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ANTIBACTERIAL PROPERTIES OF COMMERCIAL PEPPERMINT ESSENTIAL OIL AGAINST SOME GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

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ABSTRACT

The authors of this article conducted research and studied the antibacterial properties of commercial peppermint essential oil (PEO) against several gram-positive and gram-negative bacteria provided by Polish manufacturers of essential oils (Naturalne Aromaty sp. z o.o., Klaj, Poland). Therefore, to conduct research with the aim of to study the antibacterial properties of commercial peppermint essential oil (PEO), an antimicrobial susceptibility test (Kirby-Bauer diffusion test) was used to measure diameters of bacterial growth inhibition zones). In the current study, Gram-negative strains such as Escherichia coli (Migula) Castellani and Chalmers (ATCC® 25922™), Escherichia coli (Migula) Castellani and Chalmers (ATCC® 35218™), Pseudomonas aeruginosa (Schroeter) Migula (ATCC® 27853™) and Gram-positive strains such as Staphylococcus aureus subsp. aureus Rosenbach (ATCC® 29213™), methicillin-resistant (MRSA), mecA positive Staphylococcus aureus (NCTC® 12493), Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (resistant to vancomycin; sensitive to teicoplanin) and Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) were used. Results of the current study revealed that resistant to the PEO were Gram-negative bacterial strains, such as E. coli (Migula) Castellani and Chalmers (ATCC® 35218™) and P. aeruginosa (Schroeter) Migula (ATCC® 27853™) strains. The authors found that the diameters of the inhibition zones after application of PEO were similar to the control samples (96% ethanol). It was also found that after the application of REO, the increase in the diameters of the inhibition zones was 60.3% ($p < 0.05$) for the Escherichia coli strain (Migula) Castellani and Chalmers (ATCC® 25922™) compared to control samples (96% ethanol). Accordingly, Gram-positive strains such as S. aureus subsp. aureus Rosenbach (ATCC® 29213™) and methicillin-resistant S. aureus (NCTC® 12493) were equally resistant to PEO, similarly. On the other hand, Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) and Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) were sensitive to PEO. After the application of PEO, the largest diameters of inhibition zones were observed for the E. faecalis strain. The results suggest that commercial peppermint essential oil provided by Polish essential oil manufacturers (Naturalne Aromaty sp. z o.o., Kłaj, Poland) possesses some noteworthy antimicrobial properties. In vivo studies are necessary to calculate the effective dose of EOs and determine their possible side effects and toxicity.

Key words: commercial peppermint essential oil, antibacterial activity, zones of inhibition, disc diffusion technique Kirby-Bauer.

INTRODUCTION

Currently, a huge number of microorganisms, primarily hospital-acquired strains, pose a threat to life and health, due to the widespread prevalence of multidrug resistance and, as a consequence, difficulties in selecting adequate chemotherapy [21]. One of the reasons for its formation is the massive use of antimicrobial drugs, which in some cases leads to undesirable consequences: dysbiosis, anaphylactic shock, and the formation of cross-resistance [25]. In this regard, a search is underway for new drugs that, on the one hand, have antimicrobial activity with a mechanism different from conventional antibiotics, and, on the

other, are free of side effects. Recently, there has been increased interest in essential oil plants with antimicrobial activity. Such plants include peppermint (*Mentha × piperita* L.) [11,17]. This plant does not grow wild on the territory of our country, but the great need for it is satisfied through widely used cultivation.

Some species of the Genus Mentha, a genus of plants in the taxonomic family Lamiaceae (mint family), used commercially are well described with a developed cultivation process. Due to the presence of significant amounts of antioxidant phenolic compounds, extracts of this genus are traditionally used in food and are highly valued [26,38]. In addition to traditional food flavoring uses, Peppermint (*Mentha × piperita* L.) is well recognized for its traditional use to treat fever, cold, digestive, anti-viral, anti-fungal, oral mucosa, and throat in flammation [16]. Strong antioxidant, antimicrobial, antiviral, anti-inflammatory, biopesticidal, larvicidal, anticancer, radioprotective effects of Mentha species have been established and the authors report that they demonstrate genotoxicity and antidiabetic activity, which suggests the development of drugs from *Mentha* [16].

