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REVIEW ARTICLE

The effects of essential oils and their major compounds on fish bacterial pathogens – a review

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Summary

The increased resistance of fish pathogens to conventional treatments has lead researchers to investigate the antibacterial properties of natural resources, such as essential oils (EOs) of plants, in an effort to find products that are less harmful to the environment. The objective of this review is to provide an overview of the studies, *in vivo* and *in vitro*, that addressed the use of EOs and their major compounds as antimicrobial agents in fish, to identify the best EOs and compounds to investigate considering feasibility of application and suggest possible future studies. To date, studies suggest that the use of EOs in the prevention and/or treatment of infectious diseases in fish may be a promising strategy to reduce the use of conventional antibiotics in aquaculture, since several EOs effectively reduce or avoid the effects of bacterial infections in fish. The use of EOs through nanotechnology delivery systems, especially in dietary supplementation experiments, is promising. This form of application of the EOs allows a potentiation and targeting of the desired effect of the EOs and also allows the protection of EOs active constituents against enzymatic hydrolysis, deserving further study.

Antimicrobial alternatives for fish

According to the Food and Agriculture Organization of the United Nations (FAO), aquaculture is among the fastest growing production sectors, and in 2014, world aquaculture produced 49·86 million tons of fish (FAO, Food and Agriculture Organization of the United Nations 2016). Fish farms can sometimes have stressful conditions, which favour the spread of bacterial and fungal infections (Naylor *et al.* 2000), as well as viral and parasitic diseases (Walker and Winton 2010; Pantoja *et al.* 2012). Immune systems may not be fully capable of avoiding and eliminating a bacterial infection efficiently (Barton and Iwama 1991; Finlay and McFadden 2006). In order to increase fish production and reduce mortality, farmers usually administer antibiotics in the feed or water to treat and prevent bacterial diseases (Markestad and Grave 1997; Cabello 2006). The indiscriminate use of antibiotics is a hazard to the ecosystem because it can lead to the development of bacterial resistance to these drugs (Rhodes *et al.* 2000; Cabello 2006; Acar *et al.* 2009).

Some international organizations such as the World Health Organization (WHO) and FAO have presented some rules to restrict the use of antibiotics in aquaculture to minimize the impact of the indiscriminate use of antimicrobials on human and animal health as well as the appearance and the dissemination of resistant pathogens by the misuse of antibiotics (WOH, World Organization for Animal Health 2007; FAO, Food and Agriculture Organization of the United Nations 2012). According to FAO, Food and Agriculture Organization of the United Nations (2012), the most commonly used antibiotics worldwide are sulfonamides, oxytetracycline, sarafloxacin, erythromycin and florfenicol. In Brazil, only florfenicol is allowed for aquaculture practices, and florfenicol, oxytetracycline and sulfadimethoxine+ormetropim are allowed in the United States. This variation in the permission and regulation of the use of each antibiotic depends on the legislation of each country or each legal agreement determined by the country in which the use is made (WOH, World Organization for Animal Health 2007; FAO, Food and Agriculture Organization of the United Nations 2012).

Consequently, it is important to develop and utilize alternative therapies to treat bacterial infections in fish. Essential oils (EOs) are one of the alternatives being used as antibiotics and fungicides (Romero *et al.* 2012). EOs are liquid lipophilic mixtures that contain the substances responsible for the aroma of plants and are produced as secondary metabolites (Bakkali *et al.* 2008). It is believed that the primary role of these EOs in plants is to attract pollinators and to avoid pathogens, since they have antibacterial, antifungal, antiviral and insecticidal effects. The mechanisms of action of EOs depend on their chemical composition (Nazzaro *et al.* 2013), and some plant species may also have different chemotypes, characterized by the major compounds of different EOs, which would change the composition of the EO and its properties (Deering *et al.* 2017). Many EOs contain phenolic compounds that are responsible for their antimicrobial effects (Cosentino *et al.* 1999). The final effect of EOs against a pathogen may result from the synergy of distinct oil constituents or their major components (Sutili *et al.* 2016a). Consequently, the effects of an EO may change with the chemotype; therefore, studies must provide the chemical composition of the EOs analysed.

The emergence of bacterial resistance to antibiotics in aquaculture and the possible effects of antibiotics on natural microbial communities (Grenni *et al.* 2017) makes new alternatives and studies of this subject of great importance. There are reviews regarding the antibacterial properties and applicability of EOs to increase food preservation (Burt 2004; Gómez-Sánchez and López-Malo 2009) and on the effect of EOs as antibacterials against common human pathogens (Reichling *et al.* 2009; Nazzaro *et al.* 2013). Reviews concerning the use of EOs in aquaculture for the control and/or treatment of diseases have already been published (Harikrishnan and Balasundaram 2005; Murthy and Kiran 2013; Bulfon *et al.* 2015) but with a different focus from the present review. Previous reviews presented the advantages of herbal medicine based on the lack of side effects, being a biodegradable alternative, being available locally, and showing promise in replacing antibiotics to treat diseases. The objective of this review is to provide an overview of the *in vivo* and *in vitro* studies that addressed the use of EOs and their major compounds as antimicrobial agents in fish to identify the best EOs and compounds to investigate considering feasibility of application and suggest possible future studies.

Mechanisms of the antibacterial effects of EOs

The mechanisms through which different EOs are able to damage bacteria depend on their composition. Usually antimicrobial activity is derived not from only a single mechanism of action, but from a cascade of reactions involving the entire bacterial cell because EOs have several chemical structures in their composition and, consequently, several functional groups (Burt 2004; Nazzaro *et al.* 2013).

Overall, Gram-positive bacteria are more susceptible to the effects of EOs than Gram-negative bacteria (Trombetta *et al.* 2005), due to significant structural differences in the cell wall of these two groups of bacteria. Most of the cell wall of Gram-positive bacteria is composed of peptidoglycan, which allows hydrophobic molecules to easily penetrate the cell and act both on the cell wall and within the cytoplasm (Nazzaro *et al.* 2013).

In addition to the peptidoglycan layer, the cell wall of Gram-negative bacteria has an outer membrane, which is composed of a double layer of phospholipids linked to the peptidoglycan layer by lipopolysaccharides. Some hydrophobic molecules are able to penetrate the cell, but only through porin proteins that form water-filled channels distributed throughout the cell wall. Therefore, Gram-negative bacteria are more resistant to hydrophobic antibiotics (Nikaido 1994; Nazzaro *et al.* 2013).

EOs are able to affect both the cytoplasm and membrane(s) of bacteria. The mechanisms of action of EOs can include cell wall degradation, damage to the cytoplasmic membrane and membrane proteins, and reduce proton motive force and ATP synthesis. The lipophilic character of EO compounds allows them to penetrate the cell membrane and remain between the phospholipids. In addition, EOs can affect the synthesis of membrane lipids. Both effects can change membrane structure and, consequently, its permeability. EOs can also act on quorum sensing systems (i.e., the bacterial pheromones), which are important to coordinate bacterium–bacterium interactions to regulate virulence factor expression, biofilm formation, sporulation and mating (Nazzaro *et al.* 2013; Bouyahya *et al.* 2017).

Some studies indicate that the mechanism of action of OEs is dependent on their major functional groups. EOs containing mainly terpenes (*p*-cymene, limonene, terpinene, sabinene and pinenes) and some oxygenated chemical structures (borneol, camphor, 1,8 cineole, α-pinene, camphone, verbenonone and bornyl acetate) have weak or nonexistent antibacterial activity, showing more pronounced antimicrobial activity against Gram-positive bacteria. The antibacterial effect depends on their final concentration in the solution: at low concentrations, they interfere with enzymes involved in the production of energy, and they are able to cause protein denaturation at higher concentrations (Tiwari *et al.* 2009; Nazzaro *et al.* 2013). Terpenoids (thymol, carvacrol, linalool, menthol, geraniol, linalyl acetate, citronellal, and piperitone) have antibacterial activity mediated by the functional group

acting on the outer membrane of the bacterium, thus altering the permeability and/or fluidity of the membrane as well as affecting membrane proteins and periplasmic enzymes. Phenylpropenoids (eugenol, isoeugenol, vanillin, safrole and cinnamaldehyde) have antimicrobial activity conferred by free hydroxyl groups, and their mechanism of action depends on the type and number of substitutions in the aromatic ring of their structure. Usually the effect of these functional groups is on the membrane, ion transport, ATP production, and alteration of fatty acid and lipid profiles of bacteria; in addition to direct action on some bacterial enzymes such as carboxylase, protease, ATPase, and amylase as well as bacterial growth (Thoroski *et al.* 1989; Wendakoon and Sakaguchi 1995; Nazzaro *et al.* 2013).

