STUDY ON ANTI-MICROBIAL PROPERTIES OF *Enicosanthum membranifolium* SINCLAIR AND *Enicosanthum cupulare* (KING) AIRY-SHAW

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ABSTRACT

n-Hexane and ethyl acetate fractions of methanol extracts of two species of Annonaceae, *Enicosanthum membranifolium* Sinclair and *Enicosanthum cupulare* (King) Airy-Shaw, were screened for antimicrobial activity against eighteen bacterial strains using agar dilution method. The ethyl acetate fraction of the two Annonaceae plants showed higher antimicrobial activities than the *n*-hexane fraction. The extracts of the plants tested were significantly more active against gram-positive with minimum inhibitory concentration (MICs) ranging from 0.0625 to 4 mg/mL than against gram negative bacteria (MICs >4 mg/mL).

***Keywords*:** *Antibacterial activity; Enicosanthum membranifolium Sinclair; Enicosanthum cupulare (King) Airy-Shaw*

INTRODUCTION

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants. Numerous studies have been carried out to extract various natural products for screening antimicrobial activity[1-5]. Systematic screening of them may result in the discovery of novel effective compounds[6]. Annonaceae family is one of the plants which attracted much interest. Annonaceae family is a rich source of bioactive substances. Many species belonging to Annonaceae family produced antimicrobial compounds with structurally diverse. *C*-Benzylated chalcone was isolated from *Ellipeiopsis cherrevensis*[7]. Mitrephorone A, mitrephorone B and mitrephorone were isolated from *Mitrephora glabra*[8]. Reticuline, anonaine, laurelliptine and isoboldine were found in the bark of *Annona salzmanii* D.C.[9]. Cherimolin and dihydrocherimolin were obtained in *Annona cherimolia*[10]. Ent-trachyloban-19-oic acid, ent-kaur-16-en-19-oic acid, 8(14),15-pimaradien-18-oic acid and 7,15-pimaradien-18-oic acid were identified in *Mitrephora celebica*[11].

Therefore, in order to confirm value of Annonaceae plants four species belonging this family was collected in Rimbo Panti forest; they are *Enichonsanthum membranifolium* Sinclair, *Enichonsanthum cupulare* (King) Airy-Shaw. Based on preliminary screening, these species showed antimicrobial activities. As a part of our study on Annonaceae plants grown in West Sumatra, in this paper we wish to report screening of anti-microbial activities of *Enicosanthum membranifolium* Sinclair and *Enicosanthum cupulare* (King) Airy-Shaw.

MATERIALS AND METHODS

Plants Materials and Extract Preparation

Enicosanthum membranifolium Sinclair, *Enicosanthum cupulare* (King) Airy-Shaw and *Polyalthia cauliflora* var. desmantha (Hk. f. et Th.) Sinclair used in this study were collected in Rimbo Panti forest, West Sumatra, Indonesia. Voucher specimen are deposited at Herbarium Andalas University (AND), Padang, Indonesia.

Air dried plant materials were finely ground

and macerated at room temperature in methanol. The extract was subsequently filtered and concentrated *in vacuo.* Each methanol extract of the plants was partitioned successively with *n*-hexane, ethyl acetate, *n*-butyl alcohol and water. Then each fraction (*n*-hexane, ethyl acetate, *n*-butyl alcohol and water) was evaporated *in vacuo* and tested for antimicrobial activity.

Antimicrobial Screening

Micro-Organisms

The following strains of microorganisms were used: *Bacillus cereus, Bacillus subtilis*, *Streptococcus pyogenes*, *Staphylococcus epidermis, Staphylococcus aureus*, *Streptococcus agalactiae, Streptococcus salivarius*, *Enterococus faecalis, Salmonella sp., Vibrio parahaemolyticus*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Achromobacter xylosoxidans, Enterobacter aerogenes, Escherichia coli*, *Candida albicans* and *Candida sp.*

Preliminary Screening for Antimicrobial Activities

Plate diffusion assay (cup method) was performed for preliminary screening of methanol extracts and four fractions (*n*-hexane, EtOAc, *n*-butanol and water) of *Eniconsanthum membranifolium* Sinclair, *Enichonsanthum cupulare* (King) Airy-Shaw and *Polyalthia cauliflora* var. *desmantha* (Hk. f. et Th.) Sinclair. The antimicrobial assay was carried out based on an NCCLS global consensus standard[12]. In this method, *Staphylococcus aureus* and *Bacillus subtilis* were used as test organisms. The dried plant extracts were dissolved in aqueous dimethylsulfoxide (DMSO) : water (125 : 875) to a final concentration of 10 mg/L. Sterile cups were placed on the surface of agar plates inoculated with a microbial culture and then 200 µL of the solution extracts were dropped into the cups. Each extract was tested in duplicate. Control cup contained 200 µL of sterile 12.5 % aqueous DMSO. Agar plates containing bacteria were incubated at 35°C for 24 h. After incubation, the diameters of growth inhibition zones around the cups were measured.

