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

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АНТИБАКТЕРІАЛЬНІ ВЛАСТИВОСТІ КОМЕРЦІЙНОЇ ЕФІРНОЇ ОЛІЇ ГЕРАНІ ЩОДО ДЕЯКИХ ГРАМПОЗИТИВНИХ ТА ГРАМНЕГАТИВНИХ БАКТЕРІЙ

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ABSTRACT

Purpose: The purpose of the study was to evaluate the antibacterial properties of commercial geranium essential oil (Etja, Elbląg, Poland) against some Gram-positive and Gram-negative bacteria. To this intent, the antimicrobial susceptibility test was used (the Kirby–Bauer disk diffusion test for measuring zone diameters of bacterial growth inhibition).

Methodology. Natural geranium essential oil (Etja, Elbląg, Poland) was used in the current study. The testing of the antibacterial activity of geranium essential oil was carried out in vitro by the Kirby-Bauer disc diffusion technique. In the current study, Gram-positive strains such as *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (resistant to vancomycin; sensitive to teicoplanin), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™), *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 29213™), *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 25923™), *Staphylococcus aureus* (NCTC 12493™), and Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™), and *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 35218™) strains were used for the assessment of antibacterial activity of geranium essential oil.

Scientific novelty. The highest diameters of the inhibition zone around the growth of Gram-negative strains were obtained for *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) and *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™) strains. Diameters of the inhibition zone were increased by 47.6% ($p < 0.05$) and 84.1% ($p < 0.05$) compared to the control samples, respectively. Gram-positive strains were more sensitive to the impact of commercial geranium essential oil. The highest diameters of the inhibition zone around the growth of Gram-positive strains were obtained for *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) and *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 25923™). Diameters of the inhibition zone were increased by 95.1% ($p < 0.05$) and 67.7% ($p < 0.05$) compared to the control samples, respectively.

Conclusions. This study demonstrated that commercial geranium essential oil possesses potential antimicrobial properties against Gram-positive bacteria, such as *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™), *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) and *S. aureus* subsp. *aureus* Rosenbach (ATCC® 25923™) strains. *Pseudomonas aeruginosa* strain was resistant to commercial geranium essential oil. This study showed that this essential oil could be a potential preparation as a source of natural antibacterial properties.

Key words: geranium essential oil, antibacterial activity, Kirby-Bauer disc diffusion technique, Gram-positive bacteria, Gram-negative bacteria

АНОТАЦІЯ

Мета: Метою дослідження було оцінити антибактеріальні властивості комерційної ефірної олії герані (Etja, Elbląg, Польща) щодо деяких грампозитивних і грамнегативних бактерій. З цією метою використовувався тест на антимікробну чутливість (дифузійний тест Кірбі–Бауера для вимірювання діаметрів зон пригнічення росту бактерій).

Методологія. У поточному дослідженні використовувалася натуральна ефірна олія герані (Etja, Elbląg, Польща). Визначення антибактеріальної активності ефірної олії герані було проведено in vitro методом дискової

дифузії Кірбі-Бауера. У поточному дослідженні грампозитивні штами, такі як *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (стійкий до ванкоміцину; чутливий до тейкопланіну), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper- Balz (ATCC® 29212™), *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 29213™), *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 25923™), *Staphylococcus aureus* (NCTC 12493™) і грамнегативні штами, такі як *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™), а також штам *Escherichia coli* (Migula) Castellani і Chalmers (ATCC® 35218™) використовували для оцінки антибактеріальної активності ефірної олії герані.

Наукова новизна. Найбільший діаметр зони інгібування росту грамнегативних штамів отримано для штамів *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) і *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™). Діаметри зони інгібування були збільшені на 47,6 % ($p < 0,05$) і 84,1% ($p < 0,05$) порівняно з контрольними зразками, відповідно. Грампозитивні штами виявилися більш чутливими до впливу комерційної ефірної олії герані. Найбільші діаметри зони пригнічення росту грампозитивних штамів отримані для *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) і *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 25923™). Діаметри зони інгібування були збільшені на 95,1% ($p < 0,05$) і 67,7 % ($p < 0,05$) порівняно з контрольними зразками, відповідно.