In vitro experiments with isolated compounds demonstrated that eugenol exhibits higher antibacterial activity against Gram-negative bacteria (Hyldgaard *et al.* 2012), and 1,8 cineole inhibits quorum sensing (Bouyahya *et al.* 2017). β-pinene, linalool, cinnamaldehyde, geraniol, α-terpinene, β-citronellol, and estragole have deleterious effects on the structure and function of the microbial membrane and cell wall (Andrade-Ochoa *et al.* 2015). Linalyl acetate disturbs the lipid fraction, creating leakage of the intracellular bacterial material (Trombetta *et al.* 2005). Citral alters the intracellular pH, membrane integrity and potential and intracellular ATP concentration (Shi *et al.* 2016) (Fig. 1).

In vitro assessment of EOs properties against fish bacterial pathogens

It is important to perform *in vitro* assays to determine if an EO is suitable for *in vivo* antimicrobial tests. According to Ríos and Recio (2005), for an EO to be considered active, it has to kill or inhibit bacterial growth below the concentration of 100 μg ml^{-1}. However, Aligiannis *et al.* (2001) proposed a different classification where a minimum inhibitory concentration (MIC) of 500 μg ml^{-1} or less corresponds to strong inhibition, moderate inhibition is a MIC between 600 and 1500 μg ml^{-1}, and weak inhibition corresponds to a MIC above 1600 μg ml^{-1}.

According to the MIC test and classification of Ríos and Recio (2005), there are several EOs that present strong inhibition against pathogenic bacteria of fish. Among all EOs tested *in vitro*, the EO of *Syzygium aromaticum* (clove oil) has one of the most promising results, being able to inhibit bacterial growth with a MIC as low as 0·015 μg ml^{-1}. This antibacterial activity evaluated through MIC determined on 96-well microplates with U bottom wells has been shown to be effective against a variety of Gram-positive and Gram-negative bacteria, including the major pathogens of aquaculture: *Streptococcus agalactiae* (Zhang *et al.* 2013), *Flavobacterium columnare* (Sebastião *et al.* 2013), *Aeromonas hydrophila* (Griffin *et al.* 2013), *Edwardsiella tarda* and *Edwardsiella ictaluri* (Park *et al.* 2012a; Hawke *et al.* 2013). Other EOs are also effective antibacterial agents against major aquaculture pathogens, such as the EOs of *Zataria multiflora* and *Rosmarinus officinalis*, which decreased the haemolytic activity and down-regulated the transcription of sagA, a streptolysin S gene related to the secretion of virulence factors in *Streptococcus iniae* (Soltani *et al.* 2014). The EO of *Lippia sidoides* was effective against *Aer. hydrophila in vitro* (Majolo *et al.* 2017), and the EO of *Lippia nobilis* against several Gram-positive and Gram-negative bacterial species isolated from fish and shellfish products (Snuossi *et al.* 2016; Table 1). The EO of *Melaleuca alternifolia* acts on the membrane of *Pseudomonas aeruginosa*, causing bilayer expansion, directly interfering with membrane-integrated enzymes

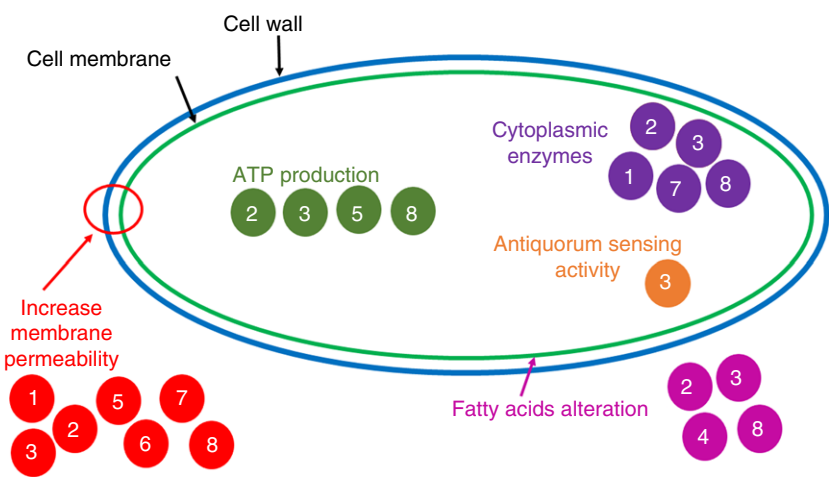

Figure 1 Mechanisms of action and target sites of isolated EOs compounds in microbial cells. 1: thymol, carvacrol, linalool, menthol, geraniol, linalyl acetate, citronellal and piperitone; 2: eugenol, isoeugenol, vanillin, safrole and cinnamaldehyde; 3: 1,8 cineole; 4: β-pinene, linalool, cinnamaldehyde, geraniol, α-terpinene, β-citronellol and estragole; 5: citral; 6: linalyl acetate; 7: *p*-cymene, limonene, terpinene, sabinene and pinenes; 8: borneol, camphor, α-pinene, camphone, verbenonone and bornyl acetate. Adapted from Nazzaro *et al.* (2013). [Colour figure can be viewed at wileyonlinelibrary.com]

Table 1 *In vitro* assays with essential oils and their antibacterial activity against fish pathogens

Bacterium	Essential oil	Major compounds (%)	MIC (μg ml^{-1})	MBC (μg ml^{-1})	Source
Aer. hydrophila *Aer. veronii*	*Ocimum gratissimum*	Eugenol (91·47)	400–1600*		Bandeira *et al.* (2017)
Aer. hydrophila *Aer. veronii*	*Hesperozygis ringens*	Pulegone (96·63)	400–1600*		Bandeira *et al.* (2017)
Cit. freundii *Raoultella ornithinolytica*	*Ocimum gratissimum* *H. ringens*	Eugenol (91·47) Pulegone (96·63)	1600 to >3200*		Bandeira *et al.* (2017)
Aer. hydrophila	*Lippia alba*	Geranial (25·4)	5000	5000	Majolo *et al.* (2017)
	Lippia origanoides	Carvacrol (49·7)	2500	2500	Majolo *et al.* (2017)
	Lippia sidoides	Thymol (76·6)	1250	1250	Majolo *et al.* (2017)
Aer. hydrophila	*Lippia alba*	Linalool (65·5)	2862	5998	Sutili *et al.* (2015a, 2015b)
Aer. hydrophila	*O. americanum*	β-linalool (32·43)	6400	NA	Sutili *et al.* (2016a, 2016b)
Aer. hydrophila	S-(+)- and R-(-)-linalool	–	3200	NA	Silva *et al.* (2017)
Aer. hydrophila	*L. nobilis*	1,8-Cineole (56)	50–200	6250 to >25 000	Snuossi *et al.* (2016)
Ent. cloacae	*L. nobilis*	1,8-Cineole (56)	50–200	>25 000 to >50 000	Snuossi *et al.* (2016)
Kl. ornithinolytica	*L. nobilis*	1,8-cineole (56)	50	6250	Snuossi *et al.* (2016)
Kl. oxytoca	*L. nobilis*	1,8-Cineole (56)	50	>12 500	Snuossi *et al.* (2016)
Staph. lentus	*L. nobilis*	1,8-Cineole (56)	50	>6250	Snuossi *et al.* (2016)
Staph. lugdunensis	*L. nobilis*	1,8-Cineole (56)	50–100	>12 500 to >25 000	Snuossi *et al.* (2016)
Ser. odorifera	*L. nobilis*	1,8-Cineole (56)	100	>12 500	Snuossi *et al.* (2016)
Staph. sciuri	*L. nobilis*	1,8-Cineole (56)	50	>6250 to >25 000	Snuossi *et al.* (2016)
Staph. xylosus	*L. nobilis*	1,8-Cineole (56)	50	>6250	Snuossi *et al.* (2016)
V. alginolyticus	*L. nobilis*	1,8-Cineole (56)	50	>6250	Snuossi *et al.* (2016)
Aer. hydrophila	*Z. officinale*	Hydrocarbon monoterpenes (32)	50–200	>780–50 000	Snuossi *et al.* (2016)
Ent. cloacae	*Z. officinale*	Hydrocarbon monoterpenes (32)	50–100	>25 000	Snuossi *et al.* (2016)
Kl. ornithinolytica	*Z. officinale*	Hydrocarbon monoterpenes (32)	200	>25 000	Snuossi *et al.* (2016)
Kl. oxytoca	*Z. officinale*	Hydrocarbon monoterpenes (32)	50	>12 500	Snuossi *et al.* (2016)
Staph. lentus	*Z. officinale*	Hydrocarbon monoterpenes (32)	50	>3130	Snuossi *et al.* (2016)
Staph. lugdunensis	*Z. officinale*	Hydrocarbon monoterpenes (32)	50–200	>12 500	Snuossi *et al.* (2016)
Ser. odorifera	*Z. officinale*	Hydrocarbon monoterpenes (32)	50	>12 500	Snuossi *et al.* (2016)
Staph. sciuri	*Z. officinale*	Hydrocarbon monoterpenes (32)	50–200	1560 to >25 000	Snuossi *et al.* (2016)
Staph. xylosus	*Z. officinale*	Hydrocarbon monoterpenes (32)	50	>12 500	Snuossi *et al.* (2016)
V. alginolyticus	*Z. officinale*	Hydrocarbon monoterpenes (32)	50	>25 000	Snuossi *et al.* (2016)
Aer. hydrophila	*A. graveolens*	Carvone (27)	50–200	12 500–50 000	Snuossi *et al.* (2016)
Ent. cloacae	*A. graveolens*	Carvone (27)	50–100	>25 000–50 000	Snuossi *et al.* (2016)
Kl. ornithinolytica	*A. graveolens*	Carvone (27)	50	>25 000	Snuossi *et al.* (2016)
Kl. oxytoca	*A. graveolens*	Carvone (27)	100	50 000	Snuossi *et al.* (2016)
Staph. lentus	*A. graveolens*	Carvone (27)	50	>25 000	Snuossi *et al.* (2016)
Staph. lugdunensis	*A. graveolens*	Carvone (27)	100–390	>25 000–50 000	Snuossi *et al.* (2016)
Ser. odorifera	*A. graveolens*	Carvone (27)	100	>12 500	Snuossi *et al.* (2016)
Staph. sciuri	*A. graveolens*	Carvone (27)	50 100	>12 500 to >50 000	Snuossi *et al.* (2016)
Staph. xylosus	*A. graveolens*	Carvone (27)	100	>12 500	Snuossi *et al.* (2016)
V. alginolyticus	*A. graveolens*	Carvone (27)	100	50 000	Snuossi *et al.* (2016)
L. garvieae	*Z. multiflora*	Carvacrol (62·82)	0·12*,†		Soltani *et al.* (2015)

