Emirates Journal of Food and Agriculture. 2022. 34(4): 289-296
doi: 10.9755/ejfa.2022.v34.i4.2843
http://www.ejfa.me/

RESEARCH ARTICLE

Physiochemical properties, antibacterial, antifungal, and antioxidant activities of essential oils from orange (*Citrus nobilis*) peel

Phong Xuan Huynh[1,*], Nhien Thi Hong Tran[1], Duyen Thi Thuy Ly[1], Thanh Ngoc Nguyen[1], and Truong Dang Le[2,3*]

[1]Biotechnology Research & Development Institute, Can Tho University, Can Tho City 94000, Vietnam, [2]Faculty of Food and Environmental Engineering, Nguyen Tat Thanh University, Ho Chi Minh City 700000, Vietnam, [3]Institute of Applied Technology and Sustainable Development, Nguyen Tat Thanh University, Ho Chi Minh City 700000, Vietnam

ABSTRACT

The continuous fresh consumption or juice production of orange fruits (*citrus nobilis*) has discarded a vast number of orange peels, which has caused significant impacts on environmental issues. This study attempted to utilize discarded orange peels to extract essential oils (EOs) and evaluated their physiochemical properties, antibacterial, antifungal, and antioxidant activities. EOs were extracted via a distillation system using a Clevenger apparatus and gas chromatography - mass spectrometry analysis was employed to characterize their chemical components. The antibacterial and antifungal test were evaluated using a well diffusion method, and antioxidant activity was determined based on DPPH radicals scavenging effect and ferric reducing antioxidant power (FRAP). The obtained EOs with the yield of 3.29 ± 0.24% in which limonene was found to be the most abundant compound in the EOs (90.42%) followed by β-myrcene (4.7%) and α-pinene (1.22%). The result showed that Gram-positive bacterium (*Bacillus cereus*) was susceptible to the 50% EOs than Gram-negative bacterium (*Escherichia coli*) with respect to inhibitory zone diameter of 15.00 ± 0.58 mm and 11.33 ± 0.58 mm. The 50% EOs also inhibited nearly 70% of the mycelial growth of *Aspergillus flavus* as well as exhibiting antioxidant activity with IC_{50} values of 0.15 ± 0.01 mg/mL and 18.29 ± 0.13 mg/mL for DPPH and FRAP assay, respectively. The orange peel EOs could be a promising alternative to synthetic preservatives in food industry due to their antimicrobial and antifungal activity as well as their antioxidant activity.

Keywords: Antibacterial activity; Antifungal activity; Antioxidant activity; Limonene; Orange peel essential oils

INTRODUCTION

Food safety is the most concerned issue of customers as well as food industry due to significantly increasing reported cases related to food spoilages (Alzoreky & Nakahara, 2003). Microorganisms are mainly responsible for the deterioration of food products in both quality and quantity. Fungi have been observed with a destruction of foodstuff by retarding nutritional values, and producing mycotoxins (Singh et al., 2010). Chemical preservatives are usually used in food products as antioxidant, antibacterial agents to extend the shelf-life but they have been found to exert many adverse side effects in long-term use. Besides, the continuous use of synthetic preservatives has been found to induce antibiotic resistance in microorganisms (Torres-Alvarez et al., 2017). To date, there has been a rising trend in finding natural alternatives to tackle the problem of antibiotic resistance in microorganisms (Balouiri et al., 2016). Essential oils have revealed a potential in antibacterial, anti-fungus, and antioxidant activities that can be promising alternatives to the chemical preservatives to prevent the food spoilages as well as lengthening the shelf-life of foodstuffs (Frassinetti et al., 2011).

According to the report of Gursoy et al. (2010), the chemical constituents of orange (*Citrus nobilis*) essential oil was limonene (76.77%) followed by γ-terpinene (8,24%), methyl chavicol (3,65%), and linalool (3,01%). The limonene content has been found to differ from different plant tissues, mostly found in citrus peels, followed by leaf and flower. The biological activities of EOs are highly dependent on cultivation, geographical location, vegetative phases and agricultural seasons of the plants (Uysal et al., 2011). Oranges are freshly

***Corresponding author:**
Phong Xuan Huynh, Biotechnology Research & Development Institute, Can Tho University, Can Tho City 94000, Vietnam. **E-mail:** hxphong@ctu.edu.vn. Truong Dang Le, Faculty of Food and Environmental Engineering, Nguyen Tat Thanh University, Ho Chi Minh City 700000, Vietnam Institute of Environmental Sciences, Nguyen Tat Thanh University, Ho Chi Minh City 700000, Vietnam. **E-mail:** ldtruong@ntt.edu.vn

