IDENTIFICATION OF THE ANTIBACTERIAL EFFICACY OF ETHANOLIC EXTRACTS FROM AGLAONEMA COMMUTATUM SCHOTT LEAVES AND ITS CULTIVARS AGAINST ESCHERICHIA COLI STRAIN

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This study aimed to evaluate the antibacterial activity of ethanolic extracts obtained from the leaves of Aglaonema commutatum Schott and its cultivars («Malay Beauty», «Silver Queen», and «Silver King») against Escherichia coli (Migula) Castellani and Chalmers (ATCC® 25922™) strain. The leaves of Aglaonema commutatum plants and its cultivars, cultivated under glasshouse conditions, were sampled at M. M. Gryshko National Botanic Garden (NBG), National Academy of Science of Ukraine (Kyiv, Ukraine). The leaves were brought into the laboratory for antimicrobial studies. Freshly sampled leaves 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. Escherichia coli (Migula) Castellani and Chalmers (ATCC® 25922™) strain was used in our study.

Antimicrobial activities of various ethanolic extracts obtained from leaves of Aglaonema commutatum plants and its cultivars («Malay Beauty», «Silver Queen», and «Silver King») against Escherichia coli (Migula) Castellani and Chalmers (ATCC® 25922™) strain was screened in the current study. The testing of the antibacterial activity of the plant extracts was carried out in vitro by the Kirby-Bauer disc diffusion technique. The leaf extracts from A. commutatum «Silver Queen» and A. commutatum 'Silver King' exhibited higher inhibitory activity than the extracts from A. commutatum and A. commutatum «Malay Beauty». Maximum in vitro inhibition was scored by A. commutatum «Silver Queen», followed by A. commutatum «Silver King», A. commutatum, and A. commutatum «Malay Beauty».

In particular, the leaf extracts from A. commutatum «Silver Queen» and A. commutatum 'Silver King' exhibited higher inhibitory activity than the extracts from A. commutatum and A. commutatum «Malay Beauty». Maximum in vitro inhibition was scored by A. commutatum «Silver Queen», followed by A. commutatum «Silver King», A. commutatum, and A. commutatum «Malay Beauty», which presented inhibition zones of (18.6±1.2) mm, (16.1±0.9) mm, (15.7±1.1) mm, and (13.5±1.0) mm, respectively. In the case of the positive controls, 96% ethanol possesses a mild anti-E. coli effect, which presented inhibition zones of (9.5±1.2) mm. The inhibition zone diameters were increased by 96% (p<0.05) for A. commutatum «Silver Queen», by 69% (p<0.05) for A. commutatum «Silver King», by 65% (p<0.05) for A. commutatum, and by 42% (p<0.05) for A. commutatum «Malay Beauty».

Thus, the use of these plants in traditional medicine and veterinary medicine
was experimentally confirmed as a potential source of raw materials for the development of medicines in the future, as well as for the development of innovative feed for farm animals.

Keywords: *Aglaonema commutatum*, antibacterial activity, *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain, Kirby-Bauer disc diffusion technique

The interest in the herbal medicines, phytochemical and biological prospecting and isolation of active compounds is growing incredibly in the few last decades. Medicinal plants are being practiced for different ailments in most of the developing countries [24]. Despite the rapid progress towards the field of medicinal chemistry, the resistance of microbes against therapeutic agents necessitates the search of novel therapeutic agents from medicinal plants [25]. Moreover, medicinal plant-based drugs can be a good alternative because of safety, biodegradability, and imposing fewer side effects [26].

The family *Araceae*, commonly known as aroids, encompasses 105 genera and more than 3,300 species that are mostly herbaceous either as terrestrial, aquatic, or epiphytic [13]. Aroids are extraordinarily diverse in appearance, with their attractive foliage being the most widely recognized feature. Some members of *Araceae* were used as ornamentals, indoor plants, food, fibers, medicinal, and biopesticide [13, 21]. It is believed that an important characteristic of aroids, which has not been completely appreciated, is that many are important medicinal plants [3].

The genus *Aglaonema* Schott (Araceae) is comprised of 21 species that inhabit humid and heavily shaded forests of many territories of Asia [3, 5, 6]. *Aglaonema*, one of the world’s most popular houseplants, has provided a rich source of variation for the breeding of different foliage forms. This genus contains many cultivars that are important tropical foliage plants due to their tolerance to drought and low light and low relative humidity levels encountered under interior conditions [4].

