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Antibacterial Activity of Some Aromatic Plant Essential Oils Against Fish Pathogenic Bacteria

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ABSTRACT

Essential oils of twenty-four plant species were obtained by hydrodistillation and investigated for their antibacterial effects against seven fish pathogenic bacteria (*Aeromonas hydrophila*, *Aeromonas salmonicida*, *Vibrio anguillarum*, *Yersinia ruckeri*, *Enterococcus faecalis*, *Lactococcus garvieae* and *Streptococcus agalactiae*). The antibacterial activity results of the essential oils obtained by disc diffusion method showed strong activities against all pathogens. In general, whole essential oils except *Artemisia absinthium* exhibited strong antibacterial effects against the most of the fish pathogens. However, the essential oil of *A. absinthium* showed weak antibacterial effect against only *A. hydrophila*. Mostly seven essential oils of the plants (*T. spicata*, *T. vulgaris*, *L. nobilis*, *C. verum*, *H. plicatum* and *A. citriodora* Paláu) among twenty-four essential oils exhibited good antibacterial activity against all fish pathogens. When compared to the tested antibiotics (furazolidon, oxytetracycline, cephalothin, and trimethoprim/sulfamethoxazole), the antibacterial effects of essential oils were mostly obtained equivalent or stronger. Considering the antibacterial activity results of the essential oils, their alternative use in lieu of antimicrobial agents against bacterial fish diseases might be convenient in the aquaculture.

Keywords: Antibacterial activity, disc diffusion, fish pathogens, essential oils

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Bazı Aromatik Bitki Esansiyel Yağlarının Patojenik Balık Bakterilerine Karşı Antibakteriyel Aktivitesi

Öz: 24 adet bitki türünün uçucu yağları hidrodistilasyon yoluyla elde edildi ve 7 çeşit balık patojenine (*Aeromonas hydrophila*, *Aeromonas salmonicida*, *Vibrio anguillarum*, *Yersinia ruckeri*, *Enterococcus faecalis*, *Lactococcus garvieae* ve *Streptococcus agalactiae*) karşı antibakteriyel etkileri araştırıldı. Uçucu yağların, disk difüzyon yöntemiyle elde edilen antibakteriyel aktivite sonuçları, tüm patojenlere karşı kuvvetli aktiviteleri olduğunu göstermektedir. Genel olarak, *Artemisia absinthium* dışında tüm uçucu yağlar, balık patojenlerinin çoğunluğuna karşı güçlü antibakteriyel etkiler göstermiştir. Bununla birlikte, *A. absinthium* uçucu yağı, sadece *A. hydrophila'* ya karşı zayıf antibakteriyel etki göstermiştir. Yirmi dört uçucu yağ arasından çoğunlukla yedi uçucu yağ (*T. spicata*, *T. vulgaris*, *L. nobilis*, *C. verum*, *H. plicatum* and *A. citriodora* Paláu) tüm balık patojenlerine karşı iyi antibakteriyel aktivite sergilemişlerdir. Test edilen antibiyotikler (furazolidon, oksitetrasiklin, sefalotin ve trimetoprim/sulfametoksazol) ile karşılaştırıldığında, uçucu yağların antibakteriyal etkileri çoğunlukla eşit veya güçlü olarak bulunmuştur. Uçucu yağların antibakteriyel aktivite sonuçları göz önünde bulundurarak, su ürünleri yetiştiriciliğinde bakteriyel balık hastalıklarına karşı antimikrobiyal ajanların yerine alternatif olarak kullanımları uygun olabilir.

