Journal of Animal and Veterinary Advances 11 (15): 2762-2768, 2012
ISSN: 1680-5593

In vivo and *In vitro* Antibacterial Activities of Some Essential Oils of Lamiaceae Species on *Aeromonas salmonicida* Isolates from Cultured Rainbow Trout, *Oncorhynchus mykiss*

[1]Gulten Okmen, [1]Aysel Ugur, [3]Nurdan Sarac and [2]Tulin Arslan
[1]Department of Biology, Faculty of Science and Arts,
[2]Department of Aquaculture, Faculty of Fisheries,
Mugla University, 48121 Kotekli-Mugla, Turkey
[3]Medical Laboratory Program, Vocational School of Health Services,
Mugla University, 48700 Marmaris-Mugla, Turkey

Abstract: In this study, usability of plant essential oils from Lamiaceae species for *Aeromonas salmonicida* infections was investigated under *in vitro* and *in vivo* conditions. A total of 18 *A. salmonicida* strains were obtained from the organs and tissues of locally cultured rainbow trout. All strains were 100% resistant to penicillin, trimethoprim and bacitrasin. All strains showed widest resistance profile to antibiotics. Antibacterial effects of essential oils obtained through water distillation of aerial parts of 4 Lamiaceae species (*Origanum onites*, *Origanum vulgare* sp. *hirtum*, *Thymbra spicata* var. *intricata* and *Satureja thymbra*) were high on 17 out of 18 strains and inhibition zones ranged between 14-25, 12-26, 10-30 and 10-33 mm, respectively but none of them was effective on *A. salmonicida* FC84 strain. The most sensitive bacteria to Origanum essential oils was *A. salmonicida* FC29 strain and the most effective essential oil was *S. thymbra's*. Therefore, MIC value of this essential oil on FC29 strain was determined. Then, the MIC value (800 μg) of this essential oil were injected to experimental fishes for *in vivo* studies. Injection of the most effective dose of *S. thymbra* essential oil and its several dilutions (400, 200, 100 μg μL^{-1}) caused toxic effect and total mortality of experimental fishes. The essential oil at the doses with low or no toxic effect did not increase the bactericidal activity of fish blood indicating that it does not protect rainbow trout against *A. salmonicida* infections. On the other hand, *S. thymbra* essential oil at non-toxic low dosages can be used as immune stimulant.

Key words: Antimicrobial activity, fish pathogenic Aeromonas species, plant essential oil, immune stimulant, trout, toxic effect

INTRODUCTION

Aquaculture industry has shown fast growth over several decades and became one the significant source of high protein food for the ever growing world human population. This fast growth and increased production intensity, however brought along some important problems such as infectious diseases causing high economical losses or their treatments with chemical agents which raise concerns on human and environmental health.

As in other organisms, fungus, parasites, viruses and bacteria can cause infections in fish but since, the expansion of aquaculture bacterial pathogens have been one of the leading factor of high economical losses (Ispir *et al*., 2004). Antimicrobial agents have been widely used to treat infections caused by bacteria including *Aeromonas hydrophila*, *A. salmonicida*, *Edwardsiella vibrio anguillarum* and *Yersinia ruckeri*. Frequent use of antimicrobial agents in aquaculture as in other uses, however causes selective pressure for antimicrobial resistance in the exposed bacterial flora consisting of both target and non-target pathogens (Midtvedt and Lingaas, 1992; World Health Organization, 1999). Development of antibiotic resistance and reduced efficacy of the contemporary drugs due to intense usage of antimicrobial agents has been identified in fish pathogens (Midtvedt and Lingaas, 1992; World Health Organization, 1999; Castro-Escarpulli *et al*., 2003). Additionally, antibiotic resistance can be transmitted horizontally from one bacterium to another and this way it can be passed through to human pathogens. Furthermore, since antimicrobial agents in aquaculture are administered via feed which is dispersed in water usage of antimicrobial

Corresponding Author: Gulten Okmen, Department of Biology, Faculty of Science and Arts, Mugla University, 4812 Kotekli-Mugla, Turkey

agents in aquaculture generates direct negative impact on the receiving aquatic environment (Aoki, 1992; Smith *et al*., 1994; Kerry *et al*., 1996; Herwig *et al*., 1997; Saavedra *et al*., 2004).

