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ANTIBACTERIAL ACTIVITY OF *Samanea saman* LEAF ETHANOL EXTRACT AGAINST *Escherichia coli* AND *Staphylococcus aureus* AND ITS TOTAL FLAVONOID AND PHENOLIC CONTENTS

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ABSTRACT

Antibacterial activity of *Samanea saman* usually shows a positif correlation to the flavonoid and phenolic contents. The aim of this study was to evaluate the antibacterial activity of the ethanol extract of *Samanea saman* against *Escherechia coli* and *Staphylococcus aureus* and determine the total flavonoid and phenolic contents of the extract. The extraction was done by ethanol 96% at room temperature. The antibacterial assay was conducted by agar disc diffusion method. The total flavonoid and phenolic contents were determined by UV-Vis Spectrofotometer with the standard of quersetin and galic acid, respectively. The extraction of 250 g of *Samanea saman* leaves resulted in 24.5 g of ethanol extracts. The ethanol extract showed a moderate inhibition of 8.33 mm towards *E. coli* and a strong inhibition of 13.6 mm towards *S. aureus* at the concentration of 4%. The Minimum Inhibitory Concentration (MIC) of the extract against *E. coli* and *S.aureus* were of 3% and 0.3%", respectively. The total flavonoid and phenolic contents were successively 1233.2991 mg QE/100g and 2544.6154 mg GAE/100g.

Keywords: *Escherechia coli*, flavonoid and phenolic content, *Samanea saman*, S*taphylococcus aureus*

INTRODUCTION

Infection is one of the most common health problems suffered by the community. Infection is an invasion of the body by microorganisms and proliferates in body tissues that cause pain. The development of tropical Indonesian infections is due to moist air, poor sanitation, densely populated environments and favorable temperatures for the development of microorganisms. One type of microorganism that can cause infection is bacteria. The bacteria that cause the most infection cases in the community are pathogenic bacteria from *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) species.

E. coli is a pathogenic bacteria that can infect any tissue or body tool and cause disease with typical signs of inflammation, necrosis, and abscess formation (Jawetz *et al*., 1995) While *S. aureus* can cause infectious diseases such as ulcers, acne, impetigo, and severe infections such as pneumonia, mastitis, plebitis, meningitis, urinary tract infections, osteomyelitis, and endocarditis. *S. aureus* is also a major cause of nosocomial infections, food poisoning, and toxic shock syndrome (Mandal, 2012).

Synthetic medicines, such as antibiotic, commonly used to overcome a diseases caused by *E. coli* and *S. aureus*, but these medicines can be resistant to the bacteria. Frieri *et al.* (2017) reported that multidrug resistant patterns in Gram-positive and -negative bacteria have resulted in difficult-to-treat or even untreatable infections with conventional antimicrobials. Therefore, an active ingredient of antibacterial plant extract is required. One of these plants is rain tree (*Samanea saman*).

Rain tree in Indonesia are used as shade trees and urban forests (Santosa *et al*., 2012; Dahlan, 2010). The rain tree leaf water extract was reported to inhibit the growth of *E. coli* at minimum concentration of 0.5 % and to *S.aureus* at 1% (Prasad *et al*., 2008). Methanol extracts of rain tree have antibacterial activity with inhibition zones ranging from 11 mm to 3.5 mm at 1 mg / mL concentration (Thippeswamy *et al*., 2012). Rain tree leaf ethanol extract inhibited the growth

of *Fusarium solani*, pathogenic fungi causing stem rot diseases on dragon fruits (Rita *et al*., 2016a; Rita *et al*., 2013). Methanol extract of *S. saman* showed measurable inhibitory activity against both *S. aureus* and *B. subtilis* (Obasi *et al*., 2010). Butanol extract of rain tree leaf collected from Bali could strongly inhibit *S. aureus* while the inhibition zone of the extract against *E. coli* was moderate at concentration of 8% (Rita *et al*., 2016b).

Antimicrobial activity of a material was associated with its chemical content, such as flavonoid and phenolic content. Mahboubi *et al*. (2015) evaluated total flavonoid and phenolic contents from extract of *Punica granatum* L. flowers and its antibacterial activity towards both Gram positive and Gram negative bacteria causing food poisoning. The total flavonoid and phenolic content was positively associated with the antibacterial activities. Phenolic and flavonoid contents of leaf extract of ten Algerian *Ficus carica* L. varieties have been investigated by Mahmoudi *et al*. (2016), the extracts have an antimicrobial effect against *Bacillus cereus* and *Staphylococcus aureus*. This study aimed to evaluate the antibacterial activity of ethanol extract of *Samanea saman* against *Escherechia coli* and *Staphylococcus aureus* and to determine the total flavonoid and phenolic contents of the extract.

