ISSN 2377-8350

ADVANCES IN
FOOD TECHNOLOGY AND NUTRITIONAL SCIENCES
OpenJournal

Openventio
PUBLISHERS

*Original Research*

# Chemical Composition of Fruit Peels Essential Oil from Citrus Species and their Antimicrobial Efficacy as Biopreservatives

**Charu Gupta, PhD***

*Amity Institute for Herbal Research and Studies, Amity University-Uttar Pradesh, Sector-125, Noida 201313, UP, India*

***Corresponding author***
**Charu Gupta, PhD**
Professor, Amity Institute for Herbal Research and Studies, Amity University - Uttar Pradesh, Sector-125, Noida 201313, UP, India; E-mail: cgupta@amity.edu

***Article information***

**Received:** November 15th, 2022; **Revised:** November 25th, 2022; **Accepted:** December 23rd, 2022; **Published:** December 29th, 2022

***Cite this article***

Gupta C. Chemical composition of fruit peels essential oil from citrus species and their antimicrobial efficacy as biopreservatives. *Adv Food Technol Nutr Sci Open J.* 2022; 8(1): 29-35. doi: 10.17140/AFTNSOJ-8-177

## ABSTRACT

**Aim**

Extraction, chemical determination and comparison of essential oil (EO) composition from the peel oil of three varieties of citrus, sweet lime (*Citrus limetta*, *Meetha Nimbu*), lemon (*C. limon*) and acid lime (*C. aurantifolia*, *Kagzi Nimbu*) and their antimicrobial efficacy as preservative in food spoilage was studied.

**Material and Methods**

The extraction from all the three types lemon peel oil was done using clevenger apparatus and the chemical constituents of lemon peel EO analyzed by GC-MS. The antagonistic activity of lemon peel EO and 50% methanolic extract from peel residue left after EO extraction was studied against common food borne pathogens like *Staphylococcus aureus*, *S. epidermidis*, *Bacillus cereus*, *B. subtilis*, *Bacillus sp.*, *Listeria monocytogenes*, *Micrococcus luteus*, *Escherichia coli*, *Klebsiella sp.* and *Pseudomonas aeruginosa* by agar well diffusion assay. The test fungal species were *Rhizopus sp.*, *Rhizomucor sp.*, *Alternaria sp.*, *Aspergillus fumigatus*, *A. niger*, *Aspergillus sp.* and *Penicillium sp.*

**Results**

The chemical constituents of lemon peel EO analyzed by GC-MS showed 22 constituents with limonene (39-92%), β-myrcene (0.08-2.57%), α-terpineol (0.3-7.3%), α- and β-pinene (0.2-25.4%) as major ingredients and 1-Octanol, cis-linalool oxide, sabinene as minor constituents. It was found that lemon peel oil exhibited wide range of antimicrobial activity against both groups of bacteria with highest inhibition of zone produced against *Bacillus mycoides* and *B. cereus* 34 mm and 28 mm respectively and against fungi *Rhizopus sp.* and *Aspergillus sp.* (34 mm and 28 mm respectively). In contrast to EO, the peel extract produced widest zone of inhibition against *Staphylococcus aureus* followed by *S. epidermidis* and *B. subtilis* (IZD of 15, 13 and 13 mm respectively) and in case of fungus, peel extract was most effective against *Alternaria sp.* and *Rhizopus sp.* (IZD 26 mm, 17 mm respectively).

**Conclusion**

These studies illustrated that antioxidant and antimicrobial activity of EO was much better than its lemon peel extract and has potential to be used as biopreservative in food spoilage.

**Keywords**

Citrus peel; Essential oil; Antimicrobial; Antioxidant; Food spoilage bacteria; Fungus.