Finally, antibiotics can accumulate in fish and the environment which creates a potential risk both for the consumers and environmental health (FAO/OIE/WHO, 2006). All these and similar outcomes reported from diverse agricultural industries increased public awareness towards the negative impacts of indirect exposures to antimicrobial agents. This increasing public awareness led to search for green solutions such as organic food products that are free of organic or synthetic chemicals. Development of alternative antibacterial treatments is necessary for organic fish production (Abutbul *et al*., 2004). Medicinal plants have been used for treatment of common infections since ancient times (Rios and Recio, 2005). In recent years, many studies were also conducted in order to evaluate the antimicrobial activity of various plant extracts on different fish pathogens (Muniruzzaman and Chowdhury, 2004; Biradar *et al*., 2007; Asha *et al*., 2008; Castro *et al*., 2008; Turker *et al*., 2009). *A. salmonicida* is one of the oldest known fish pathogen. It was first reported in the literature as the causative agent of furunculosis in 1894 (Williamson, 1928; McCarthy and Roberts, 1980). *A. salmonicida* infections range from superficial to deep skin lessions without systemic association to ulcer disease to a typical, gram negative bacterial septicemia (Noga, 1996).

Furunculosis mainly affects salmonids cultured in freshwater and can cause high mortality rates. Antibiotics and vaccines can be effectively used to control furunculosis break outs in farmed salmonids. However, chemotherapeutic administration can have drawbacks as mentioned before, it can cause the development of antibiotic resistance in bacteria. Additionally, it can decrease growth rates (Midtlyng and Lillehaug, 1998) increase production cost, produce possibility of lower market value (Manning, 1998) and cause injection risks related to vaccination (Pirhonen *et al*., 2003).

Plant genus such as *Origanum*, *Thymbra* and *Satureja* belonging to Lamiaceae family have rich (>2%) essential oil contents (Baser *et al*., 1993). Many studies conducted so far showed that their essential oils have high antimicrobial activity on microorganisms (Karaman *et al*., 2001; Yu *et al*., 2004; Sarac, 2005; Vagionas *et al*., 2007; Bendahou *et al*., 2008; Kotan *et al*., 2008; Sarac and Ugur, 2008; Sarac *et al*., 2009; Ugur *et al*., 2009) suggesting that they might be used as an alternative antibacterial agents in aquaculture industry too. Taking this proven high antimicrobial activity into consideration, the goal in this study was to evaluate antibacterial activity of Lamiaceae species on antibiotic resistant *A. salmonicida* strains isolated from locally farmed rainbow trout (*Oncorhynchus mykiss*).

MATERIALS AND METHODS

Plant material: The plant specimens *O. onites* L., *O. vulgare* sp. *hirtum* (Link) Ietswaart, *S. thymbra* L., *T. spicata* L. var. *intricata* P.H. Davis were collected at the flowering stage between May and July, 2007 from different localities of Mugla province, Turkey. All plant specimens used in the study were deposited at the Herbarium of the Biology Department of Mugla University, Mugla, Turkey.

Fish materials: Fourteen rainbow trout from the fish farms in Fethiye and Koycegiz (Mugla, Turkey) showing the symptoms of furunculosis were brought to laboratory on ice for isolation of bacteria.

Isolation of essential oils: The essential oils of air-dried aerial parts of plant samples were obtained by hydrodistillation using a Clevenger type apparatus.

Isolation and identification of *A. salmonicida*: The samples of gills, liver, kidney, spleen, skin and internal lesions of the fishes were homogenized for 2 min in stomacher (Kema Keur Pro 200) containing alkaline peptone water (1/10 w/v). They cultured on Tryptic Soy Broth (TSB) and incubated at 22°C for 48 h.

The pure culture inoculated in Tryptic Soy Agar (TSA) and Coomassie Brilliant Blue (CBB) Agar medium and incubated at 22°C for 48 h. The typical A. salmonicida colonies on TSA (Hirvela-Koski *et al*., 1988) and CBB Agar medium (Markwardt *et al*., 1989) were selected. Stock cultures of each strain were maintained for short periods at room temperature on TSA slants at +4°C and for longer storage they were frozen at -20°C in 20% (w/v) glycerol.

The isolates were identified with conventional methods. The isolates were first tested for gram reaction. Bacteria identification was restricted to gram-negative isolates. Bacterial groups were determined on the criteria of shape, motility, catalase and oxidase reactions and the Hugh and Leifson glucose oxidation-fermentation test (OF basal medium, Merck). API 20E systems (bioMerieux, France) were furthermore used in order to identify the bacteria at species level.

