ANTIMICROBIAL SCREENING OF THE VARIOUS EXTRACTS DERIVED FROM THE LEAVES AND PSEUDOBULBS OF COELOGYNE SPECIOSA (BLUME) LINDL. (ORCHIDACEAE)

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ABSTRACT

The present study was conducted to investigate 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). The leaves and pseudobulbs of C. speciosa plants, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanical Garden (NBG), National Academy of Science of Ukraine. Freshly collected leaves and pseudobulbs were washed, weighted, crushed, and homogenized in 96% ethanol, methanol, ethyl acetate, hexane, and dichloromethane (in proportion 1:19) at room temperature. Antimicrobial activity was determined using the agar disk diffusion assay (Bauer et al. 1966). The present study has shown that ethanolic extracts from leaves and pseudobulbs of C. speciosa exhibited strong activity against S. aureus (inhibition zone diameter were 21.5 mm and 19 mm, respectively), while 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 of *C. speciosa* exhibited strong activity against *E. coli* (inhibition zone diameter was 21 mm), while other extracts from pseudobulbs revealed minimum activity (inhibition zone diameter was 12 mm). Those extracts in ethyl acetate, hexane and dichloromethane both from the leaves and pseudobulbs revealed no antibacterial activity against *S. aureus*. Thus, the research showed that ethanolic extracts of *C. speciosa* possess antibacterial potency against *S. aureus* and *E. coli* and may be used as natural antiseptics and antimicrobial agents in medicine and veterinary practice. Considering the medicinal importance of the tested microorganisms, the findings of this research are considered to be very promising in the perspective of new drug development from plant sources.

**Key words:** Coelogyne speciosa Lindl., *Staphylococcus aureus*, *Escherichia coli*, antimicrobial activity, orchids, agar disk diffusion assay

**INTRODUCTION**

Living plant collections in the botanic gardens are underutilized worldwide resources for plant conservation (Cibrian-Jaramillo et al. 2013). Beside, many groups of plants are valuable sources of diverse compounds, possessing broad spectrum of biological activity. In particular, orchids have been used as a source of medicines for millennia to treat different diseases and ailments including tuberculosis, paralysis, stomach disorders, chest pain, arthritis, syphilis, jaundice, cholera, acidity, eczema, tumour, piles, boils, inflammations, menstrual disorder, spermatorrhea, leucoderma, diarrhoea, muscular pain, blood dysentery, hepatitis, dyspepsia, bone fractures, rheumatism, asthma, malaria, earache, sexually transmitted diseases, wounds and sores. In addition, many orchidaceous preparations are used as emetic, purgative, aphrodisiac, vermifuge, bronchodilator, sex stimulator, contraceptive, cooling agent and remedies in scorpion sting and snake bite (Hossain 2011). Pharmacological studies have revealed antimicrobial, antioxidant, hepatoprotective, anti-inflammatory, anti-arthritic and wound healing properties of some orchids in pre-clinical studies (Bulpitt 2005, Panda and Mandal 2013).

Orchids belong to the largest family of angiosperms, with approximately 880 genera and 27,800 species (Givnish et al. 2016). Today, nearly 50 orchid species are widely used in different systems of medicine (Panda and Mandal 2013). Some of the species like *Vanda tessellata* (Roxb.) Hook. ex G. Don, *Dactylorhiza incarnata* subsp. *incarnata*, *Dendrobium nobile* Lindl. have been already documented for their proven medicinal values (Kong et al. 2003). Pseudobulbs are most commonly used to cure ailments, followed by leaves, roots, tubers/rhizomes and flowers. Major local uses include aphrodisiacs, energizers, and treatments of skin burns, fractured or dislocated bones (both of humans and cattle), headaches, fever, and wounds. Other uses include insect repellent, blood purifier, skin fungi, antidote against snake bites and scorpion stings, induction of abortions and recovery from child birth. Orchids are mainly used as paste, powder or juice, solely or mixed with milk, honey or wheat flour. Orchid extracts are either consumed orally or applied externally. Fresh orchid
flowers are used to induce vomiting by exposure to a dominant foul smell. Local communities in India and Nepal also commonly eat freshly cut species of *Coelogyne* in the forest when they feel thirsty (Subedi et al. 2011).

A wide range of chemical compounds has been identified from medicinal orchids (Subedi et al. 2011). Studies have reported the isolation of huge number of important phytochemicals from different genera of orchids such as alkaloids, flavonoids, stilbenoids, anthocyanins, triterpenoids, orchinol, hircinol, cypripedin, bibenzyl derivatives, phenanthrenes, jibantine, nidemin and loroglossin which are present in leaves, pseudobulb, roots, flowers or in the entire plant (Pant 2013).