The composition of peppermint essential oil (PEO) includes menthol, menthone, neomenthol and iso-menthone, which is a mixture of bioactive secondary metabolites that have anti-inflammatory, antibacterial, antiviral, immunomodulatory, antitumor, neuroprotective, anti-fatigue and antioxidant activities. Cumulative evidence suggests that PEO may pharmacologically protect the gastrointestinal tract, liver, kidney, skin, respiratory, brain, and nervous systems, as well as exert hypoglycemic and lipid-lowering effects [38].

PEO is widely used in alternative medicine [11,17]. The extracts from the peppermint herb have various properties, including antiseptic [6]. Literary sources repeatedly mention the pronounced antimicrobial, in particular fungicidal, effect of peppermint [7, 22, 33, 34, 38]. Herbs included in feed additives can improve both growth performance and antioxidant activity of animals, depending on the content of phenolic compounds in them [1,10]. Authors Abdel-Waret, I. Giannenas, and their colleagues [1, 10] foundhat peppermint leaves can be used as an effective novel nutritional bio-agent of up to 15 g/kg to improve the performance of broiler chicks, mainly due to them active component [1, 10]. The authors Patra and colleagues [19, 20] studied the effect of two practically significant doses of menthol-rich plant bioactive lipid compounds (PBLC) on fermentation, microbial community composition and their interaction in the rumen of sheep and found that dietary treatment with PBLC had little effect on rumen fermentation and microbiota, but influenced the associations between some microbial taxa and short-chain fatty acids. Scientific [8] studies have found that phytogenic compounds (angelica root, capsaicin, gentian root, garlic oil, ginger extract, L-menthol, peppermint oil, thyme oil and thymol) showed various dose-dependent effects that beneficially influence chewing behavior by modulating fermentation and reducing rumen acidosis in dairy cows fed high grain diets [8]. Blood parameters associated with rumen growth or enzymatic activity in Holstein bulls were minimally affected by the addition of menthol [35].

In the current study, the antibacterial properties of commercial PEO provided by Polish essential oil manufacturers (Naturalne Aromaty sp. z o.o., Kłaj, Poland) against some Gram-positive and Gram-negative bacteria were studied. For this purpose, the authors was used an antimicrobial susceptibility test (disk - diffusion Kirby-Bauer test to measure the diameters of zones of bacterial growth inhibition).

PURPOSE OF THE WORK

Investigate of the antibacterial properties of commercial peppermint essential oil (PEO) provided by Polish essential oil manufacturers (Naturalne Aromaty sp. z o.o., Klaj, Poland), against several gram-positive and gram-negative bacteria.

MATERIALS AND METHODOLOGY

Peppermint essential oil

Commercial peppermint essential oil (PEO) was provided by Polish essential oil producers (Naturalne Aromaty sp. z o.o., Klaj, Poland). The sample that was tested did not contain additives or solvents and was confirmed by the manufacturers as natural. Samples in reusable vials were stored at 5°C in the dark but allowed to reach room temperature before testing. No information was available on geographical origin.

Disc diffusion method for determining the antibacterial activity of essential oil

Using the Kirby-Bauer disk diffusion method [4], the authors conducted a study of the antibacterial activity of peppermint EO in vitro [4]. In the current study, Gram-negative strains such as *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™), *Escherichia coli* (Migula) Castellani and Chalmers

(ATCC® 35218™), *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™) and Gram-positive strains such as *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 29213™), methicillin-resistant (MRSA), mecA positive *Staphylococcus aureus* (NCTC® 12493), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (resistant to vancomycin; sensitive to teicoplanin) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) were used.

The strains were inoculated onto Mueller-Hinton (MH) agar dishes. Sterile filter paper discs soaked in PEO were placed on each culture dishes. At 37°C for 24 hours, we incubated bacterial isolates with PEO. In the next stage, we investigated the zone of inhibition of the antibacterial activity of PEO in Petri dishes. In every experiment used a control disc soaked in 96% ethanol. At the end of the 24-h period, the inhibition zones formed were measured in millimetres using the vernier. Eight replicates (n = 8) were analyzed for each strain. The Petri dishes were observed and photographs were taken. A clear zone of inhibition around the PEO-containing discs indicated the susceptibility of the test organisms to PEO and the diameter of the clear zone was taken as an indicator of susceptibility.

