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BIOLOGY

Extraction and evaluation of antimicrobial activities of essential oils from orange peel (*Citrus nobilis*) grown in Can Tho City, Vietnam

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ABSTRACT: This study determined the extraction conditions, chemical composition, and antimicrobial activities of the essential oils of *Citrus nobilis*. The results illustrated that soaking in the 9% (w/v) NaCl solution for 2 h with a sample and solvent ratio of 1:3 (w/v) and a subsequent extraction time of 45 min yielded the highest extraction efficiency, reaching 3.66% (w/w). The main chemical components of the essential oils were limonene (90.42%), β-myrcene (4.7%), and α-pinene (1.22%). The minimum inhibitory concentration (MIC) at the density of $5x10^4$, $5x10^5$, and $5x10^6$ cells/mL of *Escherichia coli* were 100, 125, and 125 mg/mL, respectively; for *Staphylococcus aureus* were 75, 100, and 125 mg/mL, respectively; and for *Bacillus cereus* were 50, 75, and 100 mg/mL, respectively. The minimum bactericidal concentration (MBC) at densities of $5x10^4$, $5x10^5$, $5x10^6$ cells/mL of *E. coli* were 175, 225, 225 mg/mL, *S. aureus* were 150, 200, 225 mg/mL, and *B. cereus* were 125, 175, 200 mg/mL, respectively. The inhibitory activities of *Aspergillus flavus* of orange essential oils according to the agar dilution method at concentrations of 0.025-0.1%) on the 5th day were 8.84-30.61%.
Key words: antibacterial, antifungal, *Citrus nobilis*, essential oils, extraction conditions, orange peel.

Extração e avaliação de atividades antimicrobianas de óleos essenciais de casca de laranja (*Citrus nobilis*) cultivada no município de Can Tho, Vietnã

RESUMO: Este estudo teve como objetivo determinar as condições de extração, composição química e atividades antimicrobianas dos óleos essenciais de *Citrus nobilis*. Os resultados demonstram que a imersão na solução de NaCl a 9% (p/v) por 2 h com uma proporção de amostra e solvente de 1:3 (p/v) e um tempo de extração subsequente de 45 min produziu a maior eficiência de extração, atingindo 3,66 % (p/p). Os principais componentes químicos dos óleos essenciais foram limoneno (90,42%), β-mirceno (4,7%) e α-pineno (1,22%). A concentração inibitória mínima (CIM) na densidade de $5x10^4$, $5x10^5$ e $5x10^6$ células/mL de *Escherichia coli* foram 100, 125 e 125 mg/mL, respectivamente, e para *Staphylococcus aureus* foram 75, 100 e 125 mg/mL, respectivamente, e para *Bacillus cereus* foram 50, 75 e 100 mg/mL, respectivamente. A concentração bactericida mínima (MBC) em densidades de $5x10^4$, $5x10^5$, $5x10^6$ células/mL de *E. coli* foram 175, 225, 225 mg/mL, *S. aureus* foram 150, 200, 225 mg/mL e *B. cereus* foram 125, 175, 200 mg/mL, respectivamente. As atividades inibitórias de *Aspergillus flavus* dos óleos essenciais de laranja, de acordo com o método de diluição em ágar nas concentrações 0,025-0,1%, no dia cinco foram 8,84-30,61%.
Palavras-chave: antibacteriano, antifúngico, *Citrus nobilis,* óleos essenciais, condições de extração, casca de laranja.

INTRODUCTION

Orange trees are widely grown worldwide with a gross production of 73 million metric tons (AKOSAH et al., 2021). In fact, orange peel waste, accounting for 30-50% of fruit weight (ORTIZ-SANCHEZ et al., 2021), may lead to environmental pollution. The use of essential oils from plants is becoming a sustainable trend in food, cosmetics, perfumery, and many other fields because they do not affect human health (EVRENDILEK et al., 2015). Microorganisms are a major contributor to food spoilage (SINGH et al., 2010). For that, synthetic preservatives are common additives in foods such as antibacterial agents or antioxidants despite possessing potentially many side effects. There have been many studies related to the allergenicity of some preservative chemicals such as benzoic acid and sulfate, the formation of carcinogenic nitrosamines from nitrite to extend the shelf-life, but they have been found to exert many adverse side effects in long-term use (TEIXEIRA et al., 2013), in addition to possibly leading to increased antibiotic resistance in some microorganisms (TORRES-ALVAREZ et al., 2017). One of the most studied preservatives, benzoate, has been found to cause cellular damage and increase the risks of fetal deformations (SUNITHA & PREETHI, 2000) or mutagenic/genotoxic effects to human lymphocytes (PONGSAVEE, 2015). Therefore, alternative and safe preservatives such as chitosan have gained research attention for their use in fruit preservation. While chitosan has interesting antifungal and antimicrobial properties (DUAN et al., 2019), its

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production using animal shells may limit its application for vegetarian and halal food.

