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Antimicrobial Activity of Consecutive Extracts of *Etlingera brevilabrum*

(Aktiviti Antimikrob Ekstrak Berturutan *Etlingera brevilabrum*)

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ABSTRACT

Disc diffusion (DD), minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) assays were used to evaluate the antimicrobial activity of 21 consecutive extracts of different aerial parts of Etlingera brevilabrum *against 18 microorganisms that included six Gram-positive [(+)], ten Gram-negative [(-)] bacteria and two fungi. Among the plant parts, the stolon extracts showed numerous activity than the other parts in which they inhibited Gram-positive of* Staphylococcus aureus *(ethyl acetate extract: diameter of inhibition zone 12.2±0.3 mm, MIC 3.12 mg/mL, MBC 6.25 mg/mL), methicillin resistant* S. aureus *(MRSA) (ethyl acetate extract: 12.1±0.2 mm, 12.5 mg/mL, 25 mg/mL),* S. epidermidis *(ethanol extract: 11.4±0.5 mm, 3.12 mg/mL, 3.12 mg/mL),* Bacillus thuringiensis *(acetone extract: 13.3±0.5 mm, 12.5 mg/mL, 25 mg/mL) and one Gram-negative of* Vibrio paraehaemolyticus *(water extract: 14.3±0.4 mm, 1.56 mg/mL, 6.25 mg/mL). The highest activity in MIC was shown by the methanol-water (1:1) and water extracts on Gram-negative* Aeromonas hydrophila *(1.56 mg/mL: leaf water extract) and* V. paraehaemolyticus *(1.56 mg/mL: methanol-water and water extracts of stolons and leaves and stem water extract).*

Keywords: Antimicrobial; disc diffusion; Etlingera brevilabrum*; minimal bactericidal concentration; minimum inhibitory concentration*

ABSTRAK

Asai peresapan cakera (PC), kepekatan merencat minimum (KMM) dan kepekatan bakteriasid minimum (KBM) diguna bagi menilai aktiviti antimikrob 21 ekstrak berturutan bahagian udara berbeza Etlingera brevilabrum *terhadap 18 mikroorganisma termasuk enam bakteria Gram-positif [(+)], sepuluh Gram-negatif [(-)] dan dua kulat. Antara bahagian tumbuhan, ekstrak pangkal-batang memperlihat lebih banyak aktiviti daripada bahagian lain dan ia boleh merencat empat Gram-positif* Staphylococcus aureus (*ekstrak etil asetat: diameter zon perencatan 12.2±0.3 mm, KMM 3.12 mg/mL, KBM 6.25 mg/mL),* S. aureus *tahan-metisillin (SATM) (ekstrak etil asetat: 12.1±0.2 mm, 12.5 mg/mL, 25 mg/mL),* S. epidermidis *(ekstrak etanol: 11.4±0.5 mm, 3.12 mg/mL, 3.12 mg/mL),* Bacillus thuringiensis *(ekstrak aseton: 13.3±0.5 mm, 12.5 mg/mL, 25 mg/mL) dan satu Gram-negatif* Vibrio paraehaemolyticus *(ekstrak air: 14.3±0.4 mm, 1.56 mg/mL, 6.25 mg/mL). Aktiviti tertinggi dalam KMM diperlihat oleh ekstrak metanol-air (1:1) dan air terhadap Gram-negatif* Aeromonas hydrophila *(1.56 mg/mL: ekstrak air daun) dan* V. paraehaemolyticus *(1.56 mg/mL: ekstrak metanol-air dan air bagi pangkal-batang dan daun dan ekstrak air batang).*

Kata kunci: Antimikrob; Etlingera brevilabrum*; kepekatan bakteriasid minimum; kepekatan merencat minimum; peresapan cakera*

INTRODUCTION

Since ancient times, medicinal plants have been used to combat various diseases (Al-Bakri & Afifi 2007). The search for new antimicrobial drugs continues due to the rise of resistant microbes (Blondeau et al. 2001; Karaman et al. 2003). Plant extracts and compositions which possess antibacterial, antifungal and antiviral activities are prime candidates for the exploration of new and novel antimicrobial agents (Arora & Kaur 1999; Othman et al. 2011; Weckesser et al. 2007). *Etlingera brevilabrum* which belongs to the Zingiberaceae family has been known to have several uses in traditional medicine in Sarawak, Malaysia. The pounded leaves of *E. brevilabrum* were applied to dry-diseased skin of the legs, the sap of heated young stems was used to treat sore eyes and the stolons were used as medicine to cure stomach-aches (Poulsen 2006). In this study, we have evaluated the antimicrobial activity of 21 extracts from *E. brevilabrum* aerial parts of the stolons, stems and leaves according to their different medicinal uses. So far, there has been no report on the antimicrobial activity of the extracts from different parts of *E. brevilabrum* found in the literature.