## INTRODUCTION

The search for safe and novel antimicrobial agents as food bio-preservatives depend significantly on ethno-botanical data and pharmacological exploration. In order to explore potential bioactive constituents a wide variety of plant extracts, volatile oils and their constituents have been studied.[1] It has been emphasized that possible use of spices and essential oils as alternative for food preservation could be possible due to their synergistic antimicrobial effect. About 22 species of citrus are found in India besides nearly 15 exotic species introduced for experimental trials. Citrus juices are most common among fruit juices around the world and constitute a major portion of food industry.[2] In Indian traditional system of medicine, its juice is valued for curing fever, malaria and jaundice.[1] Citrus is native to tropical and subtropical areas in Southeast Asia have peculiar fragrance partly due to the presence of flavonoids and limonoids in peel. Citrus occupies a considerable importance in fruit juice industry and economy of the country. *Citrus limetta* (sweet lime, *mousambi*; family Rutaceae) fruit is commonly eaten fresh or made as juice which is rich source of vitamin

Openventio PUBLISHERS

C and instant energy.[3] *C. sinensis* (Lemon) peel is a waste produced in large quantities from various fruit processing industries. It is normally discarded and dumped in the environment that can create environmental apprehension.[4,5] One of the important products of citrus fruit peels is the essential oil (EO) which can play an important antimicrobial role in food preservation.[6] These oils are also considered as potential sources for the screening of anticancer, antimicrobial, antioxidant and free radical scavenging agents.[5] Phenols present in essential oils have been recognized as the bioactive components for the antimicrobial activity and are classified as generally recognized as safe (GRAS). Therefore, they could be used to prevent growth of many food-borne microorganisms responsible for spoilage in food industry. They are also believed to offer diverse health-promoting effects because of their free radical scavenging activities and promising potential in therapeutic applications.[4] Essential oils are gaining renewed attention due to their relatively higher safe status, wide acceptance by consumers and exploitation for potential multi-purpose functional use.

The peels of lemon and orange are good source of natural flavonoids and contain higher amount of total phenols compared to edible peeled fruit pulp.[7] The flavonoids from citrus fruit peel belong to flavones, flavanones, anthocyanidins and flavanols.[5,7] They have been reported for potential anti-oxidant, anti-cancer, anti-viral and anti-inflammatory activities. Citrus peel EO is one of the rich sources of bioactive compounds namely coumarins, flavonoids, carotenes, terpenes, linalool etc.[8] Sweet lime peel is also a source of flavonoids, pectin and EOs with strong desirable aroma and refreshing effect. They have been used as flavoring in foods, beverages, pharmaceuticals, fragrance, cosmetics and aromatherapy. They have wide spectrum of biological activities such as antimicrobial, antioxidant, anti-inflammatory and anxiolytic. The EOs from leaves and peel of *C. limetta* has been extensively studied and showed presence of varying amount of oil (0.2-2%) and D-limonene as most abundant terpene with antimicrobial properties. Several studies were performed on the composition of EOs from leaves and peel of *C. sinensis* and their biological activities.[9,10]

Present studies were undertaken for the extraction and comparison of EOs composition from peel of three varieties of citrus, sweet lime (*Citrus limetta*, *Meetha Nimbu*), lemon (*C. limon*) and Acid Lime (*C. aurantifolia*, *Kagzi Nimbu*); followed by assessment of antimicrobial activities in both EOs and 50% methanolic extract from residue left after EO extraction against ten bacterial strains (7 Gram-positive and 3 Gram-negative) and seven fungi known to cause food spoilage. Essential oil composition was determined by using gas chromatography–mass spectrometry (GC-MS), a key tool for identification of volatile secondary metabolites, and possible potential use as preservative, flavoring, beverages, pharmaceuticals, fragrance, cosmetics, nutraceutical and or functional foods.

## MATERIALS AND METHODS

### Materials and Essential Oil Extraction

All chemicals used were of analytical-reagent grade and obtained from E. Merck (Mumbai, India). The peel of different Citrus species was collected from local juice vendors of Delhi-National Capital Region (NCR) and fruit markets (India). The peels were sorted, cleaned and washed in sterile distilled water (5% aqueous-ethanol). The EO extraction was performed by hydro-distillation in a Clevenger apparatus for 6 h and residual water from EO was removed by anhydrous sodium sulphate.

