



EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACTS OBTAINED FROM *AGLAONEMA COMMUTATUM* SCHOTT AND ITS CULTIVARS AGAINST *CITROBACTER FREUNDII*

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Due to the indiscriminate use of antimicrobial drugs, the emergence of human pathogenic microorganisms resistant to antibiotics has been increased. This has caused many clinical problems in the treatment of infectious diseases. *Aglaonema* plants have been widely used in recent years because of its anti-aging and longevity properties, natural anti-allergic and anti-inflammatory properties. There is a need to evaluate extracts of this plant in order to provide scientific proof for its wide application in the traditional medicine system. In the present study, we focused on investigating the *in vitro* antibacterial activity of ethanolic extracts obtained from *Aglaonema commutatum* Schott and its cultivars (Malay Beauty, Silver Queen, and Silver King), cultivated under glasshouse conditions at M.M. Gryshko National Botanical Garden, National Academy of Science of Ukraine against *Citrobacter freundii* strain locally isolated from human materials. The testing of the antibacterial activity of the plant extracts was carried out *in vitro* by the Kirby-Bauer disc diffusion technique. The extracts from *A. commutatum* and cultivars Silver Queen exhibited higher inhibitory activity ($P < 0.05$) than the extracts from cv. Malay Beauty and cv. Silver King. The highest *in vitro* inhibition was scored by *A. commutatum*, followed by cultivars Silver Queen, Malay Beauty, and Silver King. The ethanolic extracts obtained from the leaves of *Aglaonema commutatum* and its cultivars (Malay Beauty, Silver Queen, and Silver King) has 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.

Keywords: *Aglaonema commutatum* Schott, leaf ethanolic extracts, antibacterial activity, inhibition zones, disc diffusion assay

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Introduction

Citrobacter species belong to a group of facultative, anaerobic, Gram-negative bacilli within the family Enterobacteriaceae found infrequently as normal inhabitants of the intestinal tract of humans (Plakkal et al., 2013; Liu et al., 2018). They are frequently found in water, soil, food, and the intestines of animals and humans. Previously recognized as environmental contaminants or colonizers with low virulence, they are now known to cause a wide spectrum of infections involving the urinary tract, liver, biliary tract, peritoneum, intestines, bone, respiratory tract, endocardium, wounds, soft tissue, meninges, and the bloodstream (Liu et al., 2018).

In the last decade, the demand for antimicrobial agents is increasing due to the continuous emergence of clinical bacterial strains resistant to one or several conventional antibiotics (Fassi Fehri et al., 2007). The limited effective life span of current antibiotics, the lack of compliance of patients, the unmonitored use in agriculture, and the slow rate in releasing new antimicrobial agents have led to an alarming increase in antimicrobial resistance (Othman et al., 2019). Plants promise a source of natural antimicrobial agents (Kusuma et al., 2014) because they have a great ability to produce secondary metabolites such as phenolics and polyphenols, alkaloids, terpenoids, and essential oils, lectins, and others (Othman et al., 2019). It was assumed that these metabolites can act as chemical mediators, intermediating between the plant and the environment and playing a crucial role in the protection of the plant (Taiz and Zeiger, 2010). Because of this chemical potential (a reaction against some organisms), these substances have been researched to find new molecules with antimicrobial, antimalarial, insecticidal properties. It has been reported that the antimicrobial activity of plants is related to the defense mechanism against microorganisms. Therefore, it is possible to control infectious agents using natural products responsible for the inhibitory effect on pathogenic microorganisms using various plant extracts (Fukuyama et al., 2012). Other applications for natural antioxidants may include bioactive nutraceuticals, bio-pharmaceuticals, and food additives (Kusuma et al., 2014).

