Tkaczenko H.[1], Opryshko M.[2], Antonik I.[3], Maryniuk M.[2], Gyrenko O.[2], Buyun L.[2], Kurhaluk N.[1]

IN VITRO ANTIBACTERIAL ACTIVITY OF COMMERCIAL ROSEMARY ESSENTIAL OIL AGAINST SOME GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

[1]Institute of Biology, Pomeranian University in Słupsk, Poland;
[2]M.M. Gryshko National Botanic Garden, National Academy of Science of Ukraine, Kyiv, Ukraine;
[3]Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine
e-mail: halyna.tkachenko@apsl.edu.pl

Rosemary essential oil has many compounds suitable for use in the food, cosmetic and pharmaceutical industries. Rosemary essential oils are widely used in the treatment of upper and lower respiratory tract diseases, mainly due to their antibacterial and antiviral effects.This study provides insight into the in vitro antibacterial activity of commercial rosemary essential oil against Gram-negative strains such as Escherichia coli(Migula) Castellani and Chalmers (ATCC® 25922™), E. coli (Migula) Castellani and Chalmers (ATCC® 35218™), Pseudomonas aeruginosa (Schroeter) Migula (ATCC® 27853™) and Gram-positive strains such as Staphylococcus aureus subsp. aureusRosenbach (ATCC® 29213™), methicillin-resistant (MRSA) S. aureus (NCTC® 12493), Enterococcus 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™). The results of the current study showed that Gram-negative bacterial strains such as E. coli (Migula) Castellani and Chalmers (ATCC® 35218™) and P. aeruginosa (Schroeter) Migula (ATCC® 27853™) were resistant to REO. The diameters of the inhibition zones after application of REO were similar to those of the control samples (96% ethanol). The increase in inhibition zone diameter after application of REO was 32.6% (p < 0.05) for Escherichia coli (Migula) Castellani and Chalmers (ATCC® 25922™) strains compared to control samples (96% ethanol). Similarly, the increase in inhibition zone diameters after application of REO was 50.3% (p < 0.05) for Gram-positive strains such as S. aureus subsp. aureusRosenbach (ATCC® 29213™).Methicillin-resistant S. aureus (NCTC® 12493)was resistant to REO.On the other hand, the largest inhibition zone diameters after application of REO were observed for E. faecalis strains.The increase in inhibition zone diameters after application of REO was 115.5% (p < 0.05) and 115.8% (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. The results suggest that commercial rosemary essential oil supplied by a Polish essential oil manufacturer (NaturalneAromaty sp. z o.o., Kłaj, Poland) has some significant antimicrobial properties. In vivo studies are needed to calculate the effective dose of EOs and to determine their possible side effects and toxicity.

Keywords: commercial rosemary essential oil, antibacterial properties, inhibition zones, Kirby-Bauer disc diffusion technique.

Ткаченко Г., Опришко М., Антонік І., Маринюк М., Гиренко О., Буюн Л., Кургалюк Н.

АНТИБАКТЕРІАЛЬНА АКТИВНІСТЬ КОМЕРЦІЙНОЇ ЕФІРНОЇ ОЛІЇ

РОЗМАРИНУ ПРОТИ ДЕЯКИХ ГРАМ-ПОЗИТИВНИХ ТА ГРАМ-НЕГАТИВНИХ БАКТЕРІЙ *IN VITRO*

Ефірна олія розмарину (REO) містить багато сполук, які використовують в харчовій, косметичній та фармацевтичній промисловості. Ефірні олії розмарину широко використовуються при лікуванні захворювань верхніх і нижніх дихальних шляхів, в основному завдяки їх антибактеріальним і противірусним ефектам. Це дослідження було спрямоване на дослідження *invitro* антибактеріальної активності комерційної ефірної олії розмарину щодо грамнегативних штамів, таких як *Escherichiacoli* (Migula) *Castellaniand Chalmers* (ATCC® 25922™), *E. coli* (Migula) *Castellaniand Chalmers* (ATCC® 35218™), *Pseudomonas aeruginosa* (Schroeter) *Migula* (ATCC® 27853™) і грампозитивні штами, такі як *Staphylococcus aureus subsp. aureus Rosenbach* (ATCC® 29213™), метицилін резистентний (MRSA) *S. aureus* (NCTC® 12493), *Enterococcus faecalis* (Andrewesand Horder) *Schleiferand Kilpper-Balz* (ATCC® 51299™) (стійкий до ванкоміцину; чутливий до тейкопланіну) і *E. faecalis* (Andrewesand Horder) *Schleiferand Kilpper-Balz* (ATCC® 29212™). Результати цього дослідження показали, що штами грамнегативних бактерій, такі як *E. coli* (Migula) *Castellani and Chalmers* (ATCC® 35218™) і *P. aeruginosa* (Schroeter) *Migula* (ATCC® 27853™), були стійкідо REO. Діаметри зон інгібування після нанесення REO були подібними до контрольних зразків (96% етанол). Збільшення діаметрів зони інгібування після застосування REO становило 32,6% (р<0,05) для штамів *E.coli* (Migula) *Castellani* та *Chalmers* (ATCC® 25922™) порівняно з контрольними зразками (96% етанол). Подібним чином, збільшення діаметрів зон інгібування після застосування REO становило 50,3% (р<0,05) для грампозитивних штамів, таких як *S. aureussubsp. aureusRosenbach* (ATCC® 29213™). Стійкий до метициліну *S. aureus* (NCTC® 12493) був стійким до REO. З іншого боку, найбільші діаметри зон інгібування після застосування REO спостерігалися для штамів *E. faecalis*. Збільшення діаметрів зон інгібування після застосування REO становило 115,5% (р< 0,05) і 115,8% (р< 0,05) для *E. faecalis* (AndrewesandHorder) *SchleiferandKilpper-Balz* (ATCC® 51299™) і *E. faecalis* (AndrewesandHorder) штами *Schleifer* і *Kilpper-Balz* (ATCC® 29212™) відповідно. Результати свідчать про те, що комерційна ефірна олія розмарину польського виробника ефірних олій (Naturalne Aromatysp. zo.o., Kłaj, Poland), проявляє істотне антимікробні властивості. Дослідження *invivo* необхідні для розрахунку ефективної дози REO та визначення можливих побічних ефектів і токсичності.

