The Bactericidal Effect of Essential Oils in Vietnam to *Vibrio parahaemolyticus* Causing AHPND in Shrimp

Phuong Hong Lam[1, 2], Mai Thi Le[1, 2], Anh Van Thi Le[1, 2], Dung My Thi Dang[1, 2], Chien Mau Dang[1, 2] and Ngan Nguyen Le[1, 2*]

ABSTRACT

Ocimum gratissimum (clove basil), *Cymbopogon citratus* (lemongrass), and *Mentha arvensis* (corn mint) are common Vietnamese herbs with great antibacterial activity. This study was conducted to showcase the feasibility of utilizing these herbs for disease management in aquaculture, shrimp farming in particular. Firstly, these herbs' components were identified by gas chromatography-mass spectrometry (GC-MS). Their antimicrobial activity was then assessed against three strains of *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease (AHPND) in shrimp with three colony types on CHROMagar Vibrio by the disc diffusion method, and the minimum inhibitory concentration (MIC) values were determined. The results showed that the three studied bacterial strains were sensitive to three tested essential oils. VE1 strain, which formed type 1 colony on CHROMagar Vibrio, was the most sensitive to corn mint, lemongrass and clove basil essential oils with MIC values of 0.16%, 0.08%, and 0.08% (v/v), respectively. Meanwhile, MIC values of the aforementioned essential oils in the strains forming type 2 and 3 colonies (VE2 and VE3) were 0.32%, 0.08%, and 0.16% (v/v), respectively. These results indicate a potential application of lemongrass, corn mint and clove basil essential oils in AHPND disease control in shrimp. In order to increase the applicability of these essential oils, further studies on their synthesis in micro-emulsions and evaluation of their effectiveness should be carried out.

Keywords: Antibacterial activity, Clove basil, Corn mint, Lemongrass, Minimum inhibitory concentration, *Vibrio parahaemolyticus*

INTRODUCTION

Essential oils are volatile secondary metabolites of plants (Souza *et al*., 2019). Essential oils extracted from herbs have many outstanding uses due to their antibacterial (Cazella *et al*., 2019; Man *et al*., 2019; Álvarez-Martínez *et al*., 2021), antifungal (Hu *et al*., 2019; Piras *et al*., 2019; Filho *et al*., 2021), antioxidant (Pateiro *et al*., 2018; Diniz do Nascimento *et al*., 2020), and anti-inflammatory properties (Ogunwande *et al*., 2019; Gairola *et al*., 2021), but are safe for health (Mittal *et al*., 2018; Kumar *et al*., 2020). Therefore, essential oils have been widely used in food, pharmaceuticals, hygiene, and cleaning products (Pateiro *et al*., 2018; Hu *et al*., 2019; Kumar *et al*., 2020). Most herbal essential oils (e.g., clove, oregano, thyme, basil, mustard, cinnamon) and their biologically active substances, such as eugenol, citral, linalool, thymol, carvacrol, and limonene are recognized as safe (GRAS) by the U.S. Food and Drug Administration (FDA) (Kumar *et al*., 2020). The antimicrobial activity of essential oils is one of their most remarkable biological properties. It is effective against many bacterial strains that cause diseases in humans and animals, especially multidrug-resistant bacteria (Man *et al*., 2019; Nabti *et al*., 2020) such as *Streptococcus* sp. (Li *et al*., 2019; Shanaida *et al*.,

[1]Institute for Nanotechnology, Ho Chi Minh, Vietnam
[2]Vietnam National University, Ho Chi Minh, Vietnam
*Corresponding author. E-mail address: lnngan@vnuhcm.edu.vn
Received 18 October 2022 / Accepted 20 February 2023

2021; Nabila *et al*., 2022), *Aeromonas hydrophila* (Majolo *et al*., 2019; Assane *et al*., 2020; Monteiro *et al*., 2020), *Escherichia coli* (Yuan *et al*., 2018; Li *et al*., 2019; Nabti *et al*., 2020; Shanaida *et al*., 2021), *Staphylococcus aureus* (Shanaida *et al*., 2021; Nabila *et al*., 2022), and *Enterococcus faecalis* (Shanaida *et al*., 2021). The bactericidal ability of essential oils has been found to be related to the presence of several biologically active substances such as thymol, eugenol, p-cymene, γ-terpinene, and carvacrol (Pateiro *et al*., 2018; Melo *et al*., 2019).

Ocimum gratissimum (clove basil) is a medicinal plant in Family *Lamiaceae*, widespread in Asia and Africa (Melo *et al*., 2019). The main components in clove basil essential oil include eugenol, D-germacrene, β-ocimene, and caryophyllene (Melo *et al*., 2019; Ashokkumar *et al*., 2020). *Cymbopogon citratus*, commonly known as lemongrass, is an herb of the *Poaceae* family (Yadav *et al*., 2019), and grows in sunny conditions of the tropics and subtropics (Yadav *et al*., 2019; Shendurse *et al*., 2021). The primary chemical component of lemongrass essential oil is citral, and it contains many other compounds such as geraniol, limonene, geranyl acetate, estragole, methyl eugenol, citronellal, linalool, and terpinolene (Peichel *et al*., 2019; Yadav *et al*., 2019; Shendurse *et al*., 2021). *Mentha arvensis* (corn mint) is a native plant species in Asia. The essential oil extracted from corn mint has the main components of menthol, methone, thioxanthone (Haydari *et al*., 2019; Irkin *et al*., 2021), methyl acetate, and limonene (Manh and Tuyet, 2020). The proportions of essential oil components may differ among different geographical regions or climates (Sanli and Karadoğan, 2017). However, essential oils and plant extracts from the plants mentioned above still have some main effects, including antibacterial, anti-fungal, anti-inflammatory, analgesic, antipyretic, and antidepressant (Githaiga *et al*., 2018; Haydari *et al*., 2019; Peichel *et al*., 2019; Yadav *et al*., 2019; Chagas *et al*., 2020; Shendurse *et al*., 2021; Ugbogu *et al*., 2021). In particular, their antibacterial activity has been widely studied and showed positive results against some gram-negative and gram-positive bacteria (Githaiga *et al*., 2018; Peichel *et al*., 2019; Yadav *et al*., 2019; Shendurse *et al*., 2021).

Vibrio parahaemolyticus is a gram-negative bacteria of the *Vibrio* family, found in estuaries and marine environments (Changchai and Saunjit, 2014). In particular, *Vibrio parahaemolyticus* carrying the virulence gene *PirABVP* causes acute hepatopancreatic necrosis disease (AHPND) in shrimp, which has caused significant economic losses (Zheng *et al*., 2020). Some strategies to restrict this disease in shrimp have been studied; among these efforts, herbal essential oils have been tested for their ability to eliminate bacteria and prevent this disease from occurring in shrimp, with some positive results (Jha *et al*., 2016; Bakar *et al*., 2019). The present study evaluated the antibacterial activity of essential oils extracted from three popular herbs grown in Vietnam, namely corn mint, lemongrass, and clove basil, on three strains of *Vibrio parahaemolyticus* causing AHPND with different colony appearance on CHROMagar Vibrio medium (CHROMagar, France). Besides contributing more data on the chemical compositions of these three essential oils, the results indicate their bactericidal activity against *Vibrio parahaemolyticus* causing AHPND. These findings are the basis for developing solutions using herbal essential oils as an alternative to antibiotics to prevent this disease in shrimp.

