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REVIEW

REVIEWS IN Aquaculture

Toxicity assessment and anti-*Vibrio* activity of essential oils: Potential for application in shrimp aquaculture

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Abstract

This present paper aimed to review the past 4 years (2019–2022), the inhibition of *Vibrio* spp. (including *Vibrio*'s causing AHPND) by EOs, as well as the potential toxicity of the EOs towards crustaceans, with an emphasis on *Artemia* spp. In the present review, 27 EOs from terrestrial plants are reported regarding their anti-*Vibrio* activity. Among these 27 studies, *Salvia officinalis* and *Thymus vulgaris* (Lamiaceae family) were found to be the most numerous. Among the *Vibrio* spp., *V. parahaemolyticus* (non-AHPND strain) was mostly researched. There are in total 68 publications about the toxicity of EOs in *Artemia spp*. Based on the four categories of toxicity towards *Artemia* (strongly toxic: LC_{50} < 100 μg/ml, moderately toxic: LC_{50}:100–500 μg/ml, weakly toxic: LC_{50}: 500–1000 μg/ml, and non-toxic: LC_{50} > 1000 μg/ml), strong toxicity activity was found in 37 EOs, moderate toxicity in 15 EOs, weak activity for three EO plants and 13 non-toxic extracts. In fact, LC_{50} values as low as 10.25 and 11.48 μg/ml were described in *Artemisia vulgaris* and *Euryale ferox*, respectively, showing these two plant EOs are strongly toxic to *Artemia*. Overall, and despite being generally considered "eco-friendly and natural" products and safer than antibiotics, some of the EOs are toxic to target organisms. Thus, to establish an ecologically safe application of EOs in shrimp aquaculture, the correct use of these plant EOs (in terms of concentrations and duration) in aquaculture should be considered.

KEYWORDS
essential oils, shrimp aquaculture, toxicity, vibriosis

1 | INTRODUCTION

Shrimp are decapod crustaceans, and their culture has been contributing significantly to global aquaculture output during the last decade. The production of cultured shrimp and prawns has increased from 3.58 million tons in 2010 to 6.86 million tons in 2020 (nearly 92% increase, FAO, 2022). White leg shrimp (*Penaeus vannamei*) and giant tiger prawn (*P. monodon*) are the two important penaeid shrimp species extensively cultured in a brackish and marine water environment.[1] On the other hand, giant river prawn (*Macrobrachium rosenbergii*) and oriental river prawn (*M. nipponense*) are the two main farmed freshwater species.[1] In the past decade, the intensification and expansion of shrimp farms have taken place at a rapid pace in order to achieve a higher production rate. However, under high-intensity production conditions, adverse environmental conditions might prevail (depending on the technology being used), leading to the outbreak of diseases and resulting in massive economic losses globally.[2,3] Vibriosis is one of the major disease issues in shrimp aquaculture. Pathogenic *Vibrio* spp. from the Harveyi clade is recognized as the most aggressive bacterial pathogen causing vibriosis in shrimp

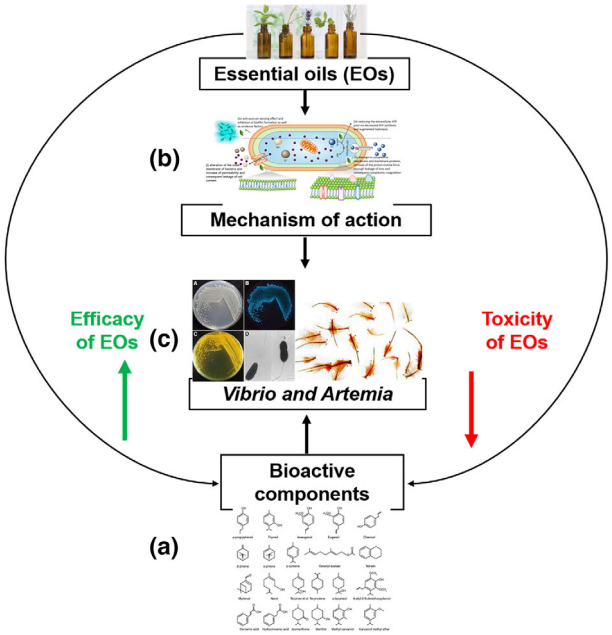


FIGURE 1 Schematic representation of the concept of this review. (a) EOs is a complex mixture containing 20–60 bioactive components (known as essential oil components. EOCs). (b) The mechanisms of action and target sites of EOs on bacterial cells are introduced. (c) The efficacy of EOs on anti-*Vibrio* activity and the toxicity of EOs on *Artemia* are investigated

farming.[4] Moreover, a special *Vibrio* strain from the Harveyi clade-*V. parahaemolyticus* (VP_{AHPND}) causing acute hepatopancreatic necrosis disease (AHPND), originally known as early mortality syndrome (EMS), has been considered a constant threat in the shrimp industry.[5]

To overcome bacterial diseases, antimicrobial agents might be used to combat their infections. However, the indiscriminate use of antibiotics in aquaculture has been banned by many countries due to the emergence of antibiotic-resistant bacteria and potentially adverse effects on the environment as well as human health.[6] As an alternative approach to antibiotics, the use of essential oils (EOs) in aquaculture has received much attention in the past decade. EOs have potential antimicrobial properties and are biodegradable and non-hazardous at certain concentrations.[7–9] Intensive research by the scientific community has already been conducted to discover new compounds and new applications of compounds extracted from plants, for instance, by identifying and characterizing the effects of EOs as a natural preservative, herbal anaesthetics, immunomodulators/immunostimulants and antimicrobial enhancers.[10–12] However, applying EOs to the shrimp aquaculture industry and possible toxicity impacts on shrimp species received far less attention from the scientific community. So far, due to a lack of sufficient data on shrimp, it is difficult to evaluate the possible toxic effects of EOs on shrimp. Luckily, the brine shrimp (*Artemia* spp.) have been studied regarding the toxic effects of EOs.[13,14] The brine shrimp (*Artemia*) genome sequence shares high homology with shrimps and other crustaceans' genomes.[15] Therefore, it might be possible that results about the potential toxicity of EOs towards *Artemia* can be a reference to apply to shrimp and even other crustaceans. For instance, a previous study reported that phloroglucinol pretreatment in the range of 5–100 μM did not have any toxic effect on the brine shrimp larvae, while the range of 1–30 μM was non-toxic to giant river prawn, *M. rosenbergii*.[16] Considering *Artemia* are more resistant, we still need to validate the result from *Artemia*, when extrapolating to the other crustaceans.

Therefore, the present review seeks to highlight the antimicrobial properties of EOs, specifically towards *Vibrio* strains that can cause vibriosis and/or AHPND, and discuss the possible mode of action mechanism involved. Furthermore, potential toxicological effects of EOs on brine shrimp (*Artemia* spp.) are researched. To achieve this goal, a literature search was performed on Google Scholar and Web of Science in August 2022 using the following search string: "essential oil(s) AND *Vibrio*" for the assessment of the anti-*Vibrio* ability of EOs, "EOCs' name AND *Vibrio*" for the assessment of the anti-*Vibrio* ability of pure compounds, "essential oil(s) AND toxicity AND *Artemia*" for evaluating the toxicological effect of EOs on *Artemia*. Time-limited to the Year 2019 to 2022. Search results were screened by title relevance with respect to EOs from the terrestrial plants. For the *Vibrio* strains, we differentiate the non-AHPND strains and AHPND strains. For AHPND strains, we further used the terms "essential oil(s) AND AHPND AND *Vibrio*" for searching the literature. In addition, research gaps and tentative future research studies are also mentioned, in order to conduct the prospective use and wide-scale application of EOs in sustainable shrimp culture (Figure 1).

