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ANTIBACTERIAL EFFECTS OF CELANDINE OIL AND EXTRACTS DERIVED FROM ROOTS AND STALKS OF GREATER CELANDINE (CHELIDONIUM MAJUS L.) COLLECTED FROM CENTRAL POMERANIAN REGION

Nataniel Stefanowski¹, Halina Tkachenko¹, Natalia Kurhaluk¹, Oleksandr Lukash², Lyudmyla Buyun³

¹ Institute of Biology and Earth Sciences, Pomeranian University in Słupsk, ul. Arciszewskiego 22b, 76-200 Słupsk, Poland e-mail: natanielstefanowski89@gmail.com, halyna.tkachenko@apsl.edu.pl ² Department of Ecology and Nature Protection, T. G. Shevchenko National University "Chernihiv Collegium", Hetmana Polubotka St, 53, 14 000 Chernihiv, Chernihiv Oblast, Ukraine e-mail: lukash2011@ukr.net ³ M. M. Gryshko National Botanic Garden, National Academy of Science of Ukraine, I, Tymiriazievska St, 01 014 Kyiv, Ukraine e-mail: orchids.lvuda@gmail.com

Abstract

A convincing number of studies indicating that alkaloids such as chelidonine and sanguinarine together with other secondary metabolites exhibit potent antibacterial, antifungal, and antiparasitic properties prompted us to examine the antimicrobial efficacy of *Chelidonium majus* L., a representative of the Papaveraceae family, against *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC[®] 29213TM) (mecA negative, Oxacillin sensitive, weak β -lactamase producing strain), *S. aureus* NCTC[®] 12493TM (mecA positive, Methicillin-resistant, EUCAST QC strain for cefoxitin), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC[®] 25922TM), *E. coli* (Migula) Castellani and Chalmers (ATCC[®] 35218TM). In the current work, we decided to evaluate the antimicrobial efficacy of ethanolic extracts derived from stalks and roots of *C. majus*, as well as commercial 100% natural fatty celandine oil (Botanica, Russia) against different *S. aureus* and *E. coli* strains. Fresh stalks and roots were washed, weighed, crushed, and homogenized in 96% ethanol (in proportion of 1:19, w/w) at room temperature. The extracts were then filtered and investigated for their antimicrobial activity. Antimicrobial activity was determined using the agar disk diffusion assay. The extracts obtained from roots and stalks of *C. majus* and commercial natural fatty celandine oil exhibited different antibacterial activities against tested strains. The ethanolic extracts of *C. majus* revealed weak antibacterial

activity against both *E. coli* (Migula) Castellani and Chalmers (ATCC[®] 25922TM) and *E. coli* (Migula) Castellani and Chalmers (ATCC[®] 35218TM) strains. The highest antibacterial activity was demonstrated for stalk extracts of *C. majus* against *S. aureus* NCTC[®] 12493TM compared to the control samples. We observed similar trends when measuring the zones of growth inhibition according to *S. aureus* subsp. *aureus* Rosenbach (ATCC[®] 29213TM) strain. Since the antimicrobial efficacy of medicinal plants varies according to the accumulation of secondary metabolites (i.e., alkaloids, flavonoids, tannins, etc.), it is not surprising that differences in this efficacy have been noted even using samples taken from the same plant but different parts of the plant (stalks, roots). The antimicrobial activity of crude ethanolic extracts obtained from stalks and roots of greater celandine can be attributed to specific compounds or a combination of compounds. The current study lays the foundation for future research to confirm the potential use of *C. majus* as a candidate for the treatment of infections caused by *S. aureus* and *E. coli* in human and veterinary medicine.

Key words: antibacterial activities, Chelidonium majus L., Staphylococcus aureus subsp. aureus, Escherichia coli strains, coastal zones, Pomeranian regions

INTRODUCTION

Coastal zones are the transition between continents and the ocean (Lakshmi 2021). A wide number of human activities take place here, some of them being potential sources of different substances toward the marine environment. The coastal zone is the site of intimate interactions between land, ocean, and atmosphere, making it extremely active, both biologically and geochemically. It is also dynamic both spatially and temporally, and thus very complex and difficult to study representatively (Kusky 2008). The coastal areas of the Baltic Sea are also home to diverse ecosystems that provide goods and services essential to human well-being. Recognizing, understanding, and appreciating the various goods and services provided by coastal ecosystems, especially provisioning and cultural services, is of great importance today (White et al. 2013). Systematic research of flora and fauna of the Kashubian-Pomeranian zone and obtaining pharmaceuticals from them are fundamentally connected with human health and nutrition, which enforces actions to protect these ecosystems (Barbier et al. 2011). For a long time, the flora of the Baltic zone has been impressed with its diversity in terms of the medicinal plants found there (Saunders et al. 2020). Plants belonging to the Papaveraceae family also consist of the flora of the coastal zone of the Baltic Sea. Recent scientific reports demonstrate the remarkable antimicrobial properties of plants belonging to the Papaveraceae family (Zielińska et al. 2018, 2021).