The authors of the article was determined and averaged the diameters of the zones. We used the following zone diameter criteria: Sussceptible (S) ≥ 15 mm, Intermediate (I) = 10–15 mm and Resistant (R) ≤ 10 mm to determine the sensitivity or resistance of bacteria to the tested phytochemicals [33].

Statistical analysis

Based on the obtained data, statistical analysis was performed using the mean ± standard error of the mean (S.E.M.) All variables were randomized according to the phytochemical activity of the PEO tested. All statistical calculation was performed on separate data from each strain [36]. The data were analyzed using a one-way analysis of variance (ANOVA) using Statistica v. 13.3 software (TIBCO Software Inc., USA) [33,37].

RESULTS AND DISCUSSION

The antibacterial activity induced by PEO estimated as diameters of growth inhibition zones of examined Gram-positive and Gram-negative strains was presented in Figures 1 and 2.

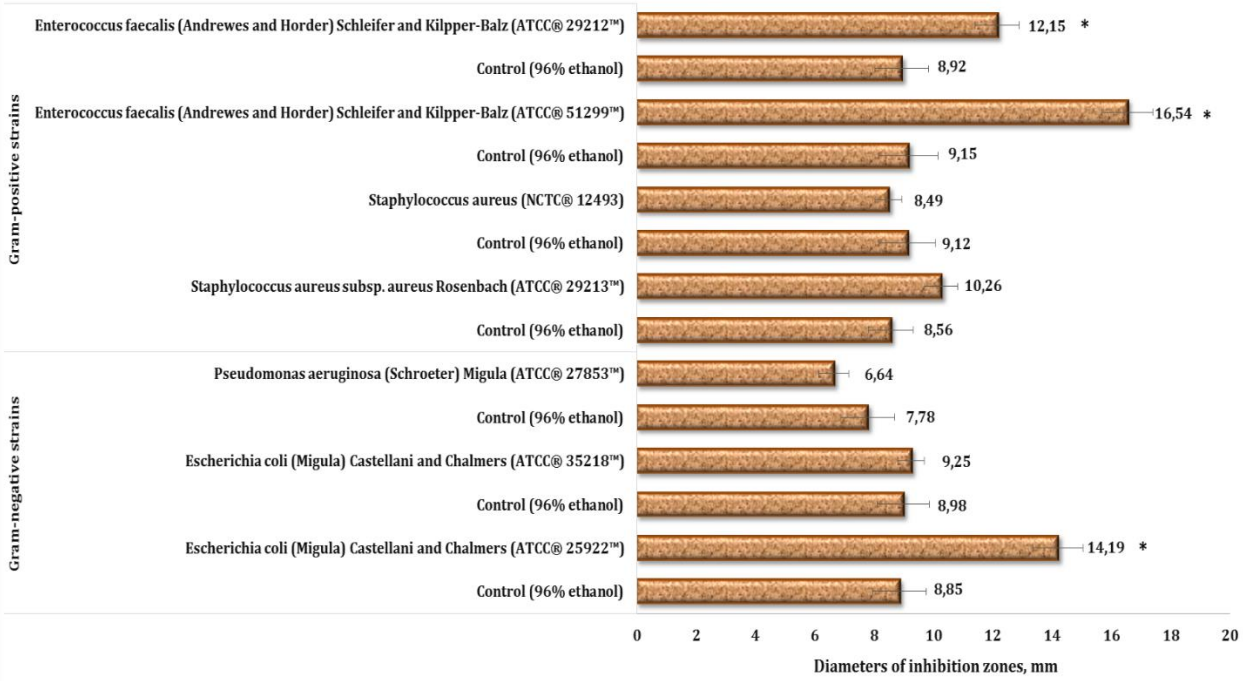

Figure 1. The antibacterial activity induced by peppermint essential oil estimated as diameters of growth inhibition zones of examined Gram-positive and Gram-negative strains.
The data were presented as the mean ± the standard error of the mean (S.E.M.).

* denote significant differences between the control (96% ethanol) and peppermint EO ($p < 0.05$).