MATERIALS AND METHODS

PLANT MATERIAL

Plant parts of *Etlingera brevilabrum* consisted of the stolons, stems and leaves were collected in December 2009 from the plant's natural habitat in Sabah, Malaysia. A

voucher specimen of WYA500 was deposited at Universiti Kebangsaan Malaysia Herbarium (UKMB). The plant species was identified by Mr. Sani Miran of Universiti Kebangsaan Malaysia.

PLANT EXTRACTS

Each of the dried parts of *Etlingera brevilabrum* was ground and extracted consecutively by using solvents of increasing polarity; namely hexane, ethyl acetate, acetone, methanol, methanol-water (1:1) and hot water. For the hexane, ethyl acetate, acetone and methanol extracts, each plant powder was macerated in the solvent for 72 h at room temperature; after filtration, similar-fresh solvent was added to the remaining powder and macerated for a further 24 h; after filtration, the next batch of new solvent was added. However after the methanol extraction was completed, methanol-water (1:1) was employed for 12 h and followed by hot water for 1 h. The fractions of similar solvent were then combined, filtered and evaporated under reduced pressure using Heidolph evaporator (Laborota 4000 eco). Fractions containing water were freeze dried. For extractions with ethanol, each powder was macerated solely with the solvent for 72 h.

MICROORGANISMS

The test bacterial strains of six Gram-positive of *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 11774, *Enterococcus faecalis* ATCC 14506, *Bacillus thuringiensis* ATCC 10792, *S. epidermidis* ATCC 12228 and Methicillin Resistant *S. aureus* (MRSA) were obtained from the Microbiology Laboratory culture collection, School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia and verified by standard microbiology method. Ten Gram-negative of *Proteus vulgaris* ATCC 33420, *Proteus mirabilis* ATCC 12453, *Serratia marcescens* ATCC 13880, *Shigella sonnei* ATCC 29930, *Pseudomonas aeruginosa* ATCC 10145, *Escherichia coli* ATCC 10536, *Enterobacter aerogenes* ATCC 13048, *Salmonella typhimurium* ATCC 51812, *Vibrio parahaemolyticus* ATCC 17802 and *Aeromonas hydrophila* ATCC 7966; two *Candida* species of *C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019 were also tested.

DD ASSAY

DD assay according to CLSI (2009a) and Scorzoni et al. (2007) was carried out to determine the antimicrobial activity of the extracts. The suspensions containing 10^8 CFU/mL were loaded on a sterile cotton swabs and streaked over the dried surface of Mueller-Hinton agar medium plates for inoculation. The 6 mm in diameter discs were impregnated with 20 µL of the extracts (100 mg/mL) and then placed on the inoculated agar. The plates were incubated at 37°C for 24 h. Antimicrobial activity was determined by measuring the diameter of inhibition zone (DIZ) against the microorganisms. All tests were carried out in triplicate. Chloramphenicol 30 µg was used as the positive control.

DETERMINATION OF MIC AND MBC

According to CLSI (2011) and Weckesser et al. (2007), MIC was evaluated on bacterial strain that showed sensitivity to the extracts in the DD assay. First the 96-well plate were dispensed with 100 µL of the culture media (Mueller-Hinton broth), next the first well was charged with 100 µL of the DMSO extract solutions. Then, 100 µL from each of their serial dilutions was transferred into consecutive wells and finally each well charged with 50 µL of the culture media and 50 µL of the inoculums which prepared after 12 h incubation at 37°C were adjusted to 0.5 McFarland standard turbidity. The final volume in each well was 200 µL with extract concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 mg/mL, respectively and positive control (chloramphenicol) concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 µg/mL, respectively. The MIC was defined as the minimum concentration that resulted in no visible growth. All tests were carried out in duplicate. For determination of the MBC, a 5 µL of the first inoculation with growth and the inoculations without growth were dispensed on a Mueller-Hinton agar plate and after 24 h incubation at 37°C, the lowest concentration without visible growth was regarded as MBC (CLSI 2009b; Kuete et al. 2007).