Antibiotic resistance and its wider implications present us with a growing healthcare crisis. Recent research points to the environment as an important component for the transmission of resistant bacteria and the emergence of resistant pathogens (Mattner et al. 2012). However, a deeper understanding of the evolutionary and ecological processes that lead to the clinical appearance of resistance genes is still lacking, as is knowledge of environmental dispersal barriers (Michael et al. 2013, O'Neill 2014). The increase in multi-drug resistance to bacterial infections is a worldwide dilemma. Recovery from systemic or local bacterial infections can be lengthy and costly, with the clinical challenges being further complicated when bacteria acquire resistance to current antibiotics. At the heart of the problem is the inability to treat established biofilms

with standard antibiotic therapy, including fluoroquinolones. One of the earliest applications of whole-genome sequencing in public health was teasing out epidemiological associations in hospital-acquired infections. Within a few years, whole-genome sequencing began to be used more widely for elucidating and interrupting transmission pathways in hospital outbreaks, such as those caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenem-resistant *Klebsiella pneumonia* (Nasr et al. 2013, Polvoy et al. 2020). Therefore, it is important to look for alternative solutions, such as medicinal plants (Lai and Roy 2004). Herbal medicine is considered to be an effective alternative to chemical medicine. Wild and introduced medicinal plants are used as a source of raw materials for obtaining a lot of effective remedies. Currently, about forty percent of medicines comprise herbal active ingredients, and the demand for medicinal herbs continues to increase (Sumner et al. 2015).

Greater celandine, *Chelidonium majus* L. (CM, Papaveraceae), is a valuable medicinal plant which is widely distributed in Europe and Asia and also introduced in Northern America. CM is a plant of great interest for its wide use in folk medicine and also in official phytotherapy (Zielińska et al. 2018, 2019). This species is known to synthesize a broad range of secondary metabolites which provide its therapeutic properties. The most common group of these secondary metabolites are isoquinoline alkaloids (1-2% in the herb and 2-4% in the roots), including chelidonine, berberine, sanguinarine, coptisine, chelerythrine and protopine. CM is a medicinal plant well-known as a valuable source of isoquinoline alkaloids, which has a variety of pharmacological properties including anti-viral and anti-bacterial effects (Amal and Banerji 2015).

Considering the points highlighted above and based on previous results obtained in our laboratory, the current study aimed to find out *in vitro* the possible antimicrobial activity of the ethanolic extracts derived from roots and stalks of CM, as well as commercial 100% natural fatty celandine oil (Botanica, Russia) against two *Escherichia coli* and *Staphylococcus aureus* strains.

MATERIALS AND METHODS

Collection of plant material

Plant materials (Fig. 1B) were harvested from natural habitats on the territory of the Kartuzy district (54°20'N 18°12'E) in the Pomeranian province (northern part of Poland) (Fig. 1A). Kartuzy is located about 32 kilometers (20 miles) west of Gdańsk and 35 km (22 miles) south-east of the town of Lębork on a plateau at an altitude of approximately 200 meters (656 feet) above sea level on average. The plateau, which is divided by the Radaune River, comprises the highest parts of the Baltic Sea Plate (www.kartuzy.pl). Plants were collected from urban (n = 5) and rural agglomerations (n = 15) on the territory of the Kartuzy district.



Fig. 1. Location of Kartuzy in the map of Poland (A), where the greater celandine (B) was collected

Preparation of plant extracts

The collected roots and stalks were brought into the laboratory for antimicrobial studies. Freshly washed samples were weighed, crushed, and homogenized in 96% ethanol (in proportion 1:19, w/w) at room temperature. The extracts were then filtered and investigated for their antimicrobial activity.

Bacterial test strain and growth conditions

For this study, a panel of organisms including *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC[®] 29213TM) (mecA negative, Oxacillin sensitive, weak β -lactamase producing strain), *S. aureus* NCTC[®] 12493TM (mecA positive, Methicillin-resistant, EUCAST QC strain for cefoxitin), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC[®] 25922TM), *E. coli* (Migula) Castellani and Chalmers (ATCC[®] 35218TM) were used. The cultivation medium was trypticase soy agar (Oxoid[®], UK), supplemented with 10% defibrinated sheep blood. Cultures were grown aerobically for 24 h at 37°C. The cultures were later diluted with a sterile solution of 0.9% normal saline to approximate the density of 0.5 McFarland standard. The McFarland standard was prepared by inoculating colonies of the bacterial test strain in sterile saline and adjusting the cell density to the specified concentration (CLSI 2014).

