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Short Communication

Therapeutic and Histopathological Effect of *Aloe vera* and *Salvia officinalis* Hydroethanolic Extracts against *Streptococcus iniae* in Rainbow Trout

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ABSTRACT

Some of the medicinal plants have antibacterial contents and appear to be proper alternatives for antibiotics in the treatment of streptococcal disease, which causes plenty of mortalities in fish farms annually. Therefore, this study investigated the therapeutic effect of *Aloe vera* and *Salvia officinalis* hydroethanolic extracts against *Streptococcus iniae* in rainbow trout. Plant extracts components were analyzed by Gas chromatography-mass spectrometry method and tested *in vitro* against *S. iniae* by disk diffusion assay. In *in vivo*, 480 rainbow trout (10±0.1 g) were distributed in 9 groups (with 3 replication), and all groups (except for the first group as the negative control that was injected with 100 µl of physiologic serum) were injected by 100 µl of LD_{50} ($3.66\times10^{8.5}$CFU/ml) of *S. iniae* suspension, intraperitoneally. The fish of groups were treated by *A. vera* and *S. officinalis* extracts in doses 0 (positive control group was fed by commercial diet without plant extract), 0.5, 1, and 1.5% (supplemented diet) and 80 mg/kg body weight erythromycin for the next 10 days. At the end of the study period, tissue samples of the gills and livers of all groups were taken for the histopathological lesion assay. The results showed that *A. vera* and *S. officinalis* had antibacterial components as Cineol, and *S. iniae* was sensitive to both *A. vera* (MBC=4.067 mg/ml) and *S. officinalis* (MBC=5.185 mg/ml) extracts. At the end of the treatment period, there were no significant differences among erythromycin, *A. vera* (1.5%), and *A. vera* (1%) in terms of the mortality of the infected fish ($P<0.05$). Moreover, *A. vera* (1.5%) showed a significantly lower mortality rate, compared to the positive control ($P<0.05$). *A. vera* (1.5%) was the best group to moderate all histopathological lesions, compared to other groups. Accordingly, *A. vera* (1.5 %) is useful to treat *streptococcosis* (caused by *S. iniae*) and alter gill and liver histopathological lesions in rainbow trout.

Keywords: Cineol, Histopathology, Medicinal herbs, *Oncorhynchus mykiss*, *Streptococcus iniae*

Effet Thérapeutique et Histopathologique des Extraits Hydroéthanoliques d'*Aloe vera* et de *Salvia officinalis* contre *Streptococcus iniae* chez la Truite Arc-en-ciel

Résumé: Certaines des plantes médicinales ont un contenu antibactérien et semblent être des alternatives appropriées aux antibiotiques dans le traitement des maladies streptococciques, à l' origine de nombreuses mortalités dans les piscicultures chaque année. Par conséquent, cette étude a examiné l'effet thérapeutique des extraits hydroéthanoliques d'*Aloe vera* et de *Salvia officinalis* contre *Streptococcus iniae* chez la truite arc-en-ciel. Les composants des extraits de plantes ont été analysés par chromatographie en phase gazeuse couplée à

l'exception du premier groupe (contrôle négatif traité avec 100µl de sérum physiologique) ont reçu 100µl de DL_{50} ($3,66 \times 10^{8.5}$CFU/ml) de suspension de *S. iniae* par voie intrapéritonéale. Les poissons des différents groupes ont reçu différentes concentrations d' extraits d' *A.* v*era* et de *S. officinalis* aux doses 0 (le groupe de contrôle positif a été nourri par un régime commercial sans extrait de plante), 0,5, 1 et 1,5% (régime complémentaire) et 80 mg/kg de corps poids d'érythromycine pour les 10 prochains jours. À la fin de la période d'étude, des échantillons de tissus des branchies et des foies de tous les groupes ont été prélevés pour l'examen des lésions histopathologiques. Les résultats ont montré que *A. vera* et *S. officinalis* avaient des composants antibactériens comme Cineol, et *S. iniae* était sensible à la fois à *A. vera* (MBC = 4,067 mg/ml) et à *S. officinalis* (MBC = 5,185 mg/ml). À la fin de la période de traitement, il n'y avait aucune différence significative entre l'érythromycine, *A. vera* (1,5%) et *A. vera* (1%) en termes de mortalité des poissons infectés ($P<0.05$). De plus, *A. vera* (1,5%) a montré un taux de mortalité significativement plus faible par rapport au contrôle positif ($P<0,05$). *A. vera* (1,5%) était le meilleur groupe pour modérer toutes les lésions histopathologiques,. En conséquence, *A. vera* (1,5%) est utile pour traiter le *streptocoque* (causée par *S. iniae*) et modifier les lésions histopathologiques des branchies et du foie chez la truite arc-en-ciel.
Mots-clés: Cineol, Histopathologie, Herbes médicinales, *Oncorhynchus mykiss*, *Streptococcus iniae*