RESULTS AND DISCUSSION

Abbreviations of various extracts from stolon (Sto), stem (S) and leaves (L) of *Etlingera brevilabrum* by using hexane (H), ethyl acetate (Ea), acetone (A), methanol (M), methanol-water 1:1 (MW), water (W) and ethanol (Et) together with the percentage yields of the extracts are shown in Table 1. The percentage yields of 21 extracts ranged from 0.15 (SA) and 6.60% (StoMW).

The results of DD, MIC and MBC for active extracts are shown in Table 2. Among 18 microorganisms used in screening the inhibition zones, the *E. brevilabrum* extracts inhibited seven test bacteria of MRSA-(+), *Staphylococcus aureus*-(+), *S. epidermidis*-(+), *Bacillus thuringiensis*-(+), *Aeromonas hydrophila*-(-), *Vibrio parahaemolyticus*-(-), *Shigella sonnei*-(-) out of 16 and none of the two *Candida* species. Six out of seven of the stolon and stem extracts of H, Ea, A, MW, W, Et (except M) and leaf extracts of MW, W showed antibacterial activity against at least one of the bacteria. According to the results, the stolon extracts possessed numerous antibacterial activities (13) compared to the stems (8) and leaves (3). The non-polar extracts of StoH and SH only exhibited weak activity against *S. sonnei*-(-) in terms of their MIC and MBC (50 mg/mL each). Among the consecutive extracts from the stolon, Ea and A extracts were active against a higher number of bacteria compared to the other extracts. MW and W extracts of each of the three parts were able to prevent the growth of *V. paraehaemolyticus*-(-) whereas

TABLE 1. Abbreviations and percentage yields of various extracts from different parts of *Etlingera brevilabrum*

	Stolon (Sto)		Stem (S)		Leaf (L)	
	Abbr.	Yield (%)	Abbr.	Yield (%)	Abbr.	Yield (%)
Hexane (H)	StoH	1.1	SH	0.35	LH	1.30
Ethyl acetate (Ea)	StoEa	0.83	SEa	0.28	LEa	1.41
Acetone (A)	StoA	0.36	SA	0.15	LA	1.18
Methanol (M)	StoM	3.80	SM	1.74	LM	2.07
Methanol :Water (1:1) (MW)	StoMW	6.60	SMW	3.33	LMW	4.13
Water (W)	StoW	3.35	SW	1.78	LW	3.18
Ethanol (Et)	StoEt	2.21	SEt	1.06	LEt	1.23

SW and LW inhibited *A. hydrophila*-(-). MRSA for the Gram-positive bacteria was inhibited by more extracts which were of StoEa, StoA, StoEt, SEa, SA and SEt. According to the results of DIZ, MIC and MBC against MRSA, StoA with 14.7 mm SA with MIC 1.56 mg/mL and MBC 6.25 mg/mL showed the strongest inhibition. All the extracts showed lower antibacterial activity compared to the positive control of chloramphenicol against all the tested bacteria. The MIC values of chloramphenicol were in the range from 1.56 to 50 μg/mL whereas those of the extracts were from 1.56 to 25 mg/mL.

The polar non-consecutive ethanolic extracts of S and Sto were not able to inhibit *Shigella sonnei*-(-) but the first in consecutive extracts of non-polar hexane inhibited the bacterium. These results are the opposite to what was previously reported in studies conducted by Eloff (1998) and Karaman et al. (2003) which had detailed that the polar extracts were more efficient than non-polar. *Etlingera brevilabrum* extracts in total have shown numerous inhibitions against Gram-positive bacteria than Gram-negative. The results obtained were in agreement with previous studies on plant extracts by Lin et al. (1999). With the MBC/MIC ratios for all the extracts being lower than 4 on corresponding bacteria, the extracts were bactericidal (Kuete et al. 2008, 2009). While the consecutive methanolic extracts of the three different parts of *E. brevilabrum* did not show any antibacterial activity, the non-consecutive methanolic leaf extracts of *E. elatior, E. fulgens, E. littoralis, E. maingayi* and *E. rubrostriata* inhibited Gram-positive bacteria of *Staphylococcus aureus, Micrococcus luteus* and *Bacillus cereus* (Chan et al. 2007).