Anahtar kelimeler: Antibakteriyel aktivite, disk difüzyon, balık patojenleri, esansiyel yağlar

Alıntılama
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Introduction

The extracts of medicinal plants had been extensively used over human beings and animals for a large number of purposes for a long time. Today, the medicinal and aromatic plants came into use in the modern medicine in contrast to synthetic ones that are regarded as unsafe to human and the environment. Besides, herbal products and plant-derived compounds present potential sources of new antibiotics, anticancer agents, and anti-HIV agents (Gurib-Fakim et al. 2005). In addition to their medicinal use in human, the medicinal plants were

also used as chemotherapeutics and food additives in aquaculture due to their ability of enhancing the fish immune system (Van Hai 2015). Aquaculture is one of the main food supply among animal food products for balanced nutrition and good health, and aquaculture fish production is the fastest growing food source sector in comparison to all other animal food sources. However, the factors such as intensification of aquaculture, periodic handling, extreme temperature changes, poor water quality and poor nutritional status contribute to adverse effects on fish health. In other word, high fish density and poor physiological environment may cause an increase in spread of pathogens in aquaculture and also an increase in the susceptibility of fish to the microbial agents (bacteria, fungi, virus etc.). So, this causes high mortality and also leads to serious economic losses (Harikrishnan et al. 2011; Reverter et al. 2014). In order to avoid and control of these pathogens, the antibiotics have been frequently used in aquaculture (Romero et al. 2012). Some antibiotics such as amoxicillin, erythromycin, enrofloxacin, oxytetracycline and furazolidone, have been used successfully to control the most of fish diseases (Harikrishnan et al. 2011). However, conscious or unconscious overdose application of antibiotics might improve the resistance of these antibiotics to the bacteria and thereby a reduced efficacy of the drugs. In addition, antibiotics possess a potential risk to consumers and the environment due to their accumulation within the environment and fishes (Harikrishnan et al. 2011; Ontas et al. 2016).

This situation prompted the scientists to search new and eco-friendly alternatives to antimicrobial agents. The most promising method to prevent fish diseases was the enhancement of the immune system by using immunostimulants derived from plants stimulating humoral and cellular defence mechanisms. Plant-derived immunostimulants are eco-friendly and easily prepared, and effective with fewer side effects during treatment of diseases and without any environmental and hazardous problems (Mousavi et al. 2011; Reverter et al. 2014) and also they do not lead to any drug resistance (Soltani et al. 2010).

Recently, this interest in natural medicine has also been increasing in fish culture (Soltani et al. 2010). Lately, the essential oils are very popular as natural antimicrobial agents due to their rich mixture of highly functional molecules (Park et al. 2011). Numerous studies have been reported about the antibacterial properties of essential oils isolated from aromatic plants for their potential bioactive principles (Romano et al. 2005). Nowadays, some studies have been reported on the antimicrobial activities of essential oils on aquatic animal diseases (Rattanachaikunsopon and Phumkhachorn 2007; Mousavi et al. 2011) and also provided a promising managemet tool for the controlling or treating aquatic fish diseases (Olusola et al. 2013). Hence, in the present study, essential oils obtained from twenty-four different Turkish plants were studied for screening their *in vitro* antibacterial activities on a fish pathogenic bacteria from aquaculture industry.

Material and Methods

Some plants were purchased from herbalists in Bolu, Turkey and some others were grown in pots to produce their essential oils. The plants used in this study were given in Table 1. Purchased plants were grounded into fine powder and some others were cut into small pieces without drying. Briefly, 100 g of each plant material (selected organ) were seperately steam-distilled by using a Clevenger type apparatus for 4 hour (Randrianarivelo et al. 2010). The obtained essential oils were collected in sealed-brown vials seperately and covered with aluminum foil and kept in a refrigerator until use. The yield of each essential oil (ml/weight) was calculated from the weight of used plant parts (Table 1).

