Int.J.Curr.Microbiol.App.Sci (2017) *6*(12): 3983-3998

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 12 (2017) pp. 3983-3998**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.612.459

Citrus sinensis and *Citrus aurantiifolia* Peel Extracts: Antibacterial, Antioxidant Activity and Total phenolic

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Keywords

Lemon peels, Orange peels, Antimicrobial, Antioxidants, Total phenolic content.

Article Info

Accepted:
28 October 2017
Available Online:
10 December 2017

A B S T R A C T

Orange and lemon peels (OP&LP) are common citrus byproducts, contain biologically active compounds. This work aimed to identify the potential of OP and LP extracts in terms of their antibacterial, antioxidant activities and total phenolic content. Antibacterial activity was determined and compared to control and Ampicilline. Ethanolic extract of microwave dried LP displayed high inhibition of some bacteria compared to methanolic extract. The air-oven dried LP extracted by methanol showed low antibacterial activity. Microwave dried OP extracted with ethanol inhibited: *L. monocytogenes*, *B. subtilis*, *P. aeruginosa* and *E. coli*, while methanolic extract inhibited *B. subtilis*, *E. coli* and *P. aeruginosa*. Inhibition zone size was less in the used extracts of dried air-oven peels. The antioxidant activity of the tested peel extracts was determined by DPPH, FRAP and OH scavenging activity methods. Total phenolics of both OP&LP extracted with ethanol were higher than in methanol extract.

Introduction

Some microorganisms cause food-borne illness problems for consumer, food industry and safety authorities' due to the phenomena of acquiring antibiotic resistance by different bacterial species (Lucera *et al.*, 2012 and Al-Sheddi, 2009). Bacteria have the ability to spread and become resistance to utilized drug as therapeutic agents (Abeysinghe, 2010 and Soković *et al.*, 2007).

Free radicals can be formed in human metabolism to deactivate the viral and bacterial presence or environmental factors like pollution, smokes, and others. Radical chain reactions with DNA, proteins and cell membrane cause harmful effects to human body. Antioxidants, enzymes and vitamins are naturally available anti-free radical defense systems used to prevent oxidative damage and to protect the body from harmful pathogens (Mantena *et al.*, 2008 and Nabavi *et al.*, 2013).

Using of natural antimicrobial in food gained much attention due to the misuse and mishandling of antibiotics as well increase consumer's awareness of the potential negative impact of synthetic preservatives on health. Compounds from natural sources have the potential to be used for food safety due to

their antimicrobial properties. Considerable efforts have been made to find natural antimicrobials that can inhibit bacterial and fungal growth in foods to improve quality and shelf-life. Studies related to antimicrobials showed the efficacy of plant-derived products in food applications and as factors influencing this effectiveness (Hayek *et al.,* 2013; Gyawali and Ibrahim, 2012, 2014; Tajkarimi *et al.,* 2010). The structural variety of plant compounds is enormous; their natural antimicrobial action depends on structural configuration and hence will be different in their antibacterial effect (Gyawali and Ibrahim, 2014 and Stojkovic *et al.,* 2013). Several plant compounds that are responsible for antimicrobial activity include phenolics, phenolic acids, quinones, saponins, flavonoids, tannins, coumarins, terpenoids, and alkaloids (Ciocan and Bara, 2007 and Lai and Roy, 2004).

Natural antimicrobials could be used to control the growth of food borne pathogens, replacing the used synthetic compounds and to control measure of the microbial growth in situations of cold chain breakdown in pasteurized food (Sanz-Puig *et al.,* 2015). Use of natural preservatives to increase the shelf-life of meat products is a promising technology since many plant-derived substances have antioxidant and antimicrobial properties (Badee *et al.,* 2013 and Gutierrez *et al.,* 2009).

Lemon and orange citrus fruits generate huge amounts of wastes (by-products) that constitute an important source of bioactive compounds for manufactured foods, and health-care (González-Molina, *et al.,* 2010).Citrus peels are primary byproducts during processing discarded as wastes and represent an environmental problem.

Total polyphenols content of citrus peel was higher than in peeled fruits (Gorinstein *et al.,* 2001 and Belitz and Grosch, 1999). Peels have biologically active compounds including natural antioxidants; and can be used as cheap source of functional ingredients and food additives (Hayat *et al.,* 2009, Marı´n *et al.,* 2002; Puupponen-Pimia *et al.,* 2002; Norah *et al.,* 2012 and Galanakis, 2012).

Extraction of phenolic compounds from citrus peels attracted interest as natural antioxidants and antimicrobial in foods (Muhammad, 2010). These compounds have high antioxidant activity and exert antimicrobial effects against food borne pathogens (El-Seedi *et al.,* 2012, Hayat *et al.,* 2010, Delgado-Adámez *et al.,* 2012a,b and Espina *et al.,* 2011) due to their high contents of terpenoids, tannins, quinones, phenolic acids and polyphenols (Calvo *et al.,* 2006 and Lee & Lee, 2010).

Antimicrobial activity of citrus peel extract is directly concerned with the components which they contain (Mehmood, *et al.,* 2015).

Antioxidant substances in citrus waste (e.g. peels) kill microbial flora in soil and increase acidity (Sharma *et al.,* 2017), as well widely used as additives for protection against oxidative degradation of foods (Kumaran and Karunakaran, 2006).

This property is connected with the ability of phenolic compounds to scavenge free radicals, break radical chain reactions and chelate metals (Nayak *et al.,* 2015).