Aglaonema (Araceae) is an important ornamental foliage plant genus, one of the most beautiful foliage plants, as are many members of this monocotyledonous family in which flowers are borne on a type of inflorescence called a spadix. The genus *Aglaonema* is comprised of 21 species that inhabit humid and heavily shaded forests of many territories of Asia (Chen et al., 2003; Govaerts and Frodin, 2002). *Aglaonema* contains many cultivars that are important tropical foliage plants due to their tolerance of drought and low light and low relative humidity levels encountered under interior conditions (Chen et al., 2002). It has a good combination of leaf color, such as green and red, green and white, pink and green, red, among others (Mariani et al., 2011). *Aglaonema* plants have been widely used in recent years because of its anti-aging and longevity properties, 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 the glycosidase inhibitor activity (Ismail and Ahmad, 2017).

The inhibitory activity of *Aglaonema modestum* Schott ex Engl. on nitric oxide synthase (iNOS) in lipopolysaccharide-activated macrophages was also investigated (Ryu et al., 2001).

The glycosidase and glycosyltransferase inhibitors have attracted considerable interest in their potential roles in curing various diseases such as cancer, diabetes and virus infection including AIDS (Winchester et al., 1992; Jacob, 1995; Asano et al., 2000). In recent years, there has been a study of genetic relationships in 9 species of *Aglaonema* by amplified fragment length polymorphism (AFLP) marker (Chen et al., 2004).

In addition, the genus *Aglaonema* has the capability to remove pollutants from the indoor air such as benzene, toluene, TCE, m-xylene, hexane, etc. (Kaur and Kumar, 2015). Song and co-workers (2007) have examined the reduction of indoor air contaminants by plants placed in an indoor space using *Aglaonema brevispathum*, *Pachira aquatica*, and *Ficus benjamiana*, which were verified as air-purifying plants by NASA. The concentration of Volatile Organic Compounds (Benzene, Toluene, Ethylbenzene, and Xylene) was monitored three hours after the plants were placed and three days after the plants were placed. The amount of reduction in the concentration of Toluene and Formaldehyde was monitored 3 hours and 3 days after the plants were placed in the space. The reduction in the concentration of Benzene, Toluene, Ethylbenzene, Xylene, and Formaldehyde was significantly greater when plants were present. When plants were placed near a window, the reduction of concentration was greater. The more plants were used, the more a reduction of indoor air contaminants occurred. The effect of reducing the concentration of air contaminants increased when the number of plants increased, and when the plants were placed in a sunny area. The concentration of Toluene was reduced by 45.6 µg/m³ when 10% of the model space was occupied by *Aglaonema brevispathum* (Song et al., 2007).

Studies have been conducted on the constituents of methanol crude extracts, derived from the leaves, stems, and roots of *Aglaonema simplex*, an aquatic plant that has been widely used as ornamental plants (Ismail and Ahmad, 2017). The results showed that the extracts contained secondary metabolites belonging to the terpenoids, steroids, phenolics, alkaloids, and glycosides. Thus, *A. simplex* is suggested as one of the potential sources of the phytochemicals for the treatment of atherosclerosis (Ismail and Ahmad, 2017).

Roy et al. (2011) have screened phytochemical substances and to assay cytotoxicity and antibacterial activities of ethanolic extracts of leaves of two medicinal plants, *Aglaonema hookerianum* Schott (Araceae) and *Lannea grandis* Engl. (Anacardiaceae) available in Bangladesh. The brine shrimp lethality bioassay showed that the ethanolic extracts of *Aglaonema hookerianum* and *Lannea grandis* possessed cytotoxic activities with LC₅₀ 5.25, 5.75 µg/mL and LC₉₀ 10.47, 9.55 µg/mL, 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 (µg/mL) 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 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 *Lansea grandis* leaves also showed significant activity against all tested bacteria with a zone of inhibition ranging from 13.93 ± 0.09 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 *Lansea grandis* showed that both plants might be useful sources for the development of new potent antibacterial agents (Roy et al., 2011).