INTRODUCTION

Infectious diseases are always regarded as hazards and may cause significant stock losses and problems for animal welfare. *Streptococcosis* is a bacterial disease that develops following the infection by *Streptococcus*. One of the main species of *Streptococcus* is *Streptococcus iniae*, which is spherical or ovoid and 0.5-2.0 µm in diameter. It occurs in pairs or chains when grown in liquid media; moreover, it is non-motile, non-spore-forming, and Gram-positive. *S. iniae* bacterium has been reported in many species of fresh, estuarine, and marine fish species, and is the main hazard to cold and warm water fish aquaculture industry in the world (Acar et al., 2015). It has been reported in many fish species throughout the world contributing to an annual loss of approximately 250 million USD in 2008 (Aboyadak et al., 2016). Infection by *S. iniae* leads to various clinical signs, which include loss of appetite, uni- or bi-lateral exophthalmia, presence of blood-tinged fluid in the body cavity, enlarged and reddened spleen, pale but enlarged liver, as well as inflammations around the heart and kidney, hemorrhages at the gill and the base of the fins and in the opercula, corneal opacity, darkening of the skin, and erratic swimming just below the surface of the water. However, in some cases, the affected fish showed no obvious clinical signs before death, and the mortality is mainly due to septicemia, as well as the infection of the brain and nervous system (Amal and Zamri-Saad, 2011). The therapeutic method which is used against *S. iniae* includes prescribing antibiotics and vaccination of fish. Vaccination is an expensive preventive method that is only administered in limited cases, such as broodstock fish, and the most common therapeutic methods use antibiotics against *streptococcosis*. Intensive aquaculture (shrimp and fish farming) has led to growing problems with bacterial diseases, the treatment of which now requires the intensive use of antibiotics. Despite the high efficacy of antibiotics, the public health hazards related to antimicrobial use in aquaculture include the development and spread of antimicrobial-resistant bacteria, resistance genes, and the presence of antimicrobial residues in aquaculture products and the environment (Defoirdt et al., 2011). The spread of antimicrobial resistance due to exposure to antimicrobial agents is well documented in veterinary medicine. It is also well documented that fish pathogens and other aquatic bacteria can develop

resistance as a result of antimicrobial exposure. The examples include *Aeromonas Salmonicida*, *Aeromonas Hydrophila*, *Edwardsiella Tarda*, *Yersinia Ruckeri*, *Photobacterium Damselae, Vibrio Anguillarum,* and *Streptococcus Iniae* (Defoirdt et al., 2011). The rise in bacterial antibiotic resistance and antibiotic residues has become a global concern, and there is a need to develop alternative therapies for bacterial pathogens in animal production, especially in aquaculture. It seems that a source of alternative treatments of antibiotics is essential oils or plant extracts, which are volatile liquid fractions that contain the substances responsible for the aromas of the plants. They are obtained from different organs, such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots. Due to their biogenic antimicrobial properties, these oils may constitute alternative prophylactic and therapeutic agents in aquaculture (Karakaya et al., 2011). *Aloe vera* (synonym: *Aloe barbadensis*) is a succulent and stemless herb found widely distributed in tropical and subtropical regions. The genus *Aloe* comprised of more than 360 species of which *A.vera* is considered to be the most popular and bioactive with more than 70 biologically active compounds (Gabriel et al., 2015). Sage (*Salvia officinalis* L.) is one of the most commercially important species within the Lamiaceae family. It is a perennial and evergreen subshrub native to the Mediterranean region. It was scientifically proven that *S. officinalis* has anti-diabetic, antioxidative, gastroprotective, anti-inflammatory, antiviral, anti-obesity, anti-spasmatic, fungicidal, bactericidal, and anticancerogenic effects. Moreover, as a natural disinfectant, *S. officinalis* essential oils could play a vital role in preventing the spread of pathogenic microorganisms and environmental problems connected with the use of synthetic chemicals (Eidi et al., 2006). With this background in mind, since there is a dearth of research on the antimicrobial activity of herbs as natural therapeutic agents for main bacterial pathogens in infected fish, the present study aimed to investigate and compare the antibacterial activity and treatment potential of *A. vera* and *S. officinalis* hydroethanolic extracts beside erythromycin antibiotics on pathogenic bacteria *S. iniae* in rainbow trout.

MATERIAL AND METHODS

Plant Extract Preparation. Aerial organs of *A. vera* and *S. officinalis* were collected from the local areas of Iran and identified in the Department of Botany, Faculty of Agriculture, Urmia University, Urmia, Iran. Plant samples were air-dried and ground in an electric mill, and 20 g of ground powders of the plants were soaked in 100 ml mixture of the equal ratio of water and alcohol for 15 min shaking at 60˚C. The resulting solution was filtered through Buchner funnel and Whatman No.1 filter paper. The solvent of the prepared extract was evaporated using a rotary evaporator, and a concentrated herbal extract was obtained in this study (Azwanida, 2015).

Identification of the Extracts' Components. Composition of the prepared concentrated herbal extracts was analyzed and identified by Gas Chromatography-Mass Spectrometer Method (GC/MS). The GC/MS analyses were performed on a Thermo Finnegan capillary gas chromatograph directly coupled to the mass spectrometer system (model GC TRACE; TRACE MS plus). The HP-5MS non-polar fused silica capillary column (30 m×0.250 mm, 0.25 µm film thickness) was also used in this study. The temperature profile was as follows: at first, the temperature of the oven was fixed at 40 ˚C for 2 min and then increased to 160 ˚C with the temperature rate of 3 ˚C/min. Finally, it increased to 280 ˚C at 5 ˚C/min for 2 min. The carrier gas was helium at a flow rate of 1 ml min^{-1}, and ionization energy was 70 eV (Gomathi et al., 2015).

