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Biological Activities of Some *Citrus* Fruit Peels extracts

Emad A. Shalaby[a], Sanaa M. M. Shanab[b], Rehab M. Hafez[b] , Abeer E. El-Ansary[a*]

[a]*Department of Biochemistry, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt*
dremad2009@yahoo.com, abeeransary@yahoo.com

[b]*Department of Botany and Microbiology, Faculty of Science, Cairo University, 12613 Giza, Egypt*
sanaashanab@sci.cu.edu.eg, rehabhafez@sci.cu.edu.eg

Abstract

The present investigation aimed to evaluate the antioxidant activity of various extracts (water, ethanol, and essential oil extracts) from orange and lemon peels, and to determine which was the most promising one using three different antioxidant assays. Gas chromatography–mass spectrometry (GC-MS) analysis was used to determine the active ingredients of the most promising extract. In addition, the present work deals with the antimicrobial activity of the most promising extract against five different strains of pathogenic bacteria (*Escherichia coli, Salmonella sp, Bacillus cereus, Staphylococcus aureus and E. coli* O157 wild type strain) and its anticancer activity of against human liver cancer cells (HepG2) using cytotoxicity assay. The tested extracts from lemon and orange (peels and residues) recorded high antioxidant activity against both radical and non-radical methods. The GC/MS analysis of most promising extract (lemon peels essential oils) showed a highly complex chemical profile, containing approximately 15 different components. The identified major compound was D-Limonene and 2,6,6-Trimethyl bicyclo(3,1,1) hept-2-ene. Also, the obtained data reported that the lemon essential oil extract showed the highest antimicrobial activity against five different strains of pathogenic bacteria followed by the lemon ethanol extract against three strains of bacteria. Furthermore, the anticancer activity results revealed that the lemon essential oil extract has a very high cytotoxicity against human liver cancer cells at different tested concentrations. Conclusively, the antibacterial and cytotoxicity efficiencies were shown to be concentration dependent of the essential oil contents.

Keywords: Citrus; peels; antioxidant activities; antibacterial activities; anticancer activity; GC/MS.

1. Introduction

Oranges (Citrus sinensis L) and Lemons (C. limon) belonging to the genus Citrus in family Rutaceae. Their fruits are mostly consumed fresh or in the form of juices or canned [1]. Like other Citrus species, fruits of orange and lemon contained high content of vitamin C and B6, sugars, dietary fiber and elements as potassium, calcium, phosphorus, magnesium, copper as well thiamin, niacin, riboflavin, folate and pantothenic acid. In addition, they also contained secondary metabolites (phytochemicals) such as Flavonoids, alkaloids, coumarins, limonoids, carotenoids, phenol acids, and essential oils [2]. These secondary metabolites possessed variable bioactivities of great importance to human health (antioxidant, anti-inflammatory, anticancer, cardiovascular and neuroprotective properties [2].Citrus fruits have also been utilized as traditional medicinal herbs in many Asian countries. Peels or entire fruits have been used to cure different diseases like dyspepsia, cough, skin irritation, muscular discomfort, and ringworm infections, as well as decrease blood pressure [2]. Non-edible parts of Citrus fruits, peels and seeds, represent 25-30% of waste produced after consuming the fruits. These waste by-products appear to exhibit significant levels of antioxidant and antimicrobial activities [3,4] . Citrus peels are rich in essential oils (0.6-1%), dietary fibers (6.30-42.13 g/100g), carotenoids and anthocyanins (0.01-0.20 g/100g). Other beneficial chemicals are found in citrus peel include vitamin C (0,109-1,150 g/100g), flavonoids and phenols (0.67-

