SYNERGISTIC EFFECT OF COMBINATION BETWEEN CYANOMACLURIN AND ARTOCARPIN FROM *ARTOCARPUS HETEROPHYLLUS* HEARTWOODS AGAINSTS *STREPTOCOCCUS PYOGENES* AND *STAPHYLOCOCCUS EPIDERMIDIS*

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ABSTRACT

Streptococcus pyogenes and *Staphylococcus epidermidis* are major problem of human health and may generate antibacterial resistance problem. Flavonoid has been used for the treatment of several ailments including bacterial infection. *Artocarpus heterophyllus* is a potential source of flavonoid compounds such as artocarpin and cyanomaclurin. The study was conducted to observe synergistic effects between flavonoid compounds against S. *pyogenes* and *S. epidermidis*. The antibacterial activity of combination of artocarpin and cyanomaclurin isolated from *A. heterophyllus* heartwoods were evaluated against *S. pyogenes* and *S. epidermidis* using broth microdilution methods. Interaction between two compounds in combination was determined using checkerboard assay. Artocarpin showed strong antibacterial activity with MIC value of 1.9 µg/mL. Cyanomaclurin only exhibited moderate activity with MIC value of 15.6 µg/mL. The mixture of compounds in several ratios tended to increase antibacterial activities of cyanomaclurin. There is no antagonistic effect when compounds used together. It can be concluded that flavonoid compounds in combination may enhance antibacterial activity to prevent development of antibacterial resistance.

Keywords: antibacterial; artocarpin; *Artocarpus heterophyllus*; cyanomaclurin; synergy

Received: April 2022, Accepted: June 2022, Published: June 2022

INTRODUCTION

Streptococcus pyogenes and *Staphylococcus epidermidis* continue to be a major public health problem. These bacteria are associated with nosocomial infection and several infectious diseases, for instance, chronic skin infectious occurred on hospitalized patients could be due to the increase of *S. epidermidis* infection (Sandel & McKillip, 2004). Reported that commercial antibiotics are probably able to control the development of bacterial infection. However, the use of antibacterial particularly in high doses and long terms will lead to resistant problem. Thus, it needs to find alternative approach to overcome antibacterial resistance problem. One of recourse strategy is the utilization of natural compounds in combination with antibiotics. Combination of antibiotic has been applied to broaden the spectrum of antibiotic as well as to obtain synergistic effect (Qin, et al., 2013).

Artocarpus heterophylus Lam. belongs to family (Moraceae), it has been used in traditional medicine in several countries. This plant has been reported to have several pharmacological activities such as antibacterial, anticancer and immunomodulator. The plant is known to produce a variety of flavonoids (Cushnie & Lamb, 2011). Flavonoids are ubiquitous compound that occurred widely in the plant. It

has been reported to possess many pharmacological activities, including antibacterial (Septama & Panichayupakaranant, 2016). Artocarpin and cyanomaclurin (Fig. 1) are flavonoids that have been successfully isolated from *A. heterophyllus* heartwood. These compounds showed the antibacterial activity against the Gram-positive and the Gram-negative bacteria (Septama & Panichayupakaranant, 2015). However, there is no report regarding the interaction of both compounds against selected bacteria. Therefore, this work aims to investigate possible synergistic effect of the two flavonoid compounds, cyanomaclurin and artocarpin against two tested bacteria such as *S. pyogenes* and *S. epidermidis.*

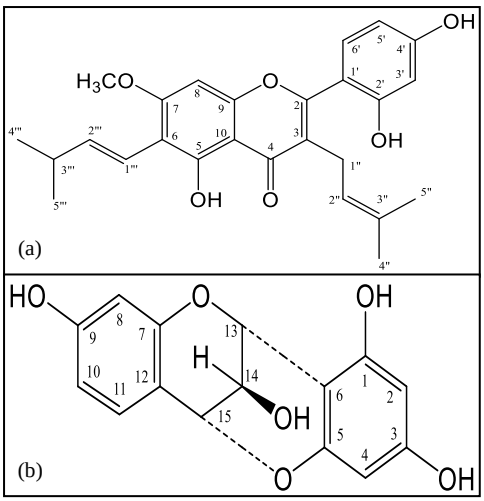

Figure 1. Chemical structure of artocarpin (a) and cyanomaclurin (b)

MATERIALS AND METHODS

a. Chemicals

Artocarpin and cyanomaclurin were isolated from of *A. heterophyllus* heartwood as previously reported by Septama and Panichayupakaranant (Septama & Panichayupakaranant, 2015). Tetracycline and dimethyl sulfoxide (DMSO) were from Sigma Aldrich (USA). Brain Heart Infusion (BHI) and agar were obtained from the Himedia (Mumbai, India). Sodium chloride (NaCl) was from Merck.

b. Bacterial Strains

Gram-positive bacteria: *Streptococcus pyogenes* (DMST 17020), and *Staphylococcus epidermidis* (ATCC 14990) were obtained from National Agency of Drug and Food Control of Indonesia.

c. The minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was evaluated using the broth microdilution standard method with minor modification (NCCLS, 2008). Tetracycline was used as positive control. DMSO was applied as negative controls. The MIC value was considered as the lowest inhibition concentration.

d. Antibacterial activity of a combination of two flavonoids

Two flavonoids, cyanomaclurin and artocarpin were combined in the ratios of 3:1, 2:1, 1:1, 1:2, and 1:3, respectively. Antibacterial activity against *S. pyogenes* and *S. epidermidis* was evaluated by broth microdilution (Milne & Gould, 2012). Interaction between two compounds in combination was monitored by calculating fractional inhibitory concentration (FIC) using the following formula:

$$FIC = \frac{MIC\ of\ compound\ in\ combintaion}{MIC\ of\ compound\ alone}$$

FIC<0.5 (synergy), 0.5≤FIC≤1 (addition), indifference (1<FIC≤4), antagonist (FIC>4) (Hamoud, et al., 2014).