Висновки. Це дослідження продемонструвало, що комерційна ефірна олія герані має потенційні антимікробні властивості щодо грампозитивних бактерій, таких як *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) і *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™), *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) і *S. aureus* subsp. *aureus* Rosenbach (ATCC® 25923™). Штам *Pseudomonas aeruginosa* був стійкий до комерційної ефірної олії герані. Це дослідження показало, що ця ефірна олія може бути потенційним препаратом як джерело природних антибактеріальних властивостей.

Ключові слова: ефірна олія герані, антибактеріальна активність, методика дискової дифузії Кірбі-Бауера, грампозитивні бактерії, грамнегативні бактерії

Introduction

Antibiotics have paved the way for today's modern medicine to prevent infections (Luzhetskyy et al., 2007). Infectious diseases were believed to be eradicated by the end of the last century. Similarly, antibiotics have been fundamental for successful invasive and high-end surgeries including organ transplantation, and immunomodulatory treatments in many medical disciplines (Wright, 2014). Also, antibiotics reduce morbidity and mortality caused by foodborne infections and other poverty-related infections in developing countries (Rossolini et al., 2014). The availability of antibiotic therapy has significantly reduced mortality in children resulting in increased life expectancy in general (Pai et al., 2015). Nevertheless, increasing numbers of bacteria are becoming resistant to multiple antibiotics currently in use resulting in multidrug-resistant (MDR) bacteria (Cerceo et al., 2016; Jara et al., 2021). Currently, the increasing resistance of microorganisms to currently used antimicrobials, combined with the emergence of emerging diseases, requires the urgent development of new, more effective drugs that could overcome this resistance (Liu et al., 2017). Plants have been used for a wide variety of purposes due to the large biological and structural diversity of their components, which constitute a unique and renewable source for the discovery of new antibacterial, antifungal, and antiparasitic compounds (Burt, 2004; Sakkas and Papadopoulou, 2017).

Herbs and essential oils derived from them have been used since the beginning of human history for various purposes (Vigan, 2010; Nerio et al., 2010; Solórzano-Santos and Miranda-Novales, 2012). Their beneficial properties have been applied to mask unpleasant odors, attract the attention of other people, and add flavor and aroma properties to prepared dishes, perfumes, cosmetics, etc. (Bassolé and Juliani, 2012; Wińska et al., 2019). Herbs and essential oils have also been used medicinally for their biological properties such as larvicidal action (Knio et al., 2008), analgesic (Sarmento-Neto et al., 2015) and anti-inflammatory properties (Lucca et al., 2022; Zhao et al., 2023), antioxidant (Valdivieso-Ugarte et al., 2019), bactericidal, virucidal, fungicidal, antiparasitic, insecticidal (Bakkali et al., 2008; Reichling, 2022) and antitumor activity (Carvalho et al., 2015; Machado et al., 2022), etc. Many essential oils exhibit antimicrobial properties, which are extremely important in fields of science and industry, such as medicine, agriculture, or cosmetology (Kalemba and Kunicka, 2003; Wińska et al., 2019; Li et al., 2022; Reichling, 2022).

Plants from the *Geranium* genus, which comprises about 400 species, have been used since ancient times in the practice of traditional medicines throughout the world (Sienkiewicz et

al., 2014a). Therefore, oil preparations based on *Geranium* species have found wide usage for the treatment of a variety of ailments (Graça et al., 2020). The results by Sienkiewicz and co-workers (2014b) suggest that geranium oil may be considered an effective component of therapy in the case of frequent recurrences of infections caused by resistant pathogens.

The *purpose* of the study was to evaluate the antibacterial properties of commercial geranium essential oil (Etja, Elbląg, Poland) against some Gram-positive and Gram-negative bacteria. To this intent, the antimicrobial susceptibility test was used (the Kirby–Bauer disk diffusion test for measuring zone diameters of bacterial growth inhibition).

