Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Antibacterial activity of ethanolic extracts of *Aglaonema commutatum* Schott and its cultivars against *Enterococcus faecalis* strains

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Article Details:
Received:2022-07-10
Accepted:2022-08-08
Available online:2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0018

The application of plants coming from tropical and subtropical regions in the management of bacterial infections can be considered a positive occurrence in most traditional medicine practices. Consequently, plants having antimicrobial activity against various pathogens can be considered great assets. Moreover, increased problems associated with side effects and bacterial resistance to chemical drugs have prompted us to focus on the antibacterial potentials of some plants belonging to the *Aglaonema* genus. The purpose of the current study was to examine the antibacterial activity of ethanolic extracts derived from *Aglaonema commutatum* Schott and its cultivars (Malay Beauty, Silver Queen, and Silver King) against two *Enterococcus faecalis* strains, i.e. *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ (resistant to vancomycin; sensitive to teicoplanin) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™. These plants were cultivated under glasshouse conditions at M.M. Gryshko National Botanical Garden, National Academy of Science of Ukraine. The testing of the antibacterial activity of the plant extracts was carried out *in vitro* by the Kirby-Bauer disc diffusion technique. Results of this study revealed that the extracts derived from leaves of *A. commutatum* and cv. Silver Queen exhibited higher inhibitory activity against the growth of *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ than the extracts from cv. Melay Beauty and Silver King. Maximum *in vitro* inhibition was scored by cultivar Silver Queen, followed by *A. commutatum*, cv. Malay Beauty and Silver King. On the other hand, extracts derived from leaves of *A. commutatum* cv. Melay Beauty and Silver Queen exhibited higher inhibitory activity against the growth of *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™ than the extracts derived from the cv. Silver King and *A. commutatum*. Maximum *in vitro* inhibition was scored by cv. Malay Beauty, followed by cv. Silver Queen and Silver King, and *A. commutatum*. Therefore, this plant can be used to treat various diseases caused by *E. faecalis* strains. There is a lot of potentials for this plant to treat infections caused by these bacteria. Therefore, these plants may be helpful in the management of infections caused by *E. faecalis*, especially in traditional medicine practices. However, further research is required to understand the mechanisms involved in antimicrobial activity.

Keywords: *Aglaonema commutatum*, leaf ethanolic extracts, antibacterial activity, inhibition zones, Kirby-Bauer disc diffusion technique

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Introduction

Aglaonema, commonly called Chinese evergreens, belongs to the family Araceae (Henny et al., 2008; Li et al., 2022). The *Aglaonema* genus comprises 21 species distributed in southeast Asia, northeast India, and southern China southward through Malaysia, New Guinea, and the Philippines (Nicolson, 1969; Govaerts and Frodin, 2002; Chen et al., 2003). *Aglaonema* contains many cultivars that are important tropical foliage plants. Plants belonging to this genus readily adapt to low light and low relative humidity levels encountered under interior conditions (Henny et al., 2008; Henny and Chen, 2010). These plants have been widely cultivated by hybridization and tissue-cultured mutation selection for ornamental and medical purposes (Chen et al., 2004; Henny and Chen, 2010; Li et al., 2022).

Aglaonema plants have been widely used in recent years because of their anti-aging and longevity properties, and natural anti-allergic and anti-inflammatory properties (Kiatsongchai, 2015; Islam et al., 2019). Moreover, a decoction of the roots is drunk to treat dropsy and fever (Perry, 1980). Anti-hyperglycemic effects of N-containing sugars from *Aglaonema treubii* Engl. in diabetic mice were noted (Nojima et al., 1998). It was shown that the genus contains polyhydroxy alkaloids that exhibit glycosidase inhibitor activity (Ismail et al., 2017).

A literature survey by Roy et al. (2013) reveals that research works on antibacterial activity have been conducted on different plants of Araceae and most of the plants under investigation have shown significant activity against different pathogenic bacteria. The available data, regarding the zone of inhibitions, indicate 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 aeruginosa*. 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* (Roy et al., 2013).