The disk diffusion method for evaluation of antibacterial activity of plant extracts

Strains tested were plated on TSA medium (Tryptone Soy Agar) and incubated for 24 h at 37°C. Then the suspension of microorganisms was suspended in sterile PBS and

the turbidity adjusted equivalent to that of a 0.5 McFarland standard. The antimicrobial susceptibility testing was done on Muller–Hinton agar by disk diffusion method (Kirby–Bauer disk diffusion susceptibility test protocol) (Bauer et al. 1966). Muller–Hinton agar plates were inoculated with 200 µl of standardized inoculum (108 CFU/mL) of the bacterium and spread with sterile swabs.

Sterile filter paper disks impregnated by extract or commercial 100% natural fatty celandine oil were applied over each of the culture plates, 15 min after bacteria suspension was placed. A negative control disk impregnated by sterile 96% ethanol was used in each experiment. After culturing bacteria on the plates with filter paper disks impregnated by extract or commercial 100% natural fatty celandine oil, they were incubated for 24 h at 37°C. The assessment of antimicrobial activity was based on the measurement of the diameter of the inhibition zone formed around the disks. The diameters of the inhibition zones were measured in millimeters and compared with those of the control disks. The activity was evidenced by the presence of a zone of inhibition surrounding the disks (CLSI 2014). The results of the disk diffusion test are "qualitative," in that a category of susceptibility (i.e., susceptible, intermediate, or resistant) is derived from the test rather than a minimum inhibitory concentration MIC (Jorgensen and Ferraro 2009).

Statistical analysis

Zone diameters were determined and averaged. Statistical analysis of the data obtained was performed by employing the mean. All variables were randomized according to the antibacterial activity of tested extracts and commercial 100% natural fatty celandine oil. The data were analyzed using one-way analysis of variance (ANOVA) using Statistica software, version 13.3 (TIBCO Software Inc., Kraków, Poland) (Zar 1999). The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (S) \geq 15 mm, Intermediate (I) = 10-15 mm, and Resistant (R) \leq 10 mm (Okoth et al. 2013).

RESULTS AND DISCUSSION

Zone of growth inhibitions of different *E. coli* and *S. aureus* strains induced by extracts derived from roots and stalks of *C. majus*, as well as commercial 100% natural fatty celandine oil (Botanica, Russia) are shown in Figs 2 and 3.

No statistically significant changes in the diameters of inhibition zones against strains studied compared to the controls were observed. The extracts derived from stalks of greater celandine showed weak antimicrobial activity against *E. coli* (Migula) Castellani and Chalmers (ATCC[®] 25922TM) strain, where the zone of growth inhibition was $(8.2 \pm 0.15 \text{ mm})$ compared to the control samples $(7.92 \pm 0.84 \text{ mm})$. This was an increase in the zone of growth inhibition only by 3.5% (p > 0.05). Root extracts also showed similar activity ($8.08 \pm 0.23 \text{ mm}$) against this strain compared to the control samples ($7.92 \pm 0.84 \text{ mm}$), where there was a 2% increase (p > 0.05). The zone of growth



Fig. 2. Zone of growth inhibitions of different *Escherichia coli* strains induced by extracts derived from roots and stalks of *C. majus* collected from different areas of Pomeranian region, as well as commercial 100% natural fatty celandine oil (Botanica, Russia) in millimeter (n = 8) compared to 96% ethanol (control samples)

inhibition against E. coli (Migula) Castellani and Chalmers (ATCC[®] 25922TM) and E. coli (Migula) Castellani and Chalmers (ATCC[®] 35218TM) strains after treatment with greater celandine oil was smaller compared to the control samples, i.e. $(7.9 \pm 0.79 \text{ mm})$ vs. $(7.92 \pm 0.84 \text{ mm}), (6.5 \pm 0.69 \text{ mm})$ vs. $(7.51 \pm 0.91 \text{ mm})$. Low antimicrobial activity (0.5% increase compared to control samples) against E. coli (Migula) Castellani and Chalmers (ATCC[®] 35218TM) strain was shown for root extracts of C. majus (7.55 \pm 0.18 mm) against control samples $(7.51 \pm 0.91 \text{ mm})$. In contrast, when analyzing the zone of growth inhibition after the application of stalk extracts against E. coli (Migula) Castellani and Chalmers (ATCC® 35218TM) strain, we did not record results that exceeded the values of the control samples, i.e. $(7.38 \pm 0.15 \text{ mm})$ vs. $(7.51 \pm 0.91 \text{ m})$ (Fig. 2). Other results were obtained after measuring the zones of growth inhibition of S. aureus NCTC® 12493TM and S. aureus subsp. aureus Rosenbach (ATCC® 29213TM) strains (Fig. 3). The highest antibacterial activity was demonstrated for stalk extracts of CM (13.65 \pm 0.6 mm) against S. aureus NCTC[®] 12493TM compared to the control samples (9.12 \pm 0.95 mm). We observed similar trends when measuring the zones of growth inhibition according to Staphylococcus aureus subsp. aureus Rosenbach (ATCC® 29213TM) strain, i.e. $(12.43 \pm 0.28 \text{ mm})$ compared to the controls $(8.56 \pm 0.75 \text{ mm})$. Root extracts also exhibited significant activity against S. aureus subsp. aureus Rosenbach (ATCC® 29213^{TM}) strain – (11.45 ± 0.91 mm vs. 8.56 ± 0.75 mm) as opposed to strain S. aureus NCTC[®] 12493TM (9.45 \pm 0.27 mm) as compared to the controls (9.12 \pm 0.95 mm). Greater celandine oil exhibited no antibacterial activity against S. aureus NCTC® 12493TM strain by measuring the zones of growth inhibition $(8.8 \pm 0.79 \text{ mm } vs. 9.12 \pm 0.95 \text{ mm})$. It showed low antimicrobial activity against strain S. aureus subsp. aureus Rosenbach (ATCC[®] 29213TM) strain, i.e. the zones of growth inhibition was $(9.5 \pm 0.79 \text{ mm})$ compared to the control samples $(8.56 \pm 0.75 \text{ mm})$ (Fig. 3).