Minimum Inhibitory Concentration (MIC)

In vitro antimicrobial activity was determined by the agar dilution method. The antimicrobial assay was carried out based on an NCCLS global consensus standard[13]. A series of concentrations (4, 2, 1, 0.5, 0.25, 0.125, 0.0625 mg/mL) from extracts were prepared in 1 mL DMSO. Each antimicrobial dilution (0.2 mL) was transferred to a tube containing 19.8 mL of BHI medium to make a 1:10 dilution. Then it was mixed well and poured into a Petri dish. It was allowed to set and dry the surface of the plates. Suspensions of microorganisms were then incubated on the plate containing of antimicrobial compounds at 35°C for 18 h. Control (just containing solvent only) was made with the same ways. Polyphenon-100 (containing epigallocatechin gallate 59.4%, epigallocatechin 11.6%, epicatechin 11.4%, epicatechin gallate 2.1% and gallocatechin gallate 4.3%) as positive control was made in the same ways. The MIC was determined as the lowest dilution which completely prevented microbial growth.

Phytochemical Screening

Identifications of secondary metabolites were carried out on the methanol extract of the plants using chemical method according to the methods described by Fransworth[14] and Harbone[15,16].

RESULT AND DISCUSSION

Screening of antimicrobial activity of methanol extracts of *Enicosanthum membranifolium* Sinclair and *Enicosanthum cupulare* (King) Airy-Shaw against *Staphyllococcus aureus* and *Bacillus subtilis* was carried out using cup method. All of methanol extracts showed activities against the microorganism tested. Inhibitory zone of *Enicosanthum membranifolium* Sinclair methanol extract was 18.3 mm (*B. subtilis*) and 12.2 mm (*S. aureus*). The inhibitory zone of *Enicosanthum cupulare* (King) Airy-Shaw was 25.7 mm (*B. subtilis*) and 9.98 mm (*S. aureus*). The *n*-hexane, ethyl acetate, *n*- butanol and water fractions of their

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extracts were tested their antimicrobial activity using cup method against *S. aureus* and *B. subtilis*. As the results the *n*-hexane and ethyl acetate fractions showed activity against microorganism tested (Table 1). The *n*-hexane and ethyl acetate fractions of each species were necessary to be continued to evaluate their potential as antimicrobial. To study their antimicrobial activities, 18 kinds of strain microorganisms were used.

Table 2 shows the MICs of the extracts tested (range of concentration of crude extracts: 0.0625 to 4 mg/mL) against a range of microorganisms. The extracts of the plant mainly inhibited against gram-positive microorganisms. Against bacterial Gram-positive, the activities of ethyl acetate fraction of *Enicosanthum membranifolium* Sinclair, *Enicosanthum cupulare* (King) Airy-Shaw and *Polyalthia cauliflora* var. desmantha Sinclair were stronger than *n*-hexane fractions. The *n*-hexane extract of *E. membranifolium* was active against *B. subtilis*, *S. pyogenes* and *E. faecalis* (gram-positive microorganisms) with range of MICs were 0.5 to 2 mg/mL.

The ethyl acetate fraction of this plant was active against *B. cereus*, *B subtilis*, *S. pyogenes*, *S. epidermis*, *S. aureus*, *S. agalactiae* and *S. salivarius* (gram-positive microorganisms) with 0.0625 to 1 mg/mL of MICs range. The n-hexane extract of *E. cupulare was active against B. cereus*, *B. subtilis*, *S. pyogenes*, *S. epidermis*, *S. aureus*, *S. agalactiae* and *S. salivarius* with MICs range were 0.25 to 1 mg/mL. MICs range of the ethyl acetate extracts of this plant against *B. cereus*, *B subtilis*, *S. pyogenes*, *S. epidermis*, *S. aureus*, *S. agalactiae* and *S. salivarius* were <0.0625 to 1 mg/mL. The *n*-hexane and ethyl acetate extracts of *P. cauliflora* were active against *B. cereus*, *B. subtilis*, *S. pyogenes*, *S. epidermis* and *S. aureus* with range of MICs were 1-2 mg/mL and <0.0625 to 0.25 mg/mL. Most of the extracts showed no inhibitory activity against *Salmonella sp*., *Yersinia enterocolitica, Achromobacter xylosoxidans, Enterobacter aerogenes, Escherichia coli, Candida albicans* and *Candida sp* (>4 mg/mL). Anti-fungal activity was tested using *Candida albicans* and *Candida sp*, most of the extract showed no significant inhibitory activity (>4 mg/mL).