HUO et al. (2019) conducted a study on extracting essential oils from *Citrus reticulate* mandarin peel using hydro-distillation. They discovered that the oil possessed antibacterial properties and could combat harmful bacteria like *Cutibacterium acnes*. *Citrus nobilis*, another member of the *Citrus* genus, is a potential source of phenolic compounds such as flavonol and flavanones (MALIK et al., 2021). Unfortunately, there are few reports on extracting essential oils from *Citrus nobilis*, with most limited to steam distillation (AGAPIN, 2020) or methanol-assisted extraction (MALIK et al., 2021).

This study aimed to take advantage of used orange peels to study the conditions affecting the extraction of essential oils and to evaluate the resistance to pathogenic microorganisms as a basis for the application of orange essential oils in the preservation of food and cosmetics, while helping to reduce environmental pollution and improve the value of oranges. Besides, this study utilized the distillation of essential oils using a solvent-free extraction method.

MATERIALS AND METHODS

Material

Orange samples used in this study were fully ripen fruits 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 a 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 a temperature of 4°C.

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

Determining conditions for extraction of orange peel essential oils

The concentration of NaCl

Orange peels (400 g) were homogenously blended using a blender (HR3652, Koninklijke Philips N.V., Netherlands). The samples were mixed with water at a 2:1 (v/w) ratio of water to sample. Sodium chloride was supplemented to the mixture at different contents of 0, 3, 6, 9, and 12% (w/v). The mixture was subjected to the distillation system using a Clevenger apparatus to obtain the crude EOs in 45 min. Disodium sulfate was added to the crude EOs to absorb the water content in EOs. The extraction efficiency was calculated as follows NDAYISHIMIYE et al. (2016).

$$E(\%) = a_x \frac{Weight\ of\ EOs\ (g)}{Orange\ peels\ weight\ (g)} x100$$

A = 0.9, the conventional density of essential oils is lighter than water.

The soaking time

The suitable soaking time (from 1 to 3h) was determined. The samples were blended and soaked with water. The mixture was supplemented with the selected sodium chloride content and subjected to distillation for the essential oils.

The ratio of sample and solvent

From the suitable soaking time and sodium chloride content, different ratios of sample and solvent (1:2, 1:3, and 1:4) were checked for essential oils extraction.

The time extraction

The extraction times (30, 45, 60, 75, 90, 105, and 120 min) were tested for essential oils production. The protocol was similar to prior steps with the determined concentration of NaCl, soaking time, and ratio of sample and solvent.

Gas chromatography-mass spectrometry analysis

The chemical compositions of the 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 employed at a flow rate of 1 mL/min. The column temperature was programmed in elevation mode from 60°C to 280°C at a rate of 2°C/min. The sample injection (1 µL) was performed in splitless mode at 230°C. Quantitative determination of the separated constituents in the EOs was quantified by total ion chromatograms (GURSOY et al., 2010).

Determine minimum inhibitory concentration (MIC)

The vials of *S. aureus*, *B. cereus*, and *E. coli* inoculum were pre-cultured in the NB medium at 37°C for 24 h. The turbidity of growth culture was

adjusted with sterile saline water to 0.5 McFarland standard to get the equivalent bacterial cells of 10^5, 10^6, and 10^7 cells/mL. The essential oils were diluted into NB medium at final concentrations of 25, 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 mg/mL. Two milliliters of bacteria cultures were blended with 2 mL of diluted essential oil samples in which the final bacterial densities were $5x10^4$; $5x10^5$, and $5x10^6$ cells/mL when the final essential oil concentrations were 12.5; 25; 50; 75; 100, 125, 150, 175, 200, 225 và 250 mg/mL. The positive control was ciprofloxacin (6 µg/mL), whereas the negative control was cultured in NB medium without adding the essential oils. After a 24-h incubation at 37°C, the number of colonies was quantified by the pour plate method (HASIKA et al., 2014).

$$\text{Inhibitory percentage (\%)} = \frac{(Ao - A)}{Ao} x100$$

A_o: the number of colonies of the positive control sample.
A: the number of colonies of samples at different concentrations.

The lowest concentration of essential oil that can inhibit bacteria by more than 90% was MIC.

Determine minimum bactericidal concentration (MBC)

Samples with the lowest concentration capable of inhibiting the growth of bacteria in the concentration range will be selected and determined by colony number by the pour plate method. The positive sample was ciprofloxacin (6 µg/mL). The negative control sample was 2 mL diluted bacteria samples mixed with 2 mL NB medium, and the number of colonies was determined by the pour plate method without incubation.

$$\text{Bactericidal percentage (\%)} = \frac{(Ao - A)}{Ao} x100$$

A_o: The number of colonies positive control sample.
A: The number of colonies of samples at different concentrations.
The lowest concentration of essential oils for the percentage of cells killed greater than or equal to 99.9% was MBC.

