ANTIMICROBIAL ACTIVITY OF FRACTIONS OF CEREMAI (*Phyllanthus acidus* (L.) Skeels) LEAVES EXTRACT AGAINST ANTIMICROBIAL RESISTANT BACTERIA

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ABSTRACT

Background: Increasing antimicrobial resistance and toxicity led to the use of herbal medicine as an alternative treatment of various diseases associated with microbes. Ethanol extract of ceremai (*Phyllanthus acidus* (L.) Skeels) leaves had antimicrobial activity against *Candida albicans*, *Esherichia coli*, and *Staphylococcus aureus* (Jagessar, 2008).

Objective: This research tested the antimicrobial activity of the ceremai leaves extract fractions against bacteria resistant to [*Methicillin-Resistant Staphylococcus aureus* (MRSA), *Methicillin-Resistant Coagulase Negative Staphylococci* (MRCNS), *Vancomycin Resistant Enterococcus* (VRE)] and test fungi (*Candida albicans*, *Microsporum gypseum*, and *Aspergillus niger*) through the determination of minimum inhibitory concentration (MIC) and the equivalence to reference antimicrobials.

Methods: Crude drug was extracted by maceration using 96% ethanol. Extracts were fractionated by liquid-liquid extraction using solvents n-hexane, ethyl acetate, and water. Determination of MIC of the ethanol extract and fractions against test bacteria and fungi were performed using agar diffusion method, then followed by determination equivalence of the most potent fractions to reference antimicrobials (tetracycline, ketoconazole).

Outcome measured : MIC and equivalence of the most potent fractions to reference antimicrobials (tetracycline, ketoconazole)

Results : MIC value of ethanol extract of ceremai leaves against VRE, MRCNS, *Candida albicans* was 1%, 0.75%, and 0.05% respectively. MIC values of ethyl acetate fraction against MRSA, VRE, MRCNS were 5%, 2.5%, and 0.63% respectively. MIC values of the most potent ethyl acetate fraction of the ceremai leaves against *Aspergillus niger*, *Candida albicans*, and *Microsporum gypseum* were 5%, 0.08%, and 2.5% respectively. Activity of 1 mg of ethyl acetate fraction was equivalent to 0.05 µg, 0.36 µg, and 0.92 µg of tetracycline respectively against MRSA, VRE, and MRCNS. Activity of 1 mg of ethyl acetate fraction of ceremai leaves was equivalent to 0.32 µg, 0.01 µg, and 0.03 µg ketoconazole respectively against *Aspergillus niger*, *Candida albicans*, and *Microsporum gypseum*.

Conclusion: In conclusion, the ceremai leaves extract had activity against VRE, MRCNS, *Candida albicans*. The ethyl acetate fraction had the most potent antimicrobial activity against MRSA, VRE, MRCNS, *Aspergillus niger*, *Candida albicans*, and *Microsporum gypseum*.

Keywords: antimicrobial, *Phyllanthus acidus* (ceremai leaves), ethyl acetate fraction

INTRODUCTION

The use of synthetic antimicrobials have many challenges due to antimicrobial resistance. Antimicrobial resistance is the ability of microbes such as bacteria, viruses, parasites, or fungi to grow even given antibiotics (NIAD, 2009). Antimicrobial resistance is caused by the improper use of antibiotics, for example, the use of inadequate doses, the use of irregular or not continuous and the treatment time is not long enough (Wattimena, 1991). Increasing antimicrobial resistance led to the use of herbal medicine as an alternative treatment of various diseases associated microbes. Search drugs derived from plants also have accelerated in recent years (Cowan, 1999).

