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Review article

An overview of the antimicrobial activity of some essential oils against fish pathogenic bacteria

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Abstract

Plant-derived essential oils as an alternative treatment method for antimicrobial-resistant bacteria have gained interest over the past few decades, particularly in the food and aquaculture industry. Essential oil is considered as a natural, cost-effective compound with a minimum impact on the environment. Studies have suggested the use of essential oils in the treatment and/or prevention of infectious diseases in fish may be a cost-effective alternative to synthetic antimicrobials. In addition to that, hundreds of different chemical profiles have been identified in essential oils. Particularly, in the same essential oil. In contrast to the synthetic antimicrobials that have unique chemical compositions between every product belonging to the same drug, the same essential oil may not perform unique antimicrobial activity every time. Therefore, more studies and concluded data are required for the effective usage of essential oils as an alternative to antimicrobial agents. In this review, we have summarized the studies about the several active essential oils that are known and tested against fish pathogenic bacteria because of their antimicrobial properties

Keywords: Antimicrobial, Bacteria, Essential oil, Fish, Pathogens

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INTRODUCTION

The natural flora is largely made up of medicinal plants, which are valued as resources in a number of fields, including pharmaceutical, flavor, fragrance and cosmetic ones. Medicinal plant-based essential oils are amongst the most popular medicinal plant products. The name "essential" has been given to these oils because they were believed to capture a plant's essence that is its odor and flavor. At least 2000 plant species have been used to make over 3000 essential oils, of which 300 are significant from a commercial standpoint (Raut and Karuppayil, 2014).

Numerous biological effects, such as antibacterial, antioxidant, antiviral and insecticidal, are demonstrated by the essential oil (Burt, 2004; Kaloustian et al., 2008). Since ancient times, essential oils have been utilized to treat a variety of infectious disorders (Irshad et al., 2019). In addition, essential oils and their chemical constituents have been shown to have antibacterial properties as recently as the present. In terms of acute toxicity, carcinogenic consequences and environmental contamination, plant-derived medications are seen as safer alternatives to synthetic treatments (Mulyaningsih et al., 2010; Mittal et al., 2019). Thus, the consumption of farmed fish that has been treated with synthetic antimicrobials may pose a health risk (Tavares-Dias, 2018). Also, the use of synthetic antimicrobials and anthelmintic drugs seems to potentially have a high cost. On the other hand, infections impact the whole population even though infections occur at the level of the individual. Therefore, the response must be applied to the whole community, not to the individual. These drawbacks which include high cost, side effects and environmental impact prompted research into using medicinal plants as a substitute for conventional medicine to treat fish diseases. (Soares and Tavares-Dias, 2013; Hashimoto et al., 2016; Soares et al., 2017).

The use of bioactive substances from medicinal plants, particularly essential oils, to control fish infections that threaten output and productivity has grown recently (Tavares-Dias, 2018). The antimicrobial efficiency of essential oils may vary with the oil as well as with the different bacterial species. For example, vetiver (*Chrysopogon zizanioides*), manukaoil (*Leptospermum scoparium*) and sandalwood (*Santalum album*) oils are highly active against Gram-positive bacteria but do not show activity against Gram-negative bacteria. As a result, the methodologies for screening the antibacterial activity of essential oils have received more attention (Hammer et al., 1999; Hammer and Carson, 2011). Several bioassays such as disk-diffusion (by measuring the diameter of the inhibition zone), well diffusion and agar or broth dilution (by determining minimum inhibitory concentration, MIC) are being used to evaluate the antimicrobial activities of essential oils (Balouiri et al., 2016). Determination of the minimum bactericidal concentration (MBC) is another method used to evaluate antimicrobial compounds. The ratio of MBC/MIC can also be used as a parameter to evaluate the antimicrobial effect of essential oils. The antimicrobial compounds which kill bacteria with MBC/MIC ratio value $\leq$ 4 are designated as bactericidal for the tested bacteria, while the antimicrobial compounds which show inhibitory action with MBC/MIC ratio value $>$ 4 are designated as bacteriostatic (Kone et al., 2004; Bulfon et al., 2014). As a compound that has wide variations in chemical composition, essential oils need more concentrated information to evaluate their antimicrobial properties. Therefore, this review is intended to provide an overview of current knowledge about the antimicrobial mode of action of several essential oils and to summarize research avenues that can help the implementation of essential oil as natural antimicrobial agents in aquaculture.

Composition of essential oils

The composition of essential oil shows considerable variation, both in quantitative and qualitative terms (Dhifi et al., 2016). This wide variation depends on the plant species, the geographical region of the plant, harvesting time and the extraction method (Dima and Dima, 2015). Essential oils are a complex blend of polar and non-polar molecules chemically. Four categories can be used to classify essential oils: Straight chain compounds not containing any side chain, terpenes related to isoprene, phenylpropanoids (benzene derivatives) and miscellaneous groups (sulfur or nitrogen-containing compounds that have varied structures have not belonged to the first 3 groups) (Morsy, 2017). Also, biological activities including antimicrobial activities of essential oils are composition-dependent (Boudjedjou et al., 2019).

