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Research Article

Antimicrobial activity of *Origanum vulgare* and *Cinnamomum verum* essential oils separately and in combination against *Streptococcus iniae*

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Abstract

The study investigates the antimicrobial effect of two herbal essential oils, the leaves of *Origanum vulgare* and the bark of *Cinnamomum verum*, separately and in combination against *S. iniae*. The essential oils were extracted and their chemical composition was analyzed using gas chromatography-mass spectrometry. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth microdilution method. The fractional inhibitory concentration (FIC) method was employed to analyze the combined effect of the essential oils. The MIC of *O. vulgare* and *C. verum* against *S. iniae* were 0.976 mg/L and 100 mg/L, respectively, and the MBC value of oregano and cinnamon essential oils against *S. iniae* were 125 mg/L and 6400 mg/L, respectively. The FIC index was 0.625, which shows that the combined performance of the two essential oils is additive. The major compounds in *Origanum* and *Cinnamomum* essential oils were phenol-2-methyl-5 (1-methylethyl) (48.61%), thymol (16.76%) and cinnamaldehyde (64.5%), respectively. Overall, the results demonstrate that both essential oils exhibit antibacterial effects against *S. iniae*. In conclusion, the combined essential oils of *O. vulgare* and *C. verum* may hold promise as a novel antibacterial agent for the potential treatment and control of streptococcosis caused by *S. iniae*.

Introduction

The most important bacterial disease in aquaculture industry is streptococcosis, known for its septicemic infectious nature (Karsidani *et al.,* 2010). It has been reported in various types of freshwater, saltwater, and brackish water fish and can even infect humans, making it a potential zoonotic disease (Soltani *et al.,* 2005). The most common cause of *streptococcosis* is *S. iniae*, which has emerged as one of the most critical pathogens in recent decades (Kamgar and Ghane, 2012). Conventionally, antibiotics have been the primary approach to control *S. iniae* infections in aquaculture . However, there are many reports of severe antibiotic resistance among aquaculture antibiotics (Caputo *et al.,* 2023; Shin *et al.,* 2023; WU *et al.,* 2023). Furthermore, *S. iniae* has gradually developed resistance to multiple antibiotics, such as kanamycin, neomycin, cephalexin, ampicillin, furazolidone, and sulfamethoxazole (Rattanachaikunsopon and Phumkhachorn, 2010; Zhang *et al.,* 2012). The use of antibiotics to combat fish diseases has drawbacks, including the risk of generating bioaccumulation and environmental pollution; Also, the development of fish vaccines is associated with obstacles such as high prices, time constraints, and a focus on specific pathogens (Ardó *et al.,* 2008; Mondal and Thomas, 2022), Given these challenges, have led to more attention being given to alternatives to antibiotic treatment.

Plant-based therapies have garnered attention due to their established therapeutic efficacy, supported by rigorous scientific investigations (Yang *et al.,* 2020). Essential oils derived from various plants have shown promising antimicrobial properties, with the ability to inhibit the growth of many microorganisms by affecting their plasma and cell membranes (Álvarez-Martínez *et al.,* 2021).

Cinnamomum verum belongs to the Lauraceae family (Jayaprakasha and Rao, 2011). The genus *Cinnamomum* comprises over 250 recognized aromatic species, predominantly distributed across Asia and Australia (Thomas and Kuruvilla, 2012). Among the various species within the genus *Cinnamomum*, *Cinnamomum zeylanicum* Blume (Ceylon cinnamon; also known as *Cinnamomum verum* J. Presl) and *Cinnamomum cassia* J. Presl (*Cassia cinnamon*) stand out as the most economically significant species. (Barceloux, 2008; Muhammad and Dewettinck, 2017). In recent years, the antibacterial properties of *Cinnamomum verum* essential oil have been proven through many studies (Nematollahi *et al.,* 2020; Wijesinghe *et al.,* 2021; dos Santos Franciscato *et al.,* 2022).

*Origanum, a genus within the Lamiaceae family, encompasses over 50 diverse species, with Origanum vulgare L. being the most famous. A prominently acknowledged plant extensively employed for medicinal purposes (*García-Beltrán and Esteban, 2016; Alekseeva et al., 2020*).* The essential oil isolated from *Origanum vulgare L.* has been shown to have biological and pharmacological activities such as antibacterial, fungicidal, and antiviral (Coccimiglio *et al.,* 2016; Leyva-López *et al.,* 2017; Vinciguerra *et al.,* 2019; Simirgiotis *et al.,* 2020; Picoli *et al.,* 2021). Moreover, Studies are demonstrating the effectiveness of *Cinnamomum verum* and

Origanum vulgare L. in controlling and preventing bacterial diseases in fish (Mabrok and Wahdan, 2018; Beltrán *et al.*, 2020; Susanti *et al.*, 2021; Alnahass *et al.*, 2023).

The combined antibacterial effects of these essential oils against *S. iniae* have not been previously reported. This study aims to analyze the chemical composition of essential oils from *Cinnamomum verum* bark and *Origanum vulgare* leaves and evaluate their individual and combined antimicrobial effects against *S. iniae*.

Materials and methods

Plant material and isolation of the essential oils

The bark of Cinnamomum verum and air-dried leaves of *Origanum vulgare* were purchased from an Herbalist in The Tohid Square in MASHHAD City, IRAN, in March 2023. The species were identified by comparison with voucher specimens kept at the Herbarium of Botany, Faculty of Agriculture, University of Tabriz, IRAN (Code 12906-TBZAH for *Cinnamomum verum* and Code 14567-TBZAH for *Origanum vulgare*). The leaves of *O. vulgare* and *C. verum* bark were submitted separately to a hydrodistillation in a Clevenger-type apparatus for four hours. Then, the essential oil was isolated and kept at four ˚C until used for antibacterial testing. Figure 1 shows the Clevenger apparatus and extracting essential oils. The extraction of essential oil from *O. vulgare* utilized 600 grams of plant material, resulting in the production of 1 ml of essential oil. Similarly, for *C. verum* bark, 500 grams were used, yielding a notable 3 mL of essential oil.

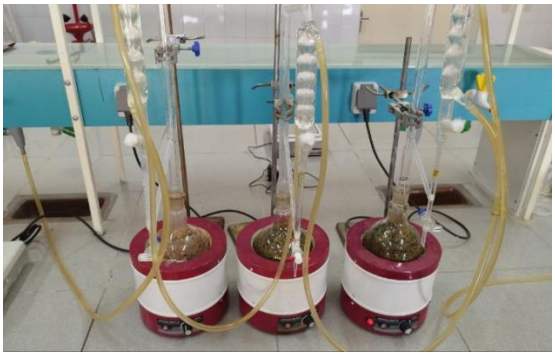

Figure 1: Extracting essential oils with Clevenger apparatus.