Ключові слова: комерційна ефірна олія розмарину, антибактеріальні властивості, зони інгібування, методика дискової дифузії Кірбі-Бауера.

INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is valued for its medicinal and aromatic properties. Rosemary is a perennial, evergreen herb with fragrant, needle-like leaves. It belongs to the Lamiaceae family. Several studies have reported that rosemary extracts have biological bioactivities such as hepatoprotective [28], antifungal [13], insecticidal [36], antioxidant [4] and antibacterial [22]. It has been used in traditional medicine as a stimulant, analgesic and against inflammatory diseases, physical and mental fatigue [25]. The antimicrobial and antioxidant

activities of rosemary extracts are attributed to phenolic acids, flavonoids and terpenoids, especially carnosic acid and rosmarinic acid [12, 16].

Rosemary essential oil (REO) is more than just aromatic magic. Behind the attractive aroma are numerous chemical components that give the oil not only a memorable flavour, but also a number of unique properties that make it valuable in the world [3, 12]. REO is a concentrated botanical extract obtained from fresh or dried rosemary by steam distillation. It contains a complex mixture of chemical compounds, including 1,8-cineol, α-pinene, camphor and others, giving it a distinctive aroma and multiple properties [18, 33]. The main components of rosemary essential oil are camphor (5.0-21%), 1,8-cineol (15-55%), α-pinene (9.0-26%), borneol (1.5-5.0%), camphene (2.5-12%), β-pinene (2.0-9.0%) and limonene (1.5-5.0%) in proportions that vary according to the vegetative stage and bioclimatic conditions [6]. Table 1 provides a detailed chemical profile of rosemary essential oil, showing the main components and their percentages. Percentages may vary depending on the variety of rosemary, growing conditions and distillation method.

Table 1

Chemical profile of rosemary essential oil showing the main components and their percentages

Compounds	Percent Composition, %	Brief Description	References
1,8-Cineole (Eucalyptol)	25-50	A key ingredient with a fresh, camphor-like aroma; known for its lipid-lowering, antioxidant, anti-inflammatory properties and respiratory benefits	[10]
α-Pinene	20-35	Contributes to the pine aroma; α-pinene exhibits antibacterial, antifungal, anti-leishmania, anti-inflammatory and antioxidative activity, neuroprotective, gastroprotective, antitumor, antimetastatic properties	[1]
Camphor	15-25	Provides a cooling sensation; possesses anti-inflammatory and analgesic properties	[17]
Borneol	2-6	Adds a minty note to the fragrance; potential antimicrobial properties	[24]
Chamazulene	5-15	Has anti-inflammatory, free radical scavenging, inhibition of cyclooxygenase-2 enzyme, and prevention of lipid peroxidation. It gives the oil a blue colour and is often associated with chamomile oil	[40]
α-Terpineol	3-8	Imparts a pleasant floral odor; known for antimicrobial and relaxing properties. α-Terpineol has pharmacological activities such as anticonvulsant, sedative, antinociceptive and hypotensive properties	[34]
β-Pinene	2-6	Contributes to the pine scent; has antibiotic resistance modulating, anticoagulant, antitumour, antimicrobial, antimalarial, antioxidant, anti-inflammatory, anti-leishmanial	[30]

Compounds	Percent Composition, %	Brief Description	References
		and analgesic effects	
Verbenone	1-5	Recognised for its skin-regenerating properties, verbenone is considered gentler on the skin than some other ingredients. It has a sweet, fruity fragrance.Possesses antimicrobial, antioxidant, cytotoxic, antinociceptive and anti-inflammatory activities	[19]
Limonene	1-4	Provides a citrus scent;possesses antioxidant, antidiabetic, anticancer, anti-inflammatory, cardioprotective, gastroprotective, hepatoprotective, immune modulatory, anti-fibrotic, anti-genotoxic activities	[2]
Myrcene	1-3	Contributes to earthy and musky notes; also found in basil and hops; possesses anxiolytic, antioxidant, anti-ageing, anti-inflammatory, analgesic properties	[35]
Terpinen-4-ol	1-3	Recognised for its anti-tumor, anti-bacterial, anti-fungal, anti-platelet, anti-oxidation, anti-senile dementia, and anti-metabolic syndrome properties, terpinen-4-ol adds a mildly sweet and floral note to the oil	[11]
Bornylacetate	1-3	Known for its anti-microbial, anti-cancer, anti-inflammatory and anti-abortion properties, bornyl acetate has a sweet, pine-like aroma	[44]
α-Humulene	1-2	Exhibits anti-cancer and anti-inflammatory properties and contributes to the herbal scent of rosemary oil	[41]
Caryophyllene	1-2	Known for its antioxidant, anti-inflammatory and anti-cancer properties, caryophyllene has a spicy, woody aroma	[32]
γ-Terpinene	1-2	Recognized for its antimicrobial and anti-inflammatory properties, γ-terpinene contributes to the overall therapeutic benefits of rosemary oil	[27]
δ-Cadinene	1-2	Contributes to the woody, earthy aroma of rosemary oil	[31]
Rosmarinicacid (non-volatile)	0.5-1.5	Although not a volatile component, rosmarinic acid is a powerful antioxidant found in rosemary. Rosmarinic acid has various biological activities such as anti-inflammatory, antioxidant, anti-diabetic, antiviral, anti-tumour, neuroprotective, hepatoprotective, etc.	[20]

Rosemary (*R. officinalis*) is a medicinal plant native to the Mediterranean region. It is cultivated throughout the world.In addition to its therapeutic uses, it is widely used as a flavouring and food preservative [14]. *R. officinalis* extracts are a promising therapeutic agent that can be added to some medical and dental formulations, such as toothpastes, mouthwashes, root canal irrigants, ointments,

soaps, for the control of pathogenic microorganisms and biofilms, with anti-inflammatory effects and without cytotoxic and genotoxic effects [15].*R. officinalis* is also widely used today as a food preservative and is known for its strong antibacterial activity [39]. REO showed the highest antimicrobial activity, with 65% of anti-infection studies from the 1990s to 2014 [3]. The antimicrobial activity of the essential oil was superior to that of the individual compounds 1,8-cineol and α-pinene [7, 22].