Phylllanthus acidus (L.) Skeels, commonly known as ceremai plant is a plant whose leaves contain saponins, flavonoids, tannins, and polyphenols, typically used to treat nausea and thrush (Hutapea, 1993). The ethanol extract of ceremai leaves have antimicrobial activity against *Candida albicans*, *Esherichia coli*, and *Staphylococcus aureus* (Jagessar, 2008). However, there has been no activity test of ethanol extract of leaf extracts and fractions ceremai against antimicrobial resistant bacteria. Therefore, in this study will test the antimicrobial activity of extracts and fractions ceremai leaf extract against antimicrobial resistant bacteria is *Methicillin-Resistant Staphylococcus aureus* (MRSA), *Vancomycin Resistant Enterococcus* (VRE), *Methicillin-Resistant Coagulase Negative Staphylococci* (MRCNS).

METHODS

Plant Extract

Leaves of ceremai collected from the District Coblong Bandung on February 2013 and determined in the Herbarium Bandungense, School of Biological Sciences and Technology, Institute of Technology Bandung. The leaves were dried with aerated and macerated with 96% ethanol for 3 x 24 hours. The extracts were concentrated under reduced pressure using *rotary vacuum evaporator* (Buchi). Extracts were fractionated by liquid-liquid extraction using solvents n-hexane, ethyl acetate, and water. A number of 25g of extract was weighed and then dissolved in 250 mL of hot water, then extracted three times with n-hexane in a 250 mL separating funnel. Then the n-hexane fraction collected in a beaker glass. Entered into a separation funnel 250 mL ethyl acetate, separation, then ethyl acetate taken part. The remaining part of the polar fraction. Three fractions were dried until the solvent exhausted. Dried fractions were weighed and then tested antimicrobial activity .

Suspension Microbes

Bacteria suspended in a liquid medium and incubated at 37°C for 18-24 hours. The suspension was shaken and turbidity set up 0,08-0,13A obtained absorbance at 625 nm wavelength (CLSI, 2010).

Determination of Antimicrobial Activity and Minimum Inhibitory Concentration

MIC determination of the ethanol extract of leaves ceremai performed by the agar diffusion method using paper discs. Paper discs containing extracts Mueller Hinton Agar affixed to the media that has been inoculated with the test bacteria. The media were incubated at 37^{o}C for 18-24 hours for MRSA, VRE, and MRCNS. MIC is determined by looking at the smallest minimum inhibitory concentration can produce zone of inhibition.

RESULTS AND DISCUSSION

The results of antimicrobial activity of ethanol extracts and fractions of ceremai leaves are shown in Tables 1 and 2.

Table 1. Results of Antimicrobial Activity of Ethanol Extract Leaf Ceremai against MRSA , VRE , and MRCNS

Concentration	Zone of Inhibition (mm)		
	MRSA	VRE	MRCNS
3%	-	6,5 ± 0,1	6,55 ± 0,5
2%	-	7,0 ± 0,1	6,75 ± 2,5
1%	-	6,75 ± 2,5	7,0 ± 0,1
0,75%	-	-	7,0 ± 0,1
0,5%	-	-	-
0,375%	-	-	-
0,25%	-	-	-

Table 2. Results of Antimicrobial Activity of Leaf Extract Fraction Ceremai against MRSA, VRE, and MRCNS

Test Material	Concentration	Zone of Inhibition (mm)		
		MRSA	VRE	MRCNS
Water fraction	10%	-	-	34,17 ± 0,27
	5%	-	-	27,45 ± 0,42
	2,5%	-	-	21,58 ± 3,99
	1,25%	-	-	11,15 ± 0,90
	0,63%	-	-	10,30 ± 0,90
	0,31%	-	-	-
	0,16%	-	-	-
	0,08%	-	-	-
Ethyl acetate Fraction	10%	9,36 ± 0,53	9,42 ± 0,6	26,9 ± 1,4
	5%	8,2 ± 0,5	7,2 ± 0,66	19,3 ± 0,23
	2,5%	-	7,2 ± 0,32	8,3 ± 0,7
	1,25%	-	-	8,3 ± 1,26
	0,63%	-	-	7,25 ± 0,22
	0,31%	-	-	-
	0,16%	-	-	-
	0,08%	-	-	-
n-Hexsan fraction	10%	-	-	8,97 ± 0,18
	5%	-	-	8,57 ± 0,17
	2,5%	-	-	-
	1,25%	-	-	-
	0,63%	-	-	-
	0,31%	-	-	-
	0,16%	-	-	-
	0,08%	-	-	-

