Phytotherapy in Lactococcosis in Aquaculture

Subjects: Veterinary Sciences

Contributor: Mehdi Soltani

Lactococcosis, particularly that caused by Lactococcus garvieae, is a major re-emerging bacterial disease seriously affecting the sustainability of aquaculture industry. Medicinal herbs and plants do not have very much in vitro antagonism and in vivo disease resistance towards lactococcosis agents in aquaculture. Most in vitro studies with herbal extractives were performed against L. garvieae with no strong antibacterial activity, but essential oils, especially those that contain thymol or carvacrol, are more effective.

Keywords: lactococcosis ; Lactococcus garvieae ; phytotherapy ; aquaculture

1. Introduction

Among the species of Lactococcus genus, L. garvieae has been highlighted as one of the most serious global bacterial pathogens in the aquaculture sector, both in freshwater and marine fish, especially at water temperatures of >15 °C, but L. lactis and L. piscium seem to be limited to some highly valuable aquaculture species, such as salmonids and sturgeons, at various water temperatures [1][2][3]. Due to the widespread sources of the bacterial agents and disease spreading, as well as the heterogenicity of the bacterial stains implicated in the disease outbreaks, both vaccination and chemotherapy require more attention in future. The application of co-friendly environmental substances, such as medicinal herbs and probiotics, are nowadays a potential best alternative to antibiotic therapy and an immune enhancer against such bacterial diseases.

2. Diseases Caused by Lactococcus Members in Aquaculture

2.1. Disease Caused by L. garvieae

Lactococcosis, caused by L. garvieae, is a systemic hyperacute bacterial disease causing general hemorrhagic symptoms in susceptible aquatic organisms [4][5]. Based on their ability to agglutinate serum raised against L. garvieae, there are two groups of bacterial serotypes: non-agglutinating (KG−) and agglutinating (KG+) phenotypes [6]. The affected fish first become relatively anorexic, with a visible darkening of skin color, showing sluggish movement and abnormal behaviors, such as erratic and spiral swimming [1][4]. In the later stages of the disease the affected fish display signs of swollen abdomen, anal prolapsus, lateral or bilateral exophthalmia (**Figure 1**A,B), cataracts (**Figure 1**C), congestion of the internal organs, spleen and liver enlargement, accumulation of turbid ascitic fluid in the peritoneal cavity, and the presence of exudates in the brain [1][2][7][8]. Acute hyperemia and or extensive hemorrhage and petechiae of the organs, including the mucosal layers of the intestine, can also be seen in the diseased fish (**Figure 1**F), and in some cases the diseased fish show signs of explosion in the eyes prior to the loss of their eyes (**Figure 1**D,E) [1][2][9]. In advanced forms of the disease, a Gram stain preparation of hematopoietic tissue imprints, including spleen and kidney, can exhibit huge numbers of Gram-positive coccoid cells in single or chain forms (**Figure 1**G).

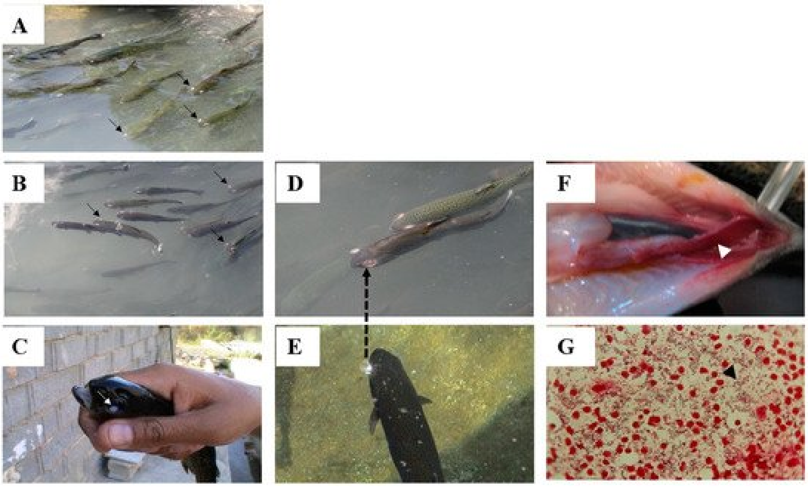

Figure 1. Rainbow trout growing in race ways and affected by L. garvieae showing: (**A**) typical bilateral exophthalmia and no change in the color skin, (**B**) typical bilateral exophthalmia and beginning of skin color change, (**C**) typical cataract and dark color, (**D**) bilateral exophthalmia and a complete loss of the eye, (**E**) darkening of body and an explosion of the eye, (**F**) hemorrhage of intestine, (**G**) direct Gram stain preparation of spleen of diseased fish showing huge numbers of Gram-positive coccoid cells confirmed as L. garvieae by phenotyping and molecular works. (photos by Professor Mehdi Soltani).

Pericarditis, peritonitis and meningitis, diffuse hemorrhage in the sclera of the eye, focal necrosis in the spleen and clumps of bacteria, hemorrhage in serosa of the swim bladder and in the interstitium of the skeletal muscles, degeneration and necrosis in epithelia of the stomach glands and their lumens full of necrotic material are among the identified histopathological findings in lactococcosis infection caused by L. garvieae [10][11][12]. Vascular changes in spleen and kidneys [13] and degenerations in the tubular epithelium with an increase in the melano-macrophage centers, hemorrhage in the form of a hematoma covering the myocardium and the bulbus arteriosa, petechial hemorrhage, vascular change, degeneration and necrosis are major histopathological findings. Lipid and ell infiltration in the liver, hemorrhage and vascular change in muscles, and petechial hemorrhage and edema in the gills are further microscopic changes reported in the infected fish by L. garvieae [11]. The severity of such pathological changes is, however, varied and depended on various factors, including level of virulent of bacterial strain, fish species and size and level of health management criteria, such as water temperature.

