

Research Article

Physicochemical, antioxidant and antimicrobial properties of citrus peel essential oils

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Abstract

Essential oils are produced as secondary metabolites of aromatic plants and can be extracted from leaves, seeds or fruit peel of the plants. Citrus peel is a rich source of limonene which is antimicrobial in nature. The present study aimed to deal with the physicochemical, antioxidant and antimicrobial properties of citrus peel essential oil from *Citrus reticulate* (mandarin orange), *Citrus limetta* (mosambi) and *Citrus limon* (lemon). The lemon peel oil had lower peroxide value (1.6 meq/kg of sample), sapon-ification value (112.2 mg KOH/g of oil) and higher iodine value (116 gl₂/100g oil) when compared with orange and mosambi peel essential oil. DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of citrus peel essential oil was measured and the result indicated that the total antioxidant activity of lemon peel oil was 89.2 % Radical Scavenging activity (RSA). The antifungal activity was assessed by agar dilution method, whereas the antibacterial property was assessed by the agar diffusion method. Lemon peel oil recorded better antimicrobial properties with minimum inhibitory concentration of 0.3% against *Aspergillus flavus MTCC 277*, 0.2% for *Penicillium MTCC 1995* and *Fusarium* oxysporum MTCC 284. Likewise, the minimum inhibitory concentration of lemon peel oil against *Staphylococcus aureus* MTCC 96 was 0.3%; *Salmonella enterica* MTCC 733 and *Erwinia* sp MTCC 2760 was 0.5%; *Pseudomonas aeruginosa* MTCC 1688 and *Escherichia coli* MTCC 443 was 0.7%. This comparative study showed that lemon peel oil had better physicochemical and antioxidant property. Lemon peel oil can be used as a preservative in the food system as it exhibited antibacterial and antifungal activity.

Keywords: Antimicrobial activity, Bacteria, Citrus fruits, Essential oil, Fungi, Lemon peel oil

INTRODUCTION

Citrus is one of the major horticulture crops produced and traded all over the world. In 2020, citrus fruit production in India was 14 million tonnes. Citrus fruit production has increased from 1.79 million tonnes during the year 1971 to 14 million tonnes in 2020 with an average annual rate of 5.16%. In the year 2021, the volume of mandarin produced was the highest among the citrus fruit production of about 6 million metric tons, followed by sweet oranges, commonly known as mosambi (3.69 million metric tons), lime/lemon (3.63 million metric tons) and others (0.69 million metric tons) (Statista, 2022).

Citrus fruits are important sources of vitamins and minerals and are protective foods with potential health promoting chemical compounds. Citrus fruits can be consumed as such or can be processed into juice. Pro-

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Article Info

https://doi.org/10.31018/ jans.v14i2.3484 Received: April 23, 2022 Revised: June 4, 2022 Accepted: June 10, 2022 cessing of citrus fruits generates huge amount of waste that includes peels, seeds and membrane residue. Around 54 billion tones of citrus peel waste is generated worldwide (Teigiserova *et al.* 2021). The citrus peel, which is the primary waste during processing are usually discarded. The citrus peel is rich in flavonoids and glycosides which has enormous health benefits such as anti-inflammatory, anti-carcinogenic and anti-microbial activity. Limonene, a biologically active compound responsible for antimicrobial property is present in the essential oil of the citrus peel which has potential application in food systems as natural preservative (Han *et al.* 2021).

Essential oils are natural extracts that contain volatile and complex compounds characterized by a highly stimulating odour. They are produced as secondary metabolites of aromatic plants and can be extracted from various parts of the plants, such as leaves, seeds or fruit peel. The annual worldwide production of citrus EO is approximately 16,000 tons (Singh et al. 2021).Essential oils are rich source of flavonoids, coumarins, limonoids, carotenoids, phenolic acid, and many polymethoxylated flavones (Bora et al., 2020). Citrus essential oil is mainly present in the flavado portion of the peel and lower the quantity of leaves, flowers, fruits, and seeds. The essential oil possesses germicidal, antioxidant, antibacterial and anticarcinogenic properties (Kademi & Garba, 2017 and Pathak et al., 2017).

