UDK 615.012.1: 582.949.2: 581.3

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THE ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OBTAINED FROM LEAVES OF *COELOGYNE BRACHYPTERA* RCHB. F. (*ORCHIDACEAE*)

В останні роки було здійснено скринінг багатьох видів лікарських рослин щодо протимікробної активності. В результаті проведених досліджень були виявлені протимікробні властивості багатьох видів орхідних, у т.ч. і *Coelogyne spp.* Висвітлено дослідження щодо вивчення антимікробної активності етанольного екстракту, отриманого з листя *Coelogyne brachyptera,* щодо специфічних грампозитивних (*Staphylococcus aureus* ATCC 25923 і локально виділений метицилін-резистентний штам *S. aureus*) і грамнегативних бактерій (*Pseudomonas aeruginosa* ATCC 27853, локально виділений штам *Pseudomonas aeruginosa*, який продукує метало-бета-лактамази (MβL), *Escherichia coli* ATCC 25922, локально виділений штам *Salmonella enteriditis*). Отримані результати свідчать, що етанольний екстракт листя *C. brachyptera* дуже активний щодо грампозитивних бактеріальних штамів (20 мм – діаметр інгібування зони росту для *S. aureus* і 26,5 мм – для метицилін-резистентного *S. aureus*) і помірно активний щодо грамнегативних мікроорганізмів (18,2 мм – для *E. coli*, 16,5 мм – для *P. aeruginosa* і 18,3 мм – для (MβL) *P. aeruginosa*, 14,8 мм – для *S. enteriditis*). Отже, наше дослідження підтверджує високу антимікробну активність етанольного екстракту, отриманого з листя *C. brachyptera*. Грампозитивні штами (*S. aureus* і метицилін-резистентний *S. aureus*) виявилися більш сприйнятливими, порівняно з грамнегативними штамами, до впливу етанольних екстрактів листя *C. brachyptera*. Крім того, екстракт листя *C. brachyptera* справляв сильний інгібуючий вплив на *S. aureus* і метицилін-резистентний *S. aureus*. Таким чином, подальші зусилля мають бути спрямовані на виявлення активних речовин, що містяться в рослинних екстрактах *C. brachyptera*, і їх фармакологічне дослідження.

Ключові слова: *Coelogyne brachyptera*, листя, екстракт, антибактеріальна активність, диско-дифузійний метод.

Introduction. Orchidaceae are one of the largest and more diverse family of flowering plants with approximately 25,000 species in 736 genera currently recognized [7]. Orchids widely distributed in various types of habitats as epiphytes, lithophytes or terrestrials, have been used all over the world in traditional healing and treatment system of a number of diseases [9, 12]. Until recently many orchids species were screened and plants with high bioactive compounds were identified, whereby it was found that medicinal orchids mainly are encompassed by the next genera: Calanthe, Coelogyne, Cymbidium, Cypipedium, Dendrobium, Ephemerantha, Eria, Galeola, Gastrodia, Gymnadenia, Habenaria, Ludisia, Luisia, Nevilia, and Thunia [16]. Unfortunately, many of these orchids have become increasingly rare due to over-collection as ornamental and medicinal plants.

Up to now a wide range of chemical compounds have been isolated from various orchids including alkaloids, bibenzyl derivatives, flavonoids, phenanthrenes and terpenoids [15]. There are some evidences that extracts and metabolites of these plants, particularly those from flowers, steam and leaves, possess a wide range of useful pharmacological activities, i.e. diuretic, antirheumatic, anti-inflammatory, anticarcinogenic, hypoglycemic activities, antimicrobial, anticonvulsive, relaxation, neuroprotective and antivirus to treat different diseases and ailments including tuberculosis, paralysis, stomach disorders, chest pain, arthritis, syphilis, jaundice, cholera, leucoderma, diarrhea, muscular pain, blood dysentery, hepatitis, dyspepsia, bone fractures, rheumatism, asthma, malaria, earache, sexually transmitted diseases, wounds etc. [13, 15].