Materials and methods

Commercial geranium essential oil. Natural geranium essential oil (Etja, Elbląg, Poland) was used in the current study. Information about this product noted that geranium oil is 100 % natural, obtained from flowers of the appropriate type of geranium. Composition: INCI: (Pelargonium Graveolens Oil) – 100 % natural geranium oil.

Geranium oil (Pelargonium Graveolens Oil) is obtained from geranium flowers by steam distillation. It has a fresh, rose fragrance. It has a soothing and calming effect on the body. It reduces tension and anxiety. It soothes the ailments associated with menstruation and climacteric, regulating the hormonal balance. Prevents water retention in the body, supports the treatment of cellulite and reduces swelling. It tightens and firms the skin, and smoothes wrinkles. It has an anti-inflammatory effect, supporting the treatment of herpes, mycosis, acne, and eczema. It is used to treat varicose veins, rheumatism, neuralgia, chickenpox, and shingles. Relieves headaches and adds energy. The geranium essential oil was stored in resalable vials at 5 °C in the dark but were allowed to adjust to room temperature prior to investigation. Geographical origins were excluded as information was mostly not available.

Determination of the antibacterial activity of essential oils by the disk diffusion method. The testing of the antibacterial activity of geranium essential oil was carried out *in vitro* by the Kirby-Bauer disc diffusion technique (Bauer et al., 1966). In the current study, Gram-positive strains such as *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (resistant to vancomycin; sensitive to teicoplanin), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™), *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 29213™), *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 25923™), *Staphylococcus aureus* (NCTC 12493™), and Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™), and *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 35218™) strains were used for the assessment of antibacterial activity of geranium essential oil.

The strains were inoculated onto Mueller-Hinton (MH) agar dishes. Sterile filter paper discs impregnated with geranium essential oil were applied over each of the culture dishes. Isolates of bacteria with geranium essential oil were then incubated at 37 °C for 24 h. The Petri dishes were then observed for the zone of inhibition produced by the antibacterial activity of geranium essential oil. A control disc impregnated with 96% ethanol was used in each experiment. At the end of the 24-h period, the inhibition zones formed were measured in millimetres using the vernier. For each strain, eight replicates were assayed (n = 8). The Petri dishes were observed and photographs were taken. The susceptibility of the test organisms to the geranium essential oil was indicated by a clear zone of inhibition around the discs containing the geranium essential oil and the diameter of the clear zone was taken as an indicator of susceptibility. Zone diameters were determined and averaged. The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (S) ≥ 15 mm, Intermediate (I) = 10–15 mm, and Resistant (R) ≤ 10 mm (Okoth et al., 2013; Tkachenko et al., 2022).

Statistical analysis. Zone diameters were determined and averaged. Statistical analysis of the data obtained was performed by employing the mean ± standard error of the mean (S.E.M.). All variables were randomized according to the phytochemical activity of the geranium essential oil tested. All statistical calculation was performed on separate data from each strain. The data were analyzed using a one-way analysis of variance (ANOVA) using Statistica v. 13.3 software (TIBCO Software Inc., Krakow, Poland) (Zar, 1999).

Results and discussion

Figures 1 and 2 summarize the results obtained by the mean diameters of the inhibition zone around the growth of Gram-positive strains such as *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (resistant to vancomycin; sensitive to teicoplanin), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™), *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 29213™), *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 25923™), *Staphylococcus aureus* (NCTC 12493™), and Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™), and *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 35218™) strains induced by geranium essential oil.