Major fish pathogens in aquaculture

Aquaculture has been affected by different kinds of pathogens such as bacteria, viruses, fungi and parasites as reviewed in Pridgeon and Phillip, (2012). Among them, bacterial infections have become a huge threat as they can survive well in the aquatic environment (Klesius and Pridgeon 2011). Gram-negative bacteria belong to genera *Aeromonas*, *Vibrio*, *Edwardsiella*, *Flavobacterium*, *Photobacterium*, *Pseudomonas*, *Francisella*, *Tenacibaculum* and so on cause infections in aquaculture (Subasinghe, 2005) Most *Aeromonas* infections are caused by the motile *Aeromonas* species (*A. hydrophila*, *A. sorbia* and *A. caviae*) in aquaculture farms, especially in freshwater aquaculture. *Aeromonas* infections differ significantly in symptoms from skin ulcers, fin and gill lesions to septicemia (Camus et al., 1998). Vibriosis is another major threat in aquaculture mainly caused by the *Vibrio anguillarum*, *V. vulnificus*, *V. ordalii*, *V. mimicus*, *V. parahaemolyticus*, *V. harveyi*, *V. alginolyticus, V. salmonicida*, *V. splendidus, V. ichthyoenteri* and so on (Ina-Salwan et al., 2019). Vibriosis has a widespread distribution and causes characteristic hemorrhagic septicemia in warm and cold-water fish species (Toranzo et al., 2005). *Edwardsiella tarda* is one of the most common species which causes Edwardsiellosis in aquatic animals including fish, amphibians and mammals (Xu and Zhang, 2013). Moreover, Gram-positive bacteria such as *Lactococcus* spp., *Streptococcus* spp. and *Renibacteritium* spp. which cause lactococcosis, streptococcosis and bacterial kidney diseases also been identified as major bacterial pathogens in aquaculture (Klesius and Pridgeon 2011; Persson et al., 2022).

Antimicrobial action of essential oils against fish pathogenic bacteria

The antimicrobial, antiviral, antifungal and insecticidal actions of essential oils make them an interesting option for the treatment or prevention of diseases in aquaculture. Hydrophobicity is the main feature of essential oils which allows them to partition into lipid compounds of the cell membrane of bacteria, disrupting the structure and making it more permeable (Dhifi et al., 2016). According to the results of the previous studies, Gram-positive bacteria have demonstrated more susceptibility to the effects of essential oils than Gram-negative bacteria due to the structurally different cell walls of these two groups of bacteria (Trombetta et al., 2005). Additionally, studies have shown that the application of essential oil to farmed fish (either through nutritional exposure or through water) can boost health and growth rates as well as treat infectious disorders (Sutili et al., 2018). However, it is essential to perform *in vitro* tests to determine if an essential oil is suitable for *in vivo* antimicrobial assays.

Lime essential oil and limonene

Antimicrobial activities of essential oils extracted from the family *Rutaceae* plants including *Citrus limon* (lemon), *C. aurantifolia* (lime), *C. reticulata* (mandarin orange), *C. aurantium* (sour orange), *C.hystrix* (kaffir lime) and *C. sinensis* have been studied. Although they are commonly called as lime essential oils, they are different in composition. Terpene derivatives (limonene, beta-pinene, gamma-terpinene and myrcene) have been identified as the major component in lime essential oils by gas chromatography-mass spectrometry (GC-MS) analysis (Chisholm et al., 2003; Craske et al., 2005). Terpene-initiated protein denaturing causes the loss of cell membrane integrity which results in membrane damage and finally bacterial death (Fisher and Phillips, 2008; Galluci et al., 2009). In the previous studies, both lemon essential oil and limonene have demonstrated higher activity against Gram-positive bacterial strains than Gram-negative bacterial strains (Nazzaro et al., 2013; Costa et al., 2014; Al-Aamri et al., 2018; Pathirana et al., 2018). The size of the inhibition zone induced by limonene and lemon essential oil in the disc distribution assays was proportionate to the concentration of limonene (Al-Aamri et al., 2018; Pathirana et al., 2018). However, both limonene and lemon essential oils are not exhibited antimicrobial properties at relatively low concentrations ranging between 1% to 10% (Pathirana et al., 2018). Lime essential oils have demonstrated more or less trivial activity against *Edwerdisella tarda* (Öntaş et al., 2016; Pathirana et al., 2018). Thus, a cost calculation is required to determine that either lemon essential oil or limonene is cost-effective as an antimicrobial agent.