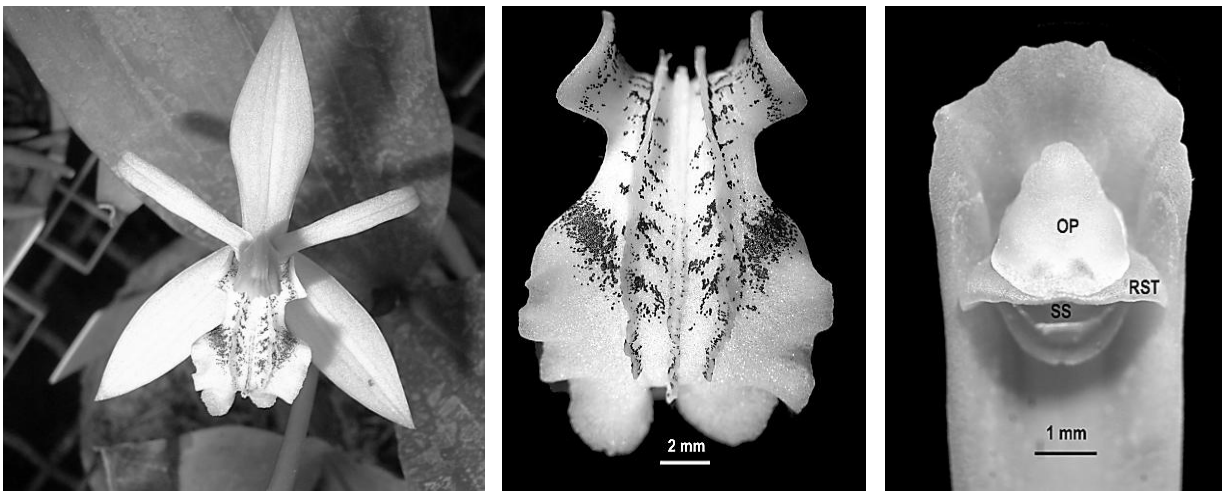
A number of medicinal orchids have been screened for antimicrobial activity against Gram-positive and Gram-negative bacteria in recent years [15]. As a result, many scientists have reported antimicrobial properties of various orchid species, including Coelogyne spp. [3-6, 8, 10, 14, 17, 18].

Therefore, we conducted a study aimed to investigate the antibacterial effects of ethanolic extract obtained from leaves of Coelogyne brachyptera Rchb.f. 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-beta-lactamases (MβL) Pseudomonas aeruginosa locally isolated, Escherichia coli ATCC 25922, Salmonella enteriditis locally isolated).

Materials and methods. Collection of Plant Materials. Coelogyne brachyptera is an epiphytic species, endemic for Indo-China, occurring in evergreen montane and highland forests on acidic substrates at elevation between 1000-2500 m a.s.l. [1] (Figs 1, 2).

A B

Fig. 1. ***Coelogyne brachyptera*** **plants, cultivated M.M. Gryshko National Botanical Garden glasshouses: a) growth habit; b) 5-7-flowered inflorescence, arising on a mature pseudobulb.**



A B C

Fig. 2. **Reproductive structures of *Coelogyne brachyptera* flower: close-up of the flower; b) labellum; c) gynostemium, pollinia are covered by operculum. Abbreviations : op – operculum; rst – rostellum; ss – stigmatic surface.**

***Preparation of Plant Extracts*.** The leaves of *C. brachyptera* plant cultivated under glasshouse conditions were sampled at M.M. Gryshko National Botanical Garden, 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 Environment, registration No. 6939/19/1-10 of 23 June 2004). The collected leaves were brought into the laboratory for antimicrobial studies. Freshly crushed leaves were washed, weighted, and homogenized in 96 %

ethanol in the (leaves to solvent) ratio of 1:10 (w/v) at room temperature. The extract was then filtered and investigated for antimicrobial activity.

Determination of antibacterial activity of plant extracts by the disk diffusion method. The extract obtained from *C. brachyptera* was screened for antimicrobial activity using disc diffusion methods [2]. Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 27853, metallo-beta-lactamases (MβL) *Pseudomonas aeruginosa* locally isolated, *Escherichia coli* ATCC 25922, *Salmonella enteriditis* locally isolated, as well as Gram-positive bacterium *Staphylococcus aureus* (ATCC 25923) and methicillin-resistant *S. aureus* locally isolated were used as test organisms. Antimicrobial activity of crude extract of the plant sample was evaluated by the paper disc diffusion method. The antimicrobial activities of the extract tested were evaluated at the end of the inoculated period by measuring the inhibition zone diameter around each paper disc in millimeters. Zone diameters were determined.