RESULTS

In this study, on the basis of broth-microdilution methods, it found that artocarpin exhibited strong antibacterial activity against *S. pyogenes* and *S. epidermidis* with MIC value of 1.9 µg/mL. It was equivalent with tetracycline as positive control. On the other hand, cyanomaclurin showed moderate antibacterial activity against both of tested bacteria with MIC value of 15.6 µg/mL (Table 1).

Table 1. Antimicrobial activity of artocarpin and cyanomaclurin against tested bacteria

Bacteria	MIC (µg/mL)		
	Artocarpin	Cyanomaclurin	Tetracycline
S. pyogenes	1.9	15.6	1.9
S. epidermidis	1.9	15.6	1.9

In order to know any interaction between artocarpin and cyanomaclurin against tested bacteria, checkerboard study was then performed. In all combinations of cyanomaclurin and artocarpin, artocarpin gave a synergistic effect to cyanomaclurin against both of tested bacteria with FICs of 0.25 (Table 2). In contrast, cyanomaclurin gave only an additional effect to artocarpin against both of tested bacteria at the combination ratios (cyanomaclurin: artocarpin) of 1:2 and 1:3, with FICs of 1. The combinations containing artocarpin less than 50% showed indifference effect.

Table 2. Fractional inhibitory concentration (FIC) of a combination of two compounds against *S. pyogenes* and *S. epidermidis*.

	S. pyogenes				*S. epidermidis*			
	MIC_a	MIC_c	FIC	Type of interaction	MIC_a	MIC_c	FIC	Type of interaction
Cyanomaclurin-Artocarpin (3:1)								
Cyanomaclurin	15.6	3.9	0.25	Synergy	15.6	3.9	0.25	Synergy
Artocarpin	1.9	3.9	2.05	Indifference	1.9	3.9	2.05	Indifference
Cyanomaclurin-Artocarpin (2:1)								
Cyanomaclurin	15.6	3.9	0.25	Synergy	15.6	3.9	0.25	Synergy
Artocarpin	1.9	3.9	2.05	Indifference	1.9	3.9	2.05	Indifference
Cyanomaclurin-Artocarpin (1:1)								
Cyanomaclurin	15.6	1.9	0.25	Synergy	15.6	3.9	0.25	Synergy
Artocarpin	1.9	1.9	1	Addition	1.9	3.9	2.05	Indifference
Cyanomaclurin-Artocarpin (1:2)								
Cyanomaclurin	15.6	1.9	0.25	Synergy	15.6	1.9	0.25	Synergy
Artocarpin	1.9	1.9	1	Addition	1.9	1.9	1	Addition
Cyanomaclurin-Artocarpin (1:3)								
Cyanomaclurin	15.6	1.9	0.25	Synergy	15.6	1.9	0.25	Synergy
Artocarpin	1.9	1.9	1	Addition	1.9	1.9	1	Addition

MIC_a : MIC of one compound alone
MIC_c : MIC of combination of two flavonoids

DISCUSSION

Along with the disc diffusion method, the broth microdilution method has been recognized as the gold standard for determining the potency and sensitivity of antibacterial activity. In this investigation, it was discovered that artocarpin demonstrated potent antibacterial activity against *S. pyogenes* and *S. epidermidis* using broth microdilution methods. It was comparable to

tetracycline, which served as a positive control. On the other hand, cyanomaclurin exhibited a low level of antibacterial activity against both bacteria tested. The results of this study validated our previous work, which shown that artocarpin possessed significant antibacterial activity against a variety of bacteria, including *S. aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Septama & Panichayupakaranant, 2015). Additionally, this chemical was found to be highly effective at altering the membrane permeability of *S. mutans* (Septama & Panichayupakaranant, 2017). In contrast, cyanomaclurin has been shown to suppress the growth of certain strains of Gram-positive bacteria, including *S. mutans* and *Bacillus subtilis* (Septama & Panichayupakaranant, 2015).

The checkerboard research was then conducted to see whether artocarpin and cyanomaclurin had any interaction with the microorganisms investigated. Artocarpin synergized with cyanomaclurin against both tested bacteria in all combinations. In comparison, cyanomaclurin had no effect when combined with artocarpin against either of the tested microorganisms. Furthermore, the results shows that the combinations containing less than 50% artocarpin had no impact against all bacteria tested. The results of this investigation corroborated our prior findings that artocarpin had a synergistic effect against tested microorganisms, including *P. aeruginosa*, when combined with many antibiotics and a natural occurring compound called lowsone methyl-ether (Panichayupakaranant, et al., 2019; Septama, et al., 2022). When two chemicals are combined, their antibacterial action may be enhanced. It has been noted that the interaction of two compounds can result in synergistic activity, although one chemical may work through a different mechanism to provide the same pharmacological effect. Additionally, when chemicals interact with the bacteria at the same active site, an agonistic effect occurs (Yang, et al., 2014).

CONCLUSION

It can be concluded that artocarpin and cyanomaclurin possessed antibacterial activity against *S. pyogenes* and *S. epidermidis.* These compounds in combination produced synergistic and additivity interaction. No antagonist effect was observed in any combinations of these two flavonoids. These results suggested that combination of cyanomalurin and artocarpin at the ratio 1:2 that gave additional antibacterial effect may be an alternative source of pure artocarpin.

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