*Coelogyne speciosa* (Blume) Lindl. (Photos 1 and 2) belongs to the section *Speciosae*, distributed from mainland Southeast Asia (Thailand), all over Malesia to the islands in the Pacific Ocean (Gravendeel 2000).

The epithet *speciosa* (which is Latin for beautiful) refers to the showy flowers. Pseudobulbs are ovoid and clustered, obtusely 4-angled when young. Leaves are one or two per pseudobulbs. Leaf blade is obovate-lanceolate to linear-lanceolate to lanceolate; apex acuminate or cuspidate; main nerves 3-5 (Gravendeel 2000). Synanthous inflorescences, characteristic feature for *C. speciosa*, are predominant in the section Speciosae: the inflorescence-bearing shoot has an immature pseudobulb hidden in the basal scales and the young leaf or leaves on top of this pseudobulb start swelling and leaf or leaves fully develop (Gravendeel and de Vogel 1999) (Figs 1 and 2).

The flowers open in succession (starting with the basal one) (www.orchidspecies.com/coelogyynespeciosa.htm).

Artificial pollination treatments, which we carried out under glasshouse conditions, have shown that *Coelogyne speciosa* is largely self-sterile. Barbara Gravendeel in Holland has initiated detailed research into *Coelogyne speciosa*, backed by extensive DNA analysis (Gravendeel and de Vogel 1999, Gravendeel 2000).
Moreover, many species of Orchidaceae family also possess antimicrobial activity (Pérez Gutiérrez 2010).

Fig. 1. The branching pattern of shoot system of *Coelogyne speciosa*
Source: own research

Fig. 2. Scheme of the structure of *C. speciosa* elementary shoot: a) leaf; b) pseudo-bulb; c) buds; d) rhizome; e) node; f) internode
Source: own research
Therefore, the aim of this study was to evaluate the antimicrobial activity of the crude extracts obtained from leaves and pseudobulbs of *C. speciosa* prepared in different solvent systems against *Staphylococcus aureus*, a clinically important microorganism responsible for many infections, as well as against *Escherichia coli* to support the use of these extracts as novel natural products for compounded bacterial-treatment modalities.

**MATERIALS AND METHODS**

*Collection of Plant Material.* The leaves and pseudobulbs of *C. speciosa* plants, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanical Garden (NBG), National Academy of Science of Ukraine. Since 1999, the whole collection of tropical and subtropical plants (including orchids) has the status of a National Heritage Collection of Ukraine. Besides that, NBG collection of tropical orchids was registered at the Administrative Organ of CITES in Ukraine (Ministry of Environmental Protection, registration No. 6939/19/1-10 of 23 June 2004).

*Preparation of Plant Extracts.* Freshly collected leaves and pseudobulbs were washed, weighted, crushed, and homogenized in 96% ethanol, methanol, ethyl acetate, hexane, and dichloromethane (in proportion 1:19) at room temperature. Consequently five kinds of solvent systems were used to extract the active ingredients from the leaves and pseudobulbs of *Coelogyne speciosa*.

*Bacterial Growth Inhibition Test of Plant Extracts by the Disk Diffusion Method.* Antimicrobial activity was determined using the agar disk diffusion assay (Bauer et al. 1966). Cultures of *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were suspended in sterile solution of 0.9% normal saline and the turbidity adjusted equivalent to that of a 0.5 McFarland standard. Culture was inoculated onto Mueller-Hinton (MH) agar plates. Sterile filter paper discs impregnated with 50 μL of extract dilutions were applied over each of the culture plates. Isolates of bacteria were then incubated at 37°C for 24 h. The plates were then observed for the zone of inhibition produced by the antifungal activity of *C. speciosa*. As negative control, disc impregnated with 50 μl of sterile ethanol, methanol, ethyl acetate, hexane, and dichloromethane was used in each experiment. The antimicrobial activities of the extracts tested were evaluated at the end of the inoculation period by measuring the inhibition zone diameter around each paper disc in millimeters. The plates were observed and photographs were taken. For each extract six replicate trials were conducted against each organism. Zone diameters were determined and averaged.

*Statistical analysis.* All statistical calculations were performed on separate data from each bacterial strains (Zar 1999). All statistical calculation was performed on separate data from each individual with STATISTICA 8.0. The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (S) ≥ 15 mm, Intermediate (I) = 11-14 mm, and Resistant (R) ≤ 10 mm (Okoth et al. 2013).
RESULTS

The present study has shown that ethanolic extracts from the leaves and pseudobulbs of *C. speciosa* exhibited strong activity against *S. aureus* (inhibition zone diameter were 21.5 mm and 19 mm, respectively), while methanolic extract from leaves and pseudobulbs revealed mild activity (8.1 and 8 mm) (Figs 3 and 4). 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*.