2 | ESSENTIAL OILS (EOS) AND EO COMPONENTS (EOCS)

The International Standard Organization (ISO), defines essential oils (EOs) as concentrated relatively hydrophobic liquids containing relatively volatile chemical compounds. They can be obtained from different parts of the plant, such as seeds, roots, buds, leaves, flowers, peels, and fruits, by the methods of steam distillation or (cold) pression.[17] The main compounds are mainly derived from three biosynthetic pathways only, (i) the mevalonate pathway leading to sesquiterpenes, (ii) the methylerythritol pathway leading to mono- and diterpenes and (iii) the shikimic acid pathway leading to phenylpropenes.[18] Generally, an EO contains about 20 to 60 chemical components (EOCs), and they are named according to their concentration in the mixture, as (i) major constituents (from 20 to 90%), (ii) secondary constituents (1–20%) and (iii) trace components (below 1%). More than 3000 distinct chemicals have been detected in EOs, with a large variety of chemical structures. Overall, the main chemical classes of EOs are classified as aliphatic (e.g., neral, citronellal), aromatic (e.g., cinnamaldehyde), carboxylic acids (e.g., isovaleric acid), coumarins (e.g., coumarin), diterpenes (e.g., phytol, taxadiene), diterpenoles (e.g., sclareol), esters (e.g., linalyl acetate), ketones (e.g., pulegone), lactones (e.g., alantolactone), monoterpenes (e.g., limonene, ocimene), monoterpenoles (e.g., linalool, thujanol), oxides (e.g., 1,8-cineol), phenols (e.g., carvacrol, thymol), phenol methyl ethers (e.g., methyl chavicol), phthalides (e.g., sedanolide), sesquiterpenes (e.g., chamazulene), sesquiterpenoles (e.g., viridiflorol, carotol) and others (e.g., allicin).[19] Chemical structure and the characteristics of commonly used EOC (ordered base on the molecular weight from the lowest to the highest) are established in Table 1. The factors determining the chemical

TABLE 1 Chemical structure and the characteristics of commonly used EOC (ordered base on the molecular weight from the lowest to the highest)

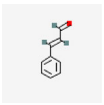
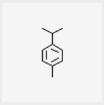
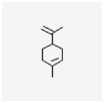
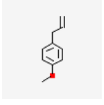
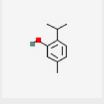
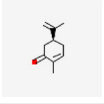
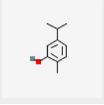
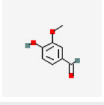
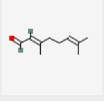
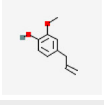
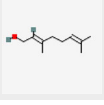
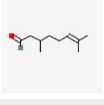
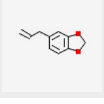
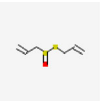
Representative EOC	Molecular Formula	Molecular weight	2D structure	Density (g/ml)	Vapour pressure (mm Hg) at 25 °C	Hydrophobicity Log *P*ow, pH 7 at 25 °C	Solubility (mg/L) at 25 °C in water
Trans-cinnamaldehyde	C_9H_8O	132.16		1.046–1.053	0.02	1.9	1420
p-Cymene	$C_{10}H_{14}$	134.22		0.853–0.855	1.50	4.10	23.4
Limonene	$C_{10}H_{16}$	136.23		0.840 (at 4 °C/20 °C)	1.55	4.23	7.57
(+)-Sabinene	$C_{10}H_{16}$	136.23		0.844	2.60	4.13	2.49
4-Allylanisole	$C_{10}H_{12}O$	148.20		0.960–0.968	0.165	3.47	178
Thymol	$C_{10}H_{14}O$	150.22		0.969	0.016	3.96	900
(+)-Carvone	$C_{10}H_{14}O$	150.22		0.956–0.961	15.5	2.4	1300 (at 18 °C)
Carvacrol	$C_{10}H_{14}O$	150.22		0.974–0.979	2.96×10^{-2}	3.49	1250
Vanillin	$C_8H_8O_3$	152.15		1.056–1.060	1.18×10^{-4}	1.37	1102
Citral	$C_{10}H_{16}O$	152.23		0.885–0.891	0.09	3.45	1340
Eugenol	$C_{10}H_{12}O_2$	164.20		1.064–1.070	0.0221	2.49	2460
Geraniol	$C_{10}H_{18}O$	154.25		0.870–0.885	0.03	3.81	100
(±)-Citronellal	$C_{10}H_{18}O$	154.25		0.850–0.860	0.25	3.53	70.2
Safrole	$C_{10}H_{10}O_2$	162.18		1.095–1.099	0.07	3.45	121

TABLE 1 (Continued)

Representative EOC	Molecular Formula	Molecular weight	2D structure	Density (g/ml)	Vapour pressure (mm Hg) at 25 °C	Hydrophobicity Log *Pow*, pH 7 at 25 °C	Solubility (mg/L) at 25 °C in water
Allicin	$C_6H_{10}OS_2$	162.3		1.109–1.112	3.8×10^{-2}	1.13	24,000 (at 10 °C)

Note: Results are extracted from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and Pesticide Properties DataBase (PPDB, http://sitem.herts.ac.uk/aeru/ppdb/en/search.htm).

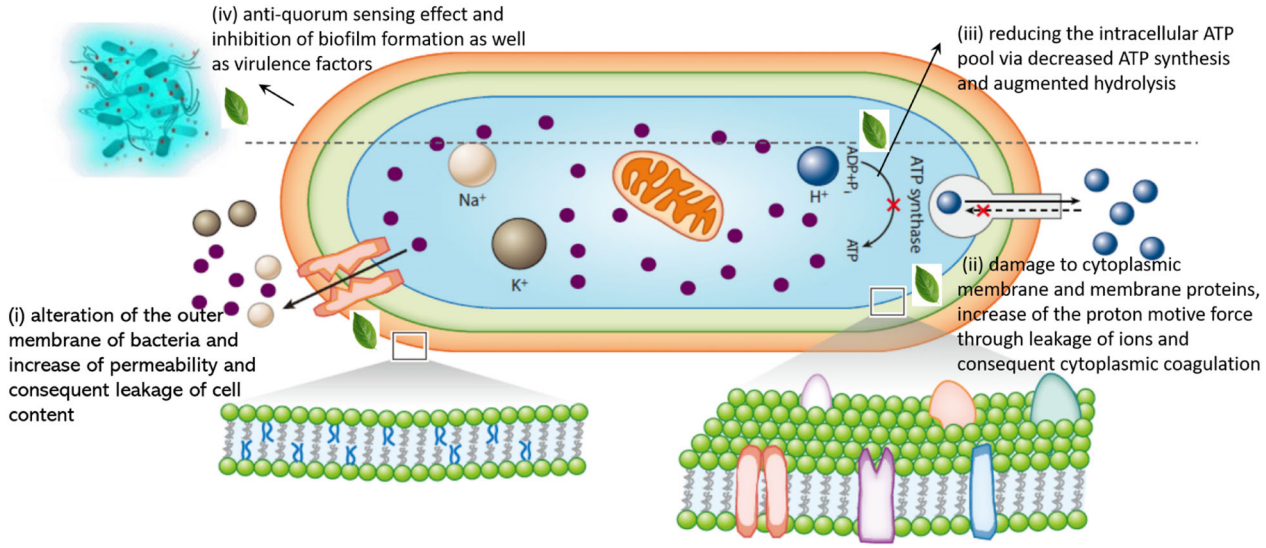


FIGURE 2 The mechanisms of action and target sites of essential oils (EOs) on bacterial cells: (i) alteration of the outer membrane, (ii) damage to cytoplasmic membrane and membrane proteins, (iii) reducing the intracellular ATP pool and (iv) anti-quorum sensing effect. Adapted from a previous study.[22]

composition of an EO are genetic characteristics of the producing plant, stage development of the plant, edaphic/environmental conditions and extraction method.[20,21]

2.1 | Mechanisms of action of the EOs

Diverse mechanisms have been put forward to explain the activity of an EO and/ or their component on bacterial cells. In brief, the mechanisms of action of the EOs can be described as follows: (i) alteration of the outer membrane, (ii) damage to cytoplasmic membrane and membrane proteins, (iii) reducing the intracellular ATP pool and (iv) anti-quorum sensing effect. (Figure 2). Examples of EOs are given based on their mechanism of action against bacteria (Table 2).

