

FICI value of *aquilaria malaccensis* leaves extract and amoxicillin against *Proteus mirabilis* and *Pseudomonas aeruginosa*

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Abstract

Infection is a common disease caused by microbes. The use of antimicrobial drugs such as Amoxicillin is most widely used in Indonesia, but has develop resistance. The resistance of Amoxicillin can be overcome by combining it with Karas leaves (*Aquilaria malaccensis* Lam.) which has an antibacterial activity. This research was conducted to know the value of Fractional Inhibitory Concentration Index (FICI) from the combination of ethanolic Karas leaves extract (*A.malaccensis* Lam.) with amoxicillin in Gram-negative bacteria test, that is, *Proteus mirabilis* and *Pseudomonas aeruginosa*. This research was used disc diffusion method by Kirby Bauer. Minimum Inhibitory Concentration (MIC) result of ethanolic karas leaves extract (*A.malaccensis* Lam.) in *P.mirabilis* and *P.aeruginosa* was 0.5mg/ml. MIC's result of amoxicillin in *P. mirabilis* and *P.aeruginosa* sequentially were 0.0039 and 0.0625 mg/ml. Then, evaluation of MIC value from combination of ethanolic karas leaves extract and amoxicillin ($\frac{1}{4}$ x MIC, $\frac{1}{2}$ x MIC, 1 x MIC, 2 x MIC and 4 x MIC) showed that the combination of ethanolic karas leaves extract with amoxicillin has characteristics of FICI value on each bacteria, *P. aeruginosa*: 0.5 (synergistic) and *P.mirabilis*: 8 (antagonist)

Keyword: antibacteria, *Aquilaria malaccensis*, amoxicillin, FICI

Introduction

Infection is a disease caused by pathogenic bacteria. Bacteria are divided into two groups based on the difference of the bacteria cell wall i.e Gram-positive bacteria and Gram-negative bacteria. Gram-negative bacteria cell wall have more toxic lipopolysaccharides (toxins) and bacterial outer membrane as its defense system (Campbell et al, 2003). Infectious bacteria such as *Proteus mirabilis* and *Pseudomonas aeruginosa* cause gastroenteritis, produce gastrointestinal disease and cause some intestinal and extra-intestinal infections such as urinary tract infections (Ebringer, 2009; Microbewiki, 2016; Porco, 1995). Infection can be healed using antibiotics. Antibacterial and antimicrobial drugs are one example of broad-spectrum antibiotics, such as amoxicillin is effective with both Gram-positive and Gram-negative (Kee and Hayes, 1996). The use of antibacterial drugs in

Indonesia is mostly amoxicillin (Pradipta et al., 2015; Saptarini, 2012).

The use of relatively high antibiotics creates problems especially in resistance. Resistance is the ability of bacteria to neutralize and weaken the working of antibiotics (Kemenkes RI, 2011) to overcome the resistance of antimicrobial compounds from plants, which found as enhancers synergistic effect combined with using antibiotics (Aiyegoro & Okoh, 2009; Stermitz et al, 2000). Ethanolic karas leaves extract (*Aquilaria malaccensis* Lam.) have been known with its antibacterial activity based on research by Robiyanto and Sari (2017). Karas (gaharu) has secondary metabolite compound as antibacterials namely alkaloids, flavonoids, phenols, steroids, tannins, saponins, and terpenoids (Huda et al, 2009). *A.malaccensis* Lam. has another name, that is *A. aggallocha* (Integrated Taxonomic Information System, 2011).

This research is done to evaluate the effect of ethanolic Karas leaves extract as antibacterial and its combination with amoxicillin using Kirby Bauer's disc diffusion method. The hypothesis of this research is the combination of ethanolic Karas leaves extract with amoxicillin can inhibit the growth of *P.aeruginosa* and *P.mirabilis* that have a synergistic characteristics.

Methods

Materials. The materials that used in this research were aquadest sterile, amoxicillin (Shadhong Bio-Technology), aqua pro injection, ethanolic Karas leaves extract (*Aquilaria malaccensis* Lam.), DMSO (Merck), Mueller-Hinton Agar (Himedia), NaCl 0,9%.

Sterilization. Sterilization of non-glass equipment are sterilized by autoclave and set at 121°C with a pressure at 15 psi (per square inch) for 15 minutes. Glass equipment sterilized in oven with temperature 160-170 °C for 2 hours. Moreover, ose needles and tweezers are sterilized using bunsen fire.

