Central Mindanao University
Journal of Science
ISSN Print: 0116-7847 ISSN Online: 2704-3703

Antibacterial activity of the crude ethanolic extracts of *Etlingera elatior and Etlingera philippinensis*

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ABSTRACT

Due to the emergence of multidrug resistant bacteria, researchers are on search for a new source of antimicrobial agents such as plants. This study reports the antibacterial activities of the leaves and rhizomes of E. *philippinensis* and E. *elatior* against Gram-positive (*M. luteus*, *S. aureus* and *S. marcescens*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*, *B. cereus*). Disc diffusion method was employed in determining the antibacterial activity of the plant ethanolic extracts with concentrations of 3000 ppm and 10000 ppm. Highest antibacterial activity was exhibited by the leaves and rhizomes of the Philippine endemic *E. philippinensis* (10000 ppm) against *M. luteus* (12.00 ± 0.56) and *P. aeruginosa* (11.50 ± 0.45), respectively. Of all extracts, only the leaves *E. elatior* (10,000 ppm) showed antibacterial activity against *S. marcescens* which is 6.57 ± 0.49.

Keywords: Antibacterial, Etlingera elatior, Etlingera philippinensis.

INTRODUCTION

The rampant emergence of multidrug resistant bacteria is one of the many ever-growing concerns that continues to challenge the healthcare sector in both developing and developed countries. In fact, multidrug-resistant microorganisms cause almost 50% of the worldwide hospital-acquired infections, thus the World Health Organization warns of a 'post-antibiotic era' (O'Neill 2014; WHO 2014; Othman et al., 2019; Khameneh et al. 2019). This has necessitated a search for a new source of antimicrobial substances such as plants as they produce a variety of bioactive compounds of known therapeutic properties (Tomovo et al., 2015; Manandhar et al., 2019). However, the composition, quantity, quality of bioactive compounds and antimicrobial activities of plants belonging to different regions of the world can be affected by many factors such as climate, age and vegetation cycle stage, plant-microbiome interaction, soil composition (macro and micro minerals) and environmental stress including drought, acidity, salinity and heavy metal contaminants (Masotti et al., 2003; Angioni, 2006; Noumedem et al., 2013). Thus, studies of bioactive components and antibacterial activities of plants in different parts of the world is greatly recommended.

Etlingera elatior (Jack) R. M. Smith, also known as torch ginger, is widely distributed and is popular in Southeast Asia (Krajarng et al., 2017) wherein its inflorescences are traditionally used for culinary and medicinal purposes. Methanolic extracts of the leaves of *E. rubrostriata* and *E. elatior* revealed antibacterial activity against Gram-positive but not Gram-negative bacteria (Chan et al., 2007). Study on the phytochemical profile of *E. elatior* revealed that it contains a high amount of vitamin C, total phenolic, and flavonoid contents (Sungthong et al., 2018; Rachkeeree et al., 2018). Essential oils from *E. elatior* could be potentially used as a new source of natural antioxidant and antibacterial in the food and pharmaceutical industries (Abdelwahab et al., 2010). Furthermore, Juwita et al. (2018) reported that both leaves and rhizome of E. elatior exhibit antibacterial, antioxidant, antiproliferative and apoptotic activities.

Etlingera philippinensis is endemic in the Philippines. It was discovered in 1905 in the district of Davao, Mindanao, Philippines by E.B. Copeland. Its basionyms include: *Hornstedtia philippinensis* Ridl., *Amomum philippinense* (Ridl.) Merr., *Achasma philippinensis* (Ridl.) B.L. Burtt & R.M. Sm. (Newman et al., 2004).

Phytochemical analysis of *E. philippinensis* plant extract revealed the presence of alkaloids, flavonoids, saponins, tannins and steroids. High DPPH radical scavenging activities were also observed in the water extracts of *E. philippinensis* (Barbosa et al. 2016). Moreover, leaves of *E. philippinensis* were found to contain chlorogenic acid (Barbosa et al., 2017). Mabini and Barbosa (2018) reported that *E. philippinensis* showed high antioxidant activity and total phenolic content.