This essential oil was used to prepare stock dilution. The stock solution of each plant was prepared by dissolving in dimethyl sulfoxide (DMSO; Sigma-Aldrich). The stock concentration of *C. verum* essential oil was 12800 mg/L, and *O. vulgare* essential oil 2000 mg/L was prepared in DMSO (final concentration of 10%).

(Approvals of the research ethical committee: IR.IAU.TABRIZ.REC.1402.187, 2023.07.04)

Chemical analysis

Gas chromatograph-mass spectrometry (GC/MS) was used to identify and detect the effective chemical composition in the essential oils of *Cinnamomum verum* and *Origanum vulgare* plants (Gas chromatograph model 7890B and mass spectrometer model 5977A manufactured by Agilent USA, equipped with split/splitless injection system and electron bombardment ionization model and has NIST and WILEY mass libraries). To analyze and identify the desired compounds, the HP5-MS column (60 m × 0.25 mm; film thickness 0.25 µm) was

used. The carrier gas was helium at 1.1 mL/min. The injection, interface, and ionization source temperatures were set to 270, 290, and 230 ˚C, respectively. The temperature program of the column started with an initial temperature of 70°C and was kept at this temperature for 3 min; then, the temperature of the column reached 180°C with a gradient of 10°C/min and remained constant at this temperature for 2 min. Finally, with a gradient of 20°C/min, it reached a temperature of 290°C and remained at this temperature for 5 min. The split mode was set as 1:20 and the injection volume was 0.5 µL. The essential oils components were identified by comparison of their retention indices and mass spectra with a NIST/ WILEY mass spectral library. The percentage content was calculated by peak area normalization. Figure 2 shows the GC/MS spectrometer and its injection method.

Figure 2: Gas chromatography/mass spectrometer (right side) and how to inject into it (left side).

Bacterial strain

S. iniae (PTCC 1887) was tested. This strain was obtained from the Persian Type Culture Collection (PTCC[1]) a subset of the Biotechnology Department at the Iranian Research Organization for Science and Technology (IROST). It was maintained on Trypticase Soy Agar with 5% defibrinated sheep blood at 30°C and supplied in a freeze-dried form. Figure 3 shows the bacterial strain.

Figure 3: *Streptococcus iniae* bactrial strain.

[1] PTCC is a member of World Federation for Culture Collections (WFCC) and the UNESCO Microbial Resources Centers Network (MIRCEN). The Site Link: https://irost.org/ptcc/en/

Antimicrobial sensitivity tests

The minimal inhibitory concentration (MIC) test

The Broth microdilution method was used to determine the minimal inhibitory Concentration (MIC) of *C. verum* and *O. vulgare* essential oils (Sarker *et al.*, 2007; Ahn *et al.*, 2012; Bazargani and Rohloff, 2016).

At first, 100 µL of Mueller Hinton Broth medium (Scharlau) was added into each well of a 96_well microtiter microplate, then 100 µL of *C. verum* and *O. vulgare* oils dissolved in DMSO (final concentration of 10%) were added to the first well, and It was diluted two-fold. In the next step, 100 µL of bacterial suspension equivalent to 0.5 McFarland (OD 600 nm=0.8) was added to each of the wells. Afterward, the plate was covered with parafilm tape to prevent dehydration and incubated at 37°C for 24 hours. After incubation, 30 microliters of resazurin 0.01% (Sigma-Aldrich) were added to each of the wells and incubated for 2 hours at 37°C. After incubation, the last well with the lowest concentration of the plant material, indicated by a blue color, was recorded as the MIC value. This test was repeated three times for each sample. A control series was also considered for each microplate, which includes the positive control: the row in which 100 µL of ciprofloxacin (1 mL/mg) was added instead of the essential oil and dilution was performed (culture medium+ antibiotic + bacteria); Negative control: the row in which instead of essential oil, 100 µL of solvent (DMSO 10%) was added in the first well and dilution was done (culture medium + DMSO + bacteria); Controlling the sterility of plant materials: the row in which 100 µL of essential oil was added in the first well and dilution was done (culture medium + essential oil); Control of bacterial growth: the row in which 100 µL of the standard strain of *S. iniae* (PTCC1887) was added in the first well and dilution was done (culture medium + bacteria).

The minimal bactericidal concentration (MBC) test

MBC is the lowest concentration of plant material that can kill 99.9% of inoculated bacteria during 24 hours of incubation at 37°C (Sasidharan *et al.*, 2014). 100 µL of the bacterial suspension from the MIC well and the wells containing essential oil concentrations higher than the MIC (all blue wells) were removed. Subsequently, the suspension corresponding to each of the mentioned dilutions was inoculated on different Mueller Hinton agar mediums (Ibresco). The plates were examined for bacterial growth after 24 hours of incubation at 37°C. The concentration of the well that killed 99.9% of the bacteria inoculated on the Mueller Hinton agar medium was recorded as MBC. This test was repeated three times for each sample.

The Fractional Inhibitory Concentration (FIC) method

The combined effect of two essential oils, *C. verum* and *O. vulgare*, against *S. iniae* bacteria was investigated and implemented based on the methods described by Pei *et al.* (2009) and Mulyaningsih *et al.* (2010). After determining the MIC of each essential oil separately, concentrations above and below the MIC (usually seven wells along

with the MIC well) were used to determine the FIC.

In this method, the final volume of each microplate well was 200 µL. Subsequently, 100 µL of bacteria, previously measured using a spectrophotometer, was added to each well in the first row. Following this, 100 µL of various concentrations of *C. verum* oil was added to each well in the same row. In the first column, different concentrations of *O. vulgare* were added to each well. This method allowed for the simultaneous determination of the single MIC and combined MIC of each compound. The remaining wells received a mixture of different dilutions of both essential oils in the first column, with 100 µL of bacteria and 50 µL of various dilutions of the oils added to each well in the first row. The microplate was then shaken for 30 seconds and incubated for 24 hours at 37°C. Following incubation, 30 µL of 0.01% resazurin was added to every well and incubated for an additional 2 hours at 37°C to determine the FIC.

Results

Chemical composition of the essential oils

The chromatograms related to the active ingredients in *C. verum* and *O. vulgare* essential oils are shown in Figures 4 and 5. Table 1 lists the constituents of *C. verum* essential oil in the order of the peaks shown in the chromatogram. According to the table, 17 compounds have been identified. The 'Area sum %' indicates the percentage composition of each compound within the total oil content. Cinnamaldehyde, representing 64.5% of the oil content and corresponding to the highest peak in the chromatogram, emerges as the most prominent compound.