Antimicrobial assay

Fish Pathogens

A. hydrophila, A. salmonicida, V. anguillarum, Y. ruckeri, E. faecalis, L. garvieae and *S. agalactiae* were used for antibacterial assay. *A. hydrophila* (ATCC 19570) and *S. agalactiae* (Pasteur Institute 55118) bacterial strains were obtained from Refik Saydam National Type Culture Collection (Ankara, Turkey). *V. anguillarum*, *Y. ruckeri* and *L. garvieae* bacterial strains were provided by Dr. İlhan Altınok, Faculty of Marine Science, Karadeniz Technical University, Surmene, Trabzon, Turkey. *E. faecalis* bacterial strain were provided by Dr. Cafer Erkin Koyuncu, Faculty of Fisheries, Mersin University, Mersin, Turkey. *A. salmonicida* bacterial strain by Dr. Şükrü Kırkan, Faculty of Veterinary Medicine, Adnan Menderes University, Aydın, Turkey.

Antibacterial assay

The antibacterial activity of twenty-four essential oil extracts was determined by using disc diffusion assay (Kirby-Bauer Method) (Andrews 2009). Agar culture plates were prepared as described before (Türker and Yıldırım 2015). Briefly, each bacterial strain was grown on Tryptic Soy Agar (TSA) (Acumedia) plates and incubated for 2 days at 28 ºC for *A. salmonicida* and *Y. ruckeri*; at 37 ºC for the other bacterial strains. The turbidity of each bacteria broth culture was adjusted to equal that of the 0.5 McFarland standard and then the broth cultures adjusted was separately inoculated on Mueller Hinton Agar plates by using cotton swabs. 10 µl of

each oil was applied to sterile filter paper discs(6 mm in diameter, Glass Microfibre filters, Whatman®). Standard antibiotic discs (furazolidone (100 µg), oxytetracycline (30 µg), cephalothin (30 µg) and trimethoprim/sulfamethoxazole (1.25 / 23.75 µg)) (Bioanalyse®) were used for positive control placed on the inoculated Muller Hinton agar plates. Hexane were used as a negative control because essential oils collected in tiny amounts in clevenger apparatus were taken with hexane. Inoculated plates with discs were incubated at 37 ºC with the exception of *A. salmonicida* and *Y. ruckeri* (at 28 ºC) for 24 hours. After incubation, inhibition zone diameter (mm) was measured. Three independent experiments were done in different times.

Statistical analysis

The Shapiro-Wilk test (Shapiro and Wilk 1965; Royston 1995) and an inspection of the skewness and kurtosis measures showed that the sample data were not approximately normally distributed ($P<0,05$). A Kruskal-Wallis H test, is a rank-based nonparametric test, showed that there was a statistically significant difference among the extract treatments ($P<0,05$) and performed a pairwise Conover test of multiple comparisons using rank sums as post-hoc test (Conover 1999). All data were analyzed by using MedCalc Statistical Software (version 15.8).

Table 1. List of the studied plant species, plant parts used and essential oil yields.

Family and plant species	Common name	Part used	Yield (ml)*
Lamiaceae			
Lavandula angustifolia	Lavender	Flower	3.3
Lavandula stoechas	French lavender	Flower	0.7
Menthax piperita	Pepper mint	Leaves	0.6
Ocimum basilicum	Sweet basil	Leaves	0.5
Origanum majorana	Wild marjoram	Leaves	0.05
Thymus vulgaris	Thyme	Leaves	0.7
Rosmarinus officinalis	Rosemary	Leaves	2.0
Thymbra spicata	Spiked thyme	Leaves	0.6
Salvia officinalis	Sage	Leaves	2.2
Lauraceae			
Laurus nobilis	Bay laurel	Leaves	1.1
Cinnamomum verum	Cinnamon	Bark	3.0
Geraniaceae			
Pelargonium graveolens	Rose geranium	Leaves	0.2
Piperaceae			
Piper nigrum	Black pepper	Seed	4.6
Verbenaceae			
Aloysia citriodora Paláu	Lemon verbena	Leaves	0.4
Zingiberaceae			
Zingiber officinale	Ginger	Root	0.7
Apiaceae			
Coriandrum sativum	Chinese parsley	Seed	0.5
Foeniculum vulgare	Common fennel	Leaves	1.2
Petroselinum sativum	Parsley	Leaves	0.1
Pimpinella anisum	Anise	Leaves	3.5
Asteraceae			
Helichrysum plicatum	Everlasting	Flower	0.03
Achillea millefolium	Yarrow	Flower	0.5
Artemisia absinthium	Wormwood	Flower	0.2
Myrtaceae			
Eucalyptus camaldulensis	River red gum	Leaves	1.6
Syzygium aromaticum	Clove	Flower buds	3.4

* Yield (ml) = weight of essential oil (ml)/ 100 g of powdered plant sample.