### Extraction of Peel Extract

The residual peel samples of Citrus left after EO extraction were dried, coarsely grounded in a mixer grinder, weighed and extracted with 50% methanol-water (5 g/50 ml) for a period of 24h at room temperature with frequent shaking. The total mixture was then centrifuged at 3,500 g for 20 min and filtered.[11,12] The filtrate was concentrated under reduced pressure in a rotary vacuum evaporator to afford a semi-solid substance and stored at 4 ºC till further use. To study different bio-activities residue was weighed and reconstituted in dimethyl sulfoxide (DMSO) to get the required end concentration. Once the extracts are dissolved in pure DMSO, these are also sterilized and thus, a very costly and time-consuming step of membrane filtration sterilization was omitted.[13]

### Gas Chromatography–Mass Spectrometry Analysis of Essential Oils

Analysis of peel EO from three different species of Citrus (*C. limetta*, *Meetha Nimbu*), Lemon (*C. limon*) and *Kagzi Nimbu* (*C. aurantifolia*) was performed on Agilent 7890A GC system 5975 C inert XL EI/CI MSD with Triple Axis Detector. The GC was equipped with a capillary column DB-HP-5MS (30 m×0.25 mm), 0.25 µm film thickness. The carrier gas flow rate was 1 mL He/min, injected volume was 10 µl and spilt ratio was 1/20. The column temperature was held at 60 ºC, programming 4 ºC/min to 180 ºC followed by 10 ºC/min to 260 ºC, detector and evaporator temperature was 260 ºC. The components of EO were identified by comparing results of mass-spectra (m/z), retention time and peak area with standard samples in GC-MS and National Institute of Standards and Technology (NIST) library mass fragmentation pattern. The components identification is based on GC retentions, computer matching with National Building Specification (NBS) library.[14]

### Total Phenolic Content and Antioxidant Activity

The powdered residue 50 mg left after EO extraction was extracted with 50% MeOH: $H_2O$ (2×10 mL), overnight at room temperature for total phenolic content (TPC) analysis. The extractive was centrifuged at 6000 g for 15 min, filtered and maintained to 20 mL and TPC was estimated by the method of Ragazzi and Veronese.[15] In 1.0 mL extract, 1.0 mL of Folin's Reagent (1 N) and 2.0 mL of $Na_2CO_3$ (20%) were added subsequently. The test mixture was mixed properly, left at room temperature for 30 min and maintained to 25 mL with water. The absorbance was measured at $\lambda_{max}$ 725 nm and reported as gallic acid equivalent (GAE) and flavonoids as quercetin equivalent (QE) mg/g.

For the estimation of antioxidant activity (AOA) of lemon peel extract (PE) residue, 2.0 mg of β-carotene was dissolved

in 20 mL of $CHCl_3$ and its 3.0 mL was added to 40 mg of linoleic acid and 400 mg of Tween 40. After removing $CHCl_3$ under reduced pressure, 100 mL of oxygenated water was added and mixed properly to obtain a stable emulsion. 3.0 mL aliquots of emulsion were mixed with 40 µl of sample extract and incubated for 1 h at 50 °C. The absorption ($\lambda_{max}$ 470 nm) of test mixture was recorded at 0 min and after 60 min of incubation at 50 °C. The AOA was expressed as percent inhibition of auto-oxidation of β-carotene and linoleic acid relative to control after 60 min by the method of Emmons and Peterson.[16] Free radical scavenging activity of the extracts (1.0 mg/mL) was assayed by using 1, 1-diphenyl-2-picryl-hydrazil (DPPH) radical ($6\times10^{-5}$ m in MeOH) according to Yen et al.[17]

### Bacterial and Fungal Strains

Ten bacterial strains (7 Gram-positive and 3 Gram-negative), mostly food borne pathogens, were selected for the study. Gram positives bacteria were *Bacillus cereus*, *B. mycoides*, *B. subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, *S. epidermidis* and *Listeria monocytogenes* while Gram negative bacteria were *Escherichia coli*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa*. The fungal species used in the present study were *Alternaria sp*, *Aspergillus fumigatus*, *A. niger*, *Aspergillus sp*, *Penicillium sp*, *Rhizopus sp. and Rhizomucor sp.* The standard bacterial and fungal stock cultures were obtained from the culture collection of Amity University UP, India. The viability tests for each isolate were carried out by resuscitating the organism in nutrient agar medium and Sabouraud's dextrose agar (SDA) medium respectively. The stock on nutrient agar medium (Hi Media, Mumbai, India) and potato dextrose agar medium was incubated at 37 °C for 24 h (bacteria) and 28 °C for 3-days (fungi) following storage at 4 °C until required for sensitivity testing.