Antifungal test

Fungal *A. flavus* was incubated on Potato Dextrose Agar at 28°C for 3-5 days. Volumes of 25, 50, 75, and 100 µL essential oils were mixed with 5 mL acetone and then supplemented with 95 mL of PDA medium pre-warmed at 40-45°C to obtain 0.025; 0.05; 0.075; 0.1% concentrations, respectively, and finally poured into the Petri dishes. The negative control samples were prepared with acetone, whereas the positive control samples were supplemented with nystatin (0.5 mg/mL). Subsequently, fungal mycelial discs (6 mm in diameter) of *A. flavus* were placed at the center of the plates. The inhibitory effect was determined by measuring the diameter of mycelial growth after 3 days and calculated as follows:

$$\text{Inhibitory percentage} = \frac{Dc - D}{Dc} x100$$

Dc: The diameter of fungal mycelium without the presence of anti-agents.
D: The zone diameter of fungal mycelium in the presence of anti-agents at different concentrations (SINGH et al., 2010).

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).

RESULT AND DISCUSSION

Determing conditions for extraction of orange peel essential oils.

The concentration of NaCl

In figure 1, extraction efficiency improved with increasing NaCl concentrations. The NaCl concentration of 9% resulted in a high extraction efficiency with an insignificant difference compared with that extracted by 12% NaCl. Sodium chloride is an inorganic salt that increases the permeability of water in cells, enhances the polarity with water, and reduces the interaction between less polar molecules with water, thereby making essential oils easy to separate and released during distillation. The promoting effect of NaCl on essential oils extraction was also confirmed in the study of HUO et al. (2019) in King mandarin (*Citrus reticulate*) peel. In this study, 2% of additional NaCl contributed a 3% improvement in essential oils yield that was better than the yield assisted by other saline such as Na_2SO_4 and $(NH_4)_2SO_4$.

The soaking time

Soaking time affects the amount of essential oils obtained. The extraction efficiency after

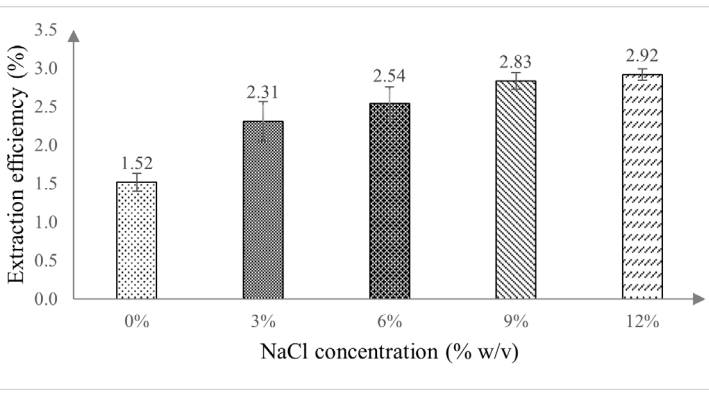

Figure 1 - Effect of concentrations of NaCl to efficiency extraction.

soaking for 2 h was not statistically different from that after soaking for 3 h (Figure 2). Water has the effect of penetrating cells and diffusing essential oil components in the cells to the outside, facilitating the distillation of essential oils and increasing the amount of oil recovered. However, due to the polar property of water, which can affect the solubility of oil and negatively impact the interaction with plant cell matrix (FILLY et al., 2016), the changing of submerging time can affect extraction efficiency. In this test, a 2-h soaking time was chosen to provide a shorter time with compatible efficiency.

The ratio of sample and solvent

Figure 3 shows that the extraction efficiency of essential oils depends on the ratio of sample and solvents, when increasing the solvent volume, the extraction efficiency of essential oils increases (at the ratio of 1:3), but when increasing to the ratio 1:4, the extraction efficiency of essential oils decreased because the amount of solvent was too much, led some components of the essential oils were dissolved in water, so the ratio of 1:3 was chosen.

This result is similar to the study of TRAN et al. (2020). Water has the effect of penetrating cells, then it will dissolve, diffuse, and attract the organic compounds in the essential oils. One possible explanation reported by SPIGNO et al. (2009) is that the ratio of water and sample affects the overall temperature of the extraction mixture. If using little water, it is not enough to dissolve the colloidal substance on the cell membrane, reducing the rate of water penetration and diffusion of essential oils, not breaking all the essential oil bags as well as not enough the amount of water needed to draw the essential oils out of the mixture.

