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Antibacterial Activity of Essential Oils and Extracts of Some Medicinal Plants against Bacterial Fish Pathogens

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Abstract

In this study, chemical compositions and in vitro antibacterial activities the components of essential oils and extracts of sage (*Salvia officinalis* L.), rosemary oil (*Rosmarinus officinalis* L.), lavandin (*Lavandula x intermedia var. Super*), and hyssop (*Hyssopus officinalis* L.) against fish pathogens: Gram-negative bacteria (*Aeromonas caviae, Aeromonas hydrophila, Vibrio anguillarum, Yersinia ruckeri, Edwardsiella tarda, Pseudomonas aeruginosa*) and Gram-positive bacteria (*Lactococcus garvieae, Staphylococcus warneri, Vagacoccus salmoninarum*) were investigated with Well Diffusion Agar assay. The composition of the essential oils and extracts were analyzed with GC/MS and HPLC, respectively. A total of 42 components in sage, 39 in rosemary, 44 in lavender, and 46 in hyssop were detected. As a result of this study, the sage and rosemary oils exhibited a broad-spectrum inhibitory effect (a strong antibacterial effect against Gram (+) and Gram (-) pathogens). Lavandin oil also showed a strong antibacterial effect against a majority of pathogens. The spectrum of inhibitory activity of hyssop oil was found narrower than other plant oils. In further studies, in vivo antibacterial effects of sage, rosemary and lavandin essential oils should be investigated.

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Introduction

The aim of this study is to characterize the phenotype, taxonomic position and antibiotic susceptibility of *P. shigelloides* pathogen isolated from enteritis-infected channel catfish. As far as we know, this is the first report of enteritis caused by *P. shigelloides* in channel catfish. In aquaculture, antimicrobial agents are widely used to prevent fish diseases caused by infectious agents (Schnick et al., 1997). It is known that the unconscious use of antimicrobial agents in the treatment of fish diseases causes many negative effects on the environment, fish, and human health (Schnick et al., 1997; Dos Santos, 2000). Therefore, herbal products' use to control fish pathogens in aquaculture is an alternative and current practice (Abutbul et al., 2005; Bansemir et al., 2006; Ekici et al., 2011; Haniffa and Kavitha, 2012; Al Laham and Al Fadel, 2014; Turker and Yıldırım, 2015; Ontas et al., 2016; Diler et al., 2017 a,b; Metin et al.,2017).

Sage, rosemary, lavender and hyssop are plants important medicinal and aromatic plants belonging to the Lamiaceae family. Due to the plants' essential oil, the odor and taste are antibacterial and antioxidant and are used in many industrial fields such as food, cosmetics, perfumery, and pharmaceutical industries.

Sage (Salvia L.) is perennial, grows up 50-100 cm in length, and has a semi-bushy structure (Baydar, 2013). Sage has economically evaluated leaves and the essential oil content of leaves varies between 1-2.5%. Sage has a very high antimicrobial and antioxidant effects and it is stated that these effects are mostly caused by components such as 1,8-cineol, α-tuyon, β-tuyon and camphor (Baricevic and Bartol 2000).

Hyssop (*Hyssopus officinalis* L.) is a perennial, grows up to 50 cm and has blue-violet flowering. Used parts of hyssop are leaves and flowers. Hyssop contains 0.1-1.8% of essential oil with maximum content at the flowering (Jankovsky and Landa, 2002). Pinocamphone, β-pinene'i, isopinocamphone were the essential oil components (Garg et al.,1999). Camphene and pinene are spasmolytics, and pinene has bactericidal effects.

Lavender (Lavandula sp.) is a perennial plant in semi-bushy form. Lavender has flowers of varying colors ranging from blue to viola, 50-100 cm in length. The ratio of essential oil in fresh lavender flowers varies between 1-3%. Commercial lavender essential oils contain more than 100 terpenoid compounds and the most important essential oil components are linalyl acetate, linalool, cineol and camphor (Kara, 2011). Rosemary (*Rosmarinus officinalis* L.) is a perennial plant, 50-100 cm height, shrub appearance; evergreen leaves in the winter, the flowers are pale blue colored (Baytop, 1984). There are secretory hairs that carry plenty of essential oil under the surface of the leaves. Rosemary leaves contain 1.0-2.0% of essential oil. The main of components of essential oil were borneol, 1,8 cineole (eucalyptol) and camphor (Baskaya et al.,2016). In rosemary, carnosic acid, carnosol, rosmarinic acid and rosmanol have antioxidant properties; borneol, 1,8-cineole, camphor and bornyl acetate are compounds used in food preservation (Sasikumar, 2004).