2.2 | Assessment of anti-*Vibrio* (non-AHPND strains) ability of EOs

Vibriosis is one of the main bacterial diseases in larval and juvenile shrimp, which is caused by several pathogenic *Vibrio* species.[35] *Vibrio* is a genus of Gram-negative bacteria, belonging to the family Vibrionaceae, the class Gammaproteobacteria.[36] *Vibrio* genus consists of over 147 species and at least 14 species of *Vibrio* have been reported as the destructive agent in shrimp cultivation, including *V. alginolyticus*, *V. anguillarum*, *V.campbellii*, *V. damsella*, *V. fischeri*, *V. harveyi*, *V. logei*, *V. mediterranei*, *V. mimicus*, *V. ordalli*, *V. orientalis*, *V. parahaemolyticus* (non-AHPND strain), *V. splendidus* and *V. vulnificus*.[36,37] *Vibrio* spp. is bacteria containing polar flagellum, surrounded with or without sheaths. These bacteria are present in marine environments, sediments, the water column, vertebrates, invertebrates, aquatic plants, free individuals or attached to the particles.[37]

The general symptoms of vibriosis include lethargy, slow growth (empty midgut and anorexia), low larval metamorphosis, body malformation (melanization, appendage necrosis, muscle opacity), reddened body (red or brown gill), abnormal swimming behaviour (swimming at the ends and /or surface of the ponds), and bioluminescence (for infection by some *Vibrio* spp.).[38,39]

In the past 4 years (2019–2022), EOs are increasingly researched as a remedy for vibriosis. There are plenty of studies showing that EOs typically possess multiple mechanisms of action due to their complex mixture components. Table 3 summarizes the available minimum inhibitory concentration (MIC) data of EOs in vitro for anti-*Vibrio* (non-AHPND strains) activities. Moreover, a brief description of the

TABLE 2 Examples of EOs are given based on their mechanism of action against bacteria

Mechanism of action	Botanical name-part used Major constituents present at >10%	Bacteria	Effective concentration[a] (μg/ml)	References
Alteration of the outer membrane	*Cinnamomum verum*-barks Cinnamaldehyde (72.81%) Benzyl alcohol (12.5%)	*Escherichia coli* (J53 R1)	0.02% (v/v)	23
	Citrus medica-fruits Limonene (45.36%), γ-terpinene (21.23%)	*E. coli* ATCC (25922) *Staphylococcus aureus* (ATCC 6538)	2500 625	24
Damage to cytoplasmic membrane	*Cinnamomum zeylanicum*-barks Cinnamaldehyde (57.97%) eugenol (19.19%)	*Porphyromonas gingivalis* (ATCC33177)	6.25	25
	Citrus medica-fruits limonene (45.36%), γ-terpinene (21.23%)	*E. coli* ATCC (25922) *S. aureus* (ATCC 6538)	2500 625	24
	Cudrania tricuspidata-fruits diethylpthalate (36.24%) scyllitol (23.94%)	*B. cereus* (ATCC 13061) *E. coli* (O157:H7 ATCC 43889)	250 500	26
	Foeniculum vulgare-seeds trans-anethole (68.53%) estragole (10.42%)	*Shigella dysenteriae* (CMCC (B) 51252)	125	27
	Kaempferia pandurate-bulbs geraniol (22.28%) ocimene (20.18%) cineole (14.97%)	*E. coli* (K1.1)	0.11% (v/v)	28
Reducing the intracellular ATP pool	*Dendranthema morifolium*-flowers β-eudesmene (19.83%) L-(−)-borneol (16.54%) camphor (14.62%)	*E. coli* (ATCC 25922) *S. aureus* (ATCC 25923)	20,000 20,000	29
	Lippia graveolens-unmentioned thymol (42.7%) carvacrol (22.2%)	*S. typhimurium* (ATCC 14028)	400	30
Anti-quorum sensing effect	*Anethum graveolens*-aerial part eugenol (49.62%) 1, 2-benzenedicarboxylic acid (12.89%) cyclohexasiloxane (12.85%)	*Chromobacterium violaceum* (CV026)	25 μl	31
	Cinnamomum verum-barks cinnamaldehyde (72.81%) benzyl alcohol (12.5%)	*E. coli* (pSb401 and pSB1075)	0.005–0.01% (v/v)	23
	Thymus vulgare-leaves thymol (55.42%)	*Pseudomonas fluorescens* (KM121)	20	32

[a]The effective concentration is presented in μg/ml; in some of the research reports the unit of the percentage (%, v/v) was used; when converting the percentage to the μg/ml, the density of the EOs needs to be considered; the density of EOs is in the range of 0.761–1.465 g/ml,[33,34] and oil specific.

effective concentration in different anti-*Vibrio* (non-AHPND strains) assays of EOs (plants are ordered alphabetically) is here provided.

Broth dilution assay is one of the most common approaches (fast and low-cost) used to determine the minimum inhibitory activity of EOs.[40] This approach depends on tested microbial inoculation at a specific inoculum density of broth media (in tubes or microtiter plates) infusing varying concentrations of potential antibacterial (usually 2-fold dilutions are used; e.g., 1, 2, 4, 8, and 16 μg/ml).[40] Following incubation, turbidity is observed either using an automated reader or visually, allowing a MIC to be established.

From the data in Table 3, we noticed that the antibacterial activity of *Syzygium aromaticum* EOs against *V. harveyi* (FP8370), *V. ichthyoenteri* (FP4004) and *V. parahaemolyticus* (ATCC33844) with a MIC of 0.125%, 0.125% and 0.07% (v/v), respectively, in the two different studies.[41,42] Eugenol and β-caryophyllene were the two main components in the *Syzygium aromaticum* EO extraction, however, the constituent's percentage of eugenol and β-caryophyllene in these two studies differ. Gang-Joon observed 58.7% of eugenol and 24.8% of β-caryophyllene in the *S. aromaticum* EO, while Mizan et al. found 86.63% of eugenol and 10.5% of β-caryophyllene in the *S. aromaticum* EO. As mentioned previously, the chemical composition of an EO is different due to many factors, for example, harvest time, extraction method, etc., even though from the same species of botanical plant.

TABLE 3 The in vitro susceptibility and inhibitory activities of EOs against *Vibrio* spp. (non-AHPND strains)

