with anhydrous Na_2SO_4 and stored in a brown bottle.

Lemon Peel Extract (LPE) was prepared using the ethanol extraction method. 100 g of lemon peel was extracted twice with 500 mL of 90% v/v ethanol containing 1% v/v propylene glycol at 40°C (4/2 h). The supernatants were combined, filtered and evaporated to obtain the extract, which was then sealed in a brown bottle.

Lemon Peel Absolute Oil (LPAO) was obtained using the petroleum ether extraction method. 100 g of lemon peel was extracted twice by reflux with 500 mL of petroleum ether at 40°C (4/2 h). The supernatants were combined, filtered and evaporated into a paste. Anhydrous ethanol in a volume ratio of 10:1 was added to the paste, stirred well and allowed to stand at -20°C for 4 h. The resulting supernatant was filtered, evaporated and the absolute oil was obtained and stored in a brown bottle [14].

Gas Chromatography-Mass Spectrometry (GC/MS) detection of components

GC conditions: The initial temperature was 50°C and maintained for 3 min, then increased by a rate of 2°C/min upto 110°C for 10 min, 2°C/min to 140°C for 2 min, 10°C/min to 250°C for 5 min (84 min total run). The carrier gas was helium (1 mL/min), injector and detector temperatures were 220°C.

Split ratio: 200:1.

MS conditions: Ionization mode was electron impact ionization source and scanning mode was full scan, with electron energy 70 eV, transfer line temperature 270°C, ion source temperature 230°C, solvent delay for 2 min, scanning range 35 u~550 u. The National Institute of Standards and Technology (NIST) MS spectral library was used to

determine the name of each volatile substance and the relative content of each component was calculated with area normalization.

Determination of total flavonoid and phenolic content

Total flavonoid content was determined in the samples through spectrophotometry using rutin as a control, following the method outlined [15]. 5.0 mL of rutin standard solution with 0 μg/mL-100 μg/mL were prepared with dimethyl sulfoxide. To this solution, 0.3 mL of 5% $NaNO_2$, 0.3 mL of 10% w/v $AlCl_3{\cdot}6H_2O$ and 4 mL of 4% w/v NaOH were added and the mixture was allowed to react at room temperature for 15 min. The absorbance value at 510 nm (A_{510nm}) was measured with deionized water as a blank. The standard curve for rutin was determined as A=0.0067C+0.0098 (r=0.9978). Subsequently, three lemon peel extracts were diluted with DMSO and analyzed using the same method. Total phenolic content was determined using the Folin-Ciocalteu colorimetric method [16]. Gallic acid standard solutions ranging from 0 μg/mL to 120 μg/mL were prepared in DMSO. 4 mL of distilled water, 0.5 mL of Folin reagent and 4 mL of 8% w/v Na_2CO_3 solution were added into each standard solution.

The reaction was at room temperature in the dark for 30 min. The absorbance at 740 nm (A_{740nm}) was measured with deionized water as a blank. The standard curve for gallic acid was determined as A=0.0104C+0.0021 (r=0.9998). Subsequently, the sample solution of lemon peel extracts were prepared in DMSO and 1 mL of the dilution solution was used to determine the total phenolic content following the same procedure.

E-nose detection of odour

2 mL of lemon peel extracts were pipetted into a 10 mL headspace vial. The vial was sealed and placed in a 2 L beaker for headspace equilibrium for 1 min. Odour was detected in headspace aspiration with PEN3 e-nose at room temperature [17]. The testing conditions included a sensor self-cleaning time of 150 s, sensor zeroing time of 5 s, sample preparation time of 5 s and signal acquisition time of 300 s. Data analysis was performed using WinMuster software in conjunction with the PEN3 e-nose.

Determination of free radical scavenging activity

The antioxidant activity of lemon peel extracts was assessed using the DPPH and ABTS methods as described [18]. The scavenging rate was calculated as $(A_0 - A_1) \times 100/A_0$, where A_0 was the absorbance of the DPPH or ABTS solution and A_1 was the absorbance after adding the sample. Half scavenging concentration (IC_{50}) refers to the concentration of lemon peel extract required to remove 50% of DPPH and ABTS free radicals.

Determination of anti-tyrosinase activity

The experiment involved four groups: Group A added 1.5 mL of 50 U/mL tyrosinase, group B did not add it, group C added 1.5 mL of 50 U/mL tyrosinase and 1 mL of sample solution and group D added 1 mL of sample solution. All groups were supplemented with PBS buffer to a total volume of 5 mL.

Then the samples were incubated for 10 min at 37°C and immediately 2.5 mmol/L tyrosine solution were added to react in the dark for 30 min at 37°C. The absorbance at 475 nm was determined for each set of samples with deionized water as a blank. Arbutin was used as a positive control. The inhibition rate of tyrosinase was calculated as follows [19].

$$\text{Inhibition rate}/\% = [(A_A - A_B) - (A_C - A_D)] \times 100/(A_A - A_B)$$

Determination of anti-bacterial activity

Preparation of bacterial suspension: *Escherichia coli* and *Staphylococcus aureus* were activated and cultured at 37°C and *Candida albicans* at 28°C to logarithmic growth phase [20].