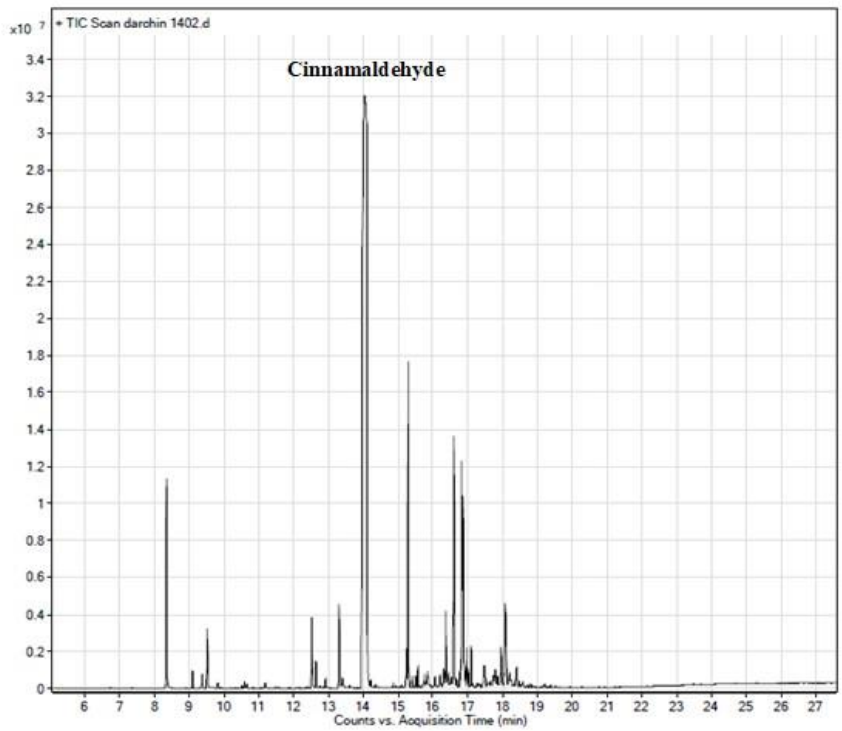

Figure 4: Chromatogram of *Cinnamomum verum* essential oil.

Table 2 lists the constituents of *O. vulgare* essential oil according to peak order in the chromatogram. This table identifies 20 effective compounds. The most prevalent compound in *O. vulgare* oil, as indicated by the percentage composition, is Phenol, 2-methyl-5 (1-methylethyl), also known as carvacrol, at 48.61%. Thymol follows at 16.76% and constitutes the highest peak in the chromatogram. The molecular structures of these compounds are depicted in Figure 6.

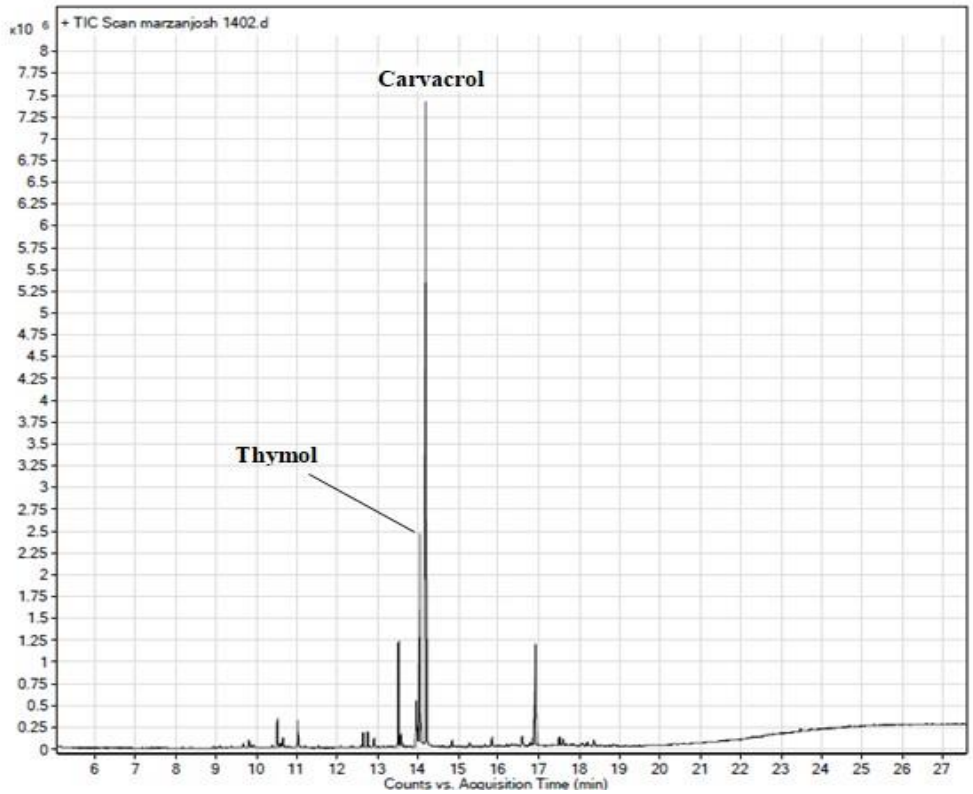

Figure 5: Chromatogram of *Origanum vulgare* essential oil.

Table 1: Compound of *Cinnamomum verum* essential oil.

Compound Label	Area sum %
Styrene	3.89
Benzaldehyde	1.18
Benzenepropanal	1.29
(Z)-3-Phenylacrylaldehyde	1.64
Cinnamaldehyde, (E)-	64.51
1,2,4-Metheno-1H-indene, octahedron-1,7adimethyl- 5-(1-methyl ethyl)-, [1S-(1.alpha.,2.alpha.,3a.beta.,4.alpha.,5.alpha.,7a. beta.,8S*)]-C15H24 10 Cpd 7: .alfa.-Copaene 15.284	0.65
.alfa.-Copaene	5.62
.gamma.-Muurolene	1.3
.alpha.-Muurolene	5.35
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7- dimethyl-1-(1-methyl ethyl)-, (1S-cis)-	3.85
cis-Calamenene	4.3
.alpha.-Calacorene	0.68
Caryophyllenyl alcohol	0.67
Di-epi-1,10-cubenol	1.09
.tau.-Muurolol	2.67
.tau.-Cadinol	0.66
Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	0.66

Table 2: Compound of *Origanum vulgare* essential oil.