In this study was investigated the in vitro antibacterial effects against the fish pathogens of medicinal plants, sage (*Salvia officinalis* L.), rosemary oil (*Rosmarinus officinalis* L.), lavandin (*Lavandula x intermedia var. Super*) and hyssop (*Hyssopus officinalis* L.) essential oils and extracts. In addition, their components were determined.

Materials and Methods

Plant materials

In this study, sage (*Salvia officinalis* L.), rosemary oil (*Rosmarinus officinalis* L.), lavandin (*Lavandula x intermedia var. Super*) and hyssop (*Hyssopus officinalis* L.) were used as plant materials. The samples during the blooming stage were taken from the experimental farm of the Agriculture Faculty, Isparta Applied Sciences University. The plants were dried in the shadow and the stem parts are separated after drying. The leaves of sage and rosemary plants, the bud of lavandin plants and leaf and flower parts of hyssop were used to obtaining plant essential oils and extracts.

Preparation of essential oil

500 g plant samples in 1.5 L water were extracted by hydro-distilllation for 3 hours

using Clevenger apparatus according to the standard procedure described in European Pharmacopoeia for determining the oil content (v/w %). The concentrations of 1000, 500, 250, 125 and 62.5 µL mL^{-1} of the essential oils in methanol were prepared (Borisutpeth et al., 2005).

Preparation of extracts

Plant extracts were prepared from a representative sample of 100 g of each powdered plant material and were taken into a 1 L capacity Erlenmeyer flask. 1 L of n-hexzane was added to it and shaken for 24 h in a horizontal shaker at 120 rpm at room temperature. Then, the n-hexane in the suspansion was removed by rotary evaporator and plant extracts were prepared. The extracts diluted at % 20 concentrations with acetone water (%10). The extract solutions were kept in a refrigerator at 4 °C until were used (Gokce et al., 2007).

Gas chromatography-mass spectroscopy analysis of the essential oil

CP-Wax 52 CB (50 m x 0.32 mm; film thickness = 0.25 µm) was used as the column for determining the essential oil components of the plants. Oven temperature program: after at 40 °C waiting for 2 minutes, reaches 4 °C degrees per minute at 250 °C. Waiting at 250 °C for 5 minutes. Injection block temperature 250 °C, Detector temperature 250 °C, Detector energy flow 70 eV, Ionization type: EI, Gas used: Helium (20 mL min^{-1}), Flow rate 1,61 psi, sample preparation 7.5 µL of essential oil + 1500 µL dichloromethane. Each component was identified by comparison from the Wiley, Nist, Tutor, FFNSC library of mass Spectra. The component amount was determined by proportioning the relative blocks of the peak areas to the total peak area.

HPLC analysis of the extracts

Agilent Eclipse XDB-C18 (250x4, 60 mm) 5-micron column was used for the determination of phenolic compounds in plant extracts. The temperature is 30 °C, the mobile phase has a flow rate of 0.8 mL min^{-1} and the injection volume is 20 µL. A solution (3% acetic acid) and B solution (Methanol) were used as mobile phase.

Bacterial fish pathogens

Pathogen strains were obtained from the culture collection in the Fish Diseases Laboratory of the Egirdir Fisheries Faculty. The bacterial fish pathogens and their origins used in the study were given in **Table 1**.

Table 1 Bacterial fish pathogens and their origins

No	Bacteria	Origin
1	*Aeromonas sobria*	Rainbow trout-Mugla-Turkey
2	*Aeromonas caviae*	ATCC 15468
3	*Aeromonas hydrophila*	ATCC 7966
4	*Vibrio anguillarum*	Rainbow trout-Mugla-Turkey
5	*Pseudomonas aeroginosa*	ATCC 27953
6	*Yersinia ruckeri*	Rainbow trout-Mugla-Turkey
7	*Edwardsiella tarda*	DSMZ 300052
8	*Lactococcus garvieae*	Rainbow trout –Mugla-Turkey
9	*Staphylococcus warneri*	Rainbow trout –Mugla-Turkey
11	*Vagococcus salmoninarum*	Rainbow trout – Isparta-Turkey

Well diffusion agar assay

Antibacterial effects of essential oils and extracts were determined by well diffusion agar assay. For *in vitro* antibacterial activity was used tryptic soy agar (TSA) for all pathogen. Salt (2%) was added to TSA and tryptic soy broth (TSB) for *V. anguillarum*. The pathogen culture was grown in 4 mL TSB for 1 d at 25°C, and 100 µL of each culture was mixed into 100 mL of melted TSA. After solidifying and drying for 15-20 minutes, wells were punched (diameter = 3 mm) and 30 µL from extract solutions or the essential oils of each plant added to the wells in triplicate. Methanol for essential oils and acetone for extracts was used as control. After incubation at 25 °C for 24 hours, the diameters of the inhibition zones were measured. Inhibition activity was considered to strong for >15mm, medium for 8-15 mm and weak for 1-8 mm zone diameters (Bansemir et al., 2006).

