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Research Article **Araştırma Makalesi**

In Vitro Antibacterial Activity of *Origanum onites* and *Mentha spicata* subs. *tomentosa* Essential Oil Nanoemulsions Against Bacterial Fish Pathogens

Origanum onites ve *Mentha spicata* subs. *tomentosa* Uçucu Yağ Nanoemülsiyonlarının Bakteriyel Balık Patojenlerine Karşı in Vitro Antibakteriyel Aktivitesi

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Abstract: The current study aimed to determine the antimicrobial activities of two different aromatic plants (*Origanum onites, Mentha spicata* subs. *tomentosa*) essential oils, and their nanoemulsion formulations against six common fish pathogens, *Pseudomonas aeruginosa, Vibrio parahaemolyticus, Aeromonas veronii, Vibrio alginolyticus, Yersinia ruckeri,* and *Lactococcus garvieae*. The main components of *Mentha spicata* subs. *tomentosa* essential oil (MEO) were piperitone (25.01%), eucalyptol (1,8-cineole) (19.53%), pulegone (14.50%) and, Piperitenone (10.98%). The major components of *Origanum onites* essential oil (OEO) which were carvacrol (46.17%) and, p-cymene (13.05%) were detected. The antibacterial effects of OEO and MEO and their nanoemulsions were determined by using the agar disc diffusion method. The OEO and its nanoemulsions were extremely effective against the Gram-negative *Aeromonas veronii* than the positive control (enrofloxacin). In addition, it was observed that OEO nanoemulsion was more effective than OEO in terms of antibacterial activity. MEO and its nanoemulsions were found to have low activity against fish pathogens, however, there was no activity on *Aeromonas veronii* and *Lactococcus garvieae.*

Keywords

- *Origanum onites*
- *Mentha spicata subs.*
- *Tomentosa*
- Bacterial fish pathogen
- Antibacterial activity

Özet: Bu çalışmanın amacı, iki farklı aromatik bitkinin (*Origanum onites, Mentha spicata* subs. *tomentosa*) uçucu yağlarının ve bunların nanoemülsiyon formülasyonlarının altı farklı balık patojeni *Pseudomonas aeruginosa, Vibrio parahaemolyticus, Aeoromonas veronii, Yersinia ruckeri, Lactococcus garvieae* ve *Vibrio alginolyticus*'a karşı antimikrobiyal aktivitelerini belirlemektir. *Mentha spicata* subs. *tomentosa* uçucu yağının (MEO) ana bileşenleri piperitone, okaliptol (1,8-cineole), pulegone, piperitenone sırasıyla %25.01, %19.53, %14.50 ve %10.98 olarak belirlenmiştir. *Origanum onites* uçucu yağının (OEO) ana bileşenleri sırasıyla karvakrol, %46.17 ile p-cymene, %13.05 oranında tespit edilmiştir. OEO ve MEO ve bu uçucu yağların nanoemülsiyonlarının antibakteriyel etkileri agar disk difüzyon yöntemi kullanılarak belirlendi. OEO nanoemülsiyonları, Gram-negatif *Aeromonas veronii*'ye karşı pozitif kontrole (enrofloksasin) göre son derece etkili olduğu tespit edilmiştir. Bunun yanında, antibakteriyel aktivite bakımından OEO nanoemülsiyonlarının, OEO'ya göre daha etkili olduğu görülmüştür. MEO ve nanoemülsiyonlarının balık patojenlerine karşı düşük bir aktiviteye sahipken *Aeromonas veronii* ve *Lactococcus garvieae* üzerinde antibakteriyel aktivite göstermemiştir.

Anahtar kelimeler

- *Origanum onites*
- *Mentha spicata* subs.
- *Tomentosa*
- Bakteriyel balık patojenleri
- Antibakteriyel aktivite

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1. INTRODUCTION

Fish pathogens have been causing severe economic losses in aquaculture for many years. In addition to financial loss, they also threaten fish health and negatively affect human health due to the zoonotic character of some pathogens. For example, the pathogen *Aeromonas veronii* is zoonotic and may play a role in developing diseases such as sepsis and gastroenteritis, especially in children (Pablos et al., 2011, Li et al. 2020).

In addition to the advantages of chemotherapeutic drugs used in treating these diseases, chemotherapeutics have disadvantages such as residual and suppressor immunity in fish. For these reasons, there has been a tendency toward treatment with medicinal plants today (Reverter et al., 2014, Hoque et al.,2016).

