



## ANTIBACTERIAL ACTIVITY OF THE ETHANOLIC EXTRACTS DERIVED FROM LEAVES AND PSEDOBULBS OF SOME ORCHIDS BELONGING TO *COELOGYNE* GENUS AGAINST *ENTEROBACTER CLOACAE* STRAIN

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Orchids belong to the largest family of angiosperms possessing the antimicrobial, antioxidant, hepatoprotective, anti-inflammatory, anti-arthritic and wound healing properties revealed in pre-clinical studies. The study of orchids also can provide health professionals with alternative, feasible, and low-cost therapies for treating bacterial and fungal diseases. For this reason, orchids may be used as new antimicrobial agents. Our previous study reported that the selected orchids belonging to the genus *Coelogyne* Lindl. exhibited fairly strong antioxidant and antimicrobial activities. This paper focused on the assessment of the antibacterial activity of eight plants, i.e. *Coelogyne cristata* Lindl., *C. fimbriata* Lindl., *C. flaccida* Lindl., *C. huettnneriana* Rchb.f., *C. ovalis* Lindl., *C. speciosa* (Blume) Lindl., *C. tomentosa* Lindl. and *C. viscosa* Lindl. towards clinical cefuroxime-resistant *Enterobacter cloacae* strain. Antibacterial activity was evaluated qualitatively and quantitatively using disc diffusion assay. The ethanolic extracts obtained from leaves and pseudobulbs of five orchids belonging to the *Coelogyne* genus were found to exhibit fairly strong antibacterial activity towards *Enterobacter cloacae* strain used, the diameter of inhibition zones varied from 8.0–25.5 mm. It has been observed that ethanolic extract obtained from pseudobulbs of eight species from the *Coelogyne* genus revealed the highest antibacterial activity (11.0–25.5 mm as the diameter of inhibition zone) as compared to ethanolic extracts obtained from leaves of the same *Coelogyne* species. These findings provide a clear demonstration of the generally overlooked importance of the collection of tropical plants accumulated at the Botanic Gardens, orchids collection, in particular, as an important source of new chemical substances with potential therapeutic effects, including antifungal activity. Further studies aimed at the isolation and identification of active substances from the extracts obtained from leaves and pseudobulbs of *Coelogyne* species could also disclose compounds with better therapeutic value. It is believed that screening of all the investigated plants for other biological activities is essential.

**Keywords:** orchids, leaf extracts, *Enterobacter cloacae*, antibacterial activity, disc diffusion technique, ethanolic extracts

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## Introduction

*Enterobacter cloacae* are part of the normal flora of the gastrointestinal tract of 40 to 80% of people and are widely distributed in the environment. Species of the genus *Enterobacter* are opportunistic pathogens and are capable of causing opportunistic infections in hospitalized patients (intensive care units, emergency units, urology department) (Michalska and Gospodarek, 2007). Species of the *Enterobacter cloacae* complex are widely encountered in nature (Mezzatesta et al., 2012). *Enterobacter aerogenes* and *E. cloacae* have been reported as important opportunistic and multidrug-resistant bacterial pathogens for humans during the last three decades in hospital wards. These Gram-negative bacteria have been largely described during several outbreaks of hospital-acquired infections (Davin-Regli et al., 2015). In recent years, *Enterobacter cloacae* have emerged as an important nosocomial pathogen (Dalben et al., 2008). The biochemical and molecular studies on *E. cloacae* have shown genomic heterogeneity, comprising six species: *Enterobacter cloacae*, *Enterobacter asburiae*, *Enterobacter hormaechei*, *Enterobacter kobei*, *Enterobacter ludwigii*, and *Enterobacter nimipressuralis*. *E. cloacae* and *E. hormaechei* are the most frequently isolated in human clinical strains (Mezzatesta et al., 2012). The dissemination of *Enterobacter* spp. is associated with the presence of redundant regulatory cascades that efficiently control the membrane permeability ensuring the bacterial protection and the expression of detoxifying enzymes involved in antibiotic degradation and inactivation. In addition, these bacterial species are able to acquire numerous genetic mobile elements that strongly contribute to antibiotic resistance. Moreover, this particular fitness helps them to colonize several environments and hosts and rapidly and efficiently adapt their metabolism and physiology to external conditions and environmental stresses (Davin-Regli et al., 2015). They are capable of overproducing AmpC  $\beta$ -lactamases by de-repression of a chromosomal gene or by the acquisition of a transferable ampC gene on plasmids conferring the antibiotic resistance (Mezzatesta et al., 2012).