In our previous study, we also investigated the antibacterial properties of ethanolic extracts derived from *Aglaonema commutatum* and its cultivars against various strains (Opryshko et al., 2019, 2020a,b). In the current study, we used two *Enterococcus faecalis* strains, i.e. *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ (resistant to vancomycin; sensitive to teicoplanin) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™. *Enterococcus faecalis* is a Gram-positive pathogen that colonizes human intestinal surfaces, forming biofilms (Oh et al., 2021). It also is a common commensal organism and a prolific nosocomial pathogen that causes biofilm-associated infections and demonstrates a high resistance to many antibiotics (Willett et al., 2021). *E. faecalis* is one of the most frequently isolated bacterial species in wounds yet little is known about its pathogenic mechanisms in this setting (Chong et al., 2017). Especially, antibiotics are less effective in eradicating biofilms and better alternatives are needed (Oh et al., 2021).

In line with the growing interest in the antibacterial potential of different tropical and subtropical plants, we examined the antibacterial properties of ethanolic extracts derived from leaves of *Aglaonema commutatum* and its cultivars against two *Enterococcus faecalis* strains.

Material and methodology

Collection of plant materials and preparation of plant extracts

The leaves of *Aglaonema commutatum* Schott and its cultivars (Malay Beauty, Silver Queen, Silver King), cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanic Garden (NBG), National Academy of Sciences of Ukraine (Kyiv) in 2020. The leaves were brought into the laboratory for antimicrobial studies. Freshly sampled leaves were washed, weighed, and homogenized in 96% ethanol (in the ratio of 1 : 19, w/w) at room temperature. The extracts were then filtered and stored in glass bottles with dark walls at 4 °C. Extracts were investigated for their antimicrobial activity in 2020–2021.

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 (Bauer et al., 1966). The antibacterial activity of these extracts was studied at the Department of Biology, Institute of Biology and Earth Sciences, Pomeranian University in Słupsk (Poland). In the current study, two *Enterococcus faecalis* strains were used, i.e. *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ (resistant to vancomycin; sensitive to teicoplanin) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™. The strains were 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 derived from the leaves of *A. commutatum* and its cultivars (Malay Beauty, Silver Queen, Silver King). A control disc impregnated with sterile 96% ethanol was used in each experiment. At the end of the period, the inhibition zones formed were measured in millimeters using the vernier. For each extract, eight replicates were assayed (n = 8). 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 paper discs containing the plant extracts and the diameter of the clear zone was taken as an indicator of susceptibility. Zone diameters were determined and averaged. The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (S) ≥15 mm, Intermediate (I) = 10–15 mm, and Resistant (R) ≤10 mm (Okoth et al., 2013).

Statistical analysis

Zone diameters were determined and averaged. Statistical analysis of the data obtained was performed by employing the mean ± standard error of the mean (S.E.M.). All variables were randomized according to the phytochemical activity of the extracts tested according to Okoth et al. (2013). 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. 13.3 software (TIBCO Software Inc., Krakow, Poland) (Zar, 1999).

Results and discussion

The ability of the selected ethanolic plant extracts derived from leaves of *A. commutatum* and its cultivars to inhibit the growth of two *E. faecalis* strains, i.e. *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ (resistant to vancomycin; sensitive to teicoplanin) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™ was determined in this study. The results revealed that four extracts exerted antibacterial activity against these strains. Moreover, the extracts derived from leaves of *A. commutatum* and cv. Silver Queen exhibited higher inhibitory activity ($p < 0.05$) against the growth of *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ than the extracts from cv. Melay Beauty and Silver King. Maximum *in vitro* inhibition was scored by cv. Silver Queen, followed by *A. commutatum*, cv. Malay Beauty and Silver King, which presented inhibition zones of (14.52 ±1.07) mm, (14.34 ±0.98) mm, (13.71 ±1.10) mm, and (13.29 ±1.16) mm, respectively. In the case of the positive controls, 96% ethanol possesses a mild anti-*E. faecalis* effect, which presented inhibition zones of (9.15 ±0.99)