Results

Antibacterial screening of 24 essential oils against 7 fish pathogens was shown in Table 1. As a result of our work, essential oils generally showed strong antibacterial effects against all bacteria. However, among bacteria, *A. hydrophila, E. faecalis, L. garviceae* and *S. agalactiae* were found as the most sensitive bacterial strains to the essential oils.

Against *A. hydrophila* and *A. salmonicida*, the strongest antibacterial effect was obtained by the oil of *Thymus vulgaris* (62.7 ± 1.5 and 40.0 ± 0.0 mm, respectively). *T. vulgaris* oil exhibited same inhibition as antibiotic furazolidon (39.0 ± 0.0 mm) against *A. salmonicida*. The second strong antibacterial effects were obtained by the oils of *Thymbra spicata* (60.0 ± 0.0 mm), *Aloysia citriodora* Paláu (51.7 ± 0.3 mm), *Cinnamomum verum* (46.0 ± 1.0 mm), *Laurus nobilis* (45.7 ± 0.7 mm), *L. angustifolia* (43.3 ± 3.3 mm) and *Mentha x piperita* (40.0 ± 0.0 mm) against *A. hydrophila* bacterial strain and also by the oils of *C. verum* (33.7 ± 0.7 mm), *T. spicata* (30.7 ± 0.7 mm) and *L. nobilis* (29.3 ± 0.3 mm) oils against *A. salmonicida* bacterial strain. Against *V. anguillarum* bacterial strain, the essential oils of *H. plicatum* (66.7 ± 0.9 mm), *C. verum* (45.7 ± 0.7 mm), *T. vulgaris* (45.0 ± 0.0 mm) and *T. spicata* (34.7 ± 0.3 mm) exhibited strong antibacterial effects. Besides, the essential oil of *H. plicatum* had also strong inhibition effect on *S. agalactiae* and *L. garviceae*. Nonetheless, *H. plicatum* essential oil had weak inhibiton effect on *E. faecalis* (15.3 ± 0.3 mm) when compared to used bacteria. In addition to *H. plicatum* essential oil, *P. nigrum* and *O.onites* showed the strongest antibacterial activity against *S. agalactiae* and this activity was followed by the strong activities of *A. citriodora* Paláu, *C. verum* and *T. spicata* essential oils. These essential oils exhibited also similar and stronger antibacterial effect than used standard antibiotics (Table 2).

Against *E. faecalis* and *L. garvieae* bacterial strains, the best antibacterial effect was obtained with essential oils of *L. nobilis* and *S. officinalis.* This effect was followed by the effects of *H. plicatum* essential oil only on *L. garvieae* bacterial strain, and the effects of *P. nigrum, A. citriodora* Paláu essential oils against both *E. faecalis* and *L. garvieae* bacterial strains. Essential oils of these plants exhibited higher inhibitory effects than all used antibiotics.

Against *Y. ruckeri* bacterial strain, the essential oils of *T. spicata* and *T. vulgaris* were found as the most effective ones (50.0 ± 0.0 mm) and this antibacterial effect was followed by the effect of *C. verum* (45.0 ± 0.0 mm), *A. citriodora* Paláu (41.7 ± 1.7 mm), *M. piperita* (37.0 ± 0.0 mm), *Coriandrum sativum* (34.3 ± 4.7 mm) and *L. nobilis* (33.3 ± 1.7 mm) oils, respectively and their inhibition zones were higher than all used standard antibiotics (Table 2).