MATERIAL AND METHODS

General Experimental Procedure

The study was initiated with the extraction of plant material, followed by antibacterial assay. After that, the total of flavonoids and phenolic content were determined by Spectrofotomer UV-vis.

Plant material

The sample of rain tree leaves (*Samanea saman*) were collected from around Denpasar Bali. The tree was identifid at LIPI-UPT Center for Plant Conservation Botanical Garden "Eka Karya" Bali. The leaves were dried at room temperature for 15 days and were powdered and stored for further analysis.

Bacterial Agents

The ethanol extract of rain tree leaves was assayed against two strains bacteria, *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). These microorganisms were obtained from culture collection of Laboratory of Microbiology Department of Biology, Faculty of Mathematic and Natural Sciences, Udayana University. The isolates were purified and maintained at 4 °C until used.

Plant extraction

Around 250 g of rain tree leaf powder was extracted with 5 L of 70% ethanol for 24 h at room temperature (25 °C). The extract was filtered through Whatman No. 4 filter paper, evaporated to dryness under vacuum and stored at 4 °C until analysis.

Antibacterial Activity Assay

Antibacterial activity assay of the rain tree leaf extract was carried out by disc diffusion method at concentrations of 4% with three repetitions (Sinarsih *et al*., 2016). The negative control used was the solvent of the extract. While positive controls are amoxicillin for *S. aureus* and meropenem for *E. coli* which were already available in paper disc form.

The assay was initiated with media preparation, 20 mL of Mueller-Hinton Nutrient Agar (NA) medium inserted into a sterile petri dish then closed and cooled to solidify. A hundread μL of suspension, having 10^8 CFU/mL of bacterial strains was dispensed on the medium using sterile cotton. The medium was then allowed to dry slightly about 5-8 minutes before the disc was attached.

As much as 20 μL the extract was dropped on disc paper with a diameter of 6 mm using a micropipette, and for negative controls, solvent was dropped at the same volume. The disc papers were then allowed to stand for ± 120 minutes. The disc paper containing extract, positive control, and negative control was then placed on top of bacterial media with tweezers. Then the media was incubated at the optimum growth temperature of *S. aureus* and *E. coli* which ranged from 35-37 °C for 24 hours. The inhibitory diameters of extract were measured after the incubation period.

Minimum inhibitory concentration (MIC) was determined at various concentrations. There were 0; 0.1; 0.2; 0.3; 0.4; 0.5; 1; 1.5; 2; 2.5; 3; 3.5;

And 4%. The concentration specified as MIC was the smallest concentration that still had the ability to inhibit bacterial growth.

Determination of Total Flavonoid and Phenolic Contents

Total Flavonoid contents

Total flavonoids were determined by aluminum chloride method (Rita *et al*., 2016b). A total of 0.0417 grams of samples were dissolved in 50% ethanol to 5 mL volume, homogenized, and centrifuged at 3000 rpm for 15 min. The filtrate was taken 25 µL, then diluted to 500 µL volume. The solution was added with 2% $AlCl_3$ (500 µL) so the total volume of the solution became 1000 µL. The mixture was allowed to stand for 90 minutes before the absorbance was measured at a wavelength of 415 nm. The total flavonoid contents were expressed as mg quercetin equivalents/100 mg extract. The total flavonoids can be calculated by the following formula:

$$F1 = \frac{C.V.F.10^{-6}}{m}\ 100\%$$

where: F1=total flavonoids, C=equality of quercetin (g/mL), V= total volume of extract (mL), F= the dilution factor, m= weight of sample (g)

Total Phenolic contents

Total phenolic contents were determined using Folin-Ciocalteu reagent (Thippeswamy *et al*., 2011; Qadir *et al*., 2017). A total of 0.025 gram samples were dissolved in 80% methanol to obtain volume of 5 mL, homogenized, and centrifuged at 3000 rpm for 15 min. The filtrate was taken 10 µL, then diluted to 100 µL volume, added with 100 µL Folin-Ciocalteu reagent, and 800 µL of 5% sodium carbonate so the total solution volume becomes 1000 µL. The mixture was allowed to stand for 90 minutes before the absorbance was measured at a wavelength of 760 nm. The total phenolic contents were expressed as mg gallic acid equivalents /100 g of extract. The total phenols can be calculated by the following formula:

$$F2 = \frac{C.V.F.10^{-6}}{m}\ 100\%$$

where: F2 = total phenol, C = equality of gallic acid (g/mL), V = total volume of extract (mL), F = the dilution factor, m = weight of sample (g)

RESULTS AND DISCUSSION

The extraction of 250 g of rain tree leaf powder resulted in 24.5 g of ethanol extract. Antibacterial activity assay against *E. coli* and *S. aureus* of rain tree leaf ethanol extract was performed at a concentrations of 4% with a positive control of meropenem for *E. coli* and amoxicillin for *S. aureus*. Antibacterial assay results are shown in Table 1.