It has been highlighted that the pathogenic mechanisms and factors contributing to the disease associated with the *E. cloacae* complex are not understood yet (Davin-Regli et al., 2015). Nevertheless, it was assumed that an ability of *E. cloacae* to form biofilms and to secrete various cytotoxins (enterotoxins, hemolysins, pore-forming toxins) is important for its pathogenicity (Mezzatesta et al., 2012). Moreover, certain *Enterobacter* spp. have been reported as plant-growth enhancers since they possess multiple growth-promoting activities (Ramesh et al., 2014).

Plant-derived antimicrobial agents have received much attention due to their effectiveness against drug-resistant strains, and diverse antimicrobial activities including antibiofilm activity (Khan et al., 2017; Lu et al., 2019).

A wide range of phytochemicals has been reported as antibacterial compounds, belonging to different classes, such as alkaloids, terpenoids, polyphenols, tannins, and phenanthrenes. It was emphasized that among them, phenanthrenes are a relatively uncommon class of aromatic metabolites in the plant kingdom, which are mainly found in the Orchidaceae and Juncaceae (Kovács et al., 2008; Chen et al., 2018; Tóth et al., 2018).

Although the antimicrobial activity of many orchid species, including *Coelogyne* species, has been effectively established against a wide spectrum of microorganisms (Kovács et al., 2008; Haque and Ghosh, 2015; Chen et al., 2018), bacterial drug resistance continues to be a worldwide public health issue in the treatment of infectious diseases, thereby stimulating search for new alternatives with fewer side effects (Mambe et al., 2019).

The orchid genus *Coelogyne* Lindl. comprises over 200 species, distributed from Southeast Asia to the south-western Pacific Islands (Clayton, 2002; Chen and Clayton 2009). It is currently divided into 4 subgenera and 19 sections (Gravendeel, 2005). *Coelogyne* species grow in the primary forest from sea level up to 4,000 m altitude. They are predominantly epiphytes, but in humid environments, some species can grow as lithophytes over rocky cliffs or even as terrestrials (Clayton, 2002). The *Coelogyne* genus is characterized by sympodial growth habit, pseudobulbs of one internode, a winged column, and massive caudices (Dressler, 1981). Within Coelogyninae subtribe this genus is distinguished by a free, never-saccate lip, with high lateral lobes over the entire length of the hypochile and smooth, papillose, toothed or warty keels on the epichile (Seidenfaden and Wood 1992).

Correspondingly, taking into consideration the development of resistance by Gram-negative bacteria the present study was aimed to compare the antibacterial activity of the leaf and pseudobulb extracts of eight orchid 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 clinical cefuroxime-resistant *Enterobacter cloacae* strain.