The use of medicinal herbs and their seconder metabolites is increasing each day worldwide because of having environmentally safe properties in aquaculture (Diler et al., 2021). Also, they do not have residue in animal tissues. Essential oils (EOs) are secondary metabolites derived from medicinal herbs. They possess biologically active properties to be used as a phytotherapeutic agent for sustainability in aquaculture. Essential oils show strong antioxidant, antiradical, and antimicrobial activities because of their constituents, such as aldehyde, terpenoid, and phenolic (Baratta et al., 1998, Chang et al., 2013).

In addition, these biologically active constituents of EOs show strong antimicrobial activity against important fish pathogens (Baydar et al., 2004, Okmen et al., 2012). The key chemical groups identified in EOs are terpenes, terpenoids, phenylpropenes, and isothiocyanates (Dawood et al., 2021). Nanoemulsions of herbal plant oils have antimicrobial activity against microorganisms. The usage of nano-emulsified EOs (NEOs) is a new approach to achieving sustainable aquaculture (Krishnamoorthy et al., 2018).

Medicinal plants have attracted attention in recent years due to their antimicrobial properties. Carvacrol and thymol are the compounds of *O. onites* essential oils, which provide the major antibacterial activity (Proestos et al., 2005), and *in vitro* activity on *A. salmonicida*, *Bacillus subtilis*, *Escherichia coli*, *P. aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, *Streptococcus mutans*, and *Micrococcus luteus* infections were also reported (Sarac & Ugur, 2008, Okmen et al., 2012). *Mentha spicata* essential oil has an antimicrobial effect due to the compounds it contains such as piperitone, pulegone, and 1,8-cineole.

There are few studies on the antibacterial activity of *M. spicata* subs. *tomentosa* EO (Sevindik et al., 2017). Moreover, there is no study on the antimicrobial activity of MEOs and nanoemulsions of MEOs and OEOs against fish pathogens. Thus, in this study, it was aimed to investigate the antimicrobial activities of two selected essential oil and their nanoemulsions, from different aromatic plants (*Origanum onites*, *Menta spicata* subs. *tomentosa*) against six common fish pathogens, *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, *Aeromonas veronii*, *Vibrio alginolyticus*, *Yersinia ruckeri*, and *Lactococcus garvieae.*

2. MATERIAL AND METHOD

2.1. Bacterial strains

Fish pathogen bacteria, *Yersinia ruckeri*, and *Lactococcus garvieae* were obtained from the culture collection of Isparta Applied Science University, Microbiology laboratory of Eğirdir Fisheries Faculty, and *Vibrio parahaemolyticus, Pseudomonas aeruginosa, Aeromonas veronii* and *Vibrio alginolyticus* were isolated from disease outbreaks in the Mediterranean Sea.

2.2. Preparation of essential oil

Origanum onites plant was purchased from Denizli province, while *Menta spicata* subs. *tomentosa* plant was obtained from Isparta province. All the essential oils were obtained using the hydro

distillation method reported by Baydar et al. (2004). Briefly, the provided plants were dried at 25°C for about two weeks in optimal conditions. The plants were then pulverized with a blender. Then, the essential oils were obtained by distillation of 200 g plant samples in 2 liters of water for three hours using the Clevenger apparatus with the hydro distillation method. All essential oils were kept in the laboratory at 4°C until the chemical composition analysis and the antibacterial studies.

2.3. Determination of volatile constituents of plant essential oil

Hewlett-Packard 6890 series gas chromatograph (Perkin Elmer (PE) Auto System XL, USA) with a flame ionization detector (FID) was used to conduct gas chromatography-mass spectroscopy analysis (GC-MS) of the essential oils. The following conditions were applied for the PE Auto System XL gas chromatography: capillary column, CPWax 52CB (50 m x 0.32 mm; film thickness ¼ 0.25 Im); oven temperature programmed, 60–220°C raised at a rate of 2°C/min and then held at 220°C for 20 min; injector and detector temperatures, 240°C; carrier gas, helium at a flow rate of 40 mL/min; and split ratio, 1/20 mL/min. Relative percentage amounts were calculated from chromatograms by the Turbo Crom Navigator computer program (Baydar et al., 2004).

2.4. Preparation of nanoemulsions

Oil-in-water nano-emulsions were prepared by mixing the individually prepared oils and water phases described by Hamouda et al. (1999) with minor modifications. Briefly, plant essential oil (14 ml), surfactant (3 ml), and ethanol (3 ml) were added to the beaker for the oil phase of nanoemulsion and then kept at 86°C for 1 hour. The mixture was cooled to room temperature, and sterilized distilled water (80 ml) was added to the cooled mixture to make up 80% of the total emulsion. It was then combined in an ice-cold beaker and homogenized in an ultrasonic homogenizer at 72 AMPL for 15 minutes.