Table 1. Antibacterial Activity of the ethanol extract of *Samanea saman* leaves against *E. coli* and S. *aureus*

Bacteria	Avarage of Inhibition Zone (mm)		
	Positive control*	Negative control	Ethanol Extract (4%)
E. coli	32.33	-	8.33 (moderate)
S. aureus	19.67	-	13.67 (strong)

*Meropenem (for *E.coli*) and Amoxycilin (for *S. aureus*)

From Table 1, it can be seen that ethanol extract of rain tree leaf could inhibit the growth of *E. coli* with moderate inhibition of 8.33 mm, while it could inhibit the growth of *S. aureus* with strong inhibition of 13.67 mm. According Davis and Stout (1971), if the extract gave an inhibition zone diameter of less than 5 mm, it was categorized as weak inhibition, between 5 and 10 mm was moderate inhibition, larger than 10 to 20 mm was strong, and higher than 20 mm was very strong inhibition. From this data, it can be shown that *S. aureus* (gram positive bacteria) was more sensitive to the extract than *E.coli* (gram negative bacteria). The different capabilities of the extract in inhibiting the growth of bacteria from different classes may be due to differences in the complexity of cell wall constituent of both types of bacteria (Pelczar *et al*., 2010).

Gram-negative bacteria have a way of protecting their cell membranes from penetrating antibacterial agents, since they have a unique outer membrane. Gram-negative bacteria have cell walls with relatively thinner peptidoglycan layers, and

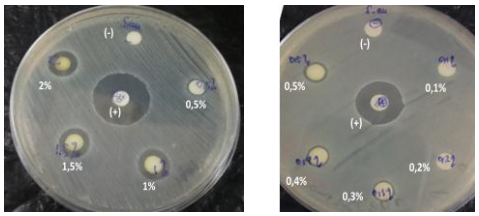
periplasmic space between cell walls and membranes. In addition, the structure of Gram-negative bacterial membrane contains Lipopoly-saccharide (LPS) or endotoxin which is a complex structure of Lipid A, short chain of sugar, and long chain carbohydrates. Polysaccharides play a role in the selective entry of hydrophobicity into cell membranes, whereas lipid properties play a role in the inclusion of hydrophilic compounds (Jawetz *et al*., 1995; Pelczar *et al*., 2010).

Gram-positive cell wall is structured by a simpler peptidoglycan layer compare to the more complex Gram-negative structure. These cause that the antibacterial compounds are more easily enter into the cell and find the target. The presence of the ability of antibacterial compounds in damaging the cell wall of bacteria causes the disruption of cell wall function as a giver of cell shape and protect cells from lysis can cause bacterial death (Dewi, 2013; Brown *et al*., 2015).

MIC was determined to recognize the smallest concentration that still had the ability to inhibit bacterial growth. It was performed at various concentrations of 0; 0.1; 0.2; 0.3; 0.4; 0.5; 1; 1.5; 2; 2.5; 3; 3.5; and 4%. The result demonstrated that MIC of the extract to inhibit the growth of *E. coli* and *S. aureus* were 3% and 0.3% respectively, with the inhibition zone of 6.50 and 6.42 mm (Figure 1 and Table 2).

These values (from Table 2) indicated that the rain tree leaf ethanol extract was ten times more effective in inhibiting the growth of *S. aureus* compared to *E. coli*. The Data also shows that the inhibitory zone increased with the increase of extract concentration. These results were consistent with the statements of Rhoades *et al*. (2000) that in general the inhibitory zone tends to increase with the increase of extract concentration. With the increasing concentration of extracts, the content of active compounds was greater so that the ability of extract inhibiting the growth of bacteria were greater. However, inhibition of ethanol extract toward *S. aureus*, at concentration of 2 and 2.5 % were not significantly different as well as that at concentration of 0.3 and 0.4 % (P < 5%).