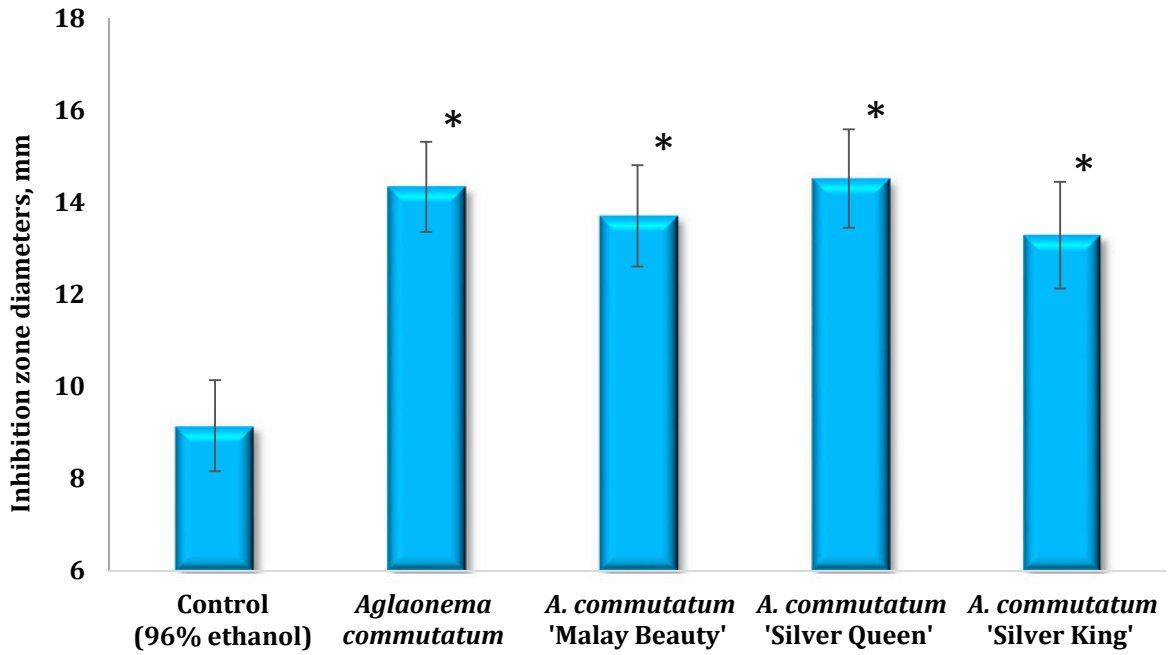

Figure 1 Antimicrobial activity of various extracts derived from leaves of *Aglaonema commutatum* Schott and its cultivars against *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ strain by disc diffusion method
*denote significant differences between the control and extract-treated groups ($p < 0.05$)

mm. The increase in inhibition zone diameters was recorded by 58.7% ($p < 0.05$) for the cv. Silver Queen, 56.7% ($p < 0.05$) for *A. commutatum*, 49.8% ($p < 0.05$) for the cv. Malay Beauty, and 45.3% ($p < 0.05$) for the cv. Silver King compared to the 96% ethanol samples (Figure 1).

Detailed photos regarding the zones of inhibition by the various plant extracts derived from leaves of *A. commutatum* and its cultivars against *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ strain were recorded and presented in Figure 2.

Assessment of antibacterial activity of extracts derived from leaves of *A. commutatum* and its cultivars against the growth of *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™ strain revealed that these extracts exerted antibacterial activity against this strain. Extracts derived from leaves of *A. commutatum* cv. Melay Beauty and Silver Queen exhibited higher inhibitory activity ($p < 0.05$) against the growth of *E. faecalis* than the extracts from the cv. Silver King and *A. commutatum*. Maximum *in vitro* inhibition was scored by cv. Malay Beauty, followed by cv. Silver Queen and Silver King, and *A. commutatum*, which presented inhibition zones of (15.74 ±1.02) mm, (14.55 ±0.97) mm, (13.64 ±0.90) mm, and (11.55 ±0.88) mm, respectively. In the case of the positive controls, 96% ethanol possesses a mild anti-*E. faecalis* effect, which presented inhibition zones of (8.92 ±0.91) mm. The increase in inhibition zone diameters was recorded by 76.5% ($p < 0.05$) for the cv. Malay Beauty, 63.1% ($p < 0.05$) for cultivar Silver Queen, 52.9% ($p < 0.05$) for the cv. Silver King, and 29.5% ($p > 0.05$) for the *A. commutatum* compared to the 96% ethanol samples (Figure 3).