Essential oils have several bioactive compounds responsible for their antimicrobial and antioxidant properties. It is effective against several foodborne pathogens such as *Aspergillus, Penicillium, Salmonella typhimurium, Staphylococcus aureus, Listeria monocytogenes,* Pseudomonas fluorescens, and others (Ju *et al.* 2019). The lipophilic fraction of the essential oil reacts with the lipid part of the cell membrane and thereby modifies the activity of the calcium ion channel. Bacterial proliferation by essential oil can be achieved by disintegeration of bacterial outer membrane, alteration in the fatty acid composition, increase in membrane fluidity resulting in leakage of potassium ions and protons, interference with glucose uptake and inhibition of enzyme activity or cell lysis (Angane *et al.* 2022)

The antioxidant activity of essential oil can preserve the food product from the toxic effects of oxidants. Essential oils can scavenge free radicals and play an important role in disease prevention such as brain dysfunction, cancer, heart disease and immune system decline that are caused by the cellular damage caused by free radicals (Miguel, 2010).

The present study deals with the physicochemical, antioxidant and antimicrobial properties of citrus peel (*Citrus reticulata*, *Citrus limetta* and *Citrus limon*) essential oil.

MATERIALS AND METHODS

Extraction of citrus peel essential oil

Citrus peels (Mandarin orange, mosambi and lemon) were procured from local juice shops of Madurai. Essential oil from citrus peels was extracted by hydrodistillation (Y. Li *et al.* 2014). 150g of dried sample was mixed with 1500 ml of water and subjected to essential oil extraction in Clevenger apparatus at 95 °C for 3 hrs. The extracted oil was evaluated for its physico chemical, antimicrobial and antioxidant properties.

Evaluation of physical properties

Colour value of the citrus peel essential oil of each species was measured using Hunter lab meter by the procedure given by Farahmandfar *et al.*, (2020). Viscosity was estimated using Brooke field viscometer by using spindle No 61 at 100 rpm; specific gravity was determined by the method given by Rao and McClements (2012). Density and solubility of citrus peel essential oil in water was determined by the procedure given by Njoku and Evbuomwan (2014).

Evaluation of chemical properties

Chemical properties such as free fatty acid value (Giwa *et al.*, 2018), iodine value (Farahmandfar *et al.*, 2018), peroxide value (Njoku and Evbuomwan, 2014), saponification value (Ezejiofor *et al.*, 2011) and thiobarbituric acid value (Okhli *et al.*, 2020) was estimated in the citrus peel essential oil.

Evaluation of antioxidant property

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of citrus peel essential oil was measured by procedure given by Olszowy and Dawidowiez (2016) and Ferric Reducing Antioxidant Potential (FRAP) of the essential oil was analysed by the method given by Farahmandfar *et al.* (2020).

Evaluation of antimicrobial property

Antimicrobial activity of citrus peel essential oil was tested against food borne pathogens. Pure culture of the bacteria (*Staphylococcus aureus subsp aureus* MTCC 96, *Escherichia coli* MTCC 443, *Salmonella enterica subsp typhi* MTCC 733, *Pseudomonas aerugino-sa* MTCC 1688 and *Erwinia* sp MTCC 2760) was obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh. Fungal strains (*Aspergillus flavus MTCC 277, Penicillium sp MTCC 1995* and *Fusarium* oxysporum MTCC 284) were collected from Department of Plant Pathology, Agricultural College and Research Institute, Madurai. The bacterial strains were grown on Nutrient Broth at 37 °C for 12-14 hrs. The strains were activated and obtained at a 10^5 CFU/ml concentration of the cell suspension by serial dilu-

tion. The fungi were grown on Potato Dextrose Agar Media at 28 °C for 4 days.