Received: 30 August 2021; **Accepted:** 19 January 2022

consumed or processed to juice, jam which produce a vast number of orange peels. The total production of orange fruits in the world reached 73 million metric tons (Akosah et al., 2021) in which orange peel waste contributed to 30-50% of fruit weight (Ortiz-Sanchez et al., 2021), leading to seriously environmental pollution. Hence, it is necessary to find an effective means such as the utilization of orange peels for the EOs production to address this widely concerned issue (Sikdar et al., 2016). There has been significantly gaining interests of EOs from natural sources to be applied on pharmaceutical, cosmetic, fragrance, or food industry due to their safe aspects for human consumption (Evrendilek, 2015). Antioxidant activity and antimicrobial activity showed the predominant effect in EOs from citrus species which has been previously discussed. *Origanum vulgare* and *Thymus vulgaris* EOs showed the bactericidal effects against *E. coli* O157:H7 in foods (S. A. Burt and Reinders, 2003). Lemon oil was reported to have the most antioxidant activity, with DPPH reduction rate of 70% as compared to orange and mandarin oil (Frassinetti et al., 2011). EOs from 14 plant species showed variations in inhibitory effects against common food pathogens (Evrendilek, 2015). Cinamon oil experienced the highest efficacy in inhibiting saprophytic and food pathogens, whereas the highest antioxidant capacity was ascribed to grapefruit zest EOs (Denkova-Kostova et al., 2021). The antibacterial activity of grapefruit EOs against pathogenic microorganisms (*E. coli, S. aureus, and Pseudomonas aeruginos*) and yeast (*Candida albicans*) showed the inhibition zone diameters of 4 – 20 mm (el Houda et al., 2020). Deng et al. (2020) noted that grapefruit essential oils exhibited an inhibitory effect against the growth of HCT116 colon and HepG2 live cancer cells. Mandarin EOs was observed with the highest antioxidant activity, whereas grapefruit and lemon EOs were considered the most effective antimicrobial agents against *Escherichia coli* and *Lactobacillus plantarum* (Raspo et al., 2020).

In Vietnam, tons of orange peels are discarded without perceiving their biological activities. From literature review, EOs in each cultivar from citrus species may have different chemical constituents, exhibiting different biological activities. Therefore, we, in this study, aimed to evaluate the biological activities of the essential oils extracted from the agro-waste of orange juice production (orange peel). The antibacterial activity against common food pathogens such as *Bacillus cereus (B. cereus), Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli),* antifungal activity against *Aspergillus flavus (A. flavus),* and antioxidant activities of EOs from orange peels were investigated and compared. The obtained results are expected to further apply in food preservation as well as easing the environmental pollution, enhancing the economic values of orange fruit.

MATERIALS AND METHODS

Materials

Orange fruits (fully ripe) were harvested in March from Can Tho city (10°11'29.0"N 105°34'53.7"E). The selected fruit, characterized by a round shape with the diameter of 10-12 cm, green and rough peel, was washed with water to remove dirt and was peeled off. The peel (flavedo) was collected and stored at the temperature of 4°C.

Bacillus cereus ATCC 10876, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Aspergillus flavus* ATCC 9643 were supplied from Microbiologics, Inc, Minnesota, USA. Mueller Hinton Agar (MHA) was purchased from HiMedia Laboratories Pvt. Ltd. (India). Lysogeny broth (LB) was supplied from Thermo Fisher Scientific Co., USA. Potato dextrose agar (PDA) was from Difco Laboratories Inc., Detroit, MI, USA. Dimethyl sulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), ciprofloxacin, nystatin, and vitamin C were purchased from Merck KGaA (Darmstadt, Germany). All other analytical chemicals were purchased from the standard commercial supplies.

Extraction of orange peel essential oils

Orange peels (300 g) was homogenously blended using a blender (HR3652, Koninklijke Philips N.V., Netherlands). The sample was mixed with water at a 2.55: 1 (v/w) ratio of water to sample. Sodium chloride was added to the mixture at 7.5% (w/v) and kept for 2 h. The mixture was subjected to the distillation system using a Clevenger apparatus to obtain the crude EOs until no more EOs were obtained. Disodium sulfate was added to the crude EOs to absorb the water content in EOs. The extraction efficiency was calculated as followed (Ferhat et al., 2006):

$$E(\%) = \frac{Volume\ of\ EOs\ (mL)}{Orange\ peels\ weigh\ (g)} \times 100$$

Characterization of physicochemical properties

Density of EOs (g/mL) was determined by weighing an equal volume of EOs in the known-weighed Eppendorf at 25°C. The acid value (AV) and saponification value of EOs were evaluated by following Vietnamese Standards (TCVN 6127: 2010 and TCVN 6126: 2015). The ester value is the abstraction of saponification value and acid value.

Gas chromatography - mass spectrometry analysis

The chemical compositions of EOs were characterized using gas chromatography - mass spectrometry (GC-MS) (Agilent-5973, Aligent Technologies Inc., USA). The HP-5 capillary column (length: 30 m, inner diameter: 0.32 mm, film thickness 0.25 μm) was used for the analysis. Carrier

gas (helium) was operated at a flow rate of 1 mL/min. The column temperature was processed at 60°C to 280°C at a rate of 2°C/min. The sample injection (1 µL) was performed in the spitless mode at 230°C. The quantitative determination of the separated constituents in the EOs were governed from total ion chromatograms (Gursoy, Tepe, and Sokmen 2010).