The bacteria was centrifuged to washed twice with 0.9% w/v normal saline, resuspended in LB medium and the bacterial concentration was adjusted to 1×10^8~5×10^8 CFU/mL.

Preparation of sample solution: The sample was first dissolved in sterile water containing 8% DMSO and then diluted with LB medium to concentrations of 0.5 mg/mL, 2.5 mg/mL, 5 mg/mL, 12.5 mg/mL and 25 mg/mL respectively and filtered through a 0.22 μm filter for later use.

Bacteria grouping: Experiments were performed in sterile 96-well plates. The control group was 30 μL bacterial suspension and 170 μL LB medium; the blank group was 200 μL LB medium; the sample group was 30 μL bacterial suspension and 170 μL sample solutions; the background group was 30 μL LB medium and 170 μL sample solutions. After incubating for 24 h at 37°C, the absorbance at 600 nm was measured using a microplate reader. The inhibition rate was the following equation:

$$\text{Inhibitionrate}/\% = [(\text{A}_{\text{control}} - \text{A}_{\text{blank}}) - (\text{A}_{\text{sample}} - \text{A}_{\text{background}})] \times 100/(\text{A}_{\text{control}} - \text{A}_{\text{blank}})$$

Determination of anti-inflammatory

Determine the cytotoxicity on HaCaT using CCK-8 method. 100 μL of 5×10^4 cells/mL HaCaT cells were seeded in a 96-well plate and incubated overnight in 5% v/v CO_2 incubator at 37°C. After the lemon peel extracts were dissolved in 2% v/v DMSO and then were diluted with DMEM medium containing 10% w/v FBS to a concentration range of 1×10^{-4}~10 mg/mL. The control group was 100 μL cells and 100 μL DMEM; the blank group was 200 μL DMEM; the sample group was 100 μL cells and 100 μL samples; the background group was 100 μL DMEM and 100 μL samples. After incubation for 24 h at 37°C, 10 μL of CCK-8 reagent was added to each well and incubated for an additional 4 h. The absorbance at 450 nm was measured on a plate reader and set three replicate wells for each group. The cell survival rate was on the following equation:

$$\text{Cell survivalrate}/\% = [(\text{A}_{\text{sample}} - \text{A}_{\text{background}})] \times 100/(\text{A}_{\text{control}} - \text{A}_{\text{blank}})$$

The content of inflammatory factors in cells was measured using an ELISA assay kit. 100 μL of 5×10^4 cells/mL logarithmic growth phase HaCaT cells were seeded into a 96-well plate and cultured in an incubator overnight. After discarding the original culture medium, 100 μL of culture medium containing 1 ng/mL TNF-α and 1 ng/mL IFN-γ was added to the model group and sample group to stimulate the cells for 6 h to induce inflammation, then the induction medium was discarded and the cells was washed three times with PBS and then 100 μL of culture medium was added to the model group, 100 μL of culture medium containing samples was added to the sample group. After cultivation in the incubator at 37°C for 24 h, the contents of TNF-α and IL-6 in the supernatant were determined according to the instructions of ELISA. Meanwhile, blank group was only added 100 μL of culture medium. The inhibition rate was on the following equation:

$$\text{Inhibitionrate}/\% = [(\text{A}_{\text{model}} - \text{A}_{\text{sample}}) \times 100/\text{A}_{\text{model}}$$

Repair experiment on scratched HaCaT cells

The samples were prepared using DMEM medium without FBS. 2 mL/well of 3×10^5 cells/mL of HaCaT cells in the logarithmic growth phase were added into a 6-well plate. When the cells were cultured to almost cover the bottom of the well, the cells were scratched by the sterile pipette head and washed multiple times with PBS solution to remove the suspended cells. 2 mL of DMEM medium containing samples was added into the cells of test groups. 2 mL of DMEM medium without FBS was added into the control group. After the scratch cells were further cultured for 0 h, 6 h, 12 h and 24 h, then were observed and took photos. The scratch area was calculated by Image J software.

$\text{Healing rate}/\% = (\text{A}_0 - \text{A}_\text{i}) \times 100/\text{A}_0$, where A_0 was the scratch area at 0 h and A_i was the scratch area at 6 h, 12 h or 24 h.

Statistical analysis

The data were expressed as $x \pm sd$. The diagrams were plotted using GraphPad Prism and differences between groups were performed using one-way Analysis of Variance (ANOVA). A value of $p<0.05$ indicates a statistically significant difference.

RESULTS AND DISCUSSION

Comparison of sensory properties of lemon peel extracts

Due to different extraction methods the three extracts had difference in colour, state and aroma as shown in Table 1.

Table 1. Sensory comparison of three types of lemon peel extracts.

Index	LPEO	LPE	LPAO
Colour	Colorless transparent	Yellow-brown	Yellow-bright
State	Liquid	Semi-paste	Liquid
Fragrance	Pure and fragrant aroma	Aroma plump	Aroma clear and lasting
Extraction rate/%	4.67 ± 0.28	31.54 ± 1.85	20.42 ± 1.03

GC/MS results of lemon peel extracts

According to the total ion chromatograms (Figure 1) of the three extracts, the detected peaks were shown in Tables 2 and 3.

Figure 1. Total ion chromatograms of three lemon peel extracts.