So, the present study aimed to investigate total phenolic content and antibacterial activity of orange and lemon peel extracts against some pathogenic bacterial strains also to evaluate the antioxidant activity of the tested peel extracts using DPPH, FRAP and OH scavenging activity assays to probe their potential use as natural value-added ingredients.

Materials and Methods

Materials

Ripened and freshly harvested citrus lemon (*Citrus aurantiifolia*) and navelat navel orange (*Citrus sinensis*) fruits were purchased from an Egyptian local market.

Chemicals

Chemicals, solvents, standards and reagents were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). All other chemicals used were of analytical grade.

Methods

Lemon and orange peel samples preparation

Lemon and orange fruits were washed by running tap water, peeled and their edible portions were carefully separated. The obtained fresh citrus peels were cut into small pieces before the drying processes.

Drying methods

Each of fresh lemon peel or orange peel pieces was divided separately into two parts and each part was dried using the following two methods**:**

Air oven-drying

The fresh citrus peels pieces were dried in an air oven (Shellab-Model 1350FX.-Made in USA) at 40 ± 2°C for ~ 48 h.

Microwave–drying

A programmable domestic microwave oven (type Samsung, 77 QH 400148, MF 2015), with a maximum output of 1500W at 2450 MHz) was used for drying the fresh lemon or orange peel pieces samples for 6 min. The dried peel samples were ground to fine powders using a mechanical laboratory grinder and passed through a 24-mesh sieve, then packaged in polyethylene bags and stored at 4±1°C until required for use.

Ethanol and methanol extraction

Ethanol and methanol solvents were applied for bioactive compounds extraction to determine and compare antioxidant and antimicrobial activities of the tested OP & LP samples according to (Jo *et al.*, 2003) and Xu *et al.*, (2008) with some modification.

Antibacterial activity assay

Media

The Mueller-Hinton agar media according to Difco-Manual, (1998) was used in the disc diffusion technique for antibacterial assays, respectively, according to Bauer *et al.*, (1996).

Microorganism

The screening of the investigated OP and LP extracts for antimicrobial activity was carried out using the disc diffusion method, which is normally used as a preliminary check in order to select between activity against the following microorganism strains: The gram-positive bacteria [*Staphylococcus aureus* (*S. aureus*) ATCC 12600, *Bacillus subtilis* (*S. subtilis*) ATCC 6051 *Listeria monocytogenes* (*L. monocytogenes*) ATCC 19115] and the gram-negative bacteria [*Pseudomonas aeruginosa* (*P. aeruginosa)* ATCC 10145, *Salmonella typhi (S. typhi)* ATCC 14028, *Escherichia coli* (*E. coli*) ATCC 11775]. The antibacterial activity of the tested extracts was measured by the inhibition zones produced. The diameter in mm of the clear zone indicated the inhibition activity.

Antibacterial assay

Antibacterial activity of the tested samples (orange or lemon peel extracts) was determined using a modified Kirby-Bauer disc diffusion method (Bauer *et al.*, 1996). One hundred µL of the tested bacteria was grown in 10 mL of fresh media Mueller-Hinton Agar medium, until they reached counts of approximately10^8 cells / mL for bacteria (NCCLS, 2002). Then, 100 µL of the antibacterial suspension was spread onto agar plates (Muller-Hinton Agar) using a sterile glass spreader, inoculated plates were inverted and incubated at 28-31°C for 30 min. Blank paper of antibacterial susceptibility disks (8mm blank, Schleicher and Schuell, Spain), that soaked with 10 µL of OP and LP extract were placed on the surface of the previously agar plates inoculated with the different tested bacteria.

Individual samples were examined in triplicate against each bacteria strain, together with a negative control (disks with 10 µL of ethanol 70% or methanol 80%), and positive control (discs with Ampicillin as antibacterial agent). Samples were incubated at 35-37°C for 24-48h then the diameters in millimeter (mm) of the inhibition zones (size of the halo formed) were measured.

Determination of the minimum inhibitory concentration (MIC)

MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Agar dilution method was adopted to find minimum inhibitory concentrations (MICs) of active extracts.

Stationary–phase cultures of tested bacterial spp. (*Staphylococcus spp* or *Bacillus. spp.*) were prepared at 37°C and used to inoculate fresh 5.0 ml culture to an OD_{600} of 0.05. The 5.0 ml cultures were then incubated at 37°C until an OD_{600} of 0.10 was achieved from which standardized bacterial suspensions were prepared to a final cell density of $6x10^5$ CFU/ml. Serial dilutions from the tested compounds samples (0 -320 µl/ml) were prepared and mixed with 5.0 ml of the standardized bacterial suspension then added to the plates and incubated for 24h at 37°C. The colony forming units (CFU) were counted for each dilution and compared to the growth of untreated controls (NCCLS: M7–A4, 1997). The smallest concentration of the plant extract that was able to kill the microorganisms was considered as the minimum inhibitory concentration (MIC).

Determination of total phenolics content

The Folin–Ciocalteu assay, adapted from (Singleton and Rossi, 1965) was used for the determination of total phenolics present in the tested citrus peel extracts. Total phenolics content were calculated with respect to Gallic acid standard curve (concentration range: 0–12µgmL^{-1}). Results were expressed in µg of Gallic acid g^{-1} fresh weight of plant material and were calculated by using the following equation:

$y = 0.0047\ x$

Where: y = Dependant factor, x = Independent factor (absorbance of sample).