Compound Label	Area sum %
1,2:4,5:9,10-Triepoxydecane	0.53
3-Octanone	0.65
o-Cymene	2.13
Eucalyptol	0.7
.gamma.-Terpinene	2.05
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1Sendo)-	1.24
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	1.2
.alpha.-Terpineol	0.84
Benzene, 1-methoxy-4-methyl-2-(1-Methyl ethyl)-	7.83
Pulegone	1.51
Cinnamaldehyde, (E)-	4.69
Thymol	16.76
*Phenol, 2-methyl-5-(1-methyl ethyl)-	48.61
2-Cyclohexen-1-one, 3-methyl-6-(1-methylethylidene)-	0.56
Caryophyllene	0.77
.beta.-Bisabolene	1.04
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyl ethyl)-, (1S-cis)-	0.53
Cyclohexene, 4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methyl-	7.14
1H-Cycloprop[e]azulen-7-ol, decahedron-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]	0.67
.alpha.-Bisabolol	0.53

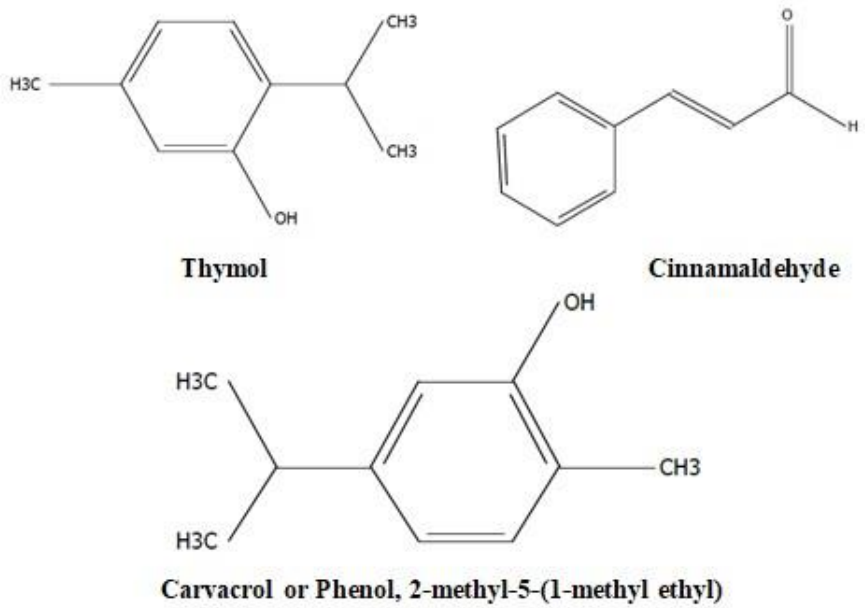

Figure 6: Chemical structures of the main antimicrobial identified in *Cinnamomum verum* and *Origanum vulgare*.

* Carvacrol

MIC and MBC of Cinnamomum verum and Origanum vulgare essential oils

Based on the results, the MIC level for *C. verum* oil in the *S. iniae* strain was 100 mg/L. Also, the MIC value of *O. vulgare* oil in the *S. iniae* strain was 0.976 mg/L. The final Figure of MIC adjusted using the broth microdilution method and using resazurin is shown in Figure 7.

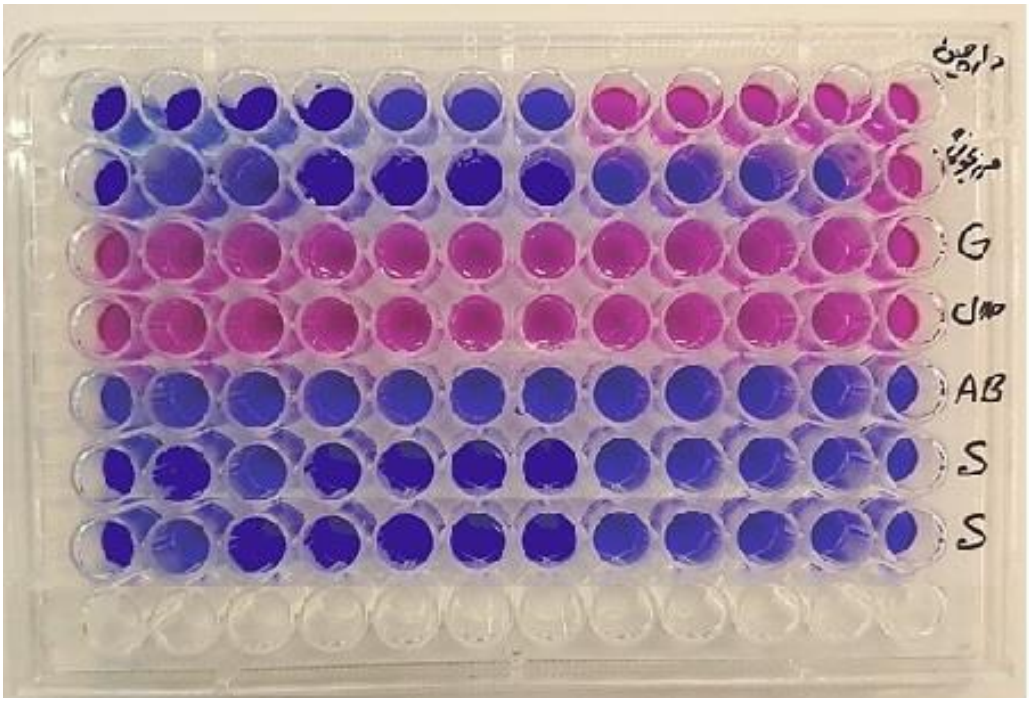

Figure 7: Minimum inhibitory concentration (MIC) test using *Cinnamomum verum* and *Origanum vulgare* essences on the Streptococcus iniae strain under study after adding the rejuvenator of resazurin. Row one: Cinnamomum verum essential oil, row two: Origanum vulgare essential oil, row G: bacterial growth control, fourth row: solvent control or DMSO 10%, row AB: positive control and row S: sterility control of essential oils.

The MBC of *C. verum* and *O. vulgare* essential oil in *S. iniae* was 6400 mg/L and 125 mg/L, respectively (Table 3). The final photo taken from the cultures carried out to determine the MBC of the studied materials is shown in Figures 8 and 9.

Table 3: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of *Cinnamomum verum* and *Origanum vulgare* essential oils against *S. iniae*.

Essential oil	MBC (mg/L)	MIC (mg/L)
Cinnamomum verum	6400	**100**
Origanum vulgare	125	**0.976**

FIC index results

Table 4 shows the result of evaluating the combined antibacterial properties of *C. verum* and *O. vulgare* essential oils using the Broth microdilution method against *S. iniae*. As shown in the table, the FIC index was 0.625, which shows that the combined performance of the two essential oils of *C. verum* and *O. vulgare* has an additive effect.

Also, the final Figure taken from the FIC result is shown in Figure 10.