*Corresponding author e-mail: abeeransary@yahoo.com.; abeeransary@agr.cu.edu.eg
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19.62 g/100g) [5,6]. Thus instead of only using the peels of Citrus fruits, as oranges and lemons, as flavoring element in biscuits, puddings, sweets, chocolates, pies, cakes, and sour sauces [3], they can be utilized for medical purposes.Lemon peels of fruits contain flavonoids, phenolic acids, carboxylic acids, amino acids, microelements (Ca, Mg, P, K, Na), carbohydrates, coumarins in addition to vitamins and their metabolites. They also constitute of essential oils in which limonene Monoterpines are dominated (69%). In addition, they include linear furanocoumarins (psoralens) and polymethoxylated flavones [7]. Oranges are high in phytochemicals such phenolics, vitamin C, and carotenoids, in addition to sugars, acids, and polysaccharides [8]. Those compounds, in both orange and lemon peels, are nutraceutical products with extra health benefits which lower the risk of chronic diseases [7,8,9]**.**Due to the rapid increase of diseases and the high usage of synthetic agents, this study attempted to evaluate the phytochemical composition of lemon and orange peels and to assess their antioxidant, antibacterial, and anticancer activities as natural alternative agents.

2. Materials and Methods

Fruits samples

Oranges (Citrus sinensis L) and Lemons (C. limon) fruits were collected from local stores in Cairo, Egypt. 500 grams of fruit peels from lemon (C. limon) and orange (Citrus sinensis L) samples were cleaned, cut into small equal sized- pieces and collected into separate vials before use.

Chemicals and reagents

Pure Methanol, Hexane, Ethyl acetate, Methylene chloride, Ethanol and Dimethyl sulfoxide (DMSO) were obtained from PubChem Co. (Darmstadt, Germany), while 2, 2 diphenyl-1-picrylhydrazyl (DPPH), Doxorubicin (DOX) and 2,2'-azino-bis (ethylbenzthiazoline-6-sulfonic acid (ABTS.+) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Bacterial strains

Anti-bacterial activities were tested using Gram–ve bacteria [Escherichia coli (ATCC 25922), E. Coli O157 wild type strain (ATCC 93111) and Salmonella sp (ATCC 14028)] and Gram + ve bacteria [Bacillus cereus (ATCC 33018) and Staphylococcus aureus (ATCC 25923)].

Cell lines

Human hepatocellular cancer cell line (HepG-2) was provided and cultivated in Vacsera (Giza, Egypt).

Extraction of essential oil

Essential oils have been extracted using steam distillation method by a Clevenger type distiller for 10 h. After extraction, essential oils are recovered, and stored, at 4°C, in sealed brown bottles. Meanwhile, the produced water extract was filtered using filter paper and the peel residues were stored at 4°C until used [10].

Ethanol extraction

Absolute Ethanol solvent was used to extract any remaining components from the residue of the steam distillation process. The extract was then filtered using filter paper and stored at 4°C [11].

Antioxidant activity methods

DPPH assay

Antioxidant activity of plant extracts was determined according to [12]. One milliliter of extract was mixed with 1 ml of a DPPH solution (0.03% w/v in methanol). After 30 min incubation at room temperature in dark, the absorbance of the solution was measured using UV/VIS spectrophotometer (T60, England, UK) at 517 nm. Control was prepared by the same procedure without extract. Ascorbic acid (0.03%, w/v) was used as a natural standard. Radical scavenging activity (%) was calculated from the following equation:

Scavenging activity (%) = [(Ac − At) / Ac] ×100, Where Ac and At are the absorption of control and tested extract, respectively.

ABTS assay

The 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS assay was carried out as described by [13]. The radical prepared by mixing equal volume (1/1, v/v) from ABTS (7mM) and potassium persulfate and leave the mixture in the dark at room temperature from 4-16 h until the reaction was completed and the absorption was stable. After incubation, The ABTS solution was diluted with distilled water to an absorbance of 0.700 ± 0.05 at 734 nm. Estimation has been made by mixing 0.9 ml of ABTS solution and 0.1 ml of extract and mixed for 45 sec. then measure the absorbance after incubation 1 min. Ascorbic acid (0.03%, w/v) was used as a natural standard. Calculate the decrease of absorption by the following equation:

Activity (%) = [(Ac − At) / Ac] ×100, where Ac and At are the absorption of control and tested extract, respectively.