Fig. 3. Antimicrobial activity of various extracts obtained from the leaves of *C. speciosa* against *Staphylococcus aureus* measured as inhibition zone diameter (n = 6)
Source: own research

Fig. 4. Antimicrobial activity of various extracts obtained from pseudobulbs of *C. speciosa* against *Staphylococcus aureus* measured as inhibition zone diameter (n = 6)
Source: own research
The results of antimicrobial activity of various extracts obtained from the leaves and pseudobulbs of *C. speciosa* against *E. coli* are presented in Figs 5 and 6. Our results also showed that ethanolic extract from leaves of *C. speciosa* exhibited strong activity against *E. coli* (inhibition zone diameter was 21 mm) (Fig. 5), while others extracts from pseudobulbs revealed minimum activity (inhibition zone diameter was 12 mm) (Fig. 6). Those extracts in ethyl acetate, hexane and dichloromethane both from the leaves and pseudobulbs revealed no antibacterial activity against *S. aureus* (Figs 5 and 6).

![Inhibition zone diameter](image)

**Fig. 5.** Antimicrobial activity of various extracts obtained from leaves of *C. speciosa* against *Escherichia coli* measured as inhibition zone diameter (n = 6)
Source: own research

![Inhibition zone diameter](image)

**Fig. 6.** Antimicrobial activity of various extracts obtained from pseudobulbs of *C. speciosa* against *Escherichia coli* measured as inhibition zone diameter (n = 6)
Source: own research
Detailed data regarding the zones of inhibition by the various plant extracts were recorded and presented in Figs 7 and 8.

**Fig. 7.** Antibacterial activity of ethanolic extracts obtained from leaves (A) and pseudobulbs (B) of *C. speciosa* against *S. aureus* measured as inhibition zone diameter

Source: own research

**Fig. 8.** Antibacterial activity of ethanolic extracts obtained from leaves (A) and pseudobulbs (B) of *C. speciosa* against *E. coli* measured as inhibition zone diameter

Source: own research

**DISCUSSION**

In the present study, the effect of extracts obtained from leaves and pseudobulbs of *C. speciosa* prepared in various solvents on the growth of *S. aureus* and *E. coli* was investigated *in vitro*. The results revealed the antimicrobial potential of ethanolic extracts. All the test organisms were susceptible to ethanolic extracts obtained from leaves and pseudobulbs of *C. speciosa* with inhibition zone diameter between 12-21.5 mm. *E. coli* was more resistant to ethanolic extracts obtained from leaves and pseudobulbs of *C. speciosa* and the diameter of inhibition zone around the rest ranged from 11-13.5 mm.

The results obtained from the present study are consistent with earlier reports, which found that other species of *Coelogyne* have noticeable phytochemical, antimicrobial...
microbial, antioxidant and anticancer activity (Sahaya Shibu et al. 2013). According to Sahaya Shibu and co-workers (2013), C. nervosa exhibits potential antimicrobial, antioxidant and anticancer properties. The previous investigations on pharmacological properties of orchid species, belonging to Coelogyne genus, have found that these plants are sources of phytochemicals which are able to initiate different biological effects including antimicrobial activity (Pérez Gutiérrez 2010, Sarmad Moin et al. 2012, Sahaya Shibu et al. 2013, Pramanick 2016).

Preliminary phytochemical screening for the presence of alkaloids, carbohydrates, glycosides, saponins, terpenoids, steroids, flavonoids, phenolic compounds, protein, gum and mucilages, phytosterol, tannins and phlobatannins were carried out. The screening of antimicrobial, antioxidant and anticancer activities was also performed. Preliminary phytochemical analysis revealed that the ethanol and aqueous extracts shown the maximum phytochemical constituents. This analysis shows the presence of alkaloids, carbohydrates, glycosides, saponins, terpenoids, steroids, flavonoids, phenolic compounds, protein, phytosterol, tannins and phlobatannins in ethanol extract. The result obtained is similar to the results of Sarmad and co-workers (2012) and Mazumder and co-workers (2010) in Coelogyne stricta and Papilionanthe teres. All the extracts of C. nervosa were tested for antimicrobial and antioxidant activities. The ethanolic extract of C. nervosa showed the maximum zone of inhibition against the bacteria Pseudomonas aeruginosa (15 mm), Enterococcus faecalis (14.3 mm), Salmonella enterica (12 mm), Bacillus subtilis (11 mm) and Corynebacteria spp. (9 mm). The ethanolic extract showed the maximum antibacterial activity against all the microorganisms tested, which shows the minimum inhibitory concentration (MIC) value in the range between 400 to 600 (μg/ml). The water extract possessed strong 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (IC$_{50}$ 126 μg/ml) and it shows cytotoxic activity towards the MCF-7 cancer cell line (IC50, 292.8 μg/ml). Several compounds like 9,10-Dihydro-5H-phenanthro-(4,5 bcd)-pyrans and pyrones were isolated from a number of species like Coelogyne, Pholidota, and Otochilus (Veerraju et al. 1989) whereas, trans-3,4,30,50-tetrahydroxystilbene is reported to possess a chemopreventative agent with anti-leukemic activity and is being extensively studied for various cancers including colorectal and lung cancer as well as in cardiovascular diseases (Wolter et al. 2002). The anticancer activity results states that the IC$_{50}$ value of water extract on MCF-7 cell line was 292.8 μg/ml which shows the plant have anticancer activity (Sahaya Shibu et al. 2013).

