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Research Article OPEN ACCESS

Evaluation of antibacterial activity of the ethanolic extracts derived from leaves of *Coelogyne brachyptera* Rchb. f. (Orchidaceae)

Lyudmyla Buyun[1], Halyna Tkachenko[2]*, Natalia Kurhaluk[2], Oleksandr Gyrenko[1], Lyudmyla Kovalska[1], Anna Góralczyk[2]

[1]M.M. Gryshko National Botanical Garden, National Academy of Sciences of Ukraine, Kyiv, Ukraine
[2]Institute of Biology and Earth Sciences, Pomeranian University in Słupsk, Poland

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The present study was aimed to assess the antibacterial activity of ethanolic extract obtained from leaves of *Coelogyne brachyptera* Rchb. f. For the current study, a panel of organisms including *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 25923™) (mecA negative), *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) (mecA negative, Oxacillin sensitive, weak β-lactamase producing strain), *S. aureus* NCTC 12493 (mecA positive, Methicillin-resistant, EUCAST QC strain for cefoxitin), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™), *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™), *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27583™) were used. The antimicrobial susceptibility testing was done on Muller-Hinton agar by the disc diffusion method. The results of our study revealed the differential efficacy of ethanolic extract obtained from leaves of *C. brachyptera* on the test organisms. The ethanolic extract obtained from leaves of *C. brachyptera* revealed significant antibacterial activity against studied strains compared to control samples (96 % ethanol). A statistically significant increase ($p < 0.05$) in the inhibition zone diameters of strain growth was 47 % for *S. aureus* subsp. *aureus* ATCC® 25923™, 40 % for *S. aureus* subsp. *aureus* ATCC® 29213™, and 44 % for *S. aureus* NCTC 12493. A non-significantly increase in inhibition zone diameters of *E. coli* strains' growth was also observed. The plant can be a source material to the herbal drug industry since it has some important antimicrobial components in the extracts that can be used for the development of therapeutic phytomedicine and phytoveterinary.

Keywords: orchids, ethanolic leaf extract, antibacterial activity, disc diffusion technique, *Staphylococcus aureus* subsp. *aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*

Introduction

Orchidaceae is one of the largest and more diverse families of flowering plants with approximately 25,000 species in 736 genera currently recognized (Chase et al., 2015). Orchids widely distributed as epiphytes, lithophytes, or terrestrials, have been used all over the world in traditional healing and treatment systems of several diseases (Kong et al., 2003; Pant, 2013). It was found that medicinal orchids mainly are encompassed by the next genera: *Anoectochilus* Blume, *Bulbophyllum* Thouars, *Calanthe* R. Br., *Coelogyne* Lindl., *Cymbidium* Sw., *Cypripedium* L., *Dendrobium* Sw., Eria Lindl., *Galeola* Lour., *Gastrodia* R. Br., *Gymnadenia* R. Br., *Habenaria* Willd., *Ludisia* A. Rich., *Luisia* Gaudich., *Nervilia* Comm. ex Gaudich., and *Thunia* Rchb. f. (Szlachetko, 2001; Kovaćs et al., 2008; Pant, 2013). Orchids have been reported to possess useful therapeutic activities like antitumor, hypoglycaemic, antimicrobial, immunomodulatory, hepatoprotective, antioxidant, and neuroprotective activities (Prasad and Koch, 2014; Biswas et al., 2016; Bhatnagar and Ghosal, 2018). It is believed that these pharmaceutical properties are due

***Corresponding Author:** Halyna Tkachenko, Institute of Biology and Earth Sciences, Pomeranian University in Słupsk, Arciszewski Str. 22b, 76-200 Słupsk, Poland
halyna.tkachenko@apsl.edu.pl

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to the activities of many phytochemicals, including alkaloids, flavonoids, phenanthrenes, terpenoids, steroids, and their derivatives, which are present in various parts of orchid plants (Zhang et al., 2015).

In recent years, the assessment of antibacterial properties of orchids has received considerable attention (Singh et al., 2012; Tkachenko et al., 2015, 2018; Buyun et al., 2016, 2017, 2018; Soumiya and Christudhas Williams, 2017). Some orchid species are used as potent inhibitors against Gram-positive and Gram-negative bacteria and also proved to be a potent antimicrobial agent (Singh et al., 2012).