Fig. 3. Zone of growth inhibitions of different *Staphylococcus aureus* strains induced by extracts derived from roots and stalks of *C. majus* collected from different areas of Pomeranian region, as well as commercial 100% natural fatty celandine oil (Botanica, Russia) in millimeter (n = 8) compared to 96% ethanol (control samples)

In our previous studies (Stefanowski et al. 2021a), we obtained different results to the zone of growth inhibitions against different strains induced by extracts derived from roots and stalks of CM collected from different areas of the Pomeranian region. We demonstrated high antimicrobial activity of both stalk and root extracts of CM collected from urban areas against S. aureus subsp. aureus Rosenbach (ATCC® 29213TM) by measuring zones of growth inhibition (for root extracts 16.9 mm, for stalks extracts 13.6 mm) compared to the control samples (8.8 mm). We also noted that stalk extracts of CM collected from both urban (15.3 mm) and rural (13.1 mm) agglomerations exhibited high antibacterial activity against S. aureus NCTC[®] 12493[™] strain compared to the control samples (9.1 mm) by statistically significantly increasing the zones of growth inhibition. Our subsequent studies against Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299TM), E. coli (Migula) Castellani and Chalmers (ATCC® 25922TM), E. coli (Migula) Castellani and Chalmers (ATCC® 35218TM) strains revealed that stem extracts of CM exhibited significant antibacterial effects measured as the zone of growth inhibition (Stefanowski et al. 2021a). In our previous study, we also demonstrated high antimicrobial activity of CM extracts against the E. coli strain, which was isolated locally from the wounds. CM extracts derived from stalks showed antimicrobial properties by a statistically significant increase in growth inhibition zone against E. coli [(12.97 ± 1.02) mm] compared to control samples $[(7.2 \pm 0.81) \text{ mm}]$. It was evident from this study that stalk extracts exhibited the highest antibacterial activity against E. coli. Other results were obtained after another series of studies on greater celandine extracts against *E. coli* strain. Other results were obtained after another series of studies on greater celandine extracts against *S. aureus* and *E. coli* strains. The extracts derived from both stalks and roots showed no antimicrobial properties against the tested strain (Stefanowski et al. 2021c, d, e). Similar results were obtained after studies on greater celandine extracts against the *S. aureus* strain. The extracts derived from stalks and roots of CM have also shown weak antibacterial activity against the tested strain, by not showing statistically significant in the zones of growth inhibition (Stefanowski et al. 2021b).

The selective antibacterial activity for alkaloids isolated from CM opens the possibility that they could be helpful for the development of new antibacterial agents for treating bacterial infections which have created nosocomial problems worldwide. CM extracts exhibit antimicrobial activity due to their complex alkaloid composition (Cheng et al. 2006, 2007, Meng et al. 2009, Zielińska et al. 2019). Zuo and co-workers (2008) have described the antibacterial effect of extracts and compounds isolated from the aerial part of CM acting against clinical strains of methicillin-resistant *Staphylococcus aureus* (MRSA). The activities were evaluated by using the macro broth dilution method and reported as the MICs/MBCs. Bioassay-guided fractionation of the most active extract from the aerial parts (EtOAc) led to the isolation of benzo[c]phenanthridine-type alkaloids 8-hydroxydihydrosanguinarine (hhS), 8-hydroxy-dihydro chelerythrine (hhC), which were potently active against MRSA strains (Zuo et al. 2008).