Evidence of bacterial cells in fish macrophage in tissues of spleen, kidney, heart (endothelial), and peritoneum are evidence of a septicemic condition, suggesting that macrophages play a key role in the host immune response to L. garvieae infection. However, intra-macrophage resistance of the bacteria can cause a spread of the pathogen to all tissues of fish by macrophages. Further, as in the young fish phagocytosis by macrophage activation may not be sufficient, thus, pathogenesis is a determinant factor, and the disease can progress.

Several factors play roles in the virulence of L. garvieae. Polysaccharide capsule is the major virulence factor in L. garvieae infection [9]. The capsulated strains resist to phagocytosis, but some non-capsulated strains are pathogenic in fish causing high mortality in rainbow trout [14], thus, the bacterial capsule may not the sole determinant of the bacterial pathogenicity. Haemolytic toxin is known to cause mortality in fish via intramuscular injection and an intracellular toxin with a low leukocidal activity reported by the bacterial isolates recovered from the diseased fish [15]. Plasmids of the virulent isolates contain a protein with an enzymatic domain corresponding to the family of actin-ADP-ribosyltransferases [16] that can kill eukaryotic cells by transferring ADP-ribose to essential proteins [17]. The toxicity of this protein in fish however, warranted future research works. The presence of a putative set of virulence factor genes (hly1, hly2, hly3, nox, sod, pavA, psaA), and proteins of enolase, lactate dehydrogenase phosphoenolpyruvate-protein phosphotransferase with roles in adhesion, cytolytic activity, oxidative stress tolerance, and metal homeostasis have been detected in strains of L. garvieae, including the avirulent reference strains ATCC® 49156 and ATCC® 4392, isolates from diseased rainbow trout in Turkey, France, Iran, Spain, and Italy [18], and fish pathogenic non-capsulated strains in South Africa [19]. These virulence lifestyle factors can indirectly contribute to host tissue damage through aiding in the infection process by evasion of the host’s innate immunity, systemic invasion, cofactor homeostasis, and spreading in the host and adhesion to host tissues. Further research works need to be directed studying the differential expression of virulence lifestyle and true virulence genes during growth in the host environment. Additionally, more studies need to assess the specific virulence factors responsible for the pathogenicity of L. garvieae, as putative virulence factor genes are present in both the fish pathogenic isolates and the avirulent isolates.

2.2. Diseases Caused by Other Species of Lactococcus Genus

L. lactis strains are genetically classified into four subspecies of lactis, cremoris, tractae, and hordniae [20]. It is not a common veterinary pathogen, although it can cause cattle mastitis and be involved in septic arthritis of the neonatal calf. For example, several variants of L. lactis have been associated with bovine mastitis [21]. In humans, it has been reported as a cause of endocarditis, arthritis, and septicemia in patients, although this requires more clarification [22][23][24][25][26].

Up to date, there are only four reports of lactococcosis by L. lactis in an aquatic organisms. The first report was an outbreak of white tail disease in cultured giant freshwater prawn (Macrobrachium rosenbergii) in Taiwan [27]. The affected prawns were cloudy and whitish in the muscles, showing remarkable edema and necrosis and inflammation in the muscles and hepatopancreas. In subsequent report by Chen et al. [3] L. lactis subsp. Lactis was isolated from affected hybrid sturgeon, Bester (Huso huso x Acipenser ruthenus) with signs of anorexia, pale body color, reddish spots on the abdomen, enteritis, enlarged abdomen, rapid respiration rate ascites, and 70%–100% mortality. Microscopically, the affected sturgeons demonstrated extensive haemorrhagic multifocal necrotic foci of spleen and liver with degeneration of hepatic cells, lipid droplets and glycogen granules, necrosis and renal tubule epithelial swelling and hydropic degeneration in kidney, skin ulcers deep in underling muscles, appearance of present of immunocompetent cells in the stomach, and small focus on tips of gills and on the myocardium [3]. No histopathological changes were, however seen in the eyeball, cerebrum and meninges of affected fish. The third report was from silver carp (Hypophthalmichthys molitrix) with extensive skin lesions near the caudal peduncle and musculoskeletal lesion in the USA [28]. The fourth outbreak of infection by L. lactis has been reported as the cause of endocarditis valvularis, parientalis thromboticans in mature allis shad (Alosa alosa) in Europe in 2018 that could be associated with the stressors, such as capturing, transport, breeding, and low oxygen level [29]. Although, in some cases the disease was reproduced experimentally, the mechanisms of pathogenesis by L. lactis in aquatic animals warranted future research works.

3. Phytotherapy of Lactococcosis in Aquaculture

3.1. In Vitro Studies

Almost all in vitro studies with vegetable and lichens extractives were performed against L. garvieae. For convenience, details of in vitro and in vivo studies have been included in **Table 1** and **Table 2**. Overall, extracts do not show strong antibacterial activity against L. garvieae, but essential oils are more effective, mainly those that contain thymol or carvacrol. There are some differences on minimum inhibitory and bactericidal concentrations for the same extractive in different studies (**Table 1**).

Table 1. Minimum inhibitory concentration (MIC) (μg mL^{-1} or μL mL^{-1}) and minimum bactericidal concentration (MBC) (μg mL^{-1} or μL mL^{-1}) of plant and lichen extractives against Lactococcus garvieae. The portion of the plant used to prepare the extractives were cited only if stated in the studies. Note: The MIC50 and MIC90 of 15 antibiotics against 146 strains of L. garvieae isolated from diseased fish are also given at the end of the table.