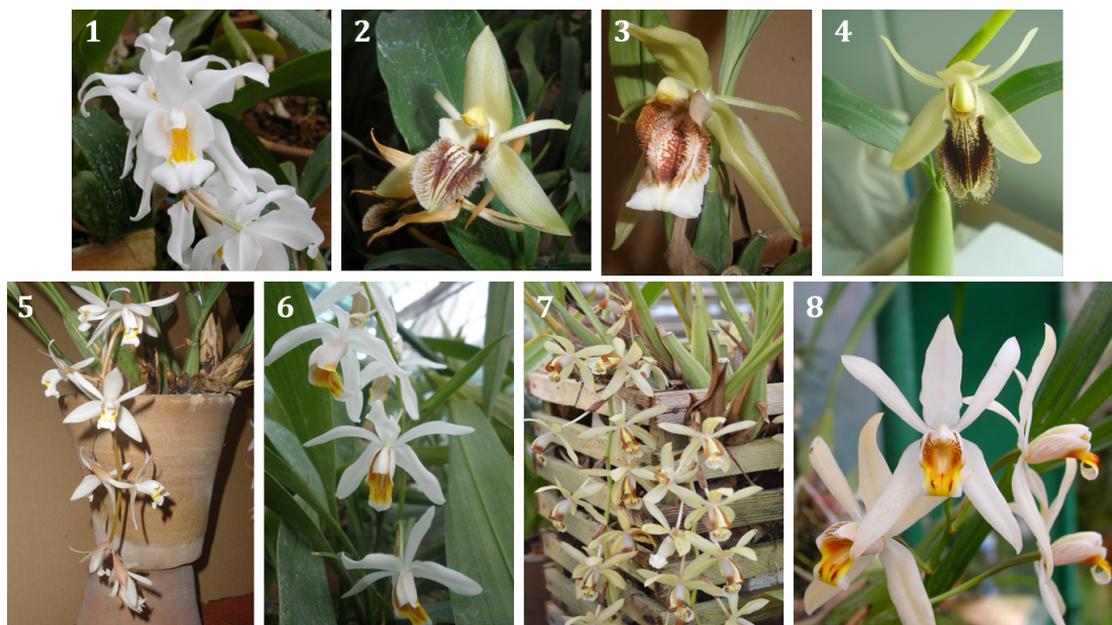
## Materials And Methodology

### Collection of Plant Material

The leaves and pseudobulbs of orchids, i.e. *C. flaccida* Lindl., *C. huettneriana* Rchb.f., *C. speciosa* (Blume) Lindl., *C. fimbriata* Lindl., *C. tomentosa* Lindl., *C. ovalis* Lindl., *C. cristata* Lindl., *C. viscosa* Rchb.f. (Figure 1) cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanic Garden (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, M.M. Gryshko National Botanic Garden 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).

### Preparation of Plant Extracts

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



**Figure 1** The general views of flowers of *Coelogyne* species from the collection of M.M. Gryshko National Botanical Garden (Kyiv, Ukraine) (Photo: Lyudmyla Buyun, Oleksandr Gyrenko)  
1 – *Coelogyne cristata* Lindl.; 2 – *Coelogyne fimbriata* Lindl.; 3 – *Coelogyne speciosa* (Blume) Lindl.; 4 – *Coelogyne ovalis* Lindl.; 5 – *Coelogyne flaccida* Lindl.; 6 – *Coelogyne huettneriana* Rchb.f.; 7 – *Coelogyne tomentosa* Lindl.; 8 – *Coelogyne viscosa* Rchb.f.

## Bacterial strain

The testing of the antibacterial activity of the plant extract was carried out *in vitro* by the Kirby-Bauer disc diffusion technique (Bauer et al., 1966). The non-repetitive clinical strain of cefuroxime-resistant *E. cloacae* isolated from bloodstream infection was collected from Koszalin Hospital during March-April, 2019. The purity, as well as the identity of isolate, was confirmed in the laboratory conditions by standard microbiological methods and were interpreted according to the guidelines of the Clinical Laboratory Standards Institute (2014).

Susceptibility testing of the isolates was performed by disk diffusion according to the Guidelines of Clinical and Laboratory Standard Institute (CLSI). The antibiotics tested were piperacillin, piperacillin-tazobactam, cefepime, cefotaxime, ceftazidime, cefuroxime, aztreonam, imipenem, meropenem, ertapenem, amikacin, gentamicin, trimethoprim-sulphamethoxazole, ciprofloxacin, levofloxacin, tetracycline, tigecycline, and polymyxin B. Results were interpreted according to CLSI criteria. MIC was determined by *E*-test strips (according to manufacturer's instruction) and agar dilution method (according to the Guidelines of Clinical and Laboratory Standard Institute). The resistance breakpoints were the same as the ones defined by the National Committee for Clinical Laboratory Standards (NCCLS, 2014).

*Enterobacter cloacae* strain studied was resistant to amoxicillin, cefuroxime, trimethoprim-sulphamethoxazole, and cefotaxime.

## 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 (108 CFU/mL) of the bacterium and spread with sterile swabs.

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.

