DOI:10.2478/arl5-2021-0023

Research Article

Antimicrobial Activity of Selected Essential Oils against Bacteria Isolated from Freshwater Fish

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Received August, 2020; Revised March, 2021; Accepted March, 2021

Abstract

The aim of study was to determine the antimicrobial activity of ten essential oils against ten microorganisms isolated from fish. The following essential oils were tested: *Cymbopogon nardus* Rendle, *Citrus limon*, *Leptospermum petersonii* Bailey, *Gaultheria procumbens*, *Pelargonium graveolens*, *Citrus paradisi*, *Citrus aurantifolia*, *Litsea cubeba* Pers., *Citrus reticulata* Blanco, *Vetiveria zizanioides*. The disc diffusion method and microdilution method were used for determination of antimicrobial activity. *Rhodotorula* spp., *Aerococcus* spp., *Aerococcus viridans*, *Enterococcus faecium*, *Enterococcus moraviensis*, *Aeromonas* spp., *Delftia acidovorans*, *Pseudomonas gessardii*, *Yersinia* spp., *Yersinia ruckeri*, *Yersinia enterocolitica* were tested in the present study. The best antimicrobial activity of *Leptospermum petersonii* Bailey were found against *Aerococcus* spp., *Enterococcus faecium*, *E. moraviensis*, *Delftia acidovorans*, *Pseudomonas gessardii*, *Yersinia ruckeri* and *Y. enterocolitica*. *Litsea cubeba* Pers. was effective against *Delftia acidovorans*, *Yersinia enterocolitica*, *Enterococcus faecium* using the disc diffusion method. The best minimal inhibition concentration (MIC 50 - 1.5 µL/mL resp. MIC 90 - 1.6 µL/mL) was found for *Litsea cubeba* Pers. against *Aerococcus* spp. and *Yersinia* spp. using microdilution method.

Keywords: essential oils, yeast, Gram-positive bacteria, Gram-negative bacteria, disc diffusion method, microdilution method

Introduction

Essential oils are multicomponent, hydrophobic plant extracts widely applied in medicine. Their antimicrobial activity has been known for a long time. They are produced from plants by only

physical means - distillation or pressing from the whole plant or from a specific part of the plant [1]. Essential oils are generally extracted by high-pressure or low-pressure distillation. Other extraction methods include solvent extraction, absolute extraction of essential oil, cold pressing, extraction with liquid carbon dioxide or the use of microwaves [2].

Citronella oil (*Cymbopogon nardus aetheroleum*) is an essential oil known for its natural insect

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repellent ability and it is important for the perfume and pharmaceutical industries [3]. The most common applications are antipyretic, diuretic, treatment of mental illnesses, perfume ingredient or as a component of aromatic tea [4].

Lemon silica (*Limonis aetheroleum*) is a hydrophobic substance extracted from lemon peel, which is used as a flavoring in soft drinks and in some foods [5].

Plants referred to as "tea trees" belong to a group of unrelated plant species, including *Camellia sinensis*, *Kunzea ericoides*, *Leptospermum scoparium* and *Leptospermum petersonii*. Some of these species have been used by Australian aborigines and early European settlers for a variety of infectious diseases, including urinary tract infections, intestinal problems, coughs, colds, burns, gum disease [6]. Gaulther oil is extracted from the leaves of the plant *Gaultheria* (family Ericaceae). The main chemical components of this essential oil are phenols (99 % methyl salicylate and gaultherin), tannin and resin. It has anti-inflammatory and antiseptic effects [7].

Pelargonium graveolens is an aromatic shrub. This essential oil is widely used in the perfumery and cosmetics industry [8].

Grapefruit oil has insecticidal and antimicrobial effects [9]. Grapefruit essential oil is obtained from the bark of grapefruit. Its main components are terpenes and oxides of terpenes - alcohols, ethers, aldehydes, ketones and esters [10].

Lime oil (*Citri aurantifoliae aetheroleum*) is one of the most widely used essential oils in the food, pharmaceutical and cosmetic industry. It is commonly added to beverages, bakery products and perfumes [11].