Statistical analysis

Data (zone diameters) were assessed by one way analysis of variance ANOVA SPSS 17.0 package program (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test were used to determine the significant variation ($p<0.05$) and the significance level was chosen as $P = 0.05$.

Results

Identification of the pathogen

GC / MS analysis

The components of sage, hyssop, rosemary and lavandin essential oils by GC-MS were given in **Table 2**. A total of 42 components in sage, 39 in rosemary, 44 in lavandin and 46 in hyssop were detected. 1,8-cineole (eucalyptol) (27.08%), thujone (20.52%), camphor (16.99%), beta pinene (4.07%) in sage; camphor (29.17%), 1,8-cineole (26.36%), borneol (7.34%), alpha pinene (6.72%) camphene (4.95%) in rosemary; linalool (42.21%), linalyl acetate (23.61%), borneol (5.38%), terpineol (5.07%) in lavandin and beta-phellandrene (49.79%), beta- myrcene (10.16%), linalool (10.04%) ve elemol- alpha (3,62%) in hyssop were determined (**Table 2**).

Table 2 Essential oil components of sage, hyssop, rosemary and lavandin (%)

Component	Retention Time	Sage	Rosemary	Lavandin	Hyssop
Tricyclene	6.36	0.12	0.16	-	-
Alpha-Thujene	6.44	0.13	-	-	-
Alpha- Pinene	6.70	3.06	6.72	0.18	0.18
Camphene	7.28	3.67	4.95	0.28	-
Verbenene	7.39	-	0.31	-	-
Sabinene	8.13	0.07	-	-	0.88
Beta- pinene	8.33	4.02	1.36	0.15	0.91
3- Octanone	8.57	0.08	0.08	0.57	-
Beta-Myrcene	8.78	1.61	0.94	1.36	10.16
Pseudolimonene	9.42	-	-	-	0.37
Phellandrene-alpha	9.50	-	0.19	-	-
1-Phellandrene	9.53	-	-	-	0.16
Delta 3-carene	9.62	-	0.77	-	-
Hexyl acetate	9.75	-	-	0.69	-
Terpinene- alpha	10.01	0.20	0.43	-	-
Cymole	10.35	-	1.79	-	-
Para-cynmene	10.36	0.64	-	0.07	-
Beta-Phellandrene	10.81	-	-	-	49.79
Limonene	10.60	0.88	2.03	0.87	-
1.8-cineole (eucalyptol)	10.74	27.08	26.36	3.31	-
Cis-ocimene	10.94	0.23	0.04	0.91	0.19
Beta-ocimene	11.45	0.10	0.04	1.50	0.94
Gamma-terpinene	12.02	0.33	0.67	-	-
Trans-sabinene hydrate	12.67	0.14	-	-	-
Linalool oxide (trans)	12.71	-	-	0.21	-
Alpha-terpinolene	13.47	0.12	0.46	0.20	-
Linalool oxide (cis)	13.61	-	-	0.11	-
Linalool	14.67	3.84	2.70	42.21	10.04
Hexyl propionate	14.74	-	-	0.24	-
Thujone	14.77	20.52	0.12	-	0.30
1-octen-3-yl acetate	14.87	-	-	0.24	-
p-menth-2- en-1-ol	15.69	-	-	-	0.31
Trans sabinene hydrate	15.72	0.09	0.06	-	0.16
Alloocimen	15.98	0.05	-	0.11	-
Cis- Sabinol	16.85	0.29	-	-	-
Camphor	17.02	16.99	29.17	2.79	0.57
3 oktyne. 2.2- dimenthyl	17.15	-	-	-	0.10
Hexyl isobutyrate	17.19	-	-	0.17	-
3- pinanone	17.80	0.28	-	-	-
Lavandulol	18.23	0.08	-	0.18	0.11
Borneol	18.65	2.67	7.34	5.38	0.72
Pinocamphone	18.76	0.12	0.74	-	-
4-Terpineol	19.13	0.62	0.94	0.17	0.18
Cyrptone	19.42	-	-	0.33	0.57
Para-cymen-8- ol	19.53	0.05	0.13	0.06	-
Terpineol-alpha	20.14	1.02	2.94	5.07	1.29
p- allylanisole	20.22	-	-	-	0.12
Berbenone	20.79	-	1.86	-	-