In the present study, the antibacterial properties of commercial REO supplied by a Polish producer of essential oils (NaturalneAromaty sp. z o.o., Kłaj, Poland) were investigated against some gram-positive and gram-negative bacteria. The antimicrobial susceptibility test was used (Kirby-Bauer disc diffusion test to measure the diameter of the zone of bacterial growth inhibition).

MATERIALS AND METHODOLOGY

Rosemary essential oil.The REO was provided by a Polish producer of essential oils (NaturalneAromaty sp. z o.o., Kłaj, Poland). The sample tested contained no additives or solvents and was confirmed by the manufacturer to be natural. Samples were stored in reclosable vials at 5°C in the dark, but allowed to reach room temperature before testing. Geographical origin was excluded as information was mostly not available.

Determination of antibacterial activity of essential oils by disc diffusion method. The antibacterial activity of REO was tested *in vitro* using the Kirby-Bauer disc diffusion technique [5]. 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.

Strains were inoculated onto Mueller-Hinton (MH) agar plates. Sterile filter paper discs impregnated with REO were placed over each culture dish. Bacterial isolates were then incubated with REO at 37 °C for 24 h. The Petri dishes were then observed for the zone of inhibition produced by the antibacterial activity of REO. A control Petri dish impregnated with 96% ethanol was used in each experiment. At the end of the 24-hour period, the inhibition zones formed were measured in millimetres using a vernier. Eight replicates were tested for each strain (n = 8). The Petri dishes were observed and photographed. The susceptibility of the test organisms to REO was indicated by a clear zone of inhibition around the discs containing REO and the diameter of the clear zone was used as an indicator of susceptibility. Zone diameters were determined and averaged. The following zone

diameter criteria were used to classify bacteria as susceptible or resistant to the tested phytochemicals: Susceptible (S) ≥ 15 mm, intermediate (I) = 10-15 mm and resistant (R) ≤ 10 mm [26, 37, 38].

Statistical analysis. Statistical analysis of the data obtained was performed using the mean ± standard error of the mean (S.E.M.). All variables were randomised according to the phytochemical activity of the REO tested. All statistical calculations were performed on separate data from each strain. The data were analysed by one-way analysis of variance (ANOVA) using Statistica v. 13.3 software (TIBCO Software Inc., USA) [43].

RESULTS AND DISCUSSION

The antibacterial activity induced by REO, estimated as the diameter of the growth inhibition zones of the Gram-positive and Gram-negative strains tested, is shown in Figures 1 and 2.
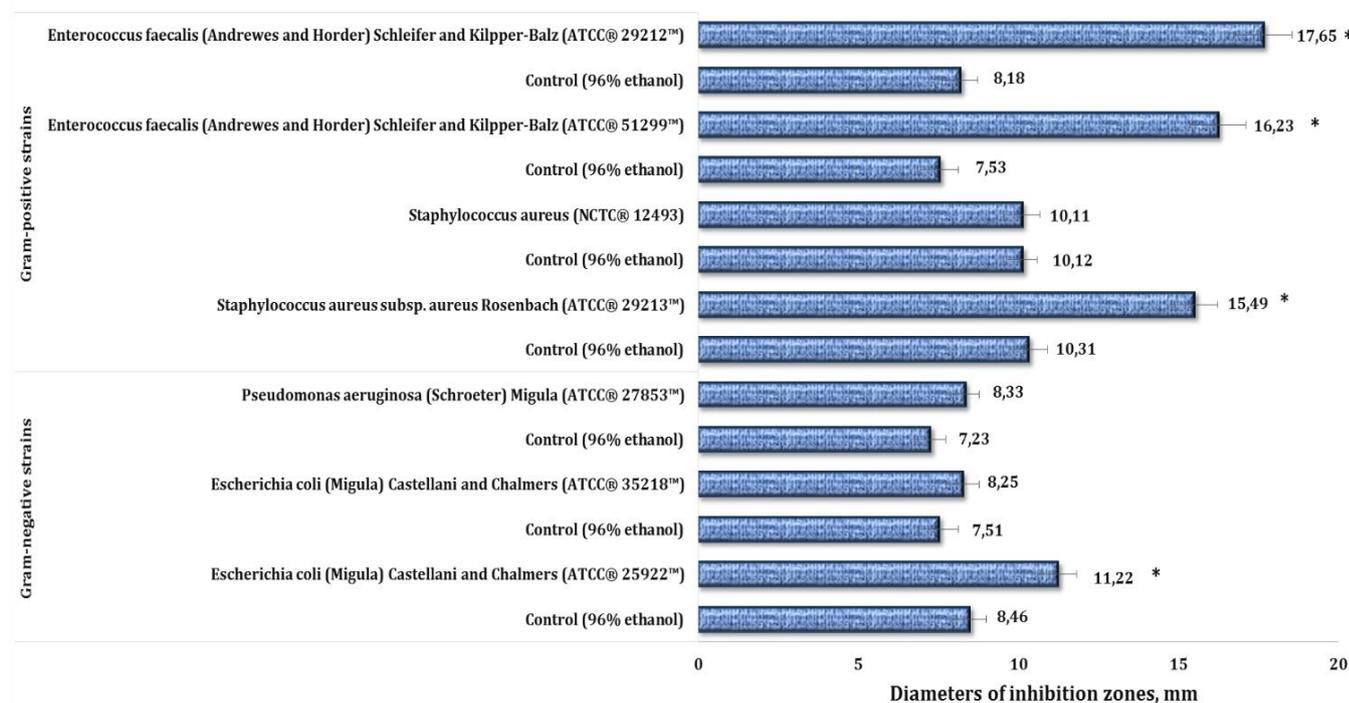

Fig. 1. The antibacterial activity induced by rosemary essential oil, estimated as the diameter of the growth inhibition zones of the Gram-positive and Gram-negative strains tested.
Data are presented as mean ± standard error of the mean (S.E.M.).
* – significant differences between control (96% ethanol) and REO (p < 0.05) are indicated.