In a study conducted by Paulomi and co-workers (2013), the bioactivity of few unexplored orchids (Aerides odorata, Acampe papilosa and Acampe ochracea that are native to North-East India) against kanamycin and ampicillin resistant E. coli was found out. All the three plants showed good antibacterial property against the selected strains of E. coli. Among the three strains, the ampicillin resistant variety showed strong inhibition (average zone diameter of 7.0 mm), the kanamycin resistant variety showed comparatively less inhibition (average zone diameter of 5.3 mm) and the (MTCC) variety showed the least (average zone diameter of 5.0 mm). The treatment in the antibiotic suspended medium showed less inhibition for acetone extract (average zone diameter of 5.3 mm) against ampicillin resistant variety than water extract (average zone diameter of 6.3 mm) against kanamycin resistant variety (Paulomi et al. 2013).
The antimicrobial effect showed by *C. speciosa* is in agreement with previous antimicrobial effects produced by numerous orchids. To screen for bioactive chemicals, Subedi and co-workers (2011) have tested the extracts of traditionally used plant parts (either stems or pseudobulbs) of a subset of the orchids used in traditional medicines or involved in trade for their antibacterial activity against common intestinal and harmful bacteria (*Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*) using both a disc diffusion method and Minimum Inhibitory Concentration Assay (MIC). Results of Subedi and co-workers (2011) suggest that traditional uses of wild orchids against infectious diseases by local communities are based on antibacterial properties which could be used for the development of alternative drugs. All the extracts showed activity against one or more test bacteria. *Aerides multiflora*, *Calanthe puberula*, *Coelogynne flaccida*, *Coelogynne nitida*, *Coelogynne punctulata*, *Coelogynne stricta*, *Cymbidium iridioides*, *Dendrobium eriiflorum*, *Dendrobium fugax* (syn. *Flickingeria fugax*), *Luisia trichorrhiza* and *Pholidota imbricata* showed a broad spectrum antibacterial activity. In contrast, *Bulbophyllum umbellatum*, *Coelogynne flaccida* and *Malaxis acuminata* reduced growth of only a single strain. The minimal inhibitory concentration ranged from 0.9375 ± 0.44194 for *Dactylorhiza hatagirea* and *Epigeneium amplum* up to 2.0833 ± 0.72169 mg/ml for *Cymbidium iridioides* and *Pholidota imbricata*, making these the most and least potent extracts, respectively. Sensitivity decreased from *E. coli* > *B. subtilis* > *S. aureus* > *P. aeruginosa* > *S. typhi* > *K. pneumonia* (Subedi et al. 2011).