Origin/ Source of L. garvieae	Plant	Extractive	Major Compounds	MIC	MBC	Reference
Rainbow trout	Camellia sinensis (green tea-leaves)	Methanolic extract	Unknown	800	1100	Akbary, 2014
Rainbow trout	Thymus vulgaris (thyme), Origanum vulgare (oregano) and Eucalyptus sp.	Mix-oil® (essential oils from leaves)	Citraconic anhydride, 1, 8-cineole, thymol	6.25	12.5	Amiri et al., 2020
Unknown	Glycyrrhiza glabra L. (black sugar-root)	n-hexane extract	Unknown	Unknown	5630	Asan-Ozusaglam et al., 2014
Unknown	Glycyrrhiza glabra L. (root)	Dichloromethane extract	Unknown	Unknown	1410	Asan-Ozusaglam et al., 2014
Type culture collection	Lavandula angustifolia (lavender)	Essential oil	Unknown	500	Unknown	Baba, 2020
Type culture collection	Eugenia caryophyllus	Essential oil	Unknown	250	Unknown	Baba, 2020

Origin/ Source of L. garvieae	Plant	Extractive	Major Compounds	MIC	MBC	Reference
Rainbow trout	Mentha piperitae (pepper mint)	Essential oil	Unknown	500	Unknown	Baba, 2020
Rainbow trout	Rosmarinus officinalis (rosemary)	Essential oil	Unknown	500	Unknown	Baba, 2020
Rainbow trout	Cinnamomum zeylanicum (cinnamon)	Essential oil	Unknown	250	Unknown	Baba, 2020
Rainbow trout	Nigella sativa (black cumin)	Essential oil	Unknown	250	Unknown	Baba, 2020
Strain O41	Lavandula officinalis (true lavender-flowers)	Ethanolic extract	Essential oil (linalyl acetate), tannins, coumarins, flavonoids, and phytosterols	4200	8400	Bulfon et al., 2014
Strain O41	Melissa officinalis (lemon balm-leaves)	Ethanolic extract	Rosmarinic acid, essential oil (citral, citronellal, β-caryophyllen), caffeic acid, and chlorogenic acidderivatives	8400	33,600	Bulfon et al., 2014
Strain O41	Ocimum basilicum (sweet basil-flowering plant)	Ethanolic extract	Essential oil (linalool, estragol, camphor, eugenol, ocimene, cineol, sesquiterpenes), tannins, favonoids, caffeic acid, and esculoside	16,800	Unknown	Bulfon et al., 2014
Strain O41	Origanum vulgare (oregano-inflorescence)	Ethanolic extract	Carvacrol, thymol, γ-terpinene, p-cymene, limonene, linolool, and borneol	4200	33,600	Bulfon et al., 2014
Strain O41	Orthosiphon stamineus (Java tea-leaves)	Ethanolic extract	Essential oil (sesquiterpenes), flavones, triterpenoid, saponins, vitamins, and organic salts	33,600	Unknown	Bulfon et al., 2014
Strain O41	Rosmarinus officinalis (leaves)	Ethanolic extract	Essential oil (eucalyptol, α-pinene, camphor, borneol), flavonoids, rosmarinic acid, and terpenes	8400	Unknown	Bulfon et al., 2014
Strain O41	Salvia officinalis (sage-leaves)	Ethanolic extract	Essential oil (thujone, monoterpenes, and sesquiterpenes), tannins, bitter substances, and flavonoids	4200	33,600	Bulfon et al., 2014

Origin/ Source of L. garvieae	Plant	Extractive	Major Compounds	MIC	MBC	Reference
Strain O41	Thymus vulgaris (leaves)	Ethanolic extract	Essential oil (thymol, carvacrol, p-cimol, and terpinene), tannins, flavonoids and triterpenes	Unknown	Unknown	Bulfon et al., 2014
Strain O41	Vaccinium vitis-idaea (lingonberry-leaves)	Ethanolic extract	Phenolic glycosides (arbutin and hydroquinone), tannins, flavonoids (iperoside, avicularin, isoquercitrin), terpenic acids (ursolic and oleanolic acids), organic acids, and mineral salts	4200	Unknown	Bulfon et al., 2014
Rainbow trout	Glycyrrhiza glabra L. (root)	Ethanolic extract	Unknown	920	Unknown	Fereidouni et al., 2013
Rainbow trout	Peganum harmala (wild rue-seed)	Methanolic extrac	Unknown	105	Unknown	Fereidouni et al., 2013
Rainbow trout	Trachyspermum copticum (carum ajowan-seed)	Ethanolic extract	Unknown	453	Unknown	Fereidouni et al., 2013
Rainbow trout	Myrtus communis (myrtle-leaves)	Essential oil	Unknown	672	Unknown	Fereidouni et al., 2013
Rainbow trout	Juglans regia (English walnut-leaves)	Ethanolic extract	Unknown	510	Unknown	Fereidouni et al., 2013
Rainbow trout	Quercus branti Lindley (Brant's oak-seed)	Ethanolic extract	Unknown	978	Unknown	Fereidouni et al., 2013
Rainbow trout	Tanacetum parthenium (feverfew-leaves)	Essential oil	Unknown	824	Unknown	Fereidouni et al., 2013
Rainbow trout	Satureja bachtiarica Bung. (savory-leaves)	Essential oil	Unknown	126	Unknown	Fereidouni et al., 2013
Rainbow trout	Glycyrrhiza glabra L.(root)	Ethanolic extract	Glycyrrhizinic acid	>1000	>1000	Goudarzi et al., 2011
Rainbow trout	Satureja bachtiarica Bung. (aerial parts)	Essential oil	Phenols: Carvacrol, thymol	8	16	Goudarzi et al., 2011
Rainbow trout	Satureja bachtiarica Bung. (aerial parts)	Ethanolic extract	Phenols: Carvacrol, thymol	>1000	>1000	Goudarzi et al., 2011
Rainbow trout	Punica granatum (pomegranate-flowers)	Ethanolic extract	Polyphenols: pomegranatate	>1000	>1000	Goudarzi et al., 2011
Rainbow trout	Quercus branti Lindley (seed/flour)	Ethanolic extract	Tannins	>1000	>1000	Goudarzi et al., 2011
Rainbow trout	Echinophora platyloba DC. (prickly parsnip-aerial parts)	Essential oil	Monoterpenes: trans-β-ocimene	>1000	>1000	Goudarzi et al., 2011