Antioxidant activity determinations

Radical Scavenging Activity (DPPH)

The effect of used (OP and LP) citrus peels extracts on 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was estimated in order to assess the antioxidant capacity according to the procedure described by (Yi *et al.*, 2008) with some modifications. The reaction mixture was incubated for 30 min in darkness

at room temperature. The absorbance of the resulting solution was measured at 517 nm using spectrophotometer (T80 UV/ Visible - PG instrument Ltd - Made in Germany). For the control, the assay was conducted in the same manner but ethanol was used instead of sample solution. DPPH scavenging capacity of the tested samples was measured as a decrease in the absorbance and was calculated by using the following equation:

Scavenging activity (%) = Ac− As / Ac X100

Where Ac and As are the absorbance's at 517 nm of the control and sample, respectively.

Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay (Benzie and Strain, 1996) was based on the ability of phenolic to reduce Fe^{3+} to Fe^{2+}. The absorbance was measured at 593 nm using a micro-plate spectrophotometer.

This analysis was performed in triplicate, using an aqueous Trolox solution as standard and the results were expressed as µ moles Trolox equivalents/100 g of fresh weight sample (OP or LP).

Hydroxyl radical (OH) scavenging activity

Hydroxyl radical scavenging activity of the tested citrus peels extracts was determined according to the method described by Halliwell *et al.*, (1987). The percentage of hydroxyl radical scavenging activity was calculated as follows:

Scavenging activity (%) = A_b - (A_s – A_{sb}) /A_b X 100

Where A_b, A_s and A_{sb} are the absorbance's at 532nm of the blank, extract, and sample blank, respectively.

Statistical analysis

All the measurements were performed in triplicate and the data are presented as mean ± SD. The obtained data were subjected to analysis of variance (ANOVA) according to PC-STAT, Version I A Copyright 1985, the university of Georgia, USA.

Results and Discussion

Antibacterial activity

Data in Table 1 and figure 1 revealed that *C. aurantiifolia* peels (LP) extract showed the highest antibacterial activities, as ethanolic extract of dried peels by microwave inhibited most of the bacteria: *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Escherichia coli* either Gram +ve or –ve, revealed inhibition zone ranging from 0-16 mm including diameter of disc paper, which 0.8 mm that showed (16, 13, 15, 16, 11, 16 mm respectively). Meanwhile, methanolic extracts displayed (11, N.I, 14, 11, N.I, 10 mm respectively) compared to the antibacterial agent (Ampicillin) and control (fresh) sample. The methanolic extract of (LP) dried with air oven showed low antibacterial activity which inhibited four bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtili* and *Escherichia coli*) while, the ethanol extract was higher than that of methanol in the inhibition of six bacteria whether Gram +ve or -ve compared to the antibiotic Ampicillin and control. Al-Ani *et al.*, (2009) illustrated that good bacterial inhibition was detected by *C. limon* especially against *S. aureus, P. aeruginosa and P. vulgaris*.

The antibacterial activity of the two extracts of *C. sinensis* orange peels is shown in terms of inhibition zone (Table 2 and Fig. 2). Fresh raw material as control did not demonstrate

any inhibition against all the tested bacteria strains except *Bacillus subtilis* in ethanolic and *Pseudomonas aeruginosa* in methanolic extracts. All extracts failed to inhibit growth of *Salmonella typhi*.

Dried orange peel by microwave extracted with ethanol inhibited most of the bacteria (*Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Listeria monocytogenes*) compared to methanolic extract which inhibited (*Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*). Only the difference was in the zone size which was less in case of using air oven drying in the two solvent extracts, while the methanolic was higher than that ethanolic extract in inhibition comparable to the standard antibiotic (Ampicillin).

The reason for the different sensitivity of the Gram-ve compared to that of Gram +ve bacteria could be due to differences in their cell wall composition. Gram-positive bacteria contain an outer peptide-glycan layer, which is an effective permeability barrier; whereas Gram-negative bacteria have an outer phospholipidic membrane (Samarakoon *et al.*, 2012). In the present study the growth of *P. aeruginosa* was strongly inhibited by lemon extracts. Such results are very interesting and with expectation, increasing the extracts concentration may produce more inhibition to this bacterium. This may inhibit bacteria by a different mechanism than that of currently used antibiotics and may have therapeutic value as an antibacterial agent against multi-drug resistant bacterial strains.

Dhanavade *et al.*, (2011) recommended that different alcoholic extracts of lemon peel gave activity against different bacterial especially *P. aeruginosa and S. typhimurium* better than the aqueous extract.

Pandey *et al.*, (2011) evaluated the antimicrobial activity of different solvent extracts (ethanolic, methanolic, ethyl acetate or hot water) of lemon fruit parts (peels, seeds, juice) against some bacteria e.g. *S. aureus, P. aeruginosa, E. coli*. The results of antimicrobial susceptibility assay showed promising evidence for the antimicrobial effects of the studied lemon fruit parts.