Agar dilution method

The food pathogenic fungi were tested by agar dilution method (Fraternale *et al.*, 2003) with some modification. The antifungal activity of the citrus peel essential oils (0.1%, 0.3%, 0.5% and 1.0%) were tested in the appropriate culture media (Potato Dextrose Agar). The oils were added to the culture medium at a temperature 40-45°C and poured into petriplates of size 10 cm (diameter). A disc (5 mm) of mycelia was taken from the edge of five-day-old fungi cultures, placed at the centre of each petriplates and incubated at 25°C. The efficacy of treatment was evaluated by observing the growth of fungi in the petriplates.

Screening method

Screening of essential oil for antibacterial activity was carried out by agar diffusion method followed by Djenane (2015) with slight modification. Nutrient agar media was used to screen the essential oil with maximum antibacterial property. 20 ml of the media was poured into the petriplates and allowed to solidify for 30 minutes in a biological safety cabinet with vertical laminar flow. 0.10 ml of standardized inoculums suspension of each bacterium was poured and spread over the plate using the L-rod and allowed to settle for 5 minutes. Sterile filter paper disc of 6mm diameter was impregnated with 5 µL essential oil using a capillary micro-pipette. The plates were left undisturbed for 15 minutes at room temperature for the diffusion of the essential oil and then incubated at 37 °C for 24 hours. The procedure was repeated for mosambi and lemon peel essential oil. At the end of 24 hours, the diameter of the clear zone around the disc was measured using a caliper and expressed in millimeters (disc diameter included) as its antimicrobial activity. The sensitivity to the different essential oil was classified by the diameter of the inhibition zone.

Determination of the minimum inhibitory concentration (MIC)

The MIC values of the best essential oil for the target bacteria were determined. The inoculum of the bacte-

Table 1. Physical properties of citrus peel essential oil

rium was prepared from 12-hour broth cultures and suspension was adjusted to 0.50 McFarland standard turbidity to give a final density of 5×10^5 cfu/mL. Citrus essential oil was dissolved in 30% glycerol at concentrations of 0.1, 0.3, 0.5, 0.7, 1, 1.5, 2, 2.5 and 3% in 10 ml sterile test tubes containing nutrient broth. MIC value of lemon peel essential oil against the five bacteria was determined by microwell dilution method. The 96well plates were prepared by adding 95 µL of nutrient broth and 5 µL of the inoculum. 100 µL of lemon peel essential oil with different concentration was added to the above wells. The last well containing only nutrient broth and inoculum without essential oil was used as negative control. The content was mixed well and incubated at 37 °C for 48 hours. After incubation, the wells were examined for the growth of the bacteria in the nutrient agar plate and the MIC (%) was determined as the lowest EO concentration that inhibited the complete growth in the nutrient agar plate.

RESULTS AND DISCUSSION

Evaluation of physical properties of citrus peel essential oil

The data of physical properties such as colour value, viscosity, specific gravity, density and solubility in water was measured and the results are given in Table 1. The color parameters (L*, a*, b*) of citrus essential oil were compared with distilled water as reference. The chromatic parameters L* (darkness/brightness), a* (greenness/redness), and b* (blueness/yellowness) values of orange peel oil was 99.8, -6.47 and -9.61, respectively. The L*, a*, b* value of mosambi (C. limetta) peel oil was 95.43, -1.09, -22.04, respectively and lemon (C. limon) peel oil was 80.38, -0.64 and -26.77, respectively. The lemon peel oil was slightly yellowish in color when compared with other citrus peel oil. Orange (C. reticulate) peel oil and mosambi (C. limetta) peel oil was more or less colorless, like that of distilled water. Viscosity is used to express the magnitude of internal

friction in a fluid. The consistency of the fluid can be evaluated using the viscometer. Viscosity of all three citrus peel oil orange peel oil- 0.99, mosambi peel oil-0.95 and lemon peel oil- 0.88 mPas) was lower than that of the viscosity of water (1.0 mPa.s). The viscosity