Nerol	22.07	-	-	0.74	-
Hexyl 2- methyl butyrate	22.82	-	-	0.11	-
z-sitral	22.83	-	-	-	0.12
Cumaldehyde	22.97	-	-	0.10	0.15
Butanoate- hexyl-3 methyl	23.24	-	-	0.10	-
Carveol	23.31	-	0.10	-	-
Linalyl Acetate	23.72	1.13	0.70	23.61	2.46
e-citral	24.79	-	-	-	0.14
Citral	24.83	-	-	0.09	-
Phellandral	25.25	-	-	-	0.17
Bornil acetate	25.84	0.75	2.83	0.07	0.10
Lavandulyl acetate	25.96	0.12	-	2.36	0.31
Sabinol	26.16	0.09	-	-	-
Bicycloelemene	28.92	-	-	-	1.17
Tiglate- hexyl	28.93	-	-	0.20	-
Piperitenone	29.19	-	0.09	-	-
Neryl acetate	30.82	0.10	0.05	0.98	0.29
Copaene- alpha	31.69	-	0.10	-	-
Geranyl Acetate	32.12	0.17	0.08	1.91	-
Bourbonene- beta	32.17	-	-	-	1.09
Methyleugenol	33.43	-	-	-	0.15
Gurjunene- alpha	33.69	-	-	-	0.46
Caryophyllene	34.42	3.32	1.49	0.57	1.26
Beta- cubenene	35.09	-	-	-	0.21
Alpha- humulene	36.68	0.79	0.17	-	0.22
Farnesene- e- beta	36.86	-	-	0.32	-
Alloaromadendrene	36.97	-	-	-	2.07
Germacrene-d	38.33	-	-	0.15	2.79
Dehydroaromadendraneh ene	38.75	-	-	-	0.16
Bicyclogermacrene	39.24	-	-	-	2.08
Geranyl ısovalerate	40.02	-	-	0.18	-
Cadinene- gamma	40.35	-	0.08	-	0.27
Delta cadinene	40.74	-	0.20	-	-
Elemol-Alpha	42.58	-	-	-	3.62
Spathulenol	44.12	-	-	-	0.62
Caryophyllene oxide	44.37	0.74	0.13	0.13	-
Ledene	45.19	3.29	-	-	-
Humulene oxide	46.02	0.29	-	-	-
Eudesmol- epi-gamma	47.43	-	-	-	0.68
Muurolol-alpha- epi	48.02	-	-	0.08	0.32
Guaiol	48.72	-	-	-	1.02
Alpha-bisabolol	50.64	-	-	0.78	-

HPLC analysis

Caffeic acid, p-coum acid, ferulic acid, hesperidine, cinnamic acid and rosmarinic acid were determined as phenolic compounds in the plant extracts (**Table 3**).

Table 3 Phenolic compounds of sage, hyssop, rosemary and lavandin extracts (μg mL^{-1})

Compounds	Sage	Rosemary	Lavandin	Hyssop
Caffeic acid	-	-	-	0.5
P - coum acid	-	1.6	2.0	0.2
Ferulic acid	-	0.3	-	0.1
Hesperidin	7.0	4.1	-	3.9
Cinnamic acid	0.1	0.2	1.8	0.4
Rosmarinic acid	1.7	0.7	-	1.3

Antimicrobial activity

Antibacterial activity of essential oils

The results of the antimicrobial screening assays were presented in **Table 4**. There were no inhibition zones in the negative controls (methanol). The oils of sage and rosemary exhibited a broad-spectrum inhibitory effect (a strong antibacterial effect against all pathogens). Lavandin oil also showed strong antibacterial effect against majority of pathogens.

Sage, rosemary and hyssop essential oils at 250-1000 μL mL^{-1} concentrations displayed the largest zones of inhibition against *V. anguillarum* compared to other pathogens ($p<0.05$). Sage and rosemary essential oils at 125 μL mL^{-1} displayed strongest antibacterial effect (19.5 mm) against *P. aeuroginosa* comparing with other pathogens ($p<0.05$). However, sage at 500 μL mL^{-1} and rosemary at 1000 μL mL^{-1} were also found strong inhibition against Gr (+) bacteria: *S. warneri, V. salmoninarum* and *L. garvieae*. Lavandin at 125 μL mL^{-1} showed strongest antibacterial effect against *P. aeuroginosa* and *V. anguillarum* ($p<005$). Hyssop oil (250-1000 μL mL^{-1}) displayed strongest effect againts *V. anguillarum* (**Table 4** see at the last page of this article). A. caviae was the most sensitive to lavandin at 250 μL mL-1; A. hydrophila to rosemary 1000 μL mL-1; E. tarda to rosemary at 250 μL mL-1; S. warneri to sage at 500-1000 μL mL-1; L. garvieae to sage at 500 μL mL-1 and rosemary at 1000 μL mL-1 compared to other oils at all concentrations ($p<0.05$).