### Antibacterial Activity Testing Using Agar well-Method (Cup Plate Method)

The antimicrobial activity of PE and EO was determined by agar well-diffusion method.[2] Pure isolate of each bacterium was first sub cultured in nutrient broth at 37 °C for 24 h. Standardized inoculum (100 µL, 106 CFU/mL; 0.5 Mac-Farland) of each test bacterium was spread with the help of sterile spreader on to a sterile Muller-Hinton Agar plate to achieve a confluent growth. The plates were allowed to dry and a sterile cork-borer (6.0 mm diameter) was used to bore the wells in the agar. Subsequently, 50 µL of PE in DMSO and 50 µL of peel essential oil (PEO) was introduced in wells of separate agar plates in triplicate. Sterile DMSO and sodium benzoate (standard food preservative) were used as negative and positive control respectively for antibacterial and antifungal studies. The plates were allowed to stand for 1 h for diffusion and then incubated at 37 °C for 24 h. The inhibition zone diameter (IZD) was recorded to the nearest size in mm and all experiments were performed in triplicate.[2]

### Antifungal Assay

For antifungal activity of PE and PEO fungal isolates were sub-cultured on Sabouraud's Dextrose Agar (SDA) at 28 °C for 3-5-days. Sterilized SDA plates and sterile cork-borer (6-mm diameter) was used to bore wells in the agar. 50 µL of PE in DMSO and 50 µL of PEO was introduced in separate plates to each peripheral well while a fungal disc was inoculated in central well. A negative control (DMSO) was used in one peripheral well to compare the activity incubated at 28 °C. The evaluations were carried out by daily measurement of colony diameter, starting after 24 h and finishing when 2/3rd of the plate surface in control was covered by fungus. The appearance of zone of inhibition was regarded as antimicrobial activity in test sample and all experiments were performed in triplicate. The results were expressed in terms of the diameter of the inhibition zone: <9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active; >18 mm, very active.[18]

### Assessment of Minimum Inhibitory Concentration

The method of Thongson et al[19] as applied; minimum inhibitory concentration (MIC) for PE and PEO was determined by agar-well-diffusion method using a two-fold serial dilution of PE in DMSO to achieve a decreasing concentration range of 1000-31.25 mg/mL. A 50 µL of EO was added aseptically into Mueller Hinton agar plates already seeded with standardized inoculum (106 CFU/mL) of test bacteria. Sodium benzoate was used as positive control and all experiments were performed in triplicate. The same method was used for fungi, except SDA plates and incubated at 28 °C. The lowest concentration of PE and PEO showing a clear zone of inhibition was considered as the MIC.[19]

## RESULTS AND DISCUSSION

The EO composition of three varieties of Citrus- *Citrus limetta*, *C. limon* and *C. aurantifolia* determined by GC-MS (Table 1) showed limonene (80.4% in *C. limetta*; 42.6% in *C. limon* and 92.7% in *C. aurantifolia*) as major constituent followed by β-myrcene, α-terpineol, β-linalool and others in varying concentrations. In earlier reports both volatile (83-99.2%) and non-volatile components (0.5-15%) have been cited.[5] The citrus EO is mainly composed of about 97% monoterpenes with alcohols, aldehydes and esters being lowest ranging from 1.8-2.2%.[12,20] Kamal et al[20] could identify about 16-27, 17-24 and 18-40 chemical constituents in the peel EO of *C. reticulate*, *C. sinensis* and *C. paradisii* respectively and limonene ranged 64.1-71.1% (*C. reticulata*), 66.8-80.9% (*C. sinensis*) and 50.8-65.5% (*C. paradisii*). Twenty-one components were identified in the peel of Hatian Pummelo (*C. drandis Osbeck*), monoterpenes and sesquiterpene hydrocarbons were main components approximately 96.64% w/w of total oil. The limonene (89.96±1.64%) was observed to be most dominant as reported. Analysis of *C. pseudolimon* and *C. grandis* PEO also revealed the presence of about thirty-three total components with limonene of 47.07% and 71.48% respectively.[20]

The PE of residue left after EO extraction showed TPCs ranging from 12.5 to 28.7 mg GAE/g and flavonoids 12.6-17.9 mg QE/g extract. Antioxidant activity of extracts were estimated using β-carotene-linoleic bleaching assay and 1,1-diphenyl-2 picrylhydrazyl (DPPH). The free radical quenching antioxidant activity varied from 38.5-69.4% and inhibited the bleaching of β-carotene and DPPH comparable to the synthetic antioxidants (BHT and BHA). The BHA and BHT have been suspected to be responsible for liver damage and carcinogenesis. Citrus peel