Family	Image	Botanical name-part used Major constituents present at > 10% MIC	*Vibrio* spp. (non-AHPND strains)	Minimum inhibitory concentration (MIC), μg/ml[a]	Major findings (effective concentration), μg/ml[a]	References
Amaryllidaceae		*Allium sativum*-bulds Allyl propyl disulfide (20.0%) Diallyl trisulfide (16.8%) Allyl sulfide (15.2%) Methyl allyl trisulfide (11.5%)	*V. parahaemolyticus* (ATCC33844)	0.09% (v/v)	-Time-kill assay (450) -Inhibit biofilm (900) -Swimming assays (1800) -Swarming motility assays (450)	41
Cupressaceae		*Calocedrus formosana*-heartwood τ-muurolol (16.1%) α-cadinol (11.1%) α-terpineol (10.6%)	*V. parahaemolyticus* (ATCC 17803)	125	-Inhibition zone (15, 30 μl)	46
Lauraceae		*Cinnamomum verum*- barks Major constituents: unmentioned	*V. parahaemolyticus*	0.0357% (v/v)	-Dynamic time kill assay (0.0357%) -bacterial morphology by FE-SEM (0.0357%)	47
Lauraceae		*Cinnamomum zeylanicum*-barks cinnamaldehyde (54.35%) eugenol (16.59%)	*V. harveyi* (FP8370) *V. ichthyoenteri* (FP4004)	0.007% (v/v)	-Antibacterial activity by disk diffusion assay (10%)	48
Rutaceae		*Citrus limon*-unmentioned D-limonene (52.85%) p-cymene (14.36%) β-pinene (13.69%)	*V. vulnificus*	25,000	-Antimicrobial activity (50 μl)	49
Rutaceae		*Citrus paradisi*-peels D-limonene (82.86%)	*V. vulnificus*	12,500	-Antimicrobial activity measured via the paper disc diffusion method (50 μl)	50
Myrtaceae		*Eucalyptus citriodora*-leaves citronellal (80.02%)	*V. campbellii* (BB120) *V. parahaemolyticus* (CAIM 170, LMG2850)	Undetermined	-Inhibition of growth (0.001%) -Vapour-phase-mediated susceptibility (20 μl)	8
Lauraceae		*Laurus nobilis*-leaves 1,8-cineole (29.58%) α-terpinyl acetate (18.08%) α-terpineol (11.78%), terpinene-4-ol (10.32%)	*V. vulnificus*	>25,000	-Antimicrobial effects by paper disc diffusion method (50 μl)	51
Lauraceae		*Lindera glauca*-fruits (E)-β-ocimene (30.54%)	*V. parahaemolyticus* (ATCC17802)	312	-Inhibition zone (10 μl)	52
Lauraceae		*Litsea citrata*-fruits citral (71.35%) limonene (11.53%)	*V. campbellii* (BB120) *V. parahaemolyticus* (CAIM 170, LMG2850)	Undetermined	-Inhibition of growth (94) -Vapour-phase-mediated susceptibility (20 μl)	8
Lauraceae		*Litsea cubeba*-unmentioned Major constituents: unmentioned	*V. parahaemolyticus* (ATCC17802)	1024	-Time kill curve (1024) -Cell membrane damage (1024) -Cell wall damage (1024) -Morphological observation (1024) -Cellular superficial hydrophobicity (256) -EPS (256) -Biofilm formation (1024)	53

(Continues)

TABLE 3 (Continued)

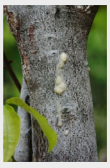
Family	Image	Botanical name-part used Major constituents present at > 10% MIC	*Vibrio* spp. (non-AHPND strains)	Minimum inhibitory concentration (MIC), μg/ml[a]	Major findings (effective concentration), μg/ml[a]	References
Lauraceae		*Litsea cubeba*- unmentioned Major constituents: unmentioned	*V. parahaemolyticus* (ATCC17802)	2500	Unmentioned	54
Myrtaceae		*Melaleuca alternifolia*-leaves terpinene-4-ol (41.35%) γ-terpinene (20.64%)	*V. campbellii* (BB120) *V. parahaemolyticus* (CAIM 170, LMG2850)	Undetermined	-Inhibition of growth (94) -Vapour-phase-mediated susceptibility (20 μl)	8
Lamiaceae		*Melissa officinalis*- unmentioned geraniol (38.31%) citronellal (27.87%) citronellol (11.38%)	*V. parahaemolyticus* (ATCC33847)	1	-Cell membrane permeability (0.25) -Cell membrane integrity (1) -Morphological alterations by SEM (0.25) -Biofilm formation (0.25) -Motility (0.25) -EPS (0.25) -Virulence gene (0.25)	55
Lamiaceae		*Mentha pulegium*-leaves D-limonene (29.35%) D-carvone (17.74%)	*V. parahaemolyticus* (ATCC17802)	20,000	Unmentioned	56
Lamiaceae		*Mesosphaerum suaveolens*- aerial part 1,8-cineole (44.5%) sabinene (13.4%)	*V. parahaemolyticus* (OCI18950)	625	-Antioxidant activity (6020)	43
Lamiaceae		*Ocimum basilicum*-aerial part linalool (74.2%)	*V. parahaemolyticus* (OCI18950)	313	-Antioxidant activity (2.75)	43
Lamiaceae		*Ocimum gratissimum*-aerial part eugenol (74.2%) 1,8-cineole (36.8%)	*V. parahaemolyticus* (OCI18950)	1250	-Antioxidant activity (18730)	43
Lamiaceae		*Origanum vulgare*- unmentioned Major constituents: unmentioned	*V. vulnificus* (ATCC 27562)	0.06	-Growth (0.06) -Intracellular ATP (0.06) -Membrane potential (0.06) -Intracellular ROS (0.015) -Intracellular MDA (0.03) -Cell membrane injury (0.06) -Cell morphology by SEM (0.06)	57
Burseraceae		*Protium heptaphyllum*-resins β-phellandrene (60.68%) p-cymene (13.63%)	*V. parahaemolyticus* (serotype K 15)	2000	-Antibiofilm activity (1000) -Cell constituent release (1000) -Cell membrane permeability (2000)	58
Asteraceae		*Rhaponticum acaule*-flowers Major constituents: unmentioned	*V. parahaemolyticus* (ATCC 43996, CECT 511) *V. vulnificus* (CECT 529)	1250 5000 2500	-Inhibition zone (20000)	59

TABLE 3 (Continued)

Family	Image	Botanical name-part used Major constituents present at > 10% MIC	*Vibrio* spp. (non-AHPND strains)	Minimum inhibitory concentration (MIC), μg/ml[a]	Major findings (effective concentration), μg/ml[a]	References
Lamiaceae		*Salvia officinalis*-unmentioned eucalyptol (14.46%) (+)-2-bornanone (14.33%) 1R-α-pinene (14.00%)	*V. vulnificus*	>25,000	-Inhibition zone (50 μl)	60
Lamiaceae		*Salvia officinalis*-aerial parts camphor (31.2%) 1,8-cineole (28.5%)	*V. parahaemolyticus* (OCI18950)	313	-Antioxidant activity (7520)	43
Myrtaceae		*Syzygium aromaticum*-buds eugenol (86.63%) β-caryophyllene (10.5%)	*V. harveyi* (FP8370) *V. ichthyoenteri* (FP4004)	0.125% (v/v)	-Antibiotic susceptibility by disk diffusion (5%)	42
Myrtaceae		*Syzygium aromaticum*-unmentioned eugenol-(58.7%) β-caryophyllene (24.8%)	*V. parahaemolyticus* (ATCC33844)	0.07% (v/v)	-Time-kill assay (0.035%) -Inhibit biofilm (0.14%) -Swimming assays (0.035%) -Swarming motility assays (0.035%)	41
Lamiaceae		*Thymus vulgaris*-unmentioned cymene (28.5%) thymol (17.8%)	*V. parahaemolyticus* (ATCC33844)	0.02% (v/v)	-Time-kill assay (0.01%) -Inhibit biofilm (0.02%) -Swimming assays (0.01%) -Swarming motility assays (0.01%)	41
Lamiaceae		*Thymus vulgaris*-aerial part Thymol (51.0%) p-cymene (26.4%)	*V. parahaemolyticus* (OCI18950)	1250	-Antioxidant activity (1150)	43

[a]The effective concentration is presented in μg/ml; in some of the research reports the unit of the percentage (%, v/v) was used; when converting the percentage to the μg/ml, the density of the EOs needs to be considered; the density of EOs is in the range of 0.761–1.465 g/ml,[33,34] and oil specific.

Two previous studies[41,43] demonstrated the antibacterial activity of *Thymus vulgaris* EO against *V. parahaemolyticus* had huge different MIC values (a 6-fold difference), with the MIC of 0.02% in the study of Mizan et al.,[41] while the MIC in the study of dos Santos Filho et al.[43] was 1250 μg/ml. The MIC units used were so different (μg/ml and percentage of dilution), leading to difficulties in comparing different studies of the same plant EOs and therefore future harmonization on the results reporting is needed. Moreover, in some of the studies listed in Table 3, the chemical composition of EO was not provided, nor was the MIC values data, which makes it hard to establish a link between the anti-*Vibrio* activities and the components in the EO responsible for these effects.