The MBC/MIC ratio of limonene and lemon essential oil can be employed to describe bactericidal and bacteriostatic properties. Both limonene and lemon essential oil have demonstrated bactericidal and bacteriostatic activities against 16 fish pathogenic bacterial strains in the study conducted by Pathirana et al., (2018). Moreover, the effect of lemon essential oil was mostly bactericidal against *Bacillus subtilis* and *Streptococcus epidermidis* according to Jafari et al. (2011). According to the research done by Vimal et al. (2013), limonene shows bactericidal activity against *S. pneuoniae*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis*. Moreover, the dietary supplementation of limonene has been suggested as an effective method to increase fish resistance against bacterial infections (Cunha et al., 2018).

Cinnamon essential oil and cinnamaldehyde

Cinnamon (*Cinnamomum zeylanicum* and *Cinnamon cassia*) is one of the most influential antimicrobial medicinal herbs belonging to the family *Lauraceae* (Rao and Gan, 2014). Cinnamaldehyde, the major component of cinnamon essential oil (50% to 90%) has 3 main mechanisms of action against microbes depending on the concentrations (Gupta et al., 2008; Wong et al., 2014; Kaskatepe et al., 2016). It inhibits enzymes in cytokine interaction and other minor cell functions and it can inhibit ATPase at high concentrations. Also, cinnamaldehyde can perturb the cell membrane at lethal concentrations (Nazzaro et al., 2013).

A significant difference ($p<0.05$) between MIC values of cinnamon essential oil and cinnamaldehyde against Gram-positive and Gram-negative fish pathogenic bacteria have been reported by Pathirana et al. (2019a). In that study, the MIC value of cinnamon essential oil for bacterial strains ranged from 0.001 to 0.031% (V/V) and the cinnamaldehyde has shown lower MIC values ranging from 0.001% to 0.015% (V/V). Besides, Firmino et al. (2018) have reported the antimicrobial activity of cinnamon essential oil and cinnamaldehyde against strains of *Pseudomonas aeruginosa*, *E. coli* and *S. aureus*. Firmino et al. (2018) have reported a strong inhibitory effect in which the MIC values of cinnamaldehyde and cinnamon essential oil were 0.3 mg/ml for Gram-negative bacterial strains and that of cinnamaldehyde was 0.25 mg/ml for *S. aureus*, with the cinnamon essential oil was of 0.6 mg/ml. The cinnamon essential oil has demonstrated only bactericidal activity (mean MBC/MIC = 2 to 4) and cinnamaldehyde (mean MBC/MIC = 7) has demonstrated both bacteriostatic and bactericidal activity against the fish pathogenic bacteria in the study conducted by Pathirana et al. (2019a) (Table 1). Pozzo et al. (2012) and Zhang et al. (2016) have reported the bactericidal activity of cinnamon essential oil and cinnamaldehyde against *S. aureus* and *E. coli*. The addition of cinnamon essential oil at the MBC levels can kill bacterial cells (Zhang et al., 2016). It has been reported that the cinnamon essential oils have a similar or stronger antimicrobial effect than some standard antibiotics including cephalothin (30 µg), oxytetracycline (30 µg) and trimethoprim/sulfamethoxazole (Yildirim and

Table 1 Size MIC and MBC values (%v/v) of essential oil against fish pathogenic bacterial strains isolated from Olive flounder (*Paralichthys olivaceus*)

Essential oil (Major component)	*Photobacterium damselae*		*Vibrio harveyi*		*Vibrio ichthyoenteri*		*Edwardsiella tarda*		*Lactococcus garvieae*		*Streptococcus iniae*		*Streptococcus parauberis*		Reference
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	
Citrus aurantifolia (Limonene)	0.062	0.125	0.125	1	0.125	0.5	0.25	1	0.125	1	0.125-0.5	04-2	0.031-0.125	0.125-1	Pathirana et al. (2018)
Lemonene	0.062	0.125	0.031	0.25	0.31	0.125	0.062	0.125-0.25	0.031	0.25	0.031-0.25	0.25-0.5	0.007-0.015	0.031-0.062	Pathirana et al. (2018)
Lavendular angustifolia (linalool)	0.125	0.25	-	-	-	-	0.5-2	4 to 8	1	4	0.063-0.5	0.5-4	0.063-0.5	0.5-1	Wimalasena et al. (2018)
Cinnamomum zeylanicum (Cinnamaldehyde)	0.003	0.031	0.007	0.031	0.007	0.031	0.001-0.003	0.003-0.015	0.015	0.031	0.015-0.031	0.031-0.062	0.007-0.015	0.015-0.031	Pathirana et al. (2019a)
Cinnamaldehyde	0.001	0.003	0.001	0.007	0.001	0.007	0.001-0.003	0.001-0.007	0.003	0.015	0.003-0.015	0.007-0.031	0.003-0.015	0.015-0.062	Pathirana et al. (2019a)
Cymbopogon flexuosus (Citral)	0.25	2	-	-	-	-	0.032-0.5	0.125-4	0.25	0.5	0.032-0.125	0.125-0.5	0.016-0.125	0.032-0.5	Pathirana et al. (2019b)
Syzygium aromaticum (Euginol)	0.125	0.25	0.125	0.25	0.125	0.25	0.125-0.5	0.25-0.5	0.5	1	0.25-0.5	0.25-1	0.25-0.5	0.5	Pathirana et al. (2019c)
Euginol	0.125	0.25	0.031	0.125	0.031	0.125	0.062-0.125	0.25-0.5	1	1	0.125-1	0.5-1	0.5-1	0.5-1	Pathirana et al. (2019c)
Zingiber officinale (Gingerols)	4	8	-	-	-	-	8	8	2	2	0.25-2	1 - 2	4	4 -8	Hossain et al. (2019)