***In vitro* antimicrobial assays:** Antimicrobial activities of the essential oils were assayed by the Disc Diffusion Method (Bauer *et al*., 1966; Collins *et al*., 1995;

Murray *et al*., 1995). The inoculum sizes of the bacteria were prepared by using No.: 0.5 McFarland tubes to give a concentration of 1×10^8 bacteria mL^{-1}. Mueller-Hinton Agar (MHA) sterilized in a flask and cooled to 45-50°C were distributed (15 mL) to 9 cm sterilized petri dishes after homogeneous injection of 0.5 mL bacteria cultures. The plates were held for 15-20 min at room temperature. Each essential oil (20 μL) was individually applied to 6 mm sterile discs (Schleicher and Schuell, Keene, NH). Discs were placed on the solid agar medium with a slight fingertip pressure. Then, plates were incubated at 22°C for 48 h. At the end of incubation period, diameters of the inhibition zones were evaluated in millimeters. Discs of penicillin (10 U), amoxicillin + clavulanic acid (20 + 10 μg), azlocillin (75 μg), chloramphenicol (5 μg), phosphomycin (50 μg), norfloxacin (10 μg), rifampin (5 μg), sulphisoxazol (300 μg), trimetoprim (5 μg), tetracycline (30 μg), bacitracin (0.04 U), trimetoprim/sulfamethoxazole (1.25/23.75 μg), ofloxacin (5 μg), oxolinic acid (2 μg) and nalidixic acid (30 μg) were used as positive controls.

Studies were performed in triplicates and the developing inhibition zones were compared with those of reference discs.

Determination of Minimum Inhibitory Concentration (MIC): Minimum Inhibitory Concentrations (MICs) of the most effective essential oil was determined by Broth Dilution Method (Collins *et al*., 1995) against the strain which was the most resistant to tested antibiotics but sensitive to the essential oil.

The essential oil were serially-diluted from 800-10 μg mL^{-1} in test tubes using 10% Tween 80 in distilled sterile water. Bacterial suspension was incubated in Brain Heart Infusion Broth (BHIB) overnight at 22°C and then standardized to 1.5×10^8 cfu mL^{-1} in No.: 0.5 McFarland tubes. About 100 μL freshly prepared bacterial suspensions were pipetted into tubes containing Mueller Hinton Broth (MHB). About 100 μL of the essential oil dilutions were then added to the culture tubes containing a final volume of 2 mL. A positive control containing the bacterial culture without the essential oil and a negative control containing only MHB were also included to the test panel. All tests were carried out in triplicates. The test tubes were incubated for 24 h at 22°C. The minimum concentrations at which no visible growth was observed were defined as the MICs.

***In vivo* antimicrobial assays:** *In vivo* studies were conducted in the research and development unit of a commercial rainbow trout farm located in Fethiye, Mugla, Turkey. The 1 week prior to injection of essential oils, fish collected from the outdoor production units were brought inside for acclimation in a concrete tank (1 m^3). Their mean weight and mean total length were 117.6±23.4 g and 21.3±1.4 cm (N = 70), respectively. After injections, fish were stocked to the previously assigned 100 L cylindrical fiberglass tanks. All tanks received spring water with a constant water temperature of 8.5±0.2°C and pH of 7.6. Water exchange rate in cylindrical tanks was kept around 5 L min^{-1}. At this exchange rate, dissolved oxygen concentration stayed above 10 mg L^{-1}.

Before the collection of blood or injection of essential oils feeding of experimental fish was ceased for 48 h and except one occasion all fish were lightly anesthetized using 2-phenoxy ethanol (1/20.000). Then, they were randomly assigned to the treatment groups and each group contained 4-5 fish. Before placing fish to their designated tanks each treatment group injected with the predetermined doses of essential oils intraperitonally. The same amount (0.2 mL) of sterile Phosphate Buffer Solution (PBS, pH = 7.0) were given to the 1st control group and the 2nd control group received no injection. The experiment was carried out in duplicates.