There was some research on the effect of pure EOCs against *Vibrio* (non-AHPND strains). In a preliminary study,[44] a wide panel of single EOC (thymol, carvacrol, vanillin, eugenol, cinnamaldehyde, geraniol, α-pinene, eucalyptol, menthol, linalool, limonene and vanillin) were used to determine their antibacterial activity against two *Vibrio* species (*V. anguillarum* and *V. harveyi*). The result showed the most effective EOC were the terpenes thymol (MIC: 1.88 mM against *V. anguillarum*, 0.94 mM against *V. harveyi*), carvacrol (MIC: 1.88 mM against *V. anguillarum*, 0.94 mM against *V. harveyi*), eugenol (MIC: 1.88 mM against *V. anguillarum* and *V. harveyi*), geraniol (MIC: 7.5 mM against *V. anguillarum* and *V. harveyi*) and the terpenic aldehydes cinnamaldehyde (MIC: 3.75 mM against *V. anguillarum*, 1.88 mM against *V. harveyi*) and vanillin (MIC: 3.75 mM against *V. anguillarum* and *V. harveyi*). Eucalyptol, linalool, menthol, α-pinene and limonene failed to inhibit the growth of *V. anguillarum* and *V. harveyi* at the tested concentrations (0.23–7.5 mM). In other previous studies, the MIC of citral against *V. parahaemolyticus* ATCC17802 was 100 μg/ml, against ATCC33847 was 150 μg/ml[45] and against *V. alginolyticus* was 125 μg/ml.[7]

2.3 | Assessment of anti-*Vibrio* (AHPND strains) ability of EOs

Acute hepatopancreatic necrosis disease (AHPND), known originally as early mortality syndrome (EMS), is a relatively new farmed penaeid shrimp bacterial disease.[61] The causative agent of AHPND is mainly some specific strains of *V. parahaemolyticus* (VP_{AHPND}). The shrimp affected with AHPND exhibits lethargy, anorexia, slow growth, an empty digestive tract and a pale to white hepatopancreas.[5] Some studies have reported management strategies to control or possible prevent AHPND outbreak in shrimp aquaculture, including supplementation of plant-derived and/or natural compounds,[8] probiotics,[62] phage therapy,[63] environmental manipulation,[64] biofloc technology,[65] and pond management.[66]

TABLE 4 Brine shrimp (*Artemia* spp.) toxicity at 24 h bathing exposure to EOs

Botanical name-part used Major constituents present at >10%	50% Lethal concentration ($LC_{50\text{-}24h}$) μg/ml	The level of toxicity[a]	References
Abies alba-needles with twigs β-Pinene (22.3%) α-Pinene (12.4%) Camphene (10.9%) Limonene (10.7%)	30.46 ± 0.02	Strongly	70
Abies × *borisii-regis*-needles with twigs β-pinene (21.1%) α-pinene (11.6%)	25.39 ± 0.04	Strongly	70
Abies cephalonica-needles with twigs β-pinene (35.5%) α-pinene (29.2%)	17.81 ± 0.03	Strongly	70
Achillea crithmifolia- aerial parts 1,8-cineole (17.7%) artemisia alcohol (16.6%)	149.35	Moderately	71
Achillea distans- aerial parts borneol (36.1%) 1,8-cineole (14.6%)	38.18	Strongly	71
Achillea grandifolia- aerial parts camphor (24.1%) ascaridole (14.6%) cis-thujone (14.1%)	94.57	Strongly	71
Achillea millefolium- aerial parts borneol (12.8%)	26.91	Strongly	71
Achillea nobilis- aerial parts artemisia ketone (38.9%)	42.87	Strongly	71
Achillea lingulata- aerial parts borneol (22.1%)	12.26	Strongly	71
Artemisia vulgaris-aerial parts germacrene D (10.6–30.5%) cis-thujone (12.9%) β-caryophyllene (5.5–16.7%)	10.25	Strongly	14
Cantinoa althaeifolia-leaves himachalene (11.62%) spathulenol (10.08%)	>1000	Non-toxic	72
Cassia singueana-flowers geranyl acetone (36.82%) phytol (18.12%) squalene (10.84%)	18.70	Strongly	73
Cinnamomum camphora-fruits linalool (13.52%) safrole (16.53%)	68.21	Strongly	13
Citrus bergamia-peels linalool (33.64%) limonene (32.29%)	>1000	Non-toxic	74
Citrus limon-peels limonene (57.65%) γ-terpinene (10.45%)	>1000	Non-toxic	74
Citrus myrtifolia-peels limonene (76.83%) linalool (10.01%)	>1000	Non-toxic	74
Cochlospermum regium-leaves copaen-4-α-ol (20.05%) β-bisabolene (11.48%) viridiflorol (10.21%)	90.17 ± 1.90	Strongly	68
Cochlospermum regium-xylopodium	625.08 ± 2.88	Weakly	68

TABLE 4 (Continued)

Botanical name-part used Major constituents present at >10%	50% Lethal concentration ($LC_{50\text{-}24h}$) μg/ml	The level of toxicity[a]	References
β-selinene (26.17%)			
Conium divaricatum-infructescences 4′-oxodecyl hexanoate (74.4%)	292	Moderately	75
Conium maculatum-aerial parts (E)-caryophyllene (15.4%) myrcene (11.7%)	497	Moderately	75
Croton rudolphianus-leaves (E)-caryophyllene (17.33%) an unknown compound (16.87%)	68.33	Strongly	76
Curcuma aeruginosa-rhizomes tropolone (18.1%) eucalyptol (17.9%)	78.2 ± 7.3	Strongly	77
Curcuma zanthorrhiza-rhizomes xanthorrhizol (26.8%) β-curcumene (17.0%) ar-curcumene (15.0%)	83.6 ± 12.1	Strongly	77
Cymbopogon citratus-leaves Sand: geranial (36.66%) neral (29.53%) geranyl propanoate (21.84%) Hydroponic: geranyl propanoate (31.70%) myrcene (19.34%) geranial (14.58%) neral (12.63%) Compost: geranyl acetate (66.41%) geranyl propanoate (25.48%) myrcene (24.31%)	83.18	Strongly	78
Cymbopogon nardus-leaves citronellal (27.34%) geraniol (23.21%) geranial (13.37%) β-citronellol (12.49%)	>1000	Non-toxic	79
Dysphania ambrosioides-aerial parts α-terpinene (50.69%) p-cymene (13.27%) ascaridole (10.26%)	86.9	Strongly	79
Eucalyptus amygdalina-leaves 1,8-cineole (35.78%) spathulenol (12.58%)	116.06	Moderately	81
Eucalyptus globulus-leaves 1,8-cineole (78.45%)	65.5	Strongly	81
Eucalyptus globulus-unmentioned eucalyptol (59.63%) p-cymene (15.55%) limonene (14.90%)	2660	Non-toxic	82
Eucalyptus gunnii-branches 1,8-cineole (74.7%) α-pinene (13.1%)	>1000	Non-toxic	83
Eucalyptus pulverulenta-branches 1,8-cineole (75.5%)	>1000	Non-toxic	83
Eugenia pyriformis- aerial parts β-caryophyllene (17.82%) bicyclogermacrene (12.84%)	125.64	Moderately	84

(Continues)

TABLE 4 (Continued)