Antibacterial test

The antibacterial activity of EOs was tested following agar well diffusion method upon a study done by Okunowo et al. (2013). The stock culture of *S. aureus, B. cereus,* and *E. coli* inoculum was grown in the LB medium at 37°C for 24 h. The turbidity of grown culture was adjusted with sterile saline water to 0.5 McFarland standard to get the equivalent bacterial cells of 1.5 x 10^8 CFU/mL. Aliquot (50 µl) of microbial suspension was spread on the surface of MHA plates. The 6 mm diameter wells were constructed by using a sterile cork borer on the MHA plates. The EOs at different concentration ranges (10%-50%) in DMSO were dispended into each agar well in an MHA plate. DMSO and ciprofloxacin (5 mg/mL) served as negative and positive controls. The antibacterial activity was evaluated according to the inhibitory zone diameter after 24 h incubation.

Antifungal test

The fungal *(A. Flavus)* was inoculated on Potato Dextrose Agar at 28°C for 3-5 days. The EOs were diluted at different concentrations in DMSO (5%, 10%, 15%, 25%, and 50%). The sterile PDA plates were prepared with a center well of 6 mm diameter and other surrounding wells for different antibacterial agents by using a 6 mm sterile cork borer. The 6 mm diameter fungal mycelial disc of *A. flavus* was placed at the center of as-prepared PDA plates. Each volume of 100 µL of EOs at different concentrations, negative control (DMSO), and positive control (nystatin 0.5 mg/mL) was poured into each well (Okunowo et al., 2013). The inhibitory effect was determined by measuring the diameter of mycelial growth after 3 days and calculated as followed:

$$Inhibitory\ percentage(\%) = \frac{D_c - D}{D_c}$$

Where D_c is the diameter of fungal mycelium without the presence of anti-agents and D is the zone diameter of fungal mycelium in the presence of anti-agents at different concentrations (Singh et al., 2010).

Measurement of DPPH radicals scavenging effect of EOs

The antioxidant activity of EOs by scavenging DPPH radicals was measured following the method of Gursoy, Tepe, and Sokmen (2010) with modifications. Four ml of EOs at different concentration in methanol (10-50 mg/mL) was mixed with one mL of 0.2 mM DPPH solution. The mixture was allowed to react for 30 min in the dark before reading the absorbance at 517 nm. Vitamin served as positive control (0.5-2.5 µg/mL). The inhibitory concentration at 50% antioxidant activity (IC_{50}) was calculated by constructing the graph of absorbance reduction versus EOs concentration or Vitamin C.

Measurement of ferric reducing antioxidant power (FRAP)

The FRAP assay was conducted upon the method of Singh et al. (2010). Briefly, the EOs (0.2 mL) at different concentrations (10-50 µg/mL) in methanol was mixed with 0.5 mL of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide (0.5 mL) followed by an incubation time of 20 min at 50°C. Aliquot (0.5 mL) of 10% trichloroacetic acid was included in the mixture. The mixture (1.25 mL) was then taken out to mix with distilled water (1.25 mL) and 0.1% ferric chloride (0.25 mL). The absorbance of final solution was recorded at the wavelength of 700 nm. Vitamin C served as a positive control. The graph of absorbance reduction versus of EOs concentration or vitamin C to calculate the IC_{50} values of EOs and vitamin C, respectively.

Statistical analysis

Each experiment was in three replicates. Data was depicted as mean ± standard deviation. One-way analysis of variance (ANOVA) and Tukey's HSD test were used to compare mean values at the level of 5% using Statgraphics centurion XVII (Statgraphics Technologies, Inc., Virginia).

RESULTS AND DISCUSSION

Characterization of orange peel essential oils

Table 1 presents the extraction efficiency and physicochemical properties of the obtained EOs by the distillation system using the Clevenger apparatus. The extraction efficiency was found at 3.29 ± 0.24%. This result was in a comparable range with previously reported studies. The extraction yield of orange peel EOs was 3.15% in the study of Gursoy, Tepe, and Sokmen (2010), whereas another study obtained the higher extraction efficiency of 3.7% (Julaeha et al., 2020). The variation of EOs in the orange peel could be ascribed to the differences in genetic

Table 1: Extraction efficiency and physicochemical properties of orange peel essential oils

Extraction efficiency (%)	3.29 ± 0.24
Density (g/mL)	0.83 ± 0.09
Acid value (mg KOH/g)	0.16 ± 0.06
Saponification value (mg KOH/g)	2.21 ± 0.12
Ester value (mg KOH/g)	2.05 ± 0.08

factors, climate, soil condition, geographical location, post-harvesting processes, or storage time (Djenane, 2015; Eleni et al., 2009). The EOs content in this study was found to 2-3 folds higher than other citrus species such as *C. sinesis L. Osbeck, C. aurantifolia, C. limon, C. amblycarpa, C. paradisi* (Farhat et al., 2011; Julaeha et al., 2020; Okunowo et al., 2013; Singh et al., 2010). This result indicated that the orange peels could be a feasible and applicable source for EOs production due to higher extraction yield compared to others.