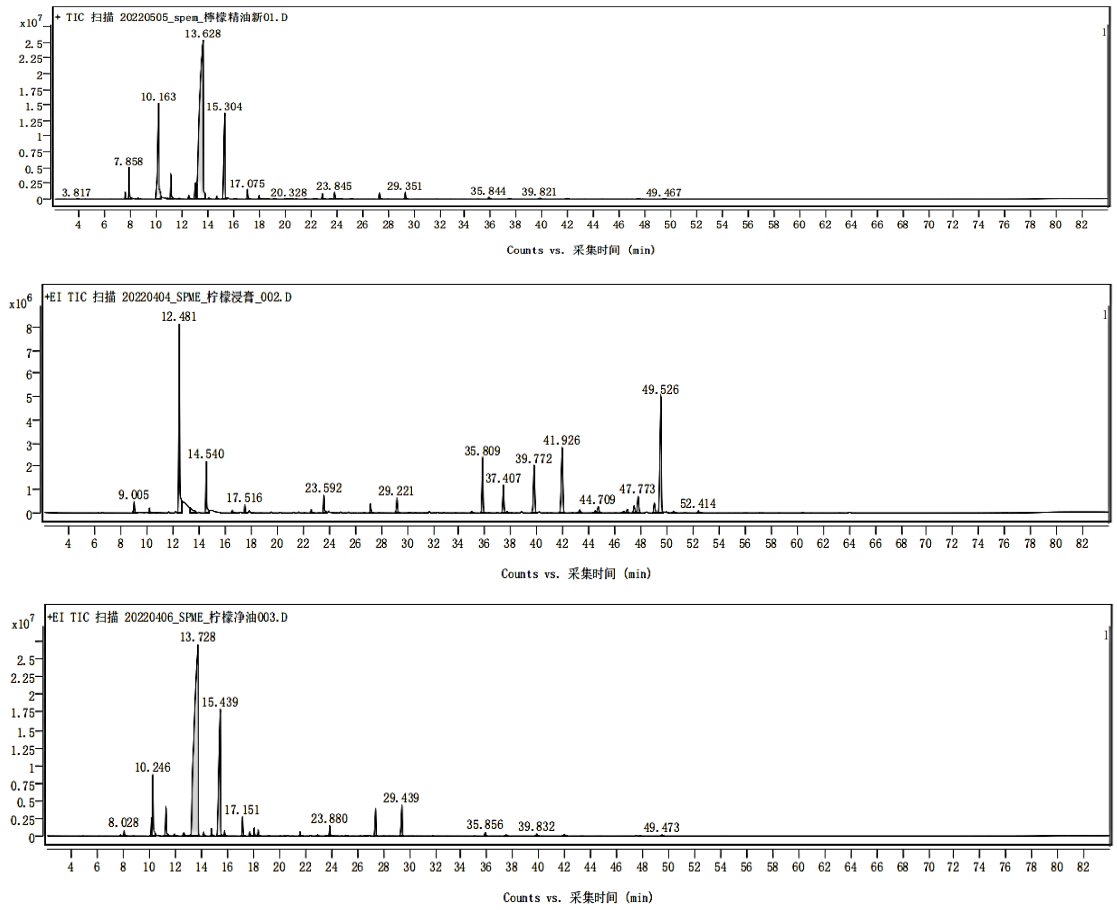


Table 2. Chemical composition and relative contents of the three lemon peel extracts.

No.	Compound	Molecular formula	CAS	Relative percentage content/%		
				LPEO	LPE	LPAO
1	m-Cymene	$C_{10}H_{14}$	535-77-3	2.93	0.14	-
2	β-Phellandrene	$C_{10}H_{16}$	555-10-2	0.67	0.16	-
3	β-Pinene	$C_{10}H_{16}$	18172-67-3	14.28	1.16	5.13
4	Myrcene	$C_{10}H_{16}$	123-35-3	2.31	0.43	2.47
5	(+)-Camphene	$C_{10}H_{16}$	79-92-5	0.1	-	-
6	D-Limonene	$C_{10}H_{16}$	5989-27-5	58.05	22.95	63.79
7	(R)-Isocarvestrene	$C_{10}H_{16}$	1461-27-4	2.20	1.87	-
8	γ-Terpinene	$C_{10}H_{16}$	99-85-4	11.98	5.74	18.82
9	Terpinolene	$C_{10}H_{16}$	586-62-9	0.87	-	2.8
10	Carene	$C_{10}H_{16}$	13466-78-9	2.91	0.29	-
11	Tetradecane	$C_{14}H_{30}$	629-59-4	-	0.19	-
12	trans-Caryophyllene	$C_{15}H_{24}$	87-44-5	0.14	7.76	-
13	Santalene	$C_{15}H_{24}$	512-61-8	-	0.23	-
14	α-Limonene	$C_{15}H_{24}$	17699-05-7	0.12	11.82	-
15	1, 5, 9, 9-tetramethyl-1, 4, 7-cyclodecatriene	$C_{15}H_{24}$	1000062-6-9	-	0.57	-
16	β-Sandalene	$C_{15}H_{24}$	25532-78-9	-	0.48	-
17	β-Fucoidin	$C_{15}H_{24}$	28973-97-9	-	1.15	-
18	α-Terpinene	$C_{15}H_{24}$	451-55-8	-	0.4	-
19	β-Sesquiphellandrene	$C_{15}H_{24}$	20307-83-9	-	0.62	-
20	Valencia Orangerene	$C_{15}H_{24}$	4630/7/3	-	1.42	-
21	Bicyclogermacrene	$C_{15}H_{24}$	24703-35-3	-	2.66	-
22	α-Himachalene	$C_{15}H_{24}$	3853-83-6	-	1.62	-
23	β-Bisabolene	$C_{15}H_{24}$	495-61-4	0.08	19.39	-
24	β-Curcumene	$C_{15}H_{24}$	28976-67-2	-	0.15	-
25	(Z)-Gamma-bisabolene	$C_{15}H_{24}$	13062-00-5	-	0.18	-
26	(+)-δ-Cadinene	$C_{15}H_{24}$	483-76-1	-	0.28	-
27	(E)-α-Bisabolene	$C_{15}H_{24}$	25532-79-0	-	0.3	-
28	Phenyl isothiocyanate	C_7H_5NS	103-72-0	-	0.22	-
29	Nonanal	$C_9H_{18}O$	124-19-6	0.07	0.23	-
30	(Z)-Citral	$C_{10}H_{16}O$	106-26-3	0.58	1.04	2.49
31	Citral	$C_{10}H_{16}O$	5392-40-5	0.05	1.9	-
32	(E)-Citral	$C_{10}H_{16}O$	141-27-5	0.62	-	2.97
33	Linalool	$C_{10}H_{18}O$	78-70-6	0.33	0.76	0.64
34	(-)-Terpinen-4-ol	$C_{10}H_{18}O$	20126-76-5	0.65	0.37	-