MATERIALS AND METHODS

Materials

Bacterial strains: the bacterium used in this research was *Vibrio parahaemolyticus* causing AHPND in shrimp, and formed three different colony types on CHROMagar Vibrio medium, namely VE1, VE2, and VE3. For VE1 (Type 1), colonies were round with a smooth edge and surface and a diameter of 2–3 mm (Figure 1a). Colonies were purple in the center, and the remaining periphery was milky. For VE2 (Type 2), colonies were round, 3–3.5 mm in diameter and dark purple (Figure 1b). The border and surface of the colonies were smooth. For VE3 (Type 3), colonies were round, purple-pink in color with a diameter of 3–3.5 mm. The edge and surface of the colonies were smooth (Figure 1c). Three bacterial strains were collected from diseased shrimp

in several southwest provinces in Vietnam between 2015 and 2017. They were stored in tryptone soya broth with the supplement of 2.5% NaCl and 20% glycerol at -20 °C. Bacterial strains were confirmed as *Vibrio parahaemolyticus* causing AHPND by detecting the presence of the *tlh* gene (gene specific for *Vibrio parahaemolyticus* species) (Adila *et al*., 2018) and *PirAVP* gene (virulence gene related to AHPND) (OIE, 2019). The confirmation was implemented by real-time PCR (real-time polymerase chain reaction) method with primers and probe for *tlh* and *PirAVP* gene described, respectively, by Sun *et al*. (2014) and OIE (2019). The real-time PCR mixture and the thermal cycling program were conducted following the protocol of Platinum Quantitative PCR SuperMix-UDG (Invitrogen).

Essential oils (EOs): the three studied essential oils were *Ocimum gratissimum* (clove basil), *Mentha arvensis* (corn mint), and *Cymbopogon citratus* (lemongrass). Essential oils were purchased from a local essential oil distillery in Vietnam and analyzed for chemical composition by gas chromatography-mass spectrometry (GC-MS). The instrument used for analysis was Agilent 5977B GC/MSD with HP-5ms capillary GC column (Agilent), with the stationary phase of 5% diphenyl 95% dimethyl polysiloxane and the mobile phase of helium gas.

Bacterial culture medium: Tryptone soya agar (HiMedia, India) supplemented with 2.5% NaCl (TSA); Tryptone soya broth (HiMedia, India) supplemented with 2.5% NaCl (TSB), CHROMagar Vibrio (CHROMagar, France) (CV); Thiosulfate citrate bile sucrose (HiMedia, India) (TCBS); and Mueller Hinton agar (HiMedia, India) supplemented with 2.5% NaCl (MH).

Antibacterial activity

The antibacterial activity of the three essential oils tested against three strains of *Vibrio parahaemolyticus* was evaluated by the disc diffusion method described by Nabti *et al*. (2020) with some modifications. A bacterial suspension of each test strain with a concentration of 10^6 CFU·mL^{-1} was spread evenly onto the surface of an MH agar plate. Subsequently, sterile filter paper discs (6 mm in diameter) containing 10 µL samples of essential oil at different dilution ratios in dimethyl sulfoxide (DMSO) of 50% and 25% (v/v) were placed on the agar surface. Sterile paper discs containing DMSO or doxycycline (30 µg) were considered negative and positive controls, respectively. These plates were incubated at 37 °C for 24 h. The diameter of the inhibition zone was measured in millimeters (mm). The experiment was conducted in triplicate; the recorded data were expressed as Mean±SD; data analyses were conducted using the one-way ANOVA followed by Tukey's test to compare the means; the difference was significant when $p<0.05$.

Determination of minimum inhibitory concentration (MIC) of the three essential oils against Vibrio parahaemolyticus causing AHPND

The MIC values of the three essential oils tested against the three *Vibrio parahaemolyticus* strains were determined by the agar dilution method (Vu *et al*., 2021) whereby 0.5% Tween-20 was added to the MH medium after being sterilized by

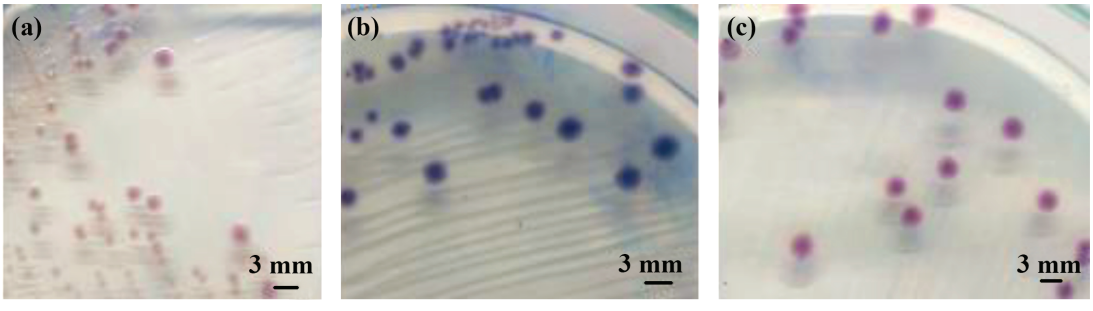

Figure 1. Three colony types of *Vibrio parahaemolyticus* grown on CHROMagar Vibrio: (a) VE1; (b) VE2; (c) VE3.

autoclave to increase the solubility of essential oils. Then the essential oil concentrations were mixed into the MH medium with a half-dilution test range from 0.32% to 0.01% (v/v). Subsequently, 5 µL of each bacterial concentration (10^6, 10^5 and 10^4 CFU·mL^{-1}) was added to each plate in three replicates. Bacterial growth was assessed after 18–24 h incubation at 37 °C.

RESULTS AND DISCUSSION

Chemical composition of essential oils tested by GC-MS method

The main component of the essential oil extracted from lemongrass was citral (70%). Other components accounted for a smaller proportion, including geraniol (3.21%), caryophyllene (3.16%), caryophyllene oxide (3.03%), β-myrcene (3.59%), α-gurjunene (1.24%), linalool (1.67%), and sulcatone (1.39%), along with some other minor components (Figure 2). According to Sharma *et al*., 2021, citral was one of the primary components of the essential oil extracted from lemongrass, with a concentration of approximately 65–85%. It was a mixture of neral and geranial (which are monoterpene aldehydes), with an odor similar to lemon. Citral in essential oils has been shown to have antibacterial, antifungal, and antiparasitic properties (Tamer *et al*., 2019). Majewska *et al*. (2019) also reported similar results from analyzing the composition of lemongrass essential oil. The results indicated that citral was the main component, followed by myrcene (accounting for 0.8–20%, varying by geographical region), geraniol (greater than 1%), and β-caryophyllene (0.1–2.46%) (Majewska *et al*., 2019).

The main components of corn mint were levomenthol (70.33%), menthone (17.70%), pulegone (2.46%), D-limonene (1.38%), 3-octanol (1.04%), and pinene (1.70%) (Figure 3). This result was similar to the results recorded by Manh and Tuyet (2020), who also analyzed the composition of corn mint harvested in Vietnam. Their research indicated that menthol was the main ingredient (accounting for about 66%), followed by methone and its isomers (about 4%), β-pinene, and limonene (Manh and Tuyet, 2020). Other studies also determined that the main components in corn mint were menthol (53-70%), and menthone. These two main components are called menthol mint (Chagas *et al*., 2020; Soltanbeigi *et al*., 2021).