The results of the current study showed that Gram-negative strains such as *E. coli* and *P. aeruginosa* were resistant to REO. The diameters of inhibition zones for *E. coli* (Migula) Castellani and Chalmers (ATCC$^®$ 25922™) strains after application of REO were increased to (11.22 ± 0.61 mm) compared to the 96% ethanol as control samples (8.46 ± 0.54 mm). Similar results were obtained for *E. coli* (Migula) Castellani and Chalmers (ATCC$^®$ 35218™) strains. The diameters of the inhibition zones after application of REO were (8.25 ± 0.52 mm) compared to the 96% ethanol control samples (7.51 ± 0.61 mm). The *P. aeruginosa* (Schroeter) Migula strain

(ATCC® 27853™) was also resistant to REO. The diameters of the inhibition zones after the application of REO were (8.33 ± 0.44 mm) compared to the 96% ethanol as control samples (7.23 ± 0.49 mm) (Fig. 1).

Gram-positive strains were more sensitive to REO than Gram-negative strains. *S. aureus* strains showed low activity against REO. The *S. aureus* (NCTC® 12493) strain was less sensitive than the *S. aureus* subsp. *aureus*Rosenbach (ATCC® 29213™) strain. Diameters of inhibition zones after application of REO were (10.11 ± 0.56 mm) compared to 96% ethanol as control samples (10.12 ± 0.48 mm) for *S. aureus* (NCTC® 12493) strain and (15.49 ± 0.75 mm) compared to 96% ethanol as control samples (10.31 ± 0.59 mm) for *S. aureus* subsp. *aureus*Rosenbach (ATCC® 29213™) strain. The increase in inhibition zone diameters after application of REO was 50.3% (p < 0.05) for *S. aureus* subsp. *aureus*Rosenbach (ATCC® 29213™) strain compared to the control samples (96% ethanol) (Fig. 1).

E. faecalis strains were more sensitive to REO (Fig. 1). Diameters of inhibition zones after application of REO were (16.23 ± 0.88 mm) compared to 96% ethanol as control samples (7.53 ± 0.60 mm) for *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) strain and (17.65 ± 8.18 mm) compared to the 96% ethanol as a control samples (8.18 ± 0.55 mm) for *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) strain. The increase in inhibition zone diameters after application of REO was 115.5% (p < 0.05) and 115.8% (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 (Fig. 1).

Detailed photographs of the zones of inhibition by REO against Gram-positive and Gram-negative bacterial strains were taken and are shown in Figure 2.

In line with our previous studies on the antibacterial potential of various plant extracts and EOs, in the current study we investigated the antibacterial potential of commercial rosemary essential oil against Gram-positive and Gram-negative bacterial strains. Gram-negative bacterial strains such as *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™) and *P. aeruginosa* (Schroeter) Migula (ATCC® 27853™) were resistant to the REO. The diameters of the inhibition zones after application of REO were similar to the control samples (96% ethanol). The increase in inhibition zone diameters after application of REO was 32.6% (p < 0.05) for *E. coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strains compared to 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 REO. On the other hand, *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) were sensitive to REO. The largest inhibition zone diameters after application of REO were observed for *E. faecalis* strains (Figures 1 and 2).
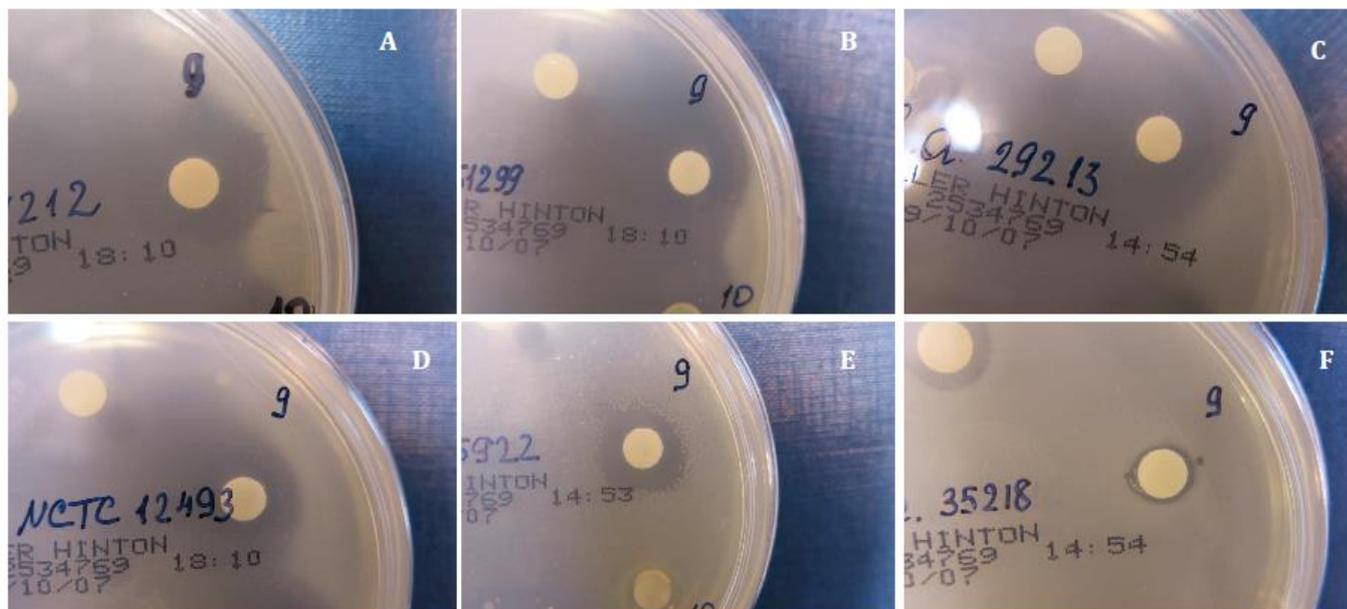
Fig. 2. Growth inhibition zones induced by rosemary essential oil against *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) (A), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (B), *Staphylococcus aureus* subsp. *aureus*Rosenbach (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)