$KMnO_4$

The scavenging effects of crude extracts were performed according to [14]. One milliliter of 0.02 M KMnO4 solution (in methanol) was added to a test tube containing 1.0 ml aliquot of extract. The mixture was vortexed for 1 min and kept at room temperature for 30 min in the dark. The absorbances of all the sample solutions and ascorbic acid (as natural antioxidant standard) were measured at 514 nm. The

percentage (%) of scavenging activity was calculated as the following: %Antioxidant activity = (control−sample×100)/control, where control is KMnO4 solution (0.02 M).

Phytochemical identification using GC/MS

The chemical composition of plant extract was performed using GC-MS QQQ 7890B GC system mass spectrometer (Agilent) with a direct capillary column HP–5MS UI (30 m × 0.25 mm × 0.25 µm film thickness). The components were identified by comparing their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

Well Diffusion Assay

The antibacterial activity of various extracts was evaluated by the well diffusion method against Escherichia coli (ATCC 25922), Salmonella sp (ATCC 14028), Bacillus cereus (ATCC 33018), Staphylococcus aureus (ATCC 25923) and E. coli O157 wild type strain (ATCC 93111). The melted agar medium (1.5% agar) was inoculated with 10% v/v of the bacterial culture broth. Wells were made in the agar plate by puncturing the gel. A 100 µL aliquot of the extract or standard drug was added into the respective wells. After incubation for 24 h at 37 °C, zones of inhibition were measured. Growth inhibition was scored positive in the presence of a detectable clear zone around the wells [15].

Assessment of anticancer activity

Cell culture

HepG-2 cells were maintained in RPMI-1640 supplemented with 100 µg/mL streptomycin, 100 units/mL penicillin and 10% heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO2 atmosphere at 37 ºC [16].

Cytotoxicity assays

The cytotoxicity of crude extracts was tested against HepG-2 cells and performed by SRB assay as previously described by [17]. Exponentially growing cells were collected using 0.25% Trypsin-EDTA and plated in 96-well plates at 1000-2000 cells/well. Cells were exposed to each test compound for 72 h and subsequently fixed with TCA (10%) for 1 h at 4 ºC. After several washings, cells were exposed to 0.4% SRB {sulforhodamine B (SRB), 2-(3-diethylamino-6-diethylazaniumylidene-xanthen-9-yl)-5-sulfo-benzenesulfonate} solution for 10 min in dark place and subsequently washed with 1% glacial acetic acid. After drying overnight, Tris-HCl was used to dissolve the SRB-stained cells and color intensity was measured at 540 nm. Doxorubicin (DOX) was used as anticancer standard.

Statistical analysis

Data were subjected to an analysis of variance and the means were compared using the least significant difference (LSD) test at 0.05 and 0.01 levels using SPSS version 22.0 computer program [18].

Results and Discussion

Antioxidant activity

DPPH assay

The antioxidant activity of different extracts (water, ethanol, and essential oil extracts) from lemon and orange peels was performed against DPPH. The obtained results in Table (1) revealed that the lemon essential oil extract had the highest antioxidant activity by 96.06% followed by the lemon ethanol extract (94.42%) and the orange ethanol extract (94.17%), respectively. Most of the extracts - except the lemon water extract and the orange essential oil extract- had a higher antioxidant activity than the positive control (76.42%).

ABTS assay

The antioxidant activity of different extracts (water, ethanol, and essential oil extracts) from lemon and orange peels was determined against ABTS.

Table 1

Antioxidant activity (%) of different extracts (water, ethanol, and essential oil extracts) from lemon and orange peels against DPPH assay

Citrus extract	Antioxidant Activity (%)
Orange /Water	85.98±0.34
Orange / Ethanol	94.17±0.21
Lemon / Water	5.0±0.15
Lemon / Ethanol	94.42±0.54
Lemon essential oil	96.06±0.23
Orange essential oil	54.23±0.39
Ascorbic acid (100 ppm)	76.42±0.31

Values are means of three replicates ± SE.

The results recorded in Table (2) demonstrated that the lemon water extract had the highest antioxidant activity against ABTS (89.65%) followed by the lemon ethanol extract (88.94%) and the orange ethanol extract (88.59%), respectively. The results revealed that the extracts mostly -except for both the orange essential oil (45.26%) and lemon essential oil extracts (69.67%) - had a higher antioxidant activity than the ascorbic acid positive control (73.93%).