There are many pieces of evidence of antioxidative [17], antimicrobial [18], phototoxic [2], etc. properties of *Aglaonema* spp. In recent years *Aglaonema* plants have been widely used because of its anti-aging and longevity properties, natural anti-allergic, and anti-inflammatory activities [8, 12]. Moreover, a decoction of the roots is drunk to treat dropsy and fever [19]. Also, the genus *Aglaonema* can remove pollutants from the indoor air such as benzene, toluene, TCE, m-xylene, hexane, etc. Anti-hyperglycemic effects of N-containing sugars from *Aglaonema treubii* Engl. in diabetic mice were noted [15].

Studies have been undertaken on the constituents of methanol crude extracts, derived from the leaves, stems, and roots of *Aglaonema simplex* (Blume) Blume, an aquatic plant that has been widely distributed as ornamental plants [10]. The results showed that the extracts contained secondary metabolites belonging to the phenolics, alkaloids, terpenoids, steroids, and glycosides. Therefore, *A. simplex* is suggested as one of the potential sources of the plant-derived compounds for the treatment of atherosclerosis [10].

A literature survey of Roy and co-workers (2013) reveals that research works on antibacterial activity have been conducted on different plants of *Araceae*. Most of the plants under investigation have shown significant activity against different pathogenic bacteria. From the available data, taking into account the zones of inhibition, it could be concluded that the bacterial strains whose activities have been inhibited most by the secondary metabolites present in the crude extracts of the plants are *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Pseudomonas aeo-
A maximum zone of inhibition has been observed in the case of ethanol extract obtained from the tuber of *Typhonium trilobatum* having a 32 mm zone of inhibition against *Staphylococcus aureus* [23]. Nevertheless, very scarce information is available concerning the antimicrobial activity of the studied plant species.

Therefore, this study aimed to evaluate the antibacterial activity of ethanolic extracts obtained from the leaves of *Aglaonema commutatum* Schott and its cultivars ('Malay Beauty', 'Silver Queen', and 'Silver King') against *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain. Materials and Methodology. Collection of Plant Materials and Preparation of Plant Extracts. The leaves of *Aglaonema commutatum* plants and its cultivars ('Malay Beauty', 'Silver Queen', and 'Silver King'), cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanic Garden (NBG), National Academy of Science of Ukraine (Kyiv, Ukraine).

The leaves were brought into the laboratory for antimicrobial studies. Freshly sampled leaves 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. *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain was used in our current study.

Determination of the antibacterial activity of plant extracts by the disk diffusion method. The testing of the antibacterial activity of the plant extracts was carried out *in vitro* by the Kirby-Bauer disc diffusion technique [1]. The strain was inoculated onto Mueller-Hinton (MH) agar plates. Sterile filter paper discs impregnated with extracts 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 antibacterial activity of ethanolic extracts screened. A negative control disc impregnated with sterile ethanol was used in each experiment. At the end of the period, the diameters of inhibition zones formed were measured in millimeters using the vernier. For each extract, eight replicates were assayed. The plates were observed and photographs were taken. The susceptibility of the test organisms to the plant extracts was indicated by a clear zone of inhibition around the holes containing the plant extracts and the diameter of the clear zone was taken as an indicator of susceptibility.

Statistical analysis. Zone diameters were determined and averaged. Statistical analysis of the data obtained was performed by employing the mean ± standard error of the mean (S.E.M.). All variables were randomized according to the phytochemical activity of extracts tested. All statistical calculation was performed on separate data from each strain. The data were analyzed using a one-way analysis of variance (ANOVA) using Statistica v. 8.0 software (StatSoft, Poland) [27]. The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (S) ≥ 15 mm, Intermediate (I) = 10–15 mm, and Resistant (R) ≤ 10 mm [16].

Results and discussion. The ability of the selected ethanolic plant extracts obtained from leaves of *A. commutatum* plants and its cultivars to inhibit *E. coli* growth was determined in this study. Data on antimicrobial activities of various ethanolic extracts obtained from leaves of *Aglaonema commutatum* plants and its cultivars ('Malay Beauty', 'Silver Queen', and 'Silver King') against *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain measured as an inhibition zone diameter are presented in Fig. 1.

