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RESEARCH ARTICLE

Evaluation on antibacterial activity of Karamunting leaf extract (*Rhodomyrtus tomentosa* (Ait) Hassk*)* with various solvents to *Shigella dysenteriae* and *Salmonella typhi*

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Abstract

Traditionally, Karamunting plants (*Rhodomyrtus tomentosa* (Ait) Hassk) are used to treat several types of infectious diseases, such as diarrheal diseases. Dysentery and typhoid fever are still common in developing countries. Dysentery is caused by the bacterium *Shigella dysenteriae* and typhus is caused by *Salmonella typhi*. This study aimed to evaluate n-hexane, ethyl acetate and ethanol extracts from Karamunting leaf as antibacterial dysentery and typhus. The extract concentrations tested were, 4000, 2000, 1000, 500, 250 and 125 µg/mL. Extraction was carried out in a Soxhlet tool in stages, starting with n-hexane, ethyl acetate and ethanol solvents. Antibacterial activity test was conducted by using the agar diffusion method. The study showed that n-hexane and ethyl acetate extracts were active against the test bacteria while the ethanol extract was inactive. The Minimum Inhibit Concentration (MIC) value of n-hexane and ethyl acetate extracts against *S. dysenteriae* and *S. typhi* bacteria was 250 µg/mL. N-hexane and ethyl acetate extracts contained phenol and flavonoid compounds. Karamunting leaf has the potential to be developed into dysentery and typhoid drugs.

***Keywords*:** karamunting, antibacterial activity test, *S. dysenteriae*, *S. typhi*

INTRODUCTION

Shigellosis is a problem in developing countries, and it is an infection caused by the genus Shigella bacteria. Shigellosis is endemic in developing countries which can cause considerable morbidity and mortality. Among the four species of Shigella, *Shigella dysenteriae* type 1 is very important because it causes the most severe diseases. The main obstacles to the treatment of shigellosis are the ease of S. to spread from person to person and the speed of resistance to antimicrobials (WHO, 2005).

Salmonellosis is also a problem for developing countries. Salmonellosis is caused by *Salmonella typhi* bacteria, in which infection by bacteria occurs from food that contaminated with faeces and faeces that contain *S. typhi* bacteria from the host organism (host). After entering the digestive tract, these bacteria will attack the intestinal wall, causing in damage and inflammation (Jawetz *et al.*, 2005)

It has been widely reported that the bacteria *S. dysenteriae* and *S. typhi* are resistant to various antibiotics such as ampicillin, tetracycline, streptomycin, and chloramphenicol. WHO determined ciprofloxacin as the first line of shigellosis treatment, but now it has been reported to be resistant to these antibiotics (Dewi, 2013). Research conducted in Calcutta, India on multidrug-resistant *S. dysenteriae* in fluoroquinolone antibiotics obtained results of minimal inhibitory levels of ciprofloxacin, norfloxacin and ofloxacin for about 4 mm, 12 mm and 12 mm, respectively. These results indicate that antibiotic resistance has occurred, where the MIC value is only effective above 20 mm (Pazhani, 2004). Medicinal plants can be used as alternative antibacterial agents. One of the potential medicinal plants for infectious drugs that are being studied is karamunting leaf (*R. tomentosa*).

Antibacterial compound from karamunting leaf has been named as rhodomyrtone. Rhodomyrtone is a derivative of phloroglucinol with the name 6,8-dihydroxy-2,4,4,4-tetramethyl-7-(3-methyl-1-oxobuthyl) -9-(2-methylpropyl)-4,9-dihydro-1H-xanthene-1,3(2H)-di-one).

Rhodomyrtone is active to *Staphylococcus aureus* and *Escherichia coli* (Dachrianus *et al.*, 2002; Salni *et al.*, 2003). It is suspected that in addition to rhodomyrtone compounds, there are also other antibacterial compounds in karamunting leaf. Research on rhodomyrtone compounds has been carried out with a focus on antibacterial activity but research on bioactive materials of active extracts as raw material for phytopharmaca drugs has not been widely carried out. In this study, extraction of karamunting leaf was carried out with several kinds of solvents and bioautography testing.