The family Orchidaceae is not only one of the most numerous, ecologically, and morphologically diverse families of flowering plants, but also one of the most endangered plant taxa (Zhang et al., 2015). Orchids are widely and illegally harvested from the wild for local, regional, and international trade as ornamental and medicinal plants. The demand for medicinal orchids is drastically increasing since the international trade of medicinal plants is becoming a major force in the global economy (Hinsley et al., 2017). However, the natural source of these plants has been significantly reduced due to indiscriminate collection, global climate changes, the specificity of life-history strategies, including specialized pollination syndromes, and association with mycorrhizal fungi (Gravendeel et al., 2004). Therefore, to conserve orchid plants in the wild and to meet the demand for medicinal plant material, assessment of biological activity of plants maintained under glasshouse conditions and developing new biotechnologies for plant reproduction *in vitro* are urgently needed.

Thus, although the antimicrobial activity of many orchid species, including *Coelogyne* species, has been effectively established against a wide spectrum of microorganisms (Majumder et al., 1995, 2001, 2011; Kovács et al., 2008; Chen et al., 2018), bacterial drug resistance continues to be a worldwide public health issue in the treatment of infectious diseases, thereby stimulating the search for new alternatives with fewer side effects (Mambe et al., 2019).

Previously, we have given considerable attention to the evaluation of the antibacterial effects of ethanolic extracts obtained from leaves and pseudobulbs of plants belonging to various *Coelogyne* species, maintained under glasshouse conditions. For example, the assessment of the antifungal potential of orchids species, i.e. *Coelogyne cristata* Lindl., *C. fimbriata* Lindl., *C. flaccida* Lindl., *C. huettneriana* Rchb.f., *C. ovalis* Lindl., *C. speciosa* (Blume) Lindl., *C. tomentosa* Lindl. and *C. viscosa* Lindl. against fungus strain, *Candida albicans* was conducted by Buyun et al. (2018). Marked antifungal efficacy was observed in the case of ethanolic extracts derived from leaves of *C. flaccida* (mean diameter of inhibition zones was 19.5 mm), *C. viscosa* (18.6 mm), *C. huettneriana* (18.2 mm), and *C. fimbriata* (17.5 mm). Extracts of *C. cristata*, *C. ovalis*, and *C. tomentosa* displayed less profound inhibitory activity against test fungus (mean diameter of inhibition zones ranging from 16 to 17.5 mm). Similarly, the ethanolic extracts from the pseudobulbs of eight *Coelogyne* species exhibited strong activity against *C. albicans* (inhibition zone diameter ranged from 16 to 23.5 mm). Moreover, it has been observed that ethanolic extract from pseudobulbs of *C. speciosa* revealed the highest antibacterial activity (21 mm as the diameter of the inhibition zone) among various *Coelogyne* species screened. The results also indicate that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results (Buyun et al., 2018).

The present study was aimed to determine the antibacterial activity of *Coelogyne brachyptera* Rchb. f. against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* strains, clinically important bacteria, which are indicator organisms commonly used in various projects to monitor antibiotic resistance (Roser et al., 2016).

Given that standardization and quality control are essential analytical steps to assure the correct identification of plant raw materials to be used as plant-derived medicines, the micromorphological investigation of *Coelogyne brachyptera* leaf has been undertaken using light microscopy. The need for constant incorporation of leaf micromorphology in pharmacological investigations has been emphasized in some recent papers (Bilić et al., 2019; Khan et al., 2020). Additionally, this investigation was conducted as part of a conservation research program focusing on preventing the extinction of rare and endangered orchid species.

Material and methodology

Collection of plant material

The leaves of *C. brachyptera* plants cultivated under glasshouse conditions were sampled at M.M. Gryshko National Botanical Garden (NBG, Kyiv, Ukraine). Since 1999 the whole collection of tropical and subtropical plants (including orchids) has had the status of a National Heritage Collection of Ukraine and is supported through State Funding. Besides, the NBG

collection of tropical orchids was registered at the Administrative Organ of CITES in Ukraine (Ministry of Environment Protection, registration No. 6939/19/1-10 of 23 June 2004).

Various databases are available for searching collections of living plants, confirming the taxonomic identity of having been reviewed, e.g. World Checklist of Orchidaceae (Govaerts et al., 2016), International Plant Names Index, The Plant List, the IUCN Red List (IUCN, 2013).

Coelogyne brachyptera is found in Burma, Thailand, Cambodia, Laos, and Vietnam. It grows epiphytically in the primary mountain forest, the most frequent at an altitude of 1000 to 2500 meters above sea level (Averyanov et al., 2003). It is a sympodial orchid with pseudobulbs of one internode, narrowly conical, 4-angled, slightly grooved, pale green, carrying 2 leaves. The leaves are elliptic to elliptic-lanceolate, subacute, plicate, 7-nerved, with an undulate margin. The flowering of *C. brachyptera* under glasshouse condition at NBG was observed in March – April (Figure 1). The duration of anthesis of a single inflorescence did not exceed 2 weeks.