Expanding the same research, the aim of this study was to evaluate the antibacterial activity of ethanolic extracts obtained from *Aglaonema commutatum* Schott and its cultivars, cultivated under glasshouse conditions at M.M. Gryshko National Botanic Garden, National Academy of Science of Ukraine against *Citrobacter freundii* strain locally isolated from human materials.

Materials and methodology

Collection of Plant Materials

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 Science of Ukraine.

Preparation of Plant Extracts

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.

Bacterial test strain and growth conditions

The non-repetitive clinical strain of *Citrobacter freundii* isolated from patients with uretic infection was collected from Koszalin Hospital during March–April, 2019. The purity, as well as the identity of isolate, was confirmed in the laboratory conditions by standard microbiological methods and were interpreted according to the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2014).

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

The cultivation medium was trypticase soy agar (Oxoid[®], UK), supplemented with 10% defibrinated sheep blood. Cultures were grown aerobically for 24 h at 37 °C. The cultures were later diluted with a sterile solution of 0.9% normal saline to approximate the density

of 0.5 McFarland standard. The McFarland standard was prepared by inoculating colonies of the bacterial test strain in sterile saline and adjusting the cell density to the specified concentration. *Citrobacter freundii* strain studied was susceptible to all antibiotics used.

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 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 obtained from the leaves of *A. commutatum* and its cultivars (Malay Beauty, Silver Queen, Silver King). A negative control disc impregnated with sterile 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. 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. Zone diameters were determined and averaged.

Statistical analysis

Zone diameters were determined and averaged. Statistical analysis of the data obtained was performed by employing the mean \pm standard error of the mean (S.E.M.). All variables were randomized according to the phytochemical activity of extracts tested. All statistical calculation was performed on separate data from each strain. The data were analyzed using a one-way analysis of variance (ANOVA) using Statistica v. 8.0 software (StatSoft, Poland) (Zar, 1999). 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).

Results and discussion

The ability of the selected ethanolic plant extracts obtained from leaves of *A. commutatum* and its cultivars to inhibit *C. freundii* growth was determined in this study. The results revealed that four extracts exert antibacterial activity against this microorganism. However, the extracts from *A. commutatum* and cv. Silver Queen exhibited higher inhibitory activity ($P < 0.05$) than the extracts from cultivars Malay Beauty and Silver King. Maximum *in vitro* inhibition was scored by *A. commutatum*, followed by cultivars Silver Queen, Malay Beauty, and Silver King, which presented inhibition zones of (14.5 \pm 0.9) mm, (13.5 \pm 0.6) mm, (11.2 \pm 0.7) mm, and (8.4 \pm 0.6) mm, respectively. In the case of the positive controls, 96% ethanol possesses a mild anti-*C. freundii* effect, which presented inhibition zones of (8.8 \pm 0.59) mm (Figure 1).

Detailed data regarding the zones of inhibition by the various plant extracts screened were recorded and presented in Figure 2.