Origin/ Source of L. garvieae	Plant	Extractive	Major Compounds	MIC	MBC	Reference
Rainbow trout	Echinophora platyloba DC. (aerial parts)	Ethanolic extract	Monoterpenes: trans-β-ocimene	>1000	>1000	Goudarzi et al., 2011
Rainbow trout	Heracleum lasiopetalum Boiss. (fruits)	Ethanolic extract	Sesquiterpene hydrocarbons: Germacrene-D	>1000	>1000	Goudarzi et al., 2011
Rainbow trout	Kelussia odoratissima Mozaff. (wild celery-leaves)	Ethanolic extract	Z-ligustilide	>1000	>1000	Goudarzi et al., 2011
Rainbow trout	Stachys lavandulifolia Vahl (wood betony-flowers)	Ethanolic extract	Sabinene, α-pinene, β-myrcene	>1000	>1000	Goudarzi et al., 2011
Rainbow trout	Thymus daenensis Celak. (common thyme-aerial part/inflorescence)	Ethanolic extract	Phenols: thymol, carvacrol	>1000	>1000	Goudarzi et al., 2011
Rainbow trout	Thymus daenensis Celak. (Aerial part/inflorescence)	Essential oil	Phenols: thymol, carvacrol	8	16	Goudarzi et al., 2011
Rainbow trout	Myrtus communis (leaves)	Ethanolic extract	α-pinene, 1,8-cineole, myrtenyl acetate	>250	>500	Goudarzi et al., 2011
Rainbow trout	Myrtus communis (leaves)	Essential oil	α-pinene, 1,8-cineole, myrtenyl acetate	>1000	>1000	Goudarzi et al., 2011
Rainbow trout	Thymbra spicata (spiked thyme-aerial part/inflorescence)	Essential oil	Phenols: thymol, carvacrol	8	16	Goudarzi et al., 2011
Rainbow trout	Bunium persicum (Boiss.) K.-Pol. (black caraway-fruits)	Essential oil	γ-terpinen-7-al, cuminaldehyde, γ terpinene	8	16	Goudarzi et al., 2011
Rainbow trout	Teucrium polium (felty germander-aerial parts)	Essential oil	α-pinene, linalool	>1000	>1000	Goudarzi et al., 2011
Rainbow trout	Alhagi maurorum (camelthorn-aerial parts)	Essential oil	Alhagidin, alhagitin, quercetin, catechin	>1000	>1000	Goudarzi et al., 2011
Rainbow trout	Zataria multifora Boiss. (Shirazi thyme)	Essential oil	Phenols: thymol, carvacrol	4	8	Goudarzi et al., 2011
Olive flounder (P. olivaceus)	Zingiber officinale (ginger)	Essential oil		2000	2000	Hossain et al., 2019
L. garvieae GQ850376	Eucalyptus globulus (southern blue gum-aerial parts)	Essential oil	1,8-eucalypol, pinene, terpineol acetae, globulol	250	250	Mahmoodi et al., 2012
L. garvieae GQ850376	Eucalyptus globulus (aerial parts)	Methanolic extract	1,8-eucalypol, pinene, terpineol acetae, globulol	500	500	Mahmoodi et al., 2012
L. garvieae GQ850376	Zataria multiflora (aerial parts)	Essential oil	phenolic monoterpene, Carvacrol, alpha-pinene	7.8	15.6	Mahmoodi et al., 2012