**Table 1.** *Chemical Constituents (%) of Peel Essential Oil from Different Citrus limetta, C. limon and C. aurantifolia*

| Component | Sweet Lime (*C. limetta*) | Lemon (*C. limon*) | Acid Lime (*C. aurantifolia*) |
|---|---|---|---|
| Limonene | 80.41 | 42.63 | 92.75 |
| β-Myrcene | 0.07 | 0.38 | 2.64 |
| α-Terpineol | 0.58 | 6.80 | 0.47 |
| Terpinen-4-ol | 0.86 | 0.65 | 0.39 |
| α-Pinene | 1.38 | 0.87 | 0.98 |
| β-Pinene | 5.64 | 26.45 | 0.43 |
| 1,8-cineol | 1.86 | 1.53 | ND |
| cis-trans-linalool oxide | 0.67 | 1.46 | ND |
| β-Linalool | 5.39 | 2.46 | 2.74 |
| Nerol | 0.56 | 1.18 | ND |
| Linalyl acetate | 0.38 | 2.78 | 1.79 |
| Geranial | 0.47 | 0.59 | 0.19 |
| Acetate neryl | ND | 2.65 | ND |
| Acétate geranyl | ND | 4.15 | ND |
| Nerolidol | ND | 5.89 | ND |
| Farnesol | 0.76 | 4.28 | 1.06 |
| Neryl acetate | 0.78 | 1.26 | 0.67 |
| γ- terpinene | ND | 12.65 | ND |
| Isocaryophyllene | ND | 1.23 | 0.58 |
| Bergamot mint oil | ND | ND | 1.20 |
| Bergamol | 6.21 | ND | ND |
| β-Bisabolol | 2.28 | ND | ND |

*ND: Not determined*

is an important source of EO, particularly limonoids that have a variety of therapeutic effects, like antifungal, antibacterial, antiviral, antimalarial and antineoplastic. Total phenolic contents ranged from 1.058 to 7.3 g/100 g dw in orange and bitter orange peel respectively have been reported.[2] Citrus peel comprises high quantities of unique flavanone glycosides like hesperidin, neohesperidin, narirutin, naringin, lower amounts of poly-methoxy flavones (sinensetin, tangeretin, nobiletin) and traces of flavonols, glycosylated flavones and hydrocinnamic acid. These citrus peel phenols have potential for use in functional food, cosmetic and pharmaceuticals as alternative to synthetic antioxidants (BHT, BHA) to prevent deterioration and extended shelf life, beverages and bakery.[21] Phytochemical from peels of orange fruit (C. sinensis) also revealed the presence of tannins, flavonoids, polyterpenes, alkaloids, saponin, terpenoids and amino acids.[22]

The EO from citrus peel has promising potential for use in food or pharmaceuticals with effective antioxidant and antimicrobial control strategy. Antioxidant phytochemicals can scavenge free radicals and increase shelf life by retarding lipid peroxidation, a major reason for deterioration of food and pharmaceuticals during storage. Antioxidants can also protect human body from free radicals and associated chronic disorders.[23]

A total of ten bacterial species and seven fungal species were used to assess the antimicrobial activity of essential oils. The antimicrobial activities of EOs and PE were determined by agar well-diffusion method against seven Gram positive bacteria and three Gram negative bacteria (Table 2). The EOs were active against both Gram-positive and Gram-negative bacteria *except Listeria monocytogenes* isolated from the spoiled food products and were most effective against *Staphylococcus aureus* followed by *Staphylococcus epidermidis* and *Bacillus subtilis* with inhibition zone diameter (IZD) of 15.0 mm, 13.0 mm and 13.0 mm respectively. Sodium benzoate generally used as food preservatives was used as a positive control and was found less effective with IZD of 16.0, 14.0 and 13.0 mm respectively. In contrast, the EOs exhibited wide range of antimicrobial activity against both groups of bacteria. The maximum zone of inhibition was observed against *Bacillus mycoides* and *Bacillus cereus* producing 34.0 mm and 28.0 mm IZD respectively. Amongst the Gram-negative bacteria, PEOs showed nearly equivalent activity against all test bacteria *Escherichia coli*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa* with IZD of 12.0, 11.0 and 11.0 mm each respectively.