Antibacterial Effect of Plant Extracts Measured by Disc Diffusion. Antibacterial activity of the herbal extracts was measured by a disc diffusion method (Mostafa et al., 2018). Based on this method, 100 µl of *Streptococcus iniae* (BCG/LMG 3740) suspension (half

McFarland 1.5×10^8CFU/ml, calculated by McFarland tubes) was spread on Mueller-Hinton Agar (MHA) growth (CONDALAB, Spain) medium plates and sterile discs (6 mm) each containing 10 µl of plant extract placed on the microbial lawns. Antibiotic discs including 15 µg erythromycin (PADTANTEB, Tehran, Iran) were also included. The tests were carried out in triplicate, and the plates were incubated at 37 °C for 24h. Inhibition zone diameters of bacteria were measured after the incubation period and reported in mm.

Determination of Minimum Inhibitory and Bactericidal Concentrations. Minimum inhibitory concentration (MIC) values were determined by broth microdilution (Kang et al., 2011). Serial two-fold dilutions were prepared from each herbal extract in the broth Brain Heart Infusion (BHI) growth medium (QUELAB, Canada) in 96-well microtiter plates. Fresh *Streptococcus iniae* suspensions prepared from overnight grown cultures in BHI were added to give a final concentration of 1.5×10^8CFU/ml. For each plant serial dilution, a control group of bacteria, a medium control group, and an extract control group were also included. The microplates were incubated at 37°C for 24h, and the first dilution with no turbidity (no growth) was considered as the MIC. Furthermore, minimum bactericidal concentrations (MBC) were determined by spreading 10 µl of the contents of the MIC wells (all wells without turbidity) that showed no bacterial growth on MHA plates followed by incubation at 37°C for 24h. The first well with colony counts of <5 was considered to be negative for growth and reported as the MBC. The MIC and MBC of erythromycin (SIGMA, CAS-NO: 114-07-8, USA) were also measured in the above methods.

Fish and Husbandry Conditions. After obtaining 1170 rainbow trout fish (mean weight=10±0.1 g) from a local farm in Urmia, Iran, they were transferred to "Artemia and Aquaculture Institute" of Urmia University, Urmia, Iran, disinfected with 3% NaCl solution for 5 min, and acclimatized to the laboratory conditions for a week in two 1000 L polyethylene tanks. Subsequently, they were fed by extruded commercial pellets (Faradaneh Co., Shahrekord, Iran) during the adaptation period. Following that, 360 fish were used to determine LD_{50} of *S. iniae*, and others (n=810) were used to investigate the bacterial challenge and therapeutic effect of the herbal extracts.

Determination of LD_{50} of *S. iniae*. The *S. iniae* (BCG/LMG 3740) provided by bacteria collection of Laboratory for Microbiology, Faculty of Sciences, Ghent University, Ghent, Belgium. After confirmation of the bacteria with biochemical tests, 360 rainbow trout fish were divided into six groups with three replications (each replication had 20 fish) and transferred into 18 polyethylene tanks filled with 80 L of dechlorinated fresh flow (3.5 L/s) water. Following that, fish were anesthetized with 150 mg/L clove powder and injected as follows: the first group was negative control (without injection), the second group was positive control (injected with 100 µl of physiologic serum intraperitoneally), and the other groups were injected intraperitoneally with 100 µl of *S. iniae* bacterial suspension containing 10^6, 10^7, 10^8, and 10^9CFU/ml by insulin syringe, respectively. Cumulative mortality of each injected fish group was recorded at the end of the next 10-day period, and LD_{50} of bacteria was measured.

Bacterial Challenge and Treatment of the Fish by Plant Extracts. After obtaining 27 polyethylene tanks filled with 80 L of dechlorinated fresh flow (3.5 L/s) water, 810 fish were randomly distributed in 9 groups with 3 replications (each replication had 30 fish) into the tanks. Fish anesthetized with 150 mg/L clove powder, and the first group (negative control) was injected with 100 µl of physiologic serum intraperitoneally, and the fish of other groups were injected with 100 µl of *S.iniae* bacterial suspension containing $3.66\times10^{8.5}$CFU/ml (measured LD_{50}) by insulin syringe intraperitoneally. Therefore, the groups were separately treated with oral administration of the commercial diet supplemented by 0% (Positive control), 0.5%,1%, and 1.5% of food *A. vera* and *S. officinalis* extracts and 80 mg kg^{-1} body weight

erythromycin (SIGMA, CAS-No.114-07-8) for the next 10 days. The control groups were fed with a commercial diet without any antibiotic and plant extract. Cumulative mortality of fish in each group was recorded daily, and the data obtained from the treatment period were compared statistically at the end of 10 days. During the trial period, water temperature and dissolved oxygens were 14±1 ˚C and 8 mg/L, respectively.

