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ANTIBACTERIAL ACTIVITY FROM ETHANOLIC EXTRACT KARAS (*AQUILARIA SP.*) LEAVES AGAINST PATHOGENIC BACTERIA

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ABSTRACT

Aquilaria microcarpa Baill. and *Aquilaria malacanecis* Lamk. are group of gaharu producing plants species that are widely cultivated in Indonesia, especially in West Borneo. Based on the empirical data the *Aquilaria sp.* leaves are usually used to treat the wound in the skin area. The purpose of this research is to determine the Minimum Inhibitory Concentration (MIC) value from ethanol extract of the leaves by disc diffusion technique. The results showed that the MIC value of ethanol extract of *Aquilaria microcarpa* Baill. Leaves to *Staphylococcus aureus* and *Bacillus cereus* bacteria were 1 mg/mL, while MIC values against *Staphyloccus epidermidis*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli* were 1,25 mg/ mL, lastly on *Salmonella typhi* and *Proteus mirabilis* were 10 mg/mL. Then, *Aquilaria malacancis* showed that MIC value against all pathogenic bacteria were 1,25 mg/ml. The conclusion of this research was that *Aquilaria microcarpa* Baill and *Aquilaria malacencis* Lamk have potencial antibacterial activity against Gram positive bacteria and Gram negative bacteria.

KEYWORDS: Aquilaria microcarpa Baill., Aquilaria malacanecis Lamk, MIC, Ethanolic extract.

INTRODUCTION

The presence of infections can occur anywhere in living organisms, such as the skin, digestion, urinary tract and others. Based on previous research there are bacteria that have become resistance to certain antibiotics that are classified as dangerous pathogens.[1,2] Indonesia is a tropical country rich in plants that have a healing effect. So it can be use as medicines, one of which is gaharu-producing plants. From the empirical study *Aquilaria sp* have potencial effect as wound therapy.[3]

The current research aimed to investigate the MIC value from two plant species there are *Aquillaria microcarpa* and *Aquilria malacencis* against 8 pathogenic bacteria like *Escherichia coli, bacillus subtillis, Pseudomonas aeruginosae, Salmonella thypi, Staphylococcus epidermidis*, *Staphylococcus aereus*, *Proteus mirabilis*, and *Bacillus cereus.*

MATERIAL AND METHOD

Ethics

The research was approved by the Ethics Committee at Health Faculty Tanjungpura University No. 7730/UN22.9/DL/2017

Material

Distilled water, ethanol (Merck), alcohol 70%, DMSO 20%, Muller Hinton Agar Medium (Oxoid), Mc. Farland's standard solution, Antibiotic as positive control.Pathogenic bacteria used in this study is a pure cultures of *Staphylococcus epidermidis*, *Staphylococcus aereus*, *Proteus mirabilis*, *Escherichia coli*, *Bacillus cereus*, *Bacillus subtilis*, *Salmonella thypi* and *Pseudomonas aeruginosa* which is a collection of Pharmaceutical Biology Laboratory, Faculty of Medicine, University of Tanjungpura Pontianak, West Borneo.

Methods

Extraction

Aquilaria sp leaves extraction was performed with a maceration method for 3x24 hours using 96% ethanol solvent. Maseration is used as an extraction method to prevent damage to heat-sensitive compounds and use immersion mechanisms to increase the contact time between the solvent and the sample which allows maximum extraction of the compounds, subsequently sieved with filter paper and white cloth. All the maserates obtained, are fed into the rotary evaporator at 40°C. Furthermore, the remaining filtrate was evaporated using a vapor plate in the waterbath to obtain a viscous extract.[4]

Antibacterial Activity Assay

A total of 20 mL MHA medium with a temperature of 45ºC -50ºC was poured into a petri dish.[5] After it solidify, each Petri dish was inoculated with *Staphylococcus epidermidis*, *Staphylococcus aereus*,

Proteus mirabilis, *Escherichia coli*, *Bacillus cereus*, *Bacillus subtilis*, *Salmonella thypi* and *Pseudomonas aeruginosa* bacteria using ose needles on the surface of the media, then placed some paper discs, each paper disc consisted of negative control of DMSO 20%, positive control of Ciprofloxacin 50µg/ml, Chloramphenicol 30 µg/ml, and ethanol extract of in various concentration with 20 µl volume for each test solution. Then incubated in an incubator at a temperature of 35 ± 2°C for 18-24 hours, after that the diameter of the inhibition zone (clear zone) around the paper disc was measured using calipers.[6]

RESULTS

Extraction

The amount of weight of simplicia used in the extraction process was 84.62 grams and macerated for 3 days with total solvent used was 3.5 liters. Furthermore, the extraction results evaporated by rotary evaporator with a temperature of 40° C, the obtained extract was 45.22 grams. Furthermore the extract was evaporated with waterbath to reduce the water content in the extract and obtained the final result of 25.27 grams. So the result of rendemen was 29,86% from total weight of simplicia used, the extract obtained was categorized as concentrated extract.