Although all used bacterial strains were mainly sensitive against tested essential oils, mostly seven essential oils of the plants (*T. spicata, T. vulgaris, L. nobilis, C. verum, H. plicatum* and *A. citriodora* Paláu) among twenty-four essential oils exhibited good antibacterial activity against all fish pathogens in present study. Nonetheless, *A. absinthium* essential oil was not effective against used bacteria except *A. hydrophila. A. absinthium* essential oil produced the smallest inhibition zone of 8.3 mm. In addition, *P. sativum* showed weaker antibacterial activities against all bacteria than those of other used essential oils. Moreover, *P. sativum* showed similar inhibition zones as antibiotic furazolidone against *L. garvieaea* and also similar inhibiton zones as antibiotic cephalothin against *Y. ruckeri* (Table 2).

In addition to plant essential oils exhibiting the best antibacterial effects, the rest of the plant essential oils exhibited good inhibitory effects against most of the tested fish pathogens and they also exhibited more stronger antibacterial effects than antibiotics used as standard drugs in the present study.

Positive controls (antibiotic discs) showed antibacterial activity to used fish pathogens. Hexane was used as a negative control and no inhibition was observed with hexane.

Discussion

Antibacterial effect of *C. lemon* and *A. spinosa* essential oils against *Y. ruckeri, A. hydrophila* and *L. garvieae* bacterial strains have been studied by Ontas et al. (2016). Their results indicated that both essential oils possessed strong antibacterial effects against *Y. ruckeri* and *A. hydrophila* whereas weak antibacterial activity was obtained against *L. garviea*. However, in our study, the essential oils of many plants showed the strong antibacterial effects against *Y. ruckeri, A. hydrophila* and *L. garviea* bacterial strains. Likewise, Cermelli et al. (2008) studied the antibacterial activity of *Eucalyptus globulus* oil and they reported that eucalyptus oil did not exhibit any antibacterial effects against *S. agalactiae*. However, the essential oil of *E. camaldulensis* possessed strong antimicrobial effect against same fish pathogens in our study.

In another study, essential oils of two *Rosmarinus officinalis* L. varieties exhibited weak to moderate antimicrobial effects against *K. pneumoniae, S. aureus, E.coli, B.subtilis* and *B.cereus* (Zaouali et al. 2010). However, Roomiani et al. (2013) reported that the essential oil of *R. officinalis* possessed very strong antibacterial effect against *Streptococcus iniae*.

Table 2. Antibacterial activities of plant essential oils.