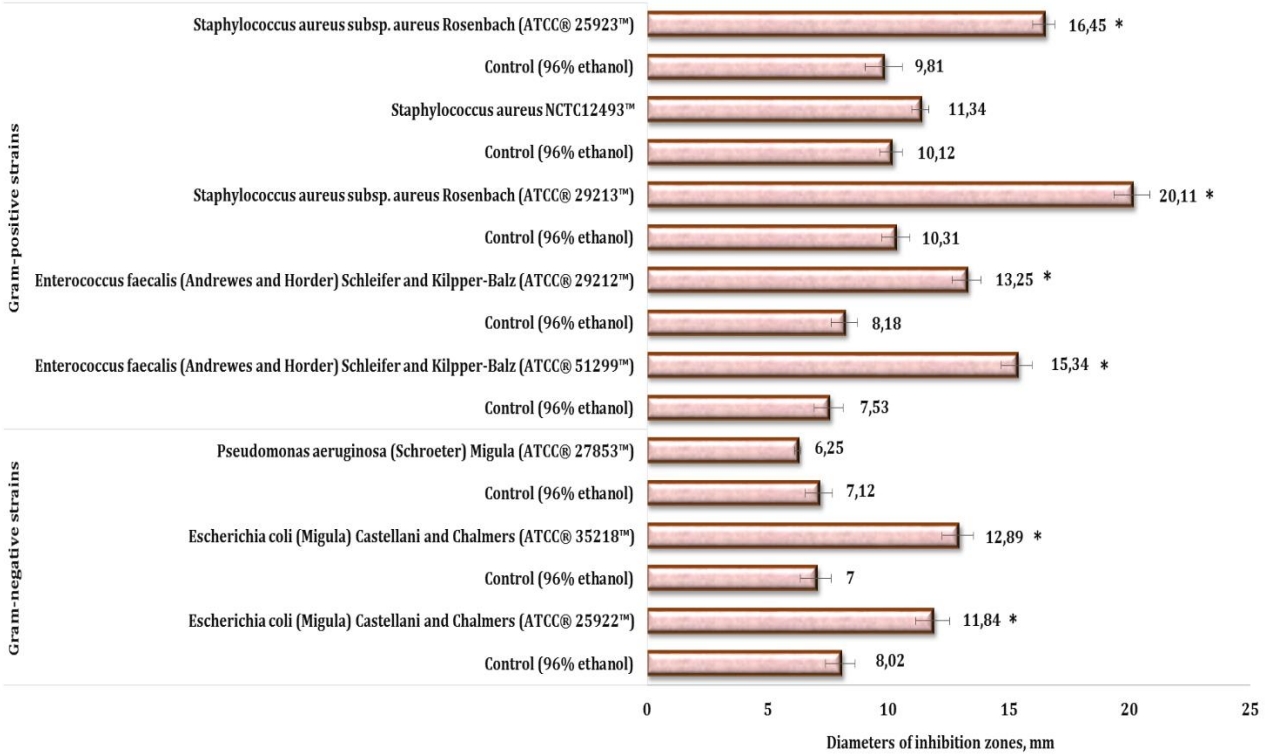


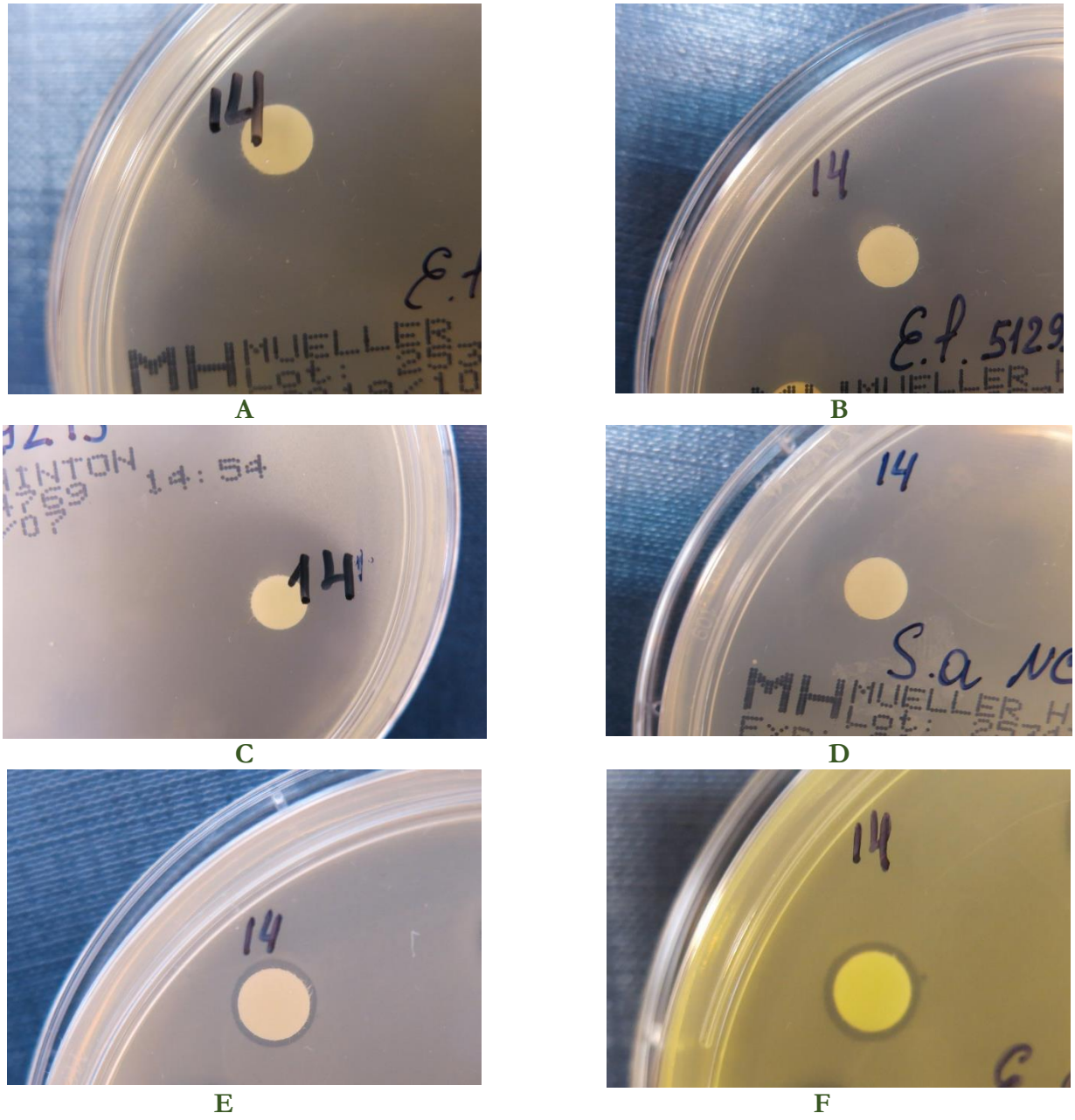
Fig. 1. The mean of inhibition zone diameters induced by geranium essential oil against some Gram-positive and Gram-negative strains (M ± m, n = 8)
*** – changes are statistically significant compared to the 96% ethanol**

We demonstrated a statistically significant increase in diameters of the zone inhibition after the application of geranium essential oil against *E. coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain by 47.6 % ($p < 0.05$) compared to the control samples (11.84 ± 0.71 mm *vs.* 8.02 ± 0.61 mm). We obtainned similar results after applying geranium essential oil to the *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™) strain, where we also observed a statistically significant increase in diameters of zone inhibition by 84.1 % ($p < 0.05$) compared to 96 % ethanol (12.89 ± 0.65 mm *vs.* 7.0 ± 0.64 mm). Diameters of zone inhibition for *P. aeruginosa* (Schroeter) Migula (ATCC® 27853™) strain after the application of geranium essential oil were at the same levels as control samples (6.25 ± 0.12 mm *vs.* 7.12 ± 0.56 mm).