Table.1 Antibacterial activity of lemon peel extracts against bacteria measured in (mm)

Inhibition zone diameter (mm / Sample)						
Strains	*B. subtilis*	*E. coli*	*P. aeruginosa*	*S. typhi*	*S. aureus*	*L. monocytogenes*
Samples						
Ampicillin	17	23	22	20	18	18
3E	15	16	16	11	16	13
4E	15	16	16	14	15	10
5E	14R	10	10	N.I	10	N.I
3M	14	10	11	N.I	11	N.I
4M	13	10	10	N.I	12	N.I
5M	10	9	9	N.I	11	M.I

3E=(Lemon/Microwavedrying/Ethanol);4E=(Lemon/Ovendrying/Ethanol);5E=(Lemon/Fresh/Ethanol**).**
3M=(Lemon/Microwave drying/Methanol); M=(Lemon/
Oven drying/Methanol); 5M= (Lemon /fresh /Methanol). NI= No inhibition; *Bacillus subtilis (B. subtilis); Escherichia coli (E. coli); Pseudomonas aeruginosa (P. aeruginosa); Salmonella typhi (S. typhi); Staphylococcus aureus (S. aureus)*; Listeria monocytogenes *(L. monocytogenes*); R= Repellent (no complete inhibition).

Table.2 Antibacterial activity of orange peel extracts against bacteria measured (mm)

Inhibition zone diameter (mm / Sample)						
Strains	*B. subtilis*	*E. coli*	*P. aeruginosa*	*S. typhi*	*S. aureus*	*L. monocytogenes*
Samples						
Ampicillin	17	23	22	20	18	18
6E	15R	14R	9	N.I	N.I	9R
1E	15R	N.I	9	N.I	N.I	N.I
2E	13R	N.I	N.I	N.I	N.I	N.I
1M	9	9	9	N.I	N.I	N.I
2M	9	9	9	N.I	10	N.I
6M	10	N.I	9	N.I	N.I	N.I

6E=(Orange/Microwave drying/Ethanol); 1E=(Orange/Oven/Ethanol); 2E =(Orange/Fresh/Ethanol); 1M=(Orange /Microwave/Methanol); 2M=(Orange /Oven/Methanol); 6M(Orange /Fresh/Methanol); R, Repellent (no complete inhibition); NI= No inhibition; *Staphylococcus aureus* (*S. aureus*); *Listeria monocytogenes* (L. *monocytogenes)*; *Bacillus subtilis* (*S. subtilis*); *Pseudomonas aeruginosa* (*P. aeruginosa); Salmonella typhi (S. typhi*); *Escherichia coli* (*E. coli*). mm= millimeter.

Table.3 Minimum inhibitory concentration (µl/ ml) of ethanolic dried orange and lemon peels by microwave

Microorganisms	*Bacillus subtilis*	*Staphylococcus aureus*
Samples	**MIC (µl/ ml)**	
Orange Peel	108 (10.8mg/ml)	----------
Lemon Peel	----------	38 (3.8mg/ml)

Table.4 Total phenolic content (mg Gallic acid/100g sample) of dried citrus peels extracted by methanol or ethanol (db)

Peel Sample	Extract Solvent	Control(Fresh)	Microwave Drying	Air oven Drying
Orange Peel	Methanol	2619.39 ± 12.72^{aB}	1535.94 ± 1.61^{bC}	1410.73 ± 5.91^{bB}
	Ethanol	5255.02 ± 24.04^{aA}	3026.34 ± 6.26^{bA}	2453.75 ± 9.72^{cA}
Lemon Peel	Methanol	1353.88 ± 2.54^{aC}	3026.34 ± 6.26^{bA}	2453.75 ± 9.72^{cA}
	Ethanol	3251.53 ± 76.67^{aB}	2632.81 ± 7.09^{bB}	2504.4 ± 7.26^{cA}

db= dry weight basis. Results are presented as means for triplicate analyses± standard deviation (SD). Means within row and column with different letters are significantly different ($P \leq 0.05$)

Table.5 Radical scavenging activities % (DPPH) of dried citrus peel extracted by methanol or ethanol (db)

Peel Sample	Extract Solvent	Control (fresh)	Microwave-Drying	Air oven-drying
Orange	Methanol	99.79 ± 0.95^{aA}	69.83 ± 0.04^{bA}	56.29 ± 0.30^{cA}
	Ethanol	98.76 ± 0.36^{aA}	68.85 ± 0.25^{bA}	53.83 ± 0.04^{cAB}
Lemon	Methanol	79.37 ± 0.25^{aB}	56.69 ± 0.02^{bB}	50.93 ± 0.01^{cC}
	Ethanol	65.56 ± 0.59^{aC}	56.01 ± 0.11^{bB}	52.64 ± 0.03^{bBC}

db = dry weight basis. Results are presented as means for triplicate analyses ± standard deviation (SD). Means within row and column with different letters are significantly different ($P \leq 0.05$)

Table.6 Ferric reducing power activities (FRAP) µM Trolox eq/100g db of citrus peel extracted by methanol or ethanol

Peel Sample	Extract solvent	Control(Fresh)	Microwave-Drying	Airoven-Drying
Orange	Methanol	1654.22 ±1.9^{aB}	760.53 ±1.43^{bA}	730.17 ±0.7^{cA}
	Ethanol	1540.13±3.11^{aD}	722.86 ±1.68^{bB}	661.59 ±1.1^{cB}
Lemon	Methanol	1674.78±3.69^{aA}	590.48 ±1.17^{bC}	550.50±1.62^{bC}
	Ethanol	1574.33 ±3.24^{aC}	552.05 ±1.35^{bD}	532.52 ±0.78^{bD}