The main components of clove basil essential oil included eugenol (42.1%), caryophyllene (31.96%), methyl eugenol (6.63%), humulene (4.36%), α-copaene (3.35%), α-pinene (2%), caryophyllene oxide (2.0%) and 3-carene (1.97%)

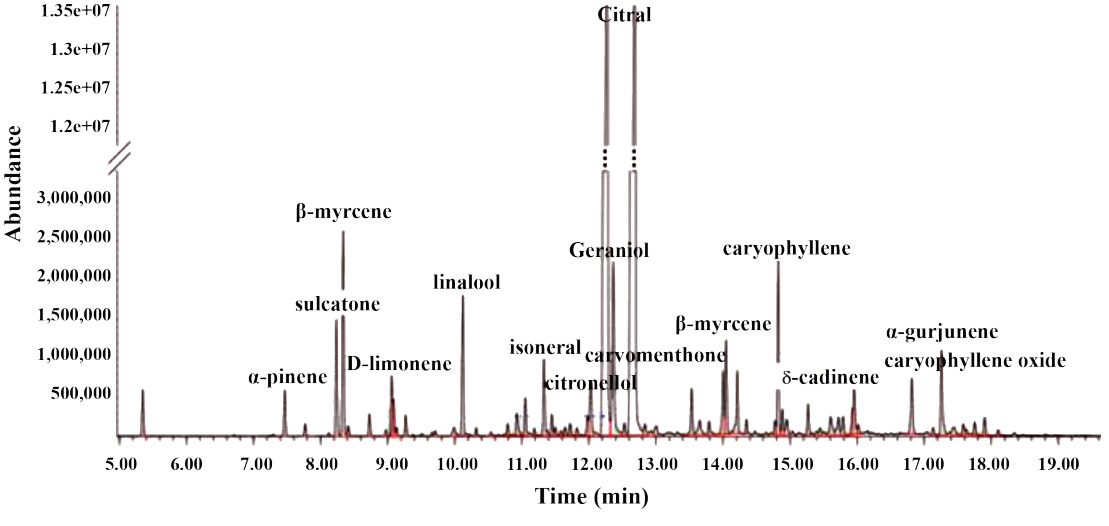

Figure 2. GC-MS chromatogram of lemongrass essential oil.

(Figure 4). According to Monteiro *et al*. (2020), eugenol (43.3%), 1,8-cineole (28.2%) and β-selinene (5.5%) were the main components in *Ocimum gratissimum* essential oil. Similar results were found in other studies (Melo *et al*., 2019; Dung *et al*., 2021), in which eugenol was shown to be the major component in *O. gratissimum* essential oil, accounting for about 65-75%, followed by other components such as caryophyllene and ocimene.

Essential oils are derived from the metabolism of plants; like in any living organism, their metabolism is influenced by many factors (Shanaida *et al*., 2021). Therefore, various factors such as harvest time, geographical origin, agro-climatic zones (Li *et al*., 2019), pathogen attack on plants, farming systems and genetic background can significantly alter the composition of essential oils through interference in secondary plant metabolism (Shanaida *et al*., 2021). The oil compounds present considerably influence the antimicrobial activity of the essential oil, and each component has a specific ability to disrupt or penetrate the bacterial structure (Monteiro *et al*., 2020). Citral, eugenol, menthol, caryophyllene, pinene, and limonene are biologically active substances with antibacterial properties (El Atki *et al*., 2019; Tamer *et al*., 2019; Ugbogu *et al*., 2021). Therefore, studying the composition of the experimental essential oils is the basis for evaluating and interpreting their bactericidal effect on the experimental bacterial strains in the following stages of this research.

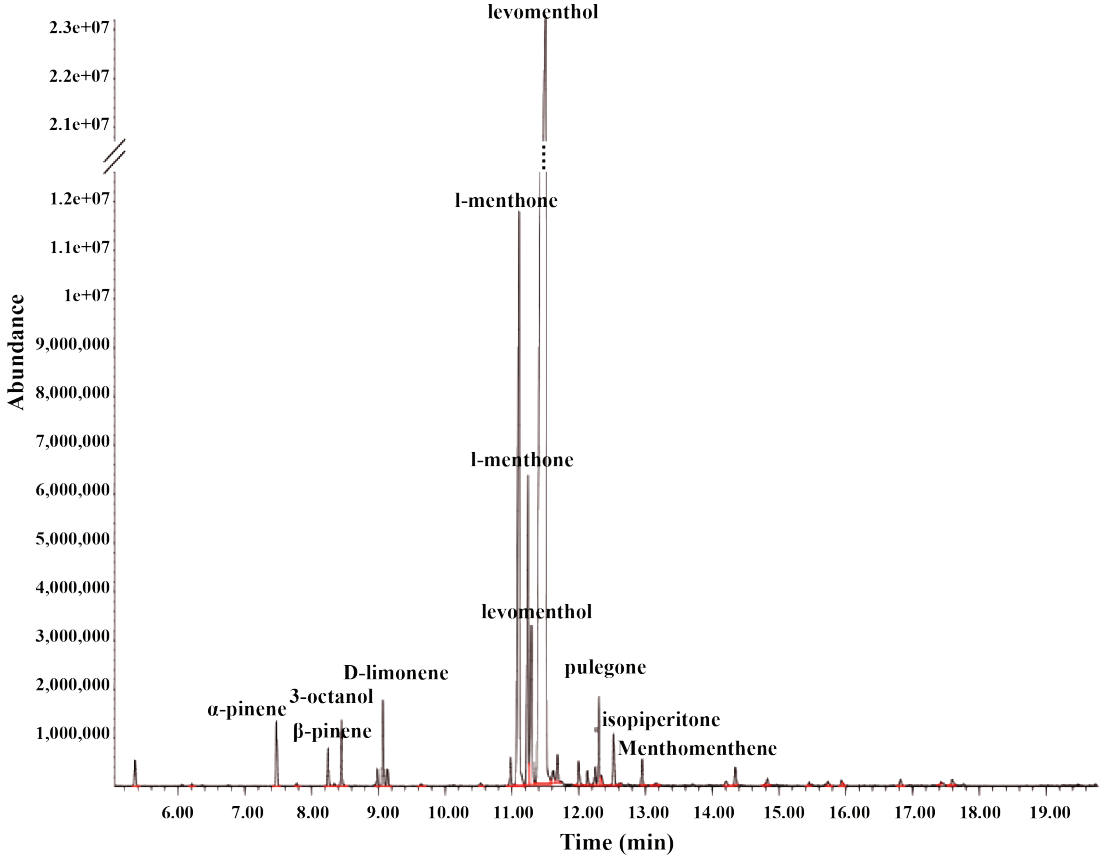

Figure 3. GC-MS chromatogram of corn mint essential oil.

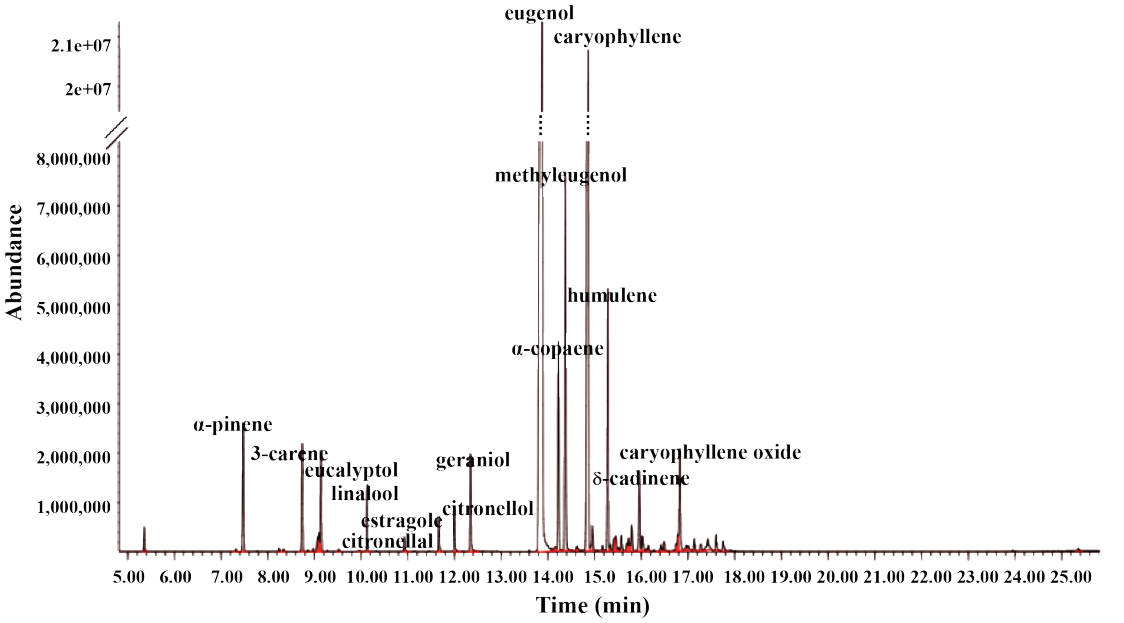

Figure 4. GC-MS chromatogram of clove basil essential oil.