Origin/ Source of L. garvieae	Plant	Extractive	Major Compounds	MIC	MBC	Reference
L. garvieae GQ850376	Zataria multiflora (aerial parts)	Methanolic extract	phenolic monoterpene Carvacrol, alpha-pinene	15.6	15.6	Mahmoodi et al., 2012
L. garvieae GQ850376	Anethum graveolens (dill-seed)	Essential oil	D-carvacrol, limonene, dill apiole, E-dihydrocarvone, Z-dihydrocarvone	62.4	125	Mahmoodi et al., 2012
L. garvieae GQ850376	Anethum graveolens (seed)	Methanolic extract	D-carvacrol, limonene, dill apiole, E-dihydrocarvone, Z-dihydrocarvone	125	125	Mahmoodi et al., 2012
L. garvieae GQ850376	Rosmarinus officinalis	Essential oil	1,8-cineole, alpha-pinene, toluene	15.6	31.2	Mahmoodi et al., 2012
L. garvieae GQ850376	Rosmarinus officinalis	Methanolic extract	1,8-cineole, alpha-pinene, toluene	31.2	31.2	Mahmoodi et al., 2012
Rainbow trout	Citrus paradisi (grapefruit), Citrus reticulata (tangerine), Citrus aurantium ssp. bergamia (bergamot), Citrus sinensis (sweet orange)	Biocitro ® (blend of citrus extracts)	Ascorbic acid, citrus bioflavonoids (hesperidin, naringin, quercetin, rutin) and organic acids	2.0	Unknown	Mora-Sánchez et al., 2020
Tilapia (O. andersonii)	Capsicum annum (Chili pepper)	Methanolic extract (capsaicin)	Unknown	Unknown	196.7	Ndashe et al., 2020
Olive flounder	Citrus aurantifolia (key lime-peel)	Essential oil	Limonene, γ-terpinene, β-pinene	0.125% (v/v)	1% (v/v)	Pathirana et al., 2018
Olive flounder	Limonene	Commercial trans-limonene (>99%)	Limonene	0.031% (v/v)	0.025% (v/v)	Pathirana et al., 2018
Olive flounder	Syzygium aromaticum (clove-buds)	Essential oil	Eugenol, β-caryophyllene, α-humulen, eugenyl-acetate	0.5% (v/v)	1% (v/v)	Pathirana et al., 2019a
Olive flounder	Commercial eugenol (>99%)	Isolated compound eugenol	Eugenol	1% (v/v)	1% (v/v)	Pathirana et al., 2019a
Olive flounder	Cinnamomum zeylanicum	Essential oil	Cinnamaldehyde, eugenol, β-Caryophyllene	0.015% (v/v)	0.031% (v/v)	Pathirana et al., 2019b
Olive flounder	Commercial trans-cinnamaldehyde (>99%) (Sigma-Aldrich)	cinnamaldehyde	Cinnamaldehyde	0.003% (v/v)	0.015% (v/v)	Pathirana et al., 2019b
Rainbow trout	Argania spinose L. (argan-oil)	Essential oil	Oleic acid, linoleic acid, palmitic acid, stearic acid	250	Unknown	Öntas et al., 2016

Origin/ Source of L. garvieae	Plant	Extractive	Major Compounds	MIC	MBC	Reference
Rainbow trout	Citrus limon L. (lemon-peel)	Essential oil	Limonene, γ-terpinene, β-pinene, α-terpineol, myrecene and terpinolene	500	Unknown	Öntas et al., 2016
Strain ATCC43921	Cinnamomum verum (cinnamon-bark)	Essential oil	Unknown	120	Unknown	Rattanachaikunsopon et al., 2009
Strain ATCC43921	Ocimum sanctum (holy basil-leaves)	Essential oil	Unknown	240	Unknown	Rattanachaikunsopon et al., 2009
Strain ATCC43921	Zingiber officinale (roots)	Essential oil	Unknown	120	Unknown	Rattanachaikunsopon et al. 2009
Strain ATCC43921	Syzygium aromaticum (flower buds)	Essential oil	Unknown	30	Unknown	Rattanachaikunsopon et al., 2009
Rainbow trout	Zataria multiflora (aerial parts)	Essential oil	Carvacrol, benzene and phenol	0.12	0.12	Soltani et al., 2014
Rainbow trout	Allium sativum (garlic-edible parts)	Essential oil	trisulfide, di-2-propenyl, disulfide, di-2-propenyl and trisulfide, methyl 2-propenyl	0.5	1	Soltani et al., 2014
Rainbow trout	Cinnamomum zeylanicum (bark)	Essential oil	cinnamic aldehyde, linalool, ortho methoxy cinnamic aldehyde and 1,8-cineole	0.5	0.5	Soltani et al., 2014
S. quinqueradiata	Chloramphenicol			0.8 [a]	1.6 [b]	Maki et al., 2008
S. quinqueradiata	Ciprofloxacin			1.6 [a]	3.13 [b]	Maki et al., 2008
S. quinqueradiata	Erythromycin			0.1 [a]	800 [b]	Maki et al., 2008
S. quinqueradiata	Enoxacin			6.25 [a]	12.5 [b]	Maki et al., 2008
S. quinqueradiata	Florfenicol			1.6 [a]	1.6 [b]	Maki et al., 2008
S. quinqueradiata	Floroxacin			12.5 [a]	12.5 [b]	Maki et al., 2008
S. quinqueradiata	Kanamycin			25 [a]	50 [b]	Maki et al., 2008
S. quinqueradiata	Lincomycin			25 [a]	800 [b]	Maki et al., 2008
S. quinqueradiata	Norfloxacin			6.25 [a]	12.5 [b]	Maki et al., 2008
S. quinqueradiata	Oxolinic acid			400 [a]	800 [b]	Maki et al., 2008
S. quinqueradiata	Orbifloxacin			1.6 [a]	1.6 [b]	Maki et al., 2008
S. quinqueradiata	Ofloxacin			3.13 [a]	6.25 [b]	Maki et al., 2008

Origin/ Source of L. garvieae	Plant	Extractive	Major Compounds	MIC	MBC	Reference
S. quinqueradiata	Penzylpenicillin			0.8 [a]	1.6 [b]	Maki et al., 2008
S. quinqueradiata	Streptomycin			25 [a]	50 [b]	Maki et al., 2008
S. quinqueradiata	Tetracycline			12.5 [a]	400 [b]	Maki et al., 2008

Letters a and b showing MIC50 and MIC90, respectively.

Table 2. Efficacy of medicinal herbs and plants on the survival of aquatic animals infected with Lactococcus garvieae. The portion of the plant used to prepare the extractives were cited only if stated in the studies.