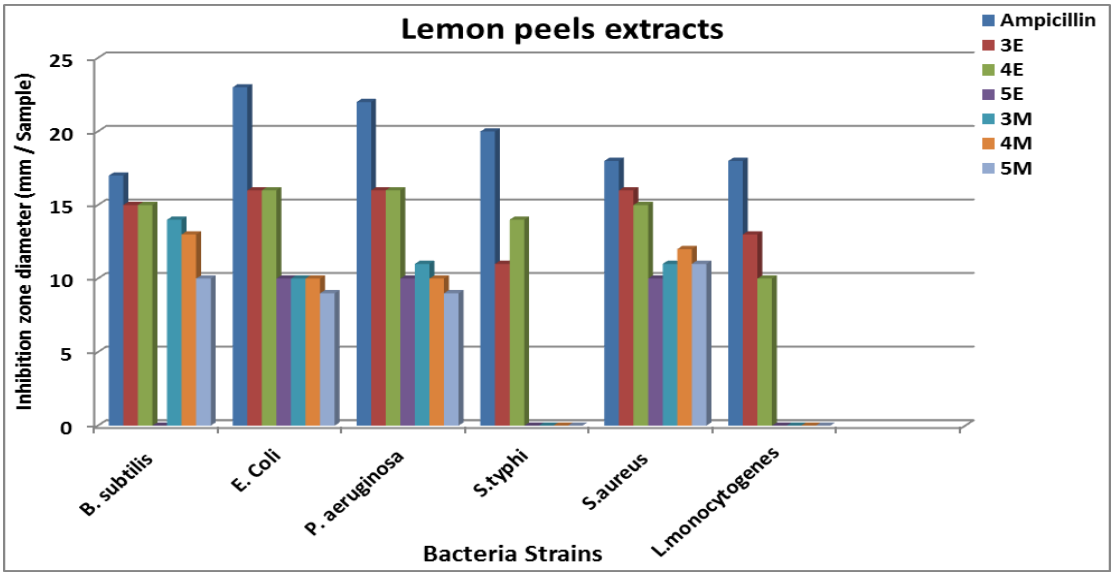
db= dry weight basis. Results are presented as means for triplicate analyses ± standard deviation (SD). Means within row and column with different letters are significantly different ($P \leq 0.0$)

Table.7 Hydroxyl radical scavenging activities (OH) of citrus peel extracted with methanol or ethanol

Peel Sample	Extract Solvent	Control(Fresh)	Microwave-drying	Airoven-Drying
Orange	**Methanol**	95.00±1.24^{aB}	93.13±1.13^{aA}	89.07±1.84^{bA}
	Ethanol	96.99±1.20^{aA}	90.57±0.43^{bB}	87.3±0.32^{cB}
Lemon	**Methanol**	70.11±0.61^{aC}	66.85±0.68^{bC}	59.62±1.08^{cC}
	Ethanol	61.9±0.63^{aD}	60.13±0.70^{bD}	55.09±1.17^{cD}

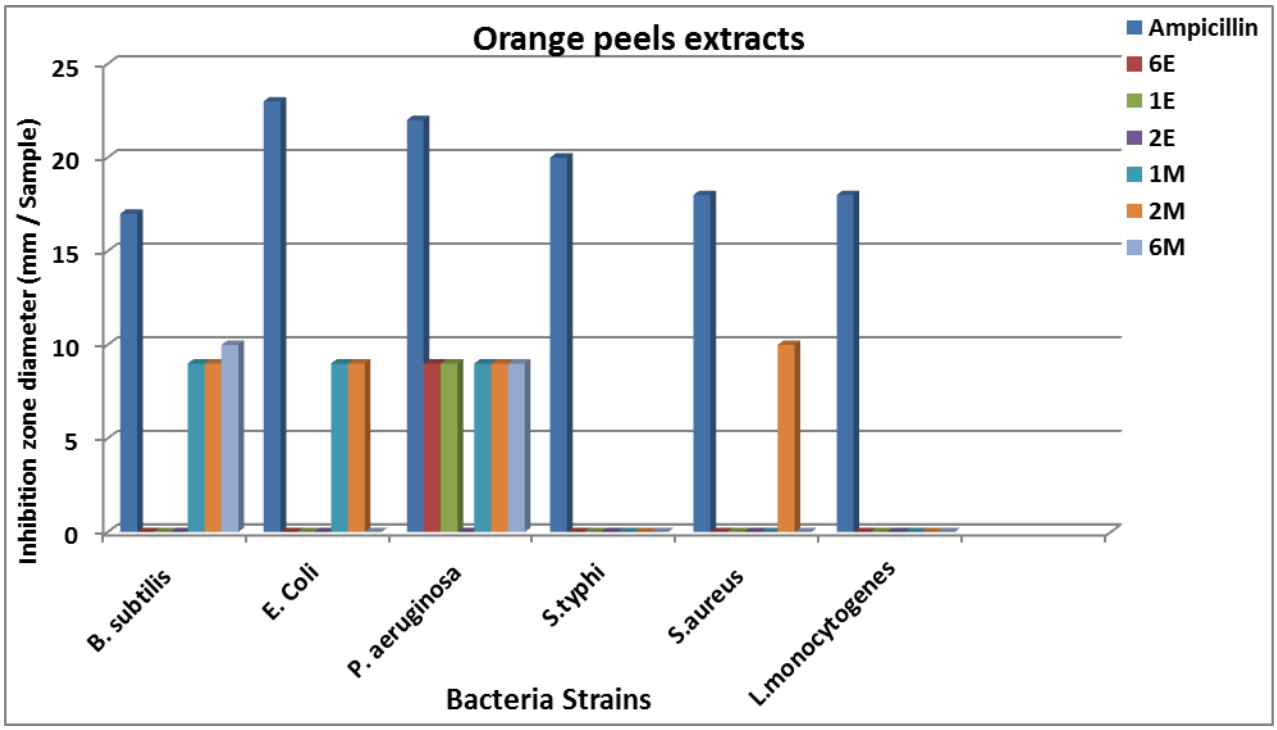
db= dry weight basis. Results are presented as means for triplicate analyses ± standard deviation (SD). Means within row and column with different letters are significantly different ($P \leq 0.0$)