The time extraction

When increasing extraction time, the amount of essential oils increased and reached a peak at 45 min, a statistically significant difference compared to 30 min (Figure 4). However, when increasing the extraction time, efficiency increased slightly, and the difference was not statistically significant compared with the extraction time at 45 min. According to the study of TOAN et al. (2020),

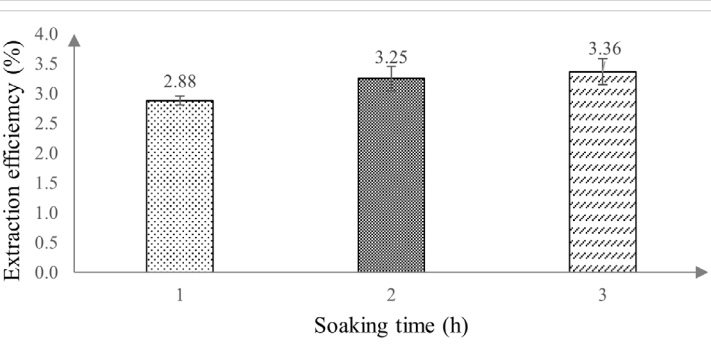

Figure 2 - Effect of soaking time to efficiency extraction.

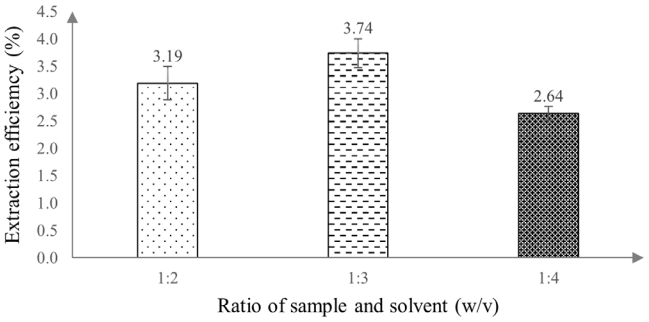

Figure 3 - Effect of the ratio and solvent to efficiency extraction.

the extraction time was also 45 min. The distillation time in different plant tissues is also different because in these tissues there are non-volatile compounds (such as waxes, resins, and long-chain fatty acids) that make the distillation process must be carried out over a long time since these compounds reduce the overall vapor pressure of the system and make diffusion difficult (MOHAMADI et al., 2013). In addition, the components of essential oils also affect the temperature of the air, so the extraction time is also different.

When extracting essential oils with a NaCl concentration of 9%, the soaking time was 2 h, the sample and solvent ratio was 1:3 (w/v), and the extraction time was 45 min for an extraction efficiency of 3.66%.

Chemical composition of essentia oils

The chemical compositions are summarized and highlighted with predominant constituents in figure 5. The orange peel EOs were observed with the dominant compound of limonene (90.42%), followed by β-myrcene (4.70%) and α-pinene (1.22%). This result was consistent with those reported in TORRES-ALVAREZ et al. (2017) with the limonene content greater than 90%. In the study of HSOUNA et al. (2017), a limonene composition in *Citrus lemon* was 39.74%, which is much lower than that of *Citrus nobilis* (Figure 5). Similarly, GURSOY et al. (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. These results indicated that *Citrus nobilis* was considered as a potential source of raw materials for limonene. 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 practices, maturity stages, or weather changes (JING et al., 2014). Besides, the type of the extraction method also partly influenced the chemical compositions of EOs (SINGH et al., 2010; RUIZ et al., 2014).

Determine minimum inhibitory concertration (MIC)

At the density of $5x10^4$ cells/mL (Figure 6), the inhibition percentage of *B. cereus* strain increased from 71.1-100% with the concentration

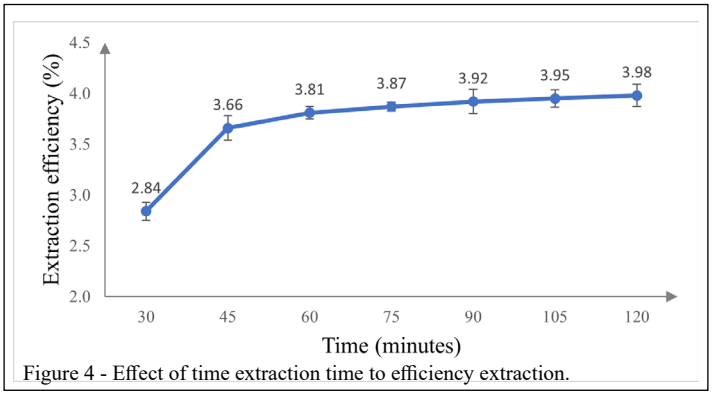

Figure 4 - Effect of time extraction time to efficiency extraction.