Detailed photos regarding the zones of inhibition by the various plant extracts derived from leaves of *A. commutatum* and its cultivars against *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™ strain were recorded and presented in Figure 4.

In line with our previous studies according to the antibacterial potential of different tropical and subtropical plants, in the current study, we examined

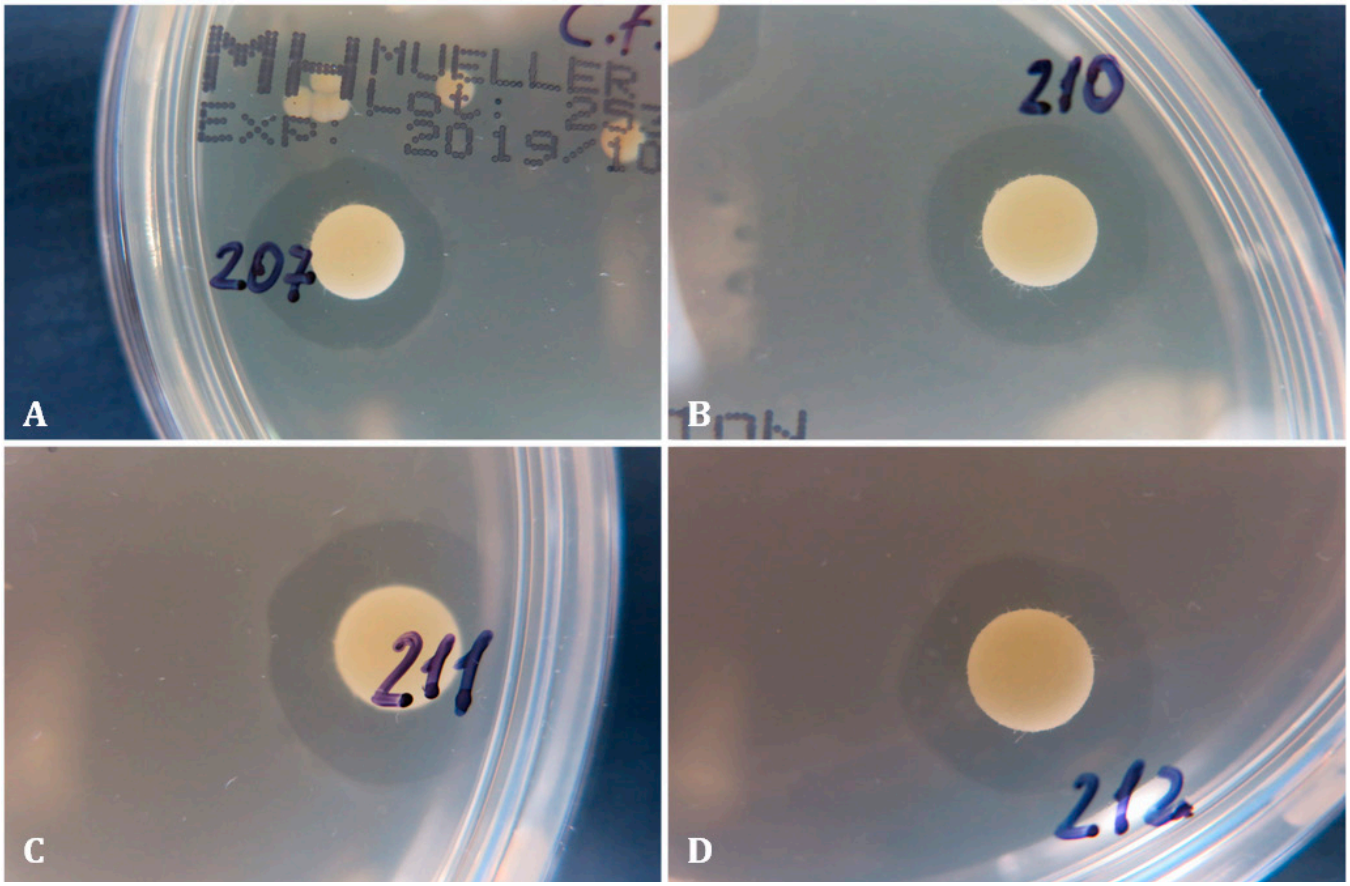

Figure 2 Inhibition growth zones induced by various ethanolic extracts derived from leaves of *Aglaonema commutatum* Schott (A) and cultivars Malay Beauty (B), Silver Queen (C), Silver King (D) against *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ strain