Antibacterial Activity Assay

Determination of MIC value of ethanol *Aquilaria microcarpa* Baill. and *Aquilaria malacanecis* Lamk leaves extract was done by using disc diffusion method. In this method the inhibition area or clear zone was formed around the paper disc. A total of 50 µl of inoculation results from MHA media, which optical density has been measured, were dispersed in a petri dish containing 20 mL of solid Nutrient Agar medium.

Based on the results of tests conducted on *Staphylococcus epidermidis*, *Staphylococcus aereus*, *Proteus mirabilis*, *Escherichia coli*, *Bacillus cereus*, *Bacillus subtilis*, *Salmonella thypi* and *Pseudomonas aeruginosa* bacteria using ethanol extract of *Aquilaria microcarpa* Baill. leaf with concentrations of 0,75 mg/ml; 1 mg / ml; 1,25 mg / ml; 2,5 mg/mL, 5 mg / Ml; 7,5 mg/mL, 10 mg/mL, and 15 mg/mL, with negative control of 20% DMSO and positive control of Ciprofloxacin and Chloramphenicol, obtained different MIC values for all bacteria. The result of MIC value determination of ethanol extract of *Aquilaria microcarpa* Baill. leaves was shown in Table 1 and *Aquiaria malacencis* Lamk leaves was shown in Table 2.

Table 1: Antibacterial activity assay *Aquilaria microcarpa* Baill.

MIC		Average diameter of inhibition zone and standard deviation (mm)
Concentration	**Bacteria strain**	
1 mg/ml	*Staphylococcus aereus*	6,12 ± 0,02
	Bacillus cereus	6,07 ± 0,02
1,25 mg/ml	*Staphylococcus epidermidis*	6,10 ± 0,02
	Escherichia coli	6,11 ± 0,02
	Pseudomonas aeruginosa	6,14 ± 0,02
10 mg/ml	*Proteus mirabilis*	6,73 ± 0,02
	Bacillus subtilis	6,82 ± 0,02
	Salmonella thypi	7,02 ± 0,04

Table 2: Antibacterial activity assay *Aquilaria malacanecis* Lamk.

MIC		Average diameter of inhibition zone and standard deviation (mm)
Concentration	**Bacteria Strain**	
	Staphylococcus epidermidis	6,86 mm ± 0,02
	Staphylococcus aereus	6,73 mm ± 0,02
	Bacillus cereus	6,81 mm ± 0,02
	Bacillus subtilis	6,68 mm ± 0,02
1,25 mg/ml	*Proteus mirabilis*	6,59 mm ± 0,02
	Escherichia coli	6,39 mm ± 0,02
	Salmonella thypi	6,65 mm ± 0,02
	Pseudomonas aeruginosa	6,59 mm ± 0,02

DISCUSSION

The MIC antibacterial activity against *Staphylococcus epidermidis*, *Staphylococcus aereus*, *Proteus mirabilis*, *Escherichia coli*, *Bacillus cereus*, *Bacillus subtilis*, *Salmonella thypi* and *Pseudomonas aeruginosa* bacteria proved the MIC value of the ethanol extract of *Aquilaria microcarpa* Baill. leaves that has antibacterial activity against the mentioned pathogenic bacteria above. Based on data of inhibition zone diameter, it shows difference of MIC value of ethanol leaf extract tested to some pathogenic bacteria, this may be due to the different characteristic of each bacteria and different response to antibacterial power of ethanol extract of *Aquilaria microcarpa* Baill. leaves which affect the MIC value.

The presents study from *Aquilaria microcarpa* Baill.

leaves and *Aquilaria malacanecis* Lamk leaves extract were also analyzed with SPSS version 23.0 for windows indicating that the data were normal and distributed homogeneously ($p > 0.05$), and have significant differences between test bacteria ($p < 0.05$) in each variation of concentration of the MIC test.

Based on this research known that MIC etanolic extract *Aquilaria malacencis* Lamk against *Escherichia coli*, *Bacillus cereus*, *Salmonella typhi*, *Bacillus subtilis*, *Staphylococcus aureus*, *Proteus mirabilis*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* were 1,25 mg/ml.The results has been showed that antibacterial activity from etanolic extract *Aquilaria malacencis* Lamk more sensitive against Gram positive bacteria than Gram negative bacteria. In the present investgation, it has been found that various caused by secondary metabolite from extract. Further studies should be undertaken to identify the active compound which can be used in drug development especially have known antibacterial activity.

CONCLUSIONS

Based on research that has been done,it can be concluded that the ethanol extract of *Aquilaria microcarpa* Baill. leaves and *Aquilaria malacencis* Lamk have different MIC value to the type of pathogen bacteria tested more sensitive against Gram positive bacteria than Gram negative bacteria. MIC value of *Aquilaria microcarpa* Baill. leaves against *Staphylococcus aereus* and *Bacillus cereus* bacteria was 1,25 mg/ml. for *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa* bacteria was 1,25 mg/ml. Lastly for *Proteus mirabilis*, *Bacillus subtilis*, and *Salmonella thypi* bacteria was 10 mg /ml.

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