Statistical analysis. All statistical calculation was performed on separate data from each bacterial strains [19]. All statistical calculation was performed on separate data from each individual with STATISTICA 8.0 (StatSoft, Poland). 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 [11].

Results. The results of antimicrobial activity of ethanolic extract obtained from *C. brachyptera* leaves are presented in Figs 3 and 4.

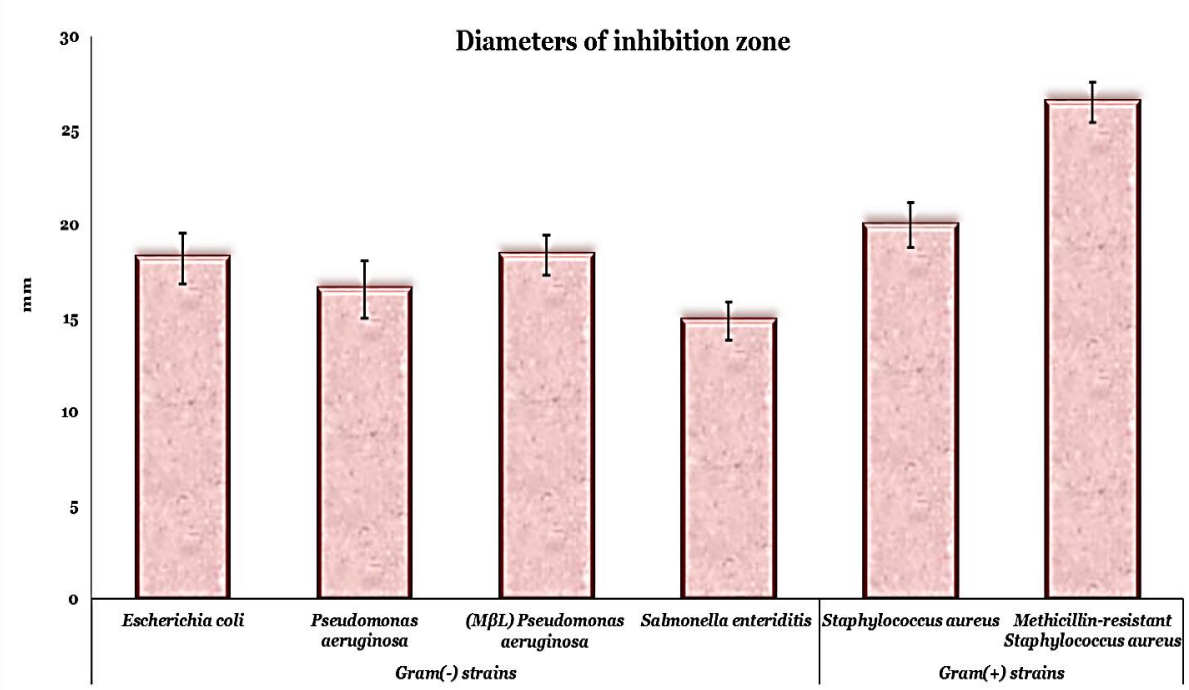
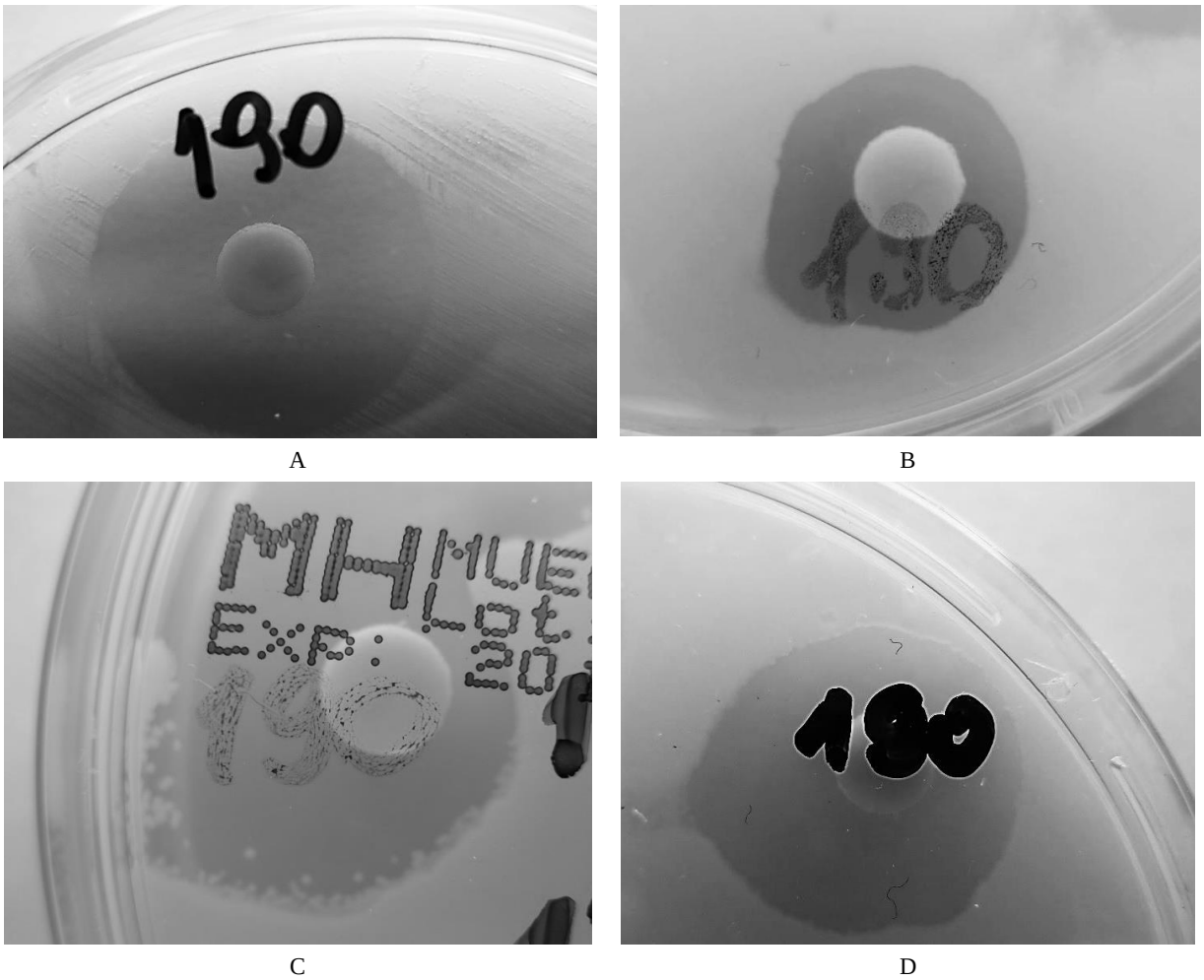