The application of plant-derived extracts from the Papaveraceae family combined with synthetic antimicrobials, absorbed into organic bacterial cellulose (BC) carriers, may be considered a promising way of fighting biofilm-forming Helicobacter pylori. Krzyżek and co-workers (2021) have determined the antibacterial activity of extracts from selected plants of the Papaveraceae family against planktonic and biofilm forms of the multidrug-resistant clinical strain of H. pylori using a broad spectrum of analytical in vitro methods. It was revealed that among the tested extracts, those obtained from Corydalis cheilanthifolia and CM were the most active, with minimal inhibitory concentrations (MICs) of 64 µg/mL and 128 µg/mL, respectively. High concentrations of both extracts showed cytotoxicity against cell lines of human hepatic origin. Therefore, researchers attempted to lower their MICs through the use of a synergistic combination with synthetic antimicrobials as well as by applying cellulose as a drug carrier. Using checkerboard assays, these researchers determined that both extracts presented synergistic interactions with amoxicillin (AMX) and 3-bromopyruvate (3-BP) and additive relationships with sertraline (SER). The antibiofilm activity of extracts and their combinations with AMX, 3-BP, or SER, was analyzed by two methods, i.e., the microcapillary overgrowth under flow conditions (the Bioflux system) and assessment of the viability of lawn biofilms after exposure to drugs released from bacterial cellulose (BC) carriers. Using both methods, these researchers observed a several-fold decrease in the level of *H. pylori* biofilm, indicating the ability of the tested compounds to eradicate the microbial biofilm (Krzyżek et al. 2021).

Zielińska and co-workers (2019) have evaluated the antimicrobial potential of CM extracts and *in vitro* cultures, as well as seven major individual alkaloids. Plant material derived from different natural habitats and *in vitro* cultures was used for the

phytochemical analysis and antimicrobial tests. The results of Zielińska and co-workers (2019) have shown that roots contained higher numbers and amounts of alkaloids in comparison to aerial parts. All tested plant extracts manifested antimicrobial activity, related to different chemical structures of the alkaloids. Root extract used at 31.25-62.5 mg/L strongly reduced bacterial biomass. From the seven individually tested alkaloids, chelerythrine was the most effective against *Pseudomonas aeruginosa* (MIC at 1.9 mg/L), while sanguinarine against *S. aureus* (MIC at 1.9 mg/L). Strong antifungal activity was observed against *Candida albicans* when chelerythrine, chelidonine, and aerial parts extract were used (Zielińska et al. 2019).

Phytochemical compositions of extracts derived from the aerial and underground parts of five Papaveraceae species (C. majus, Corydalis cava (L.) Schweigg. and Körte, C. cheilanthifolia Hemsl., C. pumila (Host) Rchb., and Fumaria vaillantii Loisel.) were examined by Zielińska and co-workers (2021). Large differences in the quality and quantity of all analyzed compounds were observed between species of different genera and also within one genus. Two groups of metabolites predominated in the phytochemical profiles. These were isoquinoline alkaloids and, in smaller amounts, non-phenolic carboxylic acids and phenolic compounds. In aerial and underground parts, 22 and 20 compounds were detected, respectively. These included: seven isoquinoline alkaloids such as protopine, allocryptopine, coptisine, berberine, chelidonine, sanguinarine, and chelerythrine; five of their derivatives as well as non-alkaloids i.e. malic acid, trans-aconitic acid, quinic acid, salicylic acid, trans-caffeic acid, p-coumaric acid, chlorogenic acid, quercetin, and kaempferol; and vanillin. The aerial parts were much richer in phenolic compounds regardless of the plant species. Characterized extracts were studied for their antimicrobial potential against planktonic and biofilm-producing cells of S. aureus, P. aeruginosa, and C. albicans. The impact of the extracts on cellular metabolic activity and biofilm biomass production was evaluated. Moreover, the antimicrobial activity of the extracts introduced to the polymeric carrier made of bacterial cellulose was assessed. Extracts of C. cheilanthifolia were found to be the most effective against all tested human pathogens. Multiple regression tests indicated a high antimicrobial impact of quercetin in extracts of aerial parts against planktonic cells of S. aureus, P. aeruginosa, and C. albicans, and no direct correlation between the composition of other bioactive substances and the results of antimicrobial activity was found (Zielińska et al. 2021).

The mechanism of antimicrobial action of secondary metabolites (e.g., CM alkaloids) is multidirectional, but the most relevant in the context of synergistic therapy is the ability to disrupt cell membrane integrity. It appears that this mechanism may also be important for positive interactions with the compounds we studied. Krzyżek and co-workers (2021) have determined the antibacterial activity of extracts from selected plants of the Papaveraceae family against planktonic and biofilm forms of the multi-drug-resistant clinical strain of *H. pylori* using a broad spectrum of analytical *in vitro* methods. Amoxicillin (AMX) and 3-bromopyruvate (3-BP) are substances that enter microbial cells via cell membrane transport proteins, so disruption of the integrity of the cell membrane(s) may open additional portals for both compounds to enter the bacteria. Due to its lipophilicity, sertraline (SER) exhibits the ability to cross the membrane

barrier independently of porins, so perhaps the presence of isoquinoline alkaloids in CM may be less important in enhancing its antimicrobial activity (Krzyżek et al. 2021). The paper by Zielińska and co-workers (2021) assumes that specific molecules or mixtures of molecules from the analyzed samples are responsible for the up-regulation of one of the energetic metabolic pathways in bacterial cells.