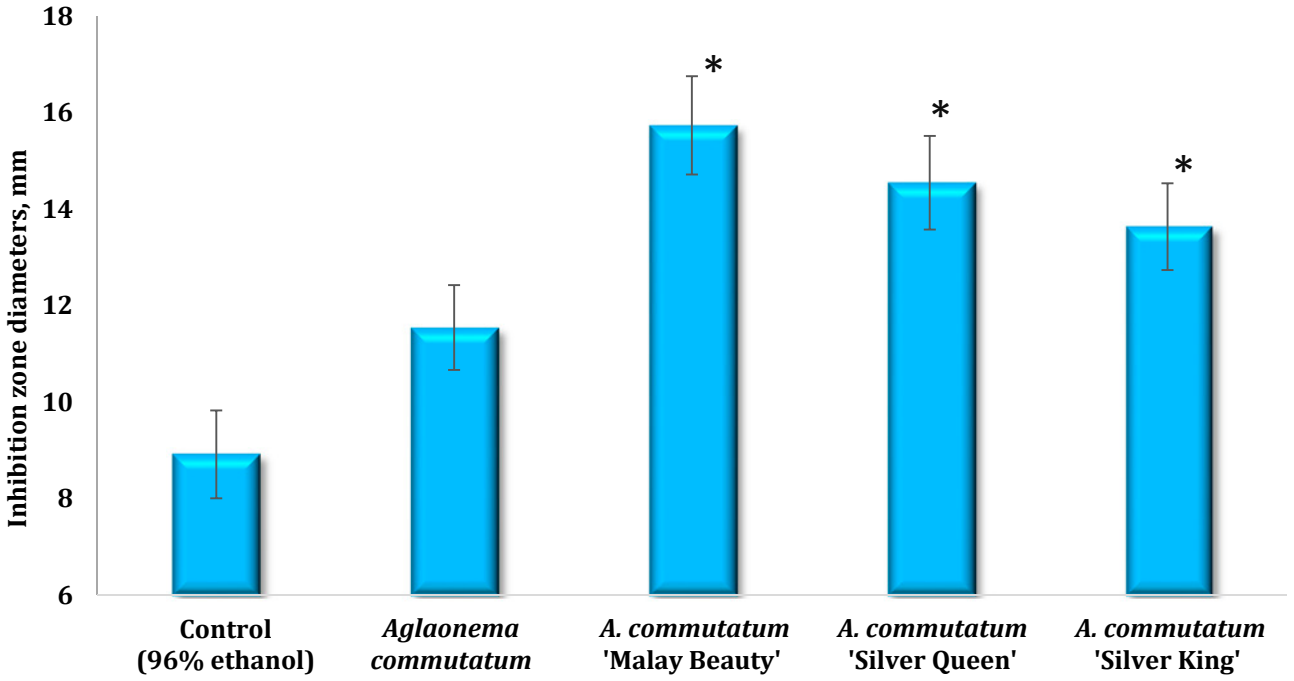

Figure 3 Antimicrobial activity of various extracts derived from leaves of *Aglaonema commutatum* Schott and its cultivars against *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™ strain by disc diffusion method
* denote significant differences between the control and extract-treated groups ($p < 0.05$).