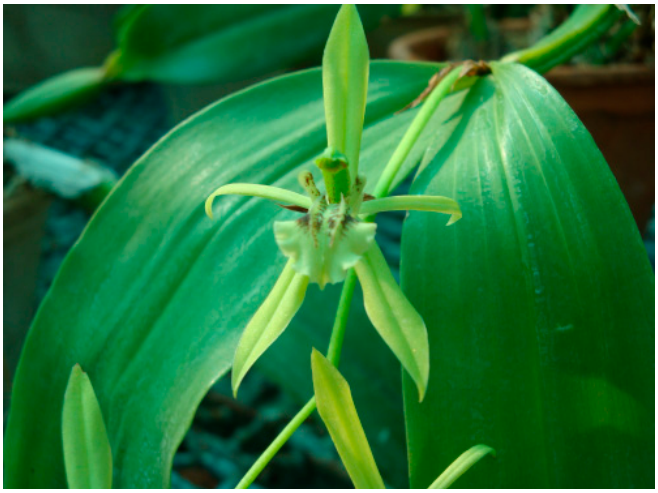

Figure 1 Vegetative shoot with inflorescence of *Coelogyne brachyptera* Rchb. f. plant, cultivated at NBG's glasshouses (Kyiv, Ukraine)

Preparation of plant extracts

The collected leaves were brought into the laboratory for antimicrobial studies. Freshly sampled leaves were washed, weighed, crushed, and homogenized in 96 % ethanol (in proportion 1 : 19) at room temperature. The extract was then filtered and investigated for antimicrobial activity.

Bacterial test strain and growth conditions

For this study, a panel of organisms including *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 25923™) (mecA negative), *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) (mecA negative, Oxacillin sensitive, weak β-lactamase producing strain), *S. aureus* NCTC 12493 (mecA positive, Methicillin-resistant, EUCAST QC strain for cefoxitin), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™), *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™), *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27583™) were used. The cultivation medium was trypticase soy agar (Oxoid®, UK), supplemented with 10 % defibrinated sheep blood. Cultures were grown aerobically for 24 hr 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, Performance Standards for Antimicrobial Susceptibility Testing, 2014).

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

Strain tested was plated on TSA medium (Tryptone Soy Agar) and incubated for 24 hr 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 the disc diffusion method (Kirby-Bauer disk diffusion susceptibility test protocol). Muller-Hinton agar plates were inoculated with 200 µl of standardized inoculum (10^8 CFU/mL) of the bacterium and spread with sterile swabs (Bauer et al., 1966).

Sterile filter paper discs impregnated by extract were applied over each of the culture plates, 15 min after bacteria suspension was placed. A negative control disc impregnated by sterile 96 % ethanol was used in each experiment. After culturing bacteria on Mueller-Hinton agar, the disks were placed on the same plates and incubated for 24 hr 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 and standard susceptibility disks. The activity was evidenced by the presence of a zone of inhibition surrounding the well.

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LM investigations of leaf surface micromorphology

For the micromorphological investigation of the leaf surface epidermal cells, Clarke's method of making impressions technique of the leaf surface has been used (Clarke, 1960). To produce the leaf epidermal impressions (imprints or replicas) the clear nail polish was applied on both surfaces of *C. brachyptera* leaf and allowed to dry for several minutes. After then the thin film was gently peeled from the leaf surface using transparent tape and the peel subsequently was mounted in water. Stomatal type, density, size were determined using nail polish imprints taken from both leaf surfaces.

For viewing epidermal features imprints light microscope Primo Star (Carl Zeiss, Jena, Germany) has been employed. The light microscopic images were captured with a digital camera Canon PowerShot A640. For measuring the dimension of epidermal cells the AxioVs40 V 4.8.2.0 software has been used (Carl Zeiss, Jena, Germany).

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 extract 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 software, v. 8.0 (StatSoft, Poland) (Zar, 1999). 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).

Results and discussion

In Figure 2, we can see that the adaxial epidermis in *C. brachyptera* leaf is composed of mostly hexagonal cells, although polygonal or isodiametric cells have been observed. The number of epidermal cells on the adaxial leaf surface varied from 160 to 224 (198.30 ±6.12) per 1 mm^2; cells are from 51.18 to 158.10 (93.44 ±1.62) µm in length and from 40.31 to 94.35 (70.32 ±0.90) µm in width.

The cells of the abaxial epidermis of the leaves are mostly rectangular or irregularly shaped; walls are straight or curved. Angles within adjacent boundaries are straight or pointed. The number of epidermal cells on the abaxial leaf surface varied from 208 to 256 (226.33 ±4.93) per 1 mm^2; cells from 50.28 to 134.15 (88.27 ±2.21) µm in length and from 32.95 to 80.58 (59.43 ±0.72) µm in width. The density of trichomes on both surfaces varied within the range of 2–4 per 1 mm^2.