When we tested the effect of geranium essential oil against Gram-positive bacterial strains, we also observed a statistically significant increase in diameters of zone inhibition of *S. aureus* subsp. *aureus* Rosenbach (ATCC® 25923™) strain by 67.7 % ($p < 0.05$) compared to the controls (16.45 ± 0.45 mm *vs.* 9.81 ± 0.77 mm). We obtained similar results after applying geranium essential oil to the *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) strain, where we also observed a statistically significant increase in diameters of zone inhibition by 95.1 % ($p < 0.05$) compared to 96 % ethanol (20.11 ± 0.74 mm *vs.* 10.31 ± 0.59 mm). Diameters of zone inhibition for *Staphylococcus aureus* (NCTC 12493™) strain after the application of geranium essential oil were at the same levels as control samples (11.34 ± 0.35 mm *vs.* 10.12 ± 0.48 mm). We recorded a similar

statistically significant increase in diameters of zone inhibition after the application of geranium essential oil against *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) strain by 103.7 % ($p < 0.05$) comparing to the control samples (15.34 ± 0.64 mm *vs.* 7.53 ± 0.6 mm). We also noted a similar statistically significant increase in diameters of zone inhibition after the application of geranium essential oil against *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) strain by 62% ($p < 0.05$) comparing to the control samples (13.25 ± 0.61 mm *vs.* 8.18 ± 0.55 mm).

Detailed photos regarding the diameters of inhibition zones of strains tested by the geranium essential oil were recorded and presented in Fig. 2.



A **B** **C** **D** **E** **F**

Fig. 2. The diameters of the inhibition zone around the growth of Gram-positive strains such as *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), and Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™) (E), and *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) (F) strains induced by geranium essential oil

In the current study, the antibacterial properties of commercial geranium essential oil (Etja, Elbląg, Poland) against some Gram-positive and Gram-negative bacteria were studied. Results of our study revealed that the highest diameters of the inhibition zone around the growth of Gram-negative strains were obtained for *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) and *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™) strains. Diameters of the inhibition zone were increased by 47.6 % ($p < 0.05$) and 84.1 % ($p < 0.05$) comparing to the control samples, respectively. Gram-positive strains were more sensitive to the impact of commercial geranium essential oil. The highest diameters of the inhibition zone around the growth of Gram-positive strains were obtained for *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) and *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 25923™). Diameters of the inhibition zone were increased by 95.1% ($p < 0.05$) and 67.7% ($p < 0.05$) comparing to the control samples, respectively (Fig. 1).

Geranium (*Pelargonium graveolens* L'Hér.) essential oil exhibits strong activity against a broad spectrum of bacterial strains (Prabuseenivasan et al., 2006; Ghannadi et al., 2012). The antibacterial activity of geranium essential oils was investigated against six bacterial species in the study of Ghannadi and co-workers (2012). Test organisms included *Listeria monocytogenes* (PTCC 1297), *Salmonella enteritidis* (PTCC 1091), *Pseudomonas aeruginosa* (PTCC 1074), *Escherichia coli* (PTCC 1330), *Staphylococcus aureus* (PTCC 1112) and *Bacillus subtilis* (PTCC 1023). The geranium essential oils were active against all of the bacteria (except *L. monocytogenes*) and the susceptibility of the strains changed with the dilution of essential oils in DMSO (Ghannadi et al., 2012). When used in solution or as an aerosol, geranium oil was effective against clinically-significant human pathogens, such as Gram-positive *S. aureus* and *Enterococcus faecalis*, and Gram-negative *P. aeruginosa*, *Proteus mirabilis*, and *Escherichia coli*, and the fungus *Candida albicans* (Rosato et al., 2007; Carmen and Hancu, 2014). Al-Jumaili and co-workers (2019) investigated the retention of inherent antibacterial activity in geranium-based plasma polymer thin films. The essential oil of *Pelargonium graveolens* and geraniol itself have shown such activity in combination with ketoconazole against *Trichophyton schoenleinii* and *T. soudanense* and in combination with Norfloxacin® against *Bacillus cereus* and *S. aureus* (Rosato et al., 2007).