The density of EOs in the orange peel was recorded at 0.83 ± 0.09 (g/mL) which was compatible with that in other EOs in citrus group (Colecio-Juárez et al., 2012; Giwa et al., 2018). In this study, the acid value and saponification value of orange peel EOs were estimated at 0.16 ± 0.06 mg KOH/g oil and 2.21 ± 0.12 mg KOH/g oil, respectively. The obtained results was significantly lower than those in prior reports (Njoku and Evbuomwan 2014; Giwa, Muhammad, and Giwa 2018). The discrepancy was possibly attributed to the plant species, climate, geography, or maturity stages. Pradhan et al. (2019) reported that the acid value and saponification value of EOs from citrus peels showed variations upon the mature stages and cultivated altitude.

The GC-MS chromatogram of orange peel EOs is depicted in Figure S1. The chemical compositions are summarized and highlighted with predominant constituents in Figure 2. The orange peel EOs were obviously observed with the dominant compound of limonene (90.42%) followed by β-myrcene (4.7%) and α-pinene (1.22%). This result was consistent with those reported in previous studies (Evrendilek, 2015; Farhat et al., 2011; Frassinetti et al., 2011; Torres-Alvarez et al., 2017) with the limonene content greater than 90%. However, Gursoy, Tepe, and Sokmen (2010) showed the variations in the chemical components in the orange peel EOs in which limonene contributed to only 76.77% followed by 8.24% and 3.01% of γ-terpinene and linalool, respectively. The discrepancy in chemical constituents of EOs could be probably attributed to differences in genetic factors between varieties and species, environmental factors such as soil types, cultivation practice, maturity stages, or weather changes (Jing et al., 2014). It was reported that the type of extraction method also partially influenced the chemical compositions of EOs (Singh et al. 2010; Ruiz and Flotats 2014).

Antibacterial activity

Antimicrobial activity of orange peel EOs is performed at different concentrations from 10% to 50% in DMSO against *E. coli, B. aureus,* and *B. cereus*. The inhibitory zone diameter of each agent is listed in table 2. In this study, DMSO was found not to induce the cytotoxic effect against tested bacteria. Thus, it could conclude that the presence of DMSO in the testing solution did not influence the result of bactericidal effects of EOs. EOs at 10% was found not to be inadequate to inactivate *S. aureus* but exerting slight inhibitory effect against *E. coli* and *B. cereus*. The bactericidal effects showed the increment when increasing the EOs concentration. The highest dosage of EOs at 50% was observed to induce the highest bactericidal effects against *E. coli, B. aureus,* and *B. cereus* with respect to 11.3 ± 0.58 mm, 11 ± 1 mm, and 15 ± 0.58 mm. Inhibitory zone diameter of 50% EOs is presented in Figure 3. The antibacterial activity could stem from the predominant limonene content in the EOs of orange peels which has been reported to induce the cytotoxic effects via disrupting bacterial membrane integrity and impairing respiration and ion transportations (Martins et al., 2000). The difference in antimicrobial activities of EOs among tested bacteria was relatively dependent on the bacterial cell wall, chemical constituents of EOs, or the synergistic effect of compounds in EOs on a certain type of microorganism (O'Bryan et al., 2008; Torres-Alvarez et al., 2017). Besides, the diffusion of EOs constituents in agar media could probably cause the significant impacts on the antimicrobial efficacy of EOs (Alzoreky and Nakahara, 2003).

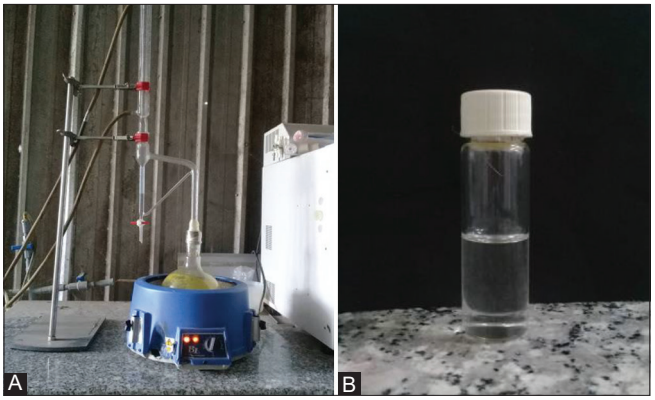

Fig 1. (A) Hydrodistillation process of essential oils using a Clevenger apparatus and (B) Visual appearance of resulting essential oils.

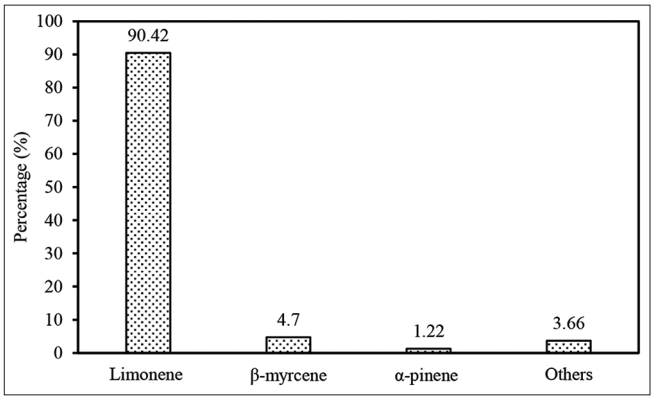

Fig 2. Chemical compositions of orange peel essential oils.