Detailed data regarding the zones of inhibition by various ethanolic extracts obtained from leaves of *A. commutatum* plants and its cultivars ('Malay Beauty', 'Silver Queen', and 'Silver King') were recorded and presented in Fig. 2.
Fig. 1. The mean inhibition zone diameters around *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) growth induced by various ethanolic extracts obtained from leaves of *Aglaonema commutatum* plants and its cultivars ('Malay Beauty', 'Silver Queen', and 'Silver King') (M ± m, n = 8).

* – statistically significant changes (p<0.05) in comparison with the control sample values (96% ethanol).

Fig. 2. Inhibition zones induced by various ethanolic extracts obtained from leaves of *A. commutatum* (207) and its cultivars ['Malay Beauty' (210), 'Silver Queen' (211), and 'Silver King' (212)] against *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) growth

*E. coli* is classified as a rod-shaped, Gram-negative bacterium in the family *Enterobacteriaceae*. The bacterium mainly inhabits the lower intestinal tract of warm-blooded animals, including humans, and is often discharged into the environment through feces or wastewater effluent [11].
It is believed, that *E. coli* is inherently susceptible to almost all clinically relevant antimicrobial agents, but this bacterial species has a great capacity to accumulate resistance genes, mostly through horizontal gene transfer [20].

The results of our study revealed that three extracts exert high antibacterial activity against strain targeted. In particular, the leaf extracts from *A. commutatum* 'Silver Queen' and *A. commutatum* 'Silver King' exhibited higher inhibitory activity than the extracts from *A. commutatum* and *A. commutatum* 'Malay Beauty'. Maximum *in vitro* inhibition was scored by *A. commutatum* 'Silver Queen', followed by *A. commutatum* 'Silver King', *A. commutatum*, and *A. commutatum* 'Malay Beauty', which presented inhibition zones of (18.6 ± 1.2) mm, (16.1 ± 0.9) mm, (15.7 ± 1.1) mm, and (13.5 ± 1.0) mm, respectively. In the case of the positive controls, 96% ethanol possesses a mild anti-*E. coli* effect, which presented inhibition zones of (9.5 ± 1.2) mm. The inhibition zone diameters were increased by 96% (p<0.05) for *A. commutatum* 'Silver Queen', by 69% (p<0.05) for *A. commutatum* 'Silver King', by 65% (p<0.05) for *A. commutatum*, and by 42% (p<0.05) for *A. commutatum* 'Malay Beauty' (Fig. 1).

Very little information is available concerning the antibacterial activity of the studied plants. For instance, Roy and co-workers (2011) have screened phytochemical substances and have assayed cytotoxicity and antibacterial activities of ethanolic extracts of leaves of two medicinal plants, *Aglaonema hookerianum* Schott (Araceae) and *Lannea coromandelica* (Houtt.) Merr. (syn. *Lannea grandis* Engl.) (Anacardiaceae) available in Bangladesh [22].

Other plants belonging to the *Araceae* family also exhibited antibacterial activity. For example, Iqbal and co-workers (2018) have assessed the antibacterial activities and phytochemical content of the methanol, n-hexane, ethyl acetate, n-butanol soluble fractions, and aqueous extracts of the tubers of *Arisaema jacquemontii* Blume against 6 bacterial strains [7]. The data suggested that different extracts showed varying degrees of growth inhibition against the tested microbes [7]. Islam and co-workers (2013) have elucidated potential antioxidant, antidiarrheal, cytotoxic, and antibacterial activities of the ethanol extract of *Alocasia indica* Schott tuber in different experimental models established *in vitro* and *in vivo* [9]. Mulla and co-workers (2011) have shown that the extracts of *A. indica* have significant antidiarrheal and *in vitro* antiprotozoal activities that support its use in traditional herbal medicine practice. The plant extracts exhibited significant *in vitro* antiprotozoal activity against both protozoa compared to standard amebicidal and giardidical drugs, metronidazole, and emetine [14].

*Aglaonema hookerianum* Schott (Araceae) and *Lannea grandis* Engl. (Anacardiaceae) extracts obtained from leaves were examined for their antibacterial activities against some Gram-positive bacteria such as *Bacillus subtilis*, *Bacillus megaterium*, and *Staphylococcus aureus*, also Gram-negative strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Salmonella paratyphi*, and *Vibrio cholerae*. Results indicated that both plant extracts (μg mL⁻¹) displayed antibacterial activity against all tested microorganisms. The ethanolic extracts of leaves of *A. hookerianum* showed significant antimicrobial activity (zone of inhibition: from 15.08±0.45 mm to 20.37±0.45 mm in diameter) against all tested bacterial strains and the highest zone of inhibition was observed against *S. paratyphi* (20.37±0.45 mm). Similarly, the ethanolic extracts of *L. grandis* leaves also showed significant activity against all tested bacteria with a zone of inhibition ranging from 13.93±0.09 mm to 18.25±0.54 mm. These results were also compared with the zones of inhibition produced by the commercially available standard antibiotic, Amoxicillin at a concentration of 10 μg per disc. Observed antibacterial properties of the ethanolic extract of *A. hookerianum* and *L. coromandelica*...
showed that both plants might be useful sources for the development of new potent antibacterial agents [22].

