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ANTIBACTERIAL ACTIVITY OF KARAMUNTING (*RHODOMYRTUS TOMENTOSA* (AITON) HASSK) LEAF EXTRACT AND FRACTIONS

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ABSTRACT

Objective: This study was to analyze the karamunting leaf extract and fractions effectiveness against *Staphylococcus aureus* and *Pseudomonas aeruginosa* infection.

Methods: Phytochemical screening is done in the extract and fractions of karamunting leaf continue with Standard agar well diffusion method for *S. aureus* and *P. aeruginosa.* Dimethyl sulfoxide was used as negative control, and standard antibiotic Kalmicetine (chloramphenicol) was used as positive control.

Result: The ethyl acetate fraction showed the highest antibacterial activity against S. aureus and P. aeruginosa.

Conclusion: Karamunting leaf can be used to treat the bacterial infection diseases especially infection from S. aureus and P. aeruginosa.

Keywords: Antibacterial, Extract, Fraction, Karamunting, Staphylococcus aureus and Pseudomonas aeruginosa.

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INTRODUCTION

It is believed that the history of humankind medicine treatment already using herbal medicine to treat with many diseases. Some of the advantages of herbal medicines are that they have fewer side effects and safe to use over time. They also inexpensive compared to the formulated drugs and they are readily available [1]. One of the common diseases is an infection from microbial. Recently, the antibiotic drugs mostly have been resistance and become an increasingly serious problem [2] and this case, making the development of alternative antibiotics a very urgent issue.

Medicinal plants have been studied extensively as alternative antimicrobial therapeutic agents [3]. One of the rising plants is Karamunting (*Rhodomyrtus tomentosa* [Aiton] Hassk). Karamunting has been used in traditional Vietnamese, Chinese and Malaysian medicine to treat diarrhea [4]. Karamunting are known to be effective in antibiofilm, antibacterial, antifungal, antioxidant, antidiarrheal, osteogenic, and anti-inflammatory [5-10]. The woods of karamunting are employed to cure wounds and injuries [11].

The purpose of this study was to explore leaf of karamunting for possible antibacterial activity, especially on *Staphylococcus aureus* and *Pseudomonas aeruginosa* in skin infection. Ethanolic extract of the leaf of the plant and its fractions in different solvents were tested against *S. aureus* and *P. aeruginosa*.

METHODS

Sample collection

Fresh leaf samples of Karamunting (*R. tomentosa* (Aiton) Hassk) were collected from Sipoholon district, North Sumatera, Indonesia, in the month of July 2016. The collected samples were brought to the laboratory on the same day and authenticated by the Indonesian Institute of Sciences: Research Center for Biology (Code: 1581/ IPH.1.01/If.07/VII/2016). The dried leaf samples were crushed and ground to obtain a finely divided powder.

Extraction and fractionation

Ethanolic extract of the powder was obtained by the maceration method for 7 days followed by filtration. The ethanolic solvent was evaporated on a rotary evaporator to obtain crude ethanolic extract and dried using freeze dryer to get the dried crude ethanolic extract. The ethanolic extract was then suspended in distilled water and partitioned with hexane and ethyl acetate to obtain fractions of these solvents. The solvents were removed on a rotary evaporator to obtain dried fractions [11,12].

Preliminary phytochemical screening

Phytochemical screening carried out on ethanolic extract, hexane fraction, and ethyl acetate fraction includes examining the chemical secondary metabolites of alkaloids, flavonoids, glycosides, saponins, tannins, triterpenoids, and steroids [13-16].

Antibacterial activity

Microorganisms used

The bacteria used are Gram-positive bacteria (*Staphylococcus aureus*/ATCC 6538) and Gram-negative bacteria (*P. aeruginosa*/ATCC 9027) which were obtained from Microbiology Laboratory, Faculty of Pharmacy, University of Sumatera Utara, with each concentration of culture test was 10^6 cfu/ml which has been likened to the turbidity standard solution of Mac Farland.