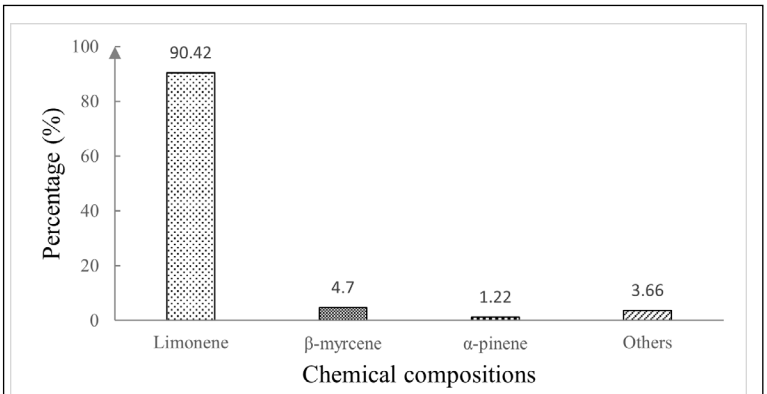

Figure 5 - Chemical compositions of orange peel essential oils.

of the essential oils from 12.5 to 125 mg/mL, and the MIC_{90} value was 50 mg/mL with the inhibition percentage of 94.1%. In strain *S. aureus*, the inhibition percentage was from 67.1-100% with the concentration of essential oils from 12.5 to 150 mg/mL, and the MIC_{90} value was 75 mg/mL with an inhibition percentage of 94.6%. In strain *E. coli,* the percentage of inhibition increased from 56.1 to 100% with the concentration of essential oils from 12.5 to 175 mg/mL, and the MIC_{90} value was 100 mg/mL with a percentage inhibition of 92.2%. In the study of HOJJATI et al. (2017), MIC_{90} of *S. aureus* was 59 mg/mL. The MIC_{90} value in the study was higher than that of THIELMANN et al. (2019) with MIC_{90} of *Citrus aurantium*, *Citrus aurantium bergamina*, *Citrus lemon*, and *Citrus paradisi* essential oils of 3.2, 6.4, 3.2, and 0.8 mg/mL, respectively. According to the study of PASHAZANOUSI et al. (2012), the MIC_{90} value was 0.128 mg/mL, which is also lower than MIC_{90} of the extracted oil in this study. From the above comparisons, it is shown that MIC_{90} values are different in the essential oil of *Citrus*. This difference may be due to the difference in the composition of the essential oil, which may be affected by the age of fruit harvest, soil, extraction method, etc.

At the density of $5x10^5$ cells/mL (Figure 7), the inhibition percentage of *B. cereus* strain increased from 57.9-100% with the essential oil concentration from 12.5 to 150 mg/mL, and the MIC_{90} value was 75 mg/mL with a percentage inhibition of 93.7%. In strain *S. aureus*, the inhibition percentage was from 57.7 to 100% with the concentration of essential oil from 12.5-200 mg/mL, and the MIC_{90} value was 100 mg/mL with the inhibition percentage of 94.9%. In *E. coli*, the percentage of inhibition increased from 31.7 to 100% with the concentration of essential oil from 12.5 to 200 mg/mL, and the MIC_{90} value was 125 mg/mL with an inhibition percentage of 96.1%.

At the density of $5x10^6$ cells/mL (Figure 8), strain *B. cereus* percentage of inhibition increased from 25.7 to 100% with essential oil concentrations from 12.5 to 175 mg/mL, and MIC_{90} value was 100 mg/mL with a percent inhibition of 90.9%. In strain *S. aureus*, the percentage inhibition was from 15-100% with the concentrations of essential oils from 12.5-200 mg/mL, and the MIC_{90} value was 125 mg/mL

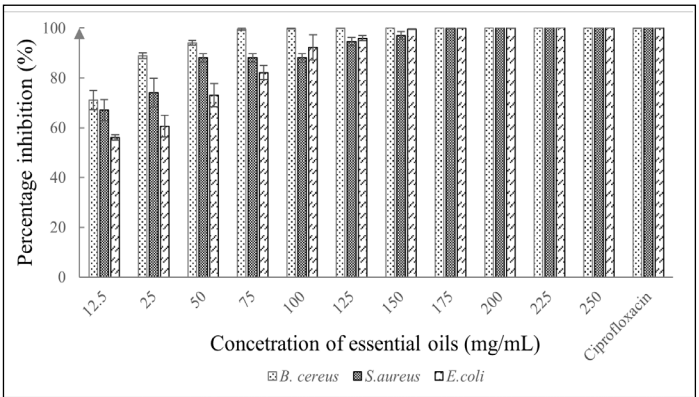

Figure 6 - Percentage inhibition of 3 microbial strains at density $5x10^4$ cells/mL.