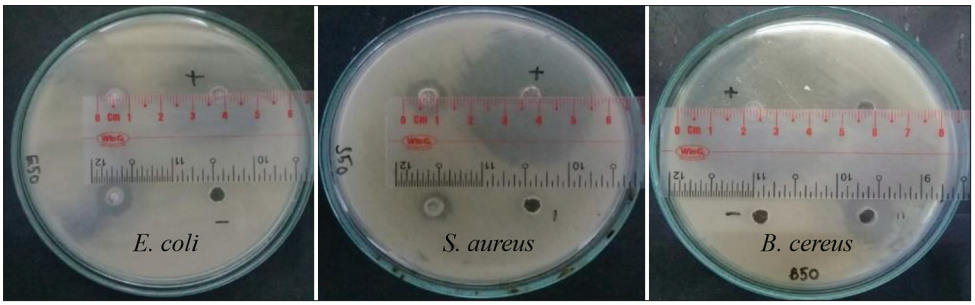

Fig 3. Inhibitory zone diameter of 50% EOs against *E. coli, S. aureus,* and *B. cereus.*

In this study, Gram-negative bacterium (*E. coli*) was found be slightly higher resistant than Gram-positive bacterium (*B. cereus*), described by the lower value of inhibitory zone diameter. This was consistent to past studies that Gram-positive bacteria were more susceptible to EOs (Burt 2004; Fancello et al. 2016). The lipopolysaccharides in the outer membrane of Gram-negative bacteria are considered a resistant factor towards anti-agents (Alzoreky and Nakahara, 2003). Chubukov et al. (2015) stated that the resistance of *E. coli* (Gram-negative bacterium) was highly correlated to a mutation of ahpC gene, which possibly attenuated the cytotoxic effect of limonene by decreasing the limonene-hydroperoxide to a more benign compound. In this study, 50% EOs showed higher bactericidal efficacy against food pathogens as compared to other studies when using 100% EOs for the antimicrobial test. Orange peel EOs in the study of Torres-Alvarez et al. (2017) exhibited the inhibitory zone diameter of 13 mm against *B. cereus* and *S. aureus.* The orange peel EOs was found to cause only 6.3 mm in inhibitory zone diameter against *E. coli O157:H7* (Evrendilek, 2015). Therefore, orange peel EOs in this study could be considered a potent antimicrobial agent, confirming the feasibility in utilizing the orange peels for the EOs production.

Antifungal activity

The antifungal activity of orange peel EOs in this study was evaluated upon the inhibitory percentage of diameter of mycelial growth. The inhibitory percentage of EOs at different concentrations against *A. flavus* is described in Figure 4. The antifungal efficacy of orange peel EOs was dose-dependent manner which was compatible with earlier reported studies (Singh et al., 2010; Viuda-Martos et al., 2008). EOs at 10% was found to inactivate 10% of diameter of mycelial growth and nearly 70% of mycelial growth diameter of *A. flavus* was inhibited by 50% EOs. The diameter of fungal mycelium in the presence of EOs at different concentrations is presented in Figure 5.

It was reported that the antifungal activity was mainly due to the presence of monoterpenes in the EOs, such as limonene, octanal, or citral (Tao, Jia, and Zhou 2014). Terpenes play a role in disrupting the cell membrane of fungi and penetrate to the bacterial cell wall, leading to the protein denaturation and cell membrane destruction (Gill and Holley 2006; Turina et al. 2006). Sharma and Tripathi (2008) also highlighted that limonene was the main compound in sweet orange peel EOs to be responsible for the inhibitory effect against *A. niger hyphae.* Therefore, it could apparently conclude that the limonene (90.42%) in the orange peel EOs showed the predominant inhibitory effect against the mycelial growth of *A. flavus.*

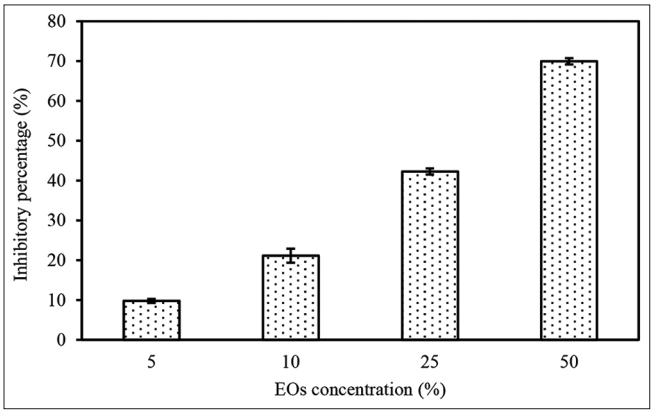

Fig 4. Inhibitory percentage of EOs at different concentrations against *A. flavus* based on the inhibition of mycelial growth diameter.