Results of testing antimicrobial activity against bacteria test showed that the ethanol extract of the leaves ceremai can inhibit bacterial growth VRE, and MRCNS. In VRE, the diameter of inhibition zone produced ethanol extract of leaves ceremai present in a concentration of 1%, 2%, and 3%. The diameter of the largest inhibition zone produced by a concentration of 2% in the amount of 7.0 ± 0.1 mm and the smallest diameter of the inhibition produced by a concentration of 3% in the amount of 6.5 ± 0.1 mm, but the minimum inhibitory concentration ceremai leaf ethanol extract against VRE bacteria present in concentration of 1%. In MRCNS, the largest inhibition zone diameters obtained in the extract concentration of 0.75% in the amount of 7.0 ± 0.1 mm, while the smallest diameter of the inhibition zones obtained at a concentration of 3% in the amount of 65.5 ± 0.5 mm , and the MIC value of ethanol extract ceremai leaves against MRCNS ie at a concentration of 0.75%.

Results of testing antimicrobial activity against tested bacteria extract fractions showed that the fraction of water and n-hexane fraction only produce inhibition zone against MRCNS. Ethyl acetate fraction is the fraction of the best because it produces a third zone of inhibition against the test bacteria. MIC values against MRSA ethyl acetate fraction produced by a concentration of 5%, and the MIC value against VRE ethyl acetate fraction produced by a concentration of 2.5%, whereas the MIC value against MRCNS ethyl acetate fraction produced by the concentration of 0.63%. The diameter of inhibition zone produced by ethyl acetate fraction of the three bacteria can be seen in Figure 1.

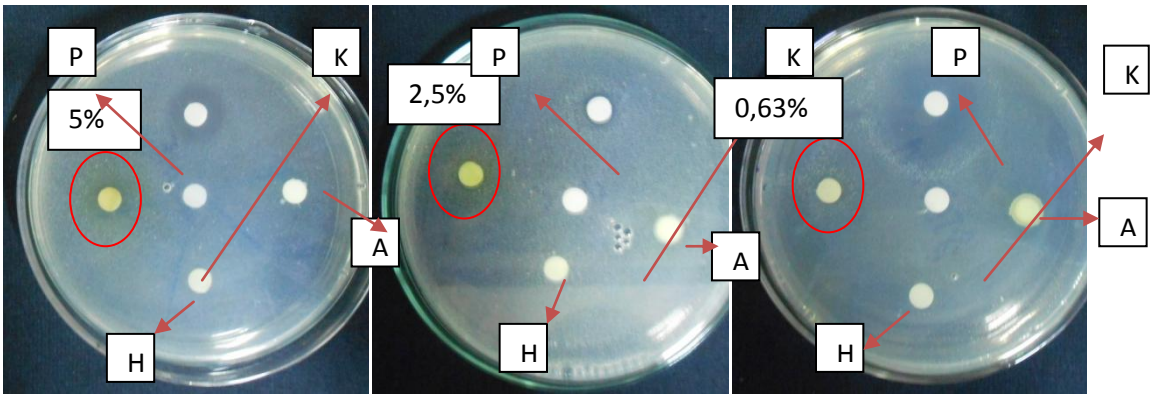

Figure.1. Results of MIC test of ethyl acetate fraction ceremai leaves against MRSA, VRE, and MRCNS

Determination equivalence of the most potent fractions to reference antimicrobials

Equivalence to reference antimicrobials is done by measuring the diameter of the inhibition produced by tetracycline against microbes in several concentration, then compared with the fraction of ethyl acetate as the most potent fractions give as antimicrobial activity against microbes. The relationship between tetracycline log concentration with zone of inhibition input to the equation of the line so we can get the equivalence, the results can be seen in Table 3 and Curve of tetracycline activity to bacteria test can be seen in Figure 2.