Antifungal effects of EOs have also been investigated and all the seven test fungi were isolated from spoiled food products (Table 3). Amongst fungus, the EOs exhibited antagonistic activity against almost all test fungi. It was most effective against fungi *Rhizopus sp.* and *Aspergillus sp.* with IZD of 34.0 mm and 28.0 mm respectively. In contrast, sodium benzoate which is used as a standard food preservative also inhibited all the test fungal species.

The MIC for the fungal species (Table 4) of EO ranged from 6.25-50 mg/mL. *Staphylococcus aureus* and *Micrococcus luteus*

**Table 2.** *Zone of Inhibition (mm) of Methanolic Extract of Peel and Essential Oil (EO) of Citrus limon on Selected Bacteria that Cause Food Spoilage*

| Bacterial species | Lemon (*C. limon*) Peel Extract | Lemon (*C. limon*) EO | Sodium benzoate (+ve C) | DMSO (-ve C) |
|---|---|---|---|---|
| *Bacillus cereus* | 12.0 | 28.0 | 14.0 | 0.0 |
| *Bacillus subtilis* | 13.0 | 14.0 | 13.0 | 0.0 |
| *Bacillus mycoides* | 12.0 | 34.0 | 22.0 | 0.0 |
| *Staphylococcus aureus* | 15.0 | 17.0 | 16.0 | 0.0 |
| *S. epidermidis* | 13.0 | 18.0 | 14.0 | 0.0 |
| *Listeria monocytogenes* | 9.0 | 14.0 | 13.0 | 0.0 |
| *Micrococcus luteus* | 12.0 | 22.0 | 18.0 | 0.0 |
| *Escherichia coli* | 11.0 | 12.0 | 11.0 | 0.0 |
| *Enterobacter aerogenes* | 11.0 | 11.0 | 11.0 | 0.0 |
| *Pseudomonas aeruginosa* | 10.0 | 11.0 | 12.0 | 0.0 |

**Table 3.** *Zone of Inhibition (mm) of Methanolic Extract of Lemon Peel and Essential Oil of Citrus limon Against Common Food Spoilage Fungi*

| Bacterial species | Lemon (*C. limon*) peel extract | Lemon (*C. limon*) EO | Sodium benzoate (+ve C) | DMSO (-ve C) |
|---|---|---|---|---|
| *Aspergillus niger* | 0.0 | 21.0 | 19.0 | 0.0 |
| *Aspergillus fumigates* | 0.0 | 26.0 | 23.0 | 0.0 |
| *Aspergillus sp.* | 9.0 | 28.0 | 24.0 | 0.0 |
| *Alternaria sp.* | 26.0 | 0.0 | 23.0 | 0.0 |
| *Rhizomucor sp.* | 0.0 | 23.0 | 21.0 | 0.0 |
| *Rhizopus sp.* | 17.0 | 34.0 | 19.0 | 0.0 |
| *Penicillium sp.* | 0.0 | 8.0 | 10.0 | 0.0 |

**Table 4.** *Minimum Inhibitory Concentration (MIC) Values of Lemon peel Essential Oil and Peel Extract (Citrus limon) Against Different Bacteria*

| Fungal species | Lemon (C. limon) peel extract | Lemon (C. limon) EO | Sodium benzoate (+ve C) | DMSO (-ve C) |
|---|---|---|---|---|
| *Aspergillus niger* | 0.0 | 21.0 | 19.0 | 0.0 |
| *Aspergillus fumigates* | 0.0 | 26.0 | 23.0 | 0.0 |
| *Aspergillus sp.* | 9.0 | 28.0 | 24.0 | 0.0 |
| *Alternaria sp.* | 26.0 | 0.0 | 23.0 | 0.0 |
| *Rhizomucor sp.* | 0.0 | 23.0 | 21.0 | 0.0 |
| *Rhizopus sp.* | 17.0 | 34.0 | 19.0 | 0.0 |
| *Penicillium sp.* | 0.0 | 8.0 | 10.0 | 0.0 |

was found to be highly sensitive to the exhibiting lowest MIC of 6.25 mg/mL followed by *Bacillus cereus* and *Bacillus mycoides* 12.5 mg/mL each. On the contrary, the MIC values for lemon PE were comparatively on the higher side and ranged between 12.5-50 mg/mL with *Staphylococcus aureus* and *Micrococcus luteus* exhibiting lowest MIC of 12.5 mg/mL (Table 4).