As a result of research, it was found that gram-negative strains such as *E. coli* and *P.aeruginosa* were resistant to the PEO. The diameters of inhibition zones for *E. coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain after the application of PEO were increased to (14.19 ±0.84 mm) compared to the 96% ethanol as control samples (8.85 ±0.91 mm). Similar results were obtained for *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™) strain. The diameters of inhibition zones after the application of PEO were (9.25 ± 0.45 mm) compared to the 96% ethanol as control samples (8.98 ± 0.88 mm). *P. aeruginosa* (Schroeter) Migula (ATCC® 27853™) strain was also resistant to the PEO. The diameters of inhibition zones after the application of PEO were (6.64 ±0.51 mm) compared to the 96% ethanol as control samples (7.78 ±0.91 mm) (Figure 1).

Gram-positive strains were sensitive to the PEO compared to the Gram-negative strains. *S. aureus* strains exhibited mild activity to the PEO. *S. aureus* (NCTC® 12493) strain was less sensitive then *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) strain. Diameters of inhibition zones after application of PEO were (10.26 ±0.56 mm) compared to the 96% ethanol as control samples (8.56 ±0.75 mm) for *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) strain and (8.49 ±0.45 mm) compared to the 96% ethanol as control samples (9.12 ±0.95 mm) for *S. aureus* (NCTC® 12493) strain. The increase of diameters of inhibition zones after the application of PEO was 19.9% ($p < 0.05$) for *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) strain compared to the control samples (96% ethanol) (Figure 1).

E. faecalis strains were more sensitive to PEO (Figure 1). Diameters of inhibition zones after application of PEO were (16.54 ±0.85 mm) compared to the 96% ethanol as control samples (9.15 ±0.99 mm) for *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) strain and (12.15 ±0.74 mm) compared to the 96% ethanol as control samples (8.92 ±0.91 mm) for *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) strain. The increase of diameters of inhibition zones after the application of PEO was 80.8% ($p < 0.05$) and 36.2% ($p < 0.05$) for *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) strains, respectively (Figure 1).

Detailed photos regarding the zones of inhibition by the PEO against Gram-positive and Gram-negative bacterial strains were recorded and presented in Figure 2.