Table 1 (*Continued*)

Bacterium	Essential oil	Major compounds (%)	MIC (μg ml^{-1})	MBC (μg ml^{-1})	Source
Aer. hydrophila	*H. ringens*	Pulegone (96·63)	800–3200*		Sutili *et al.* (2015a, 2015b)
	O. gratissimum	Eugenol (91·47)	200–1600	400–1600	Sutili *et al.* (2015a, 2015b)
	O. americanum	1,8-Cineole (21)	>than highest concentration tested		Sutili *et al.* (2015a, 2015b)
Aer. hydrophila	eugenol	—	800–3200	1600–3200	Sutili *et al.* (2014)
Aer. hydrophila	*A. monophylla*	Sabinene (23·81) and isoeugenol-*E* (23·73)‡	139·32	NA	Thirugnanasampandan *et al.* (2015)
E. coli	*A. monophylla*	Sabinene (23·81) and isoeugenol-*E* (23·73)‡	328·14	NA	Thirugnanasampandan *et al.* (2015)
Kl. pneumoniae	*A. monophylla*	Sabinene (23·81) and isoeugenol-*E* (23·73)‡	516·73	NA	Thirugnanasampandan *et al.* (2015)
Ps. aeruginosa	*A. monophylla*	Sabinene (23·81) and isoeugenol-*E* (23·73)‡	203·84	NA	Thirugnanasampandan *et al.* (2015)
Pr. mirabilis	*A. monophylla*	Sabinene (23·81) and isoeugenol-*E* (23·73)‡	189·57	NA	Thirugnanasampandan *et al.* (2015)
Pr. vulgaris	*A. monophylla*	Sabinene (23·81) and isoeugenol-*E* (23·73)‡	233·73	NA	Thirugnanasampandan *et al.* (2015)
Staph. aureus	*A. monophylla*	Sabinene (23·81) and isoeugenol-*E* (23·73)‡	541·11	NA	Thirugnanasampandan *et al.* (2015)
Aer. hydrophila	*T. vulgaris*	1,8-Cineole (53·46)	62	NA	Millezi *et al.* (2013)
	C. citratus	geranial (46·03)	31	NA	Millezi *et al.* (2013)
Strep. iniae	*Z. multiflora*	–	0·06†	0·12–0·25†	Soltani *et al.* (2014)
	R. officinalis	–	0·12–0·25†	0·5 to >1†	Soltani *et al.* (2014)
V. parahaemolyticus	*Z. officinale*	Zingiberene (27·40)	31·25†	12·5†	Debbarma *et al.* (2012)
	E. camaldulensis	α-phellandrene (27·52)	62·50†	12·5†	Debbarma *et al.* (2012)
	C. sinensis	limonene (90·94)	>1000*,†		Debbarma *et al.* (2012)
Aer. hydrophila	*Z. officinale*	Zingiberene (27·40)	31·25†	62·5†	Debbarma *et al.* (2012)
	E. camaldulensis	α-phellandrene (27·52)	125†	500†	Debbarma *et al.* (2012)
	C. sinensis	limonene (90·94)	>1000*,†		Debbarma *et al.* (2012)
V. vulnificus	*Z. officinale*	Zingiberene (27·40)	31·25†	31·25†	Debbarma *et al.* (2012)
	E. camaldulensis	α-Phellandrene (27·52)	31·25†	62·5†	Debbarma *et al.* (2012)
	C. sinensis	Limonene (90·94)	125†	250†	Debbarma *et al.* (2012)
L. monocytogenes	*Z. officinale*	Zingiberene (27·40)	15·62†	31·25†	Debbarma *et al.* (2012)
	E. camaldulensis	α-Phellandrene (27·52)	62 500*		Debbarma *et al.* (2012)
	C. sinensis	Limonene (90·94)	>1000*,†		Debbarma *et al.* (2012)
E. coli	*Z. officinale*	Zingiberene (27·40)	62·5†		Debbarma *et al.* (2012)
	E. camaldulensis	α-Phellandrene (27·52)	31·25†	500†	Debbarma *et al.* (2012)
	C. sinensis	Limonene (90·94)	1000†	>10 000†	Debbarma *et al.* (2012)
B. subtilis	*Z. officinale*	Zingiberene (27·40)	3·9†	15·62†	Debbarma *et al.* (2012)
	E. camaldulensis	α-Phellandrene (27·52)	7·812†	31·25†	Debbarma *et al.* (2012)
	C. sinensis	Limonene (90·94)	500†	>1000†	Debbarma *et al.* (2012)
Salm. typhi	*Z. officinale*	Zingiberene (27·40)	31·25†	125†	Debbarma *et al.* (2012)
	E. camaldulensis	α-Phellandrene (27·52)	125†	125†	Debbarma *et al.* (2012)
	C. sinensis	Limonene (90·94)	>10 000*,†		Debbarma *et al.* (2012)
Salm. Typhimurium	*Z. officinale*	Zingiberene (27·40)	62·5†	125†	Debbarma *et al.* (2012)
	E. camaldulensis	α-Phellandrene (27·52)	125*,†		Debbarma *et al.* (2012)
	C. sinensis	Limonene (90·94)	>1000†		Debbarma *et al.* (2012)
Salm. paratyphi	*Z. officinale*	Zingiberene (27·40)	62·5†	125†	Debbarma *et al.* (2012)
	E. camaldulensis	α-Phellandrene (27·52)	125*,†		Debbarma *et al.* (2012)
	C. sinensis	Limonene (90·94)	>1000†		Debbarma *et al.* (2012)
Y. enterocolitica	*Z. officinale*	Zingiberene (27·40)	31·25†	62·5†	Debbarma *et al.* (2012)
	E. camaldulensis	α-Phellandrene (27·52)	62·5†	125†	Debbarma *et al.* (2012)
	C. sinensis	Limonene (90·94)	>1000*,†		Debbarma *et al.* (2012)
Ps. aeruginosa	*Z. officinale*	Zingiberene (27·40)	31·25†	62·5†	Debbarma *et al.* (2012)
	E. camaldulensis	α-Phellandrene (27·52)	125*,†		Debbarma *et al.* (2012)
	C. sinensis	Limonene (90·94)	>1000*,†		Debbarma *et al.* (2012)

Table 1 (*Continued*)