Antibacterial activity of three experimental essential oils to Vibrio parahaemolyticus causing AHPND

Real-time PCR results indicated that three different colony-forming bacterial strains on CHROMagar Vibrio contained the *tlh* gene and the *PirAVP* gene (Figure 5). Therefore, the strains (VE1, VE2, and VE3) were confirmed to be *Vibrio parahaemolyticus* causing AHPND in shrimp.

The experimental results on antibacterial activity of three essential oils (lemongrass, corn mint and clove basil) against three strains of *Vibrio parahaemolyticus* causing AHPND are shown in Table 1 and Figure 6.

The results showed that the three *Vibrio parahaemolyticus* strains were sensitive to the experimental essential oils. In particular, corn mint at the concentration of 50% had stronger bactericidal ability against the VE1 strain than VE2 and VE3, shown by the significantly larger inhibition zone diameter of this oil with VE1 (24.33±0.58 mm) than with VE2 (21.00±1.00 mm) and VE3 (20.67± 0.58 mm). The bactericidal effects of lemongrass and clove basil essential oils were similar against

Table 1. Inhibition zones of three experimental essential oils against three strains of *Vibrio parahaemolyticus* causing AHPND.

	Corn mint essential oil		**Lemongrass essential oil**		**Clove basil essential oil**		**Doxycycline**
	50%	25%	50%	25%	50%	25%	
VE1	24.33 ± 0.58^{Bc}	10.67 ± 0.58^{Aa}	29.33 ± 2.08^{Ad}	16.67 ± 0.58^{Ab}	30.33 ± 0.58^{Ae}	16.33 ± 0.58^{Ab}	31.67 ± 0.58^{Ae}
VE2	21.00 ± 1.00^{Ac}	9.67 ± 0.58^{Aa}	30.00 ± 1.00^{Ad}	21.33 ± 0.58^{Bc}	31.00 ± 1.00^{Ad}	17.67 ± 0.58^{Ab}	31.33 ± 0.58^{Ad}
VE3	20.67 ± 0.58^{Ac}	9.67 ± 0.58^{Aa}	29.67 ± 0.58^{Ad}	22.00 ± 1.00^{Bc}	30.00 ± 1.00^{Ad}	17.00 ± 1.00^{Ab}	29.00 ± 2.64^{Ad}

Note: Means±SD in the same column superscripted with different uppercase letters are significantly different ($p<0.05$); different lowercase superscripts indicate significant difference ($p<0.05$) between means in the same row.

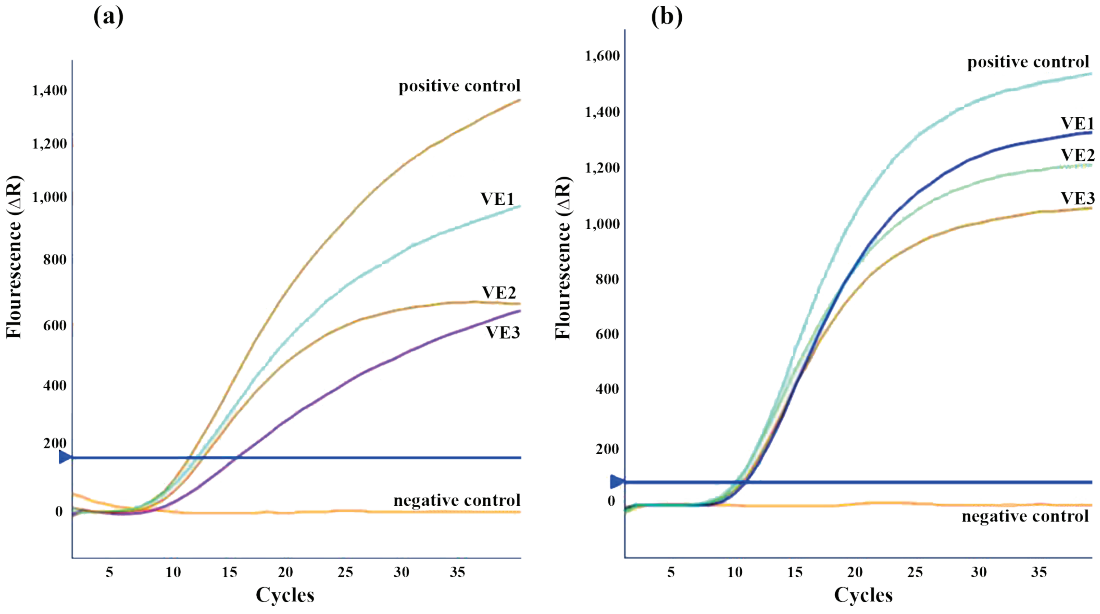

Figure 5. Real-time PCR amplification plots for *PirAVP* gene (a) and *tlh* gene (b) in three bacterial strains.

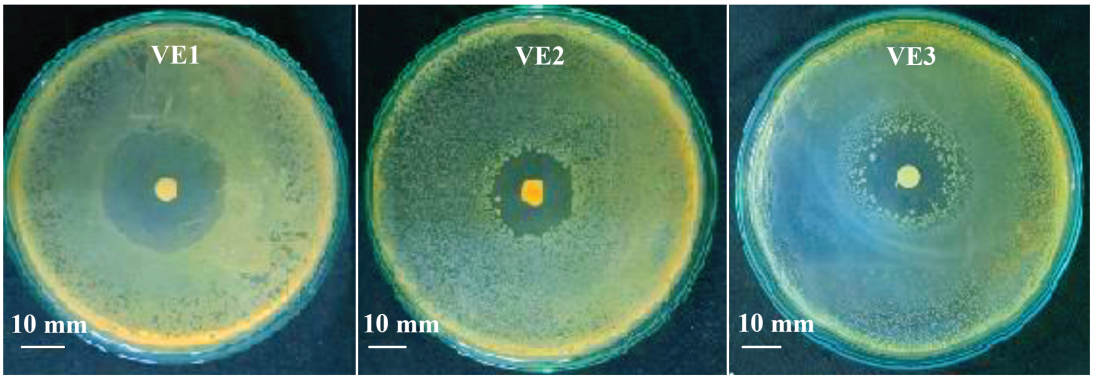

Figure 6. The inhibition zone of corn mint essential oil for three *Vibrio parahaemolyticus* strains.

the three bacterial strains, with the exception of lemongrass at 25%, which showed a larger inhibition zone against VE2 and VE3 than against VE1. Also, lemongrass and clove basil essential oils at the concentration of 50% (v/v) showed the same inhibition zone diameter as doxycycline, the positive control. In contrast, the inhibition zone diameter of corn mint against all three bacterial strains was smaller than doxycycline; the difference was significant ($p<0.05$) (Table 1). According to Muralidharan and Deecaraman (2019), the inhibition diameter of doxycycline (30 µg) against *Vibrio parahaemolyticus* strains that were isolated from several fin fishes in Chennai, India, was about 31-35 mm. The authors concluded that the isolated bacterial strains were sensitive to doxycycline. Furthermore, other research also indicated that if a *Vibrio* sp. strain had an inhibition zone diameter from doxycycline of more than 14 mm, this strain was sensitive to doxycycline (Ervia *et al*., 2021).