35	α-Terpineol	$C_{10}H_{18}O$	10482-56-1	0.72	2.05	0.89
36	Undecanal	$C_{11}H_{22}O$	112-44-7	-	0.17	-
37	4-Terpinyl acetate	$C_{12}H_{20}O_2$	4821/4/9	-	0.18	-
38	dl-Citronellol acetate	$C_{12}H_{22}O_2$	150-84-5	-	0.29	-
39	Nerylacetate	$C_{12}H_{20}O_2$	141-12-8	0.25	6.76	-
40	Acetic acid geranyl ester	$C_{12}H_{20}O_2$	105-87-3	0.09	3.67	-
41	1,1-Diethoxynonane	$C_{13}H_{28}O_2$	54815-13-3	-	0.27	-
42	3,5,5-Trimethylnonyl Hexanoic acid	$C_{18}H_{36}O_2$	1000406-06-0	-	0.13	-
Total detected components/each				22	39	9
Note: Content was less than 0.01 or not detected.						

Table 3. Components and proportion of the three lemon peel extracts.

Items	LPEO	LPE	LPAO
Terpenes/%	96.64	81.96	93.01
Alcohols/%	1.70	3.18	1.53
Aldehydes/%	1.32	3.34	5.46
Esters/%	0.34	11.12	-
Other compounds/%	0.00	0.42	-
Total	100.00	100.00	100.00

22, 39 and 9 chromatographic peaks were detected in LPEO, LPE and LPAO, respectively. The main components were terpenes, accounting for 81.96%~96.64%, as well as oxygen-containing compounds such as alcohols, aldehydes and esters. These components were the main source of the aroma of lemon peel extracts. Among them, β-pinene, myrcene, D-limonene, γ-terpinene, (Z)-citral, linalool and α-terpineol were present in all three extracts and were commonly used in the preparation of lemon and citrus flavors.

The content of D-limonene was the highest among the three extracts and it has typical orange aroma characteristics, antibacterial properties, certain anti-cancer properties, as well as effects such as relieving nerve tension, relieving pain, detoxifying, beautifying and enhancing memory [21-23]. Terpenoids were the most important aromatic components, among which β-terpenes were not only raw materials for various terpene synthetic fragrances, but also had anti-inflammatory and anti-allergic effects. γ-terpenes had strong antibacterial and antioxidant properties. (Z)-Citral had anti-inflammatory, antioxidant and apoptosis inducing properties. α-Terpineol had strong antioxidant activity [24].

Total flavonoid and phenolic content in lemon peel extracts

The total flavonoid contents in the three lemon peel extracts were more than that of total polyphenols, with LPE having the highest content, as shown in Table 4. Flavonoids and polyphenols have strong antioxidant properties, which can effectively eliminate free radicals in the body, reduce skin pigmentation caused by reactive free radicals and fatty acids, prevent cell aging, inhibit the exudation of inflammatory factors and inhibit microbial growth [25,26].

Table 4. Comparison of total flavonoids and phenolic content in three lemon peel extracts.

Extracts	Total flavonoid/μg/mL	Total phenolic/μg/mL
LPEO	42.57 ± 0.05	32.06 ± 0.08
LPE	77.94 ± 0.03	61.24 ± 0.04
LPAO	62.72 ± 0.06	48.02 ± 0.07

E-nose results of the odour of lemon peel extracts

The PEN3 E-nose consists of 10 kinds of sensors. As shown in Figure 2, the response values were mainly concentrated on the probes of S2 (sensitive to nitrogen oxides), S7 (sensitive to terpenoids and sulfur-containing compounds) and S9 (sensitive to aromatic compounds and organic sulfides). The maximum odour response peaks of three lemon peel extracts and the corresponding changes in response values after reaching the peaks of 60 s, 100 s and 140 s were shown in Table 5.