### Statistical analysis

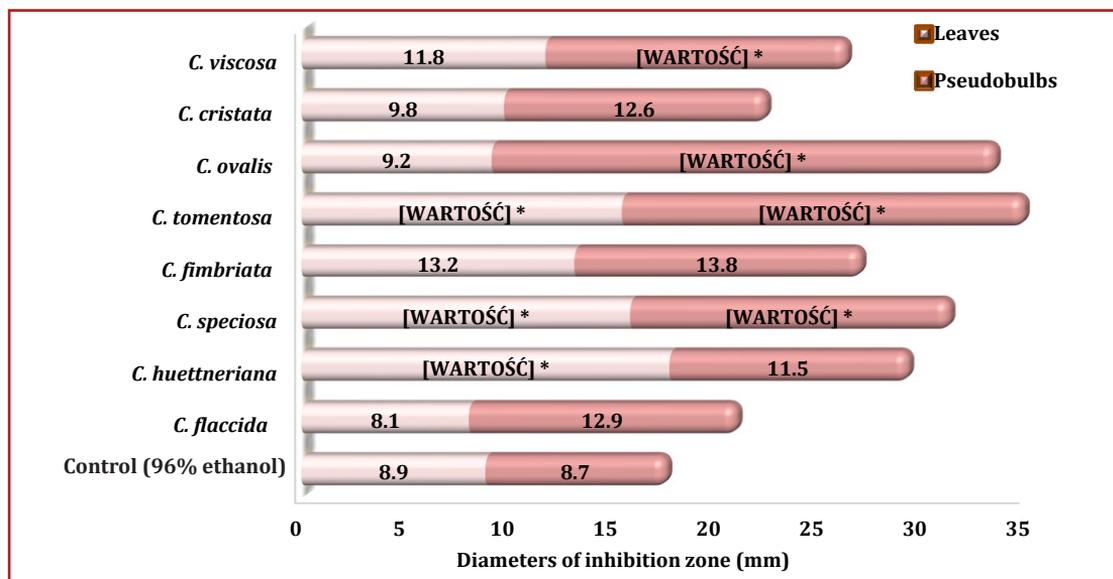
Zone diameters were determined and averaged. Statistical analysis of the data obtained was performed by employing the mean  $\pm$  standard error of the mean (S.E.M.). All variables were randomized according to the phytochemical activity of extracts tested. All statistical calculation was performed on separate data from each extract. The data were analyzed using an one-way analysis of variance (ANOVA) with Statistica software, version 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*)  $\geq 15$  mm, Intermediate (*I*) = 10–15 mm, and Resistant (*R*)  $\leq 10$  mm (Okoth et al., 2013).

## Results and discussion

Ethanollic extracts obtained from leaves and pseudobulbs of eight orchids belonging to the *Coelogyne* genus resulted in considerable suppression of clinical cefuroxime-resistant *Enterobacter cloacae* strain growth. Moreover, differential efficacy on the test organism was noted between *Coelogyne* species as well as between extracts obtained from leaves and pseudobulbs tested. Consequently, the extracts from various species displayed varied antibacterial potency against cefuroxime-resistant *Enterobacter cloacae* (Figure 2, 3).

As presented in Figure 2, tested extracts showed variable antibacterial activities with inhibition zone diameter values ranging from 8.1 to 17.8 mm (crude extracts derived from the leaves) and from 11.5 to 24.3 mm (for pseudobulb extracts). In our study, the marked antibacterial efficacy against cefuroxime-resistant *Enterobacter cloacae* strain was observed in the case of ethanollic extracts obtained from leaves of *C. tomentosa* (mean diameter of inhibition zones was  $15.5 \pm 0.9$  mm), *C. speciosa* ( $15.9 \pm 0.7$  mm), and *C. huettneriana* ( $17.8 \pm 1.2$  mm). A statistically significant increase ( $p < 0.05$ ) in inhibition zone diameters of strain growth was

100% (for *C. huettneriana*), 79% (for *C. speciosa*), and 74% (for *C. tomentosa*). Leaf extracts of *C. cristata*, *C. ovalis*, *C. fimbriata*, *C. viscosa*, and *C. flaccida* displayed less inhibitory activity against strain tested (mean diameter of inhibition zones was ranging from 8.0 to 13.5 mm) (Figure 2, 3).