S. No	Sample	Color value			Viscosity	Specific	Density	Solubility in
		L*	a*	b*	(mPa.s)	gravity	(g/ml)	water
1.	Reference (water)	84.56	-2.90	-10.00	1.0	1.0	0.99	-
2.	Orange peel oil	99.8	-6.47	-9.61	0.99	0.84	0.83	Insoluble
3.	Mosambi peel oil	95.43	-1.09	-22.04	0.95	0.86	0.85	Insoluble
4.	Lemon peel oil	80.38	-0.64	-26.77	0.88	0.85	0.85	Insoluble

of *Citrus sinensis* peel essential oil was estimated as 0.9 mPa.s by Olabanji *et al.* (2016).

Specific gravity is the ratio of a material's density with that of water. The specific gravity of orange peel oil was 0.84, mosambi peel oil was 0.86 and lemon peel oil was 0.85. This result was in agreement with that of Gi-wa *et al.* (2018), who reported the specific gravity of essential oil from orange peel waste was 0.843. All three citrus peel essential oils were insoluble and float-ed over water due to the lesser density of essential oil than water.

Evaluation of chemical properties of citrus peel essential oil

Chemical properties such as free fatty acid value, iodine value, peroxide value, saponification value, thiobarbituric acid value for all three citrus peel oils are presented in Table 2.

The hydrolysis of oils and fats produces free fatty acids (FFA). They are more prone to oxidation and result in rancidity. The lower free fatty acid value in lemon peel oil (23.5 % oleic acid equivalent) represents the quality of oil over the other two citrus essential oil (orange peel oil- 31.4 % oleic acid equivalent and mosambi peel oil-29.9 % oleic acid equivalent). Iodine value indicates the degree of unsaturation of fat or oil. It can be measured by number of grams of iodine absorbed by 100g of fat. The iodine value of orange peel oil, mosambi peel oil and lemon peel oil was 107, 103 and 116 gl₂/ 100g oil, respectively. Lemon peel oil had a significantly (5% significant) higher iodine value, representing a higher amount of unsaturation in the oil. Olabanji et al. (2016) evaluated the physico chemical properties of essential oil from C. sinensis peel and indicated that the free fatty acid value and iodine value of the essential oil were 12.61% oleic acid equivalent and 120.10 l₂g/100g, respectively. Fakayode and Abobi (2018) also mentioned that the iodine value of *C. sinensis* was $82 \text{ gl}_2/100 \text{ g}$ oil. The saponification value indicates all fatty acids present in the sample (free and esterified). It depends on the molecular weight and the percentage concentration of fatty acid components present in the oil. The saponification value of orange peel oil, mosambi peel oil, and lemon peel oil was 140.3, 121.6 and 112.2 mg KOH/g

of oil. Manuranjan *et al.* (2019) evaluated the saponification value of essential oil from *Citrus macroptera* peel as 186.96 mg KOH/g of oil. The saponification value of *Citrus sinensis, Citrus limon and Citrus aurantifolia* peel essential oil was 145.61 mg KOH/g, 158.42 mg KOH/g and 122.74 mg KOH/g of the sample, respectively (Etta - Francis et al., 2022).

Peroxide value and Thiobarbituric acid (TBA) value measure the oxidative deterioration of the oil. Peroxide value determines the concentration of hydroperoxide, the primary oxidation product. Higher number of peroxide value indicates increased formation of hydroperoxides in the oil, whereas the lower number of peroxide value indicates the good quality of oil. The peroxide value of orange peel oil was 2.2 meq/kg of the sample, mosambi peel oil was 2.6 meq/kg of sample and lemon peel oil was 1.6 meq/kg of sample. Peroxide value indicates only the amount of primary products. But these hydroperoxides can be easily broken into secondary products. TBA value is used to quantify the secondary oxidation product. It measures the malonaldehyde produced during the oxidation of fatty acids with three or more double bonds. TBA value represents the degree of oxidation in oil. The TBA value of orange peel oil, mosambi peel oil and lemon peel oil was 0.47, 0.17 and 0.03 mg melonaldehyde, respectively.