Figure 2. Response values of the odours of three lemon peel extracts.

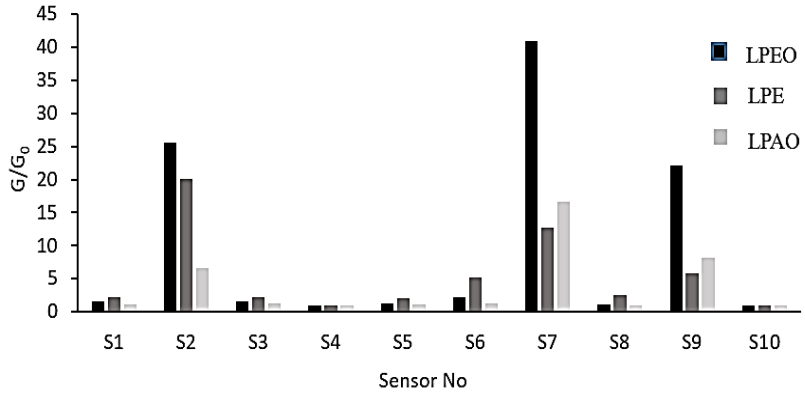


Table 5. Comparison of total flavonoids and phenolic content in three lemon peel extracts.

Extract	0 s	60 s		100 s		140 s	
	Peak value/G_0	Peak value/G	Aroma retention value/%	Peak value/G	Aroma retention value/%	Peak value/G	Aroma retention value/%
LPEO	43.4134	42.3630	97.58	41.7211	96.10	40.9111	94.24
LPE	20.3461	18.4422	90.64	17.0511	80.81	15.5736	76.54
LPAO	16.6896	16.3119	97.74	15.9618	95.64	15.3355	91.89

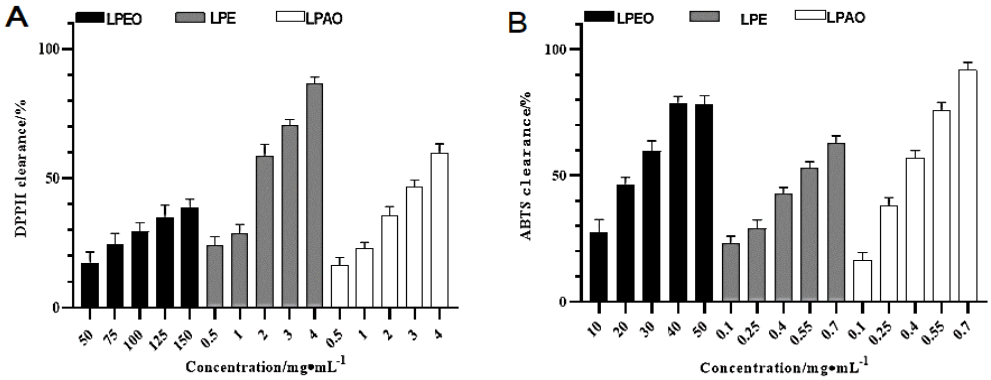
According to the maximum response peak, the intensity of the odour was: LPEO>LPE>LPAO. According to the retention value in peak values within the same time period, the persistence of odour was: LPEO, LPAO>LPE. In addition to terpenoids and aromatic components, LPE also contain some impurities such as plant wax, which affect their odour response values.

Results of antiradical scavenging of the lemon peel extracts

The scavenging activity of three lemon peel extracts against DPPH and ABTS radicals increased with concentration, as shown in Figure 3. IC_{50} of LPEO, LPE and LPAO were 237.30 mg/mL, 1.53 mg/mL and 3.15 mg/mL respectively, which the clearance rate of 4 mg/mL LPE to DPPH was 86.64 ± 1.96%. IC_{50} of LPEO, LPE and LPAO were 20.76 mg/mL, 0.49 mg/mL and 0.31 mg/mL, respectively, which the clearance rate of 0.7 mg/mL LPAO to ABTS was

91.80% ± 2.46%. Research found that D-limonene, which was the main component in lemon peel extracts, had a good antioxidant effect [27]. The contents of (Z)-citral, α-terpinene and α-terpineol were relatively high in LPE, which had a greater scavenging effect on DPPH. LPAO contained more γ-terpinene and (Z)-Citral, which had a greater scavenging effect on ABTS [28].

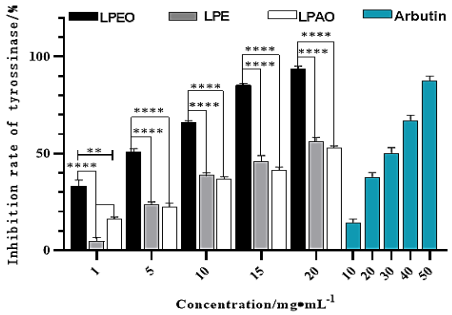
Figure 3. Scavenging rates. **Note:** (A) DPPH; (B) ABTS by three lemon peel extracts.