**Figure 2** Antimicrobial activity of ethanolic extracts obtained from leaves and pseudobulbs of eight *Coelogyne* species against cefuroxime-resistant *Enterobacter cloacae*, measured as diameters of inhibition zone ( $n = 8$ )

On the other hand, the ethanolic extracts obtained from pseudobulbs of *C. ovalis* (mean diameter of inhibition zones was  $24.3 \pm 1.3$  mm), *C. tomentosa* ( $19.4 \pm 1.1$  mm), and *C. speciosa* ( $15.4 \pm 0.8$  mm) revealed statistically significant antibacterial activity against cefuroxime-resistant *Enterobacter cloacae* strain compared to control (96% ethanol). A statistically significant increase ( $p < 0.05$ ) in inhibition zone diameters of strain growth was 179% (for *C. ovalis*), 123% (for *C. tomentosa*), and 77% (for *C. speciosa*) (Figure 2, 3).

The present study has shown that ethanolic extracts derived from the leaves and pseudobulbs of eight species from the *Coelogyne* genus exhibited marked antibacterial activity against *Enterobacter cloacae* (inhibition zone diameter were ranged from 8.0 to 25.5 mm) (Figure 2, 3). Moreover, it has been observed that ethanolic extract obtained from pseudobulbs of eight species from *Coelogyne* genus revealed the highest antibacterial activity (11.0–25.5 mm as the diameter of inhibition zone) compared to ethanolic extracts obtained from leaves of eight species from *Coelogyne* genus (Figure 2, 3).



**Figure 3** Examples of a disc diffusion assay plate showing the halos in the bacterial lawn resulting from the antibacterial activity of extracts obtained from *Coelogyne tomentosa* Lindl. leaves (A) and pseudobulbs (B), *Coelogyne ovalis* Lindl. pseudobulbs (C), *Coelogyne huettneriana* Rchb.f. leaves (D), *Coelogyne speciosa* (Blume) Lindl. leaves (E) and pseudobulbs (F)

In recent years, the assessment of antibacterial properties of orchids has received considerable attention (Majumder et al., 1995, 2001, 2011; Singh et al., 2012; Tkachenko et al., 2015; Soumiya and Christudhas, 2017; Buyun et al., 2016, 2017, 2018). A number of orchid species are used as a potent inhibitor against Gram-positive and Gram-negative

bacteria and also proved to be a potent antimicrobial agent (Singh et al., 2012). Bhatnagar and Ghosal (2018) have conducted phytochemical profiling and evaluation of the antibacterial and antimycobacterial activity of orchid of Arunachal Pradesh, *Pleione maculata*. The whole plant material was divided into diethyl ether (Et2O), n-butanol (n-BuOH) and aqueous (Aq) fractions. The five different MDR bacterial clinical isolates including *Escherichia coli* (2461), *Staphylococcus aureus* (2413), *Enterococcus* sp. (2449), *Acinetobacter* sp. (2457), *Serratia* sp. (2442), *Mycobacterium tuberculosis* H37Rv were used in the study. The screening result identified Et2O fraction as the most active fraction showing significant zone of inhibition against *E. coli* and *Staphylococcus aureus* sp. and moderate antimycobacterial activity (MIC value 104.16 µg/mL) against H37Rv strain of *Mycobacterium tuberculosis*. The largest zone of inhibition (24.1 ±0.3 and 20.7 ±0.5 mm) was demonstrated by the Et2O fraction against *E. coli* and *S. aureus* sp. while in case of n-BuOH fraction the zone of inhibition was 18.7 ±0.7 mm and 17.1 ±0.6 mm against *S. aureus* and *Serratia* sp. respectively. However, the aqueous extract was not as effective against any bacterial pathogens as compared to organic solvent fractions even though slight antibacterial activity was observed. The Et2O fraction also inhibited the growth of *Serratia* sp. and *Enterococcus* with an inhibition zone 15.2 ±0.5 mm and 13.0 ±0.5 mm. The *Acinetobacter* sp. was least inhibited by the plant fractions. Analysis of the results showed that Et2O fraction of *P. maculata* exhibited moderate antimycobacterial activity against H37Rv strains with MIC 104.16 µg/mL followed by n-BuOH fraction with MIC value 166.66 µg/mL respectively. However, the aqueous residue of the plant did not show any activity against *Mycobacterium tuberculosis* strain. Phytochemical screening of the fractions revealed the presence of triterpenes, phlobatannins, alkaloids and cardiac glycosides in the most active Et2O fraction (Bhatnagar and Ghosal, 2018).