Fig.1 Antibacterial activity of lemon peel extracts



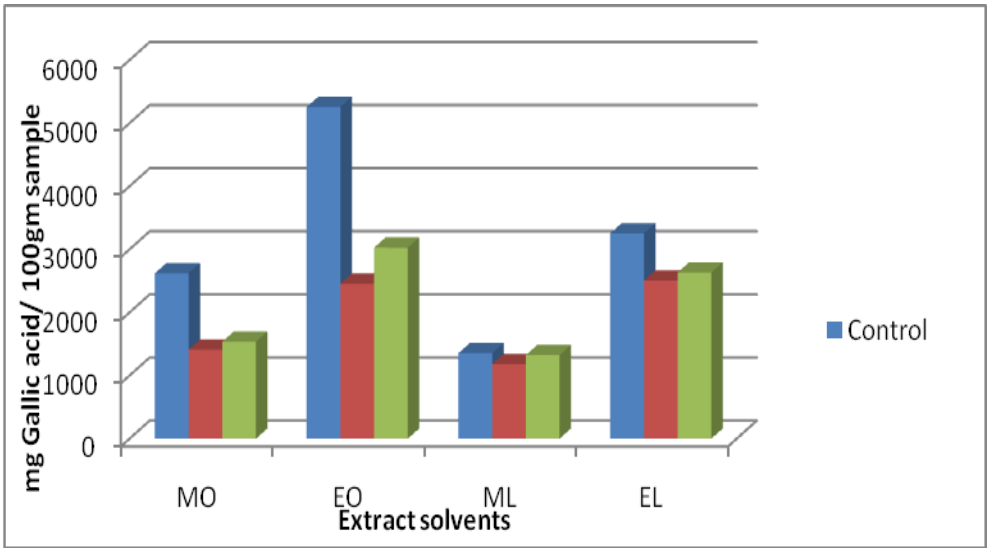
3E=(Lemon/Microwavedrying/Ethanol); 4E=(Lemon/Ovendrying/Ethanol); 5E=(Lemon/Fresh/Ethanol). 3M=(Lemon/Microwave drying/Methanol); 4M=(Lemon/Oven drying/Methanol); 5M= (Lemon /fresh / Methanol). NI= No inhibition; *Bacillus subtilis* (*B. subtilis*); *Escherichia coli* (*E. coli*); *Pseudomonas aeruginosa* (*P. aeruginosa); Salmonella typhi* (*S. typhi*); *Staphylococcus aureus* (*S. aureus*); *Listeria monocytogenes* (L. *monocytogenes)*; R= Repellent (no complete inhibition).

Fig.2 Antibacterial activity of orange peel extract



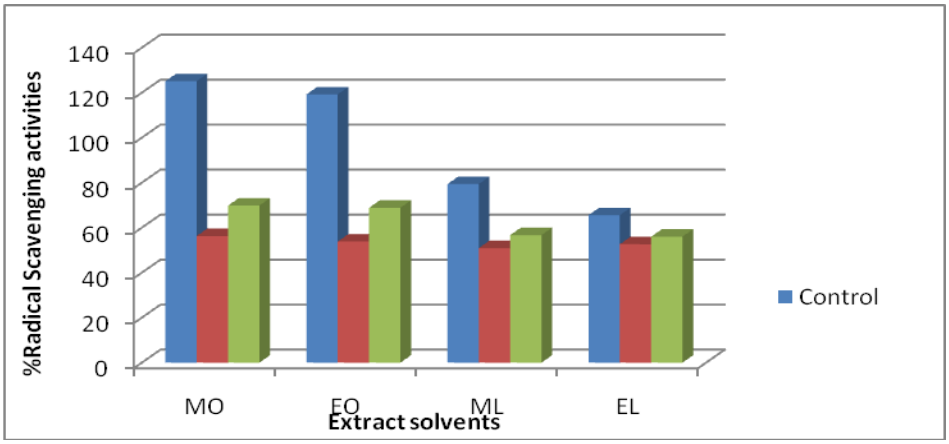
6E=(Orange/Microwave drying/Ethanol); 1E=(Orange/Oven/Ethanol); 2E =(Orange/Fresh/Ethanol); 1M=(Orange /Microwave/Methanol); 2M=(Orange /Oven/Methanol); 6M(Orange /Fresh/Methanol); R, Repellent (no complete inhibition); NI= No inhibition; *Staphylococcus aureus* (*S. aureus*); *Listeria monocytogenes* (L. *monocytogenes)*; *Bacillus subtilis* (*S. subtilis*); *Pseudomonas aeruginosa* (*P. aeruginosa); Salmonella typhi* (*S. typhi*); *Escherichia coli* (*E. coli*), mm= millimeter.