Bacterium	Essential oil	Major compounds (%)	MIC (μg ml^{-1})	MBC (μg ml^{-1})	Source
S. aureus	*Z. officinale*	Zingiberene (27·40)	7·81†	31·25†	Debbarma *et al.* (2012)
	E. camaldulensis	α-Phellandrene (27·52)	31·25†	250†	Debbarma *et al.* (2012)
	C. sinensis	Limonene (90·94)	>1000†‡		Debbarma *et al.* (2012)
Aer. salmonicida	*O. onites*	–	800	NA	Okmen *et al.* (2012)
	Origanum vulgare	–	800	NA	Okmen *et al.* (2012)
	Thymbra spicata	–	800	NA	Okmen *et al.* (2012)
	Satureja thymbra	–	800	NA	Okmen *et al.* (2012)
Strep. iniae	*Cinnamomum verum*	Cinnamaldehyde (90·24)	40	NA	Rattanachaikunsopon and Phumkhachorn (2010)
	Citrus hystrix	–	160	NA	Rattanachaikunsopon and Phumkhachorn (2010)
	Cymbopogon citratus	–	320	NA	Rattanachaikunsopon and Phumkhachorn (2010)
	Curcuma longa	–	160	NA	Rattanachaikunsopon and Phumkhachorn (2010)
V. anguillarum	*T. vulgaris*	–	80	NA	Navarrete *et al.* (2010)
Fl. psychrophilum	*T. vulgaris*	–	80–1280	NA	Navarrete *et al.* (2010)
V. ordalii	*T. vulgaris*	–	80–1280	NA	Navarrete *et al. (*2010)
V. parahaemolyticus	*T. vulgaris*	–	80–1280	NA	Navarrete *et al. (*2010)
Fl. columnare	*A. tuberosum*	–	200–800	NA	Rattanachaikunsopon and Phumkhachorn (2009a, 2009b)
V. spp.	*S. aromaticum*	Eugenol (49)	0·015	NA	Lee *et al.* (2009)
Edw. spp.	*S. aromaticum*	Eugenol (49)	0·015–0·062	NA	Lee *et al.* (2009)
Aer. spp.	*S. aromaticum*	Eugenol (49)	0·015–0·031	NA	Lee *et al.* (2009)
Fl. spp.	*S. aromaticum*	Eugenol (49)	0·031	NA	Lee *et al.* (2009)
E. coli	*S. aromaticum*	Eugenol (49)	0·062	NA	Lee *et al.* (2009)
Salm. spp.	*S. aromaticum*	Eugenol (49)	0·062	NA	Lee *et al.* (2009)
Strep. spp.	*S. aromaticum*	Eugenol (49)	0·062	NA	Lee *et al.* (2009)
Cit. freundii	*S. aromaticum*	Eugenol (49)	0·015	NA	Lee *et al.* (2009)
Y. enterocolitica	*S. aromaticum*	Eugenol (49)	0·031	NA	Lee *et al. (*2009)
Edw. spp.	*C. nardus*	Citronellal (29·6)	0·244–0·977	NA	Wei and Wee (2013)
V. spp	*C. nardus*	Citronellal (29·6)	0·244–0·488	NA	Wei and Wee (2013)
Aer. spp.	*C. nardus*	Citronellal (29·6)	0·488–0·977	NA	Wei and Wee (2013)
E. coli	*C. nardus*	Citronellal (29·6)	0·488	NA	Wei and Wee (2013)
Salm. spp.	*C. nardus*	Citronellal (29·6)	0·244–0·488	NA	Wei and Wee (2013)
Fl. spp.	*C. nardus*	Citronellal (29·6)	0·977	NA	Wei and Wee (2013)
Ps. spp.	*C. nardus*	Citronellal (29·6)	0·244	NA	Wei and Wee 2013)
Strep. spp.	*C. nardus*	Citronellal (29·6)	0·244	NA	Wei and Wee (2013)
Edw. spp.	Citronellal	–	0·244–0·977	NA	Wei and Wee (2013)
V. spp	Citronellal	–	0·244–0·977	NA	Wei and Wee (2013)
Aer. spp.	Citronellal	–	0·244–0·977	NA	Wei and Wee (2013)
E. coli	Citronellal	–	0·244–0·977	NA	Wei and Wee (2013)
Salm. spp.	Citronellal	–	0·244–0·977	NA	Wei and Wee (2013)
Fl. spp.	Citronellal	–	0·244–0·977	NA	Wei and Wee (2013)
Ps. spp.	Citronellal	–	0·244–0·977	NA	Wei and Wee (2013)
Strep. spp.	Citronellal	–	0·244–0·977	NA	Wei and Wee (2013)

– indicates that this type of analysis was not carried out. *Edw.*, *Edwardsiella*; *Ent.*, *Enterobacter* or *Enterococcus*; *Aer.*, *Aeromonas*; *B.*, *Bacillus*; *Cit.*, *Citrobacter*; *E.*, *Escherichia; Fl., Flavobacterium*; *Kl.*, *Klebsiella*; *L.*, *Lactococcus*; *L.*, *Listeria*; *Salm.*, *Salmonella*; *Ser.*, *Serratia*; *Staph.*, *Staphylococcus*; *Strep.*, *Streptococcus*; *Pr., Proteus*; *Ps., Pseudomonas; V.*, *Vibrio*; *Y.*, *Yersinia*.

NA, data not available.

*Authors indicate this range for both MIC and MBC values.

†MIC and MBC are expressed in μl ml^{-1}, because the authors did not report the EO density, and for this reason, it was not possible to convert the value to μg ml^{-1}.

‡The essential oil of leaves of *A. monophylla* collected in May 2014 had sabinene as major constituent, and the essential oil collected in December of 2013 had isoeugenol-*E* as major constituent.

Table 2 *In vitro* assays of antibacterial activity of essential oils and their compounds with the diffusion disc test

Bacterial species	Essential oil (EO)	Major compounds of EO	Concentration applied to the disk (μl)	Zone of inhibition (mm)	Source
Ps. aeruginosa	Nanoemulsion of EO of *C. aurantifolia*	NA	30	15	Thomas *et al.* (2014)
Aer. salmonicida	Nanoemulsion of EO of *A. indica*	NA	40	30	Thomas *et al.* (2013)
Staph. aureus	*Z. officinale*	NA	1000	0	Shehata *et al.* (2013)
Staph. aureus	*N. sativa*	NA	1000	0	Shehata *et al.* (2013)
Staph. aureus	*T. vulgaris*	NA	1000	0	Shehata *et al.* (2013)
Staph. aureus	*S. aromaticum*	NA	1000	4·5	Shehata *et al.* (2013)
Staph. aureus	*E. sativa*	NA	1000	0	Shehata *et al.* (2013)
Ps. aeruginosa	*Z. officinale*	NA	1000	6·7	Shehata *et al.* (2013)
Ps. aeruginosa	*N. sativa*	NA	1000	0	Shehata *et al.* (2013)
Ps. aeruginosa	*T. vulgaris*	NA	1000	13	Shehata *et al.* (2013)
Ps. aeruginosa	*S. aromaticum*	NA	1000	2	Shehata *et al.* (2013)
Ps. aeruginosa	*E. sativa*	NA	1000	10·3	Shehata *et al.* (2013)
E. coli	*Z. officinale*	NA	1000	8	Shehata *et al.* (2013)
E. coli	*N. sativa*	NA	1000	0	Shehata *et al.* (2013)
E. coli	*T. vulgaris*	NA	1000	5·7	Shehata *et al.* (2013)
E. coli	*S. aromaticum*	NA	1000	2	Shehata *et al.* (2013)
E. coli	*E. sativa*	NA	1000	5·3	Shehata *et al.* (2013)
L. monocytogenes	*Z. officinale*	NA	1000	0	Shehata *et al.* (2013)
L. monocytogenes	*N. sativa*	NA	1000	0	Shehata *et al.* (2013)
L. monocytogenes	*T. vulgaris*	NA	1000	9·5	Shehata *et al.* (2013)
L. monocytogenes	*S. aromaticum*	NA	1000	6	Shehata *et al.* (2013)
L. monocytogenes	*E. sativa*	NA	1000	6	Shehata *et al.* (2013)
L. lactis	*Z. officinale*	NA	1000	5·5	Shehata *et al.* (2013)
L. lactis	*N. sativa*	NA	1000	7·3	Shehata *et al.* (2013)
L. lactis	*T. vulgaris*	NA	1000	11·3	Shehata *et al.* (2013)
L. lactis	*S. aromaticum*	NA	1000	6·3	Shehata *et al.* (2013)
L. lactis	*E. sativa*	NA	1000	0	Shehata *et al.* (2013)
B. cereus	*Z. officinale*	NA	1000	6	Shehata *et al.* (2013)
B. cereus	*N. sativa*	NA	1000	5	Shehata *et al.* (2013)
B. cereus	*T. vulgaris*	NA	1000	7·5	Shehata *et al.* (2013)
B. cereus	*S. aromaticum*	NA	1000	5·6	Shehata *et al.* (2013)
B. cereus	*E. sativa*	NA	1000	0	Shehata *et al.* (2013)
Y. ruckeri	*O. vulgare*	Carvacrol (82)	25	8–17·5	Ekici *et al.* (2011)
Aer. hydrophila	*M. oleum*	Isopropyl myristate (34·4)	25	6·5–23·5	Ekici *et al.* (2011)
V. anguillarum	*L. romanae*	Isopropyl myristate (92·72)	25	8–32·5	Ekici *et al.* (2011)
Fl. psychrophilum	*R. officinalis*	1,8-Cineole (30·95)	25	40	Ekici *et al.* (2011)
L. garvieae	*Z. officinale*	Heneicosane (35·5)	25	6–10	Ekici *et al.* (2011)
Strep. agalactiae	*R. officinalis*	Carnosic acid (—)	–	17	Zilberg *et al.* (2010)
Aer. hydrophila	*P. brutia*	α-Pinene (90·18)	50	24·5	Ozogul *et al.* (2015)
Aer. hydrophila	*E. globulus*	Eucalyptol (59·28)	50	9·5	Ozogul *et al.* (2015)
Aer. hydrophila	*T. vulgaris*	Carvacrol (71·54)	50	12·5	Ozogul *et al.* (2015)
Aer. hydrophila	*S. officinalis*	1,8-Cineole (47·51)	50	11	Ozogul *et al.* (2015)
Aer. hydrophila	*L. officinalis*	Linalool (43·37)	50	7·5	Ozogul *et al.* (2015)
Aer. hydrophila	*C. sinensis*	Limonene (95·77)	50	11	Ozogul *et al.* (2015)
Aer. hydrophila	*C. limonum*	1,8-Cineole (29·60)	50	7·7	Ozogul *et al.* (2015)
Aer. hydrophila	*M. communis*	Limonene (71·77)	50	11·8	Ozogul *et al.* (2015)
Aer. hydrophila	*R. officinalis*	1,8-Cineole (52·17)	50	11·5	Ozogul *et al.* (2015)
Aer. hydrophila	*J. communis*	α-Pinene (90·09)	50	6	Ozogul *et al.* (2015)
E. coli	*P. brutia*	α-Pinene (90·18)	50	5·25	Ozogul *et al.* (2015)
E. coli	*E. globulus*	Eucalyptol (59·28)	50	2·75	Ozogul *et al.* (2015)
E. coli	*T. vulgaris*	Carvacrol (71·54)	50	24	Ozogul *et al.* (2015)