It was shown that blestriacin, a dihydro-biphenanthrene, extracted from the fibrous roots of *Bletilla striata* exhibited significantly anti-Gram-positive bacterial activity against *S. aureus*, *S. epidermidis*, *Enterococcus faecalis*, and *Bacillus subtilis* (Qian et al., 2015)

Phytochemical analysis revealed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, sterols in the hydro-alcoholic extract of *Coelogyne cristata* (Mitra et al., 2018). This orchid species is traditionally used in Indian subcontinent as a stimulant and tonic in aged patients suffering from persistent diseases, like asthma, degenerative changes and blood-borne diseases (Kumari et al., 1986; Singh et al., 2012; Mitra et al., 2018).

Soumiya and Christudhas (2017) have determined the phytochemical screening and antimicrobial activity of *Cymbidium aloifolium* (L.) Sw. orchids in Kanyakumari district of South India. The phytochemical screening and antimicrobial activity were performed using different extracts (methanol, ethanol, chloroform, acetone and aqueous) of *C. aloifolium*. Four different bacteria (Gram-positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram-negative: *Escherichia coli* and *Klebsiella pneumoniae*) and two fungi (*Penicillium* sp. and *Aspergillus niger*) were used in the Soumiya and Christudhas (2017) study. All the extracts showed different degrees of inhibitory action that potential against tested bacteria under this study. The antibacterial activity of *C. aloifolium* leaf extract revealed that the maximum inhibition zone of 34 mm recorded in aqueous extract against *S. aureus*. Minimum inhibitory activity was recorded in acetone and chloroform extract. The aqueous extract had the highest

activity against *Aspergillus niger* and *Rhizopus* with an inhibition zone of 45 and 42 mm, respectively. Minimum inhibitory activity was recorded in acetone and chloroform extract. The antimicrobial activity of *C. aloifolium* leaf extract revealed that the maximum inhibition zone of (43.5 ±0.84) recorded in aqueous extract against *Rhizopus* sp. followed by *Aspergillus niger* (42 ±0.62). The aqueous extract showed the inhibitory activity of (34.5 ±0.40) against *Staphylococcus aureus*. Methanol extract also exhibited some activity against *Staphylococcus aureus* (30±0.23) and *Klebsiella pneumonia* (25 ±0.47). The next activity was noticed in ethanol extract against *Bacillus subtilis*, *Aspergillus niger*, *Klebsiella pneumonia*, and *Staphylococcus aureus*. No activity was noticed in ethanol extract against *Escherichia coli* and *Rhizopus* sp. The acetone extract showed significant activity against *Bacillus subtilis*, *Aspergillus niger*, and *Rhizopus* sp. Among the different extracts, aqueous extracts showed better results than the positive control. Minimum inhibitory activity was recorded in methanol extract and acetone extracts (Soumiya and Christudhas, 2017).

The antimycobacterial, leishmanicidal and antibacterial activity of three orchids *Rhynchostylis retusa* (L.) Blume, *Tropidia curculigoides* Lindl. and *Satyrium nepalense* D. Don, traditionally used in tuberculosis, asthma and cold stage of malaria in folk Indian medicine, was demonstrated in the Bhatnagar et al. (2017) study. The dried material of each plant was divided into three parts. Solvent extraction and fractionation afforded altogether 30 extracts and fractions, which were evaluated against *Mycobacterium tuberculosis* (H37Rv and MDR strain) for antimycobacterial activity; promastigotes and amastigotes of *Leishmania donovani* for leishmanicidal activity and two Gram-positive and three Gram-negative clinical isolates for antibacterial activity. The most significant antimycobacterial activity was observed with n-hexane fraction of the flower of *Satyrium nepalense* with 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<sub>50</sub> 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. *S. nepalense* as the most promising plant followed by *R. retusa* and *T. curculioides* was demonstrated. Also, the fractions exhibited cell cytotoxicity well within the permissible limit (Bhatnagar et al., 2017).