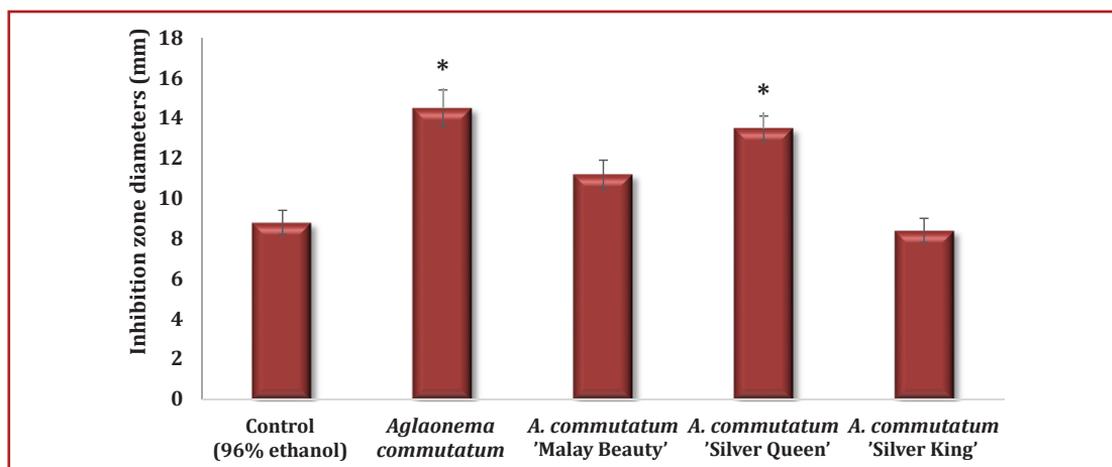


Figure 1 Antimicrobial activity of various extracts obtained from leaves of various plants belonged to the *Aglaonema* genus against *Citrobacter freundii* measured as inhibition zone diameter. * denote significant differences between the control and plant treatment groups ($p < 0.05$)

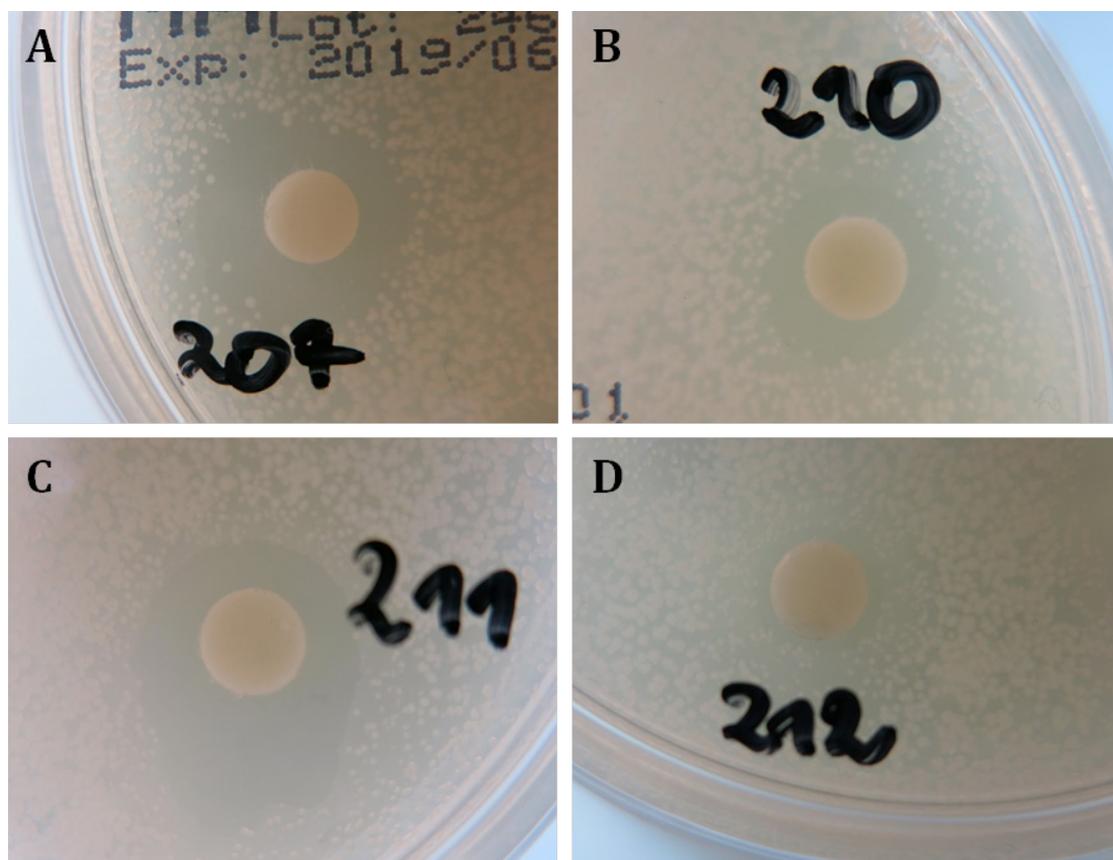


Figure 2 Inhibition zones induced by various ethanolic extracts obtained from leaves of *Aglaonema commutatum* (A) and cultivars Malay Beauty (B), Silver Queen (C), Silver King (D) against *Citrobacter freundii* growth