Making Medium for Bacteria Test. Nineteen (19) grams of MHA dissolved in aquadest sterile of 400mL, checked pH of the medium of 7,3, then sterilized in autoclave in 121°C for 15 minutes

Inoculums preparation. Bacterial colonies were taken from the stock cultures by using Ose needle and suspended in 10 mL NaCl 0.9% until the turbidity was obtained. The turbidity that obtained before then synchronized with 0.5 McFarland turbidity standard, that is, equivalent with the growth rate of $1,5 \times 10^8$ bacterial cell per mL. If the turbidity is equal, the bacterial suspension can be used as the bacterial test.

Ethanolic extract of karas leaves solution preparation. Stock solution of ethanolic extract of karas leaves was 1 mg/mL. Preparation of stock solution was done by diluting 10 mg extract with DMSO 20% and then added with aquadest up to 10 mL. Then the stock solution was diluted into 0.5 mg/mL. This concentration was based on

MIC values of *Pseudomonas aeruginosa* and *Proteus mirabilis* bacteria. (Sari, 2017)

Preparation of amoxicillin solution. Stock solution of amoxicillin was 1 mg/mL. The stock solution preparation of amoxicillin was done with diluting 10 mg amoxicillin powder in 10 mL DMSO 20%, then series of concentration was made (as represented in table 1).

Table 1. The Concentration Variant of Amoxicillin

Variant Concentrations	(mg/mL)
A	0,5
B	0,25
C	0,125
D	$6,25 \times 10^{-2}$
E	$3,12 \times 10^{-2}$
F	$1,56 \times 10^{-2}$
G	$7,8 \times 10^{-3}$
H	$3,9 \times 10^{-3}$
I	$1,9 \times 10^{-3}$
J	9×10^{-4}

Preparation of combination of ethanolic Karas leaves ethanolic extract and amoxicillin solution. The concentration of combined solutions that was used is 1, 0.5 and 0.25 from each MIC values of Karas leaves ethanolic extract and amoxicillin. The comparison of both is 1:1.

Determination of FICI values. FICI values characteristics were $\leq 0,5$ (Synergistic); $> 0,5 - \leq 1$ (Aditive); $> 1 - \leq 4$ (indiferent); > 4 (Antagonist). The FICI values of a combination of Karas leaves extract and amoxicillin was calculated based on the formula below (Dipiro et al, 2008):

$$FICI = FIC_A + FIC_B = \frac{MICCA}{MICA} + \frac{MICCB}{MICB}$$

Information:

- FICI = Fractional Inhibitory Concentration Index;
- FICA = Fractional Inhibitory Concentration of substance A;
- FICB = Fractional Inhibitory Concentration of substance B;
- MICCA = Minimum Inhibitory Concentration of substance A used in combination;
- MICCB = Minimum Inhibitory Concentration of substance B used in combination;
- MICA = Minimum Inhibitory Concentration of substance A;
- MICB = Minimum Inhibitory Concentration of substance B

Result and Discussion

The assay was begun with measurement of MIC values of amoxicillin against on pathogenic bacteria, *P. mirabilis* and *P. aeruginosa*. MIC values were 0.0039 and 0.065mg/ml. According to CLSI (2015), standardized criteria of MIC values for amoxicillin was resistance against *P. aeruginosa* ($\geq 32\mu\text{g/mL}$), but was not resistant against *P. mirabilis* ($\leq 0,008\text{mg/ml}$) (CLSI, 2015).

Futhermore, MIC value from combination of amoxicillin and ethanolic karas leaves extract was obtained (0.5mg/ml). Measurement of FICI value of combination of ethanolic Karas leaves extract and amoxicillin was represented in Table 2. Combination of concentration ethanolic Karas leaves extract and amoxicillin were

$\frac{1}{4}$ MIC, $\frac{1}{2}$ MIC, 1MIC, 2MIC and 4MIC to of each substance. It confirmed that FICI values have antagonist or indifference characteristics. Inhibition zone of each bacteria can be seen in Figure 1.

Amoxicillin belongs to penicillin (beta-lactam antibiotics), has broad-spectrum in both Gram-positive bacteria and Gram-negative bacteria. Penicillin can inhibit the growth of bacteria by interfering to transpeptidation reaction of bacterial cell wall synthesis. Because of this inhibition, it will stop the synthesis of peptidoglycan and kills the bacteria. Amoxicillin resistance against *P. aeruginosa* can be caused by its inability to reach its workplace in microbial cells. This resistance is also because of inactivation of drugs and modification of antibiotic target.