2.5. In vitro antimicrobial activity

The antibacterial effect of CEOs and MEOs and their nanoemulsions was determined against *Pseudomonas aeruginosa, Vibrio parahaemolyticus, Aeromonas veronii, Vibrio alginolyticus, Yersinia ruckeri, Lactococcus garvieae* strains by using agar disc diffusion method (Murray et al., 1995), with minor modifications. Essential oils were homogenized with ethyl alcohol (96%) at 1000, 500, 250, 125, and 62.5 µl/ml concentrations. In contrast, their nanoemulsions were diluted to 1000, 500, 250, 125, and 62.5 µl/ml in sterilized distilled water and these dilutions were absorbed into 25 µl on 6 mm diameter sterile filter paper discs.

Antibiotics which specially selected for each tested bacterium (Enrofloxacin for *Vibrio parahaemolyticus, Aeromonas veronii, Pseudomonas aeruginosa, Vibrio alginolyticus, Yersinia ruckeri,* and Amoxicillin for *Lactococcus garvieae*) were used in the positive control groups, and ethyl alcohol (96%) and sterilized distilled water was used in the negative control group. 100 µl of the bacterial culture was prepared to an optical density (OD) of 0.5 using McFarland (10^8 CFU ml^{-1}) standard tube and was added to 100 ml of the Trypticase Soy Agar (TSA). Prepared discs were placed on the TSA and incubated at 25°C for 24 hours. After the incubation, the diameters of the inhibition zones formed around the disc were measured in millimeters. All experiments were conducted in triplicate. The value that inhibition 50% of the bacterial growth was determined as the minimum inhibitory concentration (MIC). Afterward, inhibition activity was evaluated to be strong for >15 mm, medium for 8 to 15 mm, and weak for 1 to 8 mm zone diameters, according to the report (Bansemir et al., 2006).

2.6. Statistical analysis

The data obtained from each group were given as mean values and standard deviation. Duncan's multiple comparison test with SPSS version 19.0 for Windows (SPSS Inc., Chicago, IL. USA) was used to determine the significant variation ($p<0.05$) were determined using.

3. RESULTS

In the present study, the chemical composition of MEO was characterized by 82 different components. The main components were piperitone, eucalyptol (1,8-cineole), pulegone, and piperitenone with the percentage of 25.01, 19.53, 14.50, and 10.98%, respectively (Table 1). A total of 60 components in OEO were determined. The major components were carvacrol and p-cymene, with a percentage of 77.45 and 6.61%, respectively (Table 2).

Table 1. The main chemical components of MEO

Component	Retention time (Min)	Content (%)
Piperitone	21.343	25.01
1,8-Cineole	12.707	19.53
Pulegone	20.672	14.50
Piperitenone	24.286	10.98
p-Menthan-3-one, (1R,4R)-(+)-	17.485	6.06
Caryophyllene	26.991	5.59
Piperitenone oxide	24.966	2.70
Beta.-Myrcene	11.032	1.74
3-Acetoxytridecane	16.123	1.62
2-.Beta.-Pinene	10.492	1.21
Beta.-Phellandrene	10.338	1.18

Table 2. The main chemical components of OEO

Component	Retention time (Min)	Content (%)
Carvacrol	25.820	77.45
p-Cymene	9.232	6.61
1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	10.807	3.87
β-Myrcene	7.741	1.43
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	16.948	1.43
.Alpha. Terpinene	8.876	1.29
Caryophyllene	32.567	1.10

The antibacterial activities of the OEO, MEO, and their nanoemulsions are represented in Table 3. No inhibition zones were detected in the negative control groups of both essential oil and essential oil nanoemulsions. The OEO nanoemulsions displayed more strong antibacterial activity against *A. veronii* (22-40 mm) at 250-1000 µL mL^{-1} and *L. garvieae* (16-28 mm) at 250-1000 µL mL^{-1} than other fish pathogens tested. OEO nanoemulsions were found to be medium effective against *P. aeruginosa* (500 µL mL^{-1}); *V. alginolyticus* (500 µL mL^{-1}), *V. parahaemolyticus* (250 µL mL^{-1}), and *Y. ruckeri* (250-500 µL mL^{-1}). OEO nanoemulsions showed weak inhibitory activity against *V. alginolyticus*. The OEO nanoemulsions showed the most effectiveness with inhibition zone of 22-40 mm at 125-1000 µL mL^{-1} against *A. veronii* compared to positive control. MIC values of OEO nanoemulsions were 125 µL mL^{-1} at *L. garvieae*, 250 µL mL^{-1} at *A. veronii, V. alginolyticus, V. parahaemolyticus, Y. ruckeri,* and 500 µL mL^{-1} at *P. aeruginosa*.