Table 2: Inhibitory zone diameter of EOs at different concentrations

	Inhibitory zone diameter (mm) including well diameter of 6 mm		
	E. coli	***S. aureus***	***B. cereus***
Ciprofloxacin	50.33 ± 1.52^{f}	33.00 ± 0.00^{d}	36.00 ± 1.73^{f}
DMSO	6.00 ± 0.00^{a}	6.00 ± 0.00^{a}	6.00 ± 0.00^{a}
10% EOs	7.33 ± 0.58^{b}	6.00 ± 0.00^{a}	7.00 ± 0.00^{b}
20% EOs	8.67 ± 1.15^{bc}	8.67 ± 0.58^{b}	10.00 ± 0.00^{c}
30% EOs	8.67 ± 0.58^{c}	10.00 ± 0.00^{c}	10.33 ± 0.58^{c}
40% EOs	10.00 ± 0.00^{d}	9.67 ± 0.58^{bc}	13.33 ± 0.58^{d}
50% EOs	11.33 ± 0.58^{e}	11.00 ± 1.00^{c}	15.00 ± 0.58^{e}

Data are expressed as mean ± SD. Different letters (a, b, c, d, e, f) indicate the statistical difference in mean values within the same column.

Antioxidant activity

The antioxidant capacity of orange peel EOs was listed in Table 3 according to the inhibitory concentration at 50% antioxidant activity. The IC_{50} value of EOs to scavenge

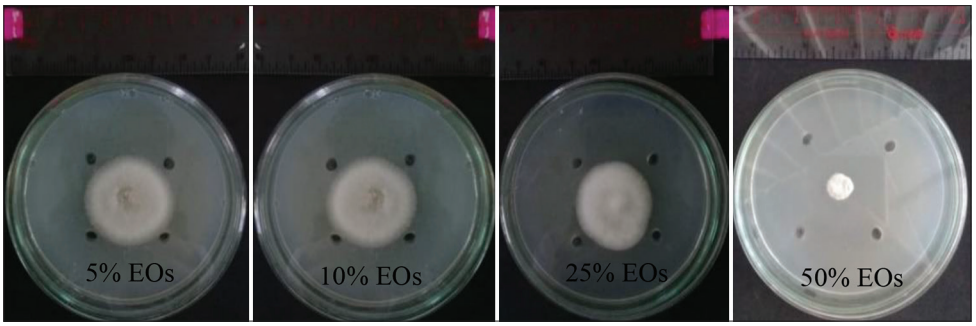

Fig 5. Zone diameter of mycelial growth of *A. flavus* in the presence of EOs at different concentrations (5%, 10%, 25%, and 50%).

Table 3: Inhibitory concentration at 50% antioxidant activity of EOs according to DPPH and FRAP assay.

	Inhibitory concentration	
	EOs (mg/mL)	Vitamin C (μg/mL)
DPPH	0.15 ± 0.01[b]	2.72 ± 0.15[a]
FRAP	18.29 ± 0.13[b]	3.74 ± 0.21[a]

Data are presented as mean ± SD. Small letters (a, b) within the same row show statistically significant difference in mean values.

50% DPPH radicals was found at 0.15 ± 0.01 mg/mL, whereas EOs needed higher IC_{50} (18.29 ± 0.13 mg/mL) in FRAP assay. This indicated that EOs showed a strong scavenging effect on DPPH radicals rather than ferric reducing capacity. A similar phenomenon was found in the study of Gursoy, Tepe, and Sokmen (2010). The variations in IC_{50} values between two methods could stem from the difference in mechanism of radicals quenching for DPPH and the potential in ferric reducing power (Huang, Ou, and Prior 2005). The free radical scavenging effect of EOs was mainly ascribed to the antioxidant activity of dominant component (limonene) in EOs which has been previously reported in past reports (Frassinetti et al., 2011; Junior et al., 2009). On the other hand, Torres-Alvarez et al. (2017) reported that the antioxidant activity was not due to only limonene but also the synergistic effect of other constituents in the EOs, promoting better antioxidant activity. As compared to the positive control (vitamin C), the IC_{50} values of vitamin C were considerably lower than those of EOs as vitamin C was considered a strong antioxidant compound. This observed trend was relatively similar to earlier report done by Fancello et al. (2016).

CONCLUSION

The physiochemical properties, antibacterial, antifungal, and antioxidant activities of orange peel EOs were successfully evaluated. Limonene was found to be a predominant component in the EOs which was mainly responsible for the biological activities of EOs. EOs showed the higher efficacy in inactivating Gram-positive bacteria than Gram-negative bacteria as well as the potential in inhibiting mycelial growth of *A. flavus*. Besides, EOs could be considered a strong DPPH radical scavenger. The result confirmed the feasibility of utilizing orange peels (an agro-waste) to produce EOs as natural alternatives to synthetic preservatives.

ACKNOWLEDGEMENT

This research was funded by Department of Science and Technology, Can Tho City, Vietnam.

Conflict of interest

Author declares that there is no conflict of interest.

Author's contribution

Nhien Thi Hong Tran, Duyen Thi Thuy Ly: Investigation, Methodologies; Phong Xuan Huynh: Conceptulization, Supervision, Reviewing and Editing Manusript; Thanh Ngoc Nguyen: Data Curation, Data Analysis; Truong Dang Le: Drafting-Manuscript, Reviewing and Editing Manuscript.