Figure 8: Minimum bactericidal concentration (MBC) test against *Streptococcus iniae* strain by *Cinnamomum verum* essential oil. The numbers inside the plates indicate the number of the well of the microplate containing essential oil and bacteria.

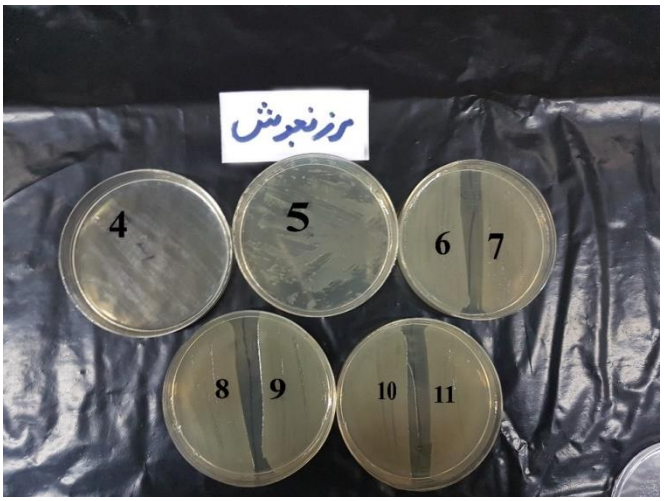

Figure 9: Minimum bactericidal concentration (MBC) test against *Streptococcus iniae* strain by *Cinnamomum verum* essential oil. The numbers inside the plates indicate the number of the well of the microplate containing essential oil and bacteria.

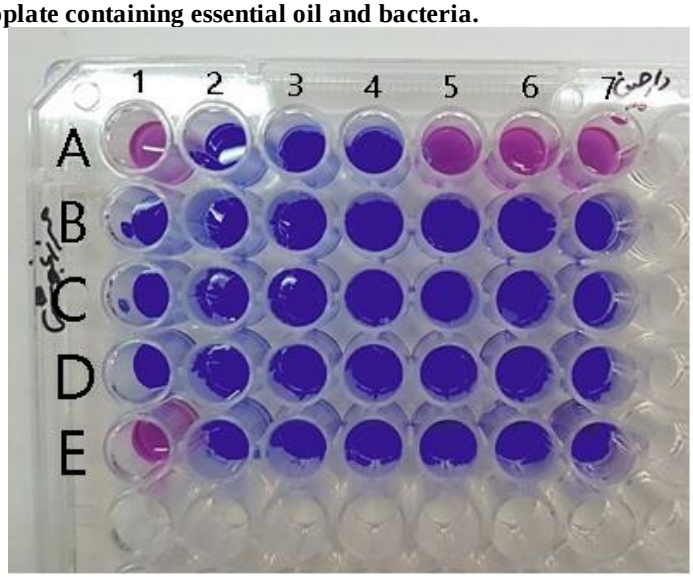

Figure 10: Fractional inhibitory concentration (FIC) index of the combination of *Cinnamomum verum* and *Origanum vulgare* essential oils. Well A1 is used as a growth control.

Discussion

Our study specifies the antimicrobioal activity of *Origanum vulgare* and *Cinnamomum verum* oils against *S. iniae*, proving their individual effectiveness through MIC and MBC determinations. These findings align with prior studies (Pathirana *et al.,* 2019; Fakharzadeh *et al.*, 2020). Genetic factors, agro-climate conditions, cultivation methods, soil attributes, and harvesting timing influence the production and quality variations of essential oils. Consequently, these oils' chemical composition and predominant compounds exhibit diversity across different environments. This diversity significantly influences their bioactivity, closely correlating with alterations in their composition (Teles *et al.,* 2019). The MIC of *O. vulgare* oil against *S. iniae* is notably low (0.976 mg/L), indicating its potent inhibitory effect on bacterial growth.

Additionally, the MBC value of 125 mg/L underscored the bactericidal capacity of this essential oil against the target pathogen; in contrast, the efficacy of *C. verum* oil against *S. iniae* exhibited higher MIC and MBC values (100 mg/L and 6400 mg/L, respectively), its inhibitory and bactericidal concentrations were comparatively higher than those of *O. vulgare*. These findings underscore the differing efficacy and suggest varying degrees of potency in inhibiting and eradicating bacterial growth; hence, an interpretation arises suggesting the superior antibacterial efficacy of *O. vulgare* over *C. verum*. The significance of this finding receives emphasis from the Ebani *et al.* (2020) study, where they observed heightened antimicrobial efficacy in *O. vulgare* compared to *Cinnamomum* against multiple strains of *Staphylococcus* bacteria. The chemical constituents of essential oils play a pivotal role in determining their antibacterial efficacy.

The GC/MS analysis revealed prominent compounds within *C. verum* and *O. vulgare* oils with *Cinnamaldehyde* identified as the primary constituent in *Cinnamomum* (Echegoyen and and Nerín, 2015; Procopio *et al.*, 2018). Consistent research findings have established a strong connection between Cinnamaldehyde and its potent antibacterial properties, highlighting its effectiveness against a diverse range of bacterial strains (Silva *et al.*, 2016; Friedman, 2017; Vasconcelos *et al.*, 2018). According to the review article by Doyle and Stephens (2019), Cinnamaldehyde exhibits action by several antibacterial mechanisms: 1- It disrupts cell division by binding to FtsZ proteins, 2- Degrades the extracellular matrix of biofilms, and 3- Interferes with quorum sensing, curtailing coordinated bacterial behaviors. Moreover,4- It compromises cell membrane integrity, 5- Alters cell morphology, 6- Cinnamaldehyde reduces intracellular ATP levels in bacteria like *E. coli* and *Listeria* (Oussalah *et al*., 2006), *Salmonella* (Silva *et al.*, 2018), *Mycobacterium avium subsp. Paratuberculosis* (Nowotarska *et al.,* 2017) disrupts vital energy processes crucial for their survival. 7- Its inhibition of membrane-bound ATPases disrupts the proton motive force, leading to compromised cellular functions and decreased ATP levels. Collectively, these actions impede bacterial replication and

survival, showcasing the multifaceted antibacterial potential of Cinnamaldehyde. The antimicrobial activity of *O. vulgare* essential oils could be associated with Carvacrol and thymol, the main component of *O. vulgare* oil (Hou *et al.,* 2020). Researchers have shown that Thymol and Carvacrol employ diverse mechanisms to combat bacterial activity. Based on the review article by Kachur and Suntres (2020), 1- Their actions involve disrupting bacterial membranes (It has been proven as a significant mechanism), 2- Inhibiting efflux pumps crucial for resistance, 3- preventing and dismantling biofilms, 4- impeding bacterial motility, and 5- Inhibiting membrane-bound ATPases vital for bacterial function. In essence, Thymol and Carvacrol's diverse antibacterial strategies highlight their potential as effective agents against bacteria.