Table 2

Antioxidant activity (%) of different extracts (water, ethanol, and essential oil extracts) from lemon and orange peels against ABTS radical assay

Citrus peels extract	Antioxidant Activity (%)
Orange /Water	85.32±0.65
Orange / Ethanol	88.59±0.33
Lemon / Water	89.65±0.53
Lemon / Ethanol	88.94±0.32
Lemon essential oil	69.67±0.54
Orange essential oil	45.26±1.01
Ascorbic acid (100 ppm)	73.93±0.21

Values are means of three replicates ± SE.

$KMnO_4$ assay

The antioxidant activity of different extracts (water, ethanol, and essential oil extracts) from lemon and orange peels was estimated against KMnO4. Table (3) showed that the lemon water extract (97.62%) had the highest antioxidant activity against KMnO4 followed by the lemon essential oil extract (88.48%) and the orange water extract (85.56%), respectively. All the extracts have a lower antioxidant activity than the positive control (99.09%).

Table 3
Antioxidant activity (%) of different extracts (water, ethanol, and essential oil extracts) from lemon and orange peels against $KMnO_4$ assay

Citrus extract	Antioxidant Activity %
Orange /Water	85.56±0.87
Orange / Ethanol	73.13±0.30
Lemon / Water	97.62±0.50
Lemon / Ethanol	73.53±0.33
Lemon essential oil	88.48±0.19
Orange essential oil	81.26±0.43
Ascorbic acid (100 ppm)	99.09±0.06

Values are means of three replicates ± SE.

The correlation coefficient between the different antioxidant assays was shown in Table (4), and the results revealed that there was a very weak positive correlation between ABTS and DPPH in all sample extracts at 0.031. However, a strong negative correlation was found between KMNO4 and DPPH at -0.55. Weak negative correlation between ABTS and KMnO4 assay was detected at -0.06.

Table 4.
Correlation coefficient between different antioxidant methods of different orange and lemon extracts

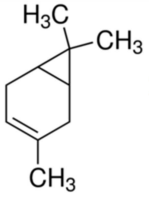
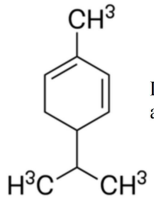
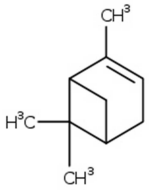
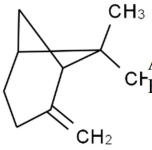
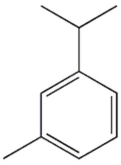
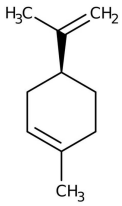
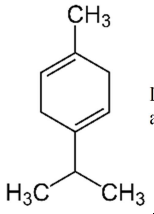
	DPPH	ABTS	$KMnO_4$
DPPH		0.031	-0.55
ABTS			-0.06
$KMnO_4$			

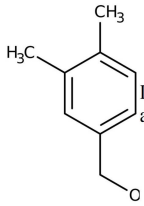
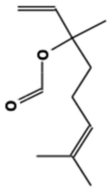
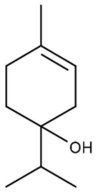
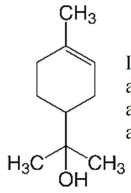
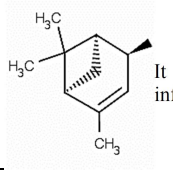
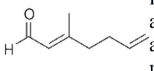
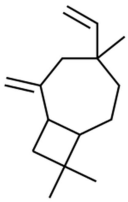
From the results illustrated in Tables (1-3), it can be concluded that the tested extracts from lemon and orange (peels and residues) recorded high antioxidant activity against both radical and non-radical assays. Lemon essential oil extract has the highest antioxidant activity against DPPH, while lemon water extract had the highest antioxidant activity against ABTS and KMnO4 when compared to ascorbic acid and all the other extracts. These results are in agreement with the findings of [19] who reported that when compared to conventional ascorbic acid, lemon essential oil demonstrated significant radical scavenging efficacy. It was found that lemon peels exhibit potent antioxidant properties [3]. This may be due to the presence of components such as flavonoids (such as hesperidin), monoterpenoids (such as limonene and α-pinene) and phenolic compounds. It was also discovered a link between antioxidant activity and α-pinene content [20]. it was suggested that the content of polyphenols can be used as an indicator of the strength of antioxidant activity [21]. Other findings indicated that antioxidant effect maybe referred to the presence of quercetin [22]. It was recommended that antioxidant activity may be attributed to naringin which is a is a flavanone-7-O-glycoside [23].