CONCLUSION

Despite the fact the leaf extracts of the other species of *Etlingera* showed antimicrobial activity on Gram-positive bacteria, the ethyl acetate, acetone, methanol and ethanol *E. brevilabrum* leaf extracts did not exhibit any antimicrobial activity on the Gram-positive bacteria. The leaf water extract inhibited both Gram-negative bacteria of *V. paraehaemolyticus* and *A. hydrophila* and the leaf methanol-water extract was active against *V. paraehaemolyticus*, with all giving the same lowest value of MIC 1.56 mg/mL. To summarize, the stolon extracts of *E. brevilabrum* showed numerous antimicrobial activities than those of the stems and leaves.

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TABLE 2. Antimicrobial activity of consecutive extracts of the stolon (Sto), stem (S), and leaves (L) of *Etlingera brevilabrum* by using hexane (H), ethyl acetate (Ea), acetone (A), methanol (M), methanol-water 1:1 (MW), water (W), and ethanol (Et)

	MRSA-(+)			*Staphylococcus aureus*-(+)			*Staphylococcus epidermidis*-(+)			*Bacillus thuringiensis*-(+)			*Aeromonas hydrophila*-(-)			*Vibrio parahaemolyticus*-(-)			*Shigella sonnei*-(-)		
	DIZa	MIC[b]	MBC[c]	DIZ	MIC	MBC	DIZ	MIC	MBC	DIZ	MIC	MBC	DIZ	MIC	MBC	DIZ	MIC	MBC	DIZ	MIC	MBC
StoH	NAd	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-	10.4±0.5	50	50
StoEa	12.1±0.2	12.5	25	12.2±0.3	3.12	6.25	12.6±0.4	6.25	25	NA	-	-	NA	-	-	NA	-	-	NA	-	-
StoA	14.7±0.5	12.5	50	NA	-	-	13.7±0.7	6.25	12.5	13.3±0.5	12.5	25	NA	-	-	NA	-	-	NA	-	-
StoMw	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-	12.0±0.6	1.56	6.25	NA	-	-
StoW	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-	14.3±0.4	1.56	6.25	NA	-	-
StoEt	11.0±0.6	12.5	50	13.4±0.7	25	25	11.4±0.5	3.12	3.12	11.0±0.4	12.5	25	NA	-	-	NA	-	-	NA	-	-
SH	N	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-	9.0±0.6	50	50
SEa	12.3±0.7	3.12	12.5	9.2±0.6	25	50	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-
SA	10.8±0.5	1.56	6.25	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-
SMW	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-	11.7±0.6	3.12	6.25	NA	-	-
SW	NA	-	-	NA	-	-	NA	-	-	NA	-	-	11.7±0.8	3.12	6.25	12.5±0.4	1.56	6.25	NA	-	-
SEt	10.7±0.6	6.25	12.5	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-
LMW	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-	11.9±0.9	1.56	3.12	NA	-	-
LW	NA	-	-	NA	-	-	NA	-	-	NA	-	-	11.8±0.6	1.56	3.12	13.5±0.4	1.56	6.25	NA	-	-
Control[e]	24	50	-	32	6.25		23	25	-	23	50	-	26	12.5	-	18	3.12	-	26	1.56	-

DIZ[a]: diameter of inhibition zone (mm); MIC[b]: minimum inhibitory concentration (mg/ml); MBC[c]: minimal bactericidal concentration (mg/ml); NA[d]: non-active.
Control[e]: Chloramphenicol. No inhibition was shown by *Bacillus subtilis*-(+), *Enterococcus faecalis*-(+), *Proteus vulgaris*-(-), *Proteus mirabilis*-(-), *Serratia marcescens*-(-), *Pseudomonas aeruginosa*-(-), *Escherichia coli*-(-), *Enterobacter aerogenes*-(-), *Salmonella typhimurium*-(-), *Candida albicans*, and *C. parapsilosis*. MIC assay in duplicate gave the same result

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