Antityrosinase activity of the lemon peel extracts

The inhibitory effect of three lemon peel extracts on tyrosinase increased with concentration, as shown in Figure 4. The intensity of inhibition on tyrosinase was LPEO (IC_{50} 3.16 mg/mL)>LPE (IC_{50} 16.02 mg/mL)>LPAO (IC50 21.73 mg/mL). The IC_{50} values of the three extracts were lower than the IC_{50} of the positive control arbutin (26.62 mg/mL). This indicated that their anti tyrosinase activities were superior to arbutin. The anti-tyrosinase activity of 20 mg/mL LPEO was 93.82% ± 1.06%. Studies shown that the bioactive substances such as flavonoids and polyphenols rich in lemon peel had a similar structure to the substrate of tyrosinase and acted as substrate analogues to bind with tyrosinase thereby inhibiting the production of melanin [29]. Meanwhile, substances such as citral, neryl acetate and pinene had a significant impact on the activity of tyrosinase [30].

Figure 4. Inhibition effect of lemon peel extract on tyrosinase. **Note:** $^{**}p \leq 0.01$; $^{***}p \leq 0.001$; $^{****}p \leq 0.0001$.

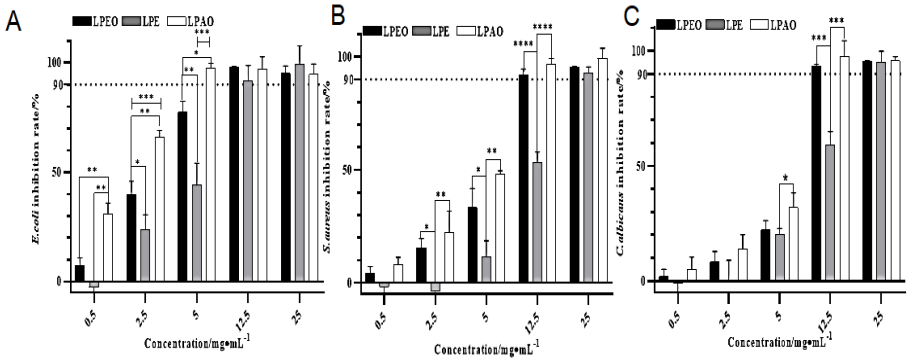


Antibacterial activity of the lemon peel extracts

The antibacterial ability of three lemon peel extracts increased with increasing concentration, as shown in Figure 5. According to the half inhibition rate, the antibacterial rates of the three lemon peel extracts were LPAO (IC_{50} 2.49 mg/mL)>LPEO (IC_{50} 3.09 mg/mL)>LPE (IC_{50} 5.47 mg/mL). The sensitivity of three lemon peel extracts to *Escherichia coli* were higher than that of *Staphylococcus aureus* and *Candida albicans*. When the concentrations of LPEO, LPAO and LPE were 11.71 mg/mL, 12.32 mg/mL and 44.13 mg/mL respectively, the antibacterial rates were over 90%. The antibacterial activity of essential oils is closely related to their chemical composition. Studies shown that the content of D-limonene in pomelo peel essential oil was positively correlated with its antibacterial activity

(r=0.811~0.923) [31]. Turpentine could damage the biofilms of *Staphylococcus aureus* and *Escherichia coli*, leading to cell permeability and thus inhibiting their growth. Neryl acetate could damage the ultrastructure of fungal cells, disrupt their cell membrane regulatory function and deformability. These substances exhibit a change of "LPAO>LPEO>LPE" in the GC/MS analysis of lemon peel extracts.

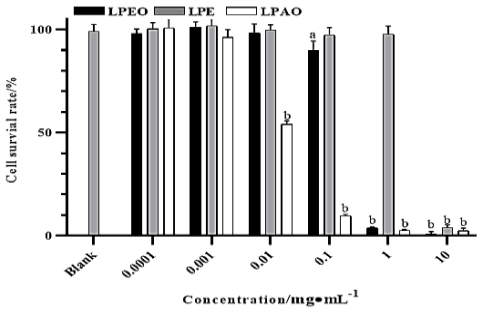
Figure 5. Antibacterial activity of three lemon peel extracts against. **Note:** (A) *Escherichia coli*; (B) *Staphylococcus aureus*; (C) *Candida albicans*, $^{*}p \le 0.05$; $^{**}p \le 0.01$; $^{***}p \le 0.001$; $^{****}p \le 0.0001$.



Anti-inflammatory effect of the lemon peel extracts

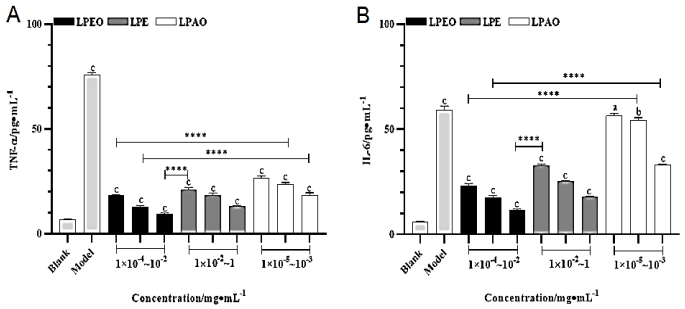
The effect of sample concentration on cell viability was shown in Figure 6. LPEO below 0.01 mg/mL, LPE below 1 mg/mL and LPAO below 0.001 mg/mL did not cause damage to HaCaT cells. Subsequent experiments will be conducted within the concentration range of non-destructive cells.