Türker, 2018).

Lemongrass essential oil

Lemongrass essential oils are the extracted product of *Cymbopogn flexuosus* and *Cymbopogon citratus* plants. Citral (65–95%) is the main active component of lemongrass essential oil (Adukwu et al., 2016). The antimicrobial activity of Lemongrass essential oil has been studied against various microbes (Adukwu et al., 2012; Naik et al., 2014; Pathirana et al., 2019b). Also, Pathirana et al. (2019b) have reported Gram-positive bacteria (*Streptococcus parauberis, S. iniae, Lactococcus garvieae*) are more sensitive than Gram-negative bacteria (*E. tarda, Photobacterium damsalae*). Gram-negative bacteria are less susceptible to the inhibitory effects of lemongrass essential oil compared to Gram-positive bacteria (Turker et al., 2009; Adukwu et al., 2012; Marino et al., 2016; Pathirana et al., 2019b). However, Gram-positive *Micrococcus* spp. and *Streptococcus* spp. and Gram-negative *E. tarda*, *E. coli* have also demonstrated susceptibility against lemongrass essential oil (Adukwu et al., 2012; Marino et al., 2016). The maximum antimicrobial effect of the lemongrass essential oil has been reported at 1:1 concentration (1 part of the lemongrass essential oil in respective parts of the methanolic solution) and the minimum antimicrobial effect has been reported at 1:10 concentration (Pathirana et al., 2019b). There was no previous report about the MBC results of lemongrass essential oil against fish pathogenic bacteria. However, in 2019, the bactericidal and bacteriostatic activity of LGO against fish pathogenic bacteria has been demonstrated by MBC and MIC values in a study conducted by Pathirana et al. (2019b) as mentioned in Table 1. Moreover, the results showed that 100% of *L. garvieae*, 75% of *S. parauberis*, 100% of *S. iniae* and 75% of *E. tarda* were sensitive to LGO. In contrast, 100% of *P. damsalae*, 25% of *E. tarda* and 25% of *S. parauberis* were resistant to LGO.

Clove essential oil and eugenol

The essential oil isolated from the buds of (*Syzygium aromaticum*) is well known for its medicinal properties and is being widely used in different industries. Eugenol is the major component of the clove essential oil followed by β-caryophyllene and other components such as benzyl alcohol in small amounts. Prashar et al. (2006) have found the concentration of eugenol to be 78%, with 13% β-caryophyllene, whereas Pawar and Thaker (2006) found that the content of eugenol was 47.64%, with the concentration of benzyl alcohol at 34.10%. Thus, the composition of the Clove essential oil shows significant variation. However, eugenol, eugenol acetate and β- caryophyllene are the constituents in clove oil that are responsible for inhibitory activity against bacteria (Seongwel et al., 2009; Cortes-rojas et al., 2014; Pathirana et al., 2019c)

Clove essential oil and eugenol can inhibit the growth of both Gram-negative and Gram-positive fish pathogenic bacteria (Pathirana et al., 2019c). However, Chaieb et al. (2007) observed extensive activity of clove essential oil against Gram-positive bacteria, producing a clear zone of inhibition.

Meanwhile, eugenol was found to be exhibited higher activity against Gram-negative strains than Gram-positive strains (Oyedemi et al., 2009; Cortes-Rojas et al., 2014; Pathirana et al., 2019c). In contrast, Rehab et al. (2016) reported the elevated effect of eugenol against *S. aureus* compared to the Gram-negative bacterial strains. Antimicrobial activity of the eugenol and clove essential oil is increased in proportion to the clove essential oil or eugenol concentration and the maximum effect has been reported at 100% (V/V) concentration of both (Fagere, 2016; Bharath et al., 2017; Pathirana et al., 2019c). Both eugenol (MBC/MIC 1-4) and clove essential oil (MBC/MIC 1-2) have demonstrated bactericidal activity against 16 fish pathogenic bacteria in the study conducted by Pathirana et al. (2019c). Fagere et al. (2016) have reported identical MBC and MIC values and reported that the effect of clove essential oil was mainly bactericidal and not bacteriostatic. Moreover, eugenol has demonstrated bacteriostatic activity at lower concentrations and bactericidal activity at slightly higher concentrations against *Salmonella* (Chitanand et al., 2010; Devi et al., 2010). Although both clove essential oil and eugenol inhibited the growth of tested fish pathogenic bacteria, eugenol could inhibit the growth of bacteria at lower concentrations than clove essential oil (Pathirana et al., 2019c).