Plant essential oils	Mean diameter of inhibitory zones (mm ± SE)						
	A. hydrophila	*A. salmonicida*	*V. anguillarum*	*Y. ruckeri*	*E. faecalis*	*L. garvieae*	*S. agalactiae*
L. angustifolia	43.3 ± 3.3^{nqrv}	12.3 ± 0.3^{agiknpru}	14.7 ± 0.3^{ainr}	17.0 ± 0.0^{gnuz}	21.3 ± 3.3^{gnrvz}	28.7 ± 1.3^{gnrä}	26.3 ± 4.7^{dfgknqrs}
L. stoechas	26.0 ± 1.0^{ci}	12.7 ± 0.3^{aginpr}	14.0 ± 0.6^{ainr}	22.7 ± 0.3^{gi}	11.0 ± 0.6^{dikosu}	14.3 ± 0.7^{hizë}	43.3 ± 1.7^{himvwy}
M. piperita	40.0 ± 0.0^{qr}	13.3 ± 0.3^{ikmnr}	16.3 ± 0.7^{akr}	37.0 ± 0.0^{fr}	19.0 ± 1.0^{gnrz}	27.0 ± 0.0^{gnqryä}	22.0 ± 1.2^{dfgknrsx}
O. basilicum	22.3 ± 0.3^{aegjs}	12.0 ± 2.5^{ainpsu}	20.0 ± 1.2^{fgmzä}	22.0 ± 0.0^{ginu}	25.0 ± 0.0^{gnrvë}	26.3 ± 2.2^{gnqry}	25.0 ± 1.0^{dfgknrsä}
T. spicata	60.0 ± 0.0^{nqrä}	30.7 ± 0.7^{dmqä}	34.7 ± 0.3^{dpqë}	50.0 ± 0.0^{lvw}	39.7 ± 1.5^{beqxyä}	50.3 ± 0.3^{lw}	48.0 ± 0.6^{ehmnqä}
T. vulgaris	62.7 ± 1.5^{lpw}	40.0 ± 0.0^{lvz}	45.0 ± 0.0^{blvw}	50.0 ± 0.0^{lvw}	41.7 ± 3.3^{jlpwë}	53.0 ± 0.0^{lpw}	60.0 ± 0.0^{bjlopvw}
R. officinalis	29.0 ± 0.6^{dmuz}	24.3 ± 0.7^{dqäë}	23.0 ± 1.0^{dmq}	28.0 ± 1.0^{dkqä}	12.7 ± 0.9^{diks}	21.7 ± 0.3^{dmv}	21.3 ± 0.9^{adfgknrsx}
O. majorana	36.7 ± 1.7^{lpw}	20.0 ± 0.0^{fvwë}	24.7 ± 0.3^{lpvwë}	29.7 ± 0.3^{fmqäë}	15.7 ± 1.3^{lpwë}	28.3 ± 0.9^{gnqrä}	31.3 ± 2.3^{ilpvwy}
S. officinalis	23.0 ± 0.0^{agjsë}	12.0 ± 0.0^{aginpsu}	16.0 ± 0.6^{aiknr}	13.0 ± 1.0^{acehsx}	65.3 ± 0.3^{afj}	66.3 ± 0.7^{abf}	16.0 ± 0.0^{adruxzë}
L. nobilis	45.7 ± 0.7^{fnprv}	29.3 ± 0.3^{fvwäë}	19.0 ± 3.5^{fgmzä}	33.3 ± 1.7^{fmqrë}	65.7 ± 1.9^{af}	66.3 ± 0.7^{abf}	23.0 ± 11.5^{dfgknrsä}
C. verum	46.0 ± 1.0^{fnpv}	33.7 ± 0.7^{flvwz}	45.7 ± 0.7^{blvw}	45.0 ± 0.0^{lpvw}	29.3 ± 0.7^{gnvë}	22.7 ± 0.9^{dmvy}	49.3 ± 0.7^{ijlpvwy}
A. citriodora Paláu	51.7 ± .3^{flpvw}	11.7 ± 0.3^{aginpsu}	28.3 ± 0.3^{pqwë}	41.7 ± 1.7^{prv}	43.3 ± 1.7^{jlpw}	61.0 ± 0.0^{bjlp}	50.7 ± 0.7^{bjlpvwy}