Litsea cubeba silica (*Litsea cubebae aetheroleum*) is widely used as a botanical insecticide, an ingredient in spices, in cosmetics and traditional Chinese medicine [12].

Mandarin green oil (*Citri reticulatae aetheroleum*) is used mainly in the perfumery and food industry because of its aroma. The production of this essential oil begins in September when the fruits are still unripe [13].

Vetiver oil (*Oleum vetiveriae*) has therapeutic, hepatoprotective, antihyperglycemic, antiseptic and antioxidant properties [14].

Bacterial pathogens associated with fish are indigenous and non-indigenous. The non-indigenous contaminants of fish or the habitat include *Escherichia coli*, *Clostridium botulinum*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella*. The indigenous bacterial pathogens are found naturally living in the fish's habitat for example *Vibrio* species and *Aeromonas* species. The bacteria from fish only cause infection when fish

are physiologically unbalanced, nutritionally deficient, or there are other stressors, i.e., poor water quality, overstocking, which allow opportunistic bacterial infections to prevail.

The aim of the study was to determine the antimicrobial activity of essential oils against bacteria isolated from fish using the disc diffusion and the microdilution method. Antimicrobial activity of EO has shown in the present study reveal the possible application of those in prevention and treatment of common bacterial pathogens in freshwater fish.

Material and Methods

Altogether 10 essential oils (Hanus a.s., Nitra, Slovakia) were used in the present study: *Cymbopogon nardus* Rendle, *Citrus limon*, *Leptospermum petersonii* Bailey, *Gaultheria procumbens*, *Pelargonium graveolens*, *Citrus paradisi*, *Citrus aurantifolia*, *Litsea cubeba* Pers., *Citrus reticulata* Blanco, *Vetiveria zizanoides* were tested.

Microorganisms tested

Fish isolates were originated from wild and aquacultured roach (*Rutilus rutilus*) and bream (*Abramis brama*) from skin, gills and gut samples. *Rhodotorula* spp., *Aerococcus* spp., *Aerococcus viridans*, *Enterococcus faecium*, *Enterococcus moraviensis*, *Aeromonas* spp., *Delftia acidovorans*, *Pseudomonas gessardii*, *Yersinia* spp., *Yersinia ruckeri*, *Yersinia enterocolitica* were isolated from fish and identified with MALDI-TOF MS Biotyper.

Antimicrobial activity

Antibacterial activity was examined by agar disc diffusion and microdilution method [16]. In our study, ticarcillin was used as positive control.

Results and Discussion

Comparing the values obtained and evaluating the results, we can conclude that the largest inhibition zone was reached by *Leptospermum petersonii* with an inhibition zone diameter of 34.00 mm against *Delftia acidovorans* (Figures 1-11). The best antimicrobial activity against *Rhodotorula* spp. was found with *L. cubeba* (15.67 mm) and *P. graveolans* EO (10.67 mm). The weak antibacterial activity showed *C. reticulata*, *C. paradisi* and *C. nardus* (3.33 mm) against *Rhodotorula* spp. (Figure 1). *L. petersonii* (25 mm) and *L. cubeba* (20.33 mm) showed the best antimicrobial activity against *Aerococcus* spp. (Figure 2). Similar results were found against *A. viridans* where *L. cubeba* (21.33 mm) showed the best antimicrobial effect (Figure 3). The best antimicrobial activity against *E. faecium* (Figure 4)

was found with *L. Cubeba* EO (27 mm). Similar results showed *L. cubeba* (Figure 5) against *E. moravensis* (30.33 mm). *Aeromonas* spp. (Figure 6) was the most sensitive against *V. zizanioides* (21.33 mm). The best antimicrobial activity of *L. petersonii* (Figure 7) was found against *D. acidovorans* (34 mm). *P. gesessardii* (Figure 8)

was the most sensitive against *L. petersonii* (17 mm). *L. cubeba* (Figure 9) showed the best antimicrobial activity against *Yersinia* spp. (31 mm) and *L. petersonii* (Figure 10-11) showed the best antimicrobial activity against *Y. enterocolitica* (26.33 mm) and *Y. ruckeri* (19.67 mm).