Table3. Linear Regression equations between Tetracycline Log Concentration with Zone of Inhibition against MRSA, VRE, and MRCNS

Bacteria Test	Reference Antimicrobials	Linear Regression equations	R^2
MRSA	Tetracycline	y = 8,47x + 3,27	0,94
MRCNS		y = 4,55x + 2,13	0,89

y = zone of inhibition
x = log concentration
R = relation coefficient

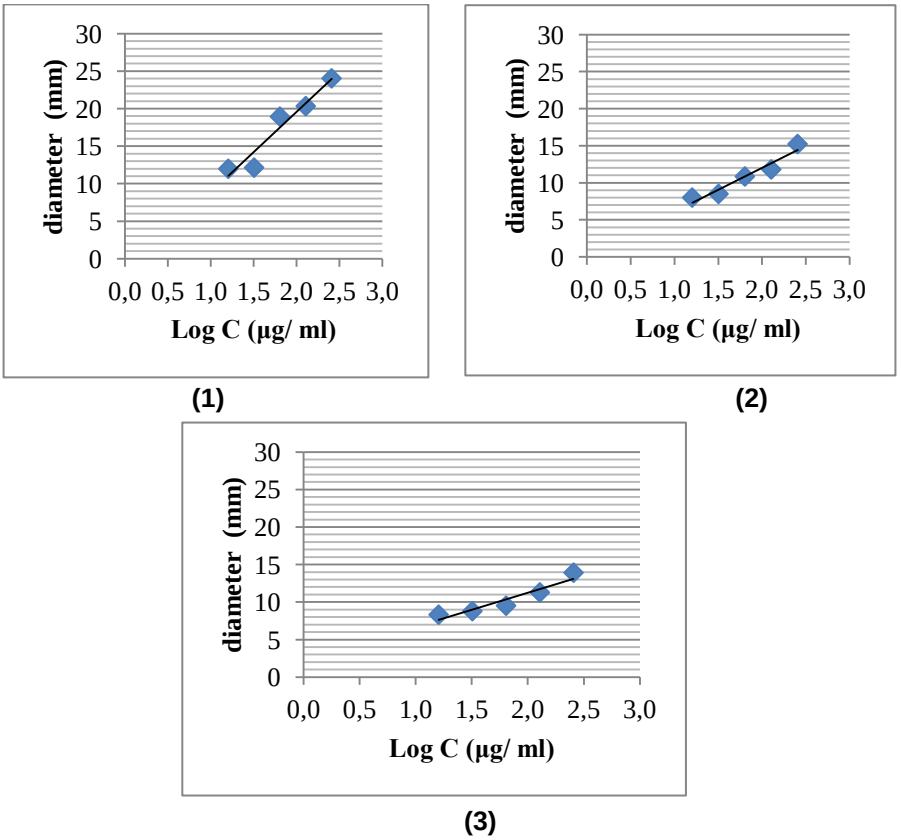

Figure 2. Curve of Tetracycline activity to bacteria test.

Equivalence of Tetracycline can be calculated through the linear regression equation. Activity of 1 mg of ethyl acetate fraction was equivalent to 0.05 µg, 0.36 µg, and 0.92 µg of tetracycline respectively against MRSA, VRE, and MRCNS.

CONCLUSION

Results of the present study suggested that ceremai leaves extract had activity against VRE and MRCNS. The ethyl acetate fraction had the most potent antimicrobial activity against MRSA, VRE, and MRCNS. MIC values of ethanol extract of ceremai leaves against VRE and MRCNS was 1%, 0.75% respectively. MIC values of ethyl acetate

fraction against MRSA, VRE, MRCNS were 5%, 2.5%, and 0.63% respectively. Activity of 1 mg of ethyl acetate fraction was equivalent to 0.05 µg, 0.36 µg, and 0.92 µg of tetracycline respectively against MRSA, VRE, and MRCNS. Results of the present study suggested that ceremai leaves extract had activity against VRE and MRCNS. The ethyl acetate fraction had the most potent antimicrobial activity against MRSA, VRE, and MRCNS.

DISCLOSURE: -

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