To the best of our knowledge, this research is the pioneering exploration into the joint effects of essential oils from *C. verum* and *O. vulgare* on *S. iniae*. The combination of essential oil compounds can result in four potential effects: synergistic, additive, indifferent, or antagonistic interactions. Specifically, an additive effect occurs when the combined impact equals the cumulative sum of the individual effects observed (Bassolé and Juliani, 2012). This report has shown that an FIC index of 0.625 indicates an additive effect when the essential oils are combined. In the study of Pouyan *et al.* (2021), a combination of *Cinnamomum camphora* and *O. vulgare* essential oils against bla CTX-M Producing *Escherichia coli* isolated from poultry *colibacillosis* demonstrated an additive effect in 40% of the samples, aligning with findings in our investigation. In addition, half of the samples demonstrated a synergistic effect, while the rest were ineffective.

Pie *et al.* (2009) examined the antibacterial characteristics of essential oil components against *E. coli*, noting both additive and synergistic effects. Similarly, El Atki *et al.* (2020) showed that mixing *cinnamon* essential oil with Carvacrol and thymol compounds resulted in an additive effect against *E. coli* and *S. aureus* pathogens, a finding supported by our study. Despite the limited number of studies investigating the combined antibacterial mechanism of essential oils, El Atki *et al.* (2020) mentioned that thymol or Carvacrol might increase cell membrane permeability, facilitating the penetration of *cinnamon* compounds into cells and enabling their interaction with proteins and nucleic acids. Furthermore, the interaction could involve the simultaneous disruption of multiple bacterial targets, such as cell division and membrane integrity, and the inhibition of efflux pumps. This coordinated attack on various bacterial processes results in an enhanced antibacterial effect against *S. iniae*.

The observed antimicrobial activities of both essential oils, individually and in combination, against the studied microorganism, advocate for their potential use in treating and preventing *Streptococcosis* diseases. However, further investigations into the safety and toxicity of these essential oils are crucial for their clinical evaluation and application in the treatment of infectious diseases.

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Conflicts of interest

The Authors declare that they have no conflict of interest.

References

Ahn, S.J., Cho, E.J., Kim, H.J., Park, S.N., Lim, Y.K. and Kook, J.K., 2012. The antimicrobial effects of deglycyrrhizinated licorice root extract on *Streptococcus* mutans UA159 in both planktonic and biofilm cultures. *Anaerobe*, 18, 590-596. DOI:10.1016/j.anaerobe.2012.10.005

Alekseeva, M., Zagorcheva, T., Atanassov, I. and Rusanov, K., 2020. *Origanum vulgare* L.-a review on genetic diversity, cultivation, biological activities and perspectives for molecular breeding. *Bulgarian Journal of Agricultural Science*, 26(**6**), 1183–1197.

Alnahass, R., Abd El-Latif, H.H., Abdel-Latif, H.M., Ibrahim, M.S. and Talat, D., 2023. Exploring antimicrobial potential of cinnamon, clove, peppermint and black cumin essential oils against fish bacterial pathogens with an emphasis on the dietary supplementation effects of cinnamon oil on striped catfish (*Pangasianodon hypophthalmus*). *Journal of Advanced Veterinary Research*, 13(**6**), 1173-1180.

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

Ardó, L., Yin, G., Xu, P., Váradi, L., Szigeti, G., Jeney, Z. and Jeney, G., 2008. Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of Nile tilapia (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila*. *Aquaculture*, 275(**1-4**), 26-33. DOI:10.1016/j.aquaculture.2007.12.022

Barceloux, D.G. 2008. Medical toxicology of natural substances: foods, fungi, medicinal herbs, plants, and venomous animals, *John Wiley and Sons*, United States of America, 1–1157.

Bassolé, I.H.N. and Juliani, H.R., 2012. Essential oils in combination and their antimicrobial properties. *Molecules*, 17(**4**), 3989-4006. DOI:10.3390/molecules17043989

Bazargani, M.M. and Rohloff, J., 2016. Antibiofilm activity of essential oils and plant extracts against *Staphylococcus aureus* and *Escherichia coli* biofilms. *Food Control*, 61, 156-164. DOI:10.1016/j.foodcont.2015.09.036

Beltrán, J.M.G., Silvera, D.G., Ruiz, C.E., Campo, V., Chupani, L., Faggio, C. and Esteban, M.Á., 2020. Effects of dietary *Origanum vulgare* on gilthead seabream (*Sparus aurata* L.) immune

and antioxidant status. *Fish and Shellfish Immunology*, 99, 452-461. DOI:10.1016/j.fsi.2020.02.040

Caputo, A., Bondad-Reantaso, M.G., Karunasagar, I., Hao, B., Gaunt, P., Verner-Jeffreys, D., Fridman, S. and Dorado-Garcia, A., 2023. Antimicrobial resistance in aquaculture: A global analysis of literature and national action plans. *Reviews in Aquaculture*, 15(**2**), 568-578. DOI: 10.1111/raq.12741

Coccimiglio, J., Alipour, M., Jiang, Z.H., Gottardo, C. and Suntres, Z., 2016. Antioxidant, antibacterial, and cytotoxic activities of the ethanolic *Origanum vulgare* extract and its major constituents. *Oxidative Medicine and Cellular Longevity*, 1404505. DOI:10.1155/2016/1404505

Dos Santos Franciscato, L.M.S., Ariati, A.M., Picolloto, A.M., Raia, R.Z., Barbosa, V.A., Bittencourt, P.R.S., Dos Reis Souza, M.R., Sakai, O.A., Ângelo, E.A. and Moritz, C.M.F., 2022. Thermal Properties of Cinnamon (*Cinnamomum verum*) Essential Oil and Its Antibacterial Activity. *Research, Society and Development*, 11(**13**), e567111335942-e567111335942. DOI:10.33448/rsd-v11i13.35942

Doyle, A.A. and Stephens, J.C., 2019. A review of cinnamaldehyde and its derivatives as antibacterial agents. *Fitoterapia*, 139, 104405. DOI:10.1016/j.fitote.2019.104405

Ebani, V.V., Bertelloni, F., Najar, B., Nardoni, S., Pistelli, L. and Mancianti, F., 2020. Antimicrobial activity of essential oils against *Staphylococcus* and *Malassezia* strains isolated from canine dermatitis. *Microorganisms*, 8(**2**), 252. DOI:10.3390/microorganisms8020252

Echegoyen, Y. and Nerín, C., 2015. Performance of an active paper based on cinnamon essential oil in mushrooms quality. *Food Chemistry*, 170, 30-36. DOI: 10.1016/j.foodchem.2014.08.032