Inoue and co-workers (2021) speculated that chlorogenic acid, sanguinarine, berberine, coptisine, p-coumaric acid, chelerythrine, chelidonine, allocryptosine, sanguinarine, and vanillin, as secondary metabolites of CM act through different mechanisms, of which two groups should be distinguished, namely, groups that destroy the bacterial membrane or inhibit DNA replication. Results of these researchers indicate that the observed antimicrobial activity is mainly determined by the composition of the active molecules in the extracts and their interactions that may occur during the process of interaction with microorganisms.

CONCLUSIONS

The ethanolic extracts of CM revealed weak antibacterial activity against both *E. coli* (Migula) Castellani and Chalmers (ATCC[®] 25922TM) and *E. coli* (Migula) Castellani and Chalmers (ATCC[®] 35218TM) strains. The highest antibacterial activity was demonstrated for stalk extracts of CM against *S. aureus* NCTC[®] 12493TM compared to the control samples. We observed similar trends when measuring the zones of growth inhibition according to *S. aureus* subsp. *aureus* Rosenbach (ATCC[®] 29213TM) strain. Greater celandine oil exhibited no antibacterial activity against studied strains by measuring the zones of growth inhibition. Our studies revealed that CM extracts can be potential agents for antiseptics. However, CM extracts showed greater antimicrobial activity in contrast to natural oil derived from greater celandine. This may be due to the concentration of secondary metabolites (i.e., alkaloids, polyphenols, tannins), which may have a high affinity for the murein structure in the bacterial cell wall. Further studies are needed to establish adequate therapeutic doses for these extracts for medical and veterinary use.

REFERENCES

- Amal K. M., Banerji P., 2015. Chelidonium majus L. (Greater celandine) A Review on its phytochemical and therapeutic perspectives. Int. J. Her. Med., 3, 10-27.
- Barbier E. B., Sally D. H., Kennedy C., Koch E. W., Stier A. C., Silliman B. R., 2011. The value of estuarine and coastal ecosystem services. *Ecological Monographs*, 81, 2, 169-193.
- Bauer A. W., Kirby W. M., Sherris J. C., Turck M., 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, 45 (4), 493-496.
- Cheng R. B., Chen X., Liu S. J., Zhang X. F., 2007. [Effect of Chelerythrine on cell surface hydrophobicity and adherence of *Streptococcus mutans*]. *Shanghai Kou Qiang Yi Xue*, 16 (1), 68-72, (in Chinese).
- Cheng R. B., Chen X., Liu S. J., Zhang X. F., Zhang G. H., 2006. [Experimental study of the inhibitory effects of *Chelidonium majus* L. extractive on *Streptococcus mutans in vitro*]. *Shanghai Kou Qiang Yi Xue*, 15 (3), 318-320, (in Chinese).