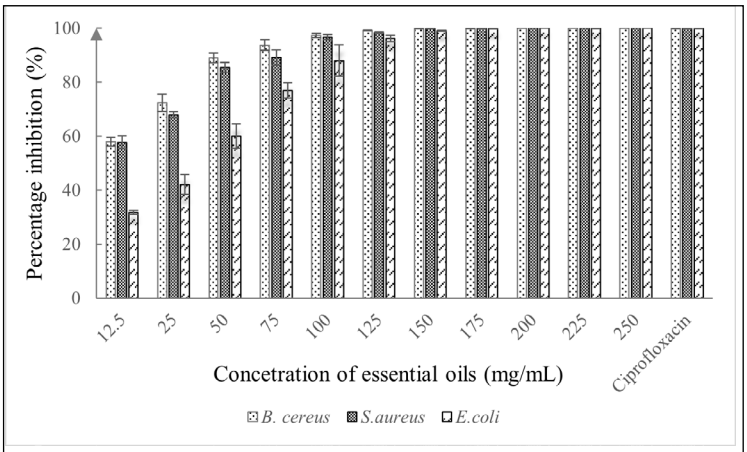

Figure 7 - Percentage inhibition of 3 microbial strains at density $5x10^5$ cells/mL.

with a percentage inhibition of 96.9%. In *E. coli*, the percentage of inhibition increased from 32.7 to 100% with the concentrations of the essential oils from 75 to 225 mg/mL, and the MIC_{90} value was 125 mg/mL with a percentage inhibition of 93.8%.

In summary, *E. coli* (Gram-negative) bacteria had higher MIC_{90} values than *B. cereus* and *S. aureus* (Gram-positive) bacteria at the same bacterial density, or Gram-positive bacteria were more sensitive to essential oils than Gram-positive bacteria with Gram-negative bacteria. The difference in susceptibility is because Gram-positive bacteria have a thick layer of peptidoglycan containing hydrophobic molecules such as proteins and teichoic acid. This hydrophobic layer surrounding the Gram-positive bacterial cell can facilitate the easy entry of hydrophobic molecules. Conversely, Gram-negative bacteria have a more complex structure consisting of an outer membrane linked to the inner peptidoglycan layer via lipoproteins. The outer membrane contains proteins and lipopolysaccharides (lipid A), making it resistant to hydrophobic molecules in the essential oils (NIKAIDO et al., 1994). This may explain the lower susceptibility of *E. coli* to essential oils than the other two strains (HYLDGAARD et al., 2012).

Determine Minimum Bactericidal Concentration (MBC)

At the density of $5x10^4$ cells/mL, the bactericidal percentage of the *B. cereus* strain increased from 27.6 to 100% with the concentration of essential oil from 50 to 150 mg/mL, and the MBC value was 125 mg/mL with a percentage inhibition of 99.9% (Figure

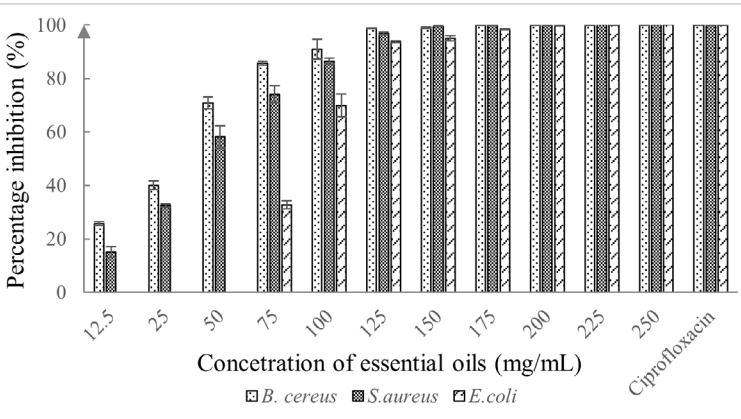

Figure 8 - Percentage inhibition of 3 microbial strains at density $5x10^6$ cells/mL.

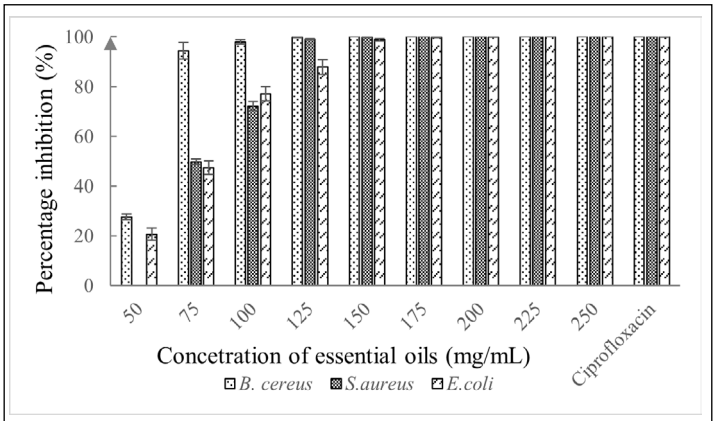

Figure 9 - Bactericidal percentage of 3 bacterial strains at the density of $5x10^4$ cells/mL.