Fig.3 Total phenolic content (mg Gallic acid/100g sample) of citrus peel samples extracted by methanol or ethanol



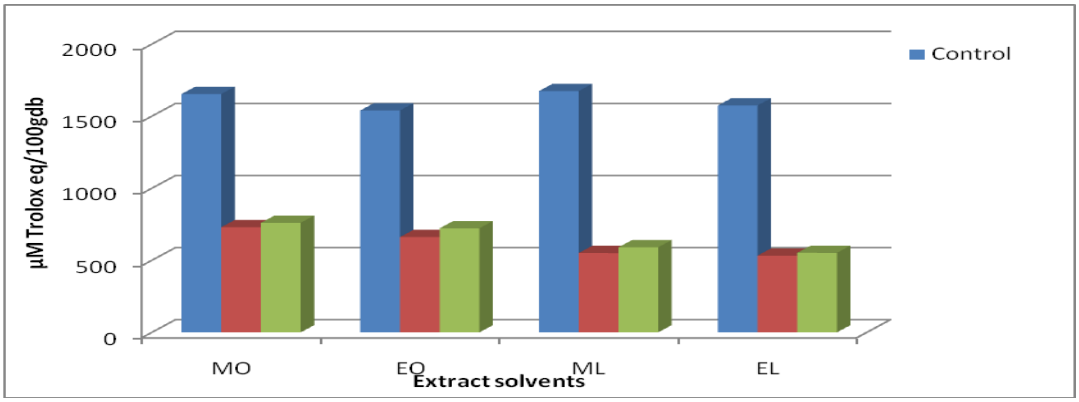
MO = Methanolic extract of orange peel; EO= Ethanolic extract of orange peel;
ML = Methanolic extract of lemon peel; EL= Ethanolic extract of lemon peel

Fig.4 Radical scavenging activities % of dried citrus peel



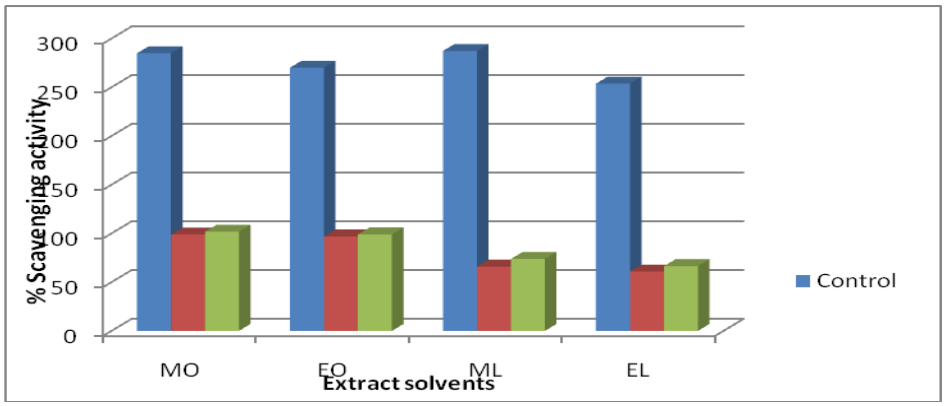
MO= Methanolic extract of orange peel; EO, Ethanolic extract of orange peel;
ML= Methanolic extract of lemon peel; EL, Ethanolic extract of lemon peel

Fig.5 Effect of drying methods on ferric reducing power activities (FRAP) of citrus peel



MO= Methanolic extract of orange peel; EO= Ethanolic extract of orange peel;
ML= Methanolic extract of lemon peel; EL=Ethanolic extract of lemon peel

Fig.6 Effect of drying methods on hydroxyl radical scavenging activities of citrus peel



MO= Methanolic extract of orange peel; EO= Ethanolic extract of orange peel;
ML= Methanolic extract of lemon peel; EL=Ethanolic extract of lemon peel

The extract in ethanol solvent was found by Abirami *et al.,* (2013) to have higher antimicrobial activity against tested microorganisms in comparison with methanol and acetone. The study revealed that the peel of lemon is a good antimicrobial agent.

Minimum inhibitory concentration (MIC)

The MIC concentration is reported in Table 3. MIC data for *B. subtilis* in orange peel was (108-10.8mg/ml) and for *S. aureus* in lemon peel was (38-3.8mg/ml). These results are supported by Pandey *et al.,* (2011) who found that ethanolic extract of lemon peels was subjected to get the MIC against *S. aureus* which was found to be 2.4 mg/ml. Abirami *et al.,* (2013) showed that the MIC (concentration) ranged between12.5 mg/mL and 200 mg/mL depending on microorganism and various extracts. The result of MIC suggested that methanolic extract of citrus fruit could possibly act as a bactericidal agent to the microorganisms (*S. aureus, K. pneumoniae, P. aeruginosa, S. typhi* and *E. coli*).