The freshly leaves of *Enicosanthum membranifolium* Sinclair and *Enicosanthum cupulare* (King) Airy-Shaw were subjected to preliminary phytochemical screening. The results showed the presence of phenolic, steroid, terpenoid and alkaloid in two plant leaves (Table 3). The test for flavonoid, however, showed negative result. The presence of secondary metabolites such as phenolics, steroid, terpenoid and alkaloid in these plants could be responsible for antimicrobial activity, because many compounds from these classes have reported as antimicrobial agents.

Table 1. Antimicrobial Activities of *n*-Hexane, Ethyl Acetate, *n*-Butanol and Water Fractions of Three Annonaceae Species

No	Plants	Fraction	Inhibitory zone (mm)	
			Bacillus subtilis	*Staphylococcus aureus*
1	*Enicosanthum membranifolium* Sinclair	*n*-Hexane	20.5	15.3
		Ethyl acetate	15.11	15.3
		n-Butanol	NA	NA
		Water	NA	NA
2	*Enicosanthum cupulare*	*n*-Hexane	14.3	13.0
		Ethyl acetate	24.1	16.8
		n-Butanol	NA	NA
		Water	NA	NA
		Ethyl acetate	18.8	11.0
		n-Butanol	NA	NA
		Water	NA	NA

NA: no activity

Table 2. MIC of *n*-Hexane and Ethyl Acetate Fractions of *Enicosanthum membranifolium* Sinclair and *Enicosanthum cupulare* Against 18 Kinds of Microorganisms

No	Microorganisms	Antimicrobial activity (mg/mL)				
		EmS		EcS		P
		Hex	EtOAc	Hex	EtOAc	
	Gram-positive spore forming rods					
1.	*Bacillus cereus*	4	0.25	0.5	0.125	0.125
2.	*Bacillus subtilis*	0.5	0.0625	0.25	<0.0625	0.5
	Gram-positive cocci					
3.	*Streptococcus pyogenes*	2	0.5	0.5	0.125	0.25
4.	*Staphylococcus epidermis*	>4	1	1	0.25	<0.0625
5.	*Staphylococcus aureus*	>4	0.5	0.5	0.25	0.125
6.	*Streptococcus agalactiae*	>4	0.25	0.25	0.5	0.5
7	*Streptococcus salivarius*	>4	1	1	0.25	0.5
8	*Enterococcus faecalis*	2	>4	>4	1	1
	Gram-negative rods					
9.	*Salmonella sp.*	>4	>4	>4	>4	0.5
10.	*Vibrio parahaemolyticus*	>4	2	>4	>4	0.125
11	*Yersinia enterocolitica*	>4	2	>4	>4	0.25
12	*Pseudomanas aeruginosa*	>4	>4	>4	>4	0.5
13	*Klebsiella pneumoniae*	>4	>4	>4	>4	1
14.	*Achromobacter xylosoxidans*	>4	2	>4	>4	0.125
15	*Enterobacter aerogenes*	>4	>4	>4	>4	1
16.	*Escherichia coli*	>4	>4	>4	>4	1
	Yeast					
17.	*Candida albicans*	>4	>4	>4	>4	0.125
18.	*Candida sp.*	>4	>4	>4	>4	0.125

EmS: *Enicosanthum membranifolium* Sinclair; EcS: *Enicosanthum cupulare*;
P: Polyphenon-100 (as positive control)

Table 3. Phytochemical Analysis of *Enicosanthum membranifolium*, *Enicosanthum cupulare*, *Polyalthia cauliflora* var. desmantha and *Goniothalamus tapis* Miq

Principles	Test applied	Plants	
		EmS	EcS
Alkaloids	Mayer	+	+
Phenolic compounds	Ferric chloride	+	+
Flavonoids	Mg/HCl	-	-
Terpenoids/steroids	H_2SO_4-Ac_2O	+	+

EmS: *Enicosanthum membranifolium*; EcS: *Enicosanthum cupulare*

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CONCLUSION

In conclusion, the three Annonaceae species used in this study exhibited selective antimicrobial activity to varying degrees. They may, therefore, provide new leads in the ongoing search for novel antimicrobial drugs. Bioassay-guided isolation of the antimicrobial active compounds will be made on the active fractions of *E. membranifolium* Sinclair, *E. cupulare* (King) Airy-Shaw and *Polyalthia cauliflora* var. *desmantha* (Hk. f. et Th.) Sinclair. We also aim to perform chemical characterization of some of the antimicrobially active compounds.

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