El Atki, Y., Aouam, I., Taroq, A., El Kamari, F., Timinouni, M., Lyoussi, B. and Abdellaoui, A., 2020. Antibacterial effect of combination of cinnamon essential oil and thymol, carvacrol, eugenol, or geraniol. *Journal of Reports in Pharmaceutical Sciences*, 9**,** 104-109. DOI:10.4103/jrptps.JRPTPS_25_19

Fakharzadeh, M.E., Haghighi, M., Sharifrouhani, M., Sharifpoor, I. and Hamidi, M., 2020. An In vitro and in vivo study on antimicrobial activity of *Origanum vulgare* extract and its nano form against *Streptococcus iniae* in rainbow trout (*Oncorhynchus mykiss*). *Iranian Journal of Fisheries Sciences*, 19(**5**), 2454-2463. DOI:10.22092/IJFS.2020.122452

Friedman, M., 2017. Chemistry, antimicrobial mechanisms, and antibiotic activities of cinnamaldehyde against pathogenic bacteria in animal feeds and human foods. *Journal of Agricultural and Food Chemistry*, 65(**48**), 10406-10423. DOI:10.1021/acs.jafc.7b04344

García-Beltrán, J.M. and Esteban, M.A., 2016. Properties and applications of plants of *Origanum* sp. genus. *SM Journal of Biology* , 2, 1006-1015.

Hou, H., Zhang, X., Zhao, T. and Zhou, L., 2020. Effects of *Origanum vulgare*

essential oil and its two main components, carvacrol and thymol, on the plant pathogen *Botrytis cinerea*. *PeerJ*, 8, e9626. DOI:10.7717/peerj.9626

Jayaprakasha, G.K. and Rao, L.J.M., 2011. Chemistry, biogenesis, and biological activities of *Cinnamomum zeylanicum*. *Critical Reviews in Food Science and Nutrition*, 51(**6**), 547-562. DOI: 10.1080/10408391003699550

Kachur, K. and Suntres, Z., 2020. The antibacterial properties of phenolic isomers, carvacrol and thymol. *Critical reviews in food science and nutrition*, 60(**18**), 3042-3053. DOI:10.1080/10408398.2019.1675585

Kamgar, M. and Ghane, M., 2012. Evaluation of *Bacillus subtilis* effect as probiotic on hematological parameters of rainbow trout, *Oncorhynchus mykiss* (Walbaum) following experimental infection with *Streptococcus iniae*. *Journal of Fisheries and Aquatic Science*, 7(**6**), 422. DOI:10.3923/jfas.2012.422.430. [In Persian]

Karsidani, S.H., Soltani, M., Nikbakhat-Brojeni, G., Ghasemi, M. and Skall, H.F., 2010. Molecular epidemiology of zoonotic *streptococcosis/lactococcosis* in rainbow trout (*Oncorhynchus mykiss*) aquaculture in Iran. *Iranian Journal of Microbiology*, 2(**4**), 198.

Leyva-López, N., Gutiérrez-Grijalva, E.P., Vazquez-Olivo, G. and Heredia, J.B., 2017. Essential oils of oregano: Biological activity beyond their antimicrobial properties. *Molecules*, 22(**6**), 989. DOI:10.3390/molecules22060989

Mabrok, M.A.E. and Wahdan, A., 2018. The immune modulatory effect of oregano (*Origanum vulgare* L.) essential oil on *Tilapia zillii* following intraperitoneal infection with *Vibrio anguillarum*. *Aquaculture International*, 26, 1147-1160. DOI:10.1007/s10499-018-0274-y

Mondal, H. and Thomas, J., 2022. A review on the recent advances and application of vaccines against fish pathogens in aquaculture. *Aquaculture International*, 30(**4**), 1971-2000. DOI:10.1007/s10499-022-00884-w

Muhammad, D.R.A. and Dewettinck, K., 2017. Cinnamon and its derivatives as potential ingredient in functional food—A review. *International journal of food properties*, 20, 2237-2263. DOI:10.1080/10942912.2017.1369102

Mulyaningsih, S., Sporer, F., Zimmermann, S., Reichling, J. and Wink, M., 2010. Synergistic properties of the terpenoids aromadendrene and 1, 8-cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. *Phytomedicine*, 17(**13**), 1061-1066. DOI:10.1016/j.phymed.2010.06.018

Nematollahi, Z., Ebrahimi, M., Raeisi, M., Dadban Shahamat, Y., Ghodsi Moghadam, M., Hashemi, M., Shabani, S. and Shirzad, H., 2020. The antibacterial activity of cinnamon essential oil against foodborne bacteria: a mini-review. *Journal of Human Environment and Health Promotion*, 6(**3**), 101-105. DOI:10.29252/jhehp.6.3.1

Nowotarska, S.W., Nowotarski, K., Grant, I.R., Elliott, C.T., Friedman, M. and Situ, C., 2017. Mechanisms of antimicrobial action of cinnamon and oregano oils, cinnamaldehyde, carvacrol, 2, 5-dihydroxybenzaldehyde, and 2-hydroxy-5-methoxybenzaldehyde against *Mycobacterium avium* subsp. *paratuberculosis* (Map). *Foods*, 6(**9**), 72. DOI:10.3390/foods6090072

Oussalah, M., Caillet, S. and Lacroix, M., 2006. Mechanism of action of Spanish oregano, Chinese cinnamon, and savory essential oils against cell membranes and walls of *Escherichia coli* O157: H7 and *Listeria monocytogenes*. *Journal of food protection*, 69(**5**), 1046-1055. DOI:10.4315/0362-028X-69.5.1046

Pathirana, H.N.K.S., Wimalasena, S.H.M.P., De Silva, B.C.J., Hossain, S. and Heo, G.J., 2019. Antibacterial activity of cinnamon (*Cinnamomum zeylanicum*) essential oil and cinnamaldehyde against fish pathogenic bacteria isolated from cultured olive flounder Paralichthys olivaceus. *Indian Journal of Fisheries*, 66(**2**), 86-92. DOI:10.21077/ijf.2019.66.2.85023-12

Pei, R.S., Zhou, F., Ji, B.P. and Xu, J., 2009. Evaluation of combined antibacterial effects of eugenol, cinnamaldehyde, thymol, and carvacrol against *E. coli* with an improved method. *Journal of food science*, 74(**7**), M379-M383. DOI:10.1111/j.1750-3841.2009.01287.x

Picoli, T., Waller, S.B., Hoffmann, J.F., Peter, C.M., da Silva Barcelos, L., Lopes, M.G., de Faria, R.O., Cleff, M.B., de Oliveira Hübner, S., de Lima, M. and Fischer, G., 2021. Antiviral and virucidal potential of *Origanum vulgare* Linn.(oregano) extracts against Bovine alphaherpesvirus 1 (BoHV-1). *Research, Society and Development*, 10(**5**), e28410514979-e28410514979. DOI:10.33448/rsd-v10i5.14979

Pouyan, S., Kafshdouzan, K. and Jebelli, A., 2021. Synergistic effect of *cinnamomum camphora* and *origanum vulgare* essential oils against bla ctx-m producing *escherichia coli* isolated from poultry *colibacillosis*. *Journal of Medical Bacteriology*, 10 (**1, 2**), 20-29.