Figure 1. Antimicrobial activity of EOs against *Rhodotorula* spp. in mm

Figure 2. Antimicrobial activity of EOs against *Aerococcus* spp. in mm

Figure 3. Antimicrobial activity of EOs against *Aerococcus viridans* in mm

Figure 4. Antimicrobial activity of EOs against *Enterococcus faecium* in mm

Figure 5. Antimicrobial activity of EOs against *Enterococcus moraviensis* in mm

Figure 6. Antimicrobial activity of EOs against *Aeromonas spp.* in mm

Figure 7. Antimicrobial activity of EOs against *Delftia acidovorans* in mm

Figure 8. Antimicrobial activity of EOs against *Pseudomonas gessardii* in mm

Figure 9. Antimicrobial activity of EOs against *Yersinia* spp. in mm

Figure 10. Antimicrobial activity of EOs against *Yersinia enterocolitica* in mm

Figure 11. Antimicrobial activity of EOs against *Yersinia ruckeri* in mm

Boukhatem et al. [17] investigated the antimicrobial effect of *Pelargonium graveolens* essential oil on various food spoilage microorganisms, including *Rhodotorula glutinis*, whose appropriate inhibition zone was 22.50 mm when disc was soaked in 10 μ L of *Pelargonium graveolens* essential oil. The antimicrobial effect became more significant when the concentration of essential oil increased: 20 μ L of essential oil per disc formed the mean inhibition zone of 27.17 mm and 30 μ L resulted in the mean inhibition zone of 29.83 mm.

Su and Ho [18] tested the antimicrobial activity of *Litsea cubeba* essential oil on various microorganisms. All essential oils (except fruit essential oil) with concentration of 5 μ L per disc showed an inhibition zone against *Bacillus cereus*, *Staphylococcus epidermis* and *Escherichia coli*.

Adel et al. [19] focused on the antimicrobial activity of the skin of the rainbow trout (*Oncorhynchus mykiss*) after a diet supplemented with essential oil from peppermint. They found that *Yersinia ruckeri* had an inhibition zone at 1% of peppermint essential oil 8.9 mm $\pm$ 0.8 mm, at 2% it was 11.2 mm $\pm$ 0.8 mm and at 3% 12.1 mm $\pm$ 1.1 mm.

In general, Gram-positive bacteria were more sensitive to essential oils than to ticarcillin. Ticarcillin was more effective against Gram-negative bacteria than essential oils. Kačániová et al. [20] found that ticarcillin was one of the

effective antimicrobials against *Pseudomonas* spp. in fish. In our study, ticarcillin was proven to be effective against *Pseudomonas gessardii*.

Chotikanatis et al. [21] tested *Delftia acidovorans* for various antibiotics and the bacteria was sensitive to ticarcillin. In our testing, we found that *Delftia acidovorans* was sensitive to ticarcillin, however, *Leptospermum petersonii* exhibited slightly stronger antimicrobial activity in comparison with ticarcillin.

Gogoi et al. [22] found that silica obtained from *Litsea cubeba* fruit has the best antimicrobial activity on *B. cereus* and *S. aureus* with inhibition zone of 7 and 11 mm at the same concentration of 500 μ g/mL.

Wang and Liu [23] showed that *Litsea cubeba* silica inhibited the growth of *B. subtilis*, *E. coli*, *E. faecalis*, *M. albicans*, *P. aeruginosa*, *S. aureus* with inhibition zone from 10.1 to 35.0 mm.

Djenane [24] studied essential oils obtained from Algerian citrus (*Citrus aurantium*, *Citrus sinensis* and *Citrus limon*) on *S. aureus*, a common pathogen of *Sardine pilchardus*. He found that lemon essential oil with an inhibition zone diameter (including a 6 mm disc) of 30.33 mm had the best antimicrobial effects on *S. aureus*.