Stomata were located only on the lower leaf surface (hypostomatous leaves). The stomatal complex is recognizable allowing us to determine the stomatal types based on nail polish imprints. The most common stomatal type was tetracytic, occasionally anomocytic stomata occurred with 5 or 6 subsidiary cells. The stomata are rounded, usually scattered or distributed in small groups of 2–3 stomata. Subsidiary cells are located parallel to the guard cells and clearly differ from the main epidermal cells of the leaves both in shape and size.

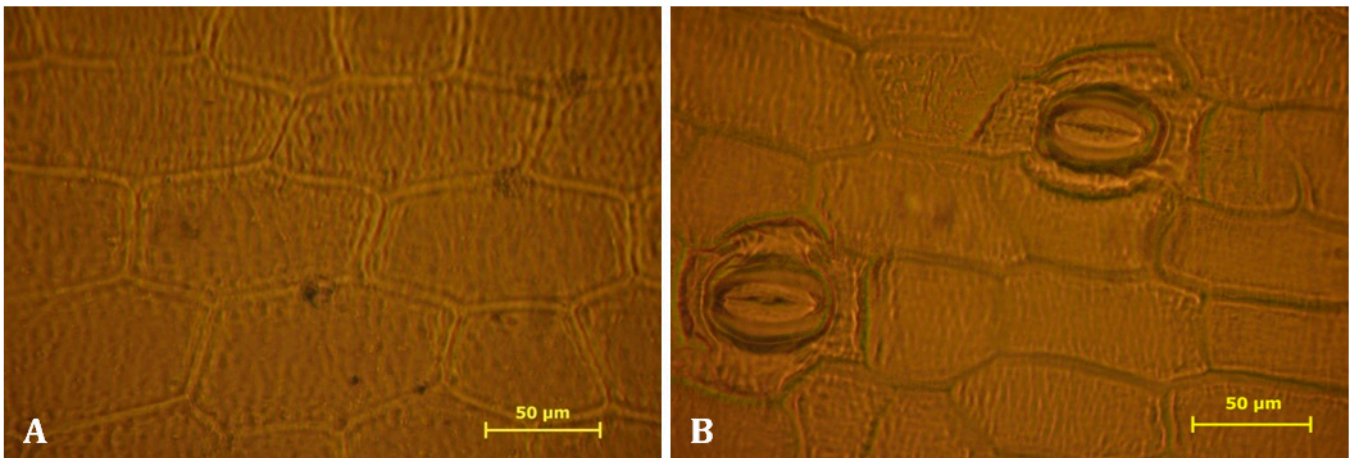

Figure 2 Adaxial (A) and abaxial (B) epidermal cells with stomata on *Coelogyne brachyptera* Rchb. f. leaf observed in nail polish imprints

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Stomata density varied from 10 mm^{-2} to 15 mm^{-2} (12.77 ±0.59). Stomatal size (guard cell length) ranged within range 47.55–68.19 µm (58.54 ±0.61 µm).

The ethanolic extract obtained from leaves of *C. brachyptera* resulted in considerable suppression of *Staphylococcus aureus* strains' growth. Moreover, the differential efficacy of ethanolic extract obtained from leaves of *C. brachyptera* on the test organisms was noted. Consequently, the extract displayed intermediate antibacterial potency against *S. aureus*, i.e. the mean of inhibition zone diameters was (12.45 ±1.18) mm, (12.51 ±0.99) mm, and (12.87 ±1.16) mm for *S. aureus* subsp. *aureus* (ATCC® 25923™), *S. aureus* subsp. *aureus* (ATCC® 29213™), and *S. aureus* NCTC 12493, respectively. On the other hand, *E. coli* exhibited lower susceptibility for the impact of the ethanolic extract obtained from leaves of *C. brachyptera*. The mean of inhibition zone diameters was (12.11 ±1.02) mm and (10.40 ±0.95) mm for *E. coli* (ATCC® 25922™) and *E. coli* (ATCC® 35218™), respectively. *P. aeruginosa* (ATCC® 27583™) strain was the most resistant to the impact of the ethanolic extract obtained from leaves of *C. brachyptera* with the mean of inhibition zone diameter (9.53 ±0.95) mm (Figure 3, 4).

Moreover, the ethanolic extract obtained from leaves of *C. brachyptera* revealed significant antibacterial activity against studied strains compared to control samples (96 % ethanol). A statistically significant increase ($p < 0.05$) in inhibition zone diameters of strain growth was 47 % (for *S. aureus* subsp. *aureus* ATCC® 25923™), 40 % (for *S. aureus* subsp. *aureus* ATCC® 29213™), and 44 % (for *S. aureus* NCTC 12493) (Figure 3, 4). A non-significantly increase ($p > 0.05$) in inhibition zone diameters of *E. coli* strains' growth was also observed (by 27 % for *E. coli* ATCC® 25922™ and by 31 % for *E. coli* ATCC® 35218™, respectively).