Histological Procedures. At the end of the treatment period of the injected fish, tissue samples were taken from gill and liver organs of survived fish as follows. The fish were anesthetized with immersion in a solution containing 150 mg/Lclove powder and then sacrificed by cervical section. Gill and liver samples were excised, rinsed in physiological saline, and fixed in aqueous Bouin's fluid for 6, 12, and 8 hours. The tissues were dehydrated in an ethyl alcohol series of ascending concentrations, embedded in paraffin, and sectioned at 5 mm. The tissue sections were stained with hematoxylin-eosin and examined by a light microscope (Camargo and Martinez, 2007). The presence of histological changes in the organs was evaluated semi-quantitatively by the degree of tissue change that was based on the severity of the lesions. For the degree of tissue alteration, the changes in each organ were classified in progressive stages of damage to the tissue. These stages include stage I alterations with no changes in the normal function of the tissue. Stage II alterations cause mild severe changes in the normal functioning of the tissue, and stage III alterations result in more severe changes and cause irreparable damage. A value of the degree of tissue change for each treatment was calculated by the following formula:

Degree of Tissue Change=(1×stage I)+(10×stage II)+(100×stage III)

(where I, II, and III correspond to the number of alterations of stages I, II, and III, respectively).

The degree of tissue change values between 0 and 10 indicates a normal functioning of the organ. Moreover, the values between 11 and 20 indicate slight damage to the organ, and the values between 21 and 50 signify moderate changes in the organ. In addition, values between 50 and 100 reveal severe lesions, and values above 100 indicate irreversible damage to the organ (Camargo and Martinez, 2007).

Statistical Analysis. The data of inhibition zone diameter of plant extracts beside erythromycin were analyzed in SPSS software (version 20) through a one-way analysis of variance (ANOVA) and Dunnett's test. A p-value less than 0.05 was considered statistically significant. Moreover, the LD_{50} data of *S. iniae* were analyzed using SPSS software (version 20) in a Probit model, and variances of cumulative mortality of fish groups were analyzed by SPSS software (version 20) through one-way ANOVA and the Tukey's test.

RESULTS AND DISCUSSION

Table 1 tabulates the main chemical properties of *A. vera* and *S. officinalis* extracts. According to the results, different biological components in *A. vera* and *S. officinalis* extracts include organic acids, alcohols, esters, and polysaccharides. The main effective antimicrobial component which exists in both *A. vera* and *S. officinalis* extracts involved in terpenoid groups as Cineol (Table 1). Moreover, the MBC of *A. vera* is less than that of *S. officinalis,* and there is no significant difference between plant extracts and erythromycin antibiotics in terms of antibacterial effect (Table 2). After statistical analysis, the LD_{50} of *S. iniae* was determined as $3.66\times10^{8.5}$ CFU/ml for rainbow trout (Table 3). Erythromycin, *A. vera* (1.5%), and *A. vera* (1%) were shown as effective therapeutic agents to treat *streptococcosis* (caused by *S. iniae* in an experimental challenge) and showed low cumulative mortality, respectively. Moreover, no difference was observed between the other groups and the positive control group (Figure 1). The severity of histopathological lesions of gills (Figure 2) and livers (Figure 3) of different groups are shown and compared with each other (Table 4). *A. vera* (1.5%) indicated the

best treatment to moderate histopathological lesions of gill and liver tissues of infected rainbow trout (Table 4).

antimicrobial agents refer to their active biogenic properties. The chemical composition of plant extracts

Table 1. The main chemical composition of *A. vera* and *S. officinalis* extracts measured by Gas chromatography-mass spectrometry

S. officinalis		*A. vera*	
Component	%	Component	%
Cineol	10.21	p-Xylene	2.25
Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, [1S-(1à,4á,5à)]-	2.14	1,5-Heptadien-4-one, 3,3,6-trimethyl-	2.41
Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (ñ)-	4.71	Oleic acid	12.28
Borneol	5.29	1-Heptanol,2-propyl-	2.32
Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-	4.75	Tetradecanoic acid	2.28
1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-	15.51	n-Hexadecanoic acid	15.22
Germacrene D	2.22	Squalene	5.37
á-Sesquiphellandrene	12.26	Hentriacontane	5.99
1-Naphthalenol, 4-methoxy-	9.55	Octacosane	10.71
1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1ar-(1aà,4á,4aá,7à,7aá,7bà)]-	5.38	1,2-Benzenedicarboxylic acid, diisooctyl ester	9.50
Butanoic acid, 3-methyl-, 1-ethenyl-1,5-dimethyl-4-hexenyl ester	3.27	Eicosane	4.87
1-Naphthaleneacetic acid, methyl ester	10.10	Heptacosane	5.19
1-Naphthalenepropanol, à-ethenyldecahydro-à,5,5,8a-tetramethyl-2-methylene-, [1S-[1à(R*),4aá,8aà]]-	3.39	Acemannan	5.97
Squalene	2.22	Cineol	12.35

Table 2. Minimum Inhibitory and Bactericidal Concentrations and inhibition zone diameter of *A. vera* and *S. officinalis* extracts against *S. iniae* beside Erythromycin

Plant extracts	Minimum Inhibitory Concentrations (mg/ml)	Minimum Bactericidal Concentrations (mg/ml)	Inhibition zone diameter (mm) (Mean±SD)*	
			Plant extracts	Erythromycin
A.vera	4.067	4.067	15.97±0.30	16.07±0.58
S. officinalis	2.592	5.185	15.67±0.25	16.07±0.58

*There is no significant difference between groups *(P<0.05)*.