In line with the growing interest in the antibacterial potential of different tropical and subtropical plants, we examined the antibacterial properties of ethanolic extracts obtained from *Aglaonema commutatum* and its cultivars against *Citrobacter freundii*. The results from the screening study were obtained with the disc diffusion method. The only extract that did not exhibit any activity against the pathogen was the ethanolic extract of *A. commutatum* 'Silver King'. The largest zone of inhibition diameter was that of the ethanolic extracts of *A. commutatum* and cv. Silver Queen against *Citrobacter freundii*.

Very little information is available concerning the antimicrobial activity of the studied plant species. A literature survey of Roy et al. (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, 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).

Yunahara et al. (2014) have determined the antibacterial and antioxidant activity of different extracts of leaves Keladi tikus [*Typhonium flagelliforme* (Lodd) Blume]. Their results showed the ethyl acetate, *n*-butanol and water fraction had antibacterial activity against *Bacillus subtilis* and *Pseudomonas aeruginosa*, while the *n*-hexane fraction had no activity against bacteria tested (Yunahara et al., 2014).

These findings were supported by other data reported in the studies conducted by Baba and Malik (2015), in which it was evaluated the antioxidant and antimicrobial activity of a methanolic extract of the roots of *Arisaema jacquemontii*. The plant belongs to the family Araceae and is commonly known as cobra lily. It is used as a food, an antihelminthic and in the treatment of respiratory infections, dermatitis and as an antidote for snakebites. The extracts had antibacterial activity against both Gram-positive and Gram-negative bacteria, with MICs of 0.24–0.41 mg/mL. The extract had the greatest activity against *Salmonella enteritidis* and *Micrococcus luteus* and the least against *Streptococcus faecalis* and *Staphylococcus aureus*. The root extract also had significant antifungal activity, with values of 28.32–36.50%, the greatest activity being seen against *Fusarium oxysporum* and the least against *Aspergillus flavus* (Baba and Malik, 2015). Out of various human cancer cell lines employed in sulphorhodamine B (SRB) assay, the tuber lectin from *Arisaema jacquemontii* was found to have appreciable inhibitory effect on the *in vitro* proliferation of HCT-15 (Dukes' type C, colorectal adenocarcinoma), HOP-62 (Lung adenocarcinoma), SW-620 (Dukes' type C, colorectal adenocarcinoma), HT-29 (colorectal adenocarcinoma), IMR-32 (neuroblastoma), SKOV-3 (adenocarcinoma), Colo-205 (Dukes' type D, colorectal adenocarcinoma), PC-3 (grade IV, adenocarcinoma), HEP-2 (Carcinoma), and A-549 (Carcinoma) cancer cell lines by 82, 77, 73, 70, 41, 41, 37, 29, 21 and 21%, respectively (Kaur et al., 2006).

Luteolin isolated from *Eminium spiculatum* (Blume) Kuntze (Araceae) exhibited moderate antibacterial activity against *Escherichia coli* and resistant strains of *Staphylococcus aureus* in 1 µg per mL concentration (Afifi and Abu-Dahab, 2012). Luteolin, luteolin-7-O-glucoside, and vitexin inhibited ADP and collagen-induced platelet aggregation in a concentration-dependent manner. For the determination of the antiproliferative activity, breast cancer cell lines MCF-7 and T47D were used in the study of Afifi and Abu-Dahab (2012). Luteolin demonstrated the highest inhibitory activity with IC₅₀ values of 14.92 and 18.49 µmol per L for MCF-7 and T47D, respectively (Afifi and Abu-Dahab, 2012).