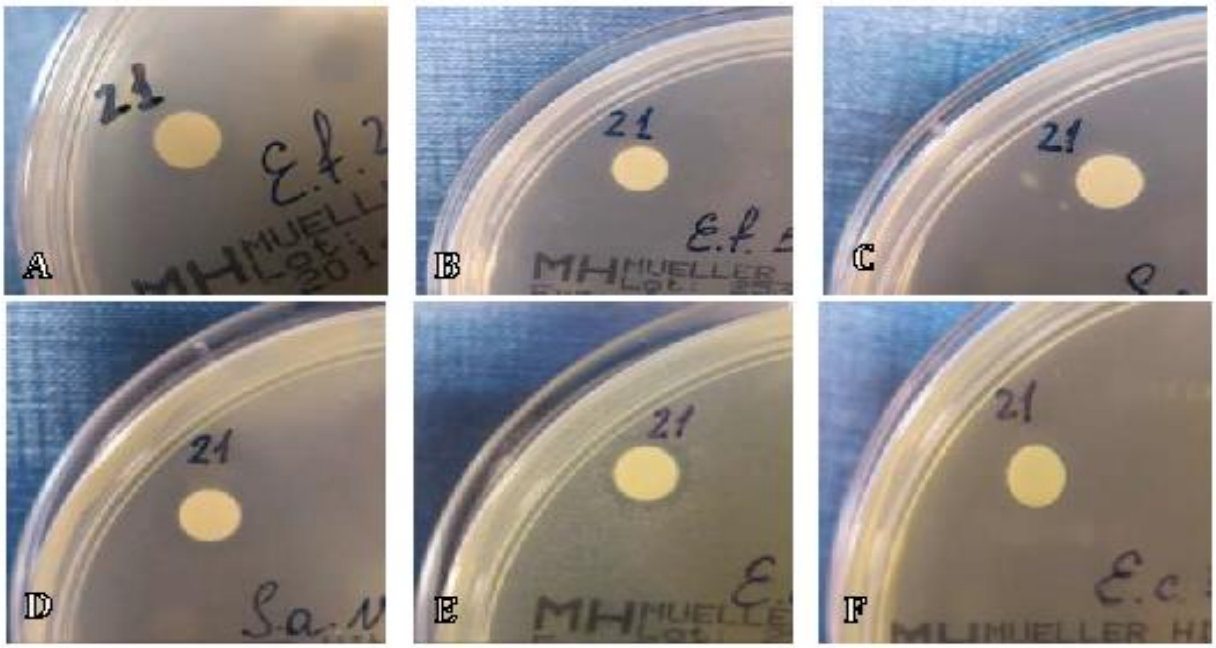

Figure 2: Inhibition growth zones induced by peppermint essential oil against *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) (A), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (B), *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) (C), *Staphylococcus aureus* (NCTC® 12493) (D), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) (E), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 35218™) (F).

In line with our previous studies according to the antibacterial potential of different plant extracts and EOs, in the current study, we examined the antibacterial potential of commercial peppermint essential oil against Gram-positive and Gram-negative bacterial strains. Resistant to the PEO were Gram-negative bacterial strains, such as *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™) and *P. aeruginosa*

(Schroeter) Migula (ATCC® 27853™) strains. The diameters of inhibition zones after the application of PEO were similar to control samples (96% ethanol). The increase of diameters of inhibition zones after the application of PEO was 60.3% ($p < 0.05$) for *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain compared to the control samples (96% ethanol). Similarly, Gram-positive strains such as *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) and methicillin-resistant *S. aureus* (NCTC® 12493) were resistant to the PEO action. On the other hand, *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) were sensitive to PEO. The highest diameters of inhibition zones after the application of PEO were observed for *E. faecalis* strains (Figures 1 and 2).

Based on some studies, the essential oils from mint species possess antimicrobial activity against microbial isolates tested and thus can be a good source of natural antimicrobial agents. For example, research scientists Zaidi and Dahiya [36] studied the antimicrobial activity in vitro, they performed phytochemical analysis and evaluated the total phenolic content of essential oil from Mentha spicata and Mentha piperita. The antimicrobial potential of mint species essential oils was evaluated by agar well diffusion method against selected clinical isolates. The antibacterial effect was investigated using the TLC-bioautographic method.

The antimicrobial activity of mint species essential oils was assessed on 11 bacterial and 4 fungal clinical isolates. Both the essential oils showed maximum activity against *S. aureus* 1, producing the maximum zone of inhibition of (21 ± 0.09 mm) in *Mentha spicata* and (19.2 ± 0.07 mm) in *Mentha piperita*.

Similarly, scientist research Desam and his colleagues [9] studied the chemical constituents and antibacterial activity of essential oils from the areal parts of Mentha × piperita L. essential oil. The research results of these authors revealed that the essential oil showed the highest antibacterial activity against microorganisms. Also, as a result of research, it was found that Staphylococcus aureus (42.44 ± 0.10 mm), Micrococcus flavus (40.01 ± 0.10 mm), Bacillus subtilis (38.18 ± 0.11 mm), Staphylococcus epidermidis (35.14 ± 0.08 mm), and Salmonella enteritidis (30.12 ± 0.12 mm) showed good inhibition zones against Mentha × piperita, according to the disc-diffusion method.