Botanical name-part used Major constituents present at >10%	50% Lethal concentration ($LC_{50\text{-}24h}$) μg/ml	The level of toxicity[a]	References
Euryale ferox-seeds hydroxytoluene (38.7%) palmitic acid (11.0%)	11.48 ± 0.51	Strongly	85
Gliricidia sepium-leaves (E)-hexadecatrienal (16.9%) pentadecanal (16.0%)	79.7	Strongly	86
Gliricidia sepium-stems humulene epoxide II (17.5%) caryophyllene oxide (10.6%)	38.7	Strongly	86
Helichrysum arenarium-inflorescences palmitic acid (23.8%) myristic acid (14.9%)	23.42	Strongly	87
Helichrysum arenarium-leaves Palmitic acid (18.8%) n-nonanal (10.4%)	21.97	Strongly	87
Helichrysum italicum-inflorescences γ-curcumene (21.5%) β-selinene (13.6%)	15.99	Strongly	87
Juniperus oxycedrus-fruits β-myrcene (37%) α-pinene (13%)	27.63	Strongly	88
Lantana camara-leaves isocaryophyllene (14.39%)	15.92	Strongly	89
Lippia alba-aerial parts sabinene (19.34%) (E)-caryophyllene (18.21%) limonene (16.47%)	53.01	Strongly	90
Litsea angulate-leaves (+)-β pinene (18.19%) cis-verbenol (11.10%)	784.24	Weakly	91
Mentha arvensis-leaves Major constituents: unmentioned	139.73	Moderately	92
Mentha spicata-leaves d-carvone (65.21%) limonene (27.28%)	245	Moderately	93
Mesosphaerum suaveolens-aerial parts Dry season: 1,8-cineole (46.31%) linalool (12.85%)	215.7 ± 12.46	Moderately	69
Mesosphaerum suaveolens-aerial parts Intermediate period: 1,8-cineole (64.44%)	167.1 ± 17.88	Moderately	69
Mesosphaerum suaveolens-aerial parts Rain season: 1,8-cineole (30.15%)	202.6 ± 19.92	Moderately	69
Murraya paniculate-friuts β-caryophyllene (20.1%) germacrene D (18.0%) α-zingiberene (15.2%)	1549.2	Non-toxic	94
Murraya paniculate-leaves β-caryophyllene (20.8%) α-zingiberene (20.0%) β-cubebene (13.2%)	1785.3	Non-toxic	94
Myrcia hatschbachii-leaves trans-calamenene (19.10%)	409.92	Moderately	95

TABLE 4 (Continued)

Botanical name-part used Major constituents present at >10%	50% Lethal concentration ($LC_{50\text{-}24h}$) μg/ml	The level of toxicity[a]	References
(E)-caryophyllene (10.96%)			
Myristica fragrans-seeds safrole (49.09%) β-phellandrene (18.27%) 3-p-menthene (10.76%)	31.05	Strongly	96
Ocotea diospyrifolia-leaves δ-elemene (25.93%) β-atlantol (15.35%) spathulenol (11.4%)	602.81	Weakly	97
Ocotea nutans-leaves Biciclogermacrene (11.41%)	71.70	Strongly	98
Piper aduncum-leaves, stems and inflorescences dillapiole (75.5%)	20.80	Strongly	99
Piper arborescens-stem barks pentadecanal (18.88%) guaiol (11.19%) β-guaiene (11.12%)	57.95	Strongly	100
Piper caninum-stem barks isocaryophyllene (20.60%) (E)-α-bergamotene (13.74%) (E)-isoeugenol (13.46%)	249.74	Moderately	100
Protium heptaphyllum-young leaves β-caryophyllene (15.1%)	490.50	Moderately	101
Protium heptaphyllum-adult leaves β-caryophyllene (15.0%)	488.30	Moderately	101
Pseudotsuga menziesii-needles and twigs α-terpinolene (22.7%) sabinene (17.9%) β-pinene (15.2%)	347.41	Moderately	102
Psidium guajava-leaves iso-caryophyllene (33.53%) veridiflorene (13.00%) farnesene (11.65%)	>1000	Non-toxic	103
Rosmarinus officinalis-leaves 1,8-cineole (17.16%) α-pinene (16.95%)	93.26 ± 7.16	Strongly	104
Rubus rosifolius-fruits Red: linalool (21.0%) α-terpineol (13.1%) α-cadinol (10.6%) Wine red: α-cadinol (17.0%)	63 (Red) 48 (Wine Red)	Strongly	105
Saussurea lappa-roots eudesma-5,11(13)-dien-8,12-olide (52.01%)	>1000	Non-toxic	106
Tagetes minuta-flowers verbenone (25%) unknown (11.69%)	>1000	Non-toxic	107
Trachyspermum ammi-fruits γ-terpinene (53.81%) thymol (29.40%)	26.20	Strongly	108
Uvaria chamae-roots benzyl benzoate (23.3%) dimethoxy-p-cymene (14.2%) s-cadinol (12.1%)	25.01	Strongly	109

(Continues)

TABLE 4 (Continued)

Botanical name-part used Major constituents present at >10%	50% Lethal concentration ($LC_{50\text{-}24h}$) μg/ml	The level of toxicity[a]	References
Vernonia Chalybaea-aerial parts β-caryophyllene (39.06%) bicyclogermacrene (19.69%)	29.96 ± 0.77	Strongly	110
Zanthoxylum armatum-fruits linalool (75.31%) E-methyl cinnamate (11.73%)	76.70	Strongly	111

[a]The level of toxicity against brine shrimp was classified into four groups: strongly toxic (LC_{50} < 100 μg/ml), moderately toxic (LC_{50}:100–500 μg/ml), weakly toxic (LC_{50}:500–1000 μg/ml), and non-toxic (LC_{50} > 1000 μg/ml).[67]

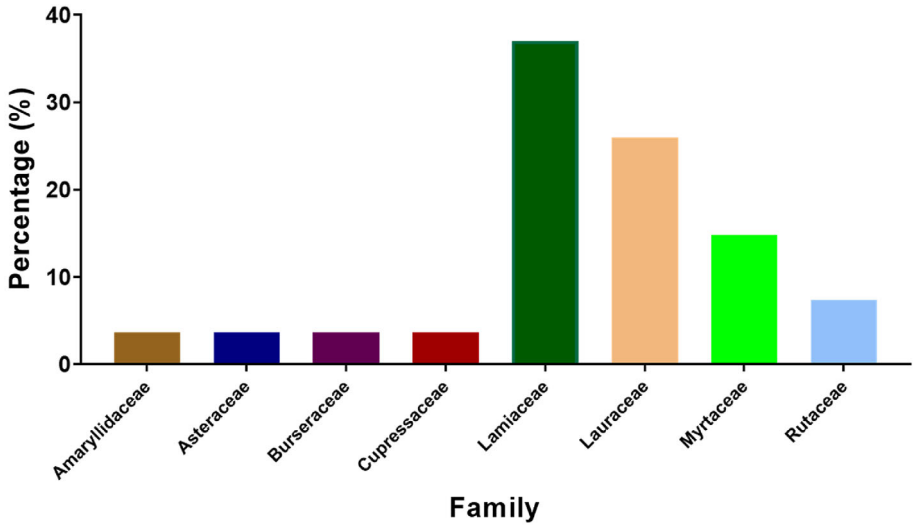


FIGURE 3 Proportion of terrestrial plants showing anti-*Vibrio* activity in vitro based on the Table 3 in the present review.

So far, it has been lacked of studies to report the effect of EO(C)s against *V. parahaemolyticus* AHPND strains. Only our previous research showed that EOs of *Litsea citrata* and *Eucalyptus citriodora* significantly inhibited the growth of *V. parahaemolyticus* (MO904, VP_{AHPND}) at 0.01 and 0.1% (v/v). While EOs of *Melaleuca alternifolia* at 0.1% can significantly inhibit the growth of MO904.[8]

2.4 | Toxicological effects of EOs with focus on brine shrimp

Besides their anti-*Vibrio* activities, EOs might also be known to exert adverse effects on aquatic animals. In this current work, we summarize the most relevant in vivo toxicity studies of EOs performed in the last 4 years (2019–2022) mostly on brine shrimp (Table 4, all plant EOs are ordered alphabetically). The level of toxicity against brine shrimp was classified into four groups: strongly toxic (LC_{50} < 100 μg/ml), moderately toxic (LC_{50}:100–500 μg/ml), weakly toxic (LC_{50}:500–1000 μg/ml), and non-toxic (LC_{50} > 1000 μg/ml).[67] Table 4 indicated the strongest toxicity towards *Artemia* after 24 h exposure with *Artemisia vulgaris* (LC_{50} = 10.25 μg/ml), *Euryale ferox* (LC_{50} = 11.48 μg/ml), *Achillea lingulate* (LC_{50} = 12.26 μg/ml) and *Abies cephalonica* (LC_{50} = 17.81 μg/ml), respectively. However, the LC_{50} can be up to 2660 μg/ml in the *Eucalyptus globulus*, showing no toxicity to *Artemia*.