REFERENCE

Akosah, D. K., S. Adjei-Nsiah and F. C. Brentu. 2021. Response of late Valencia sweet orange (*Citrus sinensis* (L.) Osbeck) to fertilization on acrisols of the semi-deciduous forest agro-ecological zone of Ghana. Commun. Soil Sci. Plant Anal. 52: 1275-1285.

Alzoreky, N. S. and K. Nakahara. 2003. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. Int. J. Food Microbiol. 80: 223-230.

Balouiri, M., M. Sadiki and S. K. Ibnsouda. 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. J. Pharm. Anal. 6: 71-79.

Burt, S. 2004. Essential oils: Their antibacterial properties and potential applications in foods a review. Int. J. Food Microbiol. 94: 223-253.

Burt, S. A. and R. D. Reinders. 2003. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. Lett. Appl. Microbiol. 36: 162-167.

Chubukov, V., F. Mingardon, W. Schackwitz, E. E. K. Baidoo,

J. Alonso-Gutierrez, Q. Hu, T. S. Lee, J. D. Keasling and A. Mukhopadhyay. 2015. Acute limonene toxicity in *Escherichia coli* is caused by limonene hydroperoxide and alleviated by a point mutation in alkyl hydroperoxidase AhpC. Appl. Environ. Microbiol. 81: 4690-4696.

Colecio-Juárez, M. C., R. E. Rubio-Núñez, J. E. Botello-Álvarez, G. M. Martínez-González, J. L. Navarrete-Bolaños and H. Jiménez-Islas. 2012. Characterization of volatile compounds in the essential oil of sweet lime (*Citrus limetta Risso*). Chil. J. Agric. Res. 72: 275-280.

Deng, W., K. Liu, S. Cao, J. Sun, B. Zhong and J. Chun. 2020. Chemical composition, antimicrobial, antioxidant, and antiproliferative properties of grapefruit essential oil prepared by molecular distillation. Molecules. 25: 1-12.

Denkova-Kostova, R., D. Teneva, T. Tomova, B. Goranov, Z. Denkova, V. Shopska, A. Slavchev and Y. Hristova-Ivanova. 2021. Chemical composition, antioxidant and antimicrobial activity of essential oils from tangerine (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.), lemon (*Citrus lemon* L.) and cinnamon (*Cinnamomum zeylanicum Blume*). Z. Naturforsch. C. J. Biosci. 76: 175-185.

Djenane, D. 2015. Chemical profile, antibacterial and antioxidant activity of Algerian citrus essential oils and their application in *Sardina pilchardus*. Foods. 4: 208-228.

el Houda, A. K. N., B. Ali, A. Ahmed, O. Salah and F. C. Yazid. 2020. Chemical composition, antimicrobial and insecticidal activities of citrus paradisi peel essential oil from Algeria. J. Microbiol. Biotechnol. Food Sci. 9: 1093-1098.

Eleni, M., M. Antonios, K. George, S. Alexios-Leandros and M. Prokopios. 2009. High quality bergamot oil from Greece: Chemical analysis using chiral gas chromatography and larvicidal activity against the West Nile virus vector. Molecules. 14: 839-849.

Evrendilek, G. A. 2015. Empirical prediction and validation of antibacterial inhibitory effects of various plant essential oils on common pathogenic bacteria. Int. J. Food Microbiol. 202: 35-41.

Fancello, F., G. L. Petretto, S. Zara, M. L. Sanna, R. Addis, M. Maldini, M. Foddai, J. P. Rourke, M. Chessa and G. Pintore. 2016. Chemical characterization, antioxidant capacity and antimicrobial activity against food related microorganisms of *Citrus limon* var. *pompia* leaf essential oil. LWT Food Sci. Technol. 69: 579-585.

Farhat, A., A. S. Fabiano-Tixier, M. E. Maataoui, J. F. Maingonnat, M. Romdhane and F. Chemat. 2011. Microwave steam diffusion for extraction of essential oil from orange peel: Kinetic data, extract's global yield and mechanism. Food Chem. 125: 255-261.

Ferhat, M. A., B. Y. Meklati, J. Smadja and F. Chemat. 2006. An improved microwave Clevenger apparatus for distillation of essential oils from orange peel. J. Chromatogr. A. 1112: 121-126.

Frassinetti, S., L. Caltavuturo, M. Cini, C. M. Croce Della and B. E. Maserti. 2011. Antibacterial and antioxidant activity of essential oils from citrus spp. J. Essent. Oil Res. 23: 27-31.

Gill, A. O. and R. A. Holley. 2006. Disruption of *Escherichia coli, Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. Int. J. Food Microbiol. 108: 1-9.

Giwa, S. O., M. Muhammad and A. Giwa. 2018. Utilizing orange peels for essential oil production. ARPN J. Eng. Appl. Sci. 13: 17-27.

Gursoy, N., B. Tepe and M. Sokmen. 2010. Evaluation of the chemical composition and antioxidant activity of the peel oil of *Citrus nobilis*. Int. J. Food Prop. 13: 983-991.

Huang, D., B. Ou and R. L. Prior. 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53: 1841-1856.