Injection of *S. thymbra* essential oil was done in two occasions. In the first occasion, fish were injected with the effective dose of 800 μg as predetermined in the MIC test. Nevertheless, fish injected with this dose never gain their balance back and died shortly after the injections. Then, two sets of 5 fish were injected again without anesthetizing in order to eliminate the possibility of over anesthesia. All these fish also, never gain their balance back their respiration stopped and they died within 15-30 min pursuing the injections. Therefore in the 2nd occasion lower doses of 400, 200, 100, 50 and 10 μg *S. thymbra* essential oil were injected. Again all fish injected with 400, 200 μg essential oil died shortly after the injections. Only one fish survived from 100 μg group. Total 80% of fish survived in 50 μg group and all fish survived in 10 μg group while no mortality occurred in the control groups. Blood samples were collected from the randomly selected half of the surviving fish at 24 and 48 h following the injections in order to determine the effects of *S. thymbra* essential oil on bactericidal activity of fish blood.

Bactericidal activity of serum: Bactericidal activity of the serum was determined by following the procedure of Kajita *et al*. (1990). An equal volume (100 mL) of serum and bacterial suspension were mixed and incubated for 1 h at 22°C. Blank control was also prepared by replacing serum with sterile PBS. The mixture was then diluted with sterile PBS at a ratio 1:10. The serum-bacterial mixture (100 mL) was plated onto nutrient agar and plates were incubated for 48 h at 22°C. The number of viable bacteria was determined by counting the growing colonies.

RESULTS AND DISCUSSION

Total 50 different presumptive *A. salmonicida* isolates were obtained on TSA medium inoculated with samples taken from the gills, liver, kidney, spleen, skin and internal lesions of the cultured rainbow trout showing the symptoms of furunculosis. Based on the colony morphologies on CBB medium, microscopic examinations and biochemical tests, 18 isolates were identified as *A. salmonicida*. Table 1 shows the antibiotic sensitivity profiles of these 18 strains against 15 antibiotics commonly used for *A. salmonicida*. All strains were found to be 100% resistant to penicillin, trimetoprim and bacitracin. Their resistances to sulphisoxazol, amoxicillin+clavulanic acid, trimetoprim/sulfamethoxazole, phosphomycin, azlocillin, nalidixic acid, oxolinic acid, chloramphenicol, rifampin, tetracycline, norfloxacin and ofloxacin were 89, 83, 78, 72, 67, 61, 55, 50, 44, 17, 11 and 6%, respectively.

Essential oils of all 4 plant species inhibited the growth of 18 out of 17 different *A. salmonicida strains* (Table 2). Inhibition zones of *O. onites*, *O. vulgare* sp. *hirtum*, *T. spicata* var. *intricata* and *S. thymbra* essential oils on these 17 strains ranged between 14-25, 12-26, 10-30 and 10-33 mm. Nonetheless, none of the 4 essential oils was effective on *A. salmonicida* FC84 strain.

Essential oil of *O. onites* was highly effective on *A. salmonicida* FC9, FC43, FC94-2 and FC95 and prevented their growth within an inhibition zone of 25 mm.It was the least effective against *A. salmonicida* FC98 (Table 2). *O. vulgare* sp. *hirtum* essential oil produced the largest inhibition zone of 26 mm for FC43 and FC94-1 strains but it was least effective against to *A. salmonicida* FC98. *T. spicata* var. *intricata* essential oil had the highest antibacterial activity against to *A. salmonicida* FC29 and its activity was much lower on FC93 and FC98 strains. *S. thymbra* essential oil were found highly effective on FC43 and FC29 strains producing inhibition zones of 33 and 32 mm, respectively.

Overall, *A. salmonicida* FC29 was determined to be the most sensitive strain to all 4 essential oils evaluated and this strain showed the highest sensitivity against the essential oil obtained from *S. thymbra*. The MIC test demonstrated that the most effective *S. thymbra* essential oil dose on *A. salmonicida* FC29 was 800 μg. For this reason *in vivo* studies were conducted by using the *S. thymbra* essential oil starting with the most effective dose. However, this dose caused total mortality of all fish due to toxic effect. Lower doses of *S. thymbra* essential oil (400, 200 and 100 μg) were also toxic to rainbow trout with a mean total weight of 117.6±23.4 g. Toxic effect of *S. thymbra* essential oil dropped at 50 μg dose and disappeared at a lower dose of 10 μg. Nevertheless, blood serum of the fish exposed to these lower doses had no bactericidal activity against *A. salmonicida* FC29. All serum bactericidal activity evaluations with blood serum collected before (at 0 h) at 24 and 48 h after injection of *S. thymbra* essential oil yielded over 300 cfu mL^{-1}.