Fig.3. **Inhibition zone diameters produced by the leaf ethanolic extract of *C. brachyptera* against Gram-positive and Gram-negative bacteria, M±m, n=6.**

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 26.5 mm for meticillin-resistant *S. aureus*), and moderate activity against the 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. enteriditis*) (Figs 3 and 4).



A B C D

Fig. 4. **Antimicrobial activity of ethanolic extract obtained from the leaves of *C. brachyptera* against Gram-negative bacteria – *E. coli* ATCC 25922 (A), *Salmonella enteriditis* locally isolated (B), *Pseudomonas aeruginosa* ATCC 27853 (C), metallo-beta-lactamases (MβL)-positive *Pseudomonas aeruginosa* locally isolated (D).**

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 26.5 mm for meticillin-resistant *S. aureus*), and moderate activity against the 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. enteriditis*) (Figs 3 and 4).

Discussion. In the present study, it was found that ethanolic extract obtained from the leaves of *C. brachyptera* showed antibacterial activity against Gram-positive and Gram-negative bacteria. It should be noted that ethanolic extract of *C. brachyptera* has displayed strong inhibitory activity against Gram-positive bacterial strains (*S. aureus* and meticillin-resistant *S. aureus*).

Our observations are in well agreement with the reports by several workers who have investigated antimicrobial properties of various orchid species previously and validate the findings that the presence of alkaloid in orchid may be the reason for this efficacy. Alkaloids are nitrogenous organic heterocyclic molecules that have pharmacological effects on humans and other animals. They are secondary metabolites of plants and the well-known alkaloids include strychnine, morphine, codeine, nicotine, atropine, cocaine, quinine, methamphetamine, reserpine, caffeine and theophylline. As it was shown previously, 214 orchid species in 64 genera contain 0.1 % or more alkaloids. The phytochemical analysis shows the presence of alkaloids, carbohydrates, glycosides, saponins, terpenoids, steroids, flavonoids, phenolic compounds, protein, phytosterol, tannins and phlobatannins in ethanol extract [14].

In addition, our finding appears to be consistent with the results of a study by Sahaya and co-workers (2013), who demonstrated antibacterial activity of the leaf extracts of *Coelogyne nervosa*,

other species from *Coelogyne* genus [14]. Of the five microbes tested (*Pseudomonas aeruginosa, Enterococcus facealis, Bacillus subtilis, Salmonella enteria, Corynebacteria* spp.) all the microbes were susceptible to the leaf extract of *C. nervosa* which includes three Gram-positive bacteria (*E. facealis, B. subtilis, Corynebacteria* spp.) and two Gram-negative bacteria (*P. aeruginosa* and *S. enteria*). Furthermore, the ethanolic extract of *C. nervosa* showed the maximum zone of inhibition against the bacteria *P. aeruginosa* (15 mm) followed by *E. facealis* (14.3 mm) and *S. enteria* (12 mm), whereas *B. subtilis, Corynebacteria* spp. had 11 and 9 mm zone, respectively [14].

In our previous study [3-6, 8, 17, 18], we have reinforced the assumption that *Coelogyne* species could be potential resource of antibacterial or antifungal agents. 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*. On the basis of these results, it was concluded that the orchid extracts from various species of *Coelogyne* genus displayed varied antifungal potency. It is interesting to note, that among orchids selected, marked antifungal efficacy was observed in case of *C. speciosa* (mean diameter of inhibition zones was 19.7 mm), *C. ovalis* (18.2 mm), *C. brachyptera* (17.2 mm), and *C. assamica* (17.1 mm). On the other hand, extract of *C. cristata* displayed least inhibitory activity against test fungus (mean diameter of inhibition zones was 14.0 mm). Orchids were shown to exhibit antifungal activity against a variety of mold species. Likewise, 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 [18]. Moreover, ethanolic orchid leaf extracts resulted in considerable suppression of growth of *S. aureus*. Consequently, 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 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* strain [17].

We also determined antibacterial and antifungal potential of ethanolic extract of *C. cristata* leaves against Gram-positive (*S. aureus)* and Gram-negative (*P. aeruginosa* and *E. coli*) bacterial strains [8]. All microorganisms tested were susceptible to the leaf extract of *C. cristata*. According to our observation, extract of *C. cristata* displayed the least inhibitory activity against test fungus (mean diameter of inhibition zones was 14.0 mm). Our results showed that the ethanolic 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). Consequently, we conclude that ethanolic extract of *C. cristata* leaves has potent antimicrobial activity against *S. aureus* [8].

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* [3]. Our study has shown that ethanolic extracts from leaves and pseudobulbs of *C. tomentosa* exhibited strong activity against *S. aureus* (diameters of inhibition zone 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 our investigation provide evidence that the crude extracts obtained from leaves and pseudobulbs of *C. tomentosa* could be considered as promising natural antimicrobial products [4]. The ethanolic extract from leaves and pseudobulbs of *C. huettneriana* exhibited strong activity against *E. coli* (diameters of inhibition zone 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 of *C. huettneriana* revealed no antibacterial activity against *S. aureus* [5].