Figure 6. Effect of the lemon peel extracts on HaCaT cell survival. **Note:** Compared with blank group, a means $p \le 0.05$, b means $p \le 0.0001$.



The results of anti-cellular inflammation (TNF-α, IL-6) were shown in Figure 7.

Figure 7. Effects of three extracts on intracellular. **Note:** (A) TNF-α; (B) IL-6 contents. Letters mean the comparison between model group and blank group between sample group and model group. The asterisk mean the comparison between different sample groups of the same concentration. a and $^{*}p \le 0.05$; b and $^{**}p \le 0.01$; c and $^{***}p \le 0.001$; $^{****}p \le 0.001$.



There was a significant difference between the model group and the blank group, indicating successful induction of cellular inflammation. Compared with the model group, all three lemon peel extracts could effectively inhibit the production of TNF-α and IL-6 and the inhibitory effect increased with dose, but the inflammatory level was not reduced to the cellular state of the blank group. LPEO had the strongest anti-inflammatory effect among the three extracts. The inhibition rates of 1 × 10^{-2} mg/mL LPEO for TNF-α and IL-6 were 87.79% ± 3.86% and 80.75% ± 2.33%, respectively.

The anti-inflammatory properties of essential oils are related to their cascade reactions to cytokines and regulatory transcription factors during signal transduction [32]. Research has confirmed that components such as limonene, carene and umbelliferon had anti-inflammatory effects. For example, limonene effectively reduced the over expression of inflammatory factors induced by doxorubicin by restoring antioxidant enzyme levels and alleviating oxidative stress [33]. Lemonene, δ-3-carene and α-pinene in the essential oil of Chinese torreya effectively inhibit the generation of pro-inflammatory factors and achieve anti-inflammatory effects [34]. Thymol in thyme essential oil had significant inhibitory effects on aging induced brain inflammation and blood telomere wear in mice [35]. The experimental results were consistent with the GC/MS detection data trends of these substances in three lemon peel extracts.

Repair results on scratched HaCaT cells

The cell scratch test is commonly used to simulate wound healing *in vitro*, and the results were shown in Figures 8 and 9 [36].

Figure 8. Imaging of repair of scratched HaCaT cells using LPEO, LPE and LPAO.

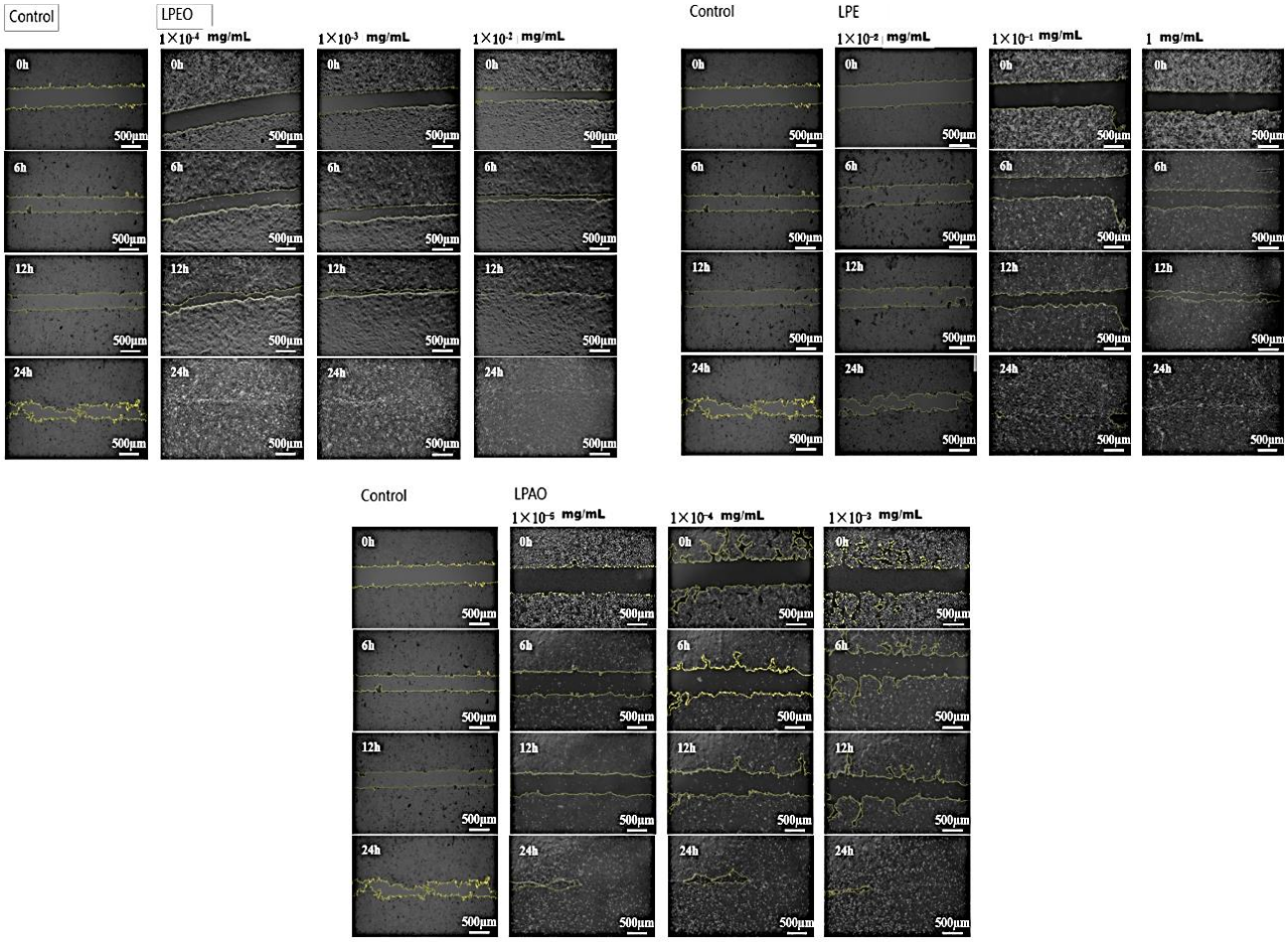
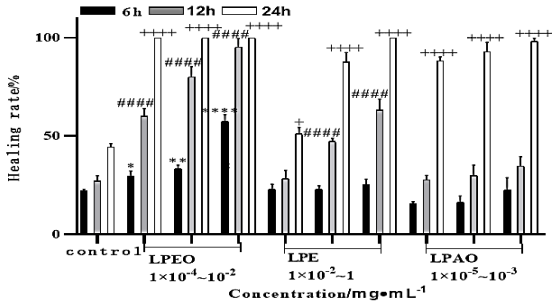