The use of antimicrobial drugs in aquaculture has well-known positive effects on the control of bacterial infections; however, several side effects influencing both the fish and the environment are associated with the excessive use of these agents (Samuelsen, 2006). The efficiency of plant extracts as alternative

depends on the extraction method which can also vary according to conditions, such as climate, soil composition, origin, season, plant organ, age, and vegetative cycle stage. The composition of plant extracts is very complex and can include more than 60 components; however, a few major components

constitute up to 85% of the plant extracts and generally determine their biological properties (Karakaya et al., 2011). In the present study, biogenic components, such as n-Hexadecanoic acid (15.22%), Cineol (12.32%), Oleic acid (12.28%), Octacosane (10.71%) for *A. vera* and 1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E) (15.51%), á-Sesquiphellandrene (12.26%), Cineol (10.21%) and 1-Naphthaleneacetic acid, and methyl ester (10.10%) for *S. officinalis* extract, were determined as the majority of biogenic components (Table 1). The essential oils of many plants contain phenolic and terpenoid compounds, and these comprise the majority of plant antimicrobial components (Garcia-Garcia et al., 2011). Therefore, in the present study, the antimicrobial activity of *A. vera* and *S. officinalis* extracts (Table 2) could refer to the presence of the monoterpenoid compound and Cineol in both of them (Table 1). *S. iniae* is sensitive to *A. vera* (MBC=4.067 mg/ml) and *S. officinalis* (MBC=5.185 mg/ml) extracts in the current study; moreover, there were no significant differences between these herbal extracts and erythromycin regarding the antibacterial activity (Table 2). Furthermore, the antibacterial activity of *A. vera* and *S. officinalis* against pathogenic bacteria has reported (Milenković et al., 2015), and their results were consistent with the findings of the present study. It has been shown that herbal-based immune stimulants are capable of enhancing nonspecific and specific defense mechanisms and reducing losses from viruses, bacteria, and parasitic infections in fish and shrimps (Randrianarivelo et al., 2010). Tafi et al. (2018) demonstrated that *A. vera* and *S. officinalis* could stimulate the immune system of rainbow trout (specifically 1.5% of feed). Moreover, *S. officinalis* significantly increased total immunoglobulin, while *A. vera* enhanced lysozyme activity, complement activity, and total immunoglobulin in the serum of rainbow trout in the intervention group, compared to those in the control group (Tafi et al., 2018). In addition, *A. vera* enhanced rainbow trout and Tilapia resistance against *S. iniae* ($P<0.05$) (Gabriel et al., 2015; Tafi et al., 2018). Chavanet al. (2015) demonstrated the antibacterial activity of *A. vera* against *Streptococcus* bacteria in an *in vitro* study. The antibacterial and immunomodulatory effects of *A. vera* and *S. officinalis* against *S. iniae* has been previously demonstrated *in vitro* and *in vivo* as an oral treatment for *S. iniae* infection in fish. The present study determined the efficacy of *A. vera* and *S. officinalis* as a therapeutant for streptococcal infection caused by *S. iniae* (Chavan et al., 2015). The hydroethanolic extracts of *A. vera* and *S. officinalis* were administrated to rainbow trout as a therapeutic agent beside erythromycin after injecting 100 µl *S. iniae* suspension ($3.66\times10^{8.5}$ CFU/ml measured in table 3) in a 10-day curing period. The results showed a significant decrease in the cumulative mortality among the infected fish which fed by *A. vera* extract (1 and 1.5%) supplemented diet. Both 1 and 1.5% *A. vera* extract with 33.33% and 26.67% cumulative mortality, respectively, had no significant effect on reducing the cumulative mortality in the infected fish beside erythromycin with 23.33% cumulative mortality (Figure 1). The *A. vera* 1.5% decreased the cumulative mortality of the infected fish more than 50%, compared to the positive control, and had the most effective antibacterial rate of plant extracts in the present study (Figure 1). In a similar study, a challenge test with *Vibrio vulnificus* showed 100% mortality in tilapia (*Oreochromismossambicus*) fed with the control diet by day 15, whereas the fish fed with the experimental diets (supplemented 1% acetone extract of medicinal plants *Cynodondactylon, Aegle marmelos*, *Withania somnifera*, and *Zingiber officinale*) registered only 63–80% mortality at the end of the challenge experiment with *Vibrio spp*. and *Photobacterium damselae* after 30 days (Immanuel et al., 2009). Although the antibacterial effect of *A. vera* and *S. officinalis* is probably due to the presence of monoterpenoidcineol, the synergistic effect of all ingredients of extracts cannot be ignored in their final properties (Olajuyigbe and Afolayan, 2012).