It is well known that toxicity can considerably vary in function of the part used of the plant and harvest time. For instance, different parts used in the plant EO affect the toxicity of *Artemia*, the LC_{50} was 90.17 μg/ml (strong toxicity), 625.08 μg/ml (weak toxicity) in the leaves, and xylopodium of *Cochlospermum regium* EO,[68] respectively. Harvest time was also one factor affecting the toxicity, collected during the dry season, intermediate period and rain season, the aerial part of *Mesosphaerum suaveolens* extractions showed that the LC_{50} values were 215.7, 167.1 and 202.6 μg/ml, showing moderate toxicity towards *Artemia*.[69]

2.5 | Analysis of data from the present review

In the current review, 27 terrestrial plants are reported regarding their anti-*Vibrio* activity (Table 3, not including those against AHPND strains). Among the families, Lamiaceae is represented the most (almost 40% of the plant species, Figure 3). In Lamiaceae family, the plant *Salvia officinalis* and *Thymus vulgaris* were used most for study.

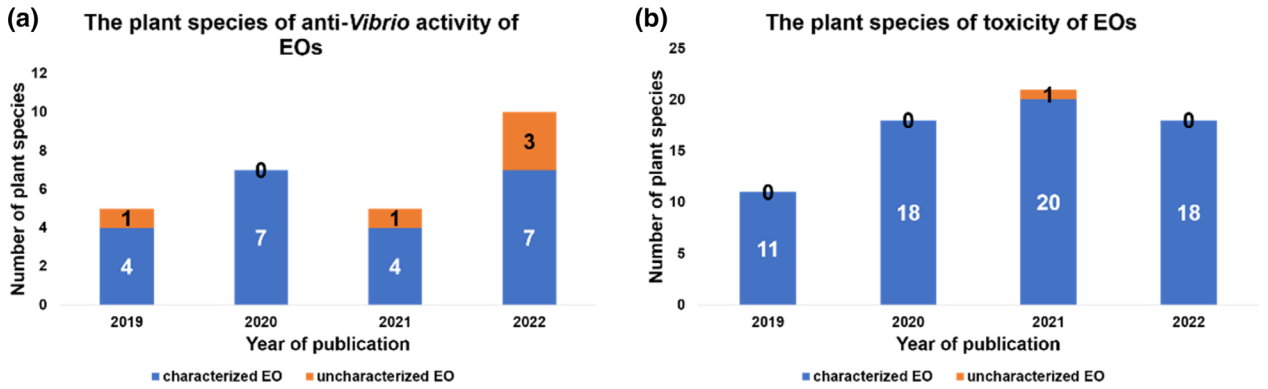


FIGURE 4 Number of plant species by year based on the Table 3 in the present review.

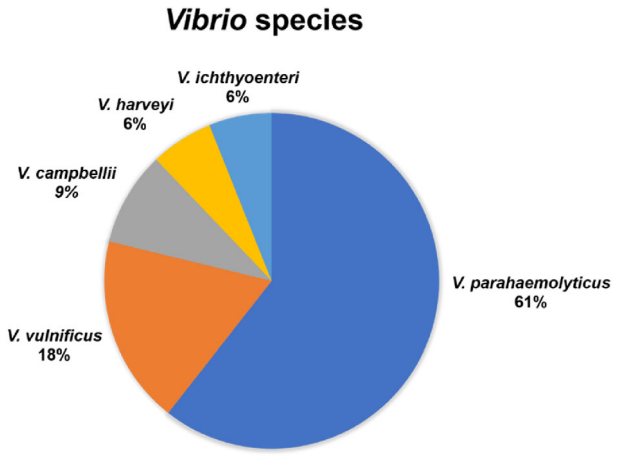


FIGURE 5 Proportion of *Vibrio* spp. covered in the present review.

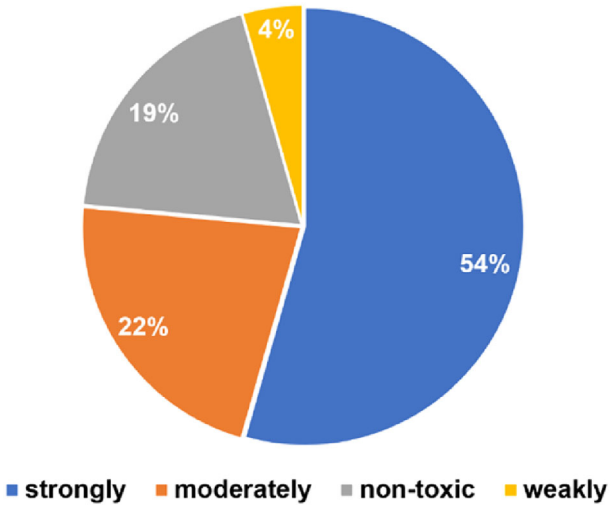


FIGURE 6 The percentage of EOs within four categories toxicity of brine shrimp.

We found quite a few publications in the past 4 years (2019–2022), which is a good indication that more attention is being paid to applying plant EOs for controlling diseases caused by *Vibrio* spp. In 2019, there were five papers (four papers about characterized EO, one paper about uncharacterized EO) were published, followed by 2020 (seven papers in total, all about characterized EO), 2021 (five papers in total, four papers about characterized EO, one paper about uncharacterized EO) and 2022 (10 papers in total, seven papers about characterized EO, three papers about uncharacterized EO) (Figure 4a). As mentioned previously, due to the lack of available data on the chemical characterization of the EO, it is difficult to pinpoint the components responsible for the observed anti-*Vibrio* activities. As shown in Figure 4b, a total of 68 EOs from different plants are represented to show different toxicity in *Artemia* spp.

Among the *Vibrio* spp. encountered in the present review, *V. parahaemolyticus* (non-AHPND strain) was included the most (61%), although *V. vulnificus* (18%) also constituted an important portion (Figure 5). *V. parahaemolyticus* (non-AHPND strain) is the most devastating bacterium. Because it is associated with food-borne infection and outbreaks linked to seafood, encoding the thermostable direct hemolysin-related hemolysin (trh) gene.[112]

The level of toxicity against *Artemia* was classified into four groups: strongly toxic (LC_{50} < 100 μg/ml), moderately toxic (LC_{50}:100–500 μg/ml), weakly toxic (LC_{50}:500–1000 μg/ml), and non-toxic (LC_{50} > 1000 μg/ml).[67] The results showed that strong toxicity activity was found in 37 plant EOs (54%), moderate toxicity in 15 plant EOs (22%), weak activity for 3 plant EOs (4%), and 13 non-toxic EOs (19%). The proportions of 68 EOs within four categories of toxicity of brine shrimp were shown in Figure 6.

The median lethal dose (LD_{50}) is the dose required to kill half the member of the tested population after a specified test duration.[113] LD_{50} figures are frequently used as a general indicator of a substance's acute toxicity. A lower LD_{50} is indicative of increased toxicity.[113] MIC is the lowest concentration of a chemical, usually a drug, which prevents the visible growth of a bacterium or bacteria.[114] One point that needs to be highlighted is that if the LD_{50} value for plant extracts is 10 times higher than the MIC value for bacteria, it might be evident that plant extracts are safe for environmental application and this baseline data support in vivo application studies on the target organisms.[115] Combined with Tables 2 and 3, only two of the plant

TABLE 5 Two of the plant EOs were chosen due to their effective data of LD_{50} and MIC

Botanical name	MIC (μg/ml)	LD_{50} in *Artemia* (μg/ml)
Citrus limon	25,000	>1000
Protium heptaphyllum	2000	488.30–490.50

Note: Presented in Tables 2 and 3 at the same time. However, there is not a single EO that meets the criterion in which the LD_{50} value for plant extracts is 10 times higher than the MIC value for *Vibrio* spp.