9). In strain *S. aureus*, the bactericidal percentage was from 49.8 to 100% with the concentrations of the essential oils from 75 to 175 mg/mL, and the MBC value was 150 mg/mL with the bactericidal percentage of 99.9%. In strain *E. coli*, the bactericidal percentage increased from 20.7 to 100% with essential oil concentrations from 50 to 200 mg/mL, and the MBC value was 175 mg/mL with a 99.9% bactericidal percentage. This result is higher than that of AJAYI-MOSES et al. (2019) recorded an MBC of *B. cereus* of 25 mg/mL with essential oils from *Citrus aurantiifolia*.

At the density of $5x10^5$ cells/mL, the percentage of *B. cereus* strain bactericidal increased from 33.9 to 100% with the concentrations of the essential oils from 75 to 200 mg/mL, and the MBC value was 175 mg/mL with the bactericidal percentage of 99.9% (Figure 10). In strain *S. aureus*, the bactericidal percentage was from 63.1 to 100% with the concentrations of essential oils from 125-200 mg/mL, and the MBC value was 200 mg/mL with 100% bactericidal. In strain *E. coli*, the percentage of bactericidal increased from 9.6-100% with essential oil concentrations from 75 to 225 mg/mL, and the MBC value was 225 mg/mL with 99.9% bactericidal percentage.

At a density of $5x10^6$ cells/mL, the bactericidal percentage of the *B. cereus* strain increased from 32.8 to 100% with the concentrations of essential oil from 100 to 225 mg/mL, and the MBC value was 200 mg/mL with a bactericidal percentage of 99.9% (Figure 11). In strain *S. aureus*, the bactericidal percentage was from 17.8 to 100% with the concentrations of the essential oils from 125 to 225 mg/mL, and the MBC value was 225 mg/mL with the bactericidal percentage of 100%. In

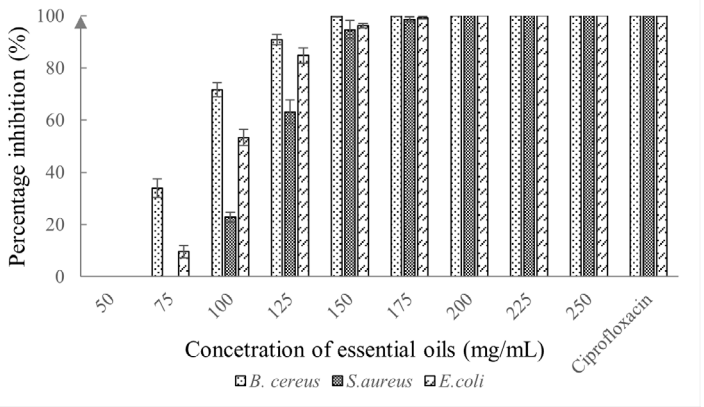

Figure 10 - Bactericidal percentage of 3 bacterial strains at the density of $5x10^5$ cells/mL.

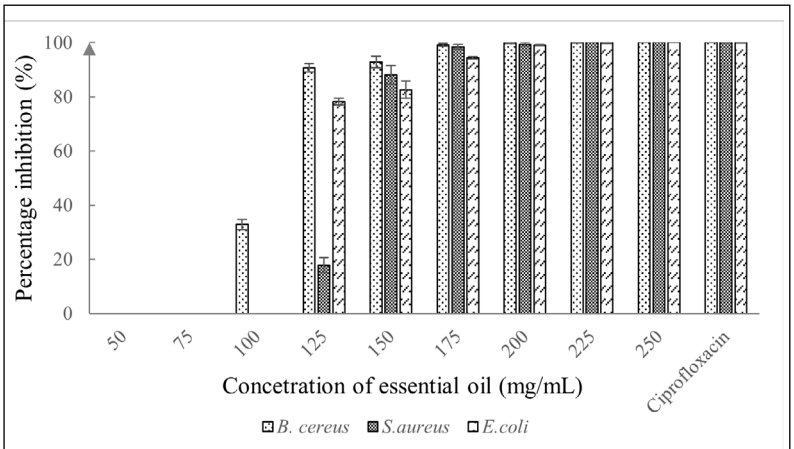

Figure 11 - Bactericidal percentage of 3 bacterial strains at the density of $5x10^6$ cells/mL.

strain *E. coli,* the bactericidal percentage increased from 78.2 to 100% with essential oil concentrations from 50 to 250 mg/mL, and the MBC value was 225 mg/mL with a 99.9% bactericidal percentage.

In general, the increase in MBC was lower than the increase in inhibition target density (log 4 to log 6), which proves the bactericidal efficiency of extracted oils over a range of bacterial densities. This is considered a useful property for application in practice. According to research by SULTANA et al. (2012), monoterpenes exert antibacterial activity through diffusion and damage cell membrane structures by increasing the concentration of lipid peroxides such as hydroxyl radicals. In addition, terpenes can disrupt and penetrate the lipid structure of the bacterial cell wall, leading to protein denaturation and cell membrane destruction, cytoplasmic leakage, cell lysis, and ultimately cell death (FISHER et al., 2008).