P. graveolens	19.0 ± 0.0^{hoxy}	8.0 ± 0.0^{eghx}	8.7 ± 0.3^{hu}	12.0 ± 0.0^{aehsx}	9.0 ± 0.6^{chou}	14.0 ± 0.6^{hize}	36.7 ± 1.7^{ehimqy}
P. nigrum	22.3 ± 1.5^{aegjs}	-	-	-	62.3 ± 1.3^{afjlp}	61.7 ± 0.3^{bjp}	63.0 ± 0.6^{bjlpv}
Z. officinale	19.0 ± 0.0^{hoxy}	9.7 ± 0.3^{ehsux}	-	12.7 ± 0.3^{acehsx}	15.3 ± 0.3^{beqxyä}	17.0 ± 1.5^{ekxë}	16.3 ± 0.7^{adruxzë}
C. sativum	30.0 ± 0.0^{dkmuz}	17.3 ± 0.3^{kmqr}	20.7 ± 0.3^{gm}	34.3 ± 4.7^{mq}	-	21.7 ± 0.9^{dmv}	37.0 ± 0.0^{ehimqy}
F. vulgare	29.7 ± 0.9^{dkmuz}	12.3 ± 3.4^{agnpsux}	10.3 ± 0.3^{ehsu}	17.0 ± 0.0^{gnuz}	9.3 ± 0.3^{chiou}	10.0 ± 0.0^{costu}	10.7 ± 0.3^{acuxzë}
P. sativum	15.0 ± 0.0^{bhotx}	-	-	10.0 ± 0.0^{oy}	9.3 ± 0.3^{chiou}	12.0 ± 0.0^{coquz}	-
P. anisum	25.0 ± 1.2^{cisë}	-	-	14.0 ± 0.6^{acxz}	7.7 ± 0.3^{chmotu}	9.7 ± 0.3^{cotu}	8.7 ± 0.3^{cotuzë}
A. millefolium	21.3 ± 0.7^{eg}	8.7 ± 0.3^{ehx}	11.0 ± 0.0^{esu}	12.0 ± 0.6^{aehsx}	14.3 ± 0.9^{beqxyä}	18.3 ± 0.9^{eksx}	34.0 ± 2.0^{ehmqä}
H. plicatum	-	-	66.7 ± 0.9^{blv}	-	15.3 ± 0.3^{beqxyä}	62.7 ± 1.2^{abfjp}	66.0 ± 1.0^{bjlp}
A. absinthium	8.3 ± 0.3^{bot}	-	-	-	-	-	-
S. aromaticum	31.3 ± 0.7^{kmuzä}	14.0 ± 0.6^{iknr}	17.0 ± 0.0^{akrz}	27.0 ± 0.6^{dkä}	12.0 ± 0.0^{diks}	16.7 ± 0.3^{eksxt}	24.3 ± 1.2^{dfgknrsä}
E. camaldulensis	23.0 ± 1.5^{acjsë}	11.0 ± 0.0^{agpsx}	11.0 ± 0.6^{su}	12.7 ± 0.7^{aehsx}	12.3 ± 1.8^{diks}	18.7 ± 1.9^{eksx}	23.3 ± 1.7^{dfgknrsä}
Positive controls							
Cephalothin	20.0 ± 0.0^{ehxy}	-	-	10.0 ± 0.0^{oy}	15.0 ± 0.0^{beqxyä}	25.0 ± 0.0^{grvy}	45.0 ± 0.0^{impvwy}
Frazolidone	30.0 ± 0.0^{dkmuz}	39.0 ± 0.0^{lvz}	19.0 ± 0.0^{fgkzä}	15.0 ± 0.0^{cnuz}	18.0 ± 0.0^{nrz}	14.0 ± 0.0^{hiozt}	10.0 ± 0.0^{acoëtuxz}
Oxytetracycline	34.0 ± 0.0^{kqä}	25.0 ± 0.0^{dfqäë}	20.0 ± 0.0^{fgmzä}	28.0 ± 0.0^{dkqä}	15.0 ± 0.0^{beqxyä}	29.0 ± 0.0^{qrä}	30.0 ± 0.0^{efgknqsä}
Trimethoprin/Sulfamethoxazole	24.0 ± 0.0^{acsë}	26.0 ± 0.0^{dfäë}	28.0 ± 0.0^{pqwë}	30.0 ± 0.0^{fqë}	32.0 ± 0.0^{glvwë}	15.0 ± 0.0^{nxzäë}	15.0 ± 0.0^{acxzë}
Negative control (Hexane)	-	-	-	-	-	-	-