The essential oil of *Eucalyptus globulus*.

Eucalyptus globulus (*E. globulus*) is well-known as a medicinal plant because of its pharmacological and biological properties. The essential oil of *E. globulus* (EOEG) is in great demand in the market (Bachir and Benali, 2012) . The main component of the EOEG is the eucalyptol, also known as terpene 1, 8-cineole. The concentration of eucalyptol varies between 44% and 84% and it is known to have significant antimicrobial activity (Goldbeck et al., 2014).

EOEG has demonstrated antimicrobial activity against *E. tarda*, *Streptococcus iniae*, *S. parauberis*, *L. garviae*, *Vibrio harveyi*, *V. ichthyoenteri* and *P. damselae* isolated from fish in the study (Park et al., 2016). Also, that study has stated that the inhibition zone increased in proportion to the EOEG concentration. Significantly, the concentration (40 μg) of EOEG has shown effective antibacterial activity similar to Amoxicillin (30 μg), Erythromycin (15 μg), Chloramphenicol (30 μg), Tetracycline (30 μg) (Park et al., 2016). The EOEG has various degrees of antimicrobial activity against different bacterial species. However, it has been suggested that the EOEG work as a significant growth inhibitor against both Gram-positive and Gram-negative bacterial species (Bachir and Benali, 2012; Park et al., 2016).

Ginger (*Zingiber officinale*) essential oil

The ginger essential oil extracted from ginger (*Zingiber officinale*) rhizome has been studied for pharmacological properties and its major components have been identified as zingiberene, ar-curcumene and β-sesquiphellandrene (Nampoothiri et al., 2012). Gingerols and shagols are the biologically active compounds of ginger essential oil and they have both antibacterial and antifungal properties (Supreetha et al., 2011; Shehata et al., 2013). The ginger essential oil has demonstrated antimicrobial activities against fish-borne bacteria such as *L. garvieae*, *Aeromonas hydrophila, Listeria monocytogenes*, *S. aureus*, *S. parauberis* and *Yersinia enterocolitica* (Nya and

Austin, 2009; Rattanachaikunsopon and Phumkhachorn, 2009; Debbarma et al., 2012; Kim et al., 2016). However, fish pathogenic *E. tarda* has shown controversial activity against ginger essential oil. *E. tarda* isolated from farmed Olive flounder in Korea were resistant to ginger essential oil (Hossain et al., 2019). In contrast, ginger essential oil has been reported as less effective against *E. tarda* isolated from *Osphronemus goramy* (Gourami) (Sarjito and Prayitno, 2015). Ginger essential oils exhibited antimicrobial properties against the tetracycline, erythromycin and nalidixic-resistant bacterial strains (Park et al., 2014) Variations in the chemical composition of ginger essential oil due to the genetic and environmental factors can make the antimicrobial activity of ginger essential oil different from that of bacteria (Snoussi et al., 2016). According to the MIC results in the study conducted by Hossain et al. (2019), ginger essential oil was effective against most of the Gram-positive bacterial strains even in lower concentrations compared to Gram-negative bacteria. The ginger essential oil has scored MBC/MIC as ≤4 against the fish pathogenic bacteria (Hossain et al., 2019). Thus, ginger essential oil has bactericidal activities

Lavender (Lavendular angustifolia) oil

Lavender (*Lavandula angustifolia*) is an ornamental flowering plant and is extensively cultivated as commercially for the extraction of lavender oil. Linalool, linalyl acetate, β-ocimene, 1,8-cineole, terpinen-4-ol and camphor are the major components of lavender essential oils (Jianu et al., 2013). The lavender essential oil is active against many bacterial species including antimicrobial-resistant bacteria such as methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococcus* spp. and fish-pathogenic *P. damselae, Yersinia ruckeri* and *L. garvieae* (Cavanagh and Wilkinson, 2002; Bulfon et al., 2014).