Antibacterial activities of different extracts of epiphytic orchids (*Eria spicata*,  *Bulbophyllum affine*, *Vanda cristata*, *Rhynchostylis retusa*, *Dendrobium nobile*, *Dendrobium amoenum*, *Coelogynne cristata*, *Pholidota imbricata*) were tested against 5 species of bacteria (Gram-positive bacteria *S. aureus* and *Bacillus subtilis* and Gram-negative bacteria *Vibrio cholerae*, *E. coli* and *Klebsiella pneumoniae*) and antifungal activities were tested against 3 species of fungi (*Candida albicans*, *Rhizopus stolonifer* and *Mucor spp.*) (Marasini and Joshi 2012). Only *Coelogynne stricta* (leaf) and *Dendrobium amoenum* were shown good activity against *Klebsiella pneumoniae* but *Pholidota articulata* and *P. imbricata* have showed good activity against *E. coli*. *Eria spicata*, *Bulbophyllum affine*, *Vanda cristata* and *Rhynchostylis retusa* have showed weak activity against all bacteria. *Dendrobium nobile* displayed good inhibition against *S. aureus* but weak one against all other bacteria. All plants extract exhibited very good inhibition against *S. aureus*. *Pholidota imbricata* and *C. cristata* have showed the highest inhibition against *V. cholerae* and *S. aureus* respectively. After evaluating the zone of inhibition values of various extracts, two extracts with highest zone of inhibition value were taken for MIC test by two fold serial dilution method. MIC of extract of *C. cristata* was tested against *S. aureus* and that of *P. imbricata* against *V. cholerae*. The MIC of *C. cristata* against *S. aureus* was observed at 31.25 mg/ml and that of *P. imbricata* against *V. cholerae* was observed at 62.5 mg/ml. The minimum bactericidal concentration (MBC) values of the extracts of *P. imbricata* against *V. cholerae* and *C. cristata* against *S. aureus* were observed at 125 mg/ml and 250 mg/ml respectively. *Coelogynne stricta* (pseudobulb), *C. stricta* (leaf) and *Dendrobium amoenum* exhibited no activity while *P. imbricata* and *P. articulata* extracts revealed good activity against fungal organisms. Rest of the extracts
showed moderate or even very weak activity against selected fungal pathogens. Antibacterial activities shown by *C. cristata*, *P. articulata* and *P. imbricata* extracts were more active than other extracts. *P. imbricata* and *P. articulata* extracts have found intermediate activity but all others extracts have showed very less activity or even failed to show activity against all different five fungal organisms (Marasini and Joshi 2012).

Rashmi and co-workers (2015) have determined antibacterial and radical scavenging activity of extracts of four epiphytic orchids, namely *Luisia zeylanica*, *Pholidota pallida*, *Dendrobium nutantiflorum* and *Coelogyne breviscapa* collected at different places of Western Ghats of Karnataka, India. Antibacterial and radical scavenging activity of orchid extracts was determined by Agar well diffusion and DPPH free radical scavenging activity. Two Gram-positive bacteria viz., *B. subtilis* and *B. coagulans* and two Gram-negative bacteria viz., *E. coli* and *Salmonella typhi* were used. In study of Rashmi and co-workers (2015), it was observed that the extracts of all orchids exhibited inhibitory effect against all tested bacteria, but to a varied extent. Among orchids, marked antibacterial activity was displayed by *C. breviscapa* as indicated by wider zones of inhibition. Other orchids exhibited more or less similar inhibitory activity against test bacteria. Among Gram-positive and Gram-negative bacteria, *B. subtilis* and *E. coli* were inhibited to higher extent by extract of orchids respectively. Extracts were shown to inhibit *B. coagulans* and *S. typhi* to more or less similar extent. Moreover, all orchid extracts displayed dose dependent scavenging of radicals i.e., on increasing the concentration of extract, scavenging efficacy was also increased. Highest and least scavenging potential was exhibited by extract of *L. zeylanica* and *P. pallida* respectively as revealed by IC$_{50}$ values. The scavenging effect of orchid extracts is in the order – *L. zeylanica* (62.62 µg/ml) > *C. breviscapa* (71.91 µg/ml) > *D. nutantiflorum* (91.46 µg/ml) > *P. pallida* (128.31 µg/ml). Reference antioxidant i.e., ascorbic acid displayed higher scavenging activity (1.12 µg/ml) when compared to orchid extracts. The observed bioactivities of orchid extracts could be ascribed to the presence of phytochemicals, in particular phenolic compounds. In study of Rashmi and co-workers (2015), a positive correlation between the phenolic content and radical scavenging activity of orchid extracts was observed, i.e., extracts possessing high phenolic content exhibited marked scavenging of DPPH free radicals.

Aqueous, ethanolic and chloroform soluble extracts of leaf and pseudobulb of *Bulbophyllum neilgherrense* (5.50 w/v) were screened for their antibacterial potential against five species of bacteria, viz., *E. coli*, *S. aureus*, *B. pumilus*, *P. aeruginosa* and *P. putida* (Priya and Krishnaveni 2005). The zone of inhibition was the largest when alcoholic extract was used. Results of Priya and Krishnaveni (2005) revealed that the extracts, though less effective than streptomycin, proved inhibitory to bacterial growth in vitro. The growth inhibitory effect was more pronounced in ethanolic extracts of leaf and pseudobulb followed by chloroform and aqueous extracts. The ethanolic leaf extract was found more effective against *E. coli*, *S. aureus* and *P. aeruginosa* than *B. pumilus* and *P. putida*. The pseudobulb extract was found more effective against *P. aeruginosa* and *P. putida* (Priya and Krishnaveni 2005).