The present study has revealed that ethanolic extract derived from the leaves *Coelogyne brachyptera* exhibited intermediated antibacterial activity against different Gram-positive and Gram-negative strains studied (inhibition zone diameter were ranged from 8.5 to 15.5 mm) (Figure 3 and 4). Moreover, it has been observed that ethanolic extract obtained from the leaves *Coelogyne brachyptera* revealed the highest antibacterial activity against *S. aureus* strains (11.0–15.5 mm as the diameter of inhibition zone) compared to *E. coli* and *P. aeruginosa* strains (Figure 3 and 4).

In our previous study (Buyun et al., 2017), we have studied the antibacterial effects of the ethanolic extract obtained from *C. brachyptera* leaves against specific Gram-positive (*Staphylococcus aureus* ATCC 25923 and methicillin-resistant *S. aureus* locally isolated) and Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, metallo-β-lactamases (MβL)-

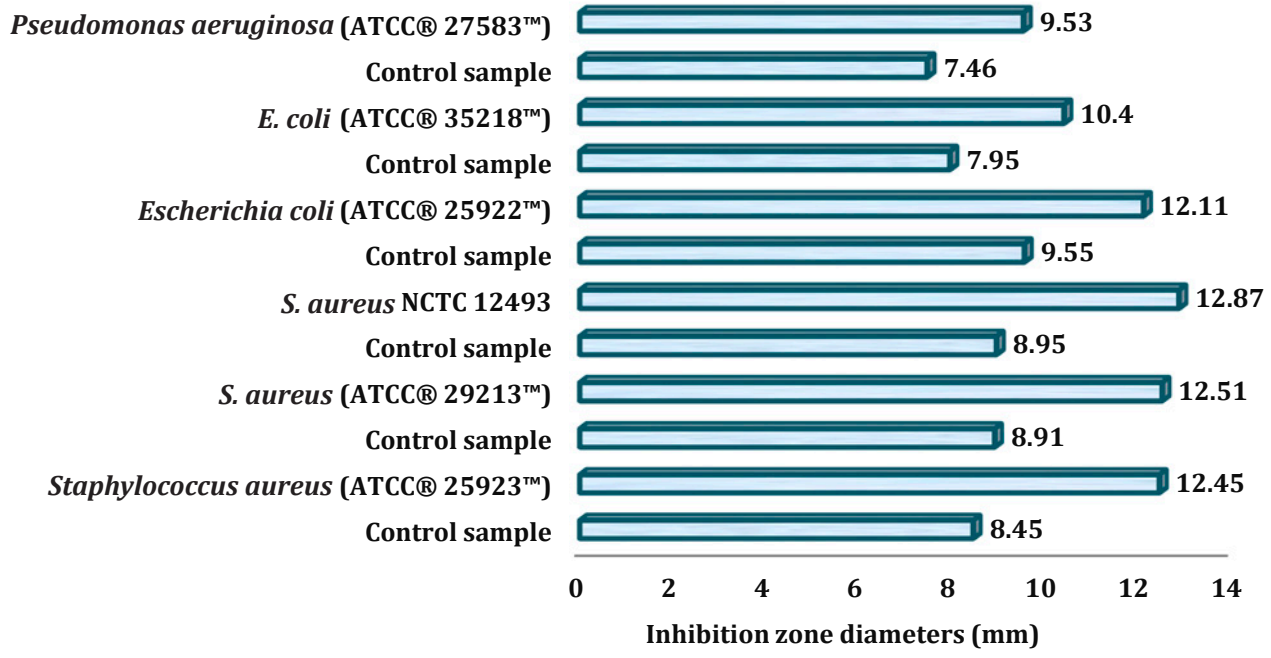

Figure 3 The mean of inhibition zone diameters achieved by an impact of the ethanolic extract obtained from leaves of *Coelogyne brachyptera* concerning *S. aureus* subsp. *aureus* (ATCC® 25923™), *S. aureus* subsp. *aureus* (ATCC® 29213™), and *S. aureus* NCTC 12493, *E. coli* ATCC® 25922™, *E. coli* ATCC® 35218™, and *P. aeruginosa* ATCC® 27583™ (n = 8)