The observed antimicrobial activity may probably be due to combination of more than one constituent of essential oils that exhibit synergistic effect.[2,21] It was observed that although lemon peel is effective against both groups of bacteria but its activity was higher in Gram positive as compared to Gram-negative bacteria. These observations are in accordance with the earlier observations that Gram-negative organisms were less susceptible to the herbal extracts than Gram-positive isolates due to the presence of high lipid in cell walls of Gram-negative bacteria.[2] Gram-positive bacteria such as *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Bacillus subtilis* contains teichoic acid in the peptidoglycan layer and is therefore inhibited by both Citrus peel extracts and EO.[14] Furthermore, the outer membrane of Gram-negative bacteria is known to present barrier to penetration of numerous antibiotic molecules and the periplasmic space contains enzymes, which are capable of breaking down foreign molecules introduced from outside thus providing greater resistance to them.[2] The antimicrobial activity could also be further attributed due to the presence of high percentage of oxygenated compounds and sesquiterpenes including limonene, α-pinene, β-myrcene and caryophyllene. These constituents from EO had been previously proved to be cytotoxic in other essential oils. Other constituent *viz.* β-pinene, α- terpineol, γ-terpinene and trans α-bergamotene

may have also imparted synergistic effects along with limonene.[24]

## CONCLUSION

Processing of large amount of citrus peel a by-product potentially represents a rich source of essential oils, phenols and dietary fiber. These residues, which are generally discarded as waste in the environment, can act as potential antimicrobial and antioxidant agents. Besides, due to their low cost and easy availability such wastes can also offer significant low-cost nutritional high fiber dietary supplements. The utilization of these bioactive rich citrus residues can provide an efficient, inexpensive and eco-friendly source of food preserving agents against food spoilage bacteria and fungi. Further, focus on more pathogenic organisms, toxicological investigations and development of cost-effective process for isolation purification still needs high attention that can be rewarding to pursue in hunt for new herbal therapeutic agent.

## ACKNOWLEDGEMENTS

The author is grateful to Dr. Ashok K. Chauhan, Founder President and Dr. Atul Chauhan, Chancellor, Dr. Balvinder Shukla VC, Amity University UP, Noida, India for the encouragement, research facilities and SERB-DST, India for the financial support.

## INSTITUTIONAL REVIEW BOARD APPROVAL

Not applicable.

## REFERENCES

1. Gupta C, Garg A.P, Gupta S. Antimicrobial and Phytochemical studies of fresh ripe pulp and dried unripe pulp of Mangifera indica (Amchur). *Middle East Journal of Scientific Research*. 2010; 5: 75-80.

2. Bozkurt T, Gülnaz O, Kaçar YA. Chemical composition of the essential oils from some citrus species and evaluation of the antimicrobial activity. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*. 2017; 11: 1-8. doi: 10.9790/2402-1110032933

3. Espina L, Somolinos M, Lorán S, Conchello P, García D, Pagán R. Chemical composition of commercial Citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes. *Food Control*. 2010; 22: 896-902. doi: 10.1016/j.foodcont.2010.11.021

4. Rafiq S, Kaul R, Sofi S.A, Bashir N, Nazir F, Nayik GA. Citrus peel as a source of functional ingredient: A review. *Journal of the Saudi Society of Agricultural Sciences*. 2016; 17: 351-358. doi: 10.1016/j.jssas.2016.07.006

5. Gupta C, Prakash D, Nazareno MA. Essential oil of some Agri-horticulture wastes: Their composition and efficacy as Biocontrol Agent. *Ind. Perfumer J*. 2019; 63: 46-58.