Host	Plant	Extractive	Dosage/Duration	Survival Increase Compared to Control (%) [2]	Reference
Giant freshwater prawn (Macrobrachium rosenbergii)	Eichhornia crassipes (water hyacinth-leaves)	Hot-water extract	1 g kg diet^{-1}, 12 days 2 g kg diet^{-1}, 12 days 3 g kg diet^{-1}, 12 days	↑57.3 ↑128.6 ↑171.4	Chang et al., 2013
Giant freshwater prawn (M. rosenbergii)	Eichhornia crassipes (leaves)	Powder	20 g kg diet^{-1}, 120 days	↑44.3	Chang et al., 2016
		Hot-water extract	20 g kg diet^{-1}, 120 days	↑89.0	
		Aqueous extract [1]	2 g kg diet^{-1}, 120 days	↑89.0	
		Dreg of aqueous extract [1]	18 g kg diet^{-1}, 120 days	↑77.7	
Giant freshwater prawn (M. rosenbergii)	Musa acuminate (banana-peel)	Aqueous extract	1 g kg diet^{-1}, 120 days 3 g kg diet^{-1}, 120 days 6 g kg diet^{-1}, 120 days	↑200 ↑300 ↑467	Rattanavichai et al., 2015
Giant freshwater prawn (M. rosenbergii)	Morinda cutrifolia (noni)	Aqueous extract	0.6 g kg diet^{-1}, 21 days 3 g kg diet^{-1}, 21 days 6 g kg diet^{-1}, 21 days	↑250 ↑50.4 NS	Halim et al., 2017
Nile tilapia (Oreochromis niloticus)	Argania spinosa (argan-seeds)	Oil	5 mL kg diet^{-1}, 45 days 10 mL kg diet^{-1}, 45 days 20 mL kg diet^{-1}, 45 days	↑66.7 ↑91.7 ↑86.1	Baba et al., 2017
Rainbow trout (Oncorhynchus mykiss)	Lentinula edodes (Shiitake mushroom)	Aqueous extract	10 g kg diet^{-1}, 45 days 20 g kg diet^{-1}, 45 days	↑79.0 ↑109.7	Baba et al., 2015
Rainbow trout (O. mykiss)	Pleurotus ostreatus (oyster mushroom)	Aqueous extract	10 g kg diet^{-1}, 42 days 20 g kg diet^{-1}, 42 days	↑40.0 ↑60.1	Uluköy et al., 2016

Host	Plant	Extractive	Dosage/Duration	Survival Increase Compared to Control (%) [2]	Reference
Rainbow trout (O. mykiss)	**Usnea barbata (beard lichen)**	**Methanolic extract**	**230 mg kg fish^{-1} (a) 460 mg kg fish^{-1} (a) 690 mg kg fish^{-1} (a)**	**↑62.4 ↑45.3 NS**	**Bilen et al., 2019**
Three spotted tilapia (Oreochromis andersonii)	**Capsaicin**	**Isolated compound**	**1.97 mg kg fish^{-1} (b)**	**80% survival vs. 0% survival in control**	**Ndashe et al., 2020**
Rainbow trout (O. mykiss)	**Origanum onites (oregano)**	**Essential oil**	**0.125 mL kg diet^{-1}, 56 days 1.5 mL kg diet^{-1}, 56 days 2.5 mL kg diet^{-1}, 56 days 3.0 mL kg diet^{-1}, 56 days**	**↑54 ↑92 ↑84 No mortality**	**Diler et al. 2017**
Nile tilapia (Oreochromis niloticus)	**Syzygium aromaticum (clove-buds)**	**Essential oil**	**5 mL kg diet^{-1}, 5 days 10 mL kg diet^{-1}, 5 days 20 mL kg diet^{-1}, 5 days 30 mL kg diet^{-1}, 5 days**	**↑40 ↑70 ↑80 No mortality**	**Rattanachaikunsopon et al., 2009**
Mullet (Mugil cephalus)	*** TCM**	**Aqueous extract of the powder**	**5 g kg fish^{-1}, 28 days 10 g kg fish^{-1}, 28 days 20 g kg fish^{-1}, 28 days**	**NS ↑230.8 ↑184.6**	**Choi et al., 2014**
Rainbow trout (O. mykiss)	**Citrus paradisi (grapefruit), Citrus reticulata (tangerine), Citrus aurantium ssp. bergamia (bergamot), Citrus sinensis (sweet orange)**	**Biocitro ® (blend of these extracts)**	**0.75 g kg diet^{-1}, 28 days**	**↑120**	**Mora-Sánchez et al., 2020**

[1] Aqueous extract was the supernatant of the hot-water extract filtered and centrifuged. The left filtered product and sediment were the dreg of aqueous extract. [2] survival increase calculated by the equation: (survival treatment × 100)/survival control–100. NS = nonsignificant difference from control. * TCM = traditional Chinese medicine: composed by the plants Rhizoma coptidis, Radix scutellariae, Cortex phellodendri, Fructus gardeniae jasminoidis, Fructus forsythiae, and Flos lonicerae japonicae. ([a]) = Gavage in infected fish twice a day for 10 days. ([b]) = Injected in the same day of infection with the bacteria.