Antibacterial activity of extracts

Rosemary and sage extracts showed strong antibacterial effect against *V. anguillarum, S. warneri, V. salmoninarum* and *L. garvieae*. However, hyssop and lavandin extracts were found to be ineffective against all tested bacteria (**Figure 1**) (**Table 5**).

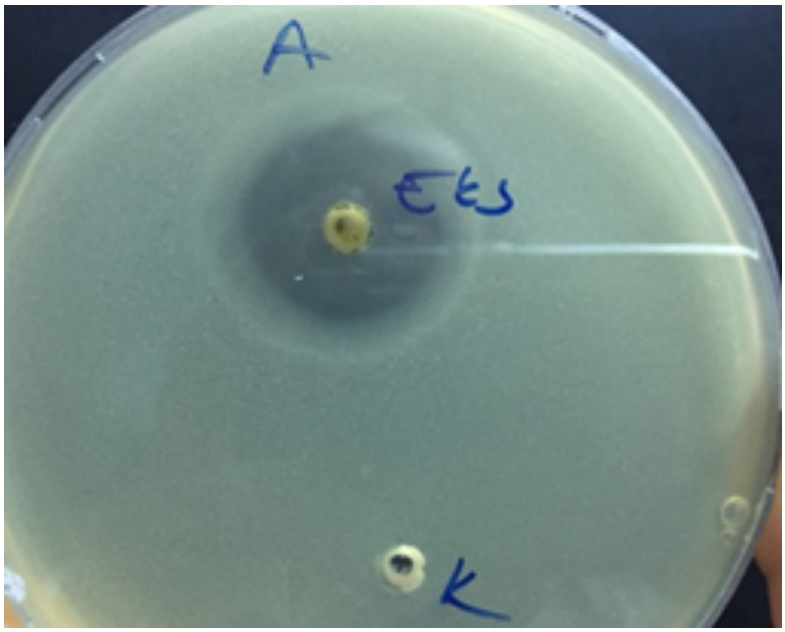

Figure 1 Inhibition zone of sage extract against *V. anguillarum*

Table 5 Antibacterial activity of rosemary, sage, hyssop and lavandin extracts against bacterial fish pathogens (inhibition zone diameter, mm)

Extracts	Rosemary	Sage	Lavandin	Hyssop
A. caviae	-	-	-	-
A. hydrophila	-	-	-	-
Y. ruckeri	6.5±0.70	-	-	-
E. tarda	11.5±0.70	-	-	-
P. aeuroginosa	-	-	-	-
V. anguillarum	35±1.41	26	-	-
S. warneri	26	20±1.41	-	-
V. salmoninarum	21.5±0.70	16±1.41	-	-
L. garviae	21.5±0.70	18.5±0.70	-	-

Discussion

Essential oils isolated from different aromatic plants are known to have a wide spectrum of antimicrobial activity (Hammer et al., 1999; Baydar et al., 2004). This activity is strongly correlated with the chemical composition of the essential oil and the chemical composition depends on climatic, season, geographic conditions and harvest period. The chemical components of plant species belonging to Lamiaceae family have been studied in literatures. Phenols belong to the largest group of secondary metabolites in plants, foremost of the family Lamiaceae and they exhibit sophisticated biological activity (Carović-StanKo et al., 2016).

R. officinalis essential oil contains mainly compounds including 1,8 cineol, camphor and α-pinene and these compounds have been identified as the most active antimicrobial components in this essential oil (Govaris et al.,2010; Jiang et al.,2011; Sienkiewicz et al.,2013). In the other study were reported that the most important constituents of the

rosemary were 1,8-cineole (78.6%), alpha-pinene (5.87%), tolüene (12.26%), camphor (8.22%) and berbonone (7.75%) (Mahmoodi et al.,2012). Bendeddouche et al. (2011), observed that the main constituents of the tested essential oil were camphor (37.6%), 1,8-cineole (10.0%), p-cymene-7-ol (7.8%) and borneol (5.4%). In this study was also determined 1,8-cineole (26.36%), camphor (29.17%), borneol (7.34%), alpha pinene (6.72%) camphene (4.95%) in rosemary essential oil.