EXPERIMENTAL

Materials

Rhodomyrtus tomentosa was collected at Tanjung Barulak, Tanah Datar, West Sumatra. *S. dysenteriae* dan *S. typhi* were from Biofarma Bandung. The solvents used for extraction were N-hexane, ethyl acetate, ethanol from Sigma-Aldrich. Nutrient agar (NA) and nutrient broth (NB), silicagel 60 GF 254, DMSO, silicagel powder for colum from Merck.

Extraction of karamunting leaf

Karamunting leaf powder of 150 g was extracted by Soxhlet using multilevel solvents, starting from 1 L n-hexane for 5x24 hours and

following by ethyl acetate and ethanol solvents. Liquid extract obtained from all three types of solvents was evaporated using a rotary evaporator until it was thickened like a paste.

Antibacterial activity test

N-hexane, ethyl acetate and ethanol extracts from karamunting leaf were tested for their activity against the bacteria *S. dysenteriae* and *S. typhi*. Antibacterial activity was tested by the diffusion method by paper disc. The diffusion method was carried out by mixing 50 µL of each bacterial suspension into 15 mL of agar medium which has been diluted in a sterile petri dish. The paper disc with 6 mm diameter was placed on a solid media surface. In the disc, a drop of 20 µl of each extract was put into incubator at 37°C for 24 h. The determination of the active extract was performed by using 4000 µg/mL concentration for each extract. The MIC determination was carried out by the agar diffusion method using 6 mm diameter paper disc. The procedure was conducted by following the active extract with concentrations of 4000, 2000, 1000, 500, 250, and 125 µg/mL. The solvent used for extraction was DMSO.

Bioautography test and determination of the group of active antibacterial compounds

The active extract was tested by bioautography to determine the Rf value of antibacterial compounds using Thin Layer Chromatography (TLC). The active extract was sprayed on a silica gel 60 GF254 plates. The active extract was doubled on the chromatogram. These two plates were inserted in a vessel containing n-hexane: ethyl acetate (8:2) eluent. The first chromatogram was used to detect active compounds and chromatogram was placed in a petri dish containing bacterial culture. The active extract in the chromatogram was allowed to stick in the medium for 1 hour. Then, the bioactive material was diffused into the media and removed carefully. The petri dish containing the bacterial culture was incubated for 24 hours then. The clear area which showed bacterial growth inhibition was observed. Then, the active compound area and its Rf value were calculated. The second chromatogram was used to detect chemical compounds by spraying the H_2SO_4 solution on a silica gel plate, followed by drying by heating on hot plate at ± 100°C until bioactive material could be seen based on the color formed. Formation of yellow, purple, and brown colors was indicated for the phenol compound, terpenoids, and tannin class, respectively (Farnsworth, 1996).

RESULTS AND DISCUSSION

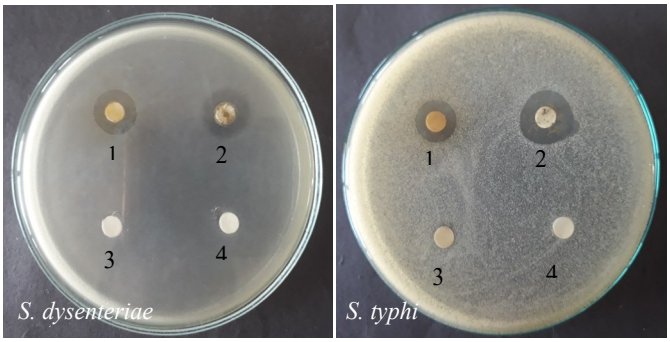
Antibacterial activity of the extract

The extracts of n-hexane, ethyl acetate and ethanol obtained from the extraction process were tested to the bacteria *S. dysenteriae* and *S. typhi* to determine extract active by the diffusion method. Antibacterial activity can be seen in Table 1.