Agar well diffusion method

Antibacterial activity was tested by the agar well diffusion method. Muller Hinton Agar was prepared and autoclaved for 15–20 min and poured in Petri plates and then cooled. The different concentrations (200, 400, and 600 mg/ml) of extract and fractions were used for this study. The Petri plates were kept for 3–4 h at low temperature and incubated at 36–37°C for 24 h. Antibacterial activity was recorded by measurement of the zone of inhibition around each disc in the plate using zone reader. Dimethyl sulfoxide was used as negative control, and standard antibiotic Kalmicetine (chloramphenicol) was used as positive control. Each assay was used triplicate for determination of antibacterial test [11].

Table 1: Phytochemical screening result of karamunting leaf extract and fractions

Screening	Ethanolic extract	Hexane fraction	Ethyl acetate fraction
Alkaloids	-	-	-
Flavonoids	+	-	+
Glycosides	+	-	+
Tannins	+	-	+
Saponins	+	-	+
Triterpenoid	+	+	-
Steroids	+	+	-

Table 2: Antibacterial activity of *karamunting* leaf extract and fractions

Samples	Zone of inhibition (mm)	
	S. aureus	P. aeruginosa
Ethanolic extract (mg/ml)		
200	12.56±0.75	14.56±0.14
400	13.46±0.18	15.4±0.3
600	14.5±0.14	16.13±0.48
Hexane fraction		
200	17.8±0.14	18.3±0.25
400	19.23±0.27	15.8±0.38
600	20.13±0.25	14.8±0.07
Ethyl acetate fraction		
200	17.75±0.27	15.73±0.25
400	20.76±0.75	19.23±0.25
600	21.5±0.75	21.43±0.24
Kalmicetine (chloramphenicol)	32.96±0.27	32.8±0.67
DMSO	0	0

S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, DMSO: Dimethyl sulfoxide

RESULTS

Phytochemical screening

Screening results of various extracts and fractions of karamunting leaf showed different chemical compound in different organic solvents.

Antibacterial evaluation

Antibacterial testing was done by agar diffusion method. The zone of inhibition was summarized in Table 1.

DISCUSSION

Karamunting leaves were analyzed for phytochemical composition such as alkaloids, flavonoids, and tannins. The phytochemical screening of crude ethanolic extract, hexane fraction, and ethyl acetate fraction of powdered karamunting leaf recorded very high values for flavonoids and tannins in ethanolic and ethyl acetate fraction. The high values that recorded for the plant extract showed that the powdered karamunting leaf maybe a good source of antimicrobial, especially as an antibacterial agent.

Historically, karamunting leaf has been used by Sipoholon district society against high bacterial infections, especially a skin infection such as acne and wound. In this study, karamunting shows effective against both Gram-positive and negative bacterial strains. Antibacterial activity of different extract and fractions (ethanolic extract, hexane fraction, and ethyl acetate fraction) of karamunting leaf were tested against *S. aureus* and *P. aeruginosa*. According to the result, all of the extract and fractions showed an inhibitory effect against both of these bacteria. It was observed that ethyl acetate fraction showed the most effective against *S. aureus* and *P. aeruginosa*.

This higher inhibition zone of the ethyl acetate fraction of karamunting leaf showed the effect of flavonoid and tannin compounds [17-19],

which are known to have antimicrobial activity. This extract, therefore, may provide a lead for an antibiotic for this pathogen, and the extract itself may be recommended for topical application. In general, less polar fractions were better antimicrobial agents than their more polar counterparts. The mechanism of phenol compounds as antibacterial by denaturing proteins and damaging the lipids on the plasma membrane of microorganisms, thus causing the content of cells coming out [20]. Further investigation into this fraction has great prospects for future antibacterial drugs.

CONCLUSIONS

Karamunting leaf showed a good potential antibacterial agent against *S. aureus* and *P. aeruginosa.* The most active fraction of antibacterial activity was ethyl acetate fraction. The phytochemical results of ethyl acetate also contain a lot of phytochemical compounds especially flavonoids and tannins. The present study supports that; this plant can be used to discover bioactive natural products that may lead to the development of new drugs for bacterial inhibition especially to treat the topical skin diseases.

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AUTHORS CONTRIBUTION

All authors have equal contribution in bringing out this article.

CONFLICT OF INTEREST

None.

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