Lavender essential oils have effectively inhibited the growth of fish pathogenic *E. tarda, P. damselae, L. garvieae, S. iniae* and *S. parauberis* even at the lower lavender essential oil concentrations (1:10) in the study of Wimalasena et al. (2018). In their study, *S. iniae* demonstrated the highest inhibition zone (30 mm) at 1:1 dilution of lavender essential oil (Table 2). However, Wimalasena et al. (2018) have reported that the antimicrobial activity of lavender essential oil against the fish-pathogenic *E. tarda* is not strong compared to the other tested fish-pathogenic bacteria. Also, Lavender essential oils have demonstrated moderate efficacy on fish pathogenic *P. damselae* where the ratio of MIC: MBC and MIC were 1:2 and 1.1 mg/ml, respectively (Bulfon et al., 2014). Moreover, *P. damselae* (FP4101) used in the study by Wimalasena et al. (2018) has shown MIC: MBC ratio of 1:2 and MIC of 0.125%. This indicates the bactericidal activity of lavender essential oil against *P. damselae*. Fish-pathogenic *L. garvieae* have exhibited high MIC values occasionally against the lavender essential oils compared to the other fish-pathogenic Gram-positive bacteria (Bulfon et al., 2014; Wimalasena et al., 2018). Similar to most of the other essential oils, the

Table 2 Size of the inhibition zone of essential oils against fish pathogenic bacteria

Bacterial species	Essential oil	Amount of essential oil applied to the disk	Zone of inhibition (mm)	Reference
E. tarda	*Eucalyptus globulus* essential oil	20 (µg)	15-24	Park et al. (2016)
V. ichthyoenteri		10 (µg)	15-24	
V. harveyi		5 (µg)	15-24	
P. damselae		5 (µg)	15-24	
S. iniae		10 (µg)	15-24	
S. parauberis		20 (µg)	15-24	
L. garviae		20 (µg)	15-24	
E. tarda	Lemon essential oil	20µl	15-17	Pathirana et al. (2018)
V. ichthyoenteri			17	
V. harveyi			16	
P. damselae			18	
S. iniae			16-20	
S. parauberis			14-22	
L. garviae			22	
E. tarda	Limonene	20µl	17-23	Pathirana et al. (2019)
V. ichthyoenteri			23	
V. harveyi			21	
P. damselae			24	
S. iniae			23-30	
S. parauberis			20-26	
L. garviae			20	
E. tarda	Ginger essential oil	20µl	0	Hossain et al. (2018)
P. damselae			8	
L. garviae			13	
S. iniae			13-22	
S. parauberis			13-7	
E. tarda	Cinnamon essential oil	20µl	19-28	Pathirana et al. (2019a)
V. ichthyoenteri			26	
V. harveyi			26	
P. damselae			30	
S. iniae			16-32	
S. parauberis			16-26	
L. garviae			17	
E. tarda	Cinamaldehyde	20µl	30-46	Pathirana et al. (2019a)
V. ichthyoenteri			30	
V. harveyi			26	
P. damselae			32	
S. iniae			24-34	
S. parauberis			20-30	
L. garviae			22	
P. damselae	Lavander essential oil	20µl	22	Wimalasena et al. (2018)
E. tarda			14-19	
L. garviae			19	
S. iniae			20-30	
S. parauberis			17-21	
P. damselae	Lemongrass essential oil	20µl	12	Pathirana et al. (2019b)
E. tarda			8-32	
L. garviae			20	
S. iniae			22-50	
S. parauberis			7-45	

Table 2 Size of the inhibition zone of essential oils against fish pathogenic bacteria (Cont.)

Bacterial species	Essential oil	Amount of essential oil applied to the disk	Zone of inhibition (mm)	Reference
E. tarda	Clove essential oil	20µl	16-18	Pathirana et al. (2019c)
V. ichthyoenteri			20	
V. harveyi			20	
P. damselae			19	
S. iniae			16-22	
S. parauberis			14-19	
L. garviae			17	
E. tarda	Eugenol	20µl	19-22	Pathirana et al. (2019c)
V. ichthyoenteri			27	
V. harveyi			24	
P. damselae			20	
S. iniae			15-25	
S. parauberis			17-19	
L. garviae			15	
A. hydrophila	Basil essential oil	10µl	22.3	Yildirim and Türker (2018)
A. salmonicida			12.5	
V. anguillarum			20	
Y. yuckeri			22	
E. faecalis			25	

lavender essential oil has also demonstrated high efficacy against the majority of Gram-positive bacteria compared to Gram-negative bacteria.

Other essential oils

Several studies have examined the antibacterial activity of some other types of essential oils (Table 3). Noel-Martinez et al. (2021) examined the antibacterial activity of *Bursera graveolens* against fish pathogenic bacteria isolated from striped merlin (*Kajikia audax*). *B. graveolens* is commonly grown in Nothern Peru and it is used for traditional medicine (Sotelo Mendez et al., 2017). D-Limonene (77.06%) is the major chemical component found in *B. graveolens*. The results showed that 1620 µg/mL for MIC and 25920 µg/mL for MBC value against *Aeromonas salmonicida* (Noel-Martinez et al., 2021). *Lippia spp.* is one of the medicinal plants which showed biological potential for antimicrobial activity (Soares and Tavares-Dias, 2013). *L. alba*, *L. origanoides* and *L. sidoide* were evaluated for their antimicrobial activity against *A. hydrophila*. The three essential oils demonstrated both bacteriostatic (MIC) and bactericidal (MBC) action against *A. hydrophila* (Majolo et al., 2017). In contrast, Sutili et al. (2015) reported the weak antimicrobial activity of *L. alba* against *A. hydrophila* isolated from infected fish. MIC and MBC values were reported as 2862 and 5998 µg mL^{-1} for *L. alba* essential oil. Moreover, *in vivo* antioxidant activity of *Hesperozygis ringens* was also evaluated in the diseased fish tissues against *A. hydrophila* and *A. Veronii* by Bandeira et al. (2017). Essential oil of *Eucalyptus camaldulensis* which contains α-phellandrene as a major component showed moderate antimicrobial activity against fish pathogenic bacteria such as *A. hydrophila*, *V. parahaemolyticus* and *V. vulnificus* (Debbarma et al., 2013). The antimicrobial activity of several essential oils is summarized in Table 3 to compare the MIC and MBC values against different fish pathogenic bacteria

