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MICROBIOLOGY

Essential oil of *Mentha suaveolens* Ehrh., composition and antibacterial activity against bacterial fish pathogens

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Abstract: In this study, it was determinated the essential oil of cultivated apple mint, *Mentha suaveolens* Ehrh. composition and *in vitro* antibacterial activity of against 11 fish pathogen bacteria including Gram-positive (*Staphylococcus warneri, Staphylococcus* sp., *Lactococcus garvieae*, *Vagococus salmoninarum*) and Gram-negative (*Aeromonas hydrophila*, *Aeromonas sobria Aeromonas cavieae, Vibrio anguillarum, Pseudomonas aeroginosa, Yersinia ruckeri, Edwardsiella tarda*) by using agar diffusion assay. The main component of *M. suaveolence* oil was obtained as piperitenone oxide. The essential oil exhibited strong inhibitory activity such as inhibition zone sizes: 30-50mm at 250-1000 µL mL^{-1} concentrations against *V. anguillarum*; 16-20mm at 31.25-125 µL mL^{-1} concentrations against *P. aeroginosa*; 15-18mm at 500-1000 µL mL^{-1} concentrations against *A. sobria*. However, it was found to be moderately effective against *E. tarda* (8-15 mm), *Y. ruckeri* (9-12mm), *S. warneri* (9-10mm), *V. salmoninarum* (9mm) and *Staphylococcus* sp. (8-9mm). The essential oil showed weak inhibitory activity against *A. cavieae* (5-8), *A. hydrophila* (6-7mm), *L. garvieae* (5-7mm). Thus, effect of essential oil of *M. suaveolens* on immune response and disease resistance against *Vibrio anguillarum, A. sobria* and *P. aeroginosa* should be investigated *in vivo* in cultured fish species in future studies.

Key words: *Mentha suaveolens,* essential oil, bacterial fish pathogens, *in vitro* antibacterial effect.

INTRODUCTION

In aquaculture, antimicrobial agents are widely used to prevent fish diseases caused by infectious agents. It is known that the unconscious use of antimicrobial agents in the treatment of fish diseases causes many negative effects on environment, fish and human health (Schnick et al. 1997). Therefore, the use of herbal products to control of fish pathogens in aquaculture is an alternative and current practice.

Phytochemicals such as phenolics, polysaccharides, proteoglycans and flavonoids may play a role in preventing or controlling infectious pathogens (Citarasu 2010). Many essential oils and plant extracts have been studied and shown to be effective against fish pathogens (Abutbul et al. 2005, Bansemir et al. 2006, Ekici et al. 2011, Haniffa & Kavitha 2012, Al Laham & Al Fadel 2014, Ontas et al. 2016, Diler et al. 2017, Metin et al. 2017). These compounds may constitute alternative prophylactic and therapeutic agents in aquaculture because of their antibacterial properties (Turker & Yıldırım 2015).

Mentha, a genus of the Lamiaceae family, is represented by about 31 species and 13 natural hybrids, mainly perennial herbs, growing wildly in damp or wet places throughout Europe, Asia, Africa, Australia and North America (Kumar et al. 2011). Mints are fast growing, invasive and generally tolerate a wide range of agro-climatic

conditions (Božović et al. 2015). The most common and popular mints for cultivation are peppermint (*M. piperita*), spearmint (*Mentha spicata*) and apple mint (*M. suaveolens*) (Chauhan & Agarwal 2013). *Mentha suaveolens* is an aromatic herb and has a spearmint flavor, which is used in the Mediterranean areas in the traditional medicine. It has an extensive range of biological activities, including cytotoxic, antimicrobial, antioxidant, anti-inflammatory, hypotensive, analgesic, sedative and insecticidal properties (Moreno et al. 2002, El-Kashoury et al. 2014, Božović et al. 2015).

The essential oil of *M. suaveolens* includes piperitone oxide and piperitenone oxide as major components. Other chemotypes of this species showed high percentage of alcohols (menthol) or ketones (pulegone, piperitenone and dihydrocarvone) (Oumzil et al. 2002, Sutour et al. 2008, El-Kashoury et al. 2014, Božović et al. 2015).