Based on some studies, the essential oils of species belonging to the Lamiaceae family have antimicrobial activity against tested microbial isolates and can therefore be a good source of natural antimicrobial agents. For example, Bernardes and co-workers (2010) reported on the antimicrobial activity of the main components of rosemary oil against oral pathogens. The antimicrobial activity of the oil and its main constituents was tested against the following microorganisms: *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Streptococcus sobrinus* and *Enterococcus faecalis*, which are potentially responsible for the formation of dental caries in humans. The microdilution method was used to determine the minimum inhibitory concentration (MIC) in the evaluation of antibacterial activity. The essential oil showed low activity against the selected microorganisms. Sixty-two constituents were identified, representing 98.06% of the total rosemary oil content. Oxygenated monoterpenes were the predominant components. The rosemary oil was characterised by prominent (> 5%) contents of camphor (18.9%), verbenone (11.3%), α-pinene (9.6%), β-myrcene (8.6%), 1,8-cineole (8.0%) and β-caryophyllene (5.1%). In this study, the pure major compounds were more active than the essential oil. Of all the microorganisms tested, the pathogen *S. mitis* was the most susceptible and *E. faecalis* the most resistant to the samples evaluated [8].

The biological activities of *R. officinalis* extract were evaluated in the study by de Oliveira and co-workers (2017) as antimicrobial effect on mono- and

polymicrobial biofilms, cytotoxicity, anti-inflammatory capacity and genotoxicity [15]. Monomicrobial biofilms of *Candida albicans*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus mutans* and *Pseudomonas aeruginosa* and polymicrobial biofilms of *C. albicans* with each bacterium were formed in microplates for 48 h and exposed to *R. officinalis* extract (200 mg/mL) for 5 min.Its cytotoxic activity was assessed on murine macrophages (RAW 264.7), human gingival fibroblasts (FMM-1), human breast carcinoma cells (MCF-7) and cervical carcinoma cells (HeLa) after exposure to different concentrations of the extract. Anti-inflammatory activity was evaluated in RAW 264.7 non-stimulated or stimulated with lipopolysaccharide (LPS) from *Escherichia coli* and treated with different concentrations of the extract for 24 h. Interleukin-1 beta (IL-1β) and tumour necrosis factor-alpha (TNF-α) were quantified by ELISA. Genotoxicity was assessed by the frequency of micronuclei (MN) in 1000 cells after exposure to the extract concentrations for 24 h.Significant reductions in colony-forming units per millilitre (CFU/mL) were observed in all biofilms. With respect to cells, it was observed that concentrations ≤ 50 mg/mL resulted in cell viability greater than 50%. The production of pro-inflammatory cytokines in the treated groups was similar or lower than in the control group. The frequency of MN in the extract-exposed groups was similar or lower than in the untreated group. *R. officinalis* extract was shown to be effective against mono- and polymicrobial biofilms; it also provided cell viability of over 50% (at ≤ 50 mg/mL), showed anti-inflammatory activity and was not genotoxic. Impact statement *R. officinalis* extract effectively contributed to the *in vitro* control of important species of microorganisms such as *Candida albicans*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus mutans* and *Pseudomonas aeruginosa* in mono- and polymicrobial biofilms, which are responsible for several infections in the oral cavity as well as in other regions of the body. Furthermore, this extract also promoted cell viability above 50% at concentrations ≤ 50 mg/mL, excellent anti-inflammatory effect, showing inhibition or reduction of the synthesis of proinflammatory cytokines, being also non-genotoxic to the cell lines studied [15].

In the study by Wang and co-workers (2012), REO and three of its major components, 1,8-cineole (27.23%), α-pinene (19.43%) and β-pinene (6.71%), were evaluated for their *in vitro* antibacterial activities and toxicological properties. REO possessed antibacterial activities similar to α-pinene and slightly better than β-pinene.Moreover, 1,8-cineole possessed the lowest antibacterial activities. REO showed the strongest cytotoxicity against three human cancer cells. Its 50% inhibition concentration (IC$_{50}$) values against SK-OV-3, HO-8910 and Bel-7402 were 0.025‰, 0.076‰ and 0.13‰ (v/v), respectively. The cytotoxicity of all test samples was significantly higher on SK-OV-3 than on HO-8910 and Bel-7402. In both antibacterial and anticancer systems, REO generally showed greater activity than its constituents [39].

The essential oils of rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.) were analysed by Bozin and co-workers (2007) using gas

chromatography-mass spectrometry and evaluated for their antimicrobial and antioxidant activities [9]. The antimicrobial activity was tested against 13 bacterial strains and 6 fungi, including *Candida albicans* and 5 dermatomycetes. The main antibacterial activity of both essential oils was expressed against *Escherichia coli*, *Salmonella typhi*, *S. enteritidis* and *Shigellasonei*. A significant rate of antifungal activity, especially of rosemary essential oil, was also exhibited. The antioxidant activity was evaluated as free radical scavenging capacity (RSC) together with the effect on lipid peroxidation (LP). RSC was assessed by measuring the scavenging activity of essential oils on 2,2-diphenyl-1-picrylhydrazil (DPPH) and hydroxyl radicals. Effects on LP were evaluated following the activities of essential oils in Fe^{2+}/ascorbate and Fe^{2+}/H_2O_2 induction systems. The essential oils studied reduced DPPH radical formation in a dose-dependent manner (IC_{50} = 3.82 µg/mL for rosemary and 1.78 µg/mL for sage). A strong inhibition of LP in both induction systems was observed especially for rosemary essential oil [9].

Luqman and co-workers (2007) evaluated the antimicrobial potential of rosemary essential oil specifically for its efficacy against the drug-resistant mutants of *Mycobacterium smegmatis*, *Escherichia coli* and *Candida albicans*. The antibacterial, antifungal and drug resistance modifying activity was evaluated both qualitatively and quantitatively using disc diffusion and broth dilution assays. Rosemary essential oil was found to be more active against Gram-positive pathogenic bacteria, except *E. faecalis* and drug-resistant mutants of *E. coli*, compared to Gram-negative bacteria. It was also found to be more active against non-filamentous, filamentous, dermatophytic pathogenic fungi and drug resistant mutants of *Candida albicans* [23].