Jing, L., Z. Lei, L. Li, R. Xie, W Xi, Y. Guan, L. W. Sumner and Z. Zhou. 2014. Antifungal activity of citrus essential oils. J. Agric. Food Chem. 62: 3011-3033.

Julaeha, E., K. S. Dewi, M. Nurzaman, T. Wahyudi and T. Herlina. 2020. Chemical Compositions and Antioxidant Activities of Indonesian Citrus Essential Oils and Their Elucidation Using Principal Component Analysis. Preprints.

Junior, M. R. M., T. A. A. e Silva, G. C. Franchi, A. Nowill, G. M. Pastore and S. Hyslop. 2009. Antioxidant potential of aroma compounds obtained by limonene biotransformation of orange essential oil. Food Chem. 116: 8-12.

Martins, A. P., L. R. Salgueiro, M. J. Gonçalves, R. Vila, F. Tomi, T. Adzet, A. P. da Cunha, S. Cañigueral and J. Casanova. 2000. Antimicrobial activity and chemical composition of the bark oil of *Croton stellulifer*, an endemic species from S. Tome e Principe. Planta Med. 66: 647-650.

Njoku, V. I. and B. O. Evbuomwan. 2014. Analysis and comparative study of essential oil extracted from Nigerian orange, lemon and lime peels. Greener J. Chem. Sci. Technol. 1: 6-14.

O'Bryan, C. A., P. G. Crandall, V. I. Chalova and S. C. Ricke. 2008. Orange essential oils antimicrobial activities against *Salmonella* spp. J. Food Sci. 73: 264-267.

Okunowo, W. O., O. Oyedeji, L. O. Afolabi and E. Matanmi. 2013. Essential oil of grape fruit (*Citrus paradisi*) peels and its antimicrobial activities Wahab. Am. J. Plant Sci. 4: 1-9.

Ortiz-Sanchez, M., J. C. Solarte-Toro, C. E. Orrego-Alzate, C. D. Acosta-Medina and C. A. Cardona-Alzate. 2021. Integral use of orange peel waste through the biorefinery concept: An experimental, technical, energy, and economic assessment. Biomass Convers. Biorefinery. 11: 645-659.

Pradhan, A., L. Sharma, S. G. Bhutia and N. D. Sherpa. 2019. Characterization of essential oil from the peel of three citrus species grown in Sikkim Himalaya. J. Appl. Hortic. 21: 157-163.

Raspo, M. A., M. B. Vignola, A. E. Andreatta and H. R. Juliani. 2020. Antioxidant and antimicrobial activities of citrus essential oils from Argentina and the United States. Food Biosci. 36: 100651.

Ruiz, B. and X. Flotats. 2014. Citrus essential oils and their influence on the anaerobic digestion process: An overview. Waste Manag. 34: 2063-2079.

Sharma, N. and A. Tripathi. 2008. Effects of *Citrus sinensis* (L.) Osbeck epicarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. Microbiol. Res. 163: 337-344.

Sikdar, D. C., R. Menon, K. Duseja, P. Kumar and P. Swami. 2016. Extraction of citrus oil from lemon (*Citrus limon*) peels by steam distillation and its characterizations. Int. J. Tech. Res. Appl. 5: 29-33.

Singh, P., R. Shukla, B. Prakash, A. Kumar, S. Singh, P. K. Mishra and N. K. Dubey. 2010. Chemical profile, antifungal, antiaflatoxigenic and antioxidant activity of *Citrus maxima* Burm. and *Citrus sinensis* (L.) Osbeck essential oils and their cyclic monoterpene, DL-limonene. Food Chem. Toxicol. 48: 1734-1740.

Tao, N., L. Jia and H. Zhou. 2014. Anti-fungal activity of *Citrus reticulata* Blanco essential oil against *Penicillium italicum* and *Penicillium digitatum*. Food Chem. 153: 265-271.

TCVN 6126:2015. (2015) Vietnamese Standard Animal and Vegetable Fats and Oils Determination of Saponification Value.

TCVN 6127: 2010. (2010) Vietnamese Standard Animal and Vegetable Fats and Oils Detemination of Acid Value and Acidity.

Torres-Alvarez, C., A. Núñez González, J. Rodríguez, S. Castillo, C. Leos-Rivas and J. G. Báez-González. 2017. Chemical composition, antimicrobial, and antioxidant activities of orange essential oil and its concentrated oils. CYTA J. Food. 15: 129-135.

Turina, A. D. V, M. V. Nolan, J. A. Zygadlo and M. A. Perillo. 2006. Natural terpenes: Self-assembly and membrane partitioning. Biophys. Chem. 122: 101-113.

Uysal, B., F. Sozmen, O. Aktas, B. S. Oksal and E. O. Kose. 2011. Essential oil composition and antibacterial activity of the grapefruit (*Citrus paradisi.* L) peel essential oils obtained by solvent-free microwave extraction: Comparison with hydrodistillation. Int. J. Food Sci. Technol. 46: 1455-1461.

Viuda-Martos, M., Y. Ruiz-Navajas, J. Fernández-López and J. Pérez-Álvarez. 2008. Antifungal activity of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) essential oils. Food Control. 19: 1130-1138.