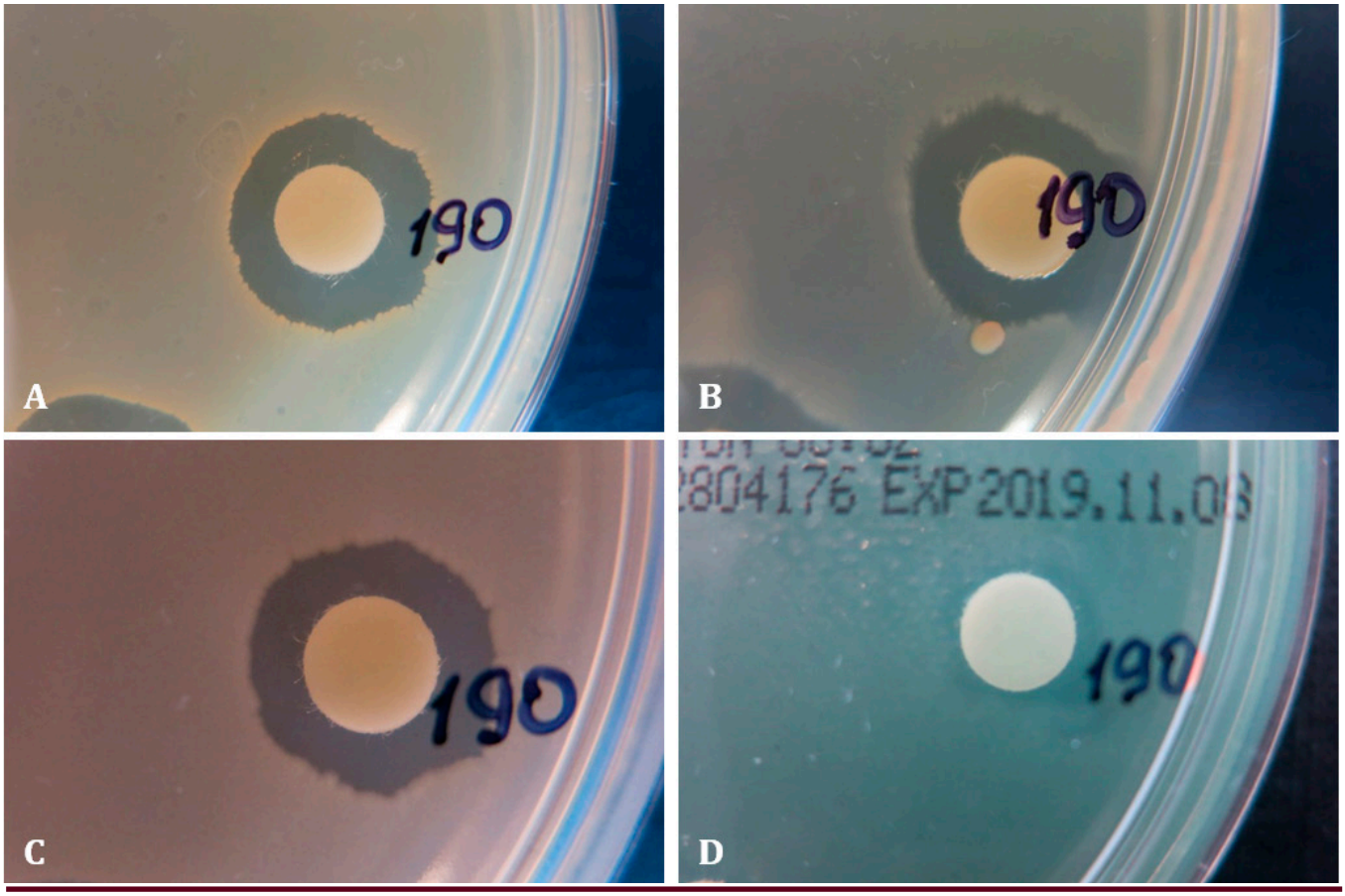

Figure 4 Examples of a disc diffusion assay plate showing the halos in the bacterial lawn resulting from the antibacterial activity of extract obtained from *C. brachyptera* leaves against *S. aureus* subsp. *aureus* ATCC® 29213™ (A), *S. aureus* NCTC 12493 (B), *E. coli* ATCC® 25922™ (C), and *P. aeruginosa* ATCC® 27583™ (D) (Photo: O. Gyrenko)

positive *Pseudomonas aeruginosa* locally isolated, *Escherichia coli* ATCC 25922, *Salmonella enteritidis* locally isolated). Our results showed that the ethanolic extract of *C. brachyptera* leaves has displayed a strong inhibitory effect against the Gram-positive bacterial strains (20 mm diameter of inhibition zone for *S. aureus* and 26.5 mm for methicillin-resistant *S. aureus*), and moderate activity against Gram-negative bacteria (18.2 mm for *E. coli*, 16.5 mm for *P. aeruginosa* and 18.3 mm for (MβL+) *P. aeruginosa*, and 14.8 mm for *S. enteritidis*). Gram-positive strains (*S. aureus* and methicillin-resistant *S. aureus*) were more susceptible to the ethanolic leaf extracts of *C. brachyptera* as compared to Gram-negative bacteria (Buyun et al., 2017).