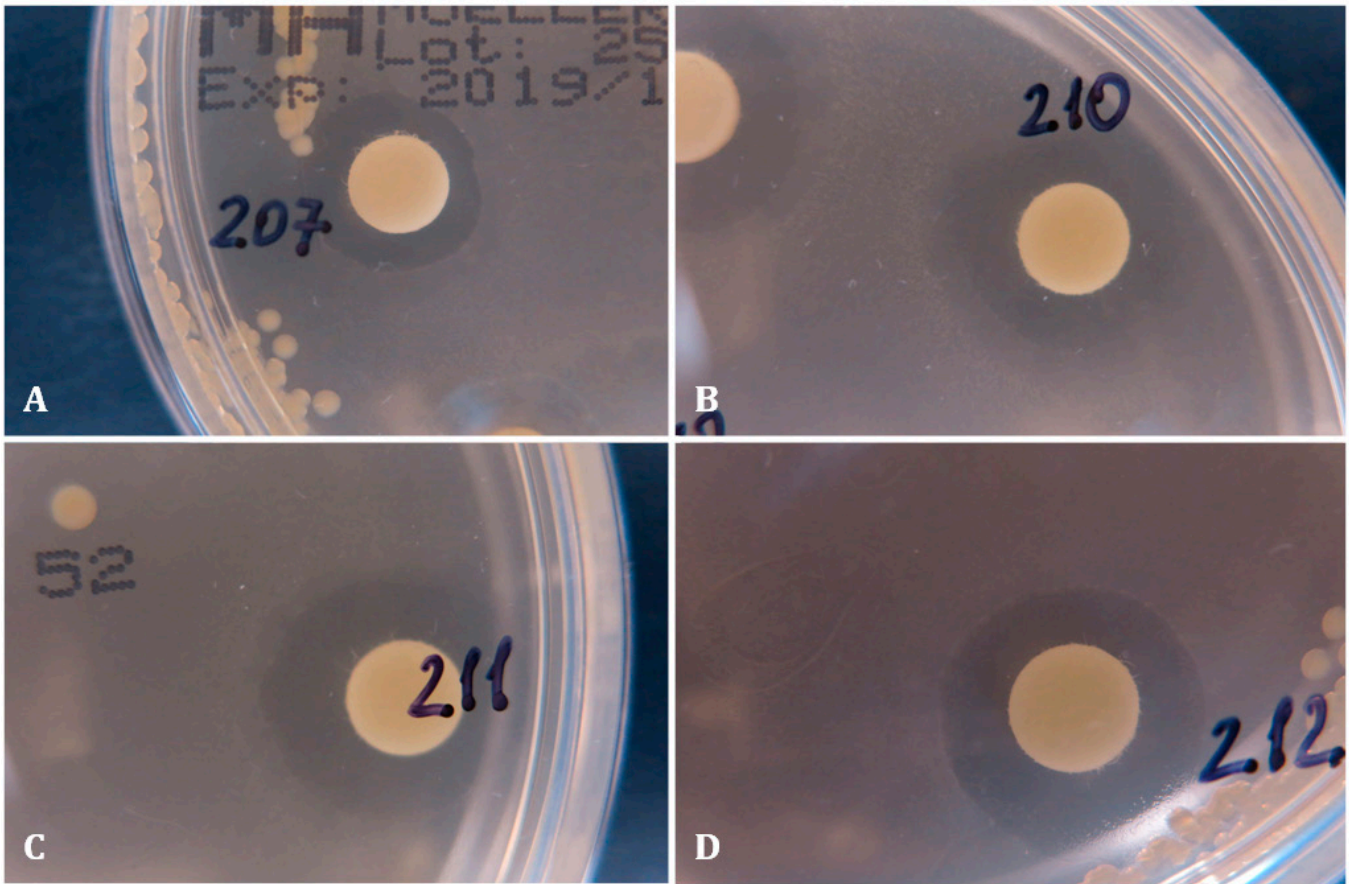

Figure 4 Inhibition growth zones induced by various ethanolic extracts derived from leaves of *Aglaonema commutatum* Schott (A) and cultivars Malay Beauty (B), Silver Queen (C), Silver King (D) against *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™ strain

the antibacterial properties of ethanolic extracts derived from leaves of *A. commutatum* and its cultivars against two *E. faecalis* strains, i.e. *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ (resistant to vancomycin; sensitive to teicoplanin) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™ using the disc diffusion method. The lowest antibacterial activity was presented by extract derived from leaves of *A. commutatum* against *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™ strain (Figure 3). The largest diameter of zone inhibition was observed for ethanolic extracts derived from *A. commutatum* cv. Silver Queen and Melay Beauty (Figure 1 and 3).