The essential oil composition of *Rosmarinus officinalis* var. *typicus* and var. *troglodytorum*, endemic to Tunisia and growing wild in different bioclimates, was determined by Zaouali and co-workers (2010). The oils were evaluated for antimicrobial and antioxidant activity. Variation in chemical composition was found to be due to cultivar rather than bioclimate. 1,8-Cineol (47.2-27.5%) and camphor (12.9-27.9%) were identified as the main components of var. *typicus* and var. *troglodytorum*, respectively. Principal component analysis of the oil constituents of all populations allowed the distinction of two distinct population groups according to varietal subdivision. Based on the determination of the diameter of inhibition and the determination of the minimum inhibitory concentration, a low to moderate antimicrobial activity was observed according to the oils against eight bacteria tested. However, oils from var. *troglodytorum*showed higher bactericidal activity than those from var. *typicus*. The antioxidant activity of the oils, determined by the 1,1-diphenyl-1-picrylhydrazyl (DPPH) assay, the ferric reduction assay (FRAP) and the β-carotene bleaching test, was relatively high. The highest activity was found in oils from var. *troglodytorum* and in a population of var. *typicus* from the upper semi-arid bioclimate [42].

Seasonal variability in essential oil composition and biological activity of *Rosmarinus officinalis* accessions in the western Himalayas was evaluated by

Rathore and co-workers (20-22). The accessions were evaluated to determine the EO content, composition, antimicrobial and cytotoxic potential of rosemary in different harvesting seasons during 2018-2019. EOs were active against both Gram-positive bacteria tested (*Micrococcus luteus* MTCC 2470 and *Staphylococcus aureus* MTCC 96). EOs showed inhibition of Gram-negative bacteria (*Salmonella typhi* MTCC 733), while *Klebsiella pneumoniae* MTCC 109 was found to be resistant. The rosemary EO of T1 (rainy season IHBT/RMAc-1) was most effective against *S. aureus* MTCC 96 with a minimum inhibitory concentration (MIC) of 4% (v/v). *In vitro* cytotoxicity evaluation did not show any potential anti-proliferative activity of EO. The EO profile of rosemary in the Western Himalayan region was influenced by harvest season and genetic variability within accessions [29].

Jardak and co-workers (2017) investigated the chemical composition of *Rosmarinus officinalis* essential oil (ROEO) and evaluated its antibiofilm activity on biofilm-forming bacteria, and its anticancer activity on cancer cell lines [21]. In this study, thirty-six compounds were identified in ROEO using GC-MS analysis. The major components were 1,8-cineol (23.56%), camphene (12.78%), camphor (12.55%) and β-pinene (12.3%). The antibacterial activity of ROEO was evaluated by the microdilution method. The oil showed inhibitory and bactericidal activity against two strains: *Staphylococcus aureus* ATCC 9144 and *Staphylococcus epidermidis* S61. It was found that the minimum inhibitory concentration (MIC) obtained for *S. aureus* and *S. epidermidis* ranged from 1.25 to 2.5 and from 0.312 to 0.625 μl·mL^{-1}, respectively, and the minimum bactericidal concentration (MBC) was in the order of 5 and 2.5 μl·mL^{-1}, respectively. Furthermore, this oil showed an inhibition of *S. epidermidis* biofilm of more than 57% at a concentration of 25 μl·mL^{-1}. The eradication of 67% of the established biofilm was observed at a concentration of 50 μl·mL^{-1} of ROEO, whereas the dose of 25 μl·mL^{-1} removed only 38% of the preformed biofilm. ROEO strongly inhibited the proliferation of Hela and MCF-7 cells with IC$_{50}$ values of 0.011 and 0.253 μl·mL^{-1}, respectively.Results from Jardak and co-workers (2017) showed that ROEO could have a potential role in the treatment of diseases associated with microbial infection or cancer cell proliferation [21].

CONCLUSIONS

In conclusion, this study provides insight into the *in vitro* antibacterial activity of commercial rosemary 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™).

The results of the current study showed that Gram-negative bacterial strains such as *E. coli* (Migula) Castellani and Chalmers (ATCC$^®$ 35218™) and *P. aeruginosa* (Schroeter) Migula (ATCC$^®$ 27853™) were resistant to REO. The diameters of the inhibition zones after application of REO were similar to those of the control samples (96% ethanol). The increase in inhibition zone diameters after application of REO was 32.6% (p < 0.05) for *E. coli* (Migula) Castellani and Chalmers (ATCC$^®$ 25922™) strains compared to control samples (96% ethanol). Similarly, the increase in inhibition zone diameters after application of REO was 50.3% (p < 0.05) for Gram-positive strains such as *S. aureus* subsp. *aureus*Rosenbach (ATCC$^®$ 29213™). Methicillin-resistant *S. aureus* (NCTC$^®$ 12493) was resistant to REO.On the other hand, the largest inhibition zone diameters after application of REO were observed for *E. faecalis* strains. The increase in inhibition zone diameters after application of REO was 115.5% (p < 0.05) and 115.8% (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.The results suggest that commercial rosemary essential oil supplied by a Polish essential oil manufacturer (NaturalneAromaty sp. z o.o., Kłaj, Poland) has some remarkable antimicrobial properties. *In vivo* studies are needed to calculate the effective dose of EOs and to determine their possible side effects and toxicity.

REFERENCES

1. Allenspach M, Steuer C. α-Pinene: A never-ending story. Phytochemistry. 2021;190:112857. doi:10.1016/j.phytochem.2021.112857.
2. Anandakumar P, Kamaraj S, Vanitha MK. D-limonene: A multifunctional compound with potent therapeutic effects. J Food Biochem. 2021;45(1):e13566. doi:10.1111/jfbc.13566.
3. Andrade JM, Faustino C, Garcia C, Ladeiras D, Reis CP, Rijo P. *Rosmarinus officinalis* L.: an update review of its phytochemistry and biological activity. Future Sci OA. 2018;4(4):FSO283. doi:10.4155/fsoa-2017-0124.
4. Bajalan I, Rouzbahani R, Pirbalouti AG, Maggi F. Antioxidant and antibacterial activities of the essential oils obtained from seven Iranian populations of *Rosmarinus officinalis*. Ind. Crops Prod. 2017;107:305–311. doi: 10.1016/j.indcrop.2017.05.063.
5. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J ClinPathol. 1966;45(4):493-496.
6. Begum A, Sandhya S, Shaffath Ali S, Vinod KR, Reddy S, Banji D. An in-depth review on the medicinal flora *Rosmarinus officinalis* (Lamiaceae). ActaSci Pol Technol Aliment. 2013;12(1):61-73.
7. Ben Arfa A, Gouja H, Hannachi H, Isoda H, Neffati M, Najjaa H. Seasonal changes in rosemary species: A chemotaxonomic assessment of two varieties based on essential oil compounds, antioxidant and antibacterial activities. PLoS One. 2022;17(8):e0273367. doi:10.1371/journal.pone.0273367.