There are a few studies on chemical the antibacterial and anti-fungal effects of *M. suaveolens* (Oumzil et al. 2002, Sutour et al. 2008, El-Kashoury et al. 2014, Božović et al. 2015). Antibacterial effects of ethanolic extract and fractions of *M. suaveolens* against human pathogenic bacteria *Staphylococcus aureus* (ATCC12600), *Streptococcus faecalis* (ATCC19433), *Bacillus subtilis* (ATCC6051), *Escherichia coli* (ATCC11775), *Neisseria gonorrhoeae* (ATCC19424), *Pseudomonas aeruginosa* (ATCC10145) (El-Kashoury et al. 2014) and major components of *Mentha suaveolens* essential oils against *Staphylococcus aureus*, *Staphylococcus simulans*, *Staphylococcus saprophyticus*, *Enterococcus* sp., *Bacillus anthracis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Proteus mirabilis*, *Enterobacter avium*, *Citrobacter freundii* (Oumzil et al. 2002) has been reported in the previous studies. Moreover, there is no study on antimicrobial activity of *M. suaveolens* against fish pathogens. Thus, in this study was identified the major constituents of essential oil of *M. suaveolens* and investigated antibacterial effects against fish pathogens.

MATERIALS AND METHODS

Plant material

Essential oil of "apple mint", a variety of *Mentha suaveolence* Ehrh., was used in the research. Cuttings from rhizomes of the cultivar were planted in experimental pilots of TARUM in Isparta University of Applied Sciences. Fertilizer was applied after planting at the rate of 50 kg ha^{-1} nitrogen, and 50 kg ha^{-1} P_2O_5. Plots were watered with drip irrigation system and kept weed free by hand hoeing. Plants were harvested at floral initiation (in mid-July). Fresh material after harvest was dried at 35ºC in drier cabin for essential oil distillation.

Preparation of essential oil

The essential oil was obtained with the distillation process using a Clevenger apparatus. Distilled water (2000 mL) was used for the distillation of dried plant samples (100 g). Distillation time was approximately 2 h at boiling point. The oil phase was separated and stored in dark glass bottle at 4 °C in fridge until both antibacterial studies and Gas chromatography-mass spectroscopy (GC-MS) analysis.

Gas chromatography-mass spectroscopy analysis of the essential oil

The GC-MS TIC (total ion chromatogram) analysis was performed using a Hewlett-Packard 6890 series gas chromatograph (Perkin Elmer (PE) Auto System XL, USA), fitted with a flame ionization detector (FID). The PE Auto System XL gas chromatograph was employed under the following conditions: capillary column, CPWax

52CB (50 m x 0.32 mm; film thickness ¼ 0.25 Im); oven temperature programmed, 60–220°C raised at a rate of 2 °C min^{-1} and then held at 220 °C for 20 min; injector and detector temperatures, 240°C; carrier gas, helium at flow rate of 40 mL min^{-1} ; and split ratio, 1/20 mL min^{-1}. Relative percentage amounts were calculated from chromatograms by the Turbo Crom Navigator computer program (Baydar et al. 2004).

In vitro antibacterial activity

Fish pathogen bacteria were obtained from the culture collection in Isparta University of Applied Sciences, Microbiology Laboratory of Fisheries Faculty. Bacteria strains; *Staphylococcus warneri, Staphylococcus* sp., *Lactococcus garvieae*, *Vagococus salmoninarum, Vibrio anguillarum* from rainbow trout; *Aeromonas sobria* from yellow tail cichlid; *Aeromonas hydrophila* ATCC 7966, *Aeromonas cavieae* ATCC 15468, *Pseudomonas aeroginosa* ATCC27953, *Yersinia ruckeri, Edwardsiella tarda* DSMZ 300052 were used in the test.

Agar disc diffusion method was performed for the antibacterial screening of the essential oil of *M. suaveolens* Ehrh. For the test, bacteria were grown in Tryptic Soy Broth for 24 h at 25 °C and then 100 µL of each culture transferred into 100 mL cooled (to 45°C) Tryptic Soy Agar. After solidifying and drying for 15–20 minute, wells were punched (diameter = 3 mm) and 25 µL of different concentrations prepared with 96% ethanol (the test concentrations: 1000, 500, 250, 125, 62.5, 31.25 µL mL^{-1}) added to wells in triplicates. Controls were prepared using 96% ethanol. Plates were incubated at 25°C for 24h and observed for clearing zones around the wells (Andrews 2004). The antibacterial activity of plant extracts was interpreted as proposed by Bansemir et al. (2006). Inhibition zones >15 mm were categorized as strong activity, from 8 to 15 mm as moderate activity, and from 1 to 8 mm as weak activity.