In our previous study (Opryshko et al., 2019), we focused on investigating the *in vitro* antibacterial activity of ethanolic extracts derived from leaves of *A. commutatum* and its cultivars (Malay Beauty, Silver Queen, and Silver King), cultivated under glasshouse conditions at M.M. Gryshko National Botanic Garden, against *Citrobacter freundii* strain locally isolated from human materials. The extracts derived from leaves of *A. commutatum* and cultivars Silver Queen exhibited higher inhibitory activity ($p < 0.05$) than the extracts from cv. Melay Beauty and cv. Silver King. The highest *in vitro* inhibition was scored by *A. commutatum*, followed by cv. Silver Queen, Malay Beauty, and Silver King (Opryshko et al., 2019).

Antimicrobial activities of various ethanolic extracts derived from leaves of *A. commutatum* plants and its cultivars (Malay Beauty, Silver Queen, and Silver King) against *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain were screened in our other study. Results obtained in this study revealed that the leaf extracts derived from *A. commutatum* cv. Silver Queen and Silver King exhibited higher inhibitory activity than the extracts from *A. commutatum* and *A. commutatum* cv. Melay Beauty. Maximum *in vitro* inhibition was scored by *A. commutatum* cv. Silver Queen, followed by *A. commutatum* cv. Silver King, *A. commutatum*, and *A. commutatum* cv. Malay Beauty (Opryshko et al., 2020a). Another our study aimed to evaluate the antibacterial activity of ethanolic extracts obtained from *A. commutatum* and its cultivars against *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™) strain (Opryshko et al., 2020b). The results obtained revealed that two extracts exerted antibacterial activity against *P. aeruginosa* strain. However, the extracts derived from leaves of *A. commutatum* and cv. Silver Queen exhibited higher inhibitory activity than the extracts of cv. Melay Beauty and Silver King. Maximum *in vitro* inhibition was scored by *A. commutatum* cv. Silver Queen, followed by *A. commutatum*, *A. commutatum* cv. Malay Beauty and Silver King, which presented inhibition zones of (11.4 ±1.0) mm, (10.2 ±1.8) mm, (9.5 ±0.9) mm, and (8.5 ±0.9) mm, respectively (Opryshko et al., 2020b).

Some information is available concerning the antimicrobial activity of the studied plant species. For example, Roy et al. (2011) have screened phytochemical substances to assay cytotoxicity and antibacterial activities of ethanolic extracts of leaves of two medicinal plants, *Aglaonema hookerianum* Schott (Family: Araceae) and *Lannea grandis* Engl. (Family: Anacardiaceae) available in Bangladesh. The brine shrimp lethality bioassay showed that the ethanolic extracts of *Aglaonema hookerianum* and *Lannea grandis* possessed cytotoxic activities with LC_{50} 5.25 ($\mu g.mL^{-1}$) and 5.75 ($\mu g.mL^{-1}$) and LC_{90} 10.47 ($\mu g.mL^{-1}$) and 9.55 ($\mu g.mL^{-1}$), respectively. Two 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*. The agar disc diffusion method was applied to observe the antibacterial efficacy of the extracts. Results indicated that both plant extracts ($\mu g.mL^{-1}$) displayed antibacterial activity against all of the tested microorganisms. The ethanolic extracts of leaves of *Aglaonema hookerianum* showed significant antimicrobial activity (zone of inhibition: 15.08 ±0.45 mm to 20.37 ±0.45 mm) against all tested bacterial strains and the highest zone of inhibition was observed against *S. paratyphi* (20.37 ±0.45 mm). The ethanolic extracts of *Lannea 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 *Aglaonema hookerianum* and *Lannea grandis* showed that both plants might be useful sources for the development of new potent antibacterial agents (Roy et al., 2011).

Some *Aglaonema* plants can also be recommended as a potent source of neuroprotective and a libido-boosting drug candidate for the management of neurological and sexual disorders. Pharmacological insights on the antidepressant, anxiolytic and aphrodisiac potentials of methanolic extract derived