Table 1 Antibacterial activity test extract at a dose of 4000 µg/mL against the bacteria *S. dysenteriae* and *S. typhi.*

Extract	Inhibition zone (mm)	
	S. dysenteriae	*S. typhi*
n-hexane extract	12.33 ± 0.57	11.67 ± 0.57
ethyl acetate extract	14.67 ± 0.57	14.33 ± 0.57
Ethanol extract	0	0

From Table 1, it shows that n-hexane and ethyl acetate extracts were active against the bacteria *S. dysenteriae* and *S. typhi*, while the ethanol extract was not active. The extraction of n-hexane has inhibitory diameters of *S. dysenteriae* and *S. typhi* with 12.33 and 11.67 mm, respectively. Ethyl acetate extract against the bacteria *S. dysenteriae* and *S. typhi* were 14.67 and 14.33 mm. Based on the diameter of the inhibitory, the bioactive ingredients of n-hexane and ethyl acetate extract were belong to the category of medium strength antibacterial ingredients, which in accordance with the provisions of the strength of antibacterial compounds. Davis and Stout (1971) reported that the antibacterial activity of active ingredients was grouped into 4 categories, namely as (> 20-30 mm) that has very strong activity, (<10-20 mm) that has strong activity, (5-10 mm) that has moderate activity and (≤5 mm) that has weak activity.



Note : 1. n-hexane extract 2. Ethyl acetate extract
3. Ethanol extract 4. Control (DMSO)

Fig. 1 Antibacterial activity of n-hexane, ethyl acetate and ethanol extract against *S. dysenteriae* and *S. typhi* bacteria.

This study was similar with the research of Sinulingga *et al.* (2018) where n-hexane and ethyl acetate fractions of ethanol extract from karamunting leaf (*R. tomentosa*) have activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria. Ethyl acetate fraction contains flavonoid compounds and tannins have the potential to treat infectious skin diseases. This study showed that n-hexane and ethyl acetate extracts of karamunting leaf have the potential to treat diarrhera shigellosis and salmonellosis.

In this study, ethanol extract of karamunting leaf was not active against the test bacteria. The extraction was carried out in stages, starting with the n-hexane as nonpolar solvent, then followed by the ethyl acetate as semi-polar solvent and the ethanol as polar solvent. Antibacterial compounds on karamunting leaf were found to be non-polar and semi-polar, so the antibacterial compounds have been extracted in n-hexane and ethyl acetate extract and there was no antibacterial compounds in ethanol extract. Compared to previous research conducted by Limsuwan *et al.* (2012), ethanol extract was carried out directly without extraction with n-hexane and ethyl acetate solvents.

Minimum inhibitory concentration (MIC)

The antibacterial tests used by various types of extracts that have antibacterial activity were n-hexane and ethyl acetate. The MIC values of two extracts were determined. Table 2 and 3 show inhibition zone value of each extract.

Table 2 The inhibition zone value of n-hexane extract on the bacteria *S. dysenteriae* and *S. typhi.*

Concentration µg/mL	Inhibition zone (mm)	
	S. dysenteriae	*S. typhi*
4000	12.00 ± 0.81	11.00 ± 1.50
2000	11.25 ± 1.50	10.25 ± 1.50
1000	10.50 ± 1.50	9.50 ± 1.50
500	9.50 ± 1.91	8.50 ± 0.81
250	8.00 ± 1.41	7.00 ± 1.50
125	0	0

In Table 2, it can be seen that n-hexane extract has the largest diameter of bacterial growth inhibition for concentration of 4000 µg/mL, which was 12.00 mm in the *S. dysenteriae* and 11.00 mm bacteria in *S. typhi* bacteria. The diameter of the inhibitor was smaller with the lower concentration of the extract. At a concentration of 250 µg/mL, the smallest resistance was found in the two types of test bacteria, so the MIC value of n-hexane extract against *S. dysenteriae* and *S. typhi* bacteria was 250 µg/mL.