- CLSI, 2014. Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute; Wayne, PA, USA. CLSI M100–S24.
- Inoue N., Terabayashi T., Takiguchi-Kawashima Y., Fujinami D., Matsuoka S., Kawano M., Tanaka K., Tsumura H., Ishizaki T., Narahara H., Kohda D., Nishida Y., Hanada K., 2021. The benzylisoquinoline alkaloids, berberine and coptisine, act against camptothecin-resistant topoisomerase I mutants. *Sci. Rep.*, 11 (1), 7718.
- Jorgensen J. H., Ferraro M. J., 2009. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin. Infect. Dis.*, 49 (11), 1749-1755.
- Krzyżek P., Junka A., Słupski W., Dołowacka-Jóźwiak A., Płachno B. J., Sobiecka A., Matkowski A., Chodaczek G., Płusa T., Gościniak G., Zielińska S., 2021. Antibiofilm and Antimicrobial-Enhancing Activity of *Chelidonium majus* and *Corydalis cheilanthifolia* Extracts against Multidrug-Resistant *Helicobacter pylori*. *Pathogens*, 10 (8), 1033.
- Kusky T., 2008. The coast: Hazardous interactions within the coastal environment. Facts On File, Inc., New York.
- Lai P. K., Roy J., 2004. Antimicrobial and chemopreventive properties of herbs and spices. *Curr*: *Med. Chem.*, 11, 1451-1460.
- Lakshmi A., 2021. Coastal ecosystem services & human wellbeing. *Indian J. Med. Res.*, 153 (3), 382-387.
- Mattner F., Bange F. C., Meyer E., Seifert H., Wichelhaus T. A., Chaberny I. F., 2012. Preventing the spread of multidrug-resistant gram-negative pathogens: recommendations of an expert panel of the German Society For Hygiene and Microbiology. *Dtsch. Arztebl. Int.*, 109 (3), 39-45.
- Meng F., Zuo G., Hao X., Wang G., Xiao H., Zhang J., Xu G., 2009. Antifungal activity of the benzo[c]phenanthridine alkaloids from *Chelidonium majus* Linn against resistant clinical yeast isolates. J. Ethnopharmacol., 125 (3), 494-496.
- Michael I., Rizzo L., McArdell C. S., Manaia C. M., Merlin C., Schwartz T., Dagot C., Fatta-Kassinos D., 2013. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review. *Water Res.*, 47 (3), 957-995.
- Nasr S. H., Radhakrishnan J., D'Agati V. D. 2013. Bacterial infection-related glomerulonephritis in adults. *Kidney Int.*, 83 (5), 792-803.
- Okoth D. A., Chenia H. Y., Koorbanally N. A., 2013. Antibacterial and antioxidant activities of flavonoids from *Lannea alata* (Engl.) Engl. (Anacardiaceae). *Phytochem. Lett.*, 6, 476-481.
- Polvoy I., Flavell R. R., Rosenberg O. S., Ohliger M. A., Wilson D. M., 2020. Nuclear Imaging of Bacterial Infection: The State of the Art and Future Directions. J. Nucl. Med., 61 (12), 1708-1716.
- O'Neill J., 2014. Review on Antimicrobial Resistance. In: Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations. (Ed.) J. O'Neill, Wellcome Trust & HM Government, London, 2-3, wellcomecollection.org/works/rdpck35v (access 16/02/2021).
- Saunders M. I., Doropoulos C., Bayraktarov E., Babcock R. C., Gorman D., Eger A. M., Vozzo M. L., Gillies C. L., Vanderklift M. A., Steven A. D. L., Bustamante R. H., Silliman B. R., 2020. Bright Spots in Coastal Marine Ecosystem Restoration. *Curr. Biol.*, 30 (24), R1500-R1510.
- Stefanowski N., Tkachenko H., Kurhaluk N., 2021a. Antibacterial efficacy of ethanolic extracts derived from roots and stalks of *Chelidonium majus* L. (Papaveraceae). In: Medicinal Herbs: from Past Experience to New Technologies: Proceedings of Ninth International Scientific and Practical Conference; June, 29-30, 2021. (Eds.) V. V. Gangur, V. F. Pocherniaeva, S. V. Klymenko, Poltava State Agricultural Academy, Poltava, Ukraine, 154-160.
- Stefanowski N., Tkachenko H., Kurhaluk N., 2021b. Antimicrobial activity of *Chelidonium* majus L. extracts against *Escherichia coli* strain. In: Proceedings of the XV All-Ukrainian

scientific-practical conference of young scientists dedicated to the 90th anniversary of the birth of Doctor of Economics, Professor, Academician of UAAS Andriy Omelyanenko, Scientific progress in animal husbandry and poultry, Kharkiv, August 26-27, 2021. Institute of Animal Husbandry NAAS, Kharkiv, 11-13.

- Stefanowski N., Tkachenko H., Kurhaluk N., 2021c. Antimicrobial efficacy of ethanolic extracts derived from stalks and roots of *Chelidonium majus* L. against different types of *Staphylo*coccus aureus and Escherichia coli strains. Scientific and Technical Bulletin of Institute of Animal Husbandry NAAS Ukraine, 126, 14-24.
- Stefanowski N., Tkachenko H., Kurhaluk N., 2021d. Evaluation of the antibacterial activity of ethanolic extracts obtained from roots and stalks of *Chelidonium majus* L. against *Escherichia coli* strains. *Agrobiodiversity for Improving Nutrition, Health, and Life Quality*, 5, 1, 126-132.
- Stefanowski N., Tkachenko H., Kurhaluk N., 2021e. Preliminarily evaluation of the antimicrobial activity of extracts derived from leaves and roots of *Chelidonium majus* L. sampled in rural and urban agglomerations of northern Poland. In: *Youth and Progress of Biology*: Abstracts of XVII International Scientific Conference for Students and Ph.D. Students, Lviv, April 19-21, 2021. LLC Romus-Poligraf, Lviv, 200-202.
- Sumner L. W., Lei Z., Nikolau B. J., Saito K., 2015. Modern plant metabolomics: Advanced natural product gene discoveries, improved technologies, and future prospects. *Nat. Prod. Rep.*, 32, 212-229.
- White M. P., Alcock I., Wheeler B. W., Depledge M. H., 2013. Coastal proximity, health and well-being: Results from a longitudinal panel survey. *Health & Place*, 23, 97-103. www.kartuzy.pl (access 16/02/2021).
- Zar J. H., 1999. Biostatistical Analysis. 4th ed., Prentice-Hall Inc., Englewood Cliffs, New Jersey.
- Zielińska S., Dziągwa-Becker M., Junka A., Piątczak E., Jezierska-Domaradzka A., Brożyna M., Paleczny J., Sobiecka A., Słupski W., Mess E., Kucharski M., Çiçek S. S., Zidorn C., Matkowski A., 2021. Screening Papaveraceae as Novel Antibiofilm Natural-Based Agents. *Molecules*, 26 (16), 4778.
- Zielińska S., Jezierska-Domaradzka A., Wójciak-Kosior M., Sowa I., Junka A., Matkowski A. M., 2018. Greater celandine's ups and downs 21 centuries of medicinal uses of *Chelidonium majus* from the viewpoint of today's pharmacology. *Front. Pharmacol.*, 9, 1-29.
- Zielińska S., Wójciak-Kosior M., Dziągwa-Becker M., Gleńsk M., Sowa I., Fijałkowski K., Rurańska-Smutnicka D., Matkowski A., Junka A., 2019. The Activity of Isoquinoline Alkaloids and Extracts from *Chelidonium majus* against Pathogenic Bacteria and *Candida* sp. *Toxins (Basel)*, 11 (7), 406.
- Zuo G. Y., Meng F. Y., Hao X. Y., Zhang Y. L., Wang G. C., Xu G. L., 2008. Antibacterial alkaloids from *Chelidonium majus* Linn (Papaveraceae) against clinical isolates of methicillin-resistant *Staphylococcus aureus*. J. Pharm. Pharm. Sci., 11 (4), 90-94.