Over the years, multidrug resistant bacteria are one of the most important threats to public health (Van Duin and Peterson, 2016; Stefanović, 2018). These multidrug resistant bacteria includes the methicillin-resistant *Staphylococcus aureus* (Van Duin and Peterson, 2016), multidrug-resistant *Pseudomonas aeruginosa* (Stefanović, 2018), *Escherichia coli* (Dautle et al., 2004), causative of catheter-associated infections *Serratia marcescens* (Ray et al., 2017), one of the common food-borne pathogen

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Received 10th March 2020; Accepted 14th May 2020

Bacillus cereus (Shawish and Tarabees, 2017; Gao et al., 2018) and *Micrococcos luteus* (Rind and Khan, 2000). According to Izah (2018) these multidrug resistant bacteria are the most commonly studied organisms for antimicrobial susceptibility pattern using plant extract. Hence, this study aims to investigate the antibacterial property of *Etlingera elatior* and *Etlingera philippinensis* against these select multidrug resistant bacteria, that is, *S. aureus*, *P. aeruginosa*, *E. coli*, *S. marcescens*, *B. cereus*, and *M. luteus*.

METHODOLOGY

Plant Materials Collection

Bulk plant leaves and rhizomes of *E. elatior* and *E. philippinensis* were collected in Musuan, Maramag, Bukidnon, and Barangay Gutapol, Kibawe, Philippines, respectively in January 2019. Representative photographs of the samples are presented in Figure 1. Identification of the collected samples were done by Dr. Florfe M. Acma , a taxonomist of the Central Mindanao University (CMU) Herbarium, Center for Biodiversity Research and Extension in Mindanao CEBREM0, CMU, University Town, Musuan, Bukidnon, Philippines.

Plant Material Preparation

The fresh leaf samples were washed with tap water then sterilized using 10% Sodium hypochlorite. Final rinsing was done with distilled water three times (Ang et al, 2019). The rhizomes were peeled and grated to fine pieces. The washed leaf and grated rhizome samples were air-dried for four weeks under shade at ambient temperature, 29 ° C. The dried leaf samples and grated rhizomes were finely powdered using a heavy duty blender and stored inside airtight Ziploc bags prior extraction.

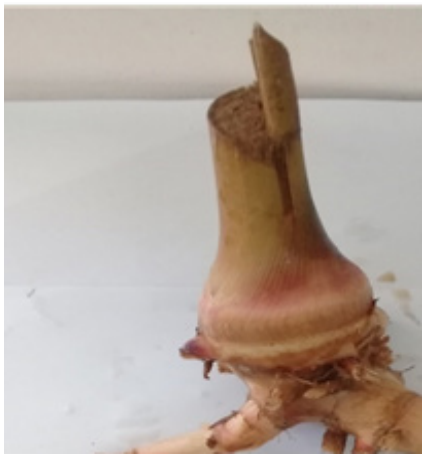
Extraction

The powdered dried leaf and rhizome samples (250g) of *E. elatior* and *E. philippinensis* were soaked in absolute ethanol (Scharlau,) for 48 hours and filtered twice, using cheese cloth first then finally with Whatman No.1 filter paper. The filtrate (ethanol extract) was rotary-evaporated under vacuum at 40 ° C to remove ethanol. Fresh crude ethanolic extracts were then used to make 3000 ppm and 10000 ppm for antibacterial analyses that were submitted to the Microbiology laboratory, College of Veterinary Medicine (CVM) in Central Mindanao University (CMU), University Town, Musuan, Bukidnon.

(A)

(B)

(C)



(D)

FIGURE 1. Photographs of E. elatior plant (A), E. philippinensis plant (B), E. elatior rhizome (C), and E. philippinensis rhizome (D).

Antibacterial Activity Determination

Test organisms

Six bacterial species were used as test organisms, namely: Gram-positive (*S. aureus*, *M. luteus* and *B. cereus*) and Gram-negative (*E. coli*, *S. marcescens* and *P. aeruginosa*). B. cereus (BIOTECH 1509) was incubated in 30 °C, aerobic for 24 hours in nutrient agar containing beef extract, peptone, and sodium chloride. *M. luteus* (BIOTECH 1793) and *E. coli* (BIOTECH 1634) were incubated at 37 °C with an incubation period of 24 hours in nutrient agar containing beef extract, peptone, and sodium chloride. *S. aureus*, *S. marcescens* and *P. aeruginosa* were isolated by Microbiology Laboratory, CVM, CMU, all in nutrient agar and incubated at 35 °C at 24 hours.

Antibacterial Assay

The antibacterial activity determination of the crude extracts was limited only to concentrations 3000 ppm and 10000 ppm when assayed against the select abovementioned bacteria. Paper Disc Diffusion Method on Mueller Hinton agar medium as described by Guevarra (2005) with slight modification was employed in this assay.