S. officinalis essential oil is generally characterized by thujones, with α-thujone usually predominating (18–43%) over β-thujone (3–8.5%), camphor (4.5–24.5%), 1,8-cineole (5.5–13%), α-humulene (0–12%), α-pinene (1–6.5%), camphene (1.5–7%), and bornyl acetate (2.5% maximum) (Bruneton, 1999). Similarly, in this study was also determined 1,8-cineole (27.08%), thujone (20.52%), camphor (16.99%), β-pinene (4.02%), linalool (3.84%), camphene (3.67%), caryophyllene (3.32%), α-pinene (3.06%), ledene (3.29%) and borneol (2.67%). Delemare et al. (2007) also determined α-thujone (24.8%), 1,8-cineole (14.8%) and camphor (10.9%). Santos-Gomes and Fernandes-Ferreira (2001) also reported that α-thujone was the main essential oil compound in the vegetative parts, representing 25.5 and 55.1% of the essential oils from sage leaves and stems, respectively.

Lavender essential oil composition was usually determined as linalool (25.0-38.0%), linalyl acetate (25.0-45.0%), cymene (4.0-10.0%), terpinen-4-ol (2.0-6.0%) and camphor (0-0.5%) (Anonymous, 2002). Baydar and Kineci (2013) noted that the major compounds of lavandin oil were linalyl acetate (47.7%), linalool (34.0%), camphor (4.8%), borneol (4.2%), and 1,8-cineole (2.6%). Similarly, Kara and Baydar (2011) reported linalool (34.3 - 54.6%), linalyl acetate (24.0 - 29.0%), borneol (1.6 - 6.7%) and camphor (1.2 - 6.0%) in lavandin oil. In the present study also was found linalool (42.21%), linalyl acetate (23.61%) and borneol (5.38%) in lavandin essential oil.

Kızıl et al. (2010) reported that the major components of *Hyssopus officinalis* were pinocamphone (57.27%), β-pinene (7.23%), terpinene-4-ol (7.13%), pinocarvone (6.49%), carvacrole (3.02%), p-cymene (2.81%) and pinocamphone (2.59%). Figueredo et al. (2012) noted the main components were pinocarvone (29.17 %), trans-pine camphone (27.19 %), β-pinene (17.63 %), cispinocamphone (4.68 %) and myrcene (2.92 %) in hyssop. In the other study was found the main constituents of the oil pinocarvone (36.3%), pinocamphone (19.6%), β -pinene (10.6%), 1,8-cineole (7.2%) and isopinocamphone (5.3%) (Özer et al., 2005). In our study, unlike other studies, beta-phellandrene (49.79%), linalool (10.04%) and elemol- alpha (3,62%) in hyssop were determined.

Based on the literatures, the family Lamiaceae seems to be a rich source of plant species containing large amounts of phenolic acids (Lamaison et al.,1991; Li et al.,1993; Kovatcheva et al.,1996; Zgórka and Kawka, 2001). The most prevalent phenolic components in sage extracts are caffeic, vanillic, ferulic, and rosmarinic acids (Pavić et al.,2019). Phenolic acid in rosemary extract was found carnosol, carnosic acid, rosmanol, 7-methyl-epirosmanol, isorosmanol, rosmadial, caffeic acid (Govaris et al.,2010). Usano-Alemany and Panjai (2015) was noted the main phenolic compounds of chlorogenic acid, rosmarinic acid, caffeic acid, ferulic acid in lavandin plant. In *H. officinalis*, the most abundant phenolic acids were considered to be ferulic acid and caffeic acid (Proestos et al.,2005). Similarly, in the present study were determined caffeic acid, p-coum acid, ferulic acid, hesperidine, cinnamic acid and rosmarinic acid as phenolic compounds in the sage, rosemary, lavandin and hyssop extracts.

Plants in Lamiaceae family have a potent antibacterial activity, mostly due to the quantity and quality of phenolic compounds present in them. In this study, rosemary and sage extracts showed strong antibacterial effect against *V. anguillarum, S. warneri, V. salmoninarum* and *L. garvieae*. The oils of sage and rosemary exhibited a strong antibacterial effect against all fish pathogens. Similary, in the previous study, *S. officinalis* and *R. officinalis* extracts exhibited a broad-spectrum inhibitory effect both on Gram-negative (*Listonella anguillarum* serotypes O1 and O2, *Y. ruckeri* and *Photobacterium damselae subsp. piscicida*) and Gram-positive bacteria (*L. garvieae*) (Bulfon et al.,2014). Mahmoodi et al. (2012) also reported extract and essential oil *R. officinalis* showed

antibacterial effect against *L. garvieae*. Similarly, in the other study was determined antibacterial activity essential oil of *R. officinalis* against *Streptococcus iniae* (Roomiani et al.,2013). However, Al Laham and Al Fadel, (2014) reported the antibacterial effect of the extract of *R. officinalis* against *A. hydrophila.* In the other study was also determined antibacterial effect against Aeromonas spp. of *R. officinalis* essential oil (Starliper et al.,2015). In contrast, Ostrand (2012) was reported rosemary oil had a limited effect on fish pathogens, *Aeromonas salmonicida* and *A. hydrophila.*