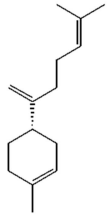
Gas chromatography–mass spectrometry (GC-MS)

The GC-MS analysis of the lemon essential oil extract promising extract revealed the presence of highly complex chemical profile containing fifteen different phytocomponents. The results shown in Table (5) and Fig (1) showed that the major components of lemon essential oil are D-Limonene (53.65%) followed by 2,6,6-Trimethyl bicyclo (3,1,1) hept-2-ene (17.4%), L-alpha-Terpineol (7.29%) and Gamma Terpinene (6.47%), respectively. Those findings were correlated to those of [24] who showed that lemon peels (C. limon) contain from 48% to 70% limonene followed by terpenoids. Also, [25] found that lemon peels contain 71.81% limonene followed by Sabinene 5.82%.

Table 5
GC-MS analysis of the lemon essential oil extract

S.N	R.T (min)	Compound name	Relative percentage	Chemical Structure	Biological activities	References
1	8.77	3-Carene	2.08		It was discovered to have antioxidant activity	[26]
2	10.37	Alpha-phellandrene	0.57		It has a role as an antimicrobial and antifungal agent.	[27,28]
3	10.57	2,6,6-Trimethyl bicyclo(3,1,1) hept-2-ene	17.4		It was discovered to have antimicrobial activities	[29]
4	11.08	Bicyclo(3,1,1)heptane, 6,6-dimethyl-2-methylene	0.79		Anti-Candida and anti-Helicobacter pylori activity	[30]
5	12.51	Benzene, 1-methyl-3-(1-methylethyl)	1.16		It has some antibacterial activity	[31]
6	12.73	D-Limonene	53.65		It shows antibacterial activity	[32]
7	13.87	Gamma Terpinene	6.47		It has antioxidant and antimicrobial properties	[33]

8	14.94	3,4-Dimethylbenzyl alcohol	1.35		It has some antimicrobial and antioxidant properties	[34, 35]
9	15.59	1,6-Octadien-3-ol, 3,7-dimethyl	0.62		It was shown to have antifungal properties	[36]
10	18.69	Terpinen-4-ol	3.34		It possesses potent antibacterial and anti-inflammatory effects.	[37]
11	19.27	L-alpha-Terpineol	7.29		It is used as an antioxidant, anticancer, anticonvulsant, antiulcer, antihypertensive, and anti-nociceptive agent.	[38]
12	20.80	Cis-Verbenol	1.44		It has anti-ischemic and anti-inflammatory activity	[39]
13	21.87	Citral	1.71		It has been shown to be antibacterial against pathogenic and food-spoilage microorganisms.	[40]
14	26.95	Bicyclo(5,2,0)nonane, 2-methylene-4,8,8-trimethyl-4-vinyl	0.6		It shows anti-inflammatory and antibacterial activity	[41]

15	29.72	Beta-Bisabolene	0.74		It was discovered to have anticancer properties.	[42]