*Aglaonema treubii* is a valuable source for glycosidase inhibitors that are antidiabetic, antimetastatic, antiviral, and immunomodulatory agents [3].

In our previous study [18], we focused on investigating the *in vitro* antibacterial activity of ethanolic extracts obtained from *A. commutatum* and its cultivars, cultivated under glasshouse conditions at M.M. Gryshko National Botanic Garden (NBG) against *Citrobacter freundii* strain locally isolated from human materials. The extracts from *A. commutatum* and *A. commutatum* 'Silver Queen' exhibited higher inhibitory activity than the extracts from *A. commutatum* 'Melay Beauty' and *A. commutatum* 'Silver King'. The highest *in vitro* inhibition was scored by *A. commutatum*, followed by *A. commutatum* 'Silver Queen', *A. commutatum* 'Malay Beauty', and *A. commutatum* 'Silver King'. The ethanolic extracts obtained from the leaves of *Aglaonema commutatum* and its cultivars have the potential for use as natural antimicrobial agents. Further *in vivo* and *in vitro* antimicrobial, phytochemical and toxicological studies are required to evaluate the chemotherapeutic effect of the plant.

**Conclusions.** Our research exhibited antibacterial properties of all tested extracts, but their activity was quite diverse. The leaf extracts from *A. commutatum* 'Silver Queen' and *A. commutatum* 'Silver King' exhibited higher inhibitory activity than the extracts from *A. commutatum* and *A. commutatum* 'Melay Beauty'. Maximum *in vitro* inhibition was scored by *A. commutatum* 'Silver Queen', followed by *A. commutatum* 'Silver King', *A. commutatum*, and *A. commutatum* 'Malay Beauty'. Thus, as a result of the research, the use of these plants in traditional medicine and veterinary medicine was experimentally confirmed as a potential source of raw materials for the development of medicines in the future, as well as for the development of innovative feed for farm animals. In considering the outcome of this study, further *in vivo* and *in vitro* antimicrobial, phytochemical and toxicological studies are required to evaluate the chemotherapeutic effect of the plant.

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гібування in vitro було відмічене A. commutatum «Silver Queen», за яким слідували A. commutatum «Silver King», A. commutatum i A. commutatum «Malay Beauty», в якому представлені зони інгібування (18,6 ± 1,2) мм, (16,1 ± 0,9) мм, (15,7 ± 1,1) мм і (13,5 ± 1,0) мм відповідно. У разі позитивного контролю 96% етанол володіє м’яким анти-E. coli ефектом, який представлений зонами інгібування (9,5 ± 1,2) мм. Діаметри зон інгібування були збільшені на 96% (p<0,05) для A. commutatum «Silver Queen», на 69% (p<0,05) для A. commutatum «Silver King», на 65% (p<0,05) для A. commutatum і на 42% (p<0,05) для A. commutatum «Malay Beauty».

Таким чином, у результаті виконаного дослідження було експериментально підтверджено використання цих рослин у традиційній медицині і ветеринарії, як потенційного джерела сировини для розробки лікарських засобів у майбутньому, а також для розробки інноваційних кормів для сільськогосподарських тварин.

Ключові слова: Aglaonema commutatum, антимікробна активність, штам Escherichia coli (Migula) Castellani and Chalmers (ATCC® 25922™), дискодифузійний метод Кірби-Бауэра

ОПРЕДЕЛЕНИЕ АНТИБАКТЕРИАЛЬНОЙ ЭФФЕКТИВНОСТИ ЭТАНОЛЬНЫХ ЭКСТРАКТОВ ЛИСТЬЕВ AGLAONEMA COMMUTATUM SCHOTT И СОРТОВ ЭТОГО ВИДА ПО ОТНОШЕНИЮ К ШТАММУ ESCHERICHIA COLI