EOs were chosen due to their effective LD_{50} and MIC values towards *Vibrio* spp. (Table 5). Among these two plant EOs, there is not a single species that meets this criterion. Despite the general perception that *Artemia* is a model animal representing shrimp and other crustaceans, it can be anticipated that there is still a big difference in sensitivity between shrimp and *Artemia* (with *Artemia* most probably being a less sensitive organism). Therefore, it is not possible to apply these toxicity results directly to the shrimp culture. Moreover, as these available toxicity data are limited to *Artemia* nauplii exposure to EOs for 24 h, there is a need to evaluate the effect of EOs on the different life stages of *Artemia* and even on these in shrimp. This evaluation should focus on, not only acute but also chronic toxicity tests.

There was no direct toxicity data on the aquaculture shrimp species in the past 4 years (2019–2022). Despite the general perception that EOs are "greener" and safer alternatives to antibiotics, there is a lack of empirical data that can sustain it, creating an imperative obligation to widen the assessment of their safety to better understand their effects on shrimp aquaculture, and even the ecosystem. Hence, more studies are required to consider the comprehensive understanding of the antibacterial properties and toxicity assessment of the EOs in the future.

2.6 | EO-based nanoemulsions used in aquaculture and their potential risk

Application to the EOs in their oil form renders them subjected to degradation during processing, storage and handling.[116] The use of nanoemulsions EOs becomes a promising trend in the field of EOs application, especially in the aquaculture sectors, preventing volatilization, low stability, low solubility in water, and associated problems of using EOs.[117] The application of EOs-based nanoemulsions can effectively inhibit the growth of *Vibrio* spp. For instance, *Citrus paradisi* EO (10% EO, w/w) nanoemulsion showed an inhibitory effect against *V. vulnificus* with a MIC value of 25,000 μg/ml.[50] The MIC value of *Thymus vulgaris* EO (10% EO, w/w) nanoemulsion against *V. vulnificus* was 12,500 μg/ml.[118] It should be noted that EO nanoemulsion contained only 10% of the EO, therefore, 10 times lower levels of bioactive EO components were present. Recalculating the results to include the real content of EO, indicates that nanoemulsification improves the antibacterial properties of EO.

Owing to its extremely small droplet size (<100 nm), EO-based nanoparticles can penetrate through the cell membranes and cause genotoxicity, becoming a public concern. Some studies have reported the toxic effects of EO-based nanomaterials for aquatic organisms, especially for *Artemia*.[79,115] In this regard, the studies of EO-based nanoemulsion need to clarify not only the regulatory aspects and bio-distribution of these components, but also to evaluate, at molecular levels, their potential risks to the fisheries and aquaculture industries.

2.7 | Biosafety, degradation and economic aspects of EO usage in aquaculture

In the appropriate concentration, EO can exert its positive biological properties.[119] However, when the concentration of EO is too high, EO may have potential cytotoxic, mutagenic and genotoxic effects.[120] Furthermore, the toxic effects are based on different species, such as *Juniperus occidentalis* EO causing relatively high toxicity to microalgae while registering no effects on crustacea. An opposite effect was observed with the *Chamaecyparis lawsoniana* EO causing toxicity at low concentrations to crustacea but not on microalgae.[121] The lipophilic properties of EOs make them pass through cell membranes easily. Therefore, a lower concentration of EOs can be effective. LC_{50} values of some EO as low as 0.0336, 0.0005 and 0.0053 μg/ml were described for microalgae, crustaceans and fish, respectively.[121] In addition, in aquaculture, a series of adverse effects have been reported when EO using for fish anaesthetics. Goulet et al. found that eugenol, the main component of clove oil, caused kidney and renal damage in frogs at an anaesthetic dose (0.35 μl/ml).[122] Some studies also found that EO can cause damage to the liver and gills in fish.[123,124]

The main components of EO are alcohols, aldehydes, acids, phenols, esters, ketones and terpenes, containing mainly carbon, oxygen and hydrogen. These properties generally make EOs biodegradable getting easily catabolized in the environment (and hence are considered to be eco-friendly). Generally speaking, the degradation of EOs can be divided into three types: physical, chemical, and biological. Light and temperature are the main factors leading to physical degradation, which may occur through different pathways which can broadly be classified as oxidative degradation, C–C bond cleavage, elimination, hydrolysis and thermal rearrangement.[116,125] For chemical degradation, oxygen, water, metal contaminants and pH play a crucial role.[116] For biodegradation, while EO can inhibit bacteria and fungi, some bacteria and fungi species can also degrade EO.[126] An aquaculture environment is a complex biological and technological system where these mechanisms can be going simultaneously, which is going to promote the degradation of EO (yet there is limited information on that, and the knowledge-based application of EO will require pharmacokinetic insight as well as insight on the biodegradation kinetics). Yet degradation products are generally considered to be environmentally friendly due to their being derived from nature.[119]

The prices of the EO depend on the plant quality, plant species, extraction methods, and area of application.[127] There is no doubt that different plant species and different extraction methods determine

the value of EOs. Kant and Kumar reviewed the extraction costs of EOs.[128] Results showed that the lowest production cost (6.71 US $/kg) was obtained for oregano EO using supercritical fluid extraction (SFE) with full energy integration. The production cost for steam distillation, water distillation, solvent extraction and SFE varies from 15.85–76.50, 7.05–86.4, 8.35–8.53 and 6.71–42.69 US$/kg, respectively.

2.8 | Research gaps and concluding remarks

Research on applying EOs by the scientific community is increasing day by day. There are some review papers mainly focusing on the beneficial effects of EOs on growth, immunity and antiparasitic activities in fish culture.[129] Some papers focus on the use as anaesthetic compounds or stress-reducing agents during fish handling and transportation.[10] Combined with the data from Tables 2, 3 and 4, despite the potential contribution of EOs to anti-*Vibrio* activity, there are still drawbacks when applying EOs to shrimp aquaculture. The shortage includes few commercially available medicinal products used in the shrimp industry and a lack of chronic and acute toxicity studies of farmed shrimp. What's more, valuable data are lacking in most cases, for instance, no precise data on optimal dose requirements, inadequate data on the effects of EOs at the molecular level, and no data about the comprehensive tests between the farm shrimp and pathogen under field conditions. In addition, possible environmental impacts of EOs received far less attention, and data on the ecotoxicological effects of EOs on different organisms across aquatic and terrestrial trophic chains are not available.

EOs are complex mixtures of a wide diversity of components and many EOs exhibit strong antimicrobial activity against pathogens in vitro, as well as have potential toxicity.[129] However, the efficiency and the toxicity of EOs depend on plant variables and the chemical composition of bioactive compounds. In addition, the chemical composition varies considerably by harvest time, collection location, plant organ or tissue, and solvent or method used for extraction.[130] The precise molecular composition of EOs plays a vital role in determining their antimicrobial efficacy and toxicity level.

Due to the limited number of studies about characterized EOs and the synergy potential of EOs, future research should focus on:

1. Discovering and identifying the composition of EOs, comparing the antimicrobial activity of EOs and the major component of EOs (without minor components) allowing to verify if the minor components of EO are critical to the antimicrobial activity.
2. Evaluating the effectiveness of different EOs or EOC combinations to determine potential synergistic activity.
3. Identifying and characterizing EOs mode of action.
4. Developing an effective delivery system of EOs, for instance, microencapsulates of EOs, to avoid the unstable condition of the water environment (low or high temperature, high pressure, O_2, pH, and so on).
5. Investigating the possible toxicity of EOs (as single or combined) or EO-based nanomaterials towards aquaculture target organisms and aquatic environment.
6. Determining optimal dose, duration, and mode of administration of EOs for shrimp species.

AUTHOR CONTRIBUTIONS

Xiaoting Zheng: Data curation; methodology; writing – original draft; writing – review and editing. **Peter Bossier:** Conceptualization; funding acquisition; supervision; writing – review and editing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors

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