Figure 9. Effects of LPEO, LPE and LPAO on the healing rate of scratched HaCaT cells. **Note:** Compared with control group, 6 h: *p<0.05, **p<0.01, ***p<0.001; 12 h: #p<0.05, ##p<0.01, ###p<0.001; 24 h: +p<0.05, ++p<0.01, +++p<0.001.



The HaCaT cell healing rate were significantly higher than that of the control group after 24 h of treatment with lemon peel extract. With the increase of lemon peel extract concentration and the extension of action time, the overall cell healing rate showed an upward trend. Among them, LPEO had a better healing effect on cells than LPE and LPAO. After treating scratched cells with 0.01 mg/mL LPEO for 12 h, the cell healing rate was 95.29% ± 3.41%. Wound repair involves processes such as coagulation, inflammation, cell proliferation and tissue remodeling. The migration of fibroblasts plays a vital role in wound healing. Lavender essential oil could promote to transfer of growth factors-β and fibroblast growth factors to accelerate wound healing [37]. *Eucalyptus globulus* labill essential oil also had a good wound healing effect on rat wound models [38].

CONCLUSION

There were differences in the composition of three lemon peel extracts, namely LPE (39 kinds)>LPEO (22 kinds)>LPAO (9 kinds), with the main components being terpenes. The content of total flavonoids in these extracts was higher than that of total polyphenols and the content of flavonoids in LPE was higher than that of LPAO and LPEO. The differences in the composition of the three extracts led to the differences in their aroma and biological functions. The aroma intensity and persistence of LPEO were the best of the three.

The three lemon peel extracts showed good antioxidant, anti tyrosinase, antibacterial, anti-inflammatory and wound healing properties. Among the extracts, LPE and LPAO showed superior scavenging abilities against DPPH and ABTS compared to LPEO, with the clearance rate exceeding 90%. Compared with LPE and LPAO, LPEO showed stronger inhibitory effect on tyrosinase and the inhibition rate at the concentration of 20 mg/mL was 93.82% ± 1.06%, which was higher than that of arbutin. The antibacterial activity of the three lemon peel extracts ranked as LPEO>LPAO>LPE and they were more sensitive to *Escherichia coli* than *Staphylococcus aureus* and *Candida albicans* and the inhibition rate could reach more than 90%. LPEO showed a stronger inhibitory effect on inflammatory factors compared to LPAO and LPE, with inhibitory rates of 87.79% ± 3.86% for TNF-α and 80.75 ± 2.33% for IL-6 at 1 × 10^{-2} mg/mL. Additionally, LPEO enhanced the migration and healing of HaCaT cells more effectively than LPE and LPAO. Following a 12 h treatment with 1 × 10^{-2} mg/mL LPEO, the healing rate of scratched cells reached 95.29% ± 3.41%.

In summary, in addition to antioxidant properties, LPEO has demonstrated superior overall performance compared to LPE and LPAO. These three extracts can also be combined to expand their application as cosmetic additives,

providing benefits such as enhancing aroma, antioxidant, whitening, antibacterial, anti-inflammatory and skin wound healing.

AUTHORS CONTRIBUTIONS

Liping Liu, Jinqing Huang and Xu Xu designed the study and drafted the manuscript. Jinqing Huang and Xu Xu performed the main experiments, Chang Liu participated in the cell experiment. All authors read and approved the final manuscript.

DECLARATION OF CONFLICTING INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

STATEMENT OF HUMAN AND ANIMAL RIGHTS

This article does not contain any studies with human or animal subjects.

STATEMENT OF INFORMED CONSENT

There are no human subjects in this article and informed consent is not applicable.

STATEMENT OF DATA AVAILABILITY

All data in the paper can be contacted by the corresponding author.

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