Evaluation of antioxidant property of citrus peel essential oil

Antioxidant activity denotes the ability to quench free radicals present in the sample. Citrus essential oil is a rich source of antioxidants with total antioxidant activity of 73.70 % for orange peel oil, 77% for mosambi peel oil and 89% for lemon peel oil. This result is in agreement with Teneva *et al.* (2019) who reported that the total antioxidant activity of *Citrus aurantinum L.* peel oil was 88.1%. The antioxidant activity of *Citrus aurantifolia* (Christm.) Swingle was 66.44% (Lin *et al.* 2019).

Evaluation of antimicrobial properties of citrus peel essential oil

Antimicrobial property of citrus essential oil evaluated against fungi (*Aspergillus flavus, Penicillium notatum* and *Fusarium oxysporum*) and bacteria

S. No	Sample	FFA (Oleic acid equiva- lent in %)	lodine value (gl₂/100g oil)	Peroxide val- ue (meq/kg of sample)	Saponification value (mg KOH/g oil)	TBA value (mg melonalde- hyde)	TAA (% RSA)
1.	Orange peel oil	31.4±0.70 ^c	107±0.63 ^b	2.2±0.05 ^c	140.3°±0.31°	0.47 ^c ±0.03 ^c	73.70±0.65 ^c
2.	Mosambi peel oil	29.9±0.67 ^b	103±0.82 ^c	2.6±0.08 ^b	121.6±0.90 ^b	0.17±0.09 ^b	77.12±0.41 ^b
3.	Lemon peel oil	23.5±0.63 ^ª	116±0.02 ^ª	1.6±0.04 ^ª	112.2±0.13 ^ª	0.03±0.05 ^a	89.20±0.09 ^ª

Table 2. Chemical properties of citrus peel essential oil

FFA- Free Fatty Acid, TBA- Thiobarbituric Acid Value, TAA- Total Antioxidant Activity

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		MIC (%	6)		
Essential oil	A. flavus	Penicillium	Fusarium		
Orange peel oil	0.4	0.5	0.2		
Mosambi peel oil	0.4	0.5	0.5		
Lemon peel oil	0.3	0.2	0.2		

Table 3. Minimum inhibitory concentration (MIC) of citrus peel essential oil against fungi

Table 4. Zone of inhibition of citrus peel essential oil against bacteria

	Zone of inhibition (mm)						
Essential oil	S. aureus	S. enterica	P. aeruginosa	E. coli	Erwinia		
Orange peel oil	18	14	10	11	14		
Mosambi peel oil	20	10	14	12	12		
Lemon peel oil	22	15	14	16	15		

(Staphylococcus aureus, Escherichia coli, Salmonella enterica, Pseudomonas aeruginosa and Erwinia sp). The minimum inhibitory concentration of citrus essential oil against fungus are given in Table 3. The MIC of orange peel oil against *A. flavus, Penicillium* and *Fusarium* was 0.4%, 0.5% and 0.25%, respectively. Likewise, the MIC of mosambi peel oil and lemon peel oil against *A. flavus* was 0.4 and 0.3% respectively, against *Penicillium* and *Fusarium* was 0.5 and 0.2%, respectively. From the results, it was inferred that lemon peel oil had lower MIC against all three fungi. This result was in agreement with Van Hung *et al.* (2013), who reported MIC of *Citrus aurantifolia* Swingle essential oil against *Penicillium expansum* as 0.2%.