In our previous study (Buyun et al. 2016, Tkachenko et al. 2015, 2016, Góralczyk et al. 2016), we have reinforced the assumption that *Coelogyne* species could be poten-
tial antibacterial or antifungal resource. We have determined antifungal potential of eleven species of orchids namely *Coelogyne viscosa* Lindl., *C. cristata* Lindl., *C. Lawrenceana* Rolfe, *C. pandurata* Lindl., *C. assamica* Linden & Rchb.f., *C. fimbriata* Lindl., *C. ovalis* Lindl., *C. asperata* Lindl., *C. speciosa* (Blume) Lindl., *C. tomentosa* Lindl., and *C. brachyptera* Rchb.f. against *Candida albicans*. Ethanolic orchid extracts resulted in considerable suppression of growth of *C. albicans*. The orchid extracts from various species of *Coelogyne* genus displayed varied antifungal potency. Among orchids selected, marked antifungal efficacy was observed in case of *C. speciosa* (mean diameter of growth of inhibition zones was 19.7 mm), *C. ovalis* (18.2 mm), *C. brachyptera* (17.2 mm), and *C. assamica* (17.1 mm). Extract of *C. cristata* displayed least inhibitory activity against test fungus (mean diameter of growth of inhibition zones was 14.0 mm). Orchids were shown to exhibit antifungal activity against a variety of mold species. Our results showed that different extracts of epiphytic orchids from *Coelogyne* genus have potent antifungal properties against *Candida albicans*. Antifungal activities shown by *C. speciosa*, *C. ovalis*, *C. brachyptera*, and *C. assamica* extracts were most active then other extracts (Tkachenko et al. 2015). Moreover, ethanolic orchid leaf extracts resulted in considerable suppression of growth of *S. aureus*. The orchid extracts from various species of *Coelogyne* genus displayed varied antimicrobial potency. Among orchids selected, marked antimicrobial efficacy was observed for *C. cristata* (mean diameter of growth of inhibition zones was 27.5 mm), *C. tomentosa* (26 mm), *C. Lawrenceana* (26 mm), *C. brachyptera* (26 mm), *C. viscosa* (25.5 mm), *C. pandurata* (24.5 mm), and *C. fimbriata* (24 mm). Thus, our results showed that different extracts of epiphytic orchids from *Coelogyne* genus have potent antimicrobial properties against *S. aureus* (Tkachenko et al. 2016).

Previously, we have investigated the antibacterial effects of ethanolic extract of *Coelogyne 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 and *Escherichia coli* ATCC 25922) and fungal strain (*Candida albicans*) (Buyun et al. 2016a). Our results showed that the ethanolic extract of *C. brachyptera* leaves showed strong activity against the Gram-positive bacterial strains (20 mm diameter of inhibition zone for *S. aureus* and 26 mm for meticillin-resistant *S. aureus*), and moderate activity against the Gram-negative bacteria (14 mm for *E. coli* and 11 mm for *P. aeruginosa*). Extract of *C. brachyptera* has displayed strong inhibitory activity against test fungus *Candida albicans* (mean diameter of inhibition zone was 17.2 mm). Our study revealed the good antimicrobial and antifungal activities of ethanolic leaf extracts of *C. brachyptera*. Yet, this research illustrates that a Gram-positive bacterium was more susceptible to the ethanolic leaf extracts of *C. brachyptera* as compared to Gram-negative bacteria species. Beside, extract of *C. brachyptera* leaves has displayed strong inhibitory effect against test fungus *C. albicans* (Buyun et al. 2016a).

We also determined antibacterial and antifungal potential of ethanolic extract of *C. cristata* leaves against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacterial strains (Góralczyk et al. 2016). All microorganisms tested were susceptible to the leaf extract of *C. cristata*. Extract of *C. cristata* displayed the least inhibitory activity against test fungus (mean diameter of growth of inhibition zones was 14 mm). Our results showed that the etha-
nolic extract of *C. cristata* leaves exhibited strong activity against the Gram-positive bacterial strain (27 mm of inhibition zone diameter for *S. aureus*), and moderate activity against the Gram-negative bacteria (13 mm for *E. coli*). *P. aeruginosa* appeared to be less sensitive to the extract (the inhibition zone was 10 mm). In our present study we conclude that ethanolic extract of *C. cristata* leaves has potent antimicrobial activity against *S. aureus* (Góralczyk et al. 2016).