Table 2 (*Continued*)

Bacterial species	Essential oil (EO)	Major compounds of EO	Concentration applied to the disk (μl)	Zone of inhibition (mm)	Source
E. coli	*S. officinalis*	1,8-Cineole (47·51)	50	6·25	Ozogul *et al.* (2015)
E. coli	*L. officinalis*	Linalool (43·37)	50	3	Ozogul *et al.* (2015)
E. coli	*C. sinensis*	Limonene (95·77)	50	1	Ozogul *et al.* (2015)
E. coli	*C. limonum*	1,8-Cineole (29·60)	50	5·25	Ozogul *et al.* (2015)
E. coli	*M. communis*	Limonene (71·77)	50	3	Ozogul *et al.* (2015)
E. coli	*R. officinalis*	1,8-Cineole (52·17)	50	3	Ozogul *et al.* (2015)
E. coli	*J. communis*	α-Pinene (90·09)	50	1·5	Ozogul *et al.* (2015)
Salm. paratyphi A	*P. brutia*	α-Pinene (90·18)	50	6	Ozogul *et al.* (2015)
Salm. paratyphi A	*E. globulus*	Eucalyptol (59·28)	50	1	Ozogul *et al.* (2015)
Salm. paratyphi A	*T. vulgaris*	Carvacrol (71·54)	50	27·5	Ozogul *et al.* (2015)
Salm. paratyphi A	*S. officinalis*	1,8-Cineole (47·51)	50	4·5	Ozogul *et al.* (2015)
Salm. paratyphi A	*L. officinalis*	Linalool (43·37)	50	3·5	Ozogul *et al.* (2015)
Salm. paratyphi A	*C. sinensis*	Limonene (95·77)	50	3	Ozogul *et al.* (2015)
Salm. paratyphi A	*C. limonum*	1,8-Cineole (29·60)	50	6·25	Ozogul *et al.* (2015)
Salm. paratyphi A	*M. communis*	Limonene (71·77)	50	4·75	Ozogul *et al.* (2015)
Salm. paratyphi A	*R. officinalis*	1,8-Cineole (52·17)	50	5·5	Ozogul *et al.* (2015)
Salm. paratyphi A	*J. communis*	α-Pinene (90·09)	50	1·25	Ozogul *et al.* (2015)
Kl. pneumonia	*P. brutia*	α-Pinene (90·18)	50	20·5	Ozogul *et al.* (2015)
Kl. pneumonia	*E. globulus*	Eucalyptol (59·28)	50	6·75	Ozogul *et al.* (2015)
Kl. pneumonia	*T. vulgaris*	Carvacrol (71·54)	50	29·75	Ozogul *et al.* (2015)
Kl. pneumonia	*S. officinalis*	1,8-Cineole (47·51)	50	4·75	Ozogul *et al.* (2015)
Kl. pneumonia	*L. officinalis*	Linalool (43·37)	50	6·75	Ozogul *et al.* (2015)
Kl. pneumonia	*C. sinensis*	Limonene (95·77)	50	16	Ozogul *et al.* (2015)
Kl. pneumonia	*C. limonum*	1,8-Cineole (29·60)	50	10·75	Ozogul *et al.* (2015)
Kl. pneumonia	*M. communis*	Limonene (71·77)	50	3·25	Ozogul *et al.* (2015)
Kl. pneumonia	*R. officinalis*	1,8-Cineole (52·17)	50	4	Ozogul *et al.* (2015)
Kl. pneumonia	*J. communis*	α-Pinene (90·09)	50	5	Ozogul *et al.* (2015)
Y. enterocolitica	*P. brutia*	α-Pinene (90·18)	50	15·5	Ozogul *et al.* (2015)
Y. enterocolitica	*E. globulus*	Eucalyptol (59·28)	50	7·5	Ozogul *et al.* (2015)
Y. enterocolitica	*T. vulgaris*	Carvacrol (71·54)	50	16	Ozogul *et al.* (2015)
Y. enterocolitica	*S. officinalis*	1,8-Cineole (47·51)	50	9	Ozogul *et al.* (2015)
Y. enterocolitica	*L. officinalis*	Linalool (43·37)	50	15	Ozogul *et al.* (2015)
Y. enterocolitica	*C. sinensis*	Limonene (95·77)	50	9·5	Ozogul *et al.* (2015)
Y. enterocolitica	*C. limonum*	1,8-Cineole (29·60)	50	6·25	Ozogul *et al.* (2015)
Y. enterocolitica	*M. communis*	Limonene (71·77)	50	4	Ozogul *et al.* (2015)
Y. enterocolitica	*R. officinalis*	1,8-Cineole (52·17)	50	3·15	Ozogul *et al.* (2015)
Y. enterocolitica	*J. communis*	α-Pinene (90·09)	50	5	Ozogul *et al.* (2015)
Ps. aeruginosa	*P. brutia*	α-Pinene (90·18)	50	15	Ozogul *et al.* (2015)
Ps. aeruginosa	*E. globulus*	Eucalyptol (59·28)	50	1·5	Ozogul *et al.* (2015)
Ps. aeruginosa	*T. vulgaris*	Carvacrol (71·54)	50	18·05	Ozogul *et al.* (2015)
Ps. aeruginosa	*S. officinalis*	1,8-Cineole (47·51)	50	7·95	Ozogul *et al.* (2015)
Ps. aeruginosa	*L. officinalis*	Linalool (43·37)	50	8	Ozogul *et al.* (2015)
Ps. aeruginosa	*C. sinensis*	Limonene (95·77)	50	2·5	Ozogul *et al.* (2015)
Ps. aeruginosa	*C. limonum*	1,8-Cineole (29·60)	50	7·5	Ozogul *et al.* (2015)
Ps. aeruginosa	*M. communis*	Limonene (71·77)	50	3·75	Ozogul *et al.* (2015)
Ps. aeruginosa	*R. officinalis*	1,8-Cineole (52·17)	50	11·5	Ozogul *et al.* (2015)
Ps. aeruginosa	*J. communis*	α-Pinene (90·09)	50	2·5	Ozogul *et al.* (2015)
Camp. jejuni	*P. brutia*	α-Pinene (90·18)	50	22·25	Ozogul *et al.* (2015)
Camp. jejuni	*E. globulus*	Eucalyptol (59·28)	50	8·75	Ozogul *et al.* (2015)
Camp. jejuni	*T. vulgaris*	Carvacrol (71·54)	50	8·75	Ozogul *et al.* (2015)
Camp. jejuni	*S. officinalis*	1,8-Cineole (47·51)	50	30	Ozogul *et al.* (2015)
Camp. jejuni	*L. officinalis*	Linalool (43·37)	50	8	Ozogul *et al.* (2015)
Camp. jejuni	*C. sinensis*	Limonene (95·77)	50	14·25	Ozogul *et al.* (2015)