from leaves of *Aglaonema hookerianum* Schott. (MEAH) in Swiss albino mice were done by Goni et al. (2021). Swiss albino mice (20–30 g) were orally administrated with MEAH at doses ranging from 100 to 400 mg.kg^{-1}, b.w. The elevated plus maze (EPM) and hole board test (HBT) were performed to determine the anxiolytic activity and the forced swimming test (FST) and tail suspension test (TST) were performed to determine the antidepressant activity of MEAH. Besides, the aphrodisiac activity of MEAH was conducted through mounting behaviour and orientation behaviour analysis. Diazepam (1 mg.kg^{-1}, b.w., i.p.) for EPM and HBT; fluoxetine HCl (20 mg.kg^{-1}, b.w., p.o.) for FST and TST, and sildenafil (5 mg.kg^{-1}, b.w., p.o.) for the mounting behaviour analysis and orientation behaviour analysis were used as reference drugs. Goni et al. (2021) revealed that the administration of the MEAH produced strong dose-dependent anxiolytic effects in both HBT and EPM tests. Likewise, the extract showed a significant reduction in the immobility time in both FST and TST compared to the control group. Besides, the MEAH was also found to possess marked aphrodisiac activity complying with several facets such as an increase in sexual performance at the highest dose (400 mg.kg^{-1}, p.o.) and the orientation toward female mice at all tested doses.

Aglaonema plants are one of the potential sources of phytochemicals for the treatment of atherosclerosis. The *Aglaonema* genus contains polyhydroxy alkaloids that exhibit glycosidase inhibitor activity. Ismail et al. (2017) have reported a phytochemical screening of *in vitro Aglaonema simplex* plantlets and the potential compounds as alternatives of SR-B1 ligands that play a role in reducing atherosclerosis. The phytochemical screening was conducted using thin-layer chromatography and attenuated total reflectance-Fourier transform infrared spectroscopy on methanol crude extracts of leaves, stems, and roots. SR-B1 ligand activities were tested on HepG2 cell line stably transfected with SR-B1 promoter. The results showed that the extracts contained secondary metabolites belonging to terpenoids, steroids, phenolics, alkaloids, and glycosides. Luciferase assay suggested that the stem and root extracts increased the expression of SR-B1 at 1.61- and 1.72-fold higher than the control, respectively (Ismail et al., 2017).

The wide variety of iminosugars present in *Aglaonema* sp. (Araceae), including homonojirimycin (HNJ), homomannojirimycin (HMJ), 2,5-dideoxy-2,5-imino-d-mannitol (DMDP), etc., makes these plants an interesting natural source of these bioactive (Asano et al., 1998, Asano et al., 2005; Rodríguez-Sánchez et al., 2016). HNJ exerted protection against influenza virus infection and produced effective immune responses *in vivo*, as revealed in Zhang and co-workers (2013a) study. HNJ was found to improve the survival rate, prolong the mean survival time, and reduce virus yields in lungs on days 4 and 6 post-infection (p.i.), after the agent had been orally administered to the mice from 2 days before infection to 6 days p.i. Administration of HNJ (1 mg.kg^{-1}) significantly increased interferon (IFN)-γ and interleukin (IL)-10 levels but decreased tumor necrosis factor (TNF)-α and IL-6 levels in serum and lungs of influenza-infected mice on days 2, 4 or 6 p.i. (Zhang et al., 2013a). HNJ showed strong antiviral activity against influenza A/PR/8/34 virus (H1N1) as measured by cytopathic effect reduction assay (Zhang et al., 2013b).

Conclusions

The present study has demonstrated that extracts derived from the leaves of *A. commutatum* and its cultivars exhibited antibacterial activity against two *E. faecalis* strains, i.e. *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ (resistant to vancomycin; sensitive to teicoplanin) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™. Ethanolic extracts derived from leaves of *A. commutatum* and the cv. Silver Queen proved to be the most effective against *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ strain. Leaf extracts derived from cultivars such as Melay Beauty, Silver Queen, and Silver King exhibited significant antibacterial activity against *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™. Therefore, this plant can be used to treat various diseases caused by *E. faecalis* strains. There is a lot of potential for this plant to treat infections caused by these bacteria, such as wound infections, bloodstream infections, and urinary tract infections. However, further research is required to understand the mechanisms involved in the antimicrobial activity of this plant for extensive clinical usage.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Funding

The authors would like to extend their sincere appreciation to The International Visegrad Fund for supporting our study.

Acknowledgements

This work was supported by Pomeranian University in Słupsk (Poland) in cooperation with M.M. Gryshko National Botanic Garden, National Academy of Science of Ukraine (Kyiv, Ukraine).

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