OEO was exhibited strong inhibitory activity against *A. veronii* (20-26 mm) at 500-1000 µL mL^{-1}, *P. aeruginosa* (16 mm) at 1000 µL mL^{-1}; *L. garvieae* (18-22 mm) at 500-1000 µL mL^{-1}; *V. parahaemolyticus* (20 mm) at 1000 µL mL^{-1}; and *Y. ruckeri* (18 mm) at 1000 µL mL^{-1}. In addition, OEO was found to be moderately effective against *A. veronii* (250 µL mL^{-1}), *P. aeruginosa* (500 µL mL^{-1}); *V. alginolyticus* (1000 µL mL^{-1}), *L. garvieae* (250 µL mL^{-1}); *V. parahaemolyticus* (500 µL mL^{-1}); and *Y. ruckeri* (250-500 µL mL^{-1}).

Table 3. Antibacterial activities of OEO, MEO and their nanoemulsions against the fish pathogen bacteria (inhibition zone, mm)

	Concentration (µl/ml)	OEO (mm)	OEO nanoemulsions (mm)	MEO (mm)	MEO nanoemulsions (mm)	Positive Control (mm)
	1000	26.2±0.3^{c}	40.4±1.9^{a}	0.0±0.0^{g}	0.0±0.0^{g}	20.0±0.0^{e}
	500	20.5±0.7^{e}	34.5±0.7^{b}	0.0±0.0^{g}	0.0±0.0^{g}	20.0±0.0^{e}
A. veronii	250	13.5±0.7^{f}	22.5±0.7^{d}	0.0±0.0^{g}	0.0±0.0^{g}	20.0±0.0^{e}
	125	0.0±0.0^{g}	0.0±0.0^{g}	0.0±0.0^{g}	0.0±0.0^{g}	20.0±0.0^{e}
	62.5	0.0±0.0^{g}	0.0±0.0^{g}	0.0±0.0^{g}	0.0±0.0^{g}	20.0±0.0^{e}
	1000	16.2±0.3^{c}	18.0±0.0^{b}	8.5±0.7^{d}	14.6±0.8^{c}	32.0±0.0^{a}
	500	10.0±1.4^{d}	13.5±0.7^{c}	0.0±0.0^{e}	0.0±0.0^{e}	32.0±0.0^{a}
P. aeruginosa	250	0.0±0.0^{e}	0.0±0.0^{e}	0.0±0.0^{e}	0.0±0.0^{e}	32.0±0.0^{a}
	125	0.0±0.0^{e}	0.0±0.0^{e}	0.0±0.0^{e}	0.0±0.0^{e}	32.0±0.0^{a}
	62.5	0.0±0.0^{e}	0.0±0.0^{e}	0.0±0.0^{e}	0.0±0.0^{e}	32.0±0.0^{a}
	1000	10.5±0.7^{e}	28.5±0.7^{b}	10.4±0.0^{e}	17.5±0.7^{c}	32.0±0.0^{a}
	500	6.6±0.8^{g}	12.4±0.6^{d}	6.6±0.6^{g}	8.4±0.6^{f}	32.0±0.0^{a}
V. alginolyticus	250	0.0±0.0^{j}	5.5±0.7^{g}	0.0±0.0^{j}	5.5±0.7^{g}	32.0±0.0^{a}
	125	0.0±0.0^{j}	0.0±0.0^{j}	0.0±0.0^{j}	4.0±0.0^{h}	32.0±0.0^{a}
	62.5	0.0±0.0^{j}	0.0±0.0^{j}	0.0±0.0^{j}	0.0±0.0^{j}	32.0±0.0^{a}
	1000	22.5±0.7^{c}	28.5±0.7^{b}	0.0±0.0^{j}	0.0±0.0^{j}	30.0±0.0^{a}
	500	18.5±0.7^{d}	19.5±0.7^{d}	0.0±0.0^{j}	0.0±0.0^{j}	30.0±0.0^{a}
L. garvieae	250	12.4±0.6^{f}	16.4±0.6^{e}	0.0±0.0^{j}	0.0±0.0^{j}	30.0±0.0^{a}
	125	4.0±0.0^{h}	9.5±0.7^{g}	0.0±0.0^{j}	0.0±0.0^{j}	30.0±0.0^{a}
	62.5	0.0±0.0^{j}	0.0±0.0^{j}	0.0±0.0^{j}	0.0±0.0^{j}	30.0±0.0^{a}
	1000	20.4±0.6^{c}	30.6±1.9^{b}	14.2±0.3^{d}	18.6±0.8^{c}	36.0±1.4^{a}
	500	12.1±2.8^{de}	18.8 ±3.5^{c}	10.2±0.3^{e}	12.4±0.6^{de}	36.0±1.4^{a}
V. parahaemolyticus	250	0.0±0.0^{f}	12.5 ±0.7^{de}	0.0±0.^{f}	0.0±0.0^{f}	36.0±1.4^{a}
	125	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.^{f}	0.0±0.0^{f}	36.0±1.4^{a}
	62.5	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.^{f}	0.0±0.0^{f}	36.0±1.4^{a}
	1000	18.2±0.3^{b}	20.5±0.7^{a}	11.5±0.7^{de}	16.5±0.7^{c}	20.0±0.0^{a}
	500	12.6±0.6^{d}	15.5±0.7^{c}	0.0±0.0^{f}	0.0±0.0^{f}	20.0±0.0^{a}
Y. ruckeri	250	10.0±1.4^{e}	10.0±0.0^{e}	0.0±0.0^{f}	0.0±0.0^{f}	20.0±0.0^{a}
	125	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{f}	20.0±0.0^{a}
	62.5	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{f}	20.0±0.0^{a}