Previous studies have described the antibacterial activity of geranium oil against *S. aureus* strains. Edwards-Jones and co-workers (2004) demonstrate the antibacterial properties of geranium oil against methicillin-resistant *S. aureus* (MRSA) strains, including those derived from the wounds of burn patients. A combination of Citricidal and geranium oil showed the greatest anti-bacterial effects against MRSA, whilst a combination of geranium and tea tree oil was most active against the methicillin-sensitive *S. aureus* (Oxford strain) (Edwards-Jones et al., 2004). Bigos and co-workers (2012) have investigated the antibacterial properties of geranium oil obtained from *Pelargonium graveolens* Ait., against one standard *S. aureus* strain ATCC 433000 and seventy clinical *S. aureus* strains. The results of the experiment showed that the oil from *P. graveolens* has strong activity against all of the clinical *S. aureus* isolates-including multidrug-resistant strains, MRSA strains, and MLS(B)-positive strains-exhibiting minimal inhibitory concentration (MIC) values of 0.25-2.50 µL/mL. *S. aureus* strains isolated from skin lesions were found to be sensitive to geranium oil at concentrations from 0.25 µl/ml to 1.5 µl/ml, and those from postoperative wounds at concentrations ranging from 0.5 µl/ml to 2.25 µl/ml. The largest number of MRSA and MSSA clinical strains, as well as those with the MLSB mechanism, were inhibited at a 1.0 µl/ml concentration of geranium oil (Bigos et al., 2012).

Coronado-López and co-workers (2018) evaluated the *in vitro* antibacterial and cytotoxic properties of the methanolic extract of *Pelargonium peltatum* (geranium) against *Streptococcus mutans* (ATCC 25175) and *Streptococcus sanguinis* (ATCC 10556). The root extract had the highest antibacterial effect with a mean result of (27.68 ± 0.97) mm and (30.80 ± 0.55) mm against *S. mutans* and *S. sanguinis*, respectively. The minimum inhibitory concentration for the leaf and root extracts was 250 mg/mL for *S. mutans* and 125 mg/mL for *S. sanguinis*. Cytotoxicity assays showed that both extracts had low cytotoxicity at high concentrations. The cellular viability was highest for the root extract at 95.3 % followed by the stem extract at 80.8 % and finally the leaf extract at 75.4 % (Coronado-López et al., 2018).

The study of Sienkiewicz and co-workers (2014b) was to determine the antimicrobial activity of geranium oil against Gram-negative bacterial clinical strains. Clinical strains were isolated from patients with difficult-to-treat wounds and a comprehensive evaluation of their sensitivity to antibiotics was carried out. The tested geranium oil was efficacious against Gram-negative pathogens responsible for problems with wound treatment. Geranium essential oil demonstrated the greatest antibacterial activity against E. coli clinical strains isolated from wound swabs: the minimal inhibitory concentration was from 3.0 µl/ml to 3.75 µl/ml. Higher MIC values, between 5.25 and 5.75 µl/ml were obtained against the isolated strains of *Citrobacter freundii*. Concentrations from 6.25 µl/ml to 8.0 µl/ml inhibited the growth of all *Enterobacter* strains. Geranium essential oil at concentrations of 6.25–7.0 µl/ml inhibited the growth of *Enterobacter sakazakii*, and *Enterobacter cloacae* were inhibited by concentrations of 7.0–8.0 µl/ml. The least sensitive to geranium oil were strains of *Pseudomonas* and *Proteus* genera, the MIC values for both genera were from 9.25 µl/ml to 10.5 µl/ml. According to the results obtained by these authors, geranium oil may be considered an effective component of therapy in the case of frequent recurrences of infections caused by resistant pathogens (Sienkiewicz et al., 2014b).

Also, Probuseenivasan and co-workers (2006) evaluated the activity of geranium oil against standard strains by using disk diffusion and the agar dilution methods. The geranium essential oil was found to inhibit the growth of Gram-positive strains such as *S. aureus* ATCC 25923 and *Bacillus subtilis* MTCC 441, as well as Gram-negative strains such as *E. coli* ATCC 25922, *K. pneumoniae* ATCC 15380, *P. aeruginosa* ATCC 27853 and *Proteus vulgaris* MTCC 1771. The MIC for geranium oil was found to be >6.4 mg/ml against *E. coli*, and >12.8 mg/ml against other Gram-negative reference strains, and it was seen to be active at a concentration of 1:5 against *Klebsiella pneumoniae* (inhibition zone of 9.0 mm) and *E. coli* (inhibition zone of 10.4 mm). *P. aeruginosa* was found to be susceptible to geranium oil at a concentration of 1:10 (inhibition zone of 9.4 mm) and *P. vulgaris* at 1:20 (inhibition zone of 8.3 mm).