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Целью исследования стала оценка антибактериальной активности этанольных экстрактов, полученных из листьев Aglaonema commutatum Schott и его сортов («Malay Beauty», «Silver Queen», и «Silver King») против штаммов Escherichia coli (Migula) Castellani и Chalmers (ATCC® 25922™). Образцы листьев растений Aglaonema commutatum и их сортов культивируемых в тепличных условиях, были отобраны в Национальном ботаническом саду им. М. М. Гришка Национальной академии наук Украины (г. Киев, Украина). Листья были доставлены в лабораторию для антимикробных исследований. Свежесобранные листья промывали, взвешивали и гомогенизовали в 96% - ном этаноле (в пропорции 1: 19) при комнатной температуре. Затем экстракты фильтровали и исследовали на предмет их антимикробной активности. В опыте использовали штаммы Escherichia coli (Migula) Castellani и Chalmers (ATCC® 25922™). Тестирование антимикробной активности растительных экстрактов осуществляли в условиях in vitro с использованием диско-диффузионного метода Кирби-Бауэра. Экстракты листьев сортов A. commutatum «Silver Queen» и A. commutatum «Silver King» оказали более высокую ингибирующее активность, чем экстракты, полученные из листьев A. commutatum и A. commutatum 'Malay Beauty'. Максимальное ингибирование роста микроорганизма in vitro было вызвано экстрактами листьев A. commutatum «Silver Queen»; далее, снижение ингибирование было отмечено в следующей последовательности: A. commutatum «Silver King», A. commutatum и A. commutatum «Malay Beauty».

В частности, экстракты листьев от A. commutatum «Silver Queen» и A. commutatum «Silver King», выставленные выше ингибирующей активностью, чем выдержки из A. commutatum и A. commutatum «Malay Beauty». Максимальное ингибирование in vitro было отмечено A. commutatum «Silver Queen», за которым
следовали A. commutatum «Silver King», A. commutatum и A. коммутатум «Malay Beauty», в котором представлены зоны ингибитирования (18,6 ± 1,2) мм, (16,1 ± 0,9) мм, (15,7 ± 1,1) мм и (13,5 ± 1,0) мм соответственно. В случае положительного контроля 96% этанол обладает мягким анти - E. coli эффектом, который представлен зонами ингибитирования (9,5 ± 1,2) мм. Диаметры зон ингибитирования были увеличены на 96% (p<0,05) для A. commutatum «Silver Queen», на 69% (p<0,05) для A. commutatum «Silver King», на 65% (p<0,05) для A. commutatum и на 42% (p<0,05) для A. commutatum «Malay Beauty».

Таким образом, в результате выполненного исследования было экспериментально подтверждено применение этих растений в традиционной медицине и ветеринарии, как потенциального источника сырья для разработки лекарственных средств в будущем, а также для разработки инновационных кормов для сельскохозяйственных животных.

Ключевые слова: Aglaonema commutatum, антимикробная активность, штамм Escherichia coli (Migula) Castellani and Chalmers (ATCC® 25922™), диско-диффузионный метод Карби-Бауэра

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IN VITRO SCREENING FOR ANTIMICROBIAL POTENTIAL OF ETHANOLIC LEAF EXTRACTS OF SOME BEGONIA SPECIES AGAINST METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) STRAIN

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The antimicrobial activities of ethanolic extracts obtained from the leaves of Begonia solimutata L.B. Sm. & Wassh., Begonia goegoensis N.E.Br., Begonia foliosa Kunth, Begonia × erythrophylla Hérincq, Begonia thiemei C.DC., Begonia peltata Otto & Dietr., Begonia heracleifolia Cham. & Schldtl., Begonia dregei Otto & Dietr., and Begonia mexicana G. Karst. ex Fotsch were assessed against methicillin-resistant Staphylococcus aureus (MRSA) strain. The leaves were brought into the laboratory for antimicrobial studies. Freshly crushed leaves 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. The testing of the antibacterial activity of the plant extracts was carried out in vitro by the Kirby-Bauer disc diffusion susceptibility test protocol. The most effective plants among species screened against S. aureus NCTC 12493 growth were B. peltata, B. thiemei, B. foliosa, Begonia × erythrophylla, and B. solimutata being highly active with the ethanolic extracts (diameters of inhibition zone ranged from 12.5 to 21 mm). The ethanolic extracts from nine Begonia plant species were evaluated for their antimicrobial activity against methicillin-resistant S. aureus (MRSA) strain. Extracts from all test Begonia plants caused a re-