Antifungal test

Figure 12 shows that the colony diameter decreases with increasing concentration of essential oils, specifically on the third day, the concentrations of essential oils from 0.025 to 0.1% create colony diameters ranging from 9.67 to 45.00 mm. However, the percentage of inhibition decreased over time because the essential oils were a mixture of volatile substances, so over time, the volatile compounds in the essential oil were lost. Therefore, the percentage of inhibition decreased over time (Figure 13).

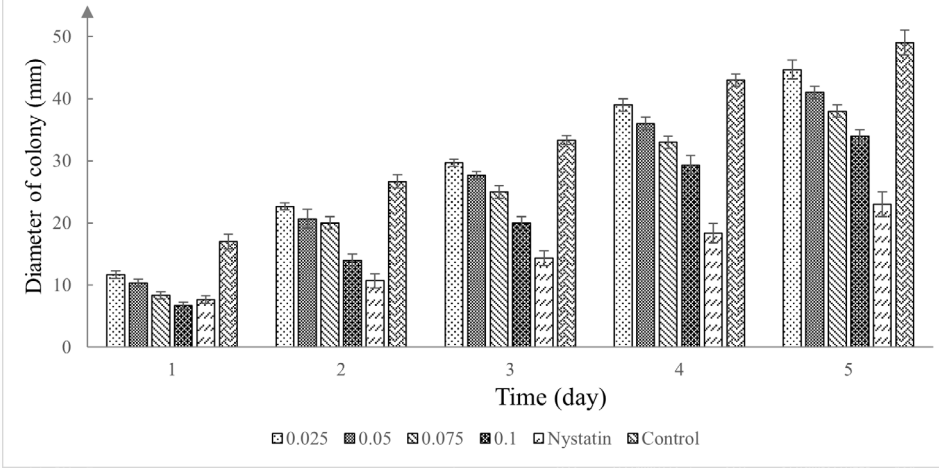

Figure 12 - Colony diameter of *A. flavus* within 5 days.

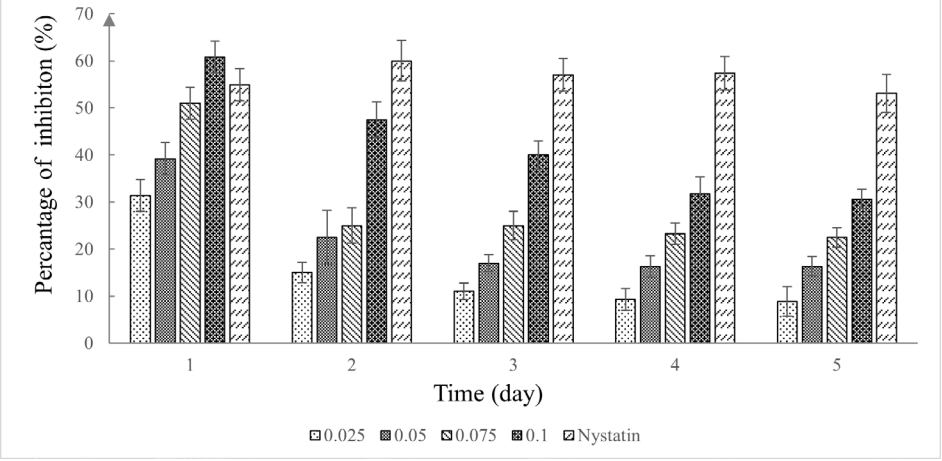

Figure 13 - Percentage of inhibition of *A. flavus* within 5 days.

According to TEPE et al. (2006), the antifungal activity of citrus essential oils in the presence of components such as D-limonene, linalool, or citral present at different concentrations in both essential oils. The amphoteric phenolic compounds can interact with the cell membrane, the hydrophilic part of the molecule interacts with the bilayer of the membrane, while the hydrophobic benzene ring is buried in the hydrophobic portion of the membrane (CRISTANI et al., 2007). These compounds cross the cell membrane, stimulate the escape of components from the cytoplasm, and lose the stiffness and integrity of mycelium cell wall, leading to death of the mycelium.

CONCLUSION

The factors affecting essential oils extraction from *C. nobilis* were successfully identified. The highest extraction yield (3.66% w/w) was achieved with the mixture of organe peel and 9% NaCl solution at the ratio of 1:3 (w/v), soaked in 2 h, and then extracted in 45 min. Limonene (90.42%) was found to be the main component in the EOs, mainly responsible for the biological activities of EOs. EOs showed higher efficiency in inactivating Gram-positive bacteria (*S. aureus* and *B. cereus*) than Gram-negative bacteria (*E. coli*) as well as the ability to inhibit the mycelial growth of mold *A. flavus*. The results confirmed the feasibility of using orange peels (agricultural waste) to produce EOs as natural alternatives to synthetic preservatives.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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