This study evaluated usability of plant essential oils from Lamiaceae species under *in vitro* and *in vivo* conditions for the treatments of *A. salmonicida* infections causing high economical losses in rainbow trout culture. Since, intensive use of antibiotics in fish farming cause development of antibiotic resistance, especially

Table 1: Antibiotic resistance of *A. salmonicida* strains from cultured rainbow trout

	Inhibition zone (mm)														
Strains	AZL	C	FF	NOR	P	RA	ST	TMP	TE	B	SXT	OFX	AMC	OA	NA
FC5	12	10	-	-	-	8	-	-	13	-	9	15	-	9	-
FC9	12	-	10	16	-	9	-	-	-	-	-	19	-	-	-
FC29	-	-	-	20	-	-	-	-	15	-	-	26	-	17	16
FC33	14	-	10	15	-	-	-	-	15	-	-	19	-	-	-
FC38	-	-	-	38	-	-	-	-	12	-	10	15	-	16	14
FC39	-	-	-	27	-	11	14	-	16	-	-	20	9	16	13
FC43	12	-	-	28	-	-	18	-	16	-	-	23	-	23	21
FC79	-	16	30	13	-	10	-	-	12	-	-	9	-	-	-
FC84	-	-	-	-	-	-	-	-	-	-	11	10	-	-	-
FC91	12	-	-	16	-	-	-	-	-	-	-	19	7	7	19
FC92	-	20	-	13	-	14	-	-	14	-	-	17	-	10	-
FC93	-	10	-	15	-	-	-	-	11	-	-	-	-	-	9
FC94-1	-	16	28	13	-	12	-	-	9	-	-	10	-	-	-
FC94-2	-	8	-	28	-	7	-	-	15	-	-	11	-	-	-
FC95	12	-	-	13	-	15	-	-	14	-	-	15	-	-	12
FC96	-	13	34	11	-	13	-	-	13	-	-	9	-	-	-
FC97	-	13	-	27	-	-	-	-	13	-	16	17	-	-	-
FC98	-	20	-	10	-	23	-	-	15	-	-	15	31	10	-

AZL: Azlocillin (75 μg); C: Chloramphenicol (5 μg); FF: Phosphomycin (50 μg); NOR: Norfloxacin (10 μg); P: Penicillin (10 U); RA: Rifampin (5 μg); ST: Sulphisoxazol (300 μg); TMP: Trimetoprim (5 μg); TE: Tetracycline (30 μg); B: Bacitracin (0.04 U); SXT: Trimetoprim/Sulfamethoxazole (1.25 μg/ 23.75 μg); OFX: Ofloxacin (5 μg); AMC: Amoxicillin+Clavulanic Acid (20 μg + 10 μg); OA: Oxolinic Acid (2 μg); NA: Nalidixic Acid (30 μg); (-): no zone

Table 2: Antibacterial activity of essential oils of *O. onites*, *O. vulgare* sp. *hirtum*, *T. spicata* var. *intricata* and *S. thymbra* against *A. salmonicida* isolated from cultured rainbow trout

	Inhibition zone (mm)			
Strains	*O. onites*	*O. vulgare* sp. *hirtum*	*T. spicata* var. *intricata*	*S. thymbra*
FC5	15	20	24	19
FC9	25	18	25	30
FC29	24	25	30	32
FC33	22	19	15	16
FC38	21	17	14	21
FC39	22	21	21	17
FC43	25	26	27	33
FC79	24	24	23	26
FC84	-	-	-	-
FC91	18	20	13	21
FC92	17	23	16	31
FC93	15	15	10	10
FC94-1	23	26	21	23
FC94-2	25	24	21	22
FC95	25	24	14	11
FC96	16	17	17	15
FC97	17	15	17	13
FC98	14	12	10	14

(-): no zone

A. salmonicida strains showing multiple antibiotic resistance were preferred for the study. Plant species of which essential oils were obtained are also chosen for their known high antimicrobial activity as demonstrated in previous studies (Karaman *et al*., 2001; Yu *et al*., 2004; Sarac, 2005; Vagionas *et al*., 2007; Bendahou *et al*., 2008; Kotan *et al*., 2008; Sarac and Ugur, 2008; Sarac *et al*., 2009; Ugur *et al*., 2009).