Table 3 The inhibition zone value of ethyl acetate extract on the bacteria *S. dysenteriae* and *S. typhi.*

Concentration µg/mL	Inhibition zone (mm)	
	S. dysenteriae	*S. typhi*
4000	13.50 ± 1.29	11,50 ± 1.50
2000	12.40 ± 1.50	10,50 ± 1.50
1000	10.50 ± 1.50	9.25 ± 1.50
500	9.50 ± 1.91	8.50 ± 1.50
250	8.00 ± 1.41	7.50 ± 0.81
125	0	0

Table 3 represents that ethyl acetate extract with a concentration of 4000 µg/mL formed the largest inhibitory diameter of 13.50 mm in the *S. dysenteriae* and 11.50 mm in *S. typhi* bacteria. The smallest concentration of ethyl acetate extract that could inhibit the growth of *S. dysenteriae* and *S. typhi* was 250 µg/mL. The concentration of n-hexane extract of 250 µg/mL could be expressed as MIC value for the *S. dysenteriae* bacteria and *S. typhi* bacteria. Based on the MIC value, the strength of both active extracts (bioactive ingredients) including the category was quite strong because it was in the range of 100-500 µg/mL. The strength of antibacterial compounds could also be seen from the MIC value. Morales *et al.* (2008) reported that based on the MIC value of antibacterial active compounds having a class level that is active compounds that have MIC value of less than 100 µg/mL are very strong, if the MIC value is between 100-500 µg/mL is strong enough, if the MIC is between 500-1000 µg/ mL it is weak and if the MIC value is more than 1000 µg/mL is the compound does not have antibacterial activity.

N-hexane and ethyl acetate extracts of karamunting leaf were more active than guava leaf extract. MIC value of karamunting leaf extract was smaller than guava leaf extract according to the research by Adnyana *et al.* (2004). The MIC value of ethanol extract of guava leaf against *Escherichia coli* was 60 mg/mL, *S. dysenteriae* was 30 mg/mL, *S. flexneri* was 40 mg/mL, and *S. typhi* was 40 mg/mL. The frequency of mice defecation which was given ethanol extract of guava leaves and white fruit peel of 150 mg/kg BW at 180-240 minutes showed different groups.

The activity of n-hexane and ethyl acetate extracts was also better compared to *Eucalyptus camaldulensis* leaf extract. Antibacterial activity of *Eucalyptus camaldulensis* leaf extract against *Klebsiella spp, S. typhi, Yersinia enterocolitica, Pseudomonas aeruginosa* (Gram-negative), *Staphylococcus aureus* and *Bacillus subtilis* had MIC value from methanol extract and dichloromethane fraction that ranged between 0.04 and 10 mg/mL with the least was *Bacillus subtilis*. Plant phytochemical screening revealed the presence of tannins, saponins and cardiac glycosides (Ayepola and Adeniyi, 2008).

The research was similar to study reported by Limsuwan *et al.* (2012) that used ethanol extract from the R. *tomentosa* leaf to be evaluated for antibacterial activity against 47 clinical isolates of *Streptococcus pyogenes*. Activity extracts of all isolates have MIC values in the range of 3.91-62.5 µg/mL and minimum bactericidal concentration (MBC) at 3.91-62.5 µg/mL. Rhodomyrtone, a compound isolated from *R. tomentosa*, showed activity against *S. pyogenes* (14 isolates) with very low MIC values (0.39-1.56 µg/mL) and MBC (0.39-1.56 µg/mL). The leaf extracts of *R. tomentosa* and rhodomyrtone have promising antibacterial activity against clinical isolates of *S. pyogenes*.

Bioautography test and determination of active compounds

Bioautography test and determination of the active compound class of n-hexane and ethyl acetate extract with the mobile phase n-hexane and ethyl acetate (8: 2) showed that n-hexane and ethyl acetate extracts contained three antibacterial compounds. After spraying with 2% H_2SO_4, the yellow spots on the n-hexane and ethyl acetate extracts were suspected to be phenol compounds with Rf of 0.50 and 0.30, respectively. The orange colour of the n-hexane and ethyl acetate extracts was thought to be flavonoids with Rf of 0.13, as can be seen from Table 4 and Fig. 2.