Moreover, the essential oils showed less inhibition zones against *Listeria monocytogenes* (17.20 ± 0.04 mm), *Enterobacter cloacae* (16.14 ± 0.13 mm), *Clavibacter michiganense* (15.05 ± 0.08 mm), *Klebsiella pneumonia* (14.24 ± 0.07 mm), *Streptococcus pyogenes* (13.26 ± 0.03 mm), *Acinetobacter baumannii* (12.08 ± 0.18 mm), *Proteus mirabilis* (12.13 ± 0.12 mm), *Enterobacter aerogenes* (12.17 ± 0.07 mm), *Bacillus megaterium* (10.03 ± 0.05 mm), *Bukholdria cepacia* (8.15 ± 0.10 mm), *Citrobacter freundii* (6.12 ± 0.02 mm), *Proteus vulgaris* (4.02 ± 0.05 mm), *Xanthomonas campestris* (3.18 ± 0.07 mm), and *Pseudomonas syringae* (3.12 ± 0.02 mm). The disc-diffusion method and other microorganisms showed moderate antibacterial activity against essential oils. The essential oil showed strong antifungal activity against yeast and fungi strains. *Alternaria alternaria* (38.16 ± 0.10 mm), *Fusarium tabacinum* (35.24 ± 0.03 mm), *Penicillium* spp. (34.10 ± 0.02 mm), *Fusarium oxyporum* (33.44 ± 0.06 mm), and *Aspergillus fumigates* (30.08 ± 0.08 mm) all show strong antifungal activity against essential oils. Furthermore, *Aspergillus variecolor* (17.23 ± 0.23), *Candida albicans* (16.34 ± 0.26), *Moliniana fructicola* (16.32 ± 0.03), *Sclorotinia selerotiorum* (15.58 ± 0.06), *Trichophyton rubrum* (15.34 ± 0.03), *Trichophyton mentagrophytes* (11.55 ± 0.06), *Sclorotinia minor* (10.22 ± 0.17), and *Fusarium solani* (10.22 ± 0.05) showed less antifungal activity against the essential oils, while *Rhizoctomia saloni*, *Fusarium acuminatum*, *Cladosporium herbarum*, and *Aspergillus flavus* show a moderate antifungal activity. The maximal and minimal inhibition concentration values were in the range of (10.22 ± 0.17) to (38.16 ± 0.10) and (0.50 ± 0.03) to (10.0 ± 0.14 μg/ml), for yeast and fungi respectively [9].

Also, PEO can be used as a supplement for caries prevention compounds. The study by Shazdehahmadi and co-workers [9] explored the antibacterial effects of *Mentha longifolia* essential oil on *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacillus* as cariogenic microorganisms and determined the compounds in it. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) ratios for *S. mutans* were 3.12% and 6.25%, for *S. sobrinus* were 6.25% and 12.5%, and *Lactobacillus* were 3.12% and 6.25%, respectively. Chemical analysis of *M. longifolia* essential oil showed 34 various compounds. *M. longifolia* essential oil has both growth-inhibitory and bactericidal effects on all three species of bacteria [9].

Our research results confirm the data obtained by the author Shazdehahmadi and his colleagues [29] indicating that this antibacterial effect was found to be similar in relation to *S. mutans* and *Lactobacillus*, which was higher than that of S. sobrinus thus, it can be used as a supplementary for caries prevention compounds [29].

Authors Işcan and co-workers [14] have conducted the antimicrobial screening of Mentha piperita essential oils against 21 human and plant pathogenic microorganisms.

The bioactivity of the oils menthol and menthone was compared using a combination of in vitro techniques such as microdilution, agar diffusion, and bioautography [14]. It was found that all of the peppermint oils screened strongly inhibited plant pathogenic microorganisms, whereas human pathogens were only moderately inhibited [14]. Using the bioautography assay, menthol was found to be responsible for the antimicrobial activity of these oils [14].

Fungal toxicity of the essential oils of *Mentha piperita* and *Lavendula angustifolia* was evaluated against three post-harvest pathogenic fungi (*Rhizopus stolonifer*, *Botrytis cinerea*, and *Aspergillus niger*) *in vitro* by Behnam and co-workers [5]. Plate assays showed that the different concentrations of essential oils have antifungal activity against these fungi [5].

The chemical composition of essential oils of Thymus and Mentha species and their antifungal activities against 17 micromycetal food poisoning, plant, animal, and human pathogens are presented by authors - researchers Soković and co-workers [30]. They established that in M. piperita oil menthol (37.4%), menthyl acetate (17.4%), and menthone (12.7%) were the main components, whereas those of M. spicata oil were carvone (69.5%) and menthone (21.9%) [30]. Mentha sp. showed strong antifungal activities, however lower than Thymus sp. The essential oils of Thymus and Mentha species possess great antifungal potential and could be used as natural preservatives and fungicides [30].

Seasonal variation in content, chemical composition, and antimicrobial and cytotoxic activities of essential oils from four *Mentha* species, i.e. *M. arvensis*, *M. piperita*, *M. longifolia*, and *M. spicata* were evaluated by Hussain and co-workers [12]. As a result of the research, it was revealed that of the Mentha essential oils tested, M. arvensis essential oil showed relatively better antimicrobial and cytotoxic activities. There was also a significant variation in the content of most of the chemical components and biological activities of seasonally collected samples was documented [12].