* Data presented as zone of inhibition of bacterial growth in mm. Means with the same letter within columns are not significantly different at $P > 0.05$

In our study, the essential oil of *R. officinalis* were found to have weak antibacterial activity against *E. faecalis* bacteria but higher inhibitory effects of *R. officinalis* essential oil were obtained against other tested fish pathogens. Adel et al. (2016) evaluated the antibacterial activity of *M. piperita* essential oils against *Y. ruckeri* bacteria and they found it had moderate effect on *Y. ruckeri* with a diameter zone of 21.6 ± 0.9 mm. Moreover, the essential oil of the same species exhibited strong antibacterial activity against *Y. ruckeri* bacteria in our work. The acetone, methanol and chloroform extracts of *O. basilicum* against the microorganisms were examined by Kaya et al. (2008). They found that three different extracts exhibited no effect against *E. faecalis*. But in our study, essential oil of same species had significant inhibitory effect against *E. faecalis*. The reason of this may be different extraction solvent and procedure that may have bacterial bioactive compounds such as essential oils in our study.

Mousavi et al. (2011) examined the combination of essential oils of *T. vulgaris*, *Salvia officinalis*, *E. globules* and *M. piperita* and reported that they have potent antibacterial effects against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeroginosa* bacterial strains. Moreover, herbal extracts have been used solely or in combination as a food additives in aquaculture systems and both administrations had the same use and practicality (Wang et al. 2015). In our study, the essential oils of *T. vulgaris*, *M. piperita* and *S. officinalis* exhibited individual good antibacterial effect against used fish pathogens. So, these essential oils in combination may be used as food additives to overcome fish diseases in aquaculture systems.

Okmen et al. (2012) investigated the inhibition activity of *T. spicata* var. *intricata* essential oil on 18 *A. salmonicida* isolates which were obtained from cultured rainbow trout organs and tissues. They found that essential oil of *T. spicata* var. *intricata* inhibited the growth of *A. salmonicida* isolates except *A. salmonicida* FC84 strain and inhibition zones changed between 10-30 mm. In the present study, *T. spicata* essential oil exhibited similar inhibition against *A. salmonicida*.

Metin et al. (2017) examined antibacterial effect of *Eugenia caryophyllata*, *M. piperita* and *Lavandula hybrida* essential oils at doses ranging from 7.8 to 1000 µl/ml against *A. salmonicida subsp. achromogenes*, *A. hydrophila*, *V. anguillarum*, *Y. ruckeri* and *L. garvieae*. As a result, they reported that *E. caryophyllata* showed strong inhibition effect *and M. piperita* and *L. hybrida* essential oil have moderate inhibition effect against used bacterial strains. But we found slightly diffferent results than their findings. *M. piperita* essential oil had weaker effect against *L. garvieae* and *A. salmonicida*, and stronger effect against *A. hydrophila* and *Y. ruckeri* in our study. In another similar study, antibacterial effects of *Origanum minutiflorum*, *A. absinthium* and *Lonicera periclymenum* essential oils against *A. hydrophila*, *Y. ruckeri* ve *L. garvieae* were examined by disc diffusion assay (Görmez and Diler 2017). They found that *O. minutiflorum* and *A. absinthium* essential oils showed good antibacterial activity against all used bacteria. But in our study, *A. absinthium* essential oils showed inhibition only against *A. hydrophila* and its inhibition was the weakest. The reason of that is possibly the application of different antibacterial method.

The essential oils isolated from aromatic plants are known to have a wide spectrum of antimicrobial effects and their effects depend upon the type, concentration and composition of the essential oils, and also the concentration of target microorganisms (Baydar et al. 2004). As we mentioned above, these studies concluded that plant essential oils have the potential for the treatment of various infections caused by gram (+) and gram (-) bacteria in aquaculture systems as an alternative to the use of synthetic antibiotics. They can also be used as food additives due to enhancement of fish immune systems (Van Hai 2015). In this research, *in vitro* antibacterial properties of essential oils from twenty-four medicinal plants have been reported against different fish pathogens. In addition, the current study did not provide information about the effects of essential oils on fish and environment, and on the effects of the essential oils in different combinations. Therefore, further researches are needed to investigate their *in vivo* tests to determine their aspects in fish laboratory.

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