(a)



(b)

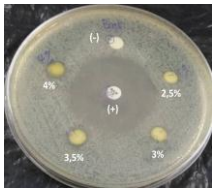


Figure 1. Inhibition zone of rain tree Leaf ethanol Extract at various concentrations. (a*) S. aureus,* (b*) E. coli*

Table 2. Inhibition zone of the growth of *E. coli and S. aureus* of rain tree leaf ethanol extract at various concentrations

Treatment	Avarage of Inhibition Zone (mm)	
(%)	*E. coli*	*S. aureus*
4.0	8.17^{a*}	13.33^{a*}
3.5	7.17^{b}	12.67^{b}
3.0	6.50^{c}	12.17^{bc}
2.5	-	11.83^{c}
2.0	-	11.67^{c}
1.5	-	10.67^{d}
1.0	-	9.50^{e}
0.5	-	7.67^{f}
0.4	-	6.92^{g}
0.3	-	6.42^{g}
0.2	-	-
0.1	-	-
0	-	-

*Values followed by the same letters in the same column are not significantly different according to the Duncan's Multiple Range Test at *P<5%*.

The calibration curve for the determination of flavonoid and phenolic contents were presented at Figure 2. Based on the calibration equation of quercetin, y = 0.070x - 0.032 and that of gallic acid, Y= 0.013x – 0.001, total flavonoid and phenolic contents could be determined using equations 1 and 2. The calculation was summarized at Table 3. From the Table 3, it could be seen that the total flavonoid and phenolic

contents were successively 1233.2991 mg QE/100g and 2544.6154 mg GAE/100 g extract.

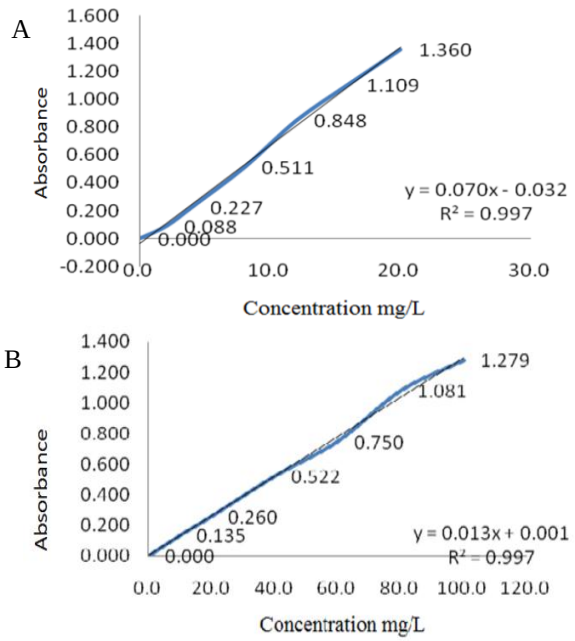


Figure 2. Calibration curve of A) Standard Quercetin B) Standard Galic acid

The data shows that the total flavonoid and phenolic contents of ethanol extract of rain tree leaf were quite high. This is related to the activity as an antibacterial. Mahboubi *et al*. (2015) stated that the antimicrobial efficacy of the herbal extracts correlates with their flavonoid contents.

Table 3. Total flavonoid and phenolic compounds of rain tree leaf ethanol extract

	Flavonoids	Phenolics
Weigh of sample (g)	0.0417	0.025
Absorbance (Y)	0.3280	0.1664
Concentration (mg/mL)	5.1429	12.7231
Volume (mL)	5	5
Dilution	20	10
Contents %	1.23	2.54
mg/100 g	1233.2991	2544.6154

Flavonoids are the result of plant metabolism that generally serves as a response to microbial infections. The theory of flavonoid mechanisms as antibacterial is similar to other phenolic compounds such as tannins in inhibiting bacterial growth, through the formation of bonds with bacterial proteins through hydrogen bonding, and the formation of covalent bonds (Cushnie and Lamb, 2005; Kumar *et al*., 2013). In addition, the mechanism of action that may occur is inactivation due to the presence of hydrogen bonds that result in cell wall protein structures and unstable cytoplasmic membranes. The instability causes selective permeability, active transport function, the control of the protein structure of the bacterial cell becomes disturbed, resulting in loss of macromolecules and ions from the cell, so that the bacterial cells lose their shape and undergo lysis (Pelczar *et al*., 2010; Naufalin and Herastuti, 2017).

CONCLUSION

Based on our study, it could be concluded that ethanol extract of rain tree (*Samanea saman*) leaf showed good antibacterial activities against *S. aureus* and moderate activity against *E. coli* which suggest that this plant could be used to treat various infections caused by bacteria. This activity is closely related to the total flavonoid and phenol contents.

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