Table 2 (*Continued*)

Bacterial species	Essential oil (EO)	Major compounds of EO	Concentration applied to the disk (μl)	Zone of inhibition (mm)	Source
Camp. jejuni	*C. limonum*	1,8-Cineole (29·60)	50	12·5	Ozogul *et al.* (2015)
Camp. jejuni	*M. communis*	Limonene (71·77)	50	9	Ozogul *et al.* (2015)
Camp. jejuni	*R. officinalis*	1,8-Cineole (52·17)	50	6	Ozogul *et al.* (2015)
Camp. jejuni	*J. communis*	α-Pinene (90·09)	50	3	Ozogul *et al.* (2015)
Ent. faecalis	*P. brutia*	α-Pinene (90·18)	50	21·25	Ozogul *et al.* (2015)
Ent. faecalis	*E. globulus*	Eucalyptol (59·28)	50	12	Ozogul *et al.* (2015)
Ent. faecalis	*T. vulgaris*	Carvacrol (71·54)	50	15	Ozogul *et al.* (2015)
Ent. faecalis	*S. officinalis*	1,8-Cineole (47·51)	50	14·5	Ozogul *et al.* (2015)
Ent. faecalis	*L. officinalis*	Linalool (43·37)	50	17·75	Ozogul *et al.* (2015)
Ent. faecalis	*C. sinensis*	Limonene (95·77)	50	12	Ozogul *et al.* (2015)
Ent. faecalis	*C. limonum*	1,8-Cineole (29·60)	50	7·25	Ozogul *et al.* (2015)
Ent. faecalis	*M. communis*	Limonene (71·77)	50	16·50	Ozogul *et al.* (2015)
Ent. faecalis	*R. officinalis*	1,8-Cineole (52·17)	50	5·25	Ozogul *et al.* (2015)
Ent. faecalis	*J. communis*	α-Pinene (90·09)	50	5·25	Ozogul *et al.* (2015)
Staph. aureus	*P. brutia*	α-Pinene (90·18)	50	28·25	Ozogul *et al.* (2015)
Staph. aureus	*E. globulus*	Eucalyptol (59·28)	50	18	Ozogul *et al.* (2015)
Staph. aureus	*T. vulgaris*	Carvacrol (71·54)	50	22·25	Ozogul *et al.* (2015)
Staph. aureus	*S. officinalis*	1,8-Cineole (47·51)	50	10·75	Ozogul *et al.* (2015)
Staph. aureus	*L. officinalis*	Linalool (43·37)	50	7·25	Ozogul *et al.* (2015)
Staph. aureus	*C. sinensis*	Limonene (95·77)	50	10	Ozogul *et al.* (2015)
Staph. aureus	*C. limonum*	1,8-Cineole (29·60)	50	10	Ozogul *et al.* (2015)
Staph. aureus	*M. communis*	Limonene (71·77)	50	15	Ozogul *et al.* (2015)
Staph. aureus	*R. officinalis*	1,8-Cineole (52·17)	50	7·25	Ozogul *et al.* (2015)
Staph. aureus	*J. communis*	α-Pinene (90·09)	50	4·25	Ozogul *et al.* (2015)
V. parahaemolyticus	*Z. officinale*	Zingiberene (27·40)	100*	15	Debbarma *et al.* (2012)
V. parahaemolyticus	*E. camaldulensis*	α-Phellandrene (27·52)	100*	13	Debbarma *et al.* (2012)
V. parahaemolyticus	*C. sinensis*	Limonene (90·94)	100*	0	Debbarma *et al.* (2012)
Aer. hydrophila	*Z. officinale*	Zingiberene (27·40)	100*	10	Debbarma *et al.* (2012)
Aer. hydrophila	*E. camaldulensis*	α-Phellandrene (27·52)	100*	14·66	Debbarma *et al.* (2012)
Aer. hydrophila	*C. sinensis*	Limonene (90·94)	100*	0	Debbarma *et al.* (2012)
V. vulnificus	*Z. officinale*	Zingiberene (27·40)	100*	16·33	Debbarma *et al.* (2012)
V. vulnificus	*E. camaldulensis*	α-Phellandrene (27·52)	100*	16·33	Debbarma *et al.* (2012)
V. vulnificus	*C. sinensis*	Limonene (90·94)	100*	7·33	Debbarma *et al.* (2012)
L. monocytogenes	*Z. officinale*	Zingiberene (27·40)	100*	20	Debbarma *et al.* (2012)
L. monocytogenes	*E. camaldulensis*	α-Phellandrene (27·52)	100*	25	Debbarma *et al.* (2012)
L. monocytogenes	*C. sinensis*	Limonene (90·94)	100*	16·33	Debbarma *et al.* (2012)
E. coli	*Z. officinale*	Zingiberene (27·40)	100*	16·33	Debbarma *et al.* (2012)
E. coli	*E. camaldulensis*	α-Phellandrene (27·52)	100*	16·66	Debbarma *et al.* (2012)
E. coli	*C. sinensis*	Limonene (90·94)	100*	8	Debbarma *et al.* (2012)
B. subtilis	*Z. officinale*	Zingiberene (27·40)	100*	46	Debbarma *et al.* (2012)
B. subtilis	*E. camaldulensis*	α-Phellandrene (27·52)	100*	29·33	Debbarma *et al.* (2012)
B. subtilis	*C. sinensis*	Limonene (90·94)	100*	30	Debbarma *et al.* (2012)
Salm. typhi	*Z. officinale*	Zingiberene (27·40)	100*	18·33	Debbarma *et al.* (2012)
Salm. typhi	*E. camaldulensis*	α-Phellandrene (27·52)	100*	15·33	Debbarma *et al.* (2012)
Salm. typhi	*C. sinensis*	Limonene (90·94)	100*	13	Debbarma *et al.* (2012)
Salm. Typhimurium	*Z. officinale*	Zingiberene (27·40)	100*	11·33	Debbarma *et al.* (2012)
Salm. Typhimurium	*E. camaldulensis*	α-Phellandrene (27·52)	100*	15·33	Debbarma *et al.* (2012)
Salm. Typhimurium	*C. sinensis*	Limonene (90·94)	100*	8·33	Debbarma *et al.* (2012)
Salm. paratyphi	*Z. officinale*	Zingiberene (27·40)	100*	14·33	Debbarma *et al.* (2012)
Salm. paratyphi	*E. camaldulensis*	α-Phellandrene (27·52)	100*	18	Debbarma *et al.* (2012)
Salm. paratyphi	*C. sinensis*	Limonene (90·94)	100	9·33	Debbarma *et al.* (2012)
Y. enterocolitica	*Z. officinale*	Zingiberene (27·40)	100*	23·33	Debbarma *et al.* (2012)
Y. enterocolitica	*E. camaldulensis*	α-Phellandrene (27·52)	100*	22·33	Debbarma *et al.* (2012)

Table 2 (*Continued*)

Bacterial species	Essential oil (EO)	Major compounds of EO	Concentration applied to the disk (μl)	Zone of inhibition (mm)	Source
Y. enterocolitica	*C. sinensis*	Limonene (90·94)	100*	14·66	Debbarma *et al.* (2012)
Ps. aeruginosa	*Z. officinale*	Zingiberene (27·40)	100*	14·33	Debbarma *et al.* (2012)
Ps. aeruginosa	*E. camaldulensis*	α-Phellandrene (27·52)	100*	16	Debbarma *et al.* (2012)
Ps. aeruginosa	*C. sinensis*	Limonene (90·94)	100*	10	Debbarma *et al.* (2012)
Staph. aureus	*Z. officinale*	Zingiberene (27·40)	100*	24	Debbarma *et al.* (2012)
Staph. aureus	*E. camaldulensis*	α-Phellandrene (27·52)	100*	25	Debbarma *et al.* (2012)
Staph. aureus	*C. sinensis*	Limonene (90·94)	100*	24·66	Debbarma *et al.* (2012)

The results varied according to the concentration of the EO tested, but, in general, the inhibition halo remained within the range described for each bacterium. *Edw.*, *Edwardsiella*; *Ent.*, *Enterobacter* or *Enterococcus*; *Aer.*, *Aeromonas*; *B.*, *Bacillus*; *Camp.*, *Campylobacter*; *Cit.*, *Citrobacter*; *E.*, *Escherichia*; *Fl.*, *Flavobacterium*; *Kl.*, *Klebsiella*; *L.*, *Lactococcus*; *L.*, *Listeria*; *Salm.*, *Salmonella*; *Ser.*, *Serratia*; *Staph.*, *Staphylococcus*; *Strep.*, *Streptococcus*; *Ps.*, *Pseudomonas*; *V.*, *Vibrio*; *Y.*, *Yersinia*. NA, data not available; –: not defined.