Conclusions. Thus, the present study revealed the good antimicrobial activities of ethanolic extract obtained from leaves 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. Beside, extract of *C. brachyptera* leaves has displayed strong inhibitory effect against *S. aureus* and meticillin-resistant *S. aureus*. Therefore, the findings of the present study support the suggestion that *Coelogyne* plants could be considered as a rich source of various bioactive compounds with antimicrobial potency. To conclude, further work is required to find out the active substances from *C. brachyptera* plant extracts and to carry out pharmacological studies.

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Антимикробная активность этанольного экстракта, полученного из листьев *Coelogyne brachyptera* Rchb. f. (*Orchidaceae*)

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В последние годы был осуществлен скрининг многих видов лекарственных растений в отношении противомикробной активности. В результате проведенных исследований были выявлены противомикробные свойства многих видов орхидных, в т.ч. и *Coelogyne* spp. Освещено исследования по изучению антимикробной активности этанольного экстракта, полученного из листьев *Coelogyne brachyptera*, в отношении специфических грамположительных (*Staphylococcus aureus* ATCC 25923 и локально выделенный метициллин-резистентный штамм *S. aureus*) и грамотрицательных бактерий (*Pseudomonas aeruginosa* ATCC 27853, локально выделенный штамм *Pseudomonas aeruginosa*, продуцирующий металло-бета-лактамазы (MβL), *Escherichia coli* ATCC 25922, локально выделенный штамм *Salmonella enteriditis*). Полученные результаты свидетельствуют о том, что этанольный экстракт листьев *C. brachyptera* оказывает сильную активность в отношении грамположительных бактериальных штаммов (20 мм – диаметр ингибирования зоны роста для *S. aureus* и 26,5 мм – для метициллин-резистентного *S. aureus*), и умеренную активность в отношении грамотрицательных микроорганизмов (18,2 мм − для *E. coli*, 16,5 мм − для *P. aeruginosa* и 18,3 мм – для (MβL) *P. aeruginosa*, 14,8 мм − для *S. enteriditis*). Следовательно, настоящее исследование показывает высокую антимикробную активность этанольного экстракта, полученного из листьев *C. brachyptera*. Грамположительные штаммы (*S. aureus* и метициллин-резистентный *S. aureus*) оказались более восприимчивыми, по сравнению с грамотрицательными штаммами, к воздействию этанольных экстрактов листьев *C. brachyptera*. Кроме того, экстракт листьев *C. brachyptera* оказал сильное ингибирующее действие в отношении *S. aureus* и метициллин-резистентного *S. aureus*. Таким образом, дальнейшие усилия должны быть направлены на выявление активных веществ, содержащихся в растительных экстрактах *C. brachyptera*, и их фармакологическое исследование.

Ключевые слова: *Coelogyne brachyptera*, листья, экстракт, антибактериальная активность, дискодиф-фузионный метод.

The antimicrobial activity of ethanolic extract obtained from leaves of *Coelogyne brachyptera* Rchb. f. (*Orchidaceae*)

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A number of medicinal orchids have been screened for antimicrobial activity in recent years. As a result, many scientists have reported antimicrobial properties of various orchid species, including *Coelogyne* spp. The aim of the present study was to investigate the antibacterial effects of ethanolic extract obtained from *Coelogyne brachyptera* Rchb.f. 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-beta-lactamases (MβL)-positive *Pseudomonas aeruginosa* locally isolated, *Escherichia coli* ATCC 25922, *Salmonella enteriditis* locally isolated). 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 26.5 mm for meticillin-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. enteriditis*). The present study revealed the good antimicrobial activities of ethanolic extract obtained from leaves of *C. brachyptera*. A Gram-positive strains (*S. aureus* and meticillin-resistant *S. aureus*) were more susceptible to the ethanolic leaf extracts of *C. brachyptera* as compared to Gram-negative bacteria. Beside, extract of *C. brachyptera* leaves has displayed strong inhibitory effect against *S. aureus* and meticillin-resistant *S. aureus*. To conclude, further work is required to find out the active substances from *C. brachyptera* plant extracts and to carry out pharmacological studies.

Key words: *Coelogyne brachyptera*, leaves, extract, antimicrobial activity, paper disc diffusion method.

Надійшла 22.05.2017 р.