All three essential oils were compared for the antibacterial activity and the oil with better antibacterial activity was selected for further analysis. The zone of inhibition of the citrus peel essential oil is given in Table 4. The zone of inhibition of orange peel oil, mosambi peel oil, and lemon peel oil against S. aureus was 18-22 mm, and S. enterica was 10-15 mm. The zone of inhibition around P. aeruginosa for orange peel oil was 10 mm, mosambi peel oil and lemon peel oil was 14 mm. The zone of inhibition around E. coli was in the range of 11-16 mm and for Erwinia was 12-15 mm. From the results, it can be inferred that the maximum zone of inhibition was exhibited by lemon peel oil against all the five bacteria, which shows the best antibacterial activity of lemon peel oil. The MIC of lemon peel oil was determined by the growth of bacteria observed in Nutrient agar plate and the results are given in Table 5. No growth was observed in plates contained 0.9% of lemon peel oil. The MIC of S. aureus was 0.3% as there was no colony formation at 0.3% concentration of oil. Likewise, the MIC of S. enterica and Erwinia was 0.5% and P. aeruginosa and E. coli were 0.7%, respectively. This result was in accordance with the study conducted

by Djenane (2015). It was reported that the MIC of lemon peel oil against *S. aureus* as 0.25%. Lemon peel oil showed better antimicrobial activity against food-borne pathogens. this may be due to the presence of Limonene along with other bioactive compounds such as linalool, γ terpinene, α pinene, β pinene and β myrcene. The synergistic effect of bio active compounds exhibit better antimicrobial property.

Antimicrobial activity of Citrus sinensis var Valencia against Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Shigella dysenteriae, Escherichia coli and Salmonella typhimurium was studied by do Evangelho et al. (2019). It was mentioned that the inhibition zone L.monocytogenes was 10.59 mm, S. aureus was 10.10 mm, B. cereus was 9.99 mm, P. Aeruginosa was 9.30 mm and S. dysenteriae was 8.73 mm. Likewise, antimicrobial activity of C. aurantium essential oil against S. aureus NBIMCC 3703, Salmonella sp. (clinical isolate), P. aeruginosa NBIMCC 1390, B. subtilis NBIMCC 1208, and E. coli NBIMCC 3702 was estimated by Teneva et al. (2019). The measured zone of inhibition and minimum inhibitory concentration against gram-positive bacteria (S. aureus NBIMCC 3703 and B. subtilis NBIMCC 1208) was 12.5 mm and 60 ppm respectively and for gram-

Table 5. Minimum inhibitory concentration (MIC) of lemon

 peel essential oil against bacteria

Bacteria	MIC (%)
S. aureus	0.3
S. enterica	0.5
P. aeruginosa	0.7
E. coli	0.7
Erwinia	0.5

negative bacteria (*Salmonella* sp, *P. aeruginosa* NBIMCC 1390 and *E. coli* NBIMCC 3702), zones of inhibition were between 9-10 mm, with a minimum inhibitory concentration of over 600 ppm. This might be due to difference in the cell wall structure and composition of the two groups of bacteria.

Conclusion

Quality evaluation of citrus peel essential oil over physicochemical properties, antioxidant and antimicrobial properties of citrus essential oil evaluated against fungi (*Aspergillus flavus, Penicillium notatum* and *Fusarium oxysporum*) and bacteria (*Staphylococcus aureus, Escherichia coli, Salmonella enterica, Pseudomonas aeruginosa* and *Erwinia* sp) exhibited that lemon (*C. limon*) peel oil had better antibacterial. antifungal and antioxidant properties when compared with orange (*C. reticulate*) and mosambi (*C. limetta*) peel oil. This may be due to the presence of mixture of bioactive components present in the lemon peel oil. Hence, lemon peel oil can be used in the food system as a natural preservative. It can also be used as edible coating material to extend the shelf life of a product.

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Conflict of interest

The authors declare that they have no conflict of interest.

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