6. Duffy CF, Power RF. Antioxidant and antimicrobial properties of some Chinese plant extracts. *Int J Antimicrob Agents*. 2001; 17(6): 527-529. doi: 10.1016/s0924-8579(01)00326-0

7. Sawalha SMS, Arráez-Román D, Segura-Carretero A, Fernández-Gutiérrez A. Quantification of main phenolic compounds in sweet and bitter orange peel using CE–MS/MS. *Food Chem*. 2009; 116: 567-574. doi: 10.1016/j.foodchem.2009.03.003

8. Fisher K, Phillips C. Potential antimicrobial uses of essential oils in food: is citrus the answer? *Trends in Food Science & Technology*. 2008; 19: 156-164. doi: 10.1016/j.tifs.2007.11.006

9. Hegazy AE, Ibrahium MI. Antioxidant activities of orange peel extracts. *World Applied Sciences Journal*. 2012; 18: 684-688. doi: 10.5829/idosi.wasj.2012.18.05.64179

10. O'Bryan CA, Pendleton SJ, Crandall PG, Ricke SC. Potential of plant essential oils and their components in animal agriculture – in vitro studies on antibacterial mode of action. *Front Vet Sci*. 2015; 2: 35. doi: 10.3389/fvets.2015.00035

11. Gupta C, Prakash D, Singh A, Singh N. Studies on the composition & potential as Biocontrol agent of Citrus limetta (Mousambi) fruit peel essential oil from Western UP. *India Perfumer J*. 2017; 61: 19-24.

12. Gupta S, Gupta C, Prakash D, Garg AP. Comparative study of antimicrobial effects of lemon oil and peel extract against food-spoilage microbes. *J Nutr Health Food Sci*. 2017; 5: 1-5. doi: 10.15226/jnhfs.2017.001110

13. Vasek OM, Cáceres LM, Chamorro ER, Velasco GA. Antibacterial activity of Citrus paradisi essential oil. *J Natural Products*. 2015; 8: 16-26.

14. Bhandari DP, Poudel DK, Satyal P, et al. Volatile compounds and antioxidant and antimicrobial activities of selected citrus essential oils originated from Nepal. *Molecules*. 2021; 26: 6683. doi: 10.3390/molecules26216683

15. Ragazzi E, Veronese G. Quantitative analysis of phenolic compounds after thin-layer chromatographic separation. *J Chromatogr*. 1973; 77: 369-375. doi: 10.1016/s0021-9673(00)92204-0

16. Emmons CL, Peterson DM. Antioxidant activity and phenolic contents of oats, groats and hulls. *Cereal Chem*. 1999; 76: 902-905. doi: 10.1094/CCHEM.1999.76.6.902

17. Yen GC, Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen. *J Agri Food Chem*. 1994; 42: 629-663. doi: 10.1021/jf00039a005

18. Velázquez-Nuñez M.J, Avila-Sosa R, Palou E, López-Malo A. Antifungal activity of orange (Citrus sinensis var. Valencia) peel essential oil applied by direct addition or vapor contact. *Food Control*. 2013; 31: 1-4. doi: 10.1016/j.foodcont.2012.09.029

19. Thongson C, Davidson P.M, Mahakarrchanakul W. Weiss J. Antimicrobial activity of ultrasound assisted solvent extracted

spices. *Lett Appl Microbiol.* 2004; 39: 401-406. doi: 10.1111/j.1472-765X.2004.01605.x

20. Kamal GM, Anwar F, Hussain AI, Sarri N, Ashraf MY. Yield and chemical composition of Citrus essential oils as affected by drying pretreatment of peels. *International Food Research Journal.* 2011; 18: 1275-1282.

21. Calo JR, Crandall PG, O'Bryan CA, Ricke SC. Essential oils as antimicrobials in food systems – A review. *Food Control.* 2015; 54: 111-119. doi: 10.1016/j.foodcont.2014.12.040

22. Hiri NM, Ioannou I, Ghoul M, Boudhrioua NM. Extraction methods of citrus peel phenolic compounds. *Food Reviews International.* 2014; 30: 265-290. doi: 10.1080/87559129.2014.924139

23. Prakash D, Upadhyay G, Gupta C. Total phenol and antioxidant activity of some fruits and their under-utilized parts. *International Food Research Journal.* 2013; 20: 1717-1724.

24. Fancello F, Petretto GL, Zara S, et al. Chemical characterization, antioxidant capacity and antimicrobial activity against food related microorganisms of Citrus limon var. pompia leaf essential oil. *LWT - Food Science and Technology.* 2016; 69: 579-585. doi: 10.1016/j.lwt.2016.02.018