*This study tested several concentrations (20, 30, 40, 50 and 100 μl), and authors determined the best concentration, this was the concentration chosen to be presented in this table.

Table 3 The use of essential oils by bath as antibacterial agents in fish

Fish species	Bacterial species	Essential oil (EO)	Major compounds (%)	Purpose/concentration/treatment/outcome	Source
R. quelen	*Ps. aeruginosa* (PA01)	*M. alternifolia* and nanoencapsulated EO of *M. alternifolia*	Terpinen-4-ol (41·98) and NA	Therapeutic/50 μl l^{-1}/1 h daily baths—7 days after, intramuscular inoculation of the bacterium and evaluation after 60 days/ therapeutic/nanoencapsulated EO showed 100% efficacy, while the free EO showed 70% efficacy	Souza *et al.* (2017a) Souza *et al.* (2017a)
R. quelen	*Aer. hydrophila*	*O. americanum*	β-linalool (46·6)	Therapeutic/10 and 20 mg l^{-1}/1 h daily baths – 5 days/increased survival 26–30%	Sutili *et al.* (2016a, 2016b)
R. quelen	*Aer. hydrophila*	*H. ringens*	Pulegone (96·63)	Therapeutic/*H. ringens*: 20 and 40 mg l^{-1}/1 h daily baths – 5 days/Increased survival 66–70%	Sutili *et al.* (2015a)
R. quelen	*Aer. hydrophila*	*O. gratissimum*	Eugenol (91·47)	Therapeutic/*O. gratissimum*: 5 and 10 mg l^{-1}/1 h daily – 5 days/did not increase fish survival	Sutili *et al.* (2015a)
R. quelen	*Aer. hydrophila*	*O. americanum*	1,8-Cineole (21)	Therapeutic/*O. americanum*: 10 and 20 mg l^{-1}/1 h daily baths – 5 days/Increased survival 66–70%	Sutili *et al.* (2015a)
R. quelen		Eugenol	–	Therapeutic/5 and 10 mg l^{-1}/1 h daily baths – 5 days/can be used to treat or prevent bacterial diseases	Sutili *et al.* (2014)
R. quelen	*Aer.* sp.	*L. alba*	Linalool (65·5)	Therapeutic/20 and 50 μl l^{-1}/1 h daily baths – 10 days/higher survival in fish treated with 50 μl l^{-1}	Sutili *et al.* (2015b)
O. mykiss	*Aer. salmonicida*	*S. thymbra*	NA	Preventive/100, 200, 400 and 800 mg l^{-1}/the MIC value (800 mg l^{-1}) and its dilutions (400, 200, 100 mg l^{-1}) were injected into experimental fish for *in vivo* studies/toxic at the effective concentration and without effect at nontoxic concentrations	Okmen *et al.* (2012)

Edw., *Edwardsiella*; *Ent.*, *Enterobacter* or *Enterococcus*; *Aer.*, *Aeromonas*; *B.*, *Bacillus*; *Cit.*, *Citrobacter*; *E.*, *Escherichia*; *Fl.*, *Flavobacterium*; *Kl.*, *Klebsiella*; *L.*, *Lactococcus*; *L.*, *Listeria*; *Salm.*, *Salmonella*; *Ser.*, *Serratia*; *Staph.*, *Staphylococcus*; *Strep.*, *Streptococcus*; *Ps.*, *Pseudomonas*; *V.*, *Vibrio*; *Y.*, *Yersinia*.

NA, data not available.

and damaging the membrane. This damage results in increased membrane fluidity and release of intracellular components (Cox and Markham 2007).

The diffusion disc test is a qualitative but nonquantitative test. Inhibition is classified as sensitive, when the diameter of the zone of inhibition is at least 3 mm smaller than the diameter found for the positive control; moderately sensitive, when the halo is greater than 2 mm but smaller than the positive control; or resistant, when the diameter of the halo is equal to or less than 2 mm (Karaman *et al.* 2003; Springfield *et al.* 2003). The EOs of *Lavandulae romanae* and *R. officinalis* (chemotype 1,8 cineole) and the nanoemulsion of the EO of *Azadirachta indica* showed a greater inhibition halo against the tested bacteria than the other EOs studied (Table 2).

In vivo assays with EOs as antibacterial agents in fish

The most common method used for the administration of antimicrobials in aquaculture is via the water in which the animals are kept or as a dietary supplement. The first system has the advantage of reaching a large number of animals at the same time; however, it has high costs as it requires a large amount of antimicrobial when applied in tanks, and a known volume of water is required. Farmers can treat the infections by bath to use smaller amounts of water and minimize these disadvantages (Park *et al.* 2012b). Dietary supplementation with EOs to feed fish has increased in recent years, but all experiments were with the use of the dietary supplementation as preventive (Sutili *et al.* 2017). The great advantage of this method is the reduction in waste when compared with application in the water. However, one of the limiting factors of this application may be the need for active feeding of the animals, because infected fish may not feed as well as healthy ones. Further limits are the effectiveness of EOs inclusion in the aquafeed (appropriate technology to be assessed) and the fact that this incorporation can sometimes negatively affect the palatability of feed. Anyway, a prophylactic medication ration is advised rather than therapeutic use. Other methods such as gavage, injection and topical application are efficient at the laboratory level; however, in fish farms, it becomes unfeasible due to the labour force necessary and stress on the animals (Park *et al.* 2012b). Another problem is the stability of the EOs through diet preparation, storage and digestion, because they may lose their biological effects (Sutili *et al.* 2017).

The EOs tested that reduced mortality of infected fish (i.e., used with therapy purpose) through baths are those from *Ocimum americanum* (both chemotypes with R-(-)-β-linalool and 1,8 cineole as major compounds), *Hesperozygis ringens* (Sutili *et al.* 2015a), *Lippia alba* (chemotype S-(+)-linalool) (Sutili *et al.* 2015b) and *M. alternifolia* (Souza *et al.* 2017a). It is noteworthy that in spite of eugenol being efficient, the EO of *Ocimum gratissimum*, which contains approximately 90% eugenol in its composition, is not as effective as eugenol in bath treatments (Table 3). This indicates that effects of an EO may not be related to its major compound, but rather due to a combination of several of its components.

Most EOs that were evaluated to treat bacterial infections using baths showed effect at concentrations below the MIC observed *in vitro*, even those that have a higher MIC than that recommended by Ríos and Recio (2005) (Table 3, Fig. 2a). An analysis of the MIC and the minimum effective bath concentrations or dietary supplementation doses of EOs found in the literature demonstrated that there is no correlation between them (Table 4, Fig. 2). This demonstrates that determination of MIC *in vitro* is not a good methodology to predict the *in vivo* effect of EOs.

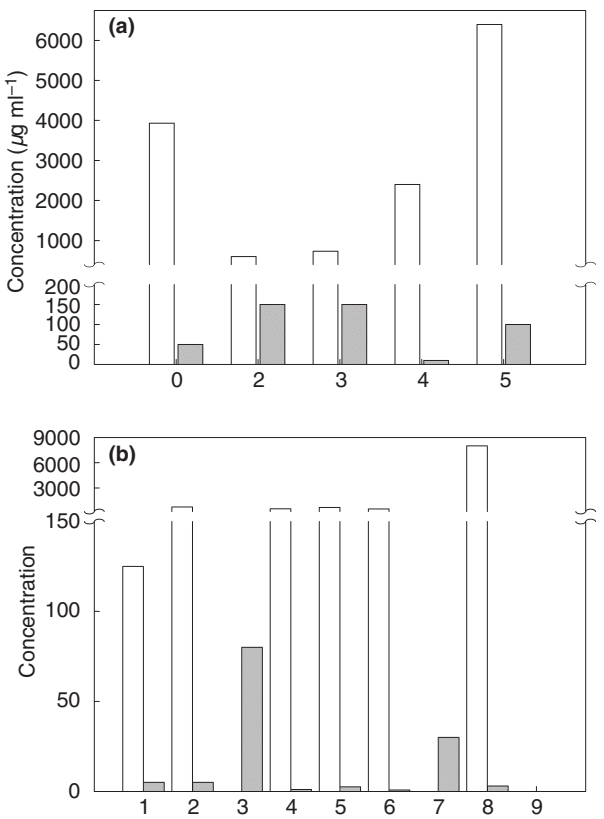

Figure 2 Comparison of *in vitro* and *in vivo* tests with EOs or compounds based on literature data. A: *in vivo* – baths; B: *in vivo* – dietary supplementation; (a) 1: *Lippia alba*, 2: *Hesperozygis ringens*, 3: *Ocimum gratissimum*, 4: eugenol, 5: *Ocimum americanum*. (b) 1: *Zingiber officinale*, 2: *Ocimum gratissimum*, 3: *Rosmarinus officinalis*, 4: *Citrus sinensis*, 5: *Thymus vulgaris*, 6: *Allium tuberosum*, 7: *Syzygium aromaticum*, 8: *Origanum onites*, 9: *Zataria multiflora*. There is no significant correlation between *in vitro* and *in vivo* tests.