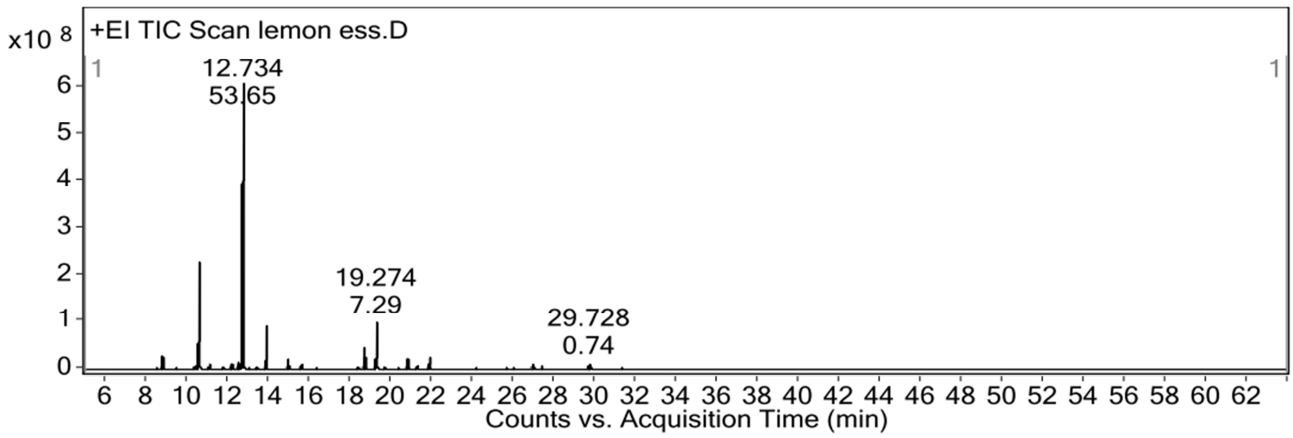


Fig. 1. GC-MS chromatogram of the lemon essential oil extract

Antimicrobial activity

Well diffusion

Antibacterial activity of different extracts from lemon and orange peels was performed against five different microorganism strains through well diffusion method.

The obtained results in Table (6) revealed that the lemon essential oil extract showed the highest antibacterial activity against five different strains of bacteria (E. coli, Salmonella sp, Bacillus cereus, Staphylococcus aureus and E. coli O157 wild type strain) followed by the lemon ethanol extract against three strains of bacteria (E. coli, Salmonella sp, Staphylococcus aureus), the lemon water extract against one strain (Staphylococcus aureus) and the orange water extract against one strain (Staphylococcus aureus), respectively. Those results were in agreements with those of [3,7, and8], who all agreed that lemon peel extracts (specifically lemon essential oil extract) have high antibacterial activities. According to [43,44] Citrus peels, particularly lemon peels, have potent antibacterial properties against a variety of medically significant organisms such as Pseudomonas aeruginosa, Salmonella typhi, and Micrococcus aureus. Furthermore, Citrus peel oils also had significant antibacterial action against the tested organisms, with lemon exhibiting the greatest activity; moreover, the phytochemical components presented in the peels oil were shown to be responsible for the antibacterial action. Because lemon essential oil and certain of its components have considerable antibacterial activity so, according to [2] all the lemon peels (C. limon) active metabolites work energetically together to provide the biological activities. The findings of [19] suggest that they might be used as natural food preservatives.

Table 6
Antibacterial activity of different extracts from lemon and orange peels against five different pathogenic bacterial strains through well diffusion method

Citrus Peels	Antimicrobial activity (Well diffusion)				
		Gram -ve		*Gram +ve*	
	E. coli 0157	*Escherichia. coli*	*Salmonella sp*	*Staphylococcus aureus*	*Basillus cereus*
Orange essential oil	>0.1mm	11.33 mm±0.47	>0.1mm	>0.1mm	>0.1mm
Lemon essential oil	14.33 mm±0.47	27 mm±2.64	21 mm±0.47	27 mm±2.64	14.33 mm±0.58
Orange water extract	>0.1mm	>0.1mm	>0.1mm	20.66 mm±0.47	>0.1mm
Lemon water extract	>0.1mm	>0.1mm	>0.1mm	21 mm±1.0	>0.1mm
Orange ethanol extract	>0.1mm	>0.1mm	>0.1mm	>0.1mm	>0.1mm
Lemon ethanol extract	>0.1mm	12.5 mm±0.70	11 mm±1.41	12.66 mm±0.57	>0.1mm
Novobiocin	ND	ND	ND	8.5mm±0.57	18.5mm±0.57
Polymyxin	30mm±0.57	18.66mm±0.57	21.33mm±0.57	ND	ND