Table 3 Carcass quality and gut relative weight and length of stressed Japanese quail influenced by dietary Car,CrM and CarCrM.

Essential oil (major component)	Bacterial species	Fish species	MIC	MBC	Reference
Bursera graveolens (D-limonene)	*Aeromonas salmonicida*	Striped marlin (*Kajikia audax*)	1620	25920	
	Pseudomonas aeruginosa		>207330	>207330	Noel-Martinez et al. (2021)
	Pichia kudriavzevii		6480	25920	
Lippia alba (Citral)			1666.7	1,666.7	
L. sidoides (Thymol)			312.5	416.7	
Mentha piperita (Menthol)	*Streptococcus alginacea*	Diseased tilapia (*Oreochromis niloticus*)	1,250	1,250	Majolo et al. (2018)
Ocimum gratissimum (Eugenol)			2,500	2,500	
Zingiber officinale (Geranial)			625	833.3	
	Aeromonas hydrophila		400	400	
	Aeromonas Veronii		800	1600	
O. gratissimum (Eugenol)	*Citrobacter freundii*		1600	1600	
	Raoltella ornithinolytica	Silver catfish (*Rhamdia quelen*)	1600	1600	Bandeira et al. (2017)
	A. hydrophila		400	400	
Hesperozygis ringens (Pulegone)	*A. Veronii*		800	800	
	Citrobacter freundii		1600	3200	
	R. ornithinolytica		3200	3200	

Table 3 Carcass quality and gut relative weight and length of stressed Japanese quail influenced by dietary Car,CrM and CarCrM. (Cont.)

Essential oil (major component)	Bacterial species	Fish species	MIC	MBC	Reference
L. alba (Geranial)	*A.hydrophila*	Not available	5000	5000	Majolo et al. (2017)
L. origanoides (Carvacrol)			2500	2500	
L. sidoides (Thymol)			1250	1250	
L. alba (Linalool)	*A.hydrophila*	Silver catfish (*R. quelen*)	2862	5998	Sutili et al. (2015)
L. nobilis (1,8-Cineole)	*A.hydrophila*	Sea bass (*D. labrax*), sea bream (*S.aurata*)	50-200	6250 to > 25000	Snuossi et al. (2016)
	Enterobacter Cloacae		50-200	>25000 to >50000	
	Klebsiella ornithinolytica		50	6250	
	K. oxytoca		50	>12500	
	Staphlococcus lentus		50	>6250	
	S. lugdunensis		50-100	>12500 to >25000	
	S. xylosus		50	>6250	
	S. sciuri		50	>6250 to >25000	
	Serratia odorifera		100	>12500	
	Vibrio alginolyticus		50	>6250	
Eucalyptus camaldulensis (α-phellandrene)	*V. parahaemolyticus*	Microbial Type Culture Collection	62.5	12.5	Debbarma et al. (2013)
	A. hydrophila		125	500	
	V. vulnificus		31.25	62.5	

isolated from different fish species.

Application of essential oils and issues in aquaculture

The widely used methods for the administration of essential oils in aquaculture are as a dietary supplement or via the culture media (immersion method) in which the fish are being kept. The whole fish population in a tank can be treated within a short period by using the immersion method. Despite this, the immersion method requires a high operational cost when applied in large-scale tanks. Therefore, treating infected fish in a small bath filled with the required concentration of essential oil is more effective than large-scale immersion efforts. (Cunha et al., 2018). The dietary supplement method is effective as a preventive method and this method requires active feeding behavior. In contrast, infected animals may not feed actively (Sutili et al., 2018). Therefore, the administration of essential oils as a dietary supplement may not be effective against infected fish. Other administration methods such as injection, gavage and topical application can be employed at the laboratory level. However, it has limitations in fish farms due to the lack of skilled labor and stress on the animals (Park et al., 2012). The most important factors that should be considered before selecting an administrative method are storage, stability in water, interaction with the other components in feed, stability against the feed manufacturing process and digestibility. Because those factors can alter the biological activities of essential oil (Cunha et al., 2018). Moreover, a literature survey conducted by (Cunha et al., 2018) has reported that there is no correlation between the analysis of the minimum effective bath concentrations and the MIC and dietary supplementation doses of essential oils. Therefore, the determination of MIC only *in vitro* is not an appropriate methodology to forecast the *in vivo* effect of essential oils every time. In addition, the usage of high-quality essential oils with genetic homogeneity was recommended because when the essential oils are exposed to oxygen, light, heat, or humidity, they can quickly break down and lose their potency (De FreitasSouza et al., 2019). According to the report by Howell (2019), it is challenging to make specific suggestions on how essential oils should be used practically in aquaculture because there is little information available on species-specific dosages or what happens when essential oils are applied to aquaculture. Also, the researchers concluded that more realistic and cost-effective studies are required before essential oils can completely replace the current health care options for farmed fish.