Table 4 Studies of fish fed with dietary essential oils supplementation as preventive against bacterial infections

Fish	Bacterium	Essential oil (EO)	Major compounds (%)	Doses (g per kg per feed)/feeding time/outcome	Source
O. niloticus	–	*T. vulgaris*	NA	1, 2·5 and 5/90 days/best 2·5	Shehata *et al.* (2013)
Oreochromis sp.	*Strep. agalactiae*	*R. officinalis*	NA	80 and 160/10 days/lower mortality	Zilberg *et al.* (2010)
O. niloticus	*Fl. columnare*	*A. tuberosum*	NA	0·2, 0·4, 0·6 and 0·8/14 days/best 0·8	Rattanachaikunsopon and Phumkhachorn (2009a)
O. niloticus	*L. garvieae*	*S. aromaticum*	NA	5, 10, 20 and 30/14 days/no mortality in fish fed diet supplemented with 30	Rattanachaikunsopon and Phumkhachorn (2009b)
O. niloticus	*Strep. agalactiae*	*O. gratissimum* and *Z. officinale*	1,8-cineole (40·4) and geranial (24)	0·5, 1·0 and 1·5/55 days/improved disease resistance and phagocytic capacity, those treated with 5 had 100% survival	Brum *et al.* (2017)
O. mossambicus	*Edw. tarda*	*C. limon*	limonene (54·4)	5, 7·5 and 10/60 days/improved nonspecific immune parameters and decreased mortality	Baba *et al.* (2016)
O. mossambicus	*Strep. iniae*	*C. sinensis*	NA	0·001, 0·003 and 0·005/90 days/ reduced mortality	Acar *et al.* (2015)
R. quelen	*Aer. hydrophila*	*Aloysia triphylla*	β-citral (20·78)	0·25 and 2/21 days/increased resistance to bacterial infection in the dose 2	Santos *et al.* (2017)
I. punctatus	*Aer. hydrophila*	*O. heracleoticum*, carvacrol, thymol and Orego-Stim®*	NA	0·5/56 days/carvacrol + thymol and OS reduced mortality	Zheng *et al.* (2009)
O. niloticus	*Strep. iniae*	*C. verum*	Cinnamaldehyde (90·2)	0·001, 0·002, 0·003 and 0·004/14 days/no mortality in fish fed with 0·004	Rattanachaikunsopon and Phumkhachorn (2010)
I. punctatus	*Edw. ictaluri*	Digestarom®†	NA	0·125, 1·5, 2·5 and 3·0/42 days/ increased survival	Peterson *et al.* (2015)
Oncorhynchus mykiss	*Lactococcus garvieae*	*O. onites*	NA	0·125, 1·5, 2·5 and 3/90 days/increased resistance to bacterial infection with dose 3	Diler *et al.* (2016)
Cyprinus carpio	*Aer. hydrophila*	*Z. multiflora*	NA	0·03, 0·06 and 0·12/8 days/ immunomodulatory effect in fish fed 0·03	Soltani *et al.* (2010)
V. labeo and *L. victorianus*	*Aer. hydrophila*	*U. dioica*	NA	10, 20 and 50/112 days/ delayed mortality	Ngugi *et al.* (2015)
O. mykiss	*Aer. salmonicida*	Digestarom†	NA	0·2/175 days/increased resistance to bacterial infection and reduced mortality in infected fish from 37 to 18%	Menanteau-Ledouble *et al.* (2015)
O. niloticus	*L. garvieae*	*A. spinosa*	NA	5, 10 and 20/45 days/increased survival in fish receiving doses 10 and 20 and immunomodulatory effect	Baba *et al.* (2017)

Edw., Edwardsiella; Ent., Enterobacter or *Enterococcus; Aer., Aeromonas; B., Bacillus; Cit., Citrobacter; E., Escherichia; Fl., Flavobacterium; Kl., Klebsiella; L., Lactococcus; L., Listeria; Salm., Salmonella; Ser., Serratia; Staph., Staphylococcus; Strep., Streptococcus; V., Vibrio; Y., Yersinia*.
*Commercial product containing natural EO of *Origanum heracleoticum*.
†Commercial product containing carvacrol, thymol, anethol and limonene.
NA, data not available.

Previously published studies on this subject indicate that increased resistance to bacterial infections provided by baths with EOs is due not only to bactericidal effects but also increased extracellular superoxide anion production by head–kidney macrophages and/or blood leucocytes, inhibition of bacterial haemolytic activity (Sutili *et al.* 2015a, 2015b, 2016b), reduction or elimination of biofilm formation (Millezi *et al.* 2013), and altered quorum sensing communication (Olivero-Verbel *et al.* 2014). Preventive baths with the EO of *M.*

alternifolia induced a potent anti-inflammatory effect mediated by the adenosinergic pathway and improved the innate immune responses through modulation of the cytokine response during *Aer. hydrophila* infection in *Rhamdia quelen*. This EO also prevents oxidative damage in liver proteins and lipids, as well as maintaining the purinergic system (Baldissera *et al.* 2017b,c). The use of the EOs of *H. ringens* and *O. americanum* in preventive baths increased the complement system activity compared with control, but there was no significant difference in survival of silver catfish challenged with *Aer. hydrophila* (Sutili *et al.* 2015a). Future studies using this methodology with other EOs are warranted, since the antibacterial effects of EOs vary according to composition.

The EOs tested so far that are effective at increasing fish resistance against bacterial infections by dietary supplementation have limonene, thymol, carvacrol, citral, cinnamaldehyde and 1,8 cineole as major compounds (Table 4). Dietary supplementation with carvacrol (Volpatti *et al.* 2013) and the combination thymol + carvacrol increased resistance to bacterial infection in fish (Zheng *et al.* 2009). Recent work indicates that dietary supplementation with EOs must be carried out several days before infection to improve immune status, enhance resistance, reduce bacterial effects and prevent outbreaks (Awad and Awaad 2017; Sutili *et al.* 2017).

Nanotechnology and EO interactions in aquaculture

The use of nanotechnology in medicine and veterinary services has increased in recent years. Because the active constituents of EOs can act only on the site of interest, nanotechnology can increase the permanence in the bloodstream, protect the substance against enzymatic hydrolysis (Nair *et al.* 2016) and even enable the transport of the active substances through the blood–brain barrier (Baldissera *et al.* 2017a). Nanotechnology systems include nanoemulsions, which consist of oil dispersions stabilized by surfactants (Anton *et al.* 2008); nanocapsules, which have an oily nucleus surrounded by a thin polymer envelope with the drug dissolved in the core, adsorbed or dispersed in the polymer wall (Vauthier and Bouchemal 2009); and nanospheres, which do not have oil in their composition, formed by a polymer matrix where the drug can be retained or adsorbed (Schaffazick *et al.* 2003).

The application of the EO of *M. alternifolia* through baths had antibacterial activity against *Ps. aeruginosa* and this effect was improved when this EO was nanoencapsulated (Souza *et al.* 2017a,b). The nanoemulsion of the EO of *A. indica* promoted 90% survival and avoided the appearance of ulcers typical of *Aer. salmonicida* infection in *Clarias batrachus* (Thomas *et al.* 2013). The nanoemulsion of the EO of *Citrus aurantifolia* reduced histological changes in the liver, skin and gills of *Oreochromis mossambicus* infected with *Ps. aeruginosa* (Thomas *et al.* 2014).

Legislation regarding the use of EO in aquaculture

Some EOs have achieved the generally recognized as safe label to be used as food additives in the United States, but no clear information existed in the European legislation (Carocho *et al.* 2015). The Brazilian legislation (interministerial normative instruction No. 28) establishes some standards for organic aquaculture production in the country and includes EOs in the list of permissible substances to be used in the control of pests and diseases (MAPA, Ministry of Agriculture, Livestock and Food Supply 2011).

Concluding remarks

To date, studies suggest that the use of EOs in the prevention and/or treatment of infectious diseases in fish may be a promising strategy to reduce the use of conventional antibiotics in aquaculture, since several EOs are effective to reduce or avoid the effects of bacterial infections in fish. Some studies used several baths to treat bacterial infections; however, this procedure may not be practical. Thus, new studies with one or two baths must be performed. Dietary supplementation with EOs also seems a good alternative to prevent disease outbreaks. The use of EOs through nanotechnology, mainly in dietary supplementation experiments, is promising and deserves further study.

Conflict of Interest

No conflict to declare.

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