Interestingly, channel catfish (*Ictaluruspunctatus*) fed with the natural oregano essential oil extracted from *Origanum heracleoticum* had the lowest mortality following an *Aeromonas hydrophila* infection, compared to fish fed with a combination of carvacrol and thymol, which are the principal active components

Table 3. Probit model analysis to measure LD_{50} of *S. iniae* by SPSS software

Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log	
	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound
0.100	213266.77	0.000	5051317.01	5.329	-44.976
0.200	2750765.981	0.000	31181974.31	6.439	-19.999
0.300	17386982.66	0.002	495802398.9	7.240	-2.621
0.400	84033635.59	1559873.550	5.711E+15	7.924	6.193
0.500	366425323.7	32478117.72	1.891E+29	8.564	7.512

Table 4. Comparative scores of histopathological lesions of gill and liver tissues of infected fish by bacteria *S. iniae*

Tissue	Lesions	Treatments								
		Control-	Control+	*A. vera* 0.5%	*A. vera*1%	*A. vera* 1.5%	*S. officinalis* 0.5%	*S. officinalis* 1%	*S. officinalis* 1.5%	Erythromycin
Gill	Epithelial separation from the lamellas membrane	-	+++	++	++	+	-	-	+	+
	Necrosis of lamella	-	+++	++	-	-	++	++	-	-
	Atrophy of gill filaments	-	+	+	-	-	+	+	+	+
	Hyperemia in the filaments	-	++	+	-	-	++	-	-	-
	Inflammatory and clubbed secondary lamellas	-	++	-	++	-	-	++	++	-
Liver	Influence of lipid vacuoles in hepatocytes	-	+++	++	++	+	++	++	++	++
	Nucleus pyknosis	-	++	+	+	+	++	++	+	+

Key to scores: -: No lesions, +: Mild, focal lesions, ++: Mild, multifocal lesions, Severe lesions +++

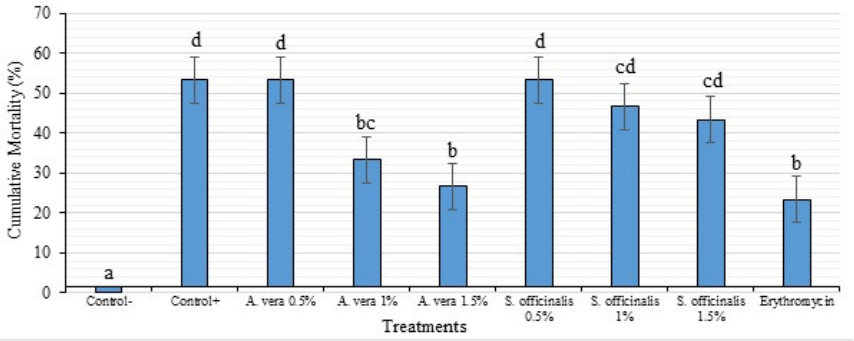

Figure 1. Comparison of cumulative mortality of the infected fish at the end of the treatment period by plant extracts and antibiotic
*Columns with different alphabets have significant difference statistically.

of oregano essential oil (Zheng et al., 2009). These results highlight the fact that the use of the entire essential oil is more effective than treatment with a combination of its principal components. Therefore, the entire extract of these herbs was used in this study in order to achieve all the therapeutic and antibacterial