RESULTS AND DISCUSSION

M. suaveolens essential oil include piperitone oxide and piperitenone oxide as major components (Božović et al. 2015). Other chemotypes of this species showed high percentage of alcohols (menthol) or ketones (pulegone, piperitenone and dihydrocarvone) (Oumzil et al. 2002, Sutour et al. 2008, El-Kashoury et al. 2014, Božović et al. 2015). In the present study, the chemical composition of *M. suaveolence* essential oil was characterized by one hundred different components and were determined 10 volatile constituents, representing 87.68% of the total composition (Table I). The main component of *M. suaveolence* oil was piperitenone oxide (66.23%).

The antimicrobial activities of the essential oil of *M. suaveolence* against fish pathogens were represented in Table II. The essential oil exhibited strong inhibitory activity against *V. anguillarum* (30-50mm) at 250-1000 µL mL^{-1} concentrations against; *P. aeroginosa*

Table I. Major chemical constituents of essential oil of the *Mentha suaveolence.*

Components	R. Time	Area %
Piperitenone oxide	31.409	66.23
Germacrene-d	38.078	5.48
Limonene	10.366	3.63
1 Octen 3YL Actate	14.542	2.75
Ledene	44.855	2.38
2-Cyclopenten-1-one, 3-methyl – 2- (2-pentenyl)-, (Z)-	32.724	1.83
Farmesene<(E),beta->	36.570	1.60
cis-Ocimene	10.665	1.34
2-Beta- Pinene	8.106	1.27
Beta-Myrcene	8.546	1.17

(16-20mm) at 31.25-125 µL mL^{-1} concentrations against; *A. sobria* (15-18mm) at 500-1000 µL mL^{-1} concentrations. In addition, the essential oil was found to be moderately effective against *E.tarda* (8-15 mm at all concentrations), *Y. ruckeri* (9-12mm at 125-1000 µL mL^{-1}), *S. warneri* (9-10mm at 125-1000 µL mL^{-1}), *V. salmoninarum* (9mm at 250 and 500 µL mL^{-1}), *Staphylococcus* sp. (8-9mm at 250-1000 µL mL^{-1}) in the present study. Similarly, in the previous studies noted *Mentha piperita* essential oil had moderate effect against *Y. ruckeri* and *V. salmoninarum* (Adel et al. 2016, Metin et al. 2017).

In the present study, *M. suaveolence* essential oil showed weak inhibitory activity against *A. hydrophila*. In contrast, Birinci Yıldırım & Türker (2018) noted strong antibacterial activity of *M. piperita* against *A. hydrophila*.

CONCLUSION

In conclusion, it was found that the main component of *M. suaveolence* essential oil was piperitenone oxide (66.23%) in this study. *M. suaveolence* essential oil exhibited strong antibacterial effect against *V. anguillarum* (30-50mm), *P. aeroginosa* (16-20mm), *A. sobria* (15-18mm); moderately antibacterial effect against *E.tarda* (8-15 mm), *Y. ruckeri* (9-12mm), *S. warneri* (9-10mm), *V. salmoninarum* (9mm), *Staphylococcus* sp. (8-9mm) and weak antibacterial effect against *A. cavieae* (5-8), *A. hydrophila* (6-7mm), *L. garvieae* (5-7mm). However, effect on immune response and disease resistance against *Vibrio anguillarum*, *A. sobria* and *P. aeroginosa* of essential oil of *M. suaveolens* should be investigated *in vivo* in cultured fish species in future studies.

Table II. Antimicrobial activities of *M. suaveolence* essential oil against fish pathogen bacteria (inhibition zone, mm).

Pathogens	Concentrations (µL mL^{-1})					
	1000	**500**	**250**	**125**	**62.5**	**31.25**
V. anguillarum	50	40	30	11	14	10
P. aeroginosa	6	13	9	17	16	20
A. sobria	18	15	12	10	8	6
E. tarda	13	15	13	11	10	8
Y. ruckeri	9	12	12	10	6	6
S. warneri	10	10	9	9	-	-
V. salmoninarum	7	9	9	7	7	6
Staphylococcus sp.	8	9	8	6	6	-
A. cavieae	5	7	8	8	6	7
A. hydrophila	7	7	7	6	-	-
L. garvieae	5	7	7	6	6	6

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SM, BID, IT and OD participated in the planning of the study, ***in vitro*** antibacterial assays and drafting the manuscript. All authors read and approved the final manuscript.