Total phenolic content

Data in Table 4 and Figure 3 illustrated that total phenolics (TPC) amount varied greatly and ranged in fresh to orange peel dried samples extracted with ethanol or methanol from 5255.02 ±24.04 to 1410.73 ±5.91 mg Gallic acid/ 100gm sample dry weight. The total phenolics content of orange peel extracted with ethanol was significantly higher ($p< 0.05$) than in methanol extract. No significant differences ($p> 0.05$) were observed in the phenolic levels of the two dried orange peels extracted with methanol. An opposite pattern was observed in dried orange peels extracted with ethanol compared to control samples. Meanwhile, ethanol extract exhibited higher phenolic content than lemon peel extracted with methanol and dried by microwave. On the contrary, a significant difference was found between TPC of air dried lemon peel and microwave dried samples. Additionally, a presence of significant differences in the TPC was noticed between all lemon peels extracted with ethanol. The noticed differences may be related to nature and characteristics of citrus fruit varieties. The differences in the values of TPC for various citrus peels types may be affected by environmental conditions, the degree of fruit ripening and genetic factors (Ladaniya, 2008). TPC of fresh peels are higher than the recovery from dried samples because the water in fresh plant cells can help phenols extraction. The reduction of phenolic compounds recovered from dried peels may be due to water evaporation and components in the cells (e.g., membranes and organelles) may hold together in the water absence and probably the extraction with solvent become more difficult. Moreover, if the citrus peel is dried before extraction, the recovery is much lower than using the fresh materials (*Li et al.,* 2006). The increase in drying temperature leads to a decrease in total polyphenols content after re-dissolution (Maria *et al.,* 2013). Worthy to note, that extraction of polyphenols from plant material is affected by the solubility of the polyphenols in the extraction solvent. Furthermore, solvent polarity plays a key role in increasing the extract contents (Naczk and Shahidi, 2006).

Radical scavenging activities (DPPH)

DPPH is a stable organic free radical with an absorption band around 515-528 nm which usually used as a reagent to measure free radical scavenging activity of antioxidants (Molyneux, 2004 and Yi *et al.,* 2008) (Fig. 4).

Table 5 revealed that antioxidant activity determination by DPPH in fresh orange peel extracted with methanol or ethanol, were 99.75 ±0.95 and 98.76 ±0.36 % respectively. The DPPH % activity of the microwave dried orange peel extracted with methanol or

ethanol were higher than air oven dried orange peel extracts. No significant differences were found between methanolic and ethanolic orange peel extracts results. Concerning lemon peel samples dried by air oven and extracted with methanol, the DPPH % was found lower than of lemon peel dried by microwave and fresh lemon peel samples as they realized 50.93 ±0.01, 56.69 ±0.02, and 79.37 ±0.25 % respectively.

Regarding ethanol extract, the microwave dried lemon peel was higher than the dried air oven peels and of lower % of fresh lemon peel. Noticeably, there are significant differences in the results of dried air oven lemon peel samples in cases of the two used extract solvents. Methanol and ethanol are the highest polar amongst the solvents. Therefore, they include high yield of phenolic compounds and highest antioxidant activity (% DPPH scavenging activity) if compared to other solvents extracts (Hegazy and Ibrahium, 2012).

Ferric ions reducing antioxidant power assay (FRAP)

FRAP assay is usually used to investigate the antioxidant capacity of plant. As shown from Table 6 and Figure 5, citrus peels had have effective and powerful reducing power when using the FRAP method and compared to the standard (Trolox). Reducing powers of tested samples were exhibited in the following order: orange peel dried microwave > orange peel dried air oven > lemon peel dried microwave > lemon peel dried air oven compared to control. These results demonstrated the electron donor properties of tested samples thereby neutralizing free radicals by forming stable products. Ramful *et al.,* (2010) reported extracts of orange were characterized by ferric reducing antioxidant power values in range (37.6 to 56.7 µmol/g FW) greater than extract of lemon (26.7 and 21.2 µmol/g FW).

Hydroxyl radical (OH) scavenging activity

Hydroxyl radicals are high reactive-oxygen species capable to attack most biological substrates, e.g. carbohydrates, DNA, polyunsaturated fatty acids, and proteins. The prevention of such harmful reactions is highly significant in terms of both human health and the shelf-life of foodstuffs, cosmetics, and pharmaceuticals.

As shown from Table 7 and Figure 6, citrus peel had scavenging hydroxyl radical using the hydroxyl radical ($OH^{\bullet}$) method. Methanolic or ethanolic extract of lemon peel dried by either microwave or air (hot) oven was lower than orange peel extracts compared to control. Fresh lemon peels was lower than either methanolic or ethanolic extracts orange peels.

Hydroxyl radical is the most unstable and reactive and showed a great oxidative power, combining rapidly with almost all molecules in its surrounding area (Sousa *et al.*, 2009).

As the most reactive oxygen species, hydroxyl radical can cause several biological consequences, including mutation, cell death, and carcinogenesis and ageing (Ragu *et al.,* 2007). Therefore the consumption of food with the ability of scavenging this radical could help to control its harmful effects (Vale *et al.*, 2014).

As a general conclusion, this study indicated that orange and lemon peels dried by microwave or air oven and extracted by methanol or ethanol have high natural phenolic contents with antimicrobial and antioxidant activities which could be recommended as useful natural value added functional ingredients, can be applied for improving and developing functional food products.

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How to cite this article:

Ibrahim M. Hassan, Hayam M. Ibrahim, Abdel Fattah A. Abdel Fattah and Ahmed, A.M.Hamed. 2017. *Citrus sinensis* and *Citrus aurantiifolia* Peel Extracts: Antibacterial, Antioxidant Activity and Total phenolic. *Int.J.Curr.Microbiol.App.Sci.* 6(12): 3983-3998.
doi: https://doi.org/10.20546/ijcmas.2017.612.459