Lavandin *(Lavandula x intermedia var. Super*) showed strong antibacterial effect against majority of pathogens in the present study. Similarly, Wimalasena et al. (2018) noted lavender (*Lavendular angustifolia*) essential oil displayed antibacterial activity of against Gram-negative and Gram-positive pathogenic bacteria isolated from cultured olive flounder (*Paralichthys olivaceus*) in Korea. Hyssop oil (250-1000 µL mL^{-1}) displayed strongest effect against *V. anguillarum* in the present study. However, there is no study on antimicrobial activity of *H. officinalis* against fish pathogens.

As a result of this study, 1,8 cineole (27.08%), thujone (20.52%), camphor (16.99%), beta pinene (4.07%) in sage; 1,8 cineole (26.36%), camphor (29.17%), borneol (7.34%), alpha pinene (6.72%) camphene (4.95%) in rosemary; linalool (42.21%), linalyl acetate (23.61%), borneol (5.38%), terpineol (5.07%) in lavandin, and beta-phellandrene (49.79%), beta-myrcene (%10.16), linalool (%10.04) and elemol-alpha (%3,62) in hyssop were determined. The oils of sage and rosemary a broad-spectrum inhibitory effect against Gram (+) and Gram (-) fish pathogens. Lavandin oil also showed strong antibacterial effect against majority of fish pathogens. The spectrum of inhibitory activity of hyssop oil was narrower than other plant oils. In the further studies, diseases resistance against fish pathogens of sage, rosemary and lavandin essential oils should be investigated. Thus, these plant essential oils can be used as an alternative to antibiotics in aquaculture industry.

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Table 4 Antibacterial activity of sage, rosemary, lavandin and hyssop essential oils against different bacterial fish pathogens (inhibition zone diameter, mm ± SD)