Data are presented as the means ± SD (n = 3); values within the same row and column having different superscripts are significantly different ($p<0.05$).

In the present study, OEO showed weak inhibitory activity against *V. alginolyicus* and *L. garvieae*. The OEO showed the most effective with inhibition zone of 26 mm at 1000 µL mL^{-1} against *A. veronii* compared to positive control. MIC values of OEO were 125 µL mL^{-1} at *L. garvieae*, 250 µL mL^{-1} at *A. veronii, Y. ruckeri* and 500 µL mL^{-1} at *P. aeruginosa*, *V. alginolyticus*, *V. parahaemolyticus*.

MEO and its nanoemulsions were effective against *P. aeruginosa*, *V. parahaemolyticus*, *V. alginolyticus*, and *Y. ruckeri*. Both MEO and its nanoemulsions did not affect the *A. veronii* and *L. garvieae*. Antimicrobial activities of the positive control group and MEO nanoemulsions were much more effective than MEO.

MEO nanoemulsions exhibited strong inhibitory activity against *V. alginolyticus* (17 mm) at 1000 µL mL^{-1}; *V. parahaemolyticus* (18 mm) at 1000 µL mL^{-1}; and *Y. ruckeri* (16 mm) at 1000 µL mL^{-1}. MEO nanoemulsions were found to be medium effective against *P. aeruginosa* (1000 µL mL^{-1}), *V. alginolyticus* (500 µL mL^{-1}), and *V. parahaemolyticus* (500 µL mL^{-1}). MEO nanoemulsions showed weak inhibitory activity against *V. alginolyticus*. MIC values of MEO nanoemulsions were 125 µL mL^{-1} at *V. alginolyticus*, 500 µL mL^{-1} at *V. parahaemolyticus* and 1000 µL mL^{-1} at *P. aeruginosa*, *Y. ruckeri*.

MEO was exhibited medium inhibitory activity against *P. aeruginosa* (1000 µL mL^{-1}); *V. alginolyticus* (1000 µL mL^{-1}), *V. parahaemolyticus* (500-1000 µL mL^{-1}), and *Y. ruckeri* (1000 µL mL^{-1}). MEO showed weak inhibitory activity against *V. alginolyticus*. MIC values of MEO were 500 µL mL^{-1} at *V. alginolyticus*, *V. parahaemolyticus* and 1000 µL mL^{-1} at *P. aeruginosa*, *Y. ruckeri*.