For the determination of antimicrobial activity by the method of minimum inhibitory concentration, the same essential oils as for the determination of antimicrobial activity were used. All tested essential oils showed antimicrobial activity on the

selected microorganisms (Table 1). Against *Aerococcus* spp., *Litsea cubeba* EO was the most effective at MICs concentrations of 50: 1.50 $\mu\text{L/mL}$ and MICs 90: 1.60 $\mu\text{L/mL}$. Growth of *Yersinia* spp. inhibited *Litsea cubeba* at MICs of 50: 1.50 $\mu\text{L/mL}$ and MICs 90: 1.60 $\mu\text{L/mL}$. Against yeast *Rhodotorula* spp., *Litsea cubeba* was the most effective with a concentration of MICs 50: 1.70 $\mu\text{L/mL}$ and MICs 90: 2.68 $\mu\text{L/mL}$. Against the growth of *Delftia acidovorans*, *Leptospermum petersonii* was the best with MICs 50: 1.70 $\mu\text{L/mL}$ and MICs 90: 2.68 $\mu\text{L/mL}$. For *Enterococcus faecium*, had the best minimum inhibitory

concentration was achieved with *Leptospermum petersonii* with MICs 50: 1.70 $\mu\text{L/mL}$ and MICs 90: 2.68 $\mu\text{L/mL}$. Against *Enterococcus moraviensis*, *Cymbopogon nardus* had the best minimum inhibitory concentration of with MICs 50: 1.70 $\mu\text{L/mL}$ and MICs 90: 2.68 $\mu\text{L/mL}$. Against *Yersinia ruckeri* *Leptospermum petersonii* was highly effective with a concentration of MICs 50: 1.70 $\mu\text{L/mL}$ and MICs 90: 2.68 $\mu\text{L/mL}$. *Litsea cubeba* silica with MICs 50: 1.70 $\mu\text{L/mL}$ and MICs 90: 2.68 $\mu\text{L/mL}$ was most effective for the growth of *Aerococcus viridans*.

Table 1.

Antimicrobial activity of essential oils with microdilution method in $\mu\text{L/mL}$

Tested microorganisms		<i>Aerococcus</i> MK 27	<i>Yersinia</i> MK 28	<i>Rhodotorula</i> MK 31	<i>Delftia acidovorans</i> MK 34	<i>Aeromonas</i> ssp. MK 47	<i>Enterococcus faecium</i> MK 50	<i>Enterococcus moraviensis</i> MK 53	<i>Yersinia ruckeri</i> MK 72	<i>Yersinia enterocolitica</i> MK 77	<i>Pseudomonas gessardii</i> MK 78	<i>Aerococcus viridans</i> MK 83
Essential oil												
<i>Cymbopogon nardus</i>	MIC 50	22.14	34.03	22.14	6.13	20.28	3.00	1.70	3.00	5.40	5.40	3.00
	MIC 90	43.79	56.41	43.79	10.83	51.13	3.20	2.68	3.20	10.55	10.55	3.20
<i>Citrus limon</i>	MIC 50	11.91	11.91	22.14	22.14	6.13	6.81	3.00	6.81	11.82	11.82	11.91
	MIC 90	20.48	20.48	43.79	43.79	10.83	10.59	3.20	10.59	20.25	20.25	20.48
<i>Leptospermum petersonii</i>	MIC 50	1.70	1.70	22.14	1.70	6.00	1.70	6.00	1.70	2.66	3.00	1.79
	MIC 90	2.68	2.68	43.79	2.68	6.38	2.68	6.38	2.68	4.64	3.20	3.79
<i>Gaultheria procumbens</i>	MIC 50	4.00	4.00	11.01	4.00	4.00	6.81	22.14	34.03	11.01	34.03	22.14
	MIC 90	4.64	4.64	18.23	4.64	64.00	10.59	43.79	56.41	18.23	56.41	43.79
<i>Pelargonium graveolens</i>	MIC 50	11.91	11.91	6.81	6.00	9.42	11.01	22.14	6.00	11.01	34.03	2.12
	MIC 90	20.48	20.48	10.59	6.38	22.32	18.23	43.79	6.38	18.23	56.41	3.93
<i>Citrus paradisi</i>	MIC 50	22.14	34.03	22.14	22.14	13.45	6.81	22.14	34.03	7.59	13.45	11.91
	MIC 90	43.79	56.41	43.79	43.79	14.47	10.59	43.79	56.41	14.59	14.47	20.48
<i>Citrus aurantifolia</i>	MIC 50	34.03	34.03	22.14	22.14	11.01	6.81	22.14	34.03	22.14	34.03	22.14
	MIC 90	56.41	56.41	43.79	43.79	18.23	10.59	43.79	56.41	43.79	56.41	43.79
<i>Litsea cubeba</i>	MIC 50	1.50	1.50	1.70	2.00	4.69	4.00	4.00	2.00	4.00	4.69	1.70
	MIC 90	1.60	1.60	2.68	2.24	7.39	4.64	4.64	2.24	4.64	7.39	2.68
<i>Citrus reticulata</i>	MIC 50	22.14	34.03	22.14	22.14	34.03	22.14	11.01	34.03	34.03	34.03	34.03
	MIC 90	43.79	56.41	43.79	43.79	56.41	43.79	18.23	56.41	56.41	56.41	56.41
<i>Vetiveria zizanioides</i>	MIC 50	3.00	3.00	22.14	6.13	10.83	6.81	22.14	9.42	11.01	20.28	3.00
	MIC 90	3.20	3.20	43.79	10.83	22.14	10.59	43.79	22.32	18.23	51.13	3.20