	Concent (µL mL^{-1})	*A. caviae*	*A. hydrophila*	*Y. ruckeri*	*E. tarda*	*P. aeuroginosa*	*V. anguillarum*	*S. warneri*	*V. salmoninarum*	*L. garviae*
Sage	1000	10±2.82$^{ab.A}$	10$^{abc.A}$	14±8.48$^{abc.A}$	9±1.41$^{a.A}$	11.5±2.12$^{ab.A}$	55±7.07$^{f.C}$	28.5±4.94$^{c.B}$	16.5±7.77$^{cde.AB}$	11.5±0.7$^{c.A}$
	500	18$^{cd.AB}$	21±5.65$^{cde.AB}$	18±2.82$^{d.AB}$	10$^{ab.A}$	21±4.$^{94bc.AB}$	47±4.24$^{e.C}$	24±5.65$^{c.B}$	20±1.41$^{de.AB}$	19±1.41$^{d.AB}$
	250	17$^{bcd.B}$	15.5±0.7$^{abcde.AB}$	15.5±2.12$^{cd.AB}$	12.5±0.7$^{ab.A}$	18.5±0.7$^{bc.B}$	39.5±6.36$^{e.C}$	12.5±2.12$^{ab.A}$	13±1.41$^{abcd.A}$	11.5±2.12$^{c.A}$
	125	14 $^{abcd.AB}$	11.5±2.12$^{abcd.A}$	14±5.65$^{abc.AB}$	14±2.$^{82ab.AB}$	19.5±4.94$^{abc.B}$	-	-	9$^{.A}$	6$^{a.A}$
	62.5	15±1.41$^{bcd.C}$	12.5±3.53$^{abcde.BC}$	10±2.82$^{ab.AB}$	10$^{ab.AB}$	9.5±2.12$^{a.AB}$	-	-	8$^{.AB}$	6$^{a.A}$
Rosemary	1000	12.5±0.7$^{abcd.AB}$	26±8.48$^{e.B}$	7$^{a.A}$	8±1.41$^{a.A}$	14.5±3.53$^{abc.AB}$	60$^{f.C}$	16±2.82$^{b.AB}$	22±2.82$^{e.B}$	16±4.24$^{d.AB}$
	500	13±2.82 $^{abcd .A}$	19±1.$^{41bcde.A}$	11.5±3.53$^{abc.A}$	13±1.41$^{ab.A}$	20.5±3.53$^{abc.A}$	60$^{f.B}$	15.5±0.7$^{ab.A}$	20±8.48$^{de.A}$	12±2.82$^{c.A}$
	250	11.5±2.$^{12abc.A}$	22±2.82$^{de.C}$	15.5±3.53$^{cd.AB}$	21±1.41$^{c.C}$	22.5±9.19$^{c.C}$	44.5±0.7$^{e.D}$	14.5±0.7$^{ab.AB}$	14±2.82$^{abcde.AB}$	10±1.41$^{bc.A}$
	125	12±1.$^{41abc.A}$	15±4.24 $^{abcde.AB}$	9±2.82 $^{ab.A}$	11.5±4.94$^{ab.A}$	21±4.24$^{cd.C}$	11±1.41$^{a.A}$	7.5±0.7$^{ab.A}$	9±1.41$^{abc.A}$	9±1.41$^{abc.A}$
	62.5	13±1.$^{41 abcd .AB}$	24^{C}	7.5±2.12 $^{a.A}$	9.5±3.53$^{a.A}$	21±9.89^{cd}	11±1.41$^{a.A}$	-	7$^{a.A}$	6$^{.A}$
Lavandin	1000	14.5±2.$^{12abcd.AB}$	10±2.82$^{abc.AB}$	7.5±0.7$^{a.A}$	10.5±2.12$^{a.AB}$	10.5±0.7$^{ab.AB}$	-	13.5±3.$^{53ab.AB}$	15.5±4.94$^{bcde.B}$	10$^{bc.AB}$
	500	15.5±6.$^{36bcd.AB}$	16.5±2.12^{AB}	11.5±0.7$^{abc.AB}$	13$^{ab.AB}$	18.5±2.12$^{abc.B}$	-	14±2.$^{82ab.AB}$	19±4.24$^{de.B}$	8.5±0.7$^{abc.A}$
	250	19.5±7.77^{e}	11±1.41^{abcd}	16±1.41^{cd}	16^{bc}	12±2.82^{abc}	-	15±4.24^{ab}	12±2.82^{abcd}	8.5±0.7^{abc}
	125	14.5±3.$^{53abcd.C}$	8.5±2.12$^{ab.A}$	10.5±0.7$^{ab.AB}$	13±2.82$^{ab.BC}$	20.5±0.7$^{abc.D}$	19$^{bc.D}$	9±1.41$^{ab.AB}$	7$^{a.A}$	7$^{ab.A}$
	62.5	18.5±0.7$^{cd.C}$	4±2.82a^{A}	10±1.41$^{ab.B}$	9±2.82$^{a.B}$	10.5±0.7$^{ab.B}$	15$^{ab.C}$	-	7$^{a.AB}$	7±1.41$^{ab.AB}$
Hyssop	1000	14±1.41 $^{abcd.B}$	9.5±0.7^{abc}	7.5±0.$^{7a.A}$	9.5±2.12$^{a.AB}$	12.5±0.$^{7abc.AB}$	26.5±2.12$^{d.C}$	10$^{ab.AB}$	7±1.41$^{a.A}$	7^{A}
	500	14.5±3.53 $^{abcd.A}$	13.5±3.53$^{abcde.A}$	9.5±3.53$^{ab.A}$	10.5±4.94^{A}	14.5±0.7$^{abc.A}$	25±2.82$^{cd.B}$	13±1.41$^{ab.A}$	10±4.24$^{abc.A}$	7.5±0.$^{7ab.A}$
	250	15$^{bcd..B}$	11±1.41$^{abcd.AB}$	10±1.$^{41ab.AB}$	13±1.41$^{ab.B}$	10.5±0.7$^{ab.AB}$	43±7.07$^{e.C}$	7±1.41$^{a.A}$	9.5±2.12$^{abc.AB}$	6.5±0.$^{7a.A}$
	125	7.5±2.12^{a}	19±5.65^{bcde}	9±2.82^{ab}	12±4.24^{ab}	12^{abc}	15±4.24^{ab}	-	8±1.41^{ab}	-
	62.5	12±1.41$^{abc.BC}$	8.5±2.12$^{ab.AB}$	6.5±2.12$^{a.A}$	9±1.41$^{a.AB}$	9.5±0.7$^{a.AB}$	14$^{ab.C}$	-	8±2.82$^{ab.AB}$	-

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