The MIC values for corn mint, lemongrass and clove basil essential oils against the three strains of *Vibrio parahaemolyticus* are shown in Table 2.

Results showed that VE1 was the most sensitive of the three bacterial strains to the essential oils tested in this experiment, based on the lower MIC values for all three essential oils. Furthermore, VE1 was more sensitive to lemongrass and clove basil essential oils than to corn mint. The corn mint, lemongrass and clove basil essential oils had similar inhibition activity against the VE2 and VE3 strains. The MIC values of corn mint, lemongrass, and clove basil were 0.32%, 0.08%, and 0.16%, respectively (Figure 7). Among the three essential oils, lemongrass showed the greatest bactericidal ability against the experimental bacterial strains.

Several other researchers have evaluated the antibacterial ability of various essential oils on *Vibrio parahaemolyticus*, and some of their results are similar to those of our experiments. According to Zheng *et al*. (2020), essential oils extracted from *Melaleuca alternifolia*, *Litsea citrata*, and *Eucalyptus citriodora* species could completely inhibit *Vibrio parahaemolyticus* at a concentration of

Table 2. MIC values of corn mint, lemongrass and clove basil essential oils against three tested strains of *Vibrio parahaemolyticus* causing AHPND.

Bacterial strain	Corn mint essential oil (% v/v)	Lemongrass essential oil (% v/v)	Clove basil essential oil (% v/v)
VE1	0.16	0.08	0.08
VE2	0.32	0.08	0.16
VE3	0.32	0.08	0.16

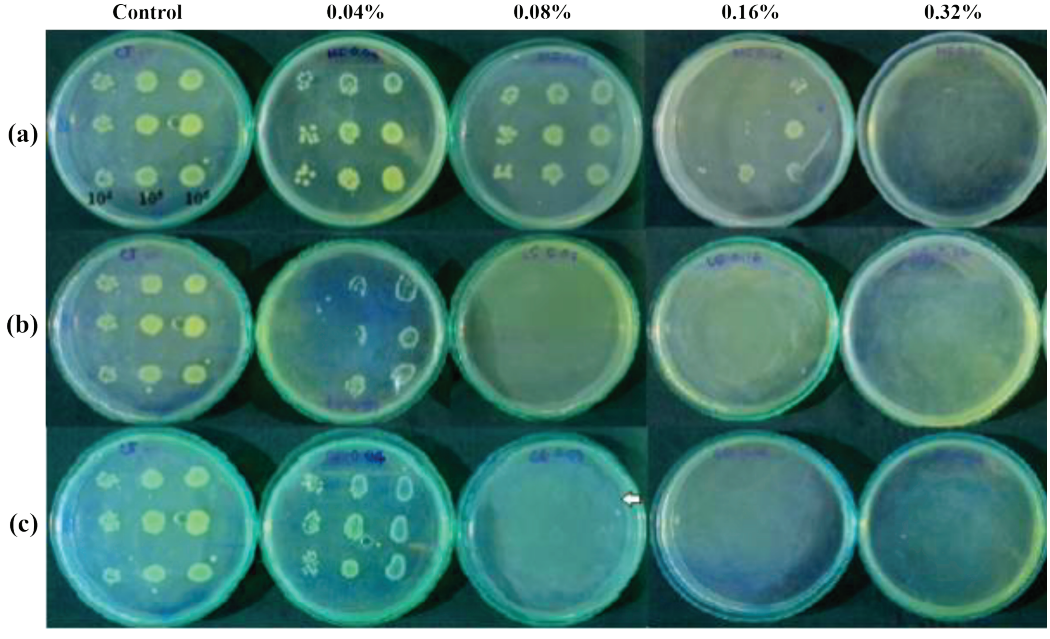

Figure 7. Minimum inhibitory concentration (at top of figure) of three essential oils against the VE3 strain: (a) corn mint essential oil; (b) lemongrass essential oil; (c) clove basil essential oil; from left to right on each plate, the bacterial suspension concentrations were 10^4, 10^5, and 10^6 CFU·mL^{-1}.

0.01%. *Litsea citrata* had the primary components of citral and limonene (Oro *et al*., 2020). Also, α-pinene, γ-terpinene, terpinen-4-ol, limonene, and o-cymene were identified as the major constituents of essential oil extracted from *M. alternifolia* (Sevik *et al*., 2021). Shanaida *et al*. (2021) reported that the essential oil from *Melissa officinalis* L. could inhibit the growth of *Vibrio parahaemolyticus* at a concentration of 0.1%. The major components of the *M. officinalis* essential oil were citronellol and geraniol (Shanaida *et al*., 2021).

The antibacterial activity mechanism of an essential oil is determined by its chemical composition (Assane *et al*., 2020). In our experiment, citral was the main ingredient in lemongrass essential oil. Citral is a monoterpene found in many herbs, plants, and citrus fruits (Lu *et al*., 2018), with antibacterial and antifungal properties (Lu *et al*., 2018). Several studies have indicated that citral plays a vital role in the antibacterial activity of essential oils, where it is a major component (Lu *et al*., 2018; Zhang *et al*., 2020). Citral causes damage and cell disruption to *Stenotrophomonas maltophilia*, a gram-negative drug-resistant bacteria (Zhang *et al*., 2020).

The main constituents of the corn mint used in our experiment were menthol (70.33%) and menthone (17.70%). Menthol is a local anti-inflammatory agent with antibacterial action through undifferentiated disruption of bacterial cells (Lykov *et al*., 2022). Martínez-Pabón and Ortega-Cuadros (2020) reported that menthol had good antibacterial properties against *Candida albicans*, *Escherichia coli*, and *Streptococcus mutans*. The lipophilicity allows menthol to move through the aqueous extracellular medium and interact with phospholipid membranes, causing damage to this structure and even inducing permeation and leakage of the internal material in cells. These effects lead to the destabilization of microorganisms (Martínez-Pabón and Ortega-Cuadros, 2020). Eugenol was the main component of clove basil essential oil, and has also been reported to have vigorous antibacterial activity (Majolo *et al*., 2019). The antibacterial mechanism of eugenol involves the rapid inhibition of the energy metabolism of bacteria (Monteiro *et al*., 2020).

Each chemical component has a specific ability to disrupt or penetrate the bacterial structure (Monteiro *et al*., 2020), leading to different bactericidal activities. For example, comparing the bactericidal ability of citral (terpenoid aldehyde), linalool (terpenoid alcohol), phenolic terpenoid (thymol and carvacrol), and terpene hydrocarbon, the results show that phenolic terpenoids and aldehydes have more potent antibacterial activity than terpenoid alcohol and terpene hydrocarbons. The reason is that phenolic terpenoids and aldehydes have strong hydrophobicity (Yuan *et al*., 2018) and the presence of highly reactive hydroxyl or acrolein functional groups.

Besides the primary components, other ingredients in essential oils also have bactericidal effect, such as linalool, limonene, pinene, terpinene, etc. Linalool produces an antibacterial effect by disrupting the cell membranes of bacteria (An *et al*., 2021; Shanaida *et al*., 2021). Limonene possesses antibacterial, antifungal, and antioxidant activities. Linalyl acetate exhibits antibacterial and antiviral properties (Li *et al*., 2019). The compounds α-terpineol, α-terpinyl acetate, and methyl eugenol also have strong antibacterial activity (Fidan *et al*., 2019; Shanaida *et al*., 2021), while p-cymene, limonene, terpinene, sabinene, and pinene are more bactericidal against gram-positive bacteria than gram-negative bacteria. The presence of chemical components in each essential oil has a synergistic effect on increasing the bioactivity of the essential oil (Li *et al*., 2019). These factors help explain why the three tested essential oils had a bactericidal effect on the three strains of *Vibrio parahaemolyticus* causing AHPND and the difference in the inhibition zone diameter and MIC value of each essential oil for each tested bacterial strain.