3.2. In Vivo Studies

All in vivo studies were related to survival against L. garvieae infection, and, in most cases, the extractives of medicinal herbs and plants were added to the diets for various periods before the treated fish being challenged with L. garvieae infection. Overall, the essential oils that showed the best in vitro antibacterial activity against L. garvieae (**Table 1**) were not tested for the in vivo bioassays yet. The extractives tested under in vivo conditions presented moderate in vitro antibacterial activity against this bacterium or even were not tested in vitro. However, the dietary supplementation with all tested extractives reduced mortality of infected animals (**Table 2**), probably because they improved immune parameters before challenging the treated fish with L. garvieae. A 12-day feeding giant freshwater prawn (Macrobrachium rosenbergii) with hot-water extract of water hyacinth (Eichhornia crassipes) leaves at 1, 2, and 3 g kg^{-1} diet induced significantly higher survival rate after challenge with L. garvieae infection, but higher disease resistance was seen in the prawn treated with higher concentration of the extract [30]. In addition, the treated animals exhibited an enhancement in the immune responses including respiratory burst, phenoloxidase activity, superoxide dismutase activity, glutathione peroxidase, total hemocyte value, differential hemocyte count, transglutaminase activity, and phagocytic activity towards L. garvtieae. In the subsequent research work by Chang and Cheng [31], dietary addition of three tested water hyacinth extracts (**Table 2**) for 120 days increased survival and immune parameters, i.e., total hemocyte count, semi-granular and granular cells counts of giant freshwater prawn while phenoloxidase activity, respiratory bursts of hemocytes were not observed only with dietary addition of powder of this plant to the diet.

4. Conclusions

Disease outbreaks by Lactococcus species specially L. garvieae is one of the major concerns faced in the aquaculture production worldwide, and various biological and environmental variables, as well as the aquaculture practices and husbandry can affect the quantity and impacts of the morbidity and mortality. Data influencing the economic losses can, thus, assist to develop policies and strategies to reduce the losses by lactococcosis outbreaks in aquaculture industry. Lactococcosis outbreaks especially by L. garvieae are increasingly recognized as a significant and re-emerging bacterial disease in aquaculture, but there is no an estimation of its economic impacts. Data describing antagonistic activity and disease resistance efficacy of potential medicinal herbs and plants towards lactococcosis caused by L. garvieae, L. lactis, L. piscium and L. raffinolactis in finfish are not very much. Almost all in vitro studies with vegetable and lichens extractives were performed against L. garvieae. Despite no strong antibacterial activity by herb extracts against L. garvieae, essential oils especially those that contain thymol and carvacrol are more effective against L. garvieae strains. The exhibited differences on minimum inhibitory and bactericidal values for the same extractive in different studies could be due to the use of different bacterial strains or parts or chemotypes of the same plant. Despite best anti-L. garvieae activity by the essential oils under in vitro assays, the in vivo bioassays need be assessed yet. The extractives tested under in vivo conditions presented moderate antibacterial activity against this bacterium or even were not tested in vitro. However, the dietary supplementation with all tested extractives reduced mortality of infected animals, probably because they improved immune parameters before challenging the treated fish with L. garvieae.

References

1. Soltani, M.; Nikbakht, G.; Ebrahimzadeh Moussavi, H.; Ahmadzadeh, N. Epizootic outbreak of lactococcosis caused by Lactococcus garvieae in farmed rainbow trout (Oncorhynchus mykiss) in Iran. Bull. Eur. Assoc. Fish Pathol. 2008, 28, 95–106.
2. Eldar, A.A.; Ghittino, C. Lactococcus garvieae and Streptococcus iniae infections in rainbow trout Oncorhynchus mykiss: Similar, but different diseases. Dis. Aquat. Org. 1999, 36, 227–231.
3. Chen, M.-H.; Hung, S.-W.; Shyu, C.-L.; Lin, C.-C.; Liu, P.-C.; Chang, C.-H.; Shia, W.-Y.; Cheng, C.-F.; Lin, S.-L.; Tu, C.-Y. Lactococcus lactis subsp. lactis infection in Bester sturgeon, a cultured hybrid of Huso huso × Acipenser ruthenus, in Taiwan. Res. Vet. Sci. 2012, 93, 581–588.
4. Austin, B.; Austin, D.A.; Austin, B.; Austin, D.A. Bacterial Fish Pathogens; Springer International Publishing: Cham, Switzerland, 2016; Volume 481.
5. Ortega, C.; Irgang, R.; Valladares-Carranza, B.; Collarte, C.; Avendaño-Herrera, R. First identification and characterization of Lactococcus garvieae isolated from rainbow trout (Oncorhynchus mykiss) cultured in mexico. Animals 2020, 10, 1609.
6. Hirono, I.; Yamashita, H.; Park, C.I.; Yoshida, T.; Aoki, T. Identification of genes in a KG—Phenotype of Lactococcus garvieae, a fish pathogenic bacterium, whose proteins react with antiKG—Rabbit serum. Microb. Pathog. 1999, 27, 407–417.
7. Bragg, R.; Broere, J. Streptococcosis in rainbow trout in South Africa. Bull. Eur. Assoc. Fish Pathol. 1986, 6, 89–91.
8. Bekker, A.; Hugo, C.; Albertyn, J.; Boucher, C.; Bragg, R. Pathogenic Gram-positive cocci in South African rainbow trout, Oncorhynchus mykiss (Walbaum). J. Fish Dis. 2011, 34, 483–487.
9. Vendrell, D.; Balcázar, J.L.; Ruiz-Zarzuela, I.; De Blas, I.; Gironés, O.; Múzquiz, J.L. Lactococcus garvieae in fish: A review. Comp. Immunol. Microbiol. Infect. Dis. 2006, 29, 177–198.
10. Chang, P.; Lin, C.; Lee, Y. Lactococcus garvieae infection of cultured rainbow trout, Oncorhynchus mykiss in Taiwan and associated biophysical characteristics and histopathology. Eur. Assoc. Fish Pathol. 2002, 22, 319–327.
11. Avci, H.; Birincioglu, S.; Tanrikul, T.; Epikmen, E.; Metin, N.; Avsever, M. Experimental Lactococcus garvieae infection in rainbow trout, Oncorhynchus mykiss, Walbaum 1792: A comparative histopathological and immunohistochemical study. J. Fish Dis. 2014, 37, 481–495.