The ethanolic extract of *C. ovalis* leaves showed strong activity against *S. aureus* (27 mm of inhibition zone diameter), while ethanolic extract from pseudobulbs revealed less activity (22 mm). Methanolic and ethyl acetate extracts obtained from *C. ovalis* leaves also showed appreciable antimicrobial activity (32 mm and 35 mm, respectively), whereas those extracts from pseudobulbs revealed no antibacterial activity against *S. aureus* (Buyun et al. 2016c). Our study has shown that ethanolic extracts from leaves and pseudobulbs of *C. tomentosa* exhibited strong activity against *S. aureus* (inhibition zone diameter were 29 mm and 30 mm, respectively), while methanolic extract from leaves and pseudobulbs revealed less profound activity (18 mm and 10 mm, respectively). Moreover, it has been observed that ethyl acetate extract obtained from *C. tomentosa* pseudobulbs also showed appreciable antimicrobial activity (25 mm), while those extracts from the leaves, as well as hexane and dichloromethane extracts both from the leaves and pseudobulbs revealed no antibacterial activity against *S. aureus*. Hence, the overall results of the present investigation provide evidence that the crude extracts obtained from leaves and pseudobulbs of *C. tomentosa* could be considered as promising natural antimicrobial products (Buyun et al. 2016b). The ethanolic extract from leaves and pseudobulbs of *C. huettneriana* exhibited strong activity against *E. coli* (inhibition zone diameter were 28 mm and 13 mm, respectively), while others extracts from leaves and pseudobulbs revealed minimum activity. Similarly, it has been observed that ethanolic extract obtained from leaves and pseudobulbs also showed appreciable antimicrobial activity against *S. aureus* (19.5 mm and 21 mm, respectively), while those extracts in ethyl acetate, hexane and dichloromethane both from the leaves and pseudobulbs revealed no antibacterial activity against *S. aureus* (Buyun et al. 2016d).

Also, the results obtained from the present research showed antimicrobial potential of *C. speciosa* extracts against *S. aureus*. So these plants extracts can be used as anti-septics and antimicrobial agents in medicine and veterinary. The antibacterial activity in *Coelogyne* may be due to presence of alkaloids, bibenzyl derivatives, flavonoids, phenanthrenes and terpenoids. Clinical studies confirmed diuretic, anti-rheumatic, anti-inflammatory, anti-carcigenic, antimicrobial, anticonvulsive, relaxation, neuroprotective and antivirus activities (Pérez Gutiérrez 2010).

Kovács and co-workers (2008) have furnished an overview of the hydroxy or and methoxy-substituted 9,10-dihydro/phenanthrenes, methylated, prenylated and other monomeric derivatives, dimeric and trimeric phenanthrenes and their biological activities. A fairly large number of phenanthrenes have been reported from higher plants, mainly in the Orchidaceae family, in the species of *Dendrobium*, *Bulbophyllum*, *Eria*, *Maxillaria*, *Bletilla*, *Coelogyne*, *Cymbidium*, *Ephemera* and *Epipendrum*. A few phenanthrenes have been found in the Hepaticae class and Dioscoreaceae, Combretaceae and Betulaceae families as well. These plants have often been used in traditional medicine, and phenanthrenes have therefore been studied for their cytotoxicity, antimicrobial, spasmylytic, anti-inflammatory, anti-platelet aggregation,
antiallergic activities and phytotoxicity (Kovács et al. 2008). Finally, it should be noted, that occurrence of phenanthrenes and dihydrophenanthrenes in orchids is considered as accumulation of orchid phytoalexins in response to infection of orchid plant by mycorrhizal fungi (Fisch et al. 1973).

It has been reported that many natural products including pigments, enzymes and bioactive components are soluble in water, which explains the highest yield of extract, while some of the solvents especially acetone are selective for tannins (Rao 2004). Different solvents have various degrees of solubility for different phytoconstituents (Manilal and Sathishkumar 1986). Therefore, we suggest that different antimicrobial activity of extracts obtained from *C. speciosa* diluted with various solvents is determined by different agents presented in extracts.