Nagananda and Satishchandra (2013) have evaluated the antibacterial and antifungal activity of medicinally important orchid *Dendrobium nodosum* Dalzell (syn. *Flickingeria nodosa* (Dalz.) 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 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 *T. mentagrophytes* (Nagananda and Satishchandra, 2013).

Previously, we have given considerable attention to 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 antifungal potential of the leaves of eight orchid species, i.e. *Coelogyne cristata*, *C. fimbriata*,

*C. flaccida*, *C. huettneriana*, *C. ovalis*, *C. speciosa*, *C. tomentosa* and *C. viscosa* against fungus strain, *Candida albicans* was conducted by Buyun et al. (2018). Marked antifungal efficacy was observed in 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 pronounced inhibitory activity against test fungus (mean diameter of inhibition zones ranging from 16.0 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 16.0 to 23.5 mm). Moreover, it has been observed that ethanolic extract obtained from pseudobulbs of *C. speciosa* revealed the highest antibacterial activity (21 mm as diameter of 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).

*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 study (Buyun et al., 2016, 2017). The ethanolic extracts from leaves and pseudobulbs of *C. speciosa* exhibited strong activity against *S. aureus* (inhibition zone diameter was 19.0 and 21.5 mm, respectively), while the methanolic extract from leaves and pseudobulbs revealed mild activity (8.0 and 8.0 mm). Moreover, it has been observed that ethyl acetate, hexane and dichloromethane extracts obtained from leaves and pseudobulbs of *C. speciosa* displayed no antibacterial activity against *S. aureus*. Our results also showed that ethanolic extract from leaves of *C. speciosa* exhibited strong activity against *E. coli* (inhibition zone diameter was 21 mm), while others extract from pseudobulbs revealed minimum activity (inhibition zone diameter did not exceed 12 mm). Those extracts in ethyl acetate, hexane, and dichloromethane both from the leaves and pseudobulbs revealed no antibacterial activity against *S. aureus* (Buyun et al., 2016, 2017).

Finally, the Global Strategy for Plant Conservation (GSPC) outlines 16 targets encompassing knowledge, conservation, sustainable use, awareness, and capacity-building activities at Botanic Gardens (Mounce et al., 2017). Therefore, our recent works have exemplified the conviction that living plant collections accumulated at Botanic Gardens as germplasm repositories, are able to provide great opportunities for multi-focused approach, aimed at both *ex situ* biodiversity conservation and pharmacological screening as well (Tkachenko et al., 2015, 2018; Buyun et al., 2018). Consequently, we believe that our activity could be considered as an implementation of the Global Strategy for Plant Conservation.

## Conclusions

In conclusion, this study has provided informative data about the antimicrobial potential of the tested plant extracts by suggesting that ethanolic extracts derived from the leaves and pseudobulbs of eight species from *Coelogyne* genus exhibited marked antibacterial activity against *Enterobacter cloacae* (inhibition zone diameter ranged from 8 to 25.5 mm). Moreover, it has been observed that ethanolic extract obtained from pseudobulbs of eight species from *Coelogyne* genus revealed the highest antibacterial activity (11.0–25.5 mm as the diameter of

inhibition zone) as compared to ethanolic extracts obtained from leaves of eight *Coelogyne* species. Consequently, the present study, which revealed the antibacterial properties of the plant extracts, is a justification of the use of orchids belonging to *Coelogyne* genus to treat some of the infectious diseases caused by antibiotic-resistant *Enterobacter cloacae* strains. This fact is withstood by the bactericidal effect of the extracts derived from the leaves and pseudobulbs. Nevertheless, there is still room for an in-depth investigation, in order to make these plants best use in medicine and veterinary and to select them as an alternative to combat bacterial resistance

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