Literature data also suggest that some members of the orchid family are used as a potent inhibitor against Gram-positive and Gram-negative bacteria and also proved to be a potent antimicrobial agent. For example, Nagananda and Satishchandra (2013) have evaluated the antibacterial and antifungal activity of *Dendrobium nodosum* Dalzell (syn. *Flickingeria nodosa* (Dalzell) Seidenf.) against human pathogens with cold and hot successive extracts. The antimicrobial activities of the plant extracts were evaluated against 7 bacterial and 6 fungal strains using the well diffusion method on Mueller Hinton agar medium. The cold water extract has antibacterial activity against *S. aureus* and *S. citreus* with a maximum zone of inhibition. The cold chloroform extract has good antifungal activity against *Trichophyton mentagrophytes* (Nagananda and Satishchandra, 2013).

Chemical analyses conducted by Majumder et al. (1995, 2001), revealed the presence of two phenanthrene derivatives in pseudobulbs of *C. cristata*: *coeloginanthridin* and *coeloginanthrin*. Phenanthrenes are the prototypical opioids that are presumably formed by oxidative coupling of the aromatic rings of stilbene precursors and possess several biological activities (Kovács et al., 2008). Phenanthrenes have been studied for cytotoxicity, antimicrobial, spasmolytic, anti-inflammatory, anti-platelet aggression, anti-allergic, immunomodulatory, anticancer, anti-aging, atherosclerosis properties (Majumder et al., 2001; Kovaćs et al., 2008; Chen et al., 2018). The anti-stress

and antioxidant activity of similar herbs from the Orchidaceae family have also been reported (Habbu et al., 2012; Sing et al., 2012; Mishra et al., 2018).

Moreover, further investigation afforded two new stilbenoids, designated *coeloginone* and *coeloginanthrone* (Majumder et al., 2011). Stilbenoids are the major secondary metabolites reported in some orchids based on previous phytochemical studies, e.g. in *Arundina graminifolia* (D. Don) Hochr. (Auberon et al., 2016). These metabolites are also known to display a wide range of biological activities such as antioxidant, antiviral, cytotoxic, and antitumoral properties (Chen and Chen, 2005; Prasad and Koch, 2014; Biswas et al., 2016; Bungtongdee et al., 2018; Mishra et al., 2018).

Mishra et al. (2018) have investigated the antioxidant and antibacterial activities of 5 different extracts and derived fractions from the tubers of *Satyrium nepalense* D. Don, a high altitude medicinal orchid of the Indian Himalayan region. Identification of the most active fractions, phytochemical characterization, total phenolic, and flavonoid contents, and biological activities were also evaluated. Petroleum ether, chloroform, ethyl acetate, methanol, water extracts, and methanol fractions were screened for their antibacterial activity at various doses (10, 50, and 100 mg/mL) against ten Gram-negative and Gram-positive bacterial strains by disc diffusion method. Methanol extract exhibited the highest antioxidant and antibacterial activities in comparison with the other extracts. Levels of phenolics and flavonoids were also the highest in the same extract. Phytochemical investigation of the active fractions of the methanol extract led to the isolation of gallic acid (19.04 mg/g) and quercetin (23.4 mg/g). Therefore, methanol extract showed interesting potential for both antioxidant and antibacterial activities (Mishra et al., 2018).

Three orchids namely, *Rhynchostylis retusa* (L.) Blume, *Tropidia curculigoides* Lindl., and *Satyrium nepalense*, traditionally used in tuberculosis, asthma, and cold stage of malaria in folk medicine, were studied by Bhatnagar et al. (2017). The most significant antimycobacterial activity was observed with the n-hexane fraction of the flower of *Satyrium nepalense* with a minimum inhibitory concentration (MIC) of 15.7 µg/mL. The most promising leishmanicidal activity was observed with diethyl ether fraction of the roots of *Rhynchostylis retusa* with IC_{50} values of 56.04 and 18.4 µg/mL against promastigotes and intracellular amastigotes respectively. Evaluation of antibacterial activity identified *S. nepalense* flower n-hexane and *R. retusa* roots diethyl ether as potential fractions with MIC values of ≤100 µg/mL against selected clinical isolates. The investigation of Bhatnagar et al. (2017) resulted in the identification of *S. nepalense* as the most promising plant, which possessed all three activities in a significant proportion.