All *A. salmonicida* strains obtained from cultured rainbow trout in this study showed multiple antibiotic resistance. *A. salmonicida* strains frequently showed multiple resistance against to antibiotics from sulphonamide, tetracycline, amoxicillin, trimetoprim+ sulphadimethoxine and quinolone groups which are commonly used in aquaculture (Tsoumas *et al*., 1989; Inglis *et al*., 1991, 1993; Barnes *et al*., 1994; Dalsgaard *et al*., 1994). In a recent study, it was also demonstrated that multiple antibiotic resistance is a common phenomenon of *A. salmonicida* isolated from Atlantic salmon with the symptoms of furunculosis (Inglis *et al*., 2006). Results of this study confirming the outcome of previous investigations illustrated once more the size of problem caused by intensive use of antibiotic in aquaculture. All these accumulated information on adverse effects of oblivious antibiotic use inevitably lead the search on alternative ways for the treatment of bacterial infections in aquaculture.

Essential oils obtained from medicinal plants can provide such an alternative. The results showed that essential oil of *O. onites* had high antibacterial activity against *A. salmonicida* strains FC9, FC43, FC94-2 and FC95, *O. vulgare* sp. *hirtum* essential oil was highly effective on FC43 and FC94-1 strains, *T. spicata* var. *intricata* essential oil had the high antibacterial activity against to FC29 strain and *S. thymbra* essential oil were found highly effective on FC43 and FC29 strains. Nevertheless *in vivo* studies demonstrated that essential oil of *S. thymbra* or perhaps the other Lamiaceae species can be toxic to rainbow trout at the most effective dose that produces the highest antibacterial activity or at much lower doses. Sadly as indicated by the serum antibacterial activity test at non-toxic low doses essential oils do not provide effective protection against *A. salmonicida* infections.

CONCLUSION

These outcomes of the *in vivo* studies indicated that essential oils of 4 Lamiaceae species could not be used as the replacement of antibiotics. Yet, these results do not exclude the possibility of their other beneficial use at non-toxic low doses. At such doses they can be used as immune stimulant. As immune stimulant, essential oils of Lamiaceae species can help rainbow trout and perhaps many other cultured species to develop resistance against disease factors in turn reducing the need of antibiotics use in aquaculture. Further studies will be conducted in near future in order to evaluate the immune stimulator effects of 4 Lamiaceae species.

ACKNOWLEDGEMENT

This study was supported by a grant from Mugla University Scientific Research Projects Fund, Mugla, Turkey (Project number: 06/14).

REFERENCES

Abutbul, S., A. Golan-Goldhirsh, O. Barazaný and D. Zýlberg, 2004. Use of I>Rosmarinus officinalis as a treatment against *Streptococcus iniae* in tilapia (*Oreochromis* sp.). Aquaculture, 238: 97-105.

Aoki, T., 1992. Present and Future Problems Concerning the Development of Resistance in Aquaculture. In: Chemotherapy in Aquaculture: From Theory to Reality, Michel, C. and D. Alderman (Eds.). Office International des Epizooties, Paris, France, pp: 254-262.

Asha, S.V., S. Anitha and R. Anantharajan, 2008. Antibacterial activity of herbal plant extracts towards the fish pathogens. Braz. J. Microbiol., 39: 756-760.

Barnes, A.C., T.S. Hasting and S.G.B. Amyes, 1994. Amoxicillin resistance in Scottish isolates of *Aeromonas salmonicida*. J. Fish Dis., 17: 357-363.

Baser, K.H.C., T. Ozek, G. Tumen and E. Sezik, 1993. Composition of the essential oils of turkish *Origanum* species with commercial importance. J. Essent. Oil Res., 5: 619-623.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Bendahou, M., A. Muselli, M. Grignon-Dubois, M. Benyoucef, J.M. Desjobert, A.F. Bernardini and J. Costa, 2008. Antimicrobial activity and chemical composition of *Origanum glandulosum* Desf. essential oil and extract obtained by microwave extraction: Comparison with hydrodistillation. Food Chem., 106: 132-139.

Biradar, S.S., N.R. Goud, U. Neogi and R. Saumya, 2007. *In vitro* and *in vivo* antibacterial studies of medicinal plant on motile aeromonad septicemia in fish caused by *Aeromonas hydrophila*. J. Fish. Aquatic Sci., 2: 417-421.