8. Bernardes WA, Lucarini R, Tozatti MG, Flauzino LG, Souza MG, Turatti IC, Andrade e Silva ML, Martins CH, da Silva Filho AA, Cunha WR. Antibacterial activity of the essential oil from *Rosmarinus officinalis* and its major components against oral pathogens. Z Naturforsch C J Biosci. 2010;65(9-10):588-593. doi:10.1515/znc-2010-9-1009.

9. Bozin B, Mimica-Dukic N, Samojlik I, Jovin E. Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., Lamiaceae) essential oils. J Agric Food Chem. 2007;55(19):7879-7885. doi:10.1021/jf0715323.

10. Cai ZM, Peng JQ, Chen Y, Tao L, Zhang YY, Fu LY, Long QD, Shen XC. 1,8-Cineole: a review of source, biological activities, and application. J Asian Nat Prod Res. 2021;23(10):938-954. doi:10.1080/10286020.2020.1839432.

11. Cao W, Tian R, Pan R, Sun B, Xiao C, Chen Y, Zeng Z, Lei S. Terpinen-4-ol inhibits the proliferation and mobility of pancreatic cancer cells by downregulating Rho-associated coiled-coil containing protein kinase 2. Bioengineered. 2022;13(4):8643-8656. doi:10.1080/21655979.2022.2054205.

12. Christopoulou SD, Androutsopoulou C, Hahalis P, Kotsalou C, Vantarakis A, Lamari FN. Rosemary Extract and Essential Oil as Drink Ingredients: An Evaluation of Their Chemical Composition, Genotoxicity, Antimicrobial, Antiviral, and Antioxidant Properties. Foods. 2021;10(12):3143. doi:10.3390/foods10123143.

13. da Silva Bomfim N, Kohiyama CY, Nakasugi LP, Nerilo SB, Mossini SAG, Romoli JCZ, Graton Mikcha JM, Abreu Filho BA, Machinski M Jr. Antifungal and antiaflatoxigenic activity of rosemary essential oil (*Rosmarinus officinalis* L.) against *Aspergillus flavus*. Food AdditContam Part A Chem Anal Control Expo Risk Assess. 2020;37(1):153-161. doi:10.1080/19440049.2019.1678771.

14. de Oliveira JR, Camargo SEA, de Oliveira LD. *Rosmarinus officinalis* L. (rosemary) as therapeutic and prophylactic agent. J Biomed Sci. 2019;26(1):5. doi:10.1186/s12929-019-0499-8

15. de Oliveira JR, de Jesus D, Figueira LW, de Oliveira FE, Pacheco Soares C, Camargo SE, Jorge AO, de Oliveira LD. Biological activities of *Rosmarinus officinalis* L. (rosemary) extract as analyzed in microorganisms and cells. ExpBiol Med (Maywood). 2017;242(6):625-634. doi:10.1177/1535370216688571.

16. Ekambaram SP, Perumal SS, Balakrishnan A, Marappan N, Gajendran SS, Viswanathan V. Antibacterial synergy between rosmarinic acid and antibiotics against methicillin-resistant *Staphylococcus aureus*. J IntercultEthnopharmacol. 2016;5(4):358-363. doi:10.5455/jice.20160906035020.

17. Fazmiya MJA, Sultana A, Rahman K, Heyat MBB, Sumbul, Akhtar F, Khan S, Appiah SCY. Current Insights on Bioactive Molecules, Antioxidant, Anti-Inflammatory, and Other Pharmacological Activities of *Cinnamomumcamphora* Linn. Oxid Med Cell Longev. 2022;2022:9354555. doi:10.1155/2022/9354555.

18. GhasemzadehRahbardar M, Hosseinzadeh H. Therapeutic effects of rosemary (*Rosmarinus officinalis* L.) and its active constituents on nervous system disorders. Iran J Basic Med Sci. 2020;23(9):1100-1112. doi:10.22038/ijbms.2020.45269.10541.

19. González-Velasco HE, Pérez-Gutiérrez MS, Alonso-Castro ÁJ, Zapata-Morales JR, Niño-Moreno PDC, Campos-Xolalpa N, González-Chávez MM. Anti-Inflammatory and Antinociceptive Activities of the Essential Oil of *Tagetesparryi* A. Gray (Asteraceae) and Verbenone. Molecules. 2022;27(9):2612. doi:10.3390/molecules27092612.

20. Guan H, Luo W, Bao B, Cao Y, Cheng F, Yu S, Fan Q, Zhang L, Wu Q, Shan M. A Comprehensive Review of Rosmarinic Acid: From Phytochemistry to Pharmacology and Its New Insight. Molecules. 2022;27(10):3292. doi: 10.3390/molecules27103292.

21. Jardak M, Elloumi-Mseddi J, Aifa S, Mnif S. Chemical composition, anti-biofilm activity and potential cytotoxic effect on cancer cells of *Rosmarinus officinalis* L. essential oil from Tunisia. Lipids Health Dis. 2017;16(1):190. doi:10.1186/s12944-017-0580-9.

22. Jiang Y, Wu N, Fu YJ, Wang W, Luo M, Zhao CJ, Zu YG, Liu XL. Chemical composition and antimicrobial activity of the essential oil of Rosemary. Environ ToxicolPharmacol. 2011 Jul;32(1):63-8. doi: 10.1016/j.etap.2011.03.011.

23. Luqman S, Dwivedi GR, Darokar MP, Kalra A, Khanuja SP. Potential of rosemary oil to be used in drug-resistant infections. AlternTher Health Med. 2007;13(5):54-59.