Essential oil of *Mentha suaveolens* Ehrh. were studied by Metin and co-workers [18] to determine the in vitro antibacterial activity 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. As a result of the research, it was revealed that essential oil exhibited strong inhibitory activity such as inhibition zone sizes: 30-50 mm at 250-1000 μL mL^{-1} concentrations against *V. anguillarum*; 16-20 mm at 31.25-125 μL mL^{-1} concentrations against *P. aeroginosa*; 15-18 mm at 500-1000 μL mL^{-1} concentrations against *A. sobria* [18].

PEOs are primarily composed of menthol in the range of 29–48%, with concentrations of menthone ranging from 20% to 31%, menthofuran at about 6.8%, and menthyl acetate at concentrations ranging from 3% to 10% [3]. Although several articles have reported the chemical composition of PEO, the chemistry of essential oils is very complex and varies significantly. As a result of research, the authors found that the relative concentrations of chemical constituents vary depending on climate, geographic location, harvest time, drying conditions, extraction method, etc. [3, 15, 28].

Inouye and co-workers [13] investigated the effects of menthol and their major constituents against pathogens affecting the respiratory tract (*Staphylococcus aureus*, *S. pneumonia*, *Streptococcus pyogenes*, *Haemohphilus influenza*) by gaseous contact. The results showed that menthol exhibited moderate activity with the minimal inhibitory dose ranging from 6.3 to 50 mg/L air [13].

The menthol may have an impact on antimicrobial resistance in gut bacteria Scientists Aperce and and his colleagues [2] determined if menthol supplementation in diets of feedlot cattle decreases the prevalence of multidrug-resistant bacteria in feces. According to research, menthol menthol was included in diets of steers at 0.3% of diet dry matter. Fecal samples were collected weekly for 4 weeks and analyzed for total coliform counts, antimicrobial susceptibilities, and the prevalence of *tet* genes in *E. coli* isolates [2]. Results revealed no effect of menthol supplementation on total coliform counts or prevalence of *E. coli* resistant to amoxicillin, ampicillin, azithromycin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, and sulfamethoxazole; however, 30 days of menthol addition to steering diets increased the prevalence of tetracycline-resistant *E. coli* [2]. Scientists-research Schelz and his colleagues [27] investigated the effects of peppermint oil and menthol *in vitro* on bacteria and their plasmids and demonstrated anti-plasmid activity similar to sodium dodecyl sulfate.

Ricci S and his colleagues found in their studies that phytogenic compounds enhancing salivary physico-chemical composition have the potential to contribute to maintaining or improving ruminal health in cattle-fed concentrate-rich rations [23]. Patra, A. K and his colleagues [19, 20] confirm in their research that menthol-rich plant bioactive lipid compounds in the applied dose range stimulate circadian eating behaviour, which cannot only be attributed to their presence during concentrated feeding hours but persist

during post-concentrate feeding hours [19, 20]. Rivera-Chacon and co-workers [24] evaluated whether supplementing a phytogenic feed additive based on L-menthol, thymol, eugenol, mint oil (*Mentha arvensis*), and cloves powder (*Syzygium aromaticum*) (PHY) can amend the ruminal fermentation profile, modulate the risk of subacute ruminal acidosis (SARA) and reduce inflammation in cattle. Research has found that PHY had beneficial effects on ruminal fermentation, reduced inflammation, and modulated the risk of SARA starting from week 3 of supplementation [24].

As a result of research by Su, Yand his colleagues [31] , it was revealed that menthone inhalation alleviates local and systemic allergic inflammation in asthmatic mice [31]. It was also revealed that menthone supplementation protects from allergic inflammation in the lungs of asthmatic mice alleviating allergic asthma through regulating airway allergic inflammation, protein overproduction, eosinophils infiltration, Th1/Th2 immune balance, CC receptor 3 and CXC receptor 1 gene expression amounts in the lungs but restoring the percentage of monocytes/macrophages in allergic asthmatic mice [32].