A number of phytochemicals such as alkaloids, bibenzyl derivatives, flavonoids, phenanthrenes etc. have been reported from orchids. Presence of these phytochemicals provides antimicrobial, antitumor, anti-inflammatory, antiviral activities, etc. A number of members of orchid family are used as potent inhibitor against bacteria and also proved to be a potent antimicrobial agents. The methanolic extract from different parts of orchids has shown antimicrobial activity. A broad spectrum antibacterial activity (24 bacteria and protozoan) was exhibited by *Pseudovanilla foliata* (syn. *Galeola foliata*) leaves and stem bark extracted with petrol, dichloromethane, ethyl acetate, butanol and methanol. A very good level of activity was demonstrated by the dichloromethane and ethyl acetate fractions (Khan and Omoloso 2004). Moreover, endophytic fungi isolated from different orchids could be of potential antibacterial or antifungal resource. Antimicrobial activity of ethanolic extract of fermentation broth of 53 endophytes (30 isolates from *Dendrobium devonianum* and 23 endophytic fungi from *D. thyrsiflorum*) was explored by Xing and co-workers (2011) using agar diffusion test. 10 endophytic fungi in *D. devonianum* and 11 in *D. thyrsiflorum* exhibited antimicrobial activity against at least one pathogenic bacterium or fungus among 6 pathogenic microbes (*E. coli*, *B. subtilis*, *S. 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). Vaz and co-workers (2009) have examine 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. The extracts of endophytic fungi isolated from leaves of terrestrial orchids in semideciduous forest were more active against *E. coli*, whereas extracts of endophytic fungi from roots of rupicolous orchids collected in rock fields were more active against *Candida krusei* and *Candida albicans*. Among the fungi that were screened in the study, 22 isolates held their antimicrobial activities after replication and were therefore selected for assessment of the MIC, which ranged from 62.5 to 250 µg/ml and 7.8 to 250 µg/ml against bacteria and fungi, re-
respectively. One isolate of *Alternaria* sp. and one isolate of *Fusarium oxysporum* presented the strongest antibacterial activity. Three *Fusarium* isolates, *Epicoccum nigrum*, and *Sclerostagonospora opuntiae* showed the greatest MIC values against the pathogenic yeasts (Vaz et al. 2009). The work also signifies the earlier findings on various antimicrobial activity of orchid (Pérez Gutiérrez 2010) and validates the findings that the presence of phytochemicals in orchid may be the reason for this efficacy.

**CONCLUSIONS**

The examined ethanolic extracts obtained from leaves and pseudobulbs of *C. speciosa* showed different antibacterial activities against *S. aureus* and *E. coli*, while methanol, ethyl acetate, hexane, and dichloromethane extracts had no antibacterial activities. The ethanolic extracts from leaves exhibited notable antimicrobial activity against *S. aureus* and *E. coli* (inhibition zone diameter were 21.5 and 20 mm). The observed activity may contribute to the reasons why the leaves are used for infectious and inflammatory conditions in ethnomedicine. The research showed that ethanolic extracts of *C. speciosa* possess antibacterial potency against *S. aureus* and *E. coli*. These findings led the authors to suggest that these extracts may be used as a natural antiseptics and antimicrobial agents in medicine and veterinary practice. Nevertheless, despite the promising results, more research should be carried out to further evaluate the roles of particular compounds, isolated from all parts of orchid plants, attributable to antimicrobial activity.

**REFERENCES**


SUMMARY

Orchids have been used all over the world in traditional healing and treatment system of a large number of diverse diseases. Extracts and metabolites of these

plants demonstrate useful pharmacological activities, i.e. diuretic, antirheumatic, anti-inflammatory, anticarcinogenic, hypoglycemic, antimicrobial, anticonvulsive, relaxation, neuroprotective, and antivirus, activities (Pérez Gutiérrez 2010). Phytochemical studies in the genus Coelogyne revealed that these plants have a wide array of secondary compounds resulting in profound biological activity, antimicrobial, in particular (Pant 2013, Sahaya et al. 2013). In this regard, it is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds. So the present study was conducted to investigate 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). The leaves and pseudobulbs of C. speciosa plants, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanical Garden (NBG), National Academy of Science of Ukraine. Freshly leaves and pseudobulbs were washed, weighted, crushed, and homogenized in 96% ethanol, methanol, ethyl acetate, hexane, and dichloromethane (in proportion 1 : 19) at room temperature. Antimicrobial activity was determined using the agar disk diffusion assay (Bauer et al. 1966). The present study has shown that ethanolic extracts from leaves and pseudobulbs of C. speciosa exhibited strong activity against S. aureus (inhibition zone diameter were 21.5 mm and 19 mm, respectively), while 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 of C. speciosa exhibited strong activity against E. coli (inhibition zone diameter was 21 mm), while others extracts from pseudobulbs revealed minimum activity (inhibition zone diameter was 12 mm). Those extracts in ethyl acetate, hexane and dichloromethane both from the leaves and pseudobulbs revealed no antibacterial activity against S. aureus. The antibacterial activity in Coelogyne may be due to presence of alkaloids, bibenzyl derivatives, flavonoids, phenanthrenes and terpenoids. Clinical studies confirmed diuretic, anti-rheumatic, anti-inflammatory, anti-carcinogenic, antimicrobial, anticonvulsive, relaxation, neuro-protective and antivirus activities (Pérez Gutiérrez 2010). The observed activity may contribute to the reasons why the leaves are used for infectious and inflammatory conditions in ethnomedicine. Thus, the research showed that ethanolic extracts of C. speciosa possess antibacterial potency against S. aureus and E. coli and may be used as a natural antiseptics and antimicrobial agents in medicine and veterinary practice. Considering the medicinal importance of the tested microorganisms, the findings of this research are considered to be very promising in the perspective of new drug development from plant sources.