From the bioautography test, there were 3 antibacterial compounds found in n-hexane and ethyl acetate extracts. Compounds with Rf 0.5 were phenol group compounds which were thought to be rhodomyrtone compounds including phloroglucinol derivatives (Salni *et al*., 2003). Rhodomyrtone, isolated from Karamunting leaf has a powerful activity against many Gram-positive and Gram-negative bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) (Limsuwan *et al.*, 2009). Rhodomyrtone showed a high level of potential against methicillin-resistant Staphylococcus aureus bacteria (MRSA). Exposure of rhodomyrtone to MRSA bacteria at subinhibit concentration (0.5 MIC; 0.5 mg/mL) showed significant modulation of gene expression, with 64 gene induction and suppression of 35 genes (Sianglum *et al.* 2012). Rhodomyrtone activity was comparable to vancomycin and daptomycin antibiotics (Leejae *et al.*, 2013).

Table 4 Bioautography test and determination of Karamunting leaf active compound.

Extract	Rf value		Colour	Compound
	S. dysenteriae	*S. typhi*		
n-hexane	0.50	0.50	Yellow	Fenol
	0.30	0.30	Yellow	Fenol
	0.13	0.13	Orange	Flavonoid
Ethyl acetate	0.50	0.50	Yellow	Fenol
	0.30	0.30	Yellow	Fenol
	0.13	0.13	Orange	Flavonoid

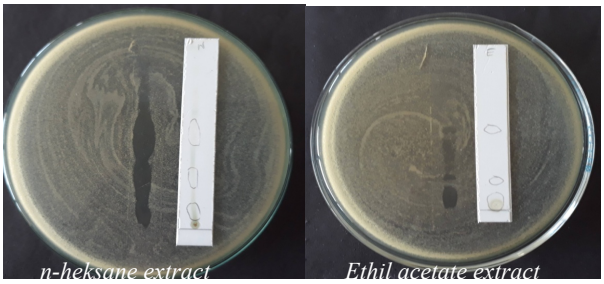


Fig. 2 Bioautography test of n-hexane and ethyl acetate extracts against *S. typhi* bacteria.

Rhodomyrtone compounds in n-hexane and ethyl acetate extracts strongly supported the antibacterial activity of these two extracts. Besides rhodomyrtone in the extracts of n-hexane and ethyl acetate, there were also phenol compounds with Rf 0.3 and flavonoid compounds with Rf 0.13. Phenol and flavonoid compounds also supported the activity of these two extracts. Phenol and flavonoid compounds were found in Myrtaceae plants. Both of these compounds have the potential to be isolated and determined for their chemical structure.

Jawetz *et al*. (2008) reported that phenol has strong antibacterial elements. Phenols and their derivatives can cause protein denaturation. Phenol can become bacteriostatic and bacteriocidal depending on its concentration. Flavonoid found in karamunting leaf according to Lai et al (2013) has three types, namely Myricetin-pentoxide, myricitrin (3-O-rhamnoside-myricetin) and isorhamnetin rhamnetin = 3-O-rhamnoside. It cannot be ascertained that among these three flavonoids which have antibacterial activity. Flavonoid has antibacterial activity in various mechanisms. Cowan (1999) also reported that flavonoids could disrupt cell function of microorganisms and inhibition of the cycle of microbial.

CONCLUSION

N-hexane and ethyl acetate extracts were active to bacterials of *S. dysenteriae* and *S. typhi*, while ethanol extract was not active. The MIC value of n-hexane and ethyl acetate extracts against *S. dysenteriae* and *S. typhi* bacteria has the same value of concentration

of 250 µg/mL. The antibacterial active compounds *S. dysenteriae* and *S. typhi* contained in the n-hexane and in the ethyl acetate extracts were phenols and flavonoids. Karamunting leaf (*R. tomentosa*) has the potential as a family medicinal plant because it has antibacterial compounds against *S. dysenteriae* and *S. typhi*.

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