Singh et al. [25] in a study of the antimicrobial activity of *Cymbopogon nardus* essential oil found that it inhibited the growth of 36 species of aqueous pathogens with MICs from 0.24 $\mu\text{g/mL}$ for *Pseudomonas* and *Salmonella* to 0.97 $\mu\text{g/mL}$ for *Edwardsiella* and *Aeromonas* strains. *Cymbopogon nardus* has been shown to be effective against *S. aureus*, *Bacillus subtilis*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. Despite of testing of another *Pseudomonas* species - *Pseudomonas gessardii*, also in our

case *Cymbopogon nardus* was very effective against the bacteria.

Nguyen et al. [26] studied the antimicrobial activity of *Litsea cubeba* essential oil on *Aeromonas hydrophila* isolated from *Caprinus caprio*. They found that essential oil (with linalool content of 95%) had bactericidal effects against *Aeromonas hydrophila* with MIC of 0.96 $\mu\text{g/mL}$. The study concluded that adding *Litsea cubeba* (with an increased linalool content) to the diet in an amount of 4-8% would reduce the use of antimicrobials to treat diseases caused by

Aeromonas hydrophila and increase the immunity of *Cyprinus carpio*.

Wei and Wee [27] discussed the antimicrobial activity of *Cymbopogon nardus* essential oil on bacteria isolated from the internal organs of aquatic animals (*Aeromonas* spp., *Pseudomonas* spp., *Aeromonas hydrophila*, *Yersinia enterocolitica*, and other microorganisms). In *Aeromonas* spp., described MICs were from 0.488 µg/mL to 0.977 µg/mL, for *Pseudomonas* spp. - MICs 0.244 µg/mL, *Aeromonas hydrophila* and *Yersinia enterocolitica* - MICs 0.488 µg/mL.

Adel et al. [19] investigated the MIC of peppermint essential oil on mucus of Rainbow trout containing, among other bacteria, *Yersinia ruckeri*. At a concentration of 1% essential oil in the diet, the MICs of *Yersinia ruckeri* was 125 µg/mL, at 2% the MICs was 100 µg/mL and at 3% the MICs was 75 µg/mL.

Conclusions

The antimicrobial activity of plant extracts was determined by the disc diffusion method. Comparing the values found and evaluating the results, it could be concluded that the largest inhibition zone was reached by *Leptospermum petersonii*. *Citrus paradise* essential oil showed the smallest zone of inhibition. When tested for antimicrobial activity by the minimum inhibitory concentration method, the essential oils showed activity against all microorganisms. The most effective was *Litsea cubeba* and followed by essential oil of *Leptospermum petersonii*. *L. petersonii* in our study showed correlation between disc diffusion method and minimum inhibitory concentration in the inhibitory effect against tested microorganisms.

Acknowledgements

This work has been supported by the grant APVV SK-BY-RD-19-0014 "The formulation of novel compositions and properties study of the polysaccharides based edible films and coatings with antimicrobial and antioxidant plant additives."

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