4. DISCUSSION

Essential oils were declared to produce the construction of various chemical compositions (such as terpenoids, sterols, flavonoids, alkaloids, phenylpropanoids, and coumarins) that show antimicrobial activity (Manandhar et al., 2019). In the present study, major components of OEO were detected as carvacrol and p-cymene. Toncer et al. (2009) reported that the OEO was characterized by higher quantities of carvacrol, thymol, p-cymene, and γ-terpinene. In another study, the main components of OEO have been reported as carvacrol, thymol, γ-terpinene, p-cymene, α-terpinene, and α-pinene (Ozkan et al., 2010). This observation is in accordance with the literature, as previous analyses concluded that carvacrol and p-cymene are typically the main constituents in OEO.

In this study, the major components of MEO were piperitone (25.01%), eucalyptol (1,8-cineole) (19.53%), pulegone (14.50%), piperitenone (10.98%). *M. spicata* subs. *tomentosa* plants gathered from Aydın province were reported to contain piperitenone oxide (25.84%), pulegone (24.72%), cis piperitenone oxide (12.55%) as major components (Sevindik et al. 2017). Sarer et al. (2011) determined that the main compounds of the *M. spicata* subs. *spicata* from eastern Turkey were carvone (48.4%), 1,8-cineole (21.3%), β-pinene (3.5%), β-caryophyllene (3.3%) and trans-dihydrocarvone (2.9%). In another study, the major compound in *Mentha spicata* was carvone (59.40%), other components present in appreciable contents were: limonène, 1,8-cinéol, germacrèneD, β-caryophyllène, β-bourbonène, α-terpinéol, terpinéne-4-ol (Boukhebti et al., 2011). In general, these findings approved that the EOs component of the medicinal herbs may vary in terms of quality and quantity. These variations could be caused by different geographical and environmental conditions, in addition to different periods of plant growth (Mazandarani et al., 2013, Dastjerdi & Mazoji, 2015, Sevindik et al., 2016).

In this study, we compared the antibacterial activities of OEOs and MEOs, and their nanoemulsions against six bacterial fish pathogens. All the essential oils and their nanoemulsions exhibited antibacterial activity against *P. aeruginosa, V. parahaemolyticus, V. alginolyticus,* and *Y. ruckeri*. However, OEO and its nanoemulsions were highly effective against the Gram-negative *A. veronii* than the positive control (enrofloxacin). This is in agreement with (Gholipourkanani et al., 2019) who reported the powerful antimicrobial activity of OEOs against *P. damselae* and *A. hydrophila*. The MIC

values of OEOs nanoemulsions are always lower than OEOs, suggesting the increase of transport mechanisms through the cell membrane of the target microorganisms. Previous studies have also found that OEO provided effective bacterial inhibition against *A. salmonicida subspecies salmonicida* (Starliper et al., 2015), and *Escherichia coli* (Shan et al., 2011). Our study is in agreement with others regarding antibacterial efficacy against Gram-positive and Gram-negative bacteria; however, these activities were further enhanced by the use of nanocarriers (Hemmila et al., 2010, Shan et al., 2011, Starliper et al., 2015, Gholipourkanani et al., 2019).

In this study, MEO and its nanoemulsions were effective against pathogens of *P. aeruginosa, V. parahaemolyticus, V. alginolyticus, and Y. ruckeri*. Still, they had no effect against the *A. veronii* and *L. garvieae*. It has been observed that the antibacterial activities of MEO and its nanoemulsions were less effective than the positive control group. Similarly, in a study, the antibacterial activity of MEO was slightly lower than that of antibiotics on tested six different bacteria genera; methicillin-resistant *Staphylococcus aureus, S. aureus, P. aeruginosa, E. faecium, E. coli,* and *B. cereus* (Sevindik et al., 2017).

5. CONCLUSION

OEO, MEO, and their nanoemulsions exhibited antibacterial activity against *Pseudomonas aeruginosa, Vibrio parahaemolyticus, Vibrio alginolyticus, Yersinia ruckeri* in this study. The antibacterial activities of *O. onites* nanoemülsions against *A. veronii* were much more effective than the positive control (enrofloxacin). This study reveals that the nanoemulsions of medicinal aromatic herbs have more species-specific antibacterial effects and can be an alternative in the treatment of infectious diseases.

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ETHICAL STATEMENT

The authors declare that no experimental animals were used in the study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Planning the study: M.N., Ö.Ö., Literature: M.N., Ö.D., Ö.Ö., A.D., Methodology: M.N., Ö.D., Ö.Ö., A.D., Performing the experiment: M.N., Data analysis: M.N., Ö.Ö., Manuscript writing: M.N., Ö.D., Ö.Ö., A.D., Supervision: Ö.D., A.D., All authors approved the final draft.

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