Recently it has been shown that some plant-derived bioactive compounds produced by fungal endophytes of orchids have been proven to possess antimicrobial and antioxidant activities (Vaz et al., 2009; Jiang et al., 2015; Bungtongdee et al., 2018). For example, Vaz et al. (2009) have examined the antimicrobial activity of endophytic fungi isolated from the leaves, stems, and roots of 54 species of Orchidaceae collected in a Brazilian tropical ecosystem. In total, 382 filamentous fungi and 13 yeast isolates were obtained and cultured to examine the production of crude extracts. Thirty-three percent of the isolates displayed antimicrobial activity against at least one target microorganism. Furthermore, the endophytic fungi isolated from different *Dendrobium* species could be of the potential antibacterial or antifungal resources (Xing et al., 2011). Thus, 10 endophytic fungi in *Dendrobium devonianum* Paxton and 11 in *D. thyrsiflorum* B.S. Williams exhibited antimicrobial activity against at least one pathogenic bacterium or fungus among 6 pathogenic microbes (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*). Out of the fungal endophytes isolated from *D. devonianum* and *D. thyrsiflorum*, *Phoma* displayed strong inhibitory activity (inhibition zones in diameter >20 mm) against pathogens. *Epicoccum nigrum* from *D. thyrsiflorum* exhibited antibacterial activity even stronger than ampicillin sodium. Fusarium isolated from the two *Dendrobium* species was effective against the pathogenic bacterial as well as fungal pathogens (Xing et al., 2011).

In vitro antimicrobial activity of various extracts obtained from vegetative parts of *Coelogyne speciosa* against Gram-positive (*Staphylococcus aureus* ATCC 25923) and Gram-negative bacteria (*Escherichia coli* ATCC 25922) was also demonstrated in our previous studies (Buyun et al., 2016, 2017) The ethanolic extracts from leaves and pseudobulbs of *C. speciosa* exhibited strong activity against *S. aureus* (inhibition zone diameters were 21.5 mm and 19 mm, respectively), while the methanolic extract from leaves and pseudobulbs revealed mild activity (8.1 and 8 mm). Moreover, it has been observed that ethyl acetate, hexane, and dichloromethane extracts obtained from leaves and pseudobulbs of *C. speciosa* revealed no antibacterial activity against *S. aureus*. Our results also showed that ethanolic extract from leaves

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of *C. speciosa* exhibited strong activity against *E. coli* (inhibition zone diameter was 21 mm), whereas other extracts from pseudobulbs revealed minimum activity (inhibition zone diameter was 12 mm) (Buyun et al., 2016, 2017). The ethanolic extracts obtained from leaves and pseudobulbs of five *Coelogyne* spp. were found to exhibit fairly strong antibacterial activity towards *Enterobacter cloacae* strain used, the diameter of inhibition zones varied within 8.0–25.5 mm (Buyun et al., 2019).

Leaf morphological traits play an important role in maintaining water balances in epiphytes (Zhang et al., 2015). It was evidenced, that the patterns of stomatal type in the studied group might represent a useful diagnostic characteristic. Data on the characteristics of the leaf surface will provide important information for the standardization of raw materials, and on the other hand is taxonomically important for the identification of samples (Song et al., 2020).

Micromorphological characteristics of leaves have been applied in systematic studies for different taxonomic groups. Recently, leaf micromorphology using microscopic analysis has also been used to facilitate accurate authentication and quality control of medicinal plants (Song et al., 2020), including orchids species such as *Dendrobium huoshanense* (Zhang et al., 2017). The results presented in the paper are potentially useful for advancing research on the assessment of medicinal properties, propagation, and conservation of *Coelogyne brachiptera*, as well as other *Coelogyne* species, under *ex situ* conservation.

Conclusions

We continued our investigations concerning the determination of the antibacterial activity of ethanolic extracts obtained from leaves and pseudobulbs of various plants belonging to the *Coelogyne* genus. In the current study, we aimed to determine the antibacterial activity of *Coelogyne brachyptera* against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* strains. The results of our study revealed the differential efficacy of ethanolic extract obtained from leaves of *C. brachyptera* on the test organisms. The extract displayed intermediate antibacterial potency against *S. aureus*. On the other hand, *E. coli* exhibited lower susceptibility for the impact of the ethanolic extract obtained from leaves of *C. brachyptera*. *P. aeruginosa* (ATCC®27583™) strain was the most resistant to the impact of the ethanolic extract obtained from leaves of *C. brachyptera*. Nevertheless, there is still room for an in-depth investigation, to make these plants best use in medicine and veterinary and to select them as an alternative to bacterial resistance. The promising results on medicinal plants screening for antibacterial activity could be considered as primary information for further phytochemical and pharmacological studies. In particular, the next step in our further investigation will be HPLC-profiling of the plant extract to find new bioactive compounds from a natural source.

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