CONCLUSIONS

Thus, the results of our researchs provide insight into the *in vitro* antibacterial activity of commercial peppermint essential oil against Gram-negative strains such as *E. coli* (Migula) Castellani and Chalmers (ATCC® 25922™), *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™), *P. aeruginosa* (Schroeter) Migula (ATCC® 27853™) and Gram-positive strains such as *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™), methicillin-resistant (MRSA) *S. aureus* (NCTC® 12493), *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (resistant to vancomycin; sensitive to teicoplanin) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™). Results of the current study revealed that resistant to the PEO were Gram-negative bacterial strains, such as *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™) and *P. aeruginosa* (Schroeter) Migula (ATCC® 27853™) strains. As a result of the research carried out by the authors of the article, it was established that diameters of inhibition zones after the application of PEO were similar to control samples (96% ethanol). The increase of diameters of inhibition zones after the application of PEO was 60.3% ($p < 0.05$) for *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain compared to the control samples (96% ethanol). Similarly, Gram-positive strains such as *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) and methicillin-resistant *S. aureus* (NCTC® 12493) were resistant to the PEO action. On the other hand, *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) were sensitive to PEO. The highest diameters of inhibition zones after the application of PEO were observed for *E. faecalis* strains. The results suggest that commercial peppermint essential oil provided by Polish essential oil manufacturers (Naturalne Aromaty sp. z o.o., Kłaj, Poland) possesses some noteworthy antimicrobial properties. *In vivo* studies are necessary to calculate the effective dose of EOs and determine their possible side effects and toxicity.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

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Антибактеріальні властивості комерційної ефірної олії м'яти проти деяких грампозитивних та грамнегативних бактерій

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Ця робота виконана за підтримки Поморського університету в Слупську (Польща) у співпраці з М.М. Національний ботанічний сад імені Гришка НАН України (Київ, Україна).
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У поточному дослідженні вивчалися антибактеріальні властивості комерційної ефірної олії м'яти перцевої (PEO), наданої польськими виробниками ефірної олії (Naturalne Aromaty sp. z o.o., Клай, Польща), проти деяких грампозитивних і грамнегативних бактерій. Для цього використовувався тест на антимікробну чутливість (дифузійний тест Кірбі–Бауера для вимірювання діаметрів зон пригнічення росту бактерій). У поточному дослідженні грамнегативні штами, такі як Escherichia coli (Migula) Castellani and Chalmers (ATCC® 25922™), Escherichia coli (Migula) Castellani and Chalmers (ATCC® 35218™), Pseudomonas aeruginosa (Schroeter) Migula (ATCC®) 27853™) і грампозитивні штами, такі як Staphylococcus aureus subsp. aureus Rosenbach (ATCC® 29213™), метицилінрезистентний (MRSA), mecA позитивний Staphylococcus aureus (NCTC® 12493), Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (стійкий до ванкоміцину; чутливий до тейкопланіну) і Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™). Результати поточного дослідження показали, що резистентними до PEO були штами грамнегативних бактерій, такі як штами E. coli (Migula) Castellani and Chalmers (ATCC® 35218™) і P. aeruginosa (Schroeter) Migula (ATCC® 27853™). Діаметри зон інгібування після нанесення PEO були подібні до контрольних зразків (96% етанол). Збільшення діаметрів зон інгібування після застосування PEO становило 60,3% ($p < 0,05$) для штаму Escherichia coli (Migula) Castellani and Chalmers (ATCC® 25922™) порівняно з контрольними зразками (96% етанол). Так само грампозитивні штами, такі як S. aureus subsp. aureus Rosenbach (ATCC® 29213™) і метицилінрезистентний S. aureus (NCTC® 12493) виявилися стійкими до дії PEO. З іншого боку, Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) і Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) були чутливі до PEO. Найбільший діаметр зон інгібування після застосування PEO спостерігався для штамів E. faecalis. Результати свідчать про те, що комерційна ефірна олія м'яти перцевої, надана польськими виробниками ефірних олій (Naturalne Aromaty sp. z o.o., Клай, Польща), має деякі варті уваги антимікробні властивості. Дослідження in vivo необхідні для розрахунку ефективної дози ЕМ та визначення їх можливих побічних ефектів і токсичності.

Ключові слова: *комерційна ефірна олія м'яти перцевої, антибактеріальна активність, зони інгібування, методика дискової дифузії Кірбі-Бауера*..