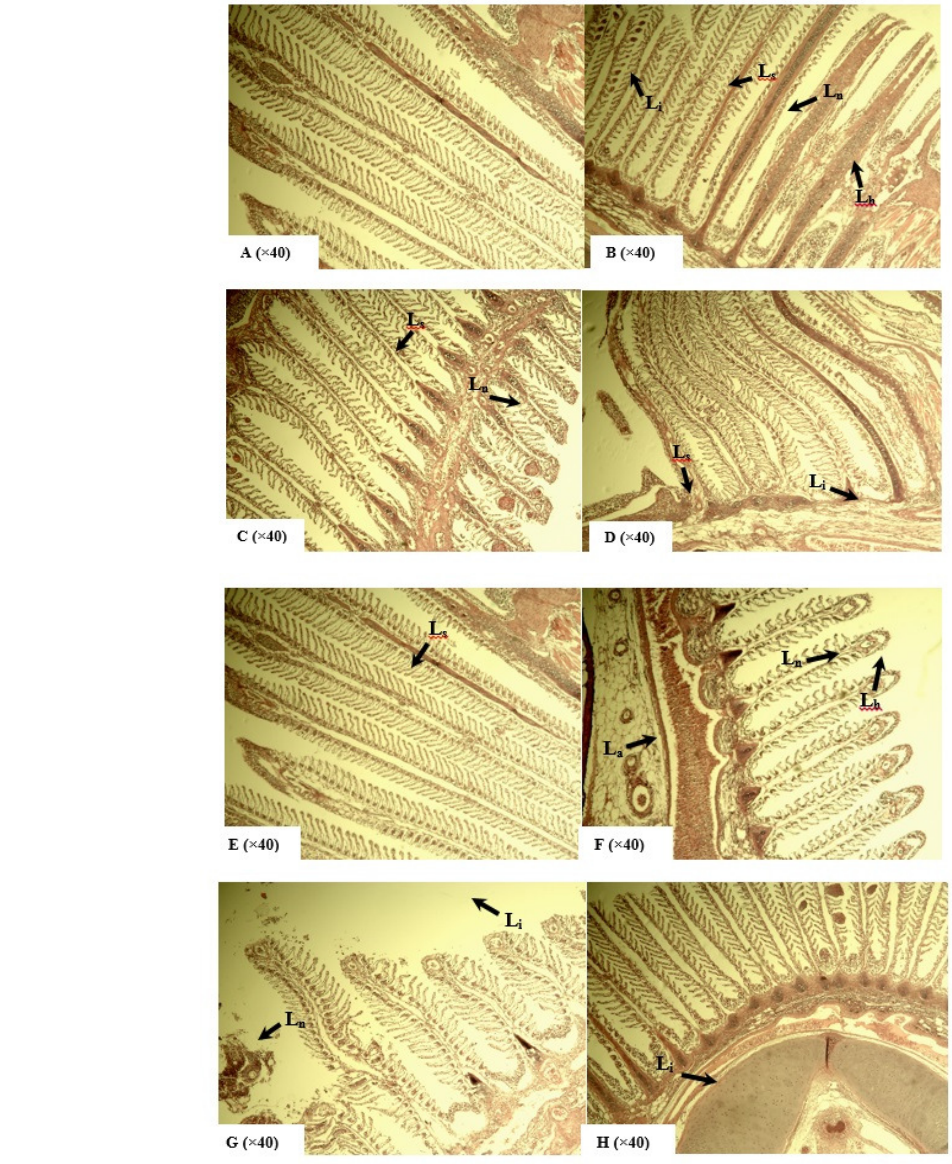

Figure 2. Histopathological changes of gill organ in controls and *S. iniae* challenged groups of rainbow trout after treatment with *A. vera* and *S. officinalis* extracts. A section of gills with normal structure, in the negative control group (A); epithelial separation from the lamellas membrane L_s, hyperemia in the filaments L_h, inflammatory and clubbed secondary lamellas L_i, necrosis of secondary lamellas L_n and Atrophy of gill filaments L_a in the positive control group (B), *A. vera* 0.5% group (C), *A. vera* 1% group (D), *A. vera* 1.5% group (D), *S. officinalis* 0.5% group (F), *S. officinalis* 1% group (G), *S. officinalis* 1.5% group (H), and Erythromycin group (I) (Mag×40; H and E staining).

potential of *A. vera* and *S. officinalis* herbal extracts. The molecular basis of the antibacterial action of essential oils is poorly understood. It has been suggested that they can disrupt the permeability of the bacterial cell membrane leading to the disruption of the proton motive force, electron flow and active transport, coagulation of cell contents, inhibition of quorum sensing, induction of heat shock proteins, and the prevention of flagella development (Reyes-Becerril et al., 2008). Rodrigues et al. (2017) demonstrated the onset of adaptive mechanisms of fish for longer exposure periods by oxytetracycline after acute (96h: 0.005-50 µg/L) and chronic (28days: 0.3125-5 µg/L) exposures. They reported that the most predominant disorders observed in gills were progressive (hypertrophy of mucous cells and hyperplasia of epithelial cells) in acute exposure and regressive (lamellar fusion, epithelial lifting of lamellae, and some changes in tissue architecture) in chronic exposure. However, only acute exposure was responsible for a significant increase in the total gill pathological index. According to their results, especially following acute exposure, a significant increase was observed in the liver pathological index, such as circulatory (hemorrhage and increase of sinusoidal space), regressive (pyknotic nucleus, vacuolization, and hepatocellular degenerations) and progressive (hypertrophy of hepatocytes) changes. Furthermore, the observed histological alterations in gill and liver appear to be the result of several physio-metabolic disorders and the consequence of the biochemical and molecular modes of action of oxytetracycline. Despite an increase in the histopathological damage in individuals exposed to oxytetracycline, the observed lesions were of minimal or moderate pathological importance, non-specific, and reversible (Rodrigues et al., 2017). The results of the present study were consistent with the findings of a study conducted by Rodrigues et al. (2017) who showed some histopathological lesions in gills and livers of rainbow trout in the erythromycin treatment (Figures 2 and 3). On the other hand, in addition to the remedial effect of erythromycin against *streptococcosis* (caused by *S. iniae*), this antibiotic could not eliminate histopathological lesions of gills and livers of infected fish. Therefore, due to the side effects of antibiotics, it is important to find and use the biologic antibacterial alternatives in aquaculture. The majority of the studies focused on the immunomodulatory potential of herbal extracts in aquaculture, and few experiments were conducted to investigate the therapeutic effect of plant extracts as alternative biological antibacterial agents instead of synthetic antibiotics for the treatment of bacterial diseases in aquatic animals. Abd El-Salam et al. (2017)reported that the clove extract was a suitable choice as an alternative bacterial therapeutic agent beside ciprofloxacin among infected catfish (*Clariasgariepinus*) with *Aeromonassobria* (Abd El-Salam et al., 2017). However, in the present study, alongside erythromycin (80 mg/kg Body Weight), two medicinal plants, namely *A. vera* and *S. officinalis* extracts were used to moderate or eliminate the histopathological lesions of gills and livers caused by *S. iniae* (Figures 2 and 3) in rainbow trout. Moreover, their potential was compared regarding the treatment of *streptococcosis* in this study. The negative effects of some herbal oils on histopathological changes may be related to the toxic constituents, excessive doses, or allergic conditions; nonetheless, they generally have no effects on health when used in the proper doses and application. Sonmez et al. (2015) reported no histopathological differences in the liver of rainbow trout fed with control, as well as 0.5% and 1% *S. officinalis* oil supplemented diets (Sönmez et al., 2015). The results of the present study demonstrated that all dosages of *A. vera* and *S. officinalis* extracts under study moderated the histopathological lesions of gill and liver organs beside positive control (*S. iniae* infected fish fed with control diet); however, *A. vera* (1.5%), erythromycin (80 mg/kg Body Weight), and *S. officinalis* (1.5%) extracts were more effective than other treatments in terms of reducing the histopathological lesions in descending order (Table 4). According to the results of the present study, the

