Antibacterial Activity of Extract Ethyl Acetate and Ethanol of Raru (Vatica pauciflora Blume)

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Abstract. This research purpose is to show the inhibition of the extract of ethanol and ethyl acetate stem bark and leaf of Raru according to the causes of bacterial E-coli (ATCC 25922) and St Aureus (ATCC 25923) with variation concentration 10%, 5%, 2.5% and 1.25%. Methods used were diffusion with disk blank paper and disk cloramphenicol as a control. The highest result observed on 10% concentration among the variation. This concentration then compared with control and the result as follow for stem bark of ethanol and ethyl acetate exctract for bacterial E-coli and St Aureus : 14.7 and 14.5 ; 11.6 and 13.5. for leaf : 14.6 and 13.5; 14.0 and 13.30

Keywords: Raru (Vatica pauciflora Blume) ; Anti Bacteria E-coli and St Aureus

1 Introduction

The Raru plants belongs to the family Dipterocarpaceae for a long time known as medicinal plant by the community Tapanuli. was used as an additional drink nira aren. The purpose of adding flavour is known as a traditional drink called Tuak (Gunawan, 2011). Once of them has been investigated In North Sumatera, Central Tapanuli, there are Raru plants types (vatica pauciflora Blume) which on phytochemical was founded flavonoid compound in activity as anti-diabetic in in vitro (Riris, 2014). Many of this plant was used as a herbal by people in the world (Al – Rubiay et al, 2008).

The bacteria have been known to cause infections. The utilization of plants are often used in traditional medicine as a preventive of infections (Vauren s. f. van, 2008). Activity antibacterial test can be done to find out if there are activities of the plant is antibacterial and the most widely method used performe by the method of diffusion discs (Natheer et al, 2012). Methods of diffusion affected many of the physical and chemical factors other than simple interactions between drugs and organism (e.g., the nature of the medium and the ability of molecular size, diffusion, and drug stability) (Jawetz et al., 2007). On researches to analyze the inhibition of plant extracts commonly used type of Gram positive bacteria and Gram negative (Palombo Enzo A, et al, 2001). On this research aim to see if extracts of leaves and bark gives the barriers against bacteria: Staphylococcus Aereus and Escherchichia Coli, with measurement results drag zone (mm) \leq 10 classified is not active; 11-15 (weak); 16-20 (medium); and \geq 20 (strong) (Greenwood, 1995).

2 Material And Method

2.1. Plant Samples

The raru (Vatica Pauciflora Blume) were collected from the Central Tapanuli of North Sumatra. Chemicals used: extract of stem bark ethyl acetate and ethanol, MHA (Mueller Hinton Agar Oxoid CM0337), physiological NaCl 0.9%, Mc Farland, 0.5.%, paper discs, chloramphenicol, DMSO, ethanol, cotton, tissue, heat-resistant plastic.

2.2. Preparation of The Sample

3 kg samples were washed, air-dried and then sift to be powder bark and leaf raru (vatica pauciflora Blume).

2.3 Extraction

Extraction was prepared with maceration in ethanol and ethyl acetate for 3×24 hours. On the process of maceration was performed 3 times then repetition was filtered by using a Buchner so obtained the filtrate and residue. The filtrate is obtained with a vacuum rotary evaporator according the boiling point of the solvent used i.e. ethanol solvents with boiling point of 78.4 ° C, and ethyl acetate with a boiling point of 77, 10 so obtained extracts.

2.4 Antibacterial Screening

2.4.1. Sterilization Equipment

Glass tools sterilized using the oven at a temperature of 180 $^{\circ}$ C during 2 hours, as for metal tools and tools which no heat resistant and medium on heating with high temperature, sterilized in an autoclave and wrapped with aluminum foil at 121 $^{\circ}$ C and 2 atm for 15 minutes.

2.4.2 The Creation of Media so that (Satish, 2008)

Media in order for the MHA (Mueller Hinton Agar) is a common medium media was used to grow the bacteria Escherichia coli and stphylococcus aureus. Weighing as much as 38 g of MHA media (Oxoid CM0337) then add 1 L aquadest ,mixed and sterilized in autoclave at 121 0 C for 15 minutes, then put the media into the BSC (Bio Safety Cabinet) then the media pour into petri dish and keep in an incubator for 24 hours at 37 0 C

2.4.3 Cultured of Bacterial Suspension (sukandar et al., 2010)

The bacteria will be used first bred prior to use for testing. By using a cotton bud taken bacteria and inserted into the impulse tube, after it suspended bacteria into the NaCl 0.9% as much as 2 mL afterwards in the vortex and levels compared with a 0.5 McFarland Standard (0.05 mL Barium Chloride in 9.95 mL of Acid Sufat, 10-8 x 1.5 mL) (Whitman and MacNair, 2010).

2.5 Methods of Paper Disc Diffusion

Weight 100 mg/mL extracted test with concentration of 10; 5; 2.5 dan1. 25mg/mL is made by doing serial dilution with 1 mL of DMSO extract (Natheeret al., 2012). Paper discs were sterilized with 20 μ L aqueous extract dried in sterile petri at room temperature. A suspension of bacteria taken with a cotton bud and smeared in the media of MHA (Oxoid CM0337) which prepared in advance. The positive control used is cloramphenikol antibiotic discs while the negative control used is solvent DMSO (Natheer et al., 2012).