Briefly, the bacterial inoculum was swabbed over the entire surface of the Mueller Hinton agar plate using a sterile cotton swab. A sterile six (6) mm filter paper disc, previously soaked in the crude extract for 24 hours, was placed on the surface of the Mueller Hinton agar. After 24 hours of incubation at 37 °C, the diameter of the zones of inhibition of bacterial growth was measured in millimeters using a caliper. The assay was done in three replicates per sample.

Statistical Analysis

The antibacterial activity test results obtained in this study were statistically analyzed using t-test to compare the antibacterial activity of *E. elatior* from *E. philippinensis* against the test organisms. The same statistical test was performed on the results to compare the antibacterial activity of the leaf extracts from the rhizome extracts of each plant species. The t-test is used to compare whether the two means differ significantly from each other at 0.05 level of significance or whether their difference can be accounted for merely by random variations.

RESULT AND DISCUSSION

There is urgency to search for more antibacterial agents from plant source due to the growing concern on antibiotic resistance of most pathogenic organisms. There are still very limited scientific data on antibacterial activity of *E. elatior* and *E. philippinensis*. To fill this gap even at least partially, this study was conducted.

The antibacterial activity of the ethanolic extracts of the leaves and rhizomes of *E. philippinensis* and *E. elatior* are presented in Table 1. Concentrations of 3,000 ppm and 10,000 ppm were based on the study of Prakash and Karmegam (2012) and Wejinyake et al., 2016 with slight modification, respectively. Thirty micrograms of amikacin (22.7±4.45-26.3±4.45) and absolute ethanol (0) were used as positive and negative control, respectively. The zone of inhibition was measured in millimeter. Antibacterial activity tests were done in three trials.

Antibacterial activity of E. philippinensis

At 3000 ppm, *E. philippinensis* was only active against *M. luteus* (6.70 ± 0.87-leaves, 6.37 ± 0.64- rhizome) and *P. aeruginosa* (7.33 ± 1.04-rhizome). Both leaves and rhizome of *E. elatior* at 3,000 ppm did not show any antibacterial activity on the select bacteria. The leaves of *E. philippinensis* at 10,000 ppm showed a considerable antibacterial activity against *P. aeruginosa* (10.60 ± 0.55), *E. coli* (4.68 ± 0.85), *M. luteus* (12.00 ± 0.56), *B. cereus* (8.23 ± 0.32). *E. philippinensis* rhizome at 10,000 ppm showed antibacterial activity against *P. aeruginosa* (11.50 ± 0.45), *M. luteus* (7.07 ± 0.38) and *B. cereus* (7.20 ± 0.30). The highest antibacterial activity was exhibited by the leaves and rhizomes of E. philippinensis (10,000 ppm) against M. luteus (12.00 ± 0.56) and P. aeruginosa (11.50 ± 0.45), respectively.

Antibacterial activity of E. elatior

For *E. elatior* at concentration 10,000 ppm, the antibacterial activities against *M. luteus* and *B. cereus* are 6.67 ± 0.58 (leaves) and 6.67 ± 0.58 (rhizome), respectively. Of all extracts only the leaves *E. elatior* (10,000 ppm) showed antibacterial activities against *S. marcescens* which is 6.57 ± 0.49. Both plants, *E. philippinensis* and *E. elatior*, were not active against *S. aureus*.

Interpretation of Antibacterial assay results

Guevarra (2005) interpreted the antibacterial activity of the crude extracts as inactive if the zone of inhibition is <10 mm; partially active if 10-13 mm; active if 14-19 mm; and very active if >19 mm. Based on this criteria, the ethanolic extracts of *E. philippinensis* at 10,000 ppm is considered partially active against *M. luteus* (12.00 ± 0.56 – leaves and *P. aeruginosa* (10.60 ± 0.55 – leaves; 11.50 ± 0.45 – rhizomes) while the rest of the extracts are inactive. It is important to note, however that these extracts are still crude and purification of the extracts may increase the antibacterial activity A study of Policegoudra et al. (2006) on mango ginger rhizome has shown that the isolated compound has a more pronounced increase in antibacterial activity than the source extract.

The interpretative range in the zone of inhibition for the antibacterial activity of 30 g Amikacin antibiotic disc was □ 14 mm for resistant, 15-16 mm for intermediate sensitivity, and □ 17 mm for sensitive. Amikacin gave □ 17 mm zones of inhibition (22.7 - 26.3) against all test organisms implying that these organisms are all sensitive to amikacin.