Values are means of three replicates ± SE; ND, not detected

Anticancer activity

Cytotoxicity assay

The results obtained from the assay as shown in Table (7) revealed that the lemon essential oil extract has a very high cytotoxicity against HepG-2 cancer cells. There is a clear indication of a directly proportional relationship between the amount of essential oil used and its cytotoxicity. According to [2] all of the active metabolites in the extract work together to provide anti-oxidative, anti-inflammatory, anti-cancer, anti-microbial, and antiallergy benefits, as well as cardiovascular protection, neuroprotection, hepatoprotection, and other benefits. At the same time, [45] stated that limonene has significant anticancer activities connected to the inhibition of tumour initiation, growth, and angiogenesis

Table 7
Anticancer activity of the lemon essential oil extract

Essential oil	Cytotoxicity %		
Conc.	500 ppm	750 ppm	1000 ppm
Lemon	81±1.41	98±1.52	99±0.57

Values are means of three replicates ± SE

Conclusion

From the obtained results, it could be concluded that citrus residues extract can be extensively used in the production of potential antioxidant, antibacterial and anticancer for biomedical application on the consumer's health.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data used and analysed in this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization: EAS, SMS, RMH and AEE Data curation; EAS, SMS, RMH and AEE Funding acquisition; EAS, SMS, RMH and AEE Investigation; EAS, SMS, RMH and AEE Methodology; EAS, SMS, RMH and AEE Resources EAS, SMS, RMH and AEE Software; EAS, SMS, RMH and AEE Validation; EAS, SMS, RMH and AEE Writing - original draft; EAS, SMS, RMH and AEE Writing - review and editing EAS, SMS, RMH and AEE Please add at the end: All authors read and approved the final manuscript.

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Arabic Abstract

الأنشطة البيولوجية للزيوت الأساسية من□عض قشور الحمضيات

هدفت الدراسة الحالية إلى تقييم النشاط المضاد للأكسدة لمختلف المستخلصات (الماء ، والإيثانول ، ومستخلصات الزيوت الأساسية) من قشور البرتقال والليمون ، وتحديد أكثرها واعدة باستخدام ثلاثة فحوصات مختلفة لمضادات الأكسدة. تم استخدام تحليل كروماتوغرافيا الغاز - قياس الطيف الكتلي (GC-MS) لتحديد المكونات الفعالة للمستخلص الواعد. بالإضافة إلى ذلك ، يتعامل العمل الحالي مع النشاط المضاد للميكروبات للمستخلص الواعد ضد خمس سلالات مختلفة من البكتيريا المسببة للأمراض (Escherichia coli و Salmonella sp و Bacillus cereus و Staphylococcus aureus و E. coli O157 wild type strain) ونشاطها المضاد للسرطان ضد خلايا سرطان الكبد البشرية (HepG2) باستخدام مقايسة السمية الخلوية. سجلت المستخلصات المختبرة من الليمون والبرتقال (قشور وبقايا) نشاطًا عاليًا لمضادات الأكسدة ضد كل من الطرق الجذرية وغير الجذرية. أظهر تحليل GC / MS لمعظم المستخلصات الواعدة (الزيوت الأساسية لقشر الليمون) صورة كيميائية معقدة للغاية ، تحتوي على ما يقرب من 15 مكونًا مختلفًا. كان المركب الرئيسي المحدد هو D-Limonene و 6،6،2-Trimethyl bicyclo (3 1،hept-2-ene-1). كما أفادت البيانات التي تم الحصول عليها أن مستخلص زيت الليمون الأساسي أظهر أعلى نشاط مضاد للميكروبات ضد خمس سلالات مختلفة من البكتيريا المسببة للأمراض يليه مستخلص الليمون الإيثانول ضد ثلاث سلالات من البكتيريا. علاوة على ذلك ، أظهرت نتائج النشاط المضاد للسرطان أن مستخلص زيت الليمون الأساسي له سمية خلوية عالية جدًا ضد خلايا سرطان الكبد البشرية بتركيزات مختلفة مختبرة. بشكل قاطع ، تبين أن الكفاءات المضادة للبكتيريا والسمية الخلوية تعتمد على تركيز محتويات الزيت العطري.