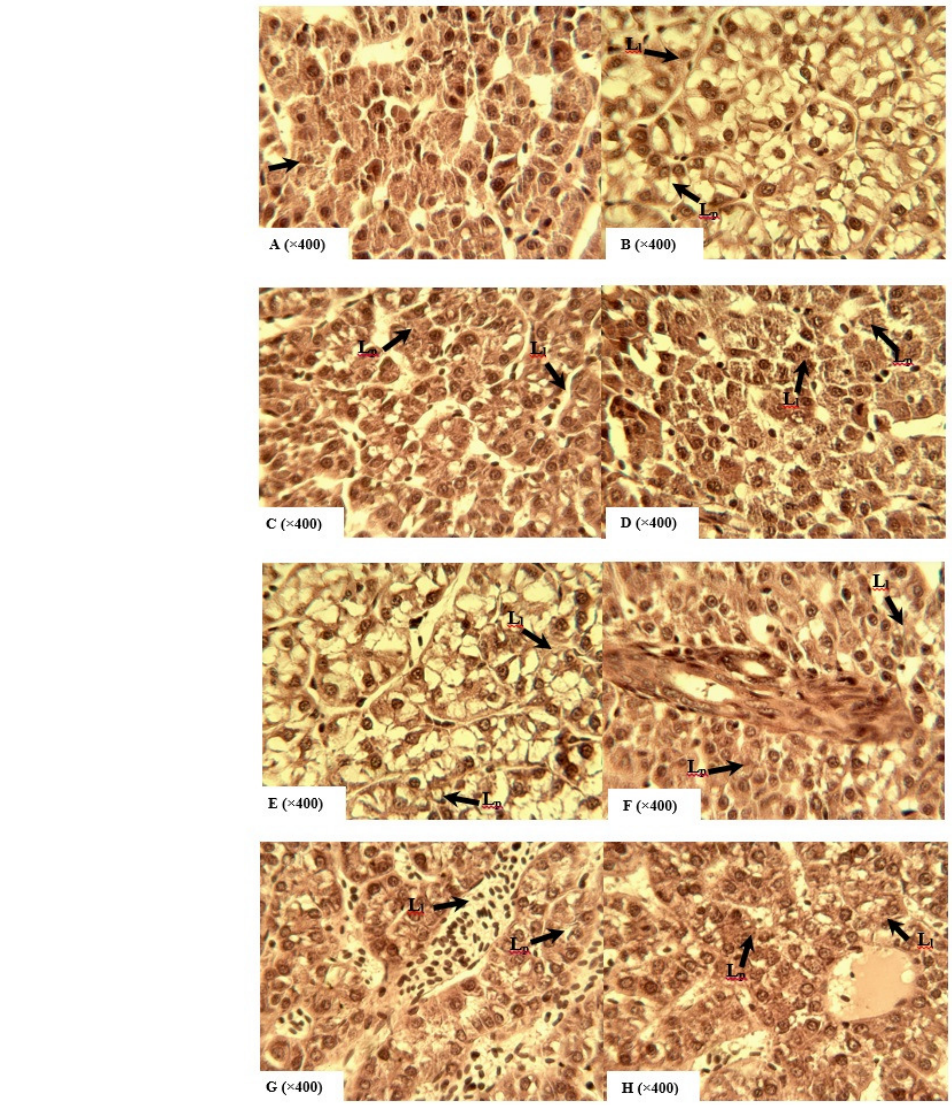

Figure 3. Histopathological changes of the liver organ in controls and *S. iniae* challenged groups of rainbow trout after treatment with *A. vera* and *S. officinalis* extracts. A section of the liver with normal structure hepatocyte (black arrow) in the negative control group (A); Influence of lipid vacuoles in hepatocytes L_l and nucleus pyknosisL_p in the positive control group (B), *A. vera* 0.5% group (C), *A. vera* 1% group (D), *A. vera* 1.5% group (E), *S. officinalis* 0.5% group (F), *S. officinalis* 1% group (G), *S. officinalis* 1.5% group (H), and Erythromycin group (I) (Mag×400; H and E staining).

alternative effect of *A. vera* (1.5%) on gills and livers of rainbow trout were effective than that of erythromycin (Table 4). In conclusion, the *A. vera* hydroethanolic extract (1.5% supplemented diet) is not toxic for the gills and livers of rainbow trout and can be used as an effective organic therapeutic alternative agent for erythromycin to treat *S. iniae* infected rainbow trout. Although the application of this alternative to aquaculture is very promising, further studies are needed to gain more insight into its mechanism of action to improve its stability and evaluate its impact on the environment and the host microbiota.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Authors' Contribution

Study concept and design: Tafi, A.A.; Meshkini, S.; Tukmechi, A.;
Acquisition of data: Tafi, A.A.; Meshkini, S.; Tukmechi, A.; Alishahi, M.
Analysis and interpretation of data: Tafi, A.A.
Drafting of the manuscript: Tafi, A.A.; Meshkini, S.
Critical revision of the manuscript for important intellectual content: Meshkini, S.; Tukmechi, A.
Statistical analysis: Tafi, A.A.; Alishahi, M.
Administrative, technical, and material support: Meshkini, S.; Noori, F.

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