The results indicated that the three essential oils of corn mint, clove basil, and lemongrass had an effective antibacterial activity on all three strains of *Vibrio parahaemolyticus* causing AHPND with different colony appearance in CHROMagar Vibrio. Several other studies have reported that these herbal essential oils could stimulate the immune system and reduce stress in aquatic animals. These promising results show the potential to use essential oils to prevent diseases in shrimp more effectively and safely

than by using other synthetic antibiotics, especially in the juvenile stage. However, the disadvantages of essential oils are that they are volatile, hydrophobic, have low stability, and are easy to oxidize (Kumar *et al*., 2020), making essential oils difficult to use in practice. Essential oil-based micro-emulsions in a transparent and soluble form can overcome these drawbacks. Essential oils in micro-emulsions can be easily mixed into feed and even be used to treat the water in shrimp culture as with other disinfectants. Therefore, further research is needed to synthesize these essential oils into micro-emulsions and evaluate their ability to prevent disease in shrimp under experimental conditions. Such studies suggest further assessments and perspectives on the effectiveness of using essential oils in disease prevention in shrimp before applying them in practice.

CONCLUSION

This study was implemented to assess the antimicrobial activity of three essential oils extracted from *Ocimum gratissimum* (clove basil), *Cymbopogon citratus* (lemongrass), and *Mentha arvensis* (corn mint) on three strains of *Vibrio parahaemolyticus* causing AHPND. The results indicated that the three experimental essential oils could inhibit the growth of the three bacterial strains, and that the antibacterial activity of corn mint was lower than the other two essential oils. Among the three *Vibrio parahaemolyticus* strains, VE1 was the most sensitive to these essential oils. MIC values of corn mint, lemongrass, and clove basil for VE1 were 0.16%, 0.08% and 0.08% (v/v), respectively, while MIC values for VE2 and VE3 were 0.32%, 0.08%, and 0.16% (v/v). The results show the potential application of these three essential oils as an alternative to antibiotics in preventing disease in shrimp. However, essential oils are volatile, so preserving and stabilizing their biological activities is challenging. The synthesis of essential oils in micro-emulsions makes them convenient to store and increases their biological activity. Therefore, the synthesis of essential oils in micro-emulsions and the evaluation of disease prevention in shrimp should be studied, which will allow for increasing the applicability of essential oils in practice.

ACKNOWLEDGEMENT

This research is funded by Vietnam National University Ho Chi Minh City (VNU-HCM) under grant number C2020-32-02.

LITERATURE CITED

Adila, N.H., B. Anto and N.J. Siti. 2018. Detection of *tlh* and *tdh* genes in *Vibrio parahaemolyticus* inhabiting farmed water ecosystem used for *L. vannamei* aquaculture. **Journal of Physics: Conference Series** 1025: 012058. DOI: 10.1088/1742-6596/1025/1/012058.

Álvarez-Martínez, F.J., E. Barrajón-Catalán, M. Herranz-López and V. Micol. 2021. Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of ction. **Phytomedicine** 90: 153626. DOI: 10.1016/j.phymed.2021.153626.

An, Q., J.N. Ren, X. Li, G. Fan, S.S. Qu, Y. Song, Y. Li and S.Y. Pan. 2021. Recent updates on bioactive properties of linalool. **Food and Function** 12(21): 10370–10389. DOI: 10.1039/D1FO02120F.

Ashokkumar, K., V. Sampathrajan, M. Murugan, M.K. Dhanya, S. Aiswarya and N. Mathews. 2020. Chemical composition of *Ocimum gratissimum* essential oil from the South Western Ghats, India. **Journal of Current Opinion in Crop Science** 1(1): 27–30.

Assane, I.M., G.M.R. Valladão and F. Pilarski. 2020. Chemical composition, cytotoxicity and antimicrobial activity of selected plant-derived essential oils against fish pathogens. **Aquaculture Research** 52(2): 793–809.

Bakar, P., I.A.A. Rafi, W.R.W. Ahmad and K.B. Chu. 2019. Antimicrobial activities of commercial essential oils against *Vibrio parahaemolyticus* from acute hepatopancreatic necrosis disease of white shrimp (*P. vannamei*). **Malaysian Fisheries Journal** 18: 102–115.

Cazella, L.N., J. Glamoclija, M. Soković, J.E. Gonçalves, G.A. Linde, N.B. Colauto and Z.C. Gazim. 2019. Antimicrobial activity of essential oil of *Baccharis dracunculifolia* DC (Asteraceae) aerial parts at flowering period. **Frontiers in Plant Science** 10: 27. DOI: 10.3389/fpls.2019.00027.

Chagas, E.C., C. Majolo, P.C. Monteiro, M.R.D. Oliveira, P.E. Gama, H.R. Bizzo and F.C.M. Chaves. 2020. Composition of essential oils of *Mentha* species and their antimicrobial activity against *Aeromonas* spp. **Journal of Essential Oil Research** 32(3): 209–215. DOI: 10.1080/10412905.2020.1741457.

Changchai, N. and S. Saunjit. 2014. Occurrence of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in retail raw oysters from the eastern coast of Thailand. **The Southeast Asian Journal of Tropical Medicine and Public Health** 45(3): 662–669.

Diniz do Nascimento, L., A.A.B.D. Moraes, K.S.D. Costa, J.M.P. Galúcio, P.S. Taube, C.M.L. Costa, J.N. Cruz, E.H.D.A. Andrade and L.J.G.D. Faria. 2020. Bioactive natural compounds and antioxidant activity of essential oils from spice plants: new findings and potential applications. **Biomolecules** 10(7): 988. DOI: 10.3390/biom10070988.

Dung, P.N.T., T.P. Dao, T.T. Le, H.T. Tran, T.T.T. Dinh, Q.L. Pham, Q.T. Tran and M.Q. Pham. 2021. Extraction and analysis of chemical composition of *Ocimum gratissimum* L essential oil in the North of Vietnam. **IOP Conference Series: Materials Science and Engineering** 1092(1): 012092. DOI: 10.1088/1757-899X/1092/1/012092.

El Atki, Y., I. Aouam, F.E. Kamari, A. Taroq, A. Gourch, B. Lyoussi and A. Abdellaoui. 2019. Antibacterial efficacy of thymol, carvacrol, eugenol and menthol as alternative agents to control the growth of nosocomial infection-bacteria. **Journal of Pharmaceutical Sciences and Research** 11(2): 306–309.

Ervia, Y., Subagiyo and A. Nuril. 2021. Antimicrobial susceptibility and minimum inhibition concentration of *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio harveyi* isolated from a white shrimp (*Litopenaeus vannamei*) pond. **IOP Conference Series: Earth and Environmental Science** 763: 012025. DOI: 10.1088/1755-1315/763/1/012025.

Fidan, H., G. Stefanova, I. Kostova, S. Stankov, S. Damyanova, A. Stoyanova and V.D. Zheljazkov. 2019. Chemical composition and antimicrobial activity of *Laurus nobilis* L. essential oils from Bulgaria. **Molecules** 24(4): 804. DOI: 10.3390/molecules24040804.

Filho, J.G.D.O., G.D.C. Silva, A.C.D. Aguiar, L. Cipriano, H.M.C.D. Azeredo, S.B. Junior and M.D. Ferreira. 2021. Chemical composition and antifungal activity of essential oils and their combinations against *Botrytis cinerea* in strawberries. **Journal of Food Measurement and Characterization** 15: 1815–1825. DOI: 10.1007/s11694-020-00765-x.