Castro, S.B.R., C.A.G. Leal, F.R. Freire, D.A. Carvalho, D.F. Oliveira and H.C.P. Figueiredo, 2008. Antibacterial activity of plant extracts from brazil against fish pathogenic bacteria. Braz. J. Microbiol., 39: 756-760.

Castro-Escarpulli, G., M.J. Figueras, G. Aguilera-Arreola, L. Soler and E. Fernandez-Rendon *et al*., 2003. Characterisation of *Aeromonas* spp. isolated from frozen fish intended for human consumption in Mexico. Int. J. Food Microbiol., 84: 41-49.

Collins, C.H., P.M. Lyne, J.M. Grange and J.O. Falkinham, 1995. Collins and Lyne's Microbiological Methods. Butterworth-Heinemann, London.

Dalsgaard, I., B. Nielsen and J.L. Larsen, 1994. Characterization of aeromonas salmonicida subsp. Salmonicida: A comparative study of strains of different geographic origin. J. Appl. Bacteriol., 77: 21-30.

FAO/OIE/WHO, 2006. Antimicrobial use in aquaculture and antimicrobial resistance. Report of a Joint FAO/OIE/WHO. Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance. Department of Food Safety, Zoonoses and Foodborne Dýseases World Health Organization.

Hirvela-Koski, V., P. Koski and T. Kuoppamaki, 1988. Enrichment in tryptic soy broth for the detection of Aeromonas salmonicida from the kidney of carrier fish. Bull. Eur. Ass. Fish Pathol., 8: 85-86.

Herwig, R.P., J.P. Gray and D.P. Weston, 1997. Antibacterial resistant bacteria in surficial sediments near salmon net-cage farms in Puget Sound, Washington. Aquaculture, 149: 263-283.

Inglis, V., G.N. Frerichs, S.D. Millar and R.H. Richards, 1991. Antibiotic resistance of Aeromonas salmonicida isolated from *Atlanta salmon*, *Salmo salar* L. in Scotland. J. Fish Dis., 14: 353-358.

Inglis, V., S.D. Millar and R.H. Richards, 1993. Resistance of aeromonas salmonicida to amoxicillin. J. Fish Dis., 16: 389-395.

Inglis, V., G.N. FrerIchs, S.D. Millar and R.H. Richards, 2006. Antibiotic resistance of Aeromonas salmonicida isolated from Atlantic salmon, Salmo salar L., in Scotland. J. Fish Dis., 14: 353-358.

Ispir, U., E. Seker, N. Saglam and M. Dorucu, 2004. A Study of flavobacterium psychrophilum infection in some cultured rainbow trout (Oncorhynchus mykiss) in Eastern anatolia. Firat Univ. J. Sci. Eng., 16: 718-724.

Kajita, Y., M. Sakai, S. Atsuta and M. Kobayash, 1990. The immunostimulatory effects of levamisole on rainbow trout, *Oncorhynchus mykiss*. Fish Pathol., 25: 93-98.

Karaman, S., M. Digraka, U. Ravidb and A. Ilcima, 2001. Antibacterial and antifungal activity of the essential oils of *Thymus revolutus* Celak from Turkey. J. Ethnopharmacol., 76: 183-186.

Kerry, J., R. Coyne, D. Gýlroy, M. Hýney and P. Smýth, 1996. Spatial distribution of oxytetracycline and elevated frequencies of oxytetracycline resistance in sediments beneath a marine salmon farm following oxytetracycline therapy. Aquaculture, 145: 31-39.

Kotan, R., S. Kordali, A. Cakir, M. Kesdek, Y. Kaya and H. Kilic, 2008. Antimicrobial and insecticidal activities of essential oil isolated from Turkish Salvia hydrangea DC. ex Benth. Biochem. System. Ecol., 36: 360-368.

Midtlyng, P.J. and A. Lillehaug, 1998. Growth of Atlantic salmon Salmo salar after intraperitoneal administration of vaccines containing adjuvants. Dis. Aquat. Org., 32: 91-97.

Manning, M.J., 1998. Immune Defence System. In: Biology of Farmed Fish, Black, K.D. and A.D. Pickering (Eds.). Sheffield Academic Press, Sheffield, pp: 180-221.

Markwardt, N.M., Y.M. Gocha and G.W. Klontz, 1989. A new application for coomassie brilliant blue agar: Detection of Aeromonas salmonicida in clinical samples. Dis. Aquat. Org., 6: 231-233.