There are many contributing factors affecting the antibacterial efficacy of plants. Some of these factors include environmental factors (time of harvesting, weather and botanical source), method of extraction, choice of solvent used metabolism and adaptation strategies of the microbes which also includes the type, genus, species

and strain, biochemistry of the plant (composition of the bioactive compounds, hydrophilicity, lipophilicity, concentration of the plant extract, pH value), plant species, age and parts used. (Li et al., 2017; Abdalla and Abdallah, 2018; Stefanović, 2018; Izah, 2018; Ellof, 2019). These best explains the variation of the results obtained in this study. For instance, E. elatior showed no antibacterial activity against all the bacteria tested at 3,000 ppm but its leaves showed antibacterial activity against M. luteus and S. marcescens at 10,000 ppm. It's rhizomes also showed antibacterial activity against B. cereus at 10,000 ppm.

Chan et al. (2007) reported no antibacterial activity of the methanol extracts of E. elatior leaves on the Gram-negative bacteria E. coli, P. aeruginosa, and Salmonella cholerasuis. In this present study, E. elatior leaves and rhizomes have no antibacterial activity in both 3000 ppm and 10000 ppm against the Gram-negative E.coli and P. aeruginosa. Interestingly, it has antibacterial activity against Gram-negative S. marcescens at 10000 ppm. Gram-negative bacteria are generally less susceptible to plant extracts than the Gram-positive bacteria (Chan et al., 2007). The outer membrane of Gram-negative bacteria consists of lipoprotein and lipopolysaccharide which is selectively permeable and thus regulates access to the underlying structures (Chopra and Greenwood, 2001).

Amikacin, positive control, is a member of aminoglycosides. A group of antibiotics used since the 1940s to primarily treat a broad spectrum of bacterial infections. In this study, amikacin exhibited greater antibacterial activity against gram-positive; *S. aureus*, *M. luteus* and *B. cereus* and Gram-negative; *E. coli*, *S. marcescens* and *P. aeruginosa*, ranges from 22.7±4.45-26.3±4.45 mm, as compared to both the root and leaf extracts of *philippinensis* and *E. elatior*. This is to be expected since the extracts have various impurities as compared to the drug that is a purified and semi-synthetically processed molecule (Ramirez and Tolmasky, 2017; Otto et al., 2014).

Furthermore, it is interesting to note, that despite being just a crude extract, both plants displayed antibacterial activity on selected test organisms. This result warrants further purification and biological activity testing of *E. philippinensis* and *E. elatior*.

CONCLUSION AND RECOMMENDATION

Crude ethanolic extracts of E. elatior and E. philippinensis showed antibacterial activity against selected test organisms. The Philippine endemic E. philippinensis leaves and rhizomes possessed the highest antibacterial activity against M. luteus and P. aeruginosa, respectively. Further purification and biological activity testing of E. elatior and E. philippinensis is recommended. Moreover, it is recommended that antibacterial studies on more varied extract concentrations and antifungal studies be done on these plants.

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Table 1. Antimicrobial activity as zone of inhibition (mm) of E. philippinensis and E. elatior ethanolic extracts against selected bacterial strains. Results are expressed as Mean of three replicates ± SD; 0.00 = No Zone of Inhibition; Gram-negative (G-); Gram-positive (G)

Microorganims	Plant Extract Concentration								Zone of Inhibition, mm	
	3000 ppm				10000 ppm				Positive Control (Amikacin, 30mcg)	Negative Control (Ethanol, Absolute)
	E. philippinensis		*E. elatior*		*E. philippinensis*		*E. elatior*			
	L	R	L	R	L	R	L	R		
P. aeruginosa (G-)	0.00	7.33± 1.04	0.00	0.00	10.60± 0.55	11.50± 0.45	0.00	0.00	23.0± 4.81	0.00
E. coli (G-)	0.00	0.00	0.00	0.00	4.68 ± 0.85	0.00	0.00	0.00	22.7± 4.45	0.00
M. luteus (G+)	6.70± 0.87	6.37± 0.64	0.00	0.00	12.00± 0.56	7.07± 0.38	6.67± 0.58	0.00	24.2± 2.33	0.00
S. marcescens (G-)	0.00	0.00	0.00	0.00	0.00	0.00	6.57± 0.49	0.00	23.5± 3.32	0.00
B. cereus (G+)	0.00	0.00	0.00	0.00	8.23± 0.32	7.20± 0.30	0.00	6.67± 0.58	26.3± 4.45	0.00
S. aureus (G+)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.4± 1.98	0.00

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