A total of 36 compounds were observed by Lohani and co-workers (2019) in the gas chromatogram of geranium essential oil. The highly abundant constituent of the oil was citronellol (37.01 %) and geraniol (17.99 %). Other constituents were citronellyl formate (5.51 %), linalool (4.11 %), rose oxide (2.40 %), geranyl formate (2.19 %), citronellyl propionate (1.90 %), geranyl tiglate (1.59 %), α-pinene (1.59 %), geranyl propionate (1.10 %), and limonene (1.02 %). All the other components were present in an amount lower than 1.0 %. From the results, it was observed that oxygenated monoterpenes were present in the majority, that is, the main reason that geranium essential oil is characterized by its sweet rose-like (citronellol) and flowery rose-like odor (geraniol) with important demand in perfumery (Lohani et al., 2019). Previous researchers reported citronellol and geraniol as the main constituents of geranium essential oil (Sanja and Maksimović, 2012; Sharopov et al., 2014), According to a study by Sharopov and co-workers (2014), the main constituents of the geranium (*Pelargonium graveolens* L'Hér.) essential oil were citronellol (37.5 %), geraniol (6.0 %), caryophyllene oxide (3.7 %), menthone (3.1 %), linalool (3.0 %), β-bourbonene (2.7 %), isomenthone (2.1 %) and geranyl formate (2.0 %). It may be said that the major activities of geranium essential oil are due to the presence of its major constituents such as citronellol and geraniol (Lohani et al., 2019).

Silva and co-workers (2020) evaluated the behavior of positive and negative enantiomers of β-citronellol on strains of *Candida albicans* and *C. tropicalis* involved in candidemia and revealed that both isomers of β-citronellol presented a similar profile of antifungal activity. Derivatives of geraniol-grafted chitosan oligosaccharide exhibited good solubility, thermal stability, and antibacterial properties in the study of Yue and co-workers (2017). The results obtained by Feng and co-workers (2022) indicated that nanoemulsification of geraniol oil enhanced the stability and antibacterial activity of geraniol to some extent, which will promote the utilization of geraniol in food preservation. Geraniol was investigated for its antiulcer and anti-*Helicobacter pylori* activity in rats (Bhattamisra et al., 2018). In the rapid urease test, treatment with geraniol (30 mg/kg) and the standard drugs produced a 33 % and 67 % cure respectively from *H. pylori* infection. Further, the reduction in bacterial load in the gastric mucosa was confirmed using modified Giemsa staining. Geraniol was observed to exhibit significant antiulcer and

anti-H. pylori activity in a rodent model (Bhattamisra et al., 2018). Geraniol administered in the aerosol inhibited the development of such dangerous human pathogens as *Haemophilus influenzae*, *Streptococcus pneumoniae*, *S. pyogenes*, and *Staphylococcus aureus* (Chen and Viljoen, 2010; Mączka et al., 2020).

Conclusions

In the current study, we assessed *in vitro* antimicrobial profiling of commercial geranium essential oil (Etja, Elbląg, Poland) against some Gram-positive and Gram-negative strains. This study demonstrated that commercial geranium essential oil possesses potential antimicrobial properties against Gram-positive bacteria, such as *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™), *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) and *S. aureus* subsp. *aureus* Rosenbach (ATCC® 25923™) strains. *Pseudomonas aeruginosa* strain was resistant to commercial geranium essential oil. This study showed that this essential oil could be a potential preparation as a source of natural antibacterial properties. However, further studies are needed to clarify the mechanisms involved in their antimicrobial properties. Future pharmacological studies and development in other areas are thus warranted.

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