24. Mei Y, Li L, Fan L, Fan W, Liu L, Zhang F, Hu Z, Wang K, Yang L, Wang Z. The history, stereochemistry, ethnopharmacology and quality assessment of borneol. J Ethnopharmacol. 2023;300:115697. doi:10.1016/j.jep.2022.115697.

25. Nieto G, Ros G, Castillo J. Antioxidant and Antimicrobial Properties of Rosemary (*Rosmarinus officinalis*, L.): A Review. Medicines (Basel). 2018;5(3):98. doi: 10.3390/medicines5030098.

26. Okoth DA, Chenia HY, Koorbanally NA. Antibacterial and antioxidant activities of flavonoids from *Lanneaalata* (Engl.) Engl. (Anacardiaceae). Phytochemistry Letters, 2013;6:476–481. doi:10.1016/j.phytol.2013.06.003.

27. Ramalho TR, Oliveira MT, Lima AL, Bezerra-Santos CR, Piuvezam MR. Gamma-Terpinene Modulates Acute Inflammatory Response in Mice. Planta Med. 2015;81(14):1248-54. doi: 10.1055/s-0035-1546169.

28. Rašković A, Milanović I, Pavlović N, Ćebović T, Vukmirović S, Mikov M. Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) essential oil and its hepatoprotective potential. BMC Complement Altern Med. 2014;14:225. doi:10.1186/1472-6882-14-225.

29. Rathore S, Mukhia S, Kapoor S, Bhatt V, Kumar R, Kumar R. Seasonal variability in essential oil composition and biological activity of *Rosmarinus officinalis* L. accessions in the western Himalaya. Sci Rep. 2022;12(1):3305. doi:10.1038/s41598-022-07298-x

30. Salehi B, Upadhyay S, Erdogan Orhan I, Kumar Jugran A, L D Jayaweera S, A Dias D, Sharopov F, Taheri Y, Martins N, Baghalpour N, Cho WC, Sharifi-Rad J. Therapeutic Potential of α- and β-Pinene: A Miracle Gift of Nature. Biomolecules. 2019;9(11):738. doi:10.3390/biom9110738.

31. Satyal P, Jones TH, Lopez EM, McFeeters RL, Ali NA, Mansi I, Al-Kaf AG, Setzer WN. Chemotypic Characterization and Biological Activity of *Rosmarinus officinalis*. Foods. 2017;6(3):20. doi: 10.3390/foods6030020.

32. Scandiffio R, Geddo F, Cottone E, Querio G, Antoniotti S, Gallo MP, Maffei ME, Bovolin P. Protective Effects of (E)-β-Caryophyllene (BCP) in Chronic Inflammation. Nutrients. 2020;12(11):3273. doi:10.3390/nu12113273.

33. Shahina Z, Al Homsi R, Price JDW, Whiteway M, Sultana T, Dahms TES. Rosemary essential oil and its components 1,8-cineole and α-pinene induce ROS-dependent lethality and ROS-independent virulence inhibition in *Candida albicans*. PLoS One. 2022;17(11):e0277097. doi:10.1371/journal.pone.0277097.

34. Souza R, Cardoso M, Menezes C, Silva J, De Sousa D, Batista J. Gastroprotective activity of α-terpineol in two experimental models of gastric ulcer in rats. Daru. 2011;19(4):277-281.

35. Surendran S, Qassadi F, Surendran G, Lilley D, Heinrich M. Myrcene-What Are the Potential Health Benefits of This Flavouring and Aroma Agent?. Front Nutr. 2021;8:699666. doi:10.3389/fnut.2021.699666.

36. Tak JH, Isman MB. Enhanced cuticular penetration as the mechanism for synergy of insecticidal constituents of rosemary essential oil in Trichoplusiani. Sci Rep. 2015;5:12690. doi: 10.1038/srep12690.

37. Tkachenko H, Opryshko M, Gyrenko O, Maryniuk M, Buyun L, Kurhaluk N. Antibacterial properties of commercial lavender essential oil against some Gram-positive and Gram-negative bacteria. Agrobiodiversity for Improving Nutrition, Health and Life Quality, 2022;6(2):220–228. doi:10.15414/ainhlq.2022.0023.

38. Tkaczenko H, Kurhaluk N, Opryshko M, Maryniuk M, Gyrenko O, Buyun L. *In vitro* antibacterial efficacy of the commercial wintergreen (*Gaultheria procumbens* L.) essential oil against some Gram-positive and Gram-negative strains. Agrobiodiversity for Improving Nutrition, Health and Life Quality, 2023;7(1):115-122. doi:10.15414/ainhlq.2023.0013.

39. Wang W, Li N, Luo M, Zu Y, Efferth T. Antibacterial activity and anticancer activity of *Rosmarinus officinalis* L. essential oil compared to that of its main components. Molecules. 2012;17(3):2704-2713. doi:10.3390/molecules17032704.

40. Wang X, Dong K, Ma Y, Jin Q, Yin S, Wang S. Hepatoprotective effects of chamazulene against alcohol-induced liver damage by alleviation of oxidative stress in rat models. Open Life Sci. 2020;15(1):251-258. doi:10.1515/biol-2020-0026.

41. Yeo D, Hwang SJ, Song YS, Lee HJ. Humulene Inhibits Acute Gastric Mucosal Injury by Enhancing Mucosal Integrity. Antioxidants (Basel). 2021;10(5):761. doi:10.3390/antiox10050761.

42. Zaouali Y, Bouzaine T, Boussaid M. Essential oils composition in two *Rosmarinus officinalis* L. varieties and incidence for antimicrobial and antioxidant activities. Food ChemToxicol. 2010;48(11):3144-3152. doi:10.1016/j.fct.2010.08.010.

43. Zar JH. *Biostatistical Analysis*. 4th ed., Prentice-Hall Inc., Englewood Cliffs, New Jersey, 1999.

44. Zhao ZJ, Sun YL, Ruan XF. Bornyl acetate: A promising agent in phytomedicine for inflammation and immune modulation. Phytomedicine. 2023;114:154781. doi: 10.1016/j.phymed.2023.154781.

Стаття надійшла до редакції / The article was received   25.11.2023