Gairola, K., S. Gururani, R. Kumar, O. Prakash, S. Agarwal and S.K. Dubey. 2021. Phytochemical composition, antioxidant, and anti-inflammatory activities of essential oil of *Acmella uliginosa* (Sw.) Cass. grown in North India Terai region of Uttarakhand. **Trends in Phytochemical Research** 5(1): 44–52. DOI: 10.30495/tpr.2021.680495.

Githaiga, B.M., E.M. Gathuru, P.N. Waithaka and L.W. Kiarie. 2018. Determination of antibacterial activity of essential oils from mint (*Mentha spicata*) leaves on selected pathogenic bacteria. **Journal of Drugs and Pharmaceutical Science** 2(2): 8–14. DOI: 10.31248/JDPS2018.015.

Haydari, M., V. Maresca, D. Rigano, A. Taleei, A.A. Shahnejat-Bushehri, J. Hadian, S. Sorbo, M. Guida, C. Manna, M. Piscopo, R. Notariale, F.D. Ruberto, L. Fusaro and A. Basile. 2019. Salicylic acid and melatonin alleviate the effects of heat stress on essential oil composition and antioxidant enzyme activity in *Mentha×Piperita* and *Mentha Arvensis* L. **Antioxidants** 8(11): 547. DOI: 10.3390/antiox8110547.

Hu, F., X.F. Tu, K. Thakur, F. Hu, X.L. Li, Y.S. Zhang, J.G. Zhang and Z.J. Wei. 2019. Comparison of antifungal activity of essential oils from different plants against three fungi. **Food and Chemical Toxicology** 134(3): 110821. DOI: 10.1016/j.fct.2019.110821.

Irkin, R., S. Carikci, S. Akalin and Z. Batu. 2021. Decontamination effect of lemon peel and mint leaf extracts on salad vegetables. **Journal of Food and Nutrition Research** 60(2): 146–152.

Jha, R.K., Y.H. Babikian, H.Y. Babikian, L.V. Khoa, D. Wisoyo, S. Srisombat and B. Jiaravanon. 2016. Efficacy of natural herbal formulation against acute hepatopancreatic necrosis disease (AHPND) causing *Vibrio parahaemolyticus* in *Penaeus vannamei*. **Veterinary Medicine** 2(1): 1–6. DOI: 10.17140/VMOJ-2-109.

Kumar, A., P. Singh, V. Gupta and B. Prakash. 2020. **8-Application of nanotechnology to boost the functional and preservative properties of essential oils.** In: Functional and Preservative Properties of Phytochemicals (ed. B. Prakash), pp. 241–267. Academic Press, Cambridge, Massachusetts, USA. DOI: 10.1016/B978-0-12-818593-3.00008-7.

Li, Z.H., M. Cai, Y.S. Liu, P.L. Sun and S.L. Luo. 2019. Antibacterial activity and mechanisms of essential oil from *Citrus medica* L. *var. sarcodactylis*. **Molecules** 24(8): 1577. DOI: 10.3390/molecules24081577.

Lu, W.C., D.W. Huang, C.C.R. Wang, C.H. Yeh, J.C. Tsai, Y.T. Huang and P.H. Li. 2018. Preparation, characterization, and antimicrobial activity of nanoemulsions incorporating citral essential oil. **Journal of Food and Drug Analysis** 26(1): 82–89. DOI: 10.1016/j.jfda.2016.12.018.

Lykov, A., L. Rachkovskaya, E. Rachkovsky and O. Poveshchenko. 2022. **Effect of the composition of aluminum-silica carrier with menthol, camphor, acidum boricum, and colloidal silver on monocytes lines function.** Proceedings of Ural-Siberian Conference on Computational Technologies in Cognitive Science, Genomics and Biomedicine (CSGB) 2022: 22009851. DOI: 10.1109/CSGB56354.2022.9865261.

Majewska, E., M. Kozłowska, E. Gruczyńska-Sękowska, D. Kowalska and K. Tarnowska. 2019. Lemongrass (*Cymbopogon citratus*) essential oil: extraction, composition, bioactivity and uses for food preservation–a review. **Polish Journal of Food and Nutrition Sciences** 69(4): 327–341. DOI: 10.31883/pjfns/113152.

Majolo, C., P.C. Monteiro, A.V.P.D. Nascimento, F.C.M. Chaves, P.E. Gama, H.R. Bizzo and E.C. Chagas. 2019. Essential oils from five Brazilian piper species as antimicrobials against strains of *Aeromonas hydrophila*. **Journal of Essential Oil Bearing Plants** 22(3): 746–761. DOI: 10.1080/0972060X.2019.1645047

Man, A., L. Santacroce, R. Jacob, A. Mare and L. Man. 2019. Antimicrobial activity of six essential oils against a group of human pathogens: A comparative study. **Pathogens** 8(1): 15. DOI: 10.3390/pathogens8010015.

Manh, H.D. and O.T. Tuyet. 2020. Larvicidal and repellent activity of *Mentha arvensis* L. essential oil against *Aedes aegypti*. **Insects** 11(3): 198. DOI: 10.3390/insects11030198.

Martínez-Pabón, M.C. and M. Ortega-Cuadros. 2020. Thymol, menthol and eucalyptol as agents for microbiological control in the oral cavity: A scoping review. **Revista Colombiana de Ciencias Químico-Farmacéuticas** 49(1): 44–69. DOI: 10.15446/rcciquifa.v49n1.87006.

Melo, R.S., Á.M.A. Azevedo, A.M.G. Pereira, R.R. Rocha, R.M.B. Cavalcante, M.N.C. Matos, P.H.R. Lopes, G.A. Gomes, T.H.S. Rodrigues, H.S.D. Santos, I.L. Ponte, R.A. Costa, G.S. Brito, F.E.A.C. Júnior and V.A. Carneiro. 2019. Chemical composition and antimicrobial effectiveness of *Ocimum gratissimum* L. essential oil against multidrug-resistant isolates of *Staphylococcus aureus* and *Escherichia coli*. **Molecules** 24(21): 3864. DOI: 10.3390/molecules24213864.

Mittal, R., R. Kumar and H.S. Chahal. 2018. Antimicrobial activity of *Ocimum sanctum* leaves extracts and oil. **Journal of Drug Delivery and Therapeutics** 8(6): 201–204. DOI: 10.22270/jddt.v8i6.2166.

Monteiro, P.C., C. Majolo, F.C.M. Chaves, H.R. Bizzo, F.L.A. O'Sullivan and E.C. Chagas. 2020. Antimicrobial activity of essential oils from *Lippia sidoides*, *Ocimum gratissimum* and *Zingiber officinale* against *Aeromonas* spp. **Journal of Essential Oil Research** 33(2): 152–161. DOI: 10.1080/10412905.2020.1848653.

Muralidharan, V. and M. Deecaraman. 2019. *In vitro* susceptibility of commonly used antibiotics against *Vibrio parahaemolyticus*, isolated from finfishes, Chennai, India. **Journal of Infectious Diseases and Treatment** 5(1): 1–4.

Nabila, B., A. Piras, B. Fouzia, D. Falconieri, G. Kheira, F.F. Fedoul and S.R. Majda. 2022. Chemical composition and antibacterial activity of the essential oil of *Laurus nobilis* leaves. **Natural Product Research** 36(4): 989–993. DOI: 10.1080/14786419.2020.1839450.