Toxicity of essential oils on the fish

Essential oils are natural, but that doesn't mean they can be used without studying the possible side effect. Before using any essential oil as an antimicrobial agent, it should be tested for effects that can be harmful to the host organism. In aquaculture, the toxicological assessment of essential oil against cultured organisms is an essential process that should be done before use in the field. Toxic effects in fish can be verified through toxicological assessment, by obtaining data such as induced adverse effects concentration and clinical signs (Tavares – dias et al., 2018). The toxicity of a particular essential oil can be evaluated as a chronic, sub-chronic, or acute effect in a 24–96 h test. The concentration of the used essential oil, the organs affected, the severity of the effects and the reversion of toxic effects should be recorded. Such assays determine safe limits for the use of essential oils in the immersion treatment method. However, the recommended therapeutic concentrations may vary according to exposure time, species and size of the fish, bath quality and temperature (Doleželová et al., 2011; Malheiros et al., 2016). Eugenol, the active ingredient in clove essential oil has been studied extensively for its toxicity against fish and it ranged from 1.8 to 184.3 mg/L. Also, exposure time and fish species are the determining factors of the observed wide concentration range. In addition to that, eugenol is the most widely used for fish as a natural anesthetic and is approved in several countries, including Chile, Australia, and New Zealand (Trushenski et al., 2013). However, the US Food and Drug Administration has not recommended the use of clove oil in fish because of its carcinogenic potential in rats (Spanghero et al., 2019). Cunha et al. (2016) reported that the mortality rate among silver catfish *Rhamdia quelen* was 10% after 96 h of exposure to *Tagetes minuta* essential oil at 50 mg/L, 70% and 80% after 24 h and 96 h, respectively. LC50 values of rainbow trout within 96-h against the thyme, cumin, thymol and caraway essential oils have been reported as 6.6, 35,2.6, and 14 mg/L, respectively with histopathological changes such as hyperemia and edema in an organ. Especially, necrosis and inflammation in the brain and cellular hyperplasia and fusion of lamellae in the gills (Tabarraei et al., 2019). The mean lethal concentration (LC50) of *Lippia origanoides* essential oil for tambaqui (*Colossoma macropomum*) was reported as 15.2 mg/L. However. Tambaqui infected with *A. hydrophila* (experimentally) had a higher survival rate after the immersion treatment at 10 mg/ *L. origanoides* bath. This information reveals that the acute toxicity of essential oils is significant and their use in the aquaculture industry should be cautious. Further studies on acute and chronic toxicity and the harmful effects of essential oils on treated organisms, non-target organisms and the environment are encouraged. Therefore, further studies are needed to enable the replacement of the current synthetic antimicrobial treatments with essential oils.

CONCLUSIONS

In this review study, we summarized several active essential oils that have been tested and shown to be effective against fish pathogenic bacteria due to their antibacterial capabilities. Studies have suggested that the usage of essential oils instead of synthetic antimicrobials may be a more affordable alternative for the treatment and/or prevention of infectious illnesses in fish. It has been demonstrated that certain elements in essential oils have bactericidal effects in the literature. Most studies cited the activity of the antibacterial and antioxidant properties of essential oil. However, not like synthetic antimicrobials that have unique chemical compositions between every product belonging to the same drug, the same essential oil may not perform unique antimicrobial activity every time. In addition, the safety of the usage of essential oils and how they react with other antibiotics needs to be studied further before applying them as an alternative to antimicrobials in aquaculture. Therefore, the characterization of essential oils at the molecular level using advanced technology is recommended to proceed in future studies.

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AUTHOR CONTRIBUTIONS

M.V.K.S. Wickramanayake and **P.M. Kumarage** contributed equally to this work. M.V.K.S. Wickramanayake, P.M. Kumarage and Gang Joon-Heo contributed to the study conception and design. The literature search was performed by M.V.K.S. Wickramanayake, P.M. Kumarage, and Sana Majeed. The first draft of the manuscript was written by M.V.K.S. Wickramanayake and P.M. Kumarage. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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