3 Results And Discussion

 Table 1. Diameter zone of inhibition (mm) of ethanol and ethyl acetate Extract of stem bark of to bacteria Escherichia coli

Extractstem bark of	Concentration	n Diameter zone of inhibition			
raru	(%)	D1	D2	D3	D
	0	0	0	0	0
	10	13.2	13	13.3	13.3
Ethyl acetat	5	9.6	9.9	9.5	9.9
	2.5	8.5	8	8	8.5
	1.25	7	7	7	7
	0,02	18,8	18.6	18.5	18.8
	0	0	0	0	0
Chloramphenocol	10	13	13	13.5	13.5
	5	11	12	12.45	12.5
	2.5	8.4	9	10	10
	1.25	6	6.5	6	6.5
Chloramphenicol	0.02	18.8	18.6	18.4	18.8

Extract stem bark ofraru	Concent ration (%)	Diameter zone of inhibition (mm) St. aureus			
		Dı	D ₂	\mathbf{D}_3	D
	0	0	0	0	0
	10	14	13.5	13	14
Ethyl ac etate	5	10	10.5	10	10.5
	2,5	9	9	8.5	9
	1,25	6	6	ó	6
Ch loramphenic ol	0,02	17,8	18	18,5	18,5
	0	0	0	0	0
	10	14.5	14	14.6	14.6
Ethanol	5	12,5	12,4	12,4	12.5
	2.5	10.8	10.5	10	10.8
	1,25	8	8.7	8.5	8.7
Ch loramphenic ol	0,02	20	20 <i>,</i> 3	20,1	20,3

Table 2. Diameter zone of inhibition (mm) of ethanol and ethyl acetate Extract of stem bark of to bacteria *Staphylococcus aureus*

Table 3.	Diameter zone of inhibition (mm) of raru leaf ethanol and ethyl acetate extract to
	bacteria Escherichia coli

Raru leaf extract	Concent ration (%)	Diameter zone of inhibition Escherichia coli				
		D1	D2	D3	D	
Ethyl acetate	10	11,1	11,6	11,6	11,6	
	5	9,5	9,6	9,5	9,6	
	2,5	9,3	9	9	9,3	
	1,25	6,5	6	6	6,5	
Chloramphenicol	24,2					
	10	14,7	14,6	14	14,7	
Ethanol	5	11,2	11	10	11,2	
	2,5	9	8,7	8,5	9	
	1,25	6,1	6,1	6,2	6,2	
Chloramphenicol	20,5					

Raru leaf of extract	Concent ration (%)	Diameter zone of inhibition (mm) St. aureus				
		D1	D2	D3	D	
	10	12,5	13	13,5	13,5	
Ethyl acetate	5	10,3	12	12,2	12,2	
	2,5	9	9,3	9,5	9,5	
	1,25	7,5	7	7,6	7,6	
chloramphenicol	20,3	20,3				
	10	14	14,5	14	14,5	
	5	11	10,8	11,5	11,5	
Ethanol	2,5	8	9	8,8	9	
	1,25	8	7,5	7	8	
chloramphenicol	23,2					

Tabel 4. Diameter zone of inhibition (mm) of raru leaf ethanol and ethyl acetate extract to bacteria *Stahylococcus aureus*.

Following are the results of the image zone the resistance of each extract and test bacteria.



A B Figure 1. (A) Zone inhibition extract EA and (B) ET leaf to E.coli



Figure 2. (A) Zone inhibition extract ET and (B) EA to E.coli Staphylococcus aureus



A B Figure 3. (A) Zone inhibition extract EA and (B) ET leaf to E.coli *E. coli*



Figure 4. (A) Zone inhibition extract EA and (B) ET leaf to *Staphylococcus aureus*.



Figure 5. Zone inhibition antibacterial extract of raru stem bark against E.coli





Figure 6. Zone inhibition antibacterial extract of raru stem bark against *staphylococcus* aureus

Figure 7. Zone inhibition antibacterial extract of raru leaf against Escherichia coli.



Figure 8. Zone inhibition antibacterial extract of raru leaf against Staphylococcus aureus.

It shown inhibition zone widest at concentrations of 10% ethanol extract of the leaves and bark. Antibacterial activity of ethyl acetate extract of raru stem bark on staphylococcus aureus bacteria has a larger diameter compared to Escherichia coli bacteria. While the ethanol extract of raru stem bark on Escherichia coli bacteria has a clear zone diameter greater than that of staphylococcus aureus. From the results obtained in the extract, it can be seen that the higher the concentration, the clearer zones produced are also wider, the bark and leaf extracts have strong antibacterial activity (Greenwood, 1995).

The activity test of ethanol and ethyl acetate extracts from the bark and leaves of Raru (Vatica pauciflora Blume) inhibited E. coli as gram negative bacteria and St Aureus gram positive bacteria, a greater variation in concentration gave a barrier to wider bacteria.

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