The antimicrobial, phytotoxic, haemagglutination and antioxidant potential of crude methanolic extract (Crd. MeOH Ext.) and four organic fractions of *Arisaema tortuosum* were investigated by Azam et al. (2016). All fractions have been screened for antimicrobial properties against eight bacterial pathogens and six fungal pathogens using agar well diffusion and tube dilution method, respectively. Furthermore, the organic fractions were also screened for its phytotoxicity against *Lemna minor*. Haemagglutination was performed against all human blood groups while free radical scavenging activity was performed to investigate the antioxidant potential of *A. tortuosum*. Results obtained for antibacterial activity exhibited a various degree of the zone of inhibition and significant activity was observed for *Pseudomonas aeruginosa* (27.16 ±0.60) followed by *Bacillus cereus* (18.55 ±0.69) for Crd. MeOH Ext. and chloroform (CHCl₃) fraction, respectively while some strains showed resistant at the same concentration. Similarly, non-significant antifungal activity was observed for the plant extracts. However, the highest activity among the strains was observed for *Alternaria alternata* (22 ±1.24%) and *Aspergillus niger* (20 ±1.00%) for ethyl acetate (EtOAc) fraction and Crd. MeOH Ext., respectively. Based on the results of Azam et al. (2016) study, it can be concluded that *A. tortuosum* has significant antimicrobial and moderate phytotoxic potential and therefore can lead to antibiotics and herbicide production.

Dzotam et al. (2016) have investigated *in vitro* antibacterial activity of the methanol extracts of three Cameroonian food plants, *Moringa oleifera* Lam. (Moringaceae), *Xanthosoma mafaffa* (L.) Schott (Araceae), *Passiflora edulis* Sims (Passifloraceae) against multidrug-resistant (MDR) bacteria. The study was extended to the ability of the studied extracts to potentiate the activity of some commonly used antibiotics against some of the tested MDR bacteria. The studied microorganisms included sensitive and resistant strains of *Escherichia coli* (ATCC8739, AG100, AG100A, AG102, AG100ATet, W3110), *Enterobacter aerogenes* (ATCC13048, EA289, EA27, EA298, CM64), *Klebsiella pneumoniae* (ATCC11296, KP55, KP63, K24), *Pseudomonas aeruginosa* (PA01, PA124), *Providencia stuartii* (ATCC29914, NEA16) obtained clinically or from the American Type Culture Collection (ATCC). The broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs) of the extracts, as well as those of antibiotics in association with the extracts. It appears that the extracts from *P. edulis* inhibited the growth of 17/19 (89.5%) bacteria with a concentration ranged from 128 to 1,024 µg/mL. The two other samples showed selective activities, their inhibitory activity being recorded on 13/19 (68.4%) and 11/19 (57.9%) tested bacteria for *M. oleifera* and *X. mafaffa* extracts respectively. The lowest MIC value (128 µg/mL) was obtained with *P. edulis* and *M. oleifera* extracts on *Escherichia*

coli AG100. Extracts showed antibacterial activities with minimum inhibitory concentrations ranging from 128–1,024 µg/mL on the majority of the 19 tested Gram-negative bacterial strains. Extract from the pericarp of *P. edulis* inhibited the growth of 89.5% of the 19 tested bacterial strains, the lowest minimum inhibitory concentration (MIC) value of 128 µg/mL being recorded against *Escherichia coli* AG100 strain (Dzotam et al., 2016).

Conclusions

The ethanolic extracts obtained from the leaves of *Aglaonema commutatum* Schott and its cultivars (*A. commutatum* 'Malay Beauty', *A. commutatum* 'Silver Queen', and *A. commutatum* 'Silver King') have the potential for use as natural antimicrobial agents. Considering our 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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