McCarthy, D.H. and R.J. Roberts, 1980. Furunculosis of Fish the Present State of our Knowledge. In: Advances in Aquatic Microbiology, Droop, M.A. and H.W. Jannasch, (Eds.)., Academic Press, London, pp: 293-341.

Midtvedt, T. and E. Lingaas, 1992. Putative Public Health Risks of Antibiotic Resistance Development in Aquatic Bacteria. In: Chemotherapy in Aquaculture: From Theory to Reality, Michael, C. and D.J. Alderman (Eds.). Office International de Epizooties, Paris, pp: 302-314.

Muniruzzaman, M. and M.B.R. Chowdhury, 2004. Sensitivity of fish pathogenic bacteria to various medicinal herbs. Bangladesh J. Vet. Med., 2: 75-82.

Murray, P.R., E.J. Baron and N.A. Pfaller, 1995. Manual of Clinic Microbiology. ASM Press, Washington, DC.

Noga, E.J., 1996. Fish Disease: Diagnosis and Treatment. Mosby-Yearbook, Inc., St. Louis, Missouri, pp: 142-146.

Pirhonen, J., C.B. Schreck, P.W. Reno and H. Ogut, 2003. Effect of fasting on feed intake, growth and mortality of Chinook salmon, Oncorhynchus tshawytscha, during an induced Aeromonas salmonicida epizootic. Aquaculture, 216: 31-38.

Rios, J.L. and M.C. Recio, 2005. Medicinal plants and antimicrobial activity: Perspectives of ethno-pharmacology. J. Ethnopharma, 100: 80-84.

Saavedra, M.J., S. Guedes-Novais, A. Alves, P. Rema, M. Tacao, A. Correia and A. Martinez-Murcia, 2004. Resistance to β-lactam antibiotics in Aeromonas hydrophila isolated from rainbow trout (Oncorhynchus mykiss). Int. Microbiol., 7: 207-211.

Sarac, N. and A. Ugur, 2008. Antimicrobial Activities of the Essential Oils of *Origanum onites* L., *Origanum vulgare* L. subspecies *hirtum* (Link) Ietswaart, *Satureja thymbra* L. and *Thymus cilicicus* Boiss. and Bal. Growing Wild in Turkey. J. Med. Food, 11: 568-573.

Sarac, N., 2005. The determination of antimicrobial effect of some lamiaceae species which growing wild in mugla region. M.Sc. Thesis, Mugla University Institute of Science and Technology.

Sarac, N., A. Ugur, M.E. Duru and O. Varol, 2009. Antimicrobial activity, antioxidant activity and chemical composition of *Origanum onites* L. and .*Origanum vulgare* L. ssp. hirtum (Link) Ietswaart from Mugla (Turkey). Acta Horticul. (ISHS), 826: 397-404.

Smith, P., M.P. Hiney and O.B. Samuelsen, 1994. Bacterial resistance to antimicrobial agents used in fish farming: A critical evaluation of method and meaning. Ann. Rev. Fish Dis., 4: 273-313.

Tsoumas, A., D.J. Alderman and C.J. Rodgers, 1989. Aeromonas salmonicida: Development of resistance to 4- quinolone antimicrobials. J. Fish Dis., 12: 493-507.

Turker, H., A.B. Yildirim, F.P. Karakas and H. Koyluoglu, 2009. Antibacterial activities of extreacts from some Turkish endemic plants on common fish pathogens. Turk. J. Biol., 33: 73-78.

Ugur, A., N. Sarac and M.E. Duru, 2009. Antimicrobial activity and chemical composition of the essential oil of *Satureja thymbra* L. from Mugla (Turkey). Acta Horticul. (ISHS), 826: 405-412.

Vagionas, K., K. Graikou, O. Ngassapa, D. Runyoro and I. Chinou, 2007. Composition and antimicrobial activity of the essential oils of three *Satureja* species growing in Tanzania. Food Chem., 103: 319-332.

Williamson, I.J.F., 1928. Furunculosis of the salmonidae, Fish. Board Scotland Salmon Fish, 5: 1-17.

World Health Organization, 1999. Joint FAO/NACA/WHO study group on food safety issues associated with products from aquaculture. WHO Technical Report Series. No. 883.

Yu, J., J. Lei, H. Yu, X. Cai and G. Zou, 2004. Chemical composition and antimicrobial activity of the essential oil of *Scutellaria barbata*. Phytochemistry, 65: 881-884.