WŁAŚCIWOŚCI PRZECIWBAKTERYJNE OLEJKU GLISTNIKOWEGO ORAZ EKSTRAKTÓW Z KORZENI I ŁODYG GLISTNIKA JASKÓŁCZE ZIELE (*CHELIDOINIUM MAJUS* L.) ZEBRANEGO Z PÓŁNOCNEJ CZĘŚCI POMORZA

Streszczenie

Przekonująca liczba badań wskazujących, że alkaloidy takie, jak chelidonina i sangwinaryna wraz z innymi metabolitami wtórnymi wykazują silne właściwości przeciwbakteryjne, przeciwgrzybicze i przeciwpasożytnicze, skłoniła nas do zbadania skuteczności przeciwbakteryjnej glistnika jaskółcze ziele (Chelidonium majus L.), przedstawiciela rodziny Papaveraceae, wobec szczepów Staphylococcus aureus subsp. aureus Rosenbach (ATCC® 29213TM) (szczep mecA ujemny, wrażliwy na oksacylinę, wytwarzający słabą β-laktamazę), S. aureus NCTC® 12493™ (mecA dodatni, metycylinooporny, szczep EUCAST QC dla cefoksytyny), Escherichia coli (Migula) Castellani i Chalmers (ATCC® 25922TM), E. coli (Migula) Castellani i Chalmers (ATCC® 35218TM). W obecnej pracy postanowiliśmy ocenić skuteczność przeciwdrobnoustrojową ekstraktów etanolowych uzyskanych z łodyg i korzeni C. majus, jak również komercyjnego tłustego oleju glistnikowego (Botanica, Rosja) wobec różnych szczepów S. aureus i E. coli. Świeże łodygi i korzenie umyto, zważono, rozdrobniono i homogenizowano w 96% etanolu (w proporcji 1:19) w temperaturze pokojowej. Następnie ekstrakty filtrowano i badano ich aktywność przeciwdrobnoustrojową, którą określano za pomocą testu dyfuzyjnego. Ekstrakty uzyskane z korzeni i łodyg C. majus oraz komercyjny naturalny olejek tłuszczowy z glistnika wykazywały różną aktywność przeciwbakteryjną wobec badanych szczepów. Etanolowe ekstrakty z C. majus wykazały słabą aktywność przeciwbakteryjną zarówno wobec szczepów E. coli (Migula) Castellani i Chalmers (ATCC[®] 25922™), jak i E. coli (Migula) Castellani i Chalmers (ATCC[®] 35218TM). Najwyższą aktywność przeciwbakteryjną wykazano dla ekstraktów z łodygi C. majus wobec S. aureus NCTC® 12493™ w porównaniu z próbkami kontrolnymi. Podobne tendencje zaobserwowano podczas pomiaru stref zahamowania wzrostu szczepu S. aureus subsp. aureus Rosenbach (ATCC® 29213TM). Ponieważ skuteczność przeciwdrobnoustrojowa roślin leczniczych różni się w zależności od nagromadzenia metabolitów wtórnych (tj. alkaloidów, flawonoidów, tanin itp.), nie jest zaskakujące, że różnice w tej skuteczności odnotowano nawet przy użyciu próbek pobranych z tej samej rośliny, ale z różnych jej części (łodygi, korzenie). Aktywność przeciwdrobnoustrojowa surowych ekstraktów etanolowych uzyskanych z łodyg i korzeni glistnika jaskółcze ziele może być przypisana konkretnym związkom lub ich kombinacji. Obecne badania stanowią podstawę przyszłych badań mających na celu potwierdzenie potencjalnego zastosowania C. majus jako kandydata do leczenia infekcji wywołanych przez S. aureus i E. coli w medycynie i weterynarii.