Procopio, F.R., Oriani, V.B., Paulino, B.N., Do Prado-Silva, L., Pastore, G.M., Sant'Ana, A.S. and Hubinger, M.D., 2018. Solid lipid microparticles loaded with cinnamon oleoresin: Characterization, stability and antimicrobial activity. *Food Research International*, 113, 351-361. DOI:10.1016/j.foodres.2018.07.026

Rattanachaikunsopon, P. and Phumkhachorn, P., 2010. Potential of cinnamon (*Cinnamomum verum*) oil to control *Streptococcus iniae* infection in tilapia (*Oreochromis niloticus*). *Fisheries Science*, 76, 287-293. DOI:10.1007/s12562-010-0218-6

Sarker, S.D., Nahar, L. and Kumarasamy, Y., 2007. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods*, 42(**4**), 321-324. DOI:10.1016/j.ymeth.2007.01.006

Sasidharan, N.K., Sreekala, S.R., Jacob, J. and Nambisan, B., 2014. In vitro synergistic effect of curcumin in

combination with third generation cephalosporins against bacteria associated with infectious diarrhea. *BioMed Research International*, 2014, 561456. DOI:10.1155/2014/561456

Shin, S.B., Cho, S.R. and Mok, J.S., 2023. Antimicrobial Resistance of Fecal-Indicator Bacteria Isolated from Aquatic Animal Farms along the Korean Coast. *Aquaculture Research*, 5014754. DOI:10.1155/2023/5014754

Silva, L.N., Zimmer, K.R., Macedo, A.J. and Trentin, D.S., 2016. Plant natural products targeting bacterial virulence factors. *Chemical reviews*, 116(**16**), 9162-9236. DOI:10.1021/acs.chemrev.6b00184

Silva, A.F., Dos Santos, A.R., Trevisan, D.A.C., Ribeiro, A.B., Campanerut-Sá, P.A.Z., Kukolj, C., de Souza, E.M., Cardoso, R.F., Svidzinski, T.I.E., de Abreu Filho, B.A. and Junior, M.M., 2018. Cinnamaldehyde induces changes in the protein profile of *Salmonella Typhimurium* biofilm. *Research in microbiology*, 169(**1**), 33-43. DOI:10.1016/j.resmic.2017.09.007

Simirgiotis, M.J., Burton, D., Parra, F., López, J., Muñoz, P., Escobar, H. and Parra, C., 2020. Antioxidant and antibacterial capacities of *Origanum vulgare* L. essential oil from the arid Andean Region of Chile and its chemical characterization by GC-MS. *Metabolites*, 10(**10**), 414. DOI:10.3390/metabo10100414

Soltani, M., Jamshidi, S. and Sharifpour, I., 2005. *Streptococcosis* caused by *Streptococcus iniae* in farmed rainbow trout (*Oncorhynchys mykiss*) in Iran: biophysical characteristics and pathogenesis. *Bulletin of the European Association of Fish Pathologists*, 25(**3**), 95-106.

Susanti, E., Wahjuningrum, D., Nuryati, S. and Setiawati, M., 2021. The effectiveness of cinnamon powder and cinnamon leaf extract to prevent Aeromonas hydrophila infection on striped catfish Pangasianodon hypophthalamus. *Jurnal Akuakultur Indonesia*, 20(**2**), 163-173. DOI:10.19027/jai.20.2.163-173

Teles, A.M., Rosa, T.D.D.S., Mouchrek, A.N., Abreu-Silva, A.L., Calabrese, K.D.S. and Almeida-Souza, F., 2019. *Cinnamomum zeylanicum*, *Origanum vulgare*, and *Curcuma longa* essential oils: chemical composition, antimicrobial and antileishmanial activity. *Evidence-Based Complementary and Alternative Medicine*, 2421695. DOI:10.1155/2019/2421695

Thomas, j., and Kuruvilla, K.M., 2012. cinnamon. In k. v. peter (Ed.), *Handbook of herbs and spices* (Second ed., Vol. 1, pp. 182-196). Woodhead Publishing.

Vasconcelos, N.G., Croda, J. and Simionatto, S., 2018. Antibacterial mechanisms of cinnamon and its constituents: A review. *Microbial Pathogenesis*, 120, 198-203. DOI:10.1016/j.micpath.2018.04.036

Vinciguerra, V., Rojas, F., Tedesco, V., Giusiano, G. and Angiolella, L., 2019. Chemical characterization and antifungal activity of *Origanum vulgare*, *Thymus vulgaris* essential oils and carvacrol against *Malassezia furfur*. *Natural Product*

Research, 33(**22**), 3273-3277. DOI:10.1080/14786419.2018.1468325

Wijesinghe, G.K., Feiria, S.B., Maia, F.C., Oliveira, T.R., Joia, F., Barbosa, J.P., Boni, G.C. and HÖfling, J.F., 2021. In-vitro antibacterial and antibiofilm activity of *Cinnamomum verum* leaf oil against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. *Anais da Academia Brasileira de Ciências*, 93, e20201507. DOI:10.1590/0001-3765202120201507

Wu, J., Ye, F., Qu, J. and Dai, Z., 2023. Insight into the Antibiotic Resistance of Bacteria Isolated from Popular Aquatic Products Collected in Zhejiang, China. *Polish Journal of Microbiology*, 72(**1**), 61-67. DOI:10.33073/pjm-2023-010

Yang, Y., Islam, M.S., Wang, J., Li, Y. and Chen, X., 2020. Traditional Chinese medicine in the treatment of patients infected with 2019-new coronavirus (SARS-CoV-2): a review and perspective. *International journal of biological sciences*, 16(**10**), 1708. DOI:10.7150/ijbs.45538

Zhang L., Jie X., Jingang H.,Yunxia X., Wenting M., Shouming F., 2012. Isolation and identification of pathogenetic *Streptococcus iniae* from *Selenotoca multifasciata*. *Jorunal of Huazhong Agricultural University*, 31(**1**), 95-99. DOI: 1000-24212012）01-0095-05