Table 2. The inhibition zones Diameter of FICI

Bacteria	The Concentration (mg/ml)	The inhibition zones Diameter (mm)			$\bar{x} \pm \text{SD}$	FICI values
	A + E	I	II	III		
<i>Pseudomonas aeruginosa</i>	0,0625 + 0,5	12,25	13,625	13,675	13,18 \pm 0,8	2
	0,0312 + 0,25	12	13,45	12,5	12,65 \pm 0,73	1
	0,0156 + 0,125	11,7	11,6	10,6	11,3 \pm 0,6	0,5
<i>Proteus mirabilis</i>	0,0156 + 2	9	9,8	10,5	9,76 \pm 0,75	8
	0,0078 + 1	0	0	0	0 \pm 0	4
	0,0039 + 0,5	0	0	0	0 \pm 0	2
	0,0019 + 0,25	0	0	0	0 \pm 0	1
	0,0009 + 0,125	0	0	0	0 \pm 0	0,5

Information: A (Amoxicillin); E (Extract); FICI values = FICI ≤ 0.5 (synergistic); FICI $> 0,5 - \leq 1$ (additive); FICI $> 1 - \leq 4$ (indifferent) and FICI > 4 (antagonist).

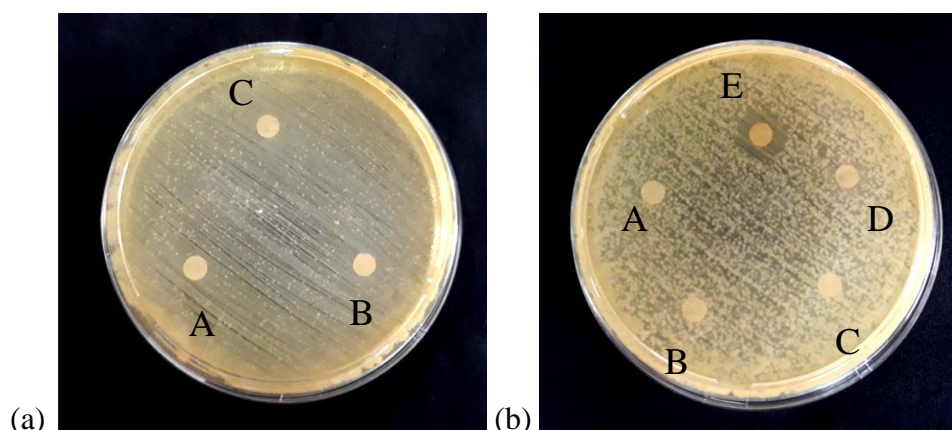


Figure 1. Inhibit zone of combination *Aquilaria malaccensis* extract and amoxicillin

Information: bacteria (a) *Pseudomonas aeruginosa*, (b) *Proteus mirabilis*, Combined concentration A = $\frac{1}{4}$ x MIC extract and $\frac{1}{4}$ x MIC amoxicillin; B = $\frac{1}{2}$ x MIC extract and $\frac{1}{2}$ x MIC amoxicillin; C = 1 x MIC extract and 1 x MIC amoxicillin; D = 2 x MIC extract and 2 x MIC amoxicillin; E = 4 x MIC extract and 4 x MIC amoxicillin

The FICI values of combination of amoxicillin and ethanolic Karas leaves extract on *Pseudomonas aeruginosa* yields synergistic effect (FICI value = 0.5). This synergistic effect is greater than individual effect of amoxicillin or Karas leaves ethanolic extract. Karas leaves extract contains steroid that can decrease the bacteria membrane integrity, so that it can change the morphology of bacteria cell membranes and causes brittle cells and lysis (Muhani et al, 2017).

FICI value of the combination of amoxicillin and Karas leaves extract on *Proteus mirabilis* is 8, and it is categorized as an antagonist, so that single use of either amoxicillin or karas leaves extract is better than combination of both. This result probably because *Proteus mirabilis* membrane only has one porin. Porin is needed for beta-lactam antibiotics as their pathway inserting into bacteria. Karas leaves contains several secondary metabolites such as alkaloids, flavonoids, phenols, steroids, tannins, saponins and terpenoids (Huda et al , 2009). This secondary metabolites is suspected blocking the entry of antibiotics into bacterial cells (Mitsuyama et al, 1987).

The difference mechanism of action between extract and amoxicillin as antibacterial can cause different criteria of their combination. The antagonist or reduction effect of combination between the extract and amoxicillin may occur due to incompatibility of extract metabolites and antibiotics or competitive properties in inhibiting bacteria (Ofokansi et al, 2013). The use of single extracts can show a decrease in bacterial action. The extract contains several bioactive compounds differently, which makes the ability of microbial inhibition difficult compared to a single antibiotic.

Conclusion

Inhibitory zone diameter and FICI value of a combination of ethanol extract of Karas leaves (*Aquilaria malaccensis* Lam.) and amoxicillin in *Pseudomonas aeruginosa* were 11.3 ± 0.6 mm and 0.5 (synergistic), and in

Proteus mirabilis were $9.76 \pm 0, 75$ mm and 8 (antagonists).

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