Nabti, L.Z., F. Sahli, H. Laouer, A. Olowo-Okere, J.G.N. Wandjou and F. Maggi. 2020. Chemical composition and antibacterial activity of essential oils from the algerian endemic *Origanum glandulosum* Desf. against multidrug-resistant uropathogenic *E. coli* isolates. **Antibiotics** 9(1): 29. DOI: 10.3390/antibiotics9010029.

Ogunwande, I.A., O.N. Avoseh, K.N. Olasunkanmi, O.A. Lawal, R. Ascrizzi and G. Flamini. 2019. Chemical composition, anti-nociceptive and anti-inflammatory activities of essential oil of *Bougainvillea glabra*. **Journal of Ethnopharmacology** 232: 188–192. DOI: 10.1016/j.jep.2018.12.017.

Oro, V., S. Krnjajic, M. Tabakovic, J. Stanojević and S. Ilic-Stojanovic. 2020. Nematicidal activity of essential oils on a psychrophilic *Panagrolaimus* sp. (Nematoda: Panagrolaimidae). **Plants** 9(11): 1588. DOI: 10.3390/plants9111588.

Pateiro, M., F.J. Barba, R. Domínguez, A.S. Sant'Ana, A.M. Khaneghah, M. Gavahian, B. Gómez and J.M. Lorenzo. 2018. Essential oils as natural additives to prevent oxidation reactions in meat and meat products: A review. **Food Research International** 113: 156–166. DOI: 10.1016/j.foodres.2018.07.014.

Peichel, C., D.V.T. Nair, G. Dewi, A.M. Donoghue, K.M. Reed and A.K. Johny. 2019. Effect of Lemongrass (*Cymbopogon citratus*) Essential oil on the survival of multidrug-resistant *Salmonella enterica* serovar Heidelberg in contaminated poultry drinking water. **Journal of Applied Poultry Research** 28(4): 1121–1130. DOI: 10.3382/japr/pfz076.

Piras, A., S. Porcedda, D. Falconieri, A. Maxia, M. Gonçalves, C. Cavaleiro and L. Salgueiro. 2019. Antifungal activity of essential oil from *Mentha spicata* L. and *Mentha pulegium* L. growing wild in Sardinia island (Italy). **Natural Product Research** 35(6): 993–999. DOI: 10.1080/14786419.2019.1610755.

Sanli, A. and T. Karadoğan. 2017. Geographical impact on essential oil composition of endemic *Kundmannia anatolica* Hub.-Mor. (Apiaceae). **African Journal of Traditional, Complementary and Alternative Medicines** 14(1): 131–137.

Sevik, R., G. Akarca, M. Kilinc and Ç. Ascioglu. 2021. Chemical composition of tea tree (*Melaleuca alternifolia*) (Maiden & Betche) Cheel essential oil and its antifungal effect on foodborne molds isolated from meat products. **Journal of Essential Oil Bearing Plants** 24(3): 561–570. DOI: 10.1080/0972060X.2021.1942232.

Shanaida, M., N. Hudz, M. Białoń, M. Kryvtsowa, L. Svydenko, A. Filipska and P.P. Wieczorek. 2021. Chromatographic profiles and antimicrobial activity of the essential oils obtained from some species and cultivars of the Mentheae tribe (Lamiaceae). **Saudi Journal of Biological Sciences** 28(11): 6145–6152. DOI: 10.1016/j.sjbs.2021.06.068.

Sharma, S., S. Habib, D. Sahu and J. Gupta. 2021. Chemical properties and therapeutic potential of citral, a monoterpene isolated from lemongrass. **Medicinal Chemistry** 17(1): 2–12. DOI: 10.2174/1573406416666191227111106.

Shendurse, A.M., R.B. Sangwan, A. Kumar, R. Venkateshappa, A.C. Patel, G. Gopikrishna and S.K. Roy. 2021. Phytochemical screening and antibacterial activity of lemongrass (*Cymbopogon citratus*) leaves essential oil. **Journal of Pharmacognosy and Phytochemistry** 10(2): 445–449.

Soltanbeigi, A., M. Özgüven and M.B. Hassanpouraghdam. 2021. Planting-date and cutting-time affect the growth and essential oil composition of *Mentha*× *piperita* and *Mentha arvensis*. **Industrial Crops and Products** 170(7): 113790. DOI: 10.1016/j.indcrop.2021.113790.

Souza, C.F., M.D. Baldissera, B. Baldisserotto, B.M. Heinzmann, J.A. Martos-Sitcha and J.M. Mancera. 2019. Essential oils as stress-reducing agents for fish aquaculture: A review. **Front in Physiol** 10: 785. DOI: 10.3389/fphys.2019.00785.

Sun, H., Y. Li, X. Shi, Y. Lin, Y. Qiu, J. Zhang, Y. Liu, M. Jiang, Z. Zhang, Q. Chen, Q. Sun and Q. Hu. 2014. Association of CRISPR/Cas evolution with *Vibrio parahaemolyticus* virulence factors and genotypes. **Foodborne Pathogens and Disease** 12(1): 68–73. DOI: 10.1089/fpd.2014.1792.

Tamer, C.E., S. Suna and G. Özcan-Sinir. 2019. **14-Toxicological aspects of ingredients used in nonalcoholic beverages.** In: Non-Alcoholic Beverages (eds. A. Mihai Grumezescu and A.M. Holban), pp. 441–481. Woodhead Publishing, Cambridge, UK.

Ugbogu, O.C., E. Okezie, G.O. Agi, C. Ibe, C.N. Ekweogu, V.C. Ude, M.E. Uche, R.O. Nnanna and E.A. Ugbogu. 2021. A review on the traditional uses, phytochemistry, and pharmacological activities of clove basil (*Ocimum gratissimum* L.). **Heliyon** 7(11): e08404. DOI: 10.1016/j.heliyon.2021.e08404.

Vu, T.X., T.B. Tran, C.Q. Hoang, H.T. Nguyen, M.X.B. Phan, A.N. Dao, M.T. Dinh, K. Soytong and H.Q. Nguyen. 2021. Chemical compositions and anti-malassezia properties of Vietnamese *Mentha arvensis* and *Piper betle* essential oils. **International Journal of Agricultural Technology** 17(4): 1619–1630.

World Organisation for Animal Health (OIE). 2019. **Manual of diagnostic test for aquatic animals. Chapter 2.2.1: Acute hepatopancreatic necrosis disease.** https://www.woah.org/en/what-we-do/standards/codes-and-manuals/aquatic-manual-online-access/. Cited 1 Oct 2022.

Yadav, M., R. Dhar, S. Singh and T. Allen. 2019. Comparative study of antimicrobial activity of lemongrass (*Cymbopogon citratus*), clove (*Syzygium aromaticum*), and tulsi (*Ocimum*) essential oils against foodborne pathogens. **Asian Journal of Pharmaceutical and Clinical Research** 12(4): 230–234. DOI: 10.22159/ajpcr.2019.v12i4.29451.

Yuan, W., C.H.M. Teo and H.G. Yuk. 2018. Combined antibacterial activities of essential oil compounds against *Escherichia coli* O157: H7 and their application potential on fresh-cut lettuce. **Food Control** 96: 112–118. DOI: 10.1016/j.foodcont.2018.09.005.

Zhang, Y., J. Wei, H. Chen, Z. Song, H. Guo, Y. Yuan and T. Yue. 2020. Antibacterial activity of essential oils against *Stenotrophomonas maltophilia* and the effect of citral on cell membrane. **LWT–Food Science and Technology** 117(7): 108667. DOI: 10.1016/j.lwt.2019.108667.

Zheng, X., A.F. Feyaerts, P.V. Dijck and P. Bossier. 2020. Inhibitory activity of essential oils against *Vibrio campbellii* and *Vibrio parahaemolyticus*. **Microorganisms** 8(12): 1946. DOI: 10.3390/microorganisms812 1946.