12. Chen, S.-C.; Lin, Y.-D.; Liaw, L.-L.; Wang, P.-C. Lactococcus garvieae infection in the giant freshwater prawn Macrobranchium rosenbergii confirmed by polymerase chain reaction and 16S rDNA sequencing. Dis. Aquat. Org. 2001, 45, 45–52.

13. Algöet, M.; Bayley, A.; Roberts, E.; Feist, S.; Wheeler, R.; Verner-Jeffreys, D. Susceptibility of selected freshwater fish species to a UK Lactococcus garvieae isolate. J. Fish Dis. 2009, 32, 825–834.

14. Türe, M.; Haliloğlu, H.İ.; Altuntaş, C.; Boran, H.; Kutlu, İ. Comparison of experimental susceptibility of rainbow trout (Oncorhynchus mykiss), turbot (Psetta maxima), black sea trout (Salmo trutta labrax) and sea bass (Dicentrarchus labrax) to Lactococcus garvieae. Turk. J. Fish. Aquat. Sci. 2014, 14, 507–513.

15. Kusuda, R.; Hamaguchi, M. Extracellular and intracellular toxins of Streptococcus sp. isolated from yellowtail. Bull. Eur. Assoc. Fish Pathol. 1988, 8, 9–10.

16. Aguado-Urda, M.; López-Campos, G.H.; Gibello, A.; Cutuli, M.T.; López-Alonso, V.; Fernández-Garayzábal, J.F.; Blanco, M.M. Genome sequence of Lactococcus garvieae 8831, isolated from rainbow trout lactococcosis outbreaks in Spain. J. Bacteriol. 2011, 193, 4263–4264.

17. Holbourn, K.P.; Shone, C.C.; Acharya, K. A family of killer toxins. FEBS J. 2006, 273, 4579–4593.

18. Ture, M.; Altinok, I. Detection of putative virulence genes of Lactococcus garvieae. Dis. Aquat. Org. 2016, 119, 59–66.

19. Meyburgh, C.; Bragg, R.; Boucher, C. Lactococcus garvieae: An emerging bacterial pathogen of fish. Dis. Aquat. Org. 2017, 123, 67–79.

20. Saraoui, T.; Leroi, F.; Björkroth, J.; Pilet, M.-F. Lactococcus piscium: A psychrotrophic lactic acid bacterium with bioprotective or spoilage activity in food—A review. J. Appl. Microbiol. 2016, 121, 907–918.

21. Rodrigues, M.; Lima, S.; Higgins, C.; Canniatti-Brazaca, S.; Bicalho, R. The Lactococcus genus as a potential emerging mastitis pathogen group: A report on an outbreak investigation. J. Dairy Sci. 2016, 99, 9864–9874.

22. Mannion, P.; Rothburn, M. Diagnosis of bacterial endocarditis caused by Streptococcus lactis and assisted by immunoblotting of serum antibodies. J. Infect. 1990, 21, 317–318.

23. Clark, I.; Burnie, J. Immunoblotting and culture positive endocarditis. J. Clin. Pathol. 1991, 44, 152–156.

24. Campbell, P.; Dealler, S.; Lawton, J. Septic arthritis and unpasteurised milk. J. Clin. Pathol. 1993, 46, 1057–1058.

25. Durand, J.M.; Rousseau, M.C.; Gandois, J.M.; Kaplanski, G.; Mallet, M.N.; Soubeyrand, J. Streptococcus lactis septicemia in a patient with chronic lymphocytic leukemia. Am. J. Hematol. 1995, 50, 64–65.

26. Zechini, B.; Cipriani, P.; Papadopoulou, S.; Di Nucci, G.; Petrucca, A.; Teggi, A. Endocarditis caused by Lactococcus lactis subsp. lactis in a patient with atrial myxoma: A case report. Diagn. Microbiol. Infect. Dis. 2006, 56, 325–328.

27. Wang, P.-C.; Lin, Y.-D.; Liaw, L.-L.; Chern, R.-S.; Chen, S.-C. Lactococcus lactis subspecies lactis also causes white muscle disease in farmed giant freshwater prawns Macrobrachium rosenbergii. Dis. Aquat. Org. 2008, 79, 9–17.

28. Khoo, L.H.; Austin, F.W.; Quiniou, S.M.; Gaunt, P.S.; Riecke, D.K.; Jacobs, A.M.; Meals, K.O.; Dunn, A.W.; Griffin, M.J. Lactococcosis in silver carp. J. Aquat. Anim. Health 2014, 26, 1–8.

29. Wünnemann, H.; Eskens, U.; Prenger-Berninghoff, E.; Ewers, C.; Lierz, M. Lactococcus lactis, causative agent of an endocarditis valvularis and parietalis thromboticans in the allis shad, Alosa alosa (L.). J. Fish Dis. 2018, 41, 1207–1215.

30. Chang, C.-C.; Tan, H.-C.; Cheng, W. Effects of dietary administration of water hyacinth (Eichhornia crassipes) extracts on the immune responses and disease resistance of giant freshwater prawn, Macrobrachium rosenbergii. Fish Shellfish Immunol. 2013, 35, 92–100.

31. Chang, C.C.; Cheng, W. Multiple dietary administrating strategies of water hyacinth (Eichhornia crassipes) on enhancing the immune responses and disease resistance of giant freshwater prawn, Macrobrachium rosenbergii. Aquac. Res. 2016, 47, 140–152.