Total flavonoid and phenolic contents of n-butanol extract of Samanea saman leaf and the antibacterial activity towards Escherichia coli and Staphylococcus aureus

Wiwik Susanah Rita, I. Made Dira Swantara, I. A. Raka Astiti Asih, Ni Ketut Sinarsih, and I. Kadek Pater Suteja

Citation: AIP Conference Proceedings 1718, 060005 (2016); doi: 10.1063/1.4943327

Articles you may be interested in

Antimicrobial activity of honey of stingless bees, tiúba (Melipona fasciculata) and jandaira (Melipona subnitida) compared to the strains of Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa

Isolation, identification, and antibacterial activity of chemical compounds from ethanolic extract of suji leaf (Pleomele angusifolia NE Brown)

Antibacterial efficacy of silver nanoparticles against Escherichia coli

Interaction Of ZnO Nanoparticles With Food Borne Pathogens Escherichia coli DH5α and Staphylococcus aureus 5021 & Their Bactericidal Efficacy

Fine Encapsulated ZnO Nanophosphors And Their Potential Antibacterial Evaluation On The Gram Negative Bacillus Escherichia coli
AIP Conf. Proc. 1147, 528 (2009); 10.1063/1.3183485
Total Flavonoid and Phenolic Contents of n-Butanol Extract of Samanea saman leaf and The Antibacterial Activity towards Escherichia coli and Staphylococcus aureus

Wiwik Susanah Rita¹,², a), I Made Dira Swantara¹,², b), I A Raka Astiti Asih², Ni Ketut Sinarsih¹, and I Kadek Pater Suteja²

¹ Department of Applied Chemistry, Postgraduate Program Udayana University, Denpasar, Bali, Indonesia
² Department of Chemistry Udayana University, Jimbaran, Bali, Indonesia

a) Email: wiwiksr@yahoo.com
b)m_dira_swantara@yahoo.co.id

Abstract. Total flavonoid and phenolic contents in some natural products was suspected of having a positive correlation to its activity in inhibiting the growth of bacteria. The aim of this study was to determine the total flavonoid and phenolic contents of n-butanol extract of Samanea saman leaf, and to evaluate the antibacterial activity towards Escherechia coli and Staphylococcus aureus. Extraction of compounds was done by ethanol 96%, followed by fractionation into n-hexane, ethyl acetate, and n-butanol. Determination of total flavonoid and phenolic contents was done by UV-Vis Spectrophotometer using standard of quersetin and galic acid respectively. In addition, antibacterial activity was evaluated by agar disc diffusion method. Extraction of 1000 g of Samanea saman leaf was obtained 80 g of ethanol extracts, fractionation of the extract was obtained 8.02 g of n-hexane extracts, 7.11 g of ethyl acetate extracts, 13.5 g of n-butanol extracts, and 14.16 g of aqueous extracts. Phytochemical screening of the n-butanol extracts revealed the presence of flavonoid and phenolic compounds. Total flavonoid and phenolic contents were successively 43.5798 mg QE/100g and 34.0180 mg GAE/100g. The butanol extracts inhibited the growth of S.aureus higher than the growth of E. coli. At the concentration of 2, 4, 6, 8 % (b/v), and positive control (meropenem 10 μg/disc), inhibition zone towards S. aureus was successively 5.67, 9.33, 10.33, 12.00, and 32.33 mm, while the inhibition zone towards E. coli was1.33, 3.33, 4.33, 5.43, and 34.00 mm.

Keywords: Flavonoid contents; Phenolic content; Samanea saman; Escherechia coli; Staphylococcus aureus

1. INTRODUCTION

Diarrhea is a clinical symptoms and gastrointestinal (gut) that are characterized by increased frequency of defecation more than usual (repeatedly). Jawetz et al. [1] reported that one of the bacteria that can cause diarrhea was Escherichia coli. Other diseases caused by E. coli were urinary tract infections, sepsis, and meningitis. According to Melliawati [2], the spread of E. coli can occur by means of direct contact (touching, shaking hands, and so on) is then passed through the mouth.

Given the Escherichia coli bacteria harmful to human health, it is necessary to mitigation or prevention of the development of E. coli, one of which is to utilize the active ingredients from plants that can be used as an antibacterial or suppress the growth of E. coli. One of the plants is rain tree (Samanea saman). Prasad et al. [3] has studied that rain tree leaf extract can inhibit the growth of bacteria (Escherichia coli, and Staphylococcus aureus). Based on phytochemical screening indicated the presence of tannin, flavonoids, saponins, steroids, cardiac glycosides, and terpenoids in tamarind leaf extract. Rita [4] reported that rain tree leaf collected from Denpasar contained triterpenoids, steroids, flavonoids, tannins, alkaloids, and saponins.

According to Staples et al. [5], rain tree leaves can be used as a traditional medicine like drug fever, diarrhea, headache, and abdominal pain. Tamarind leaf usefulness as diarrhea medicine, closely related to the growth of Escherichia coli in the gut. Orhan et al. [6] revealed that some flavonoids were formed as antimicrobial barriers in plants response to microbial infection. Parubak [7] reported that flavonoids in the leaves of Akway (Drimys beccariana Gibbs) has antibacterial...
activity against *Escherichia coli* and *Bacillus subtilis*. Hendra et al. [8] also reported that the presence of flavonoids in the fruit of the gods crown contribute to its activity as an antibacterial.

Considering the antimicrobial activity of rain tree leaf, antibacterial activity of flavonoids extracted from some plants, as well as the presence of flavonoids in leaf of rain tree, there is a possibility that antibacterial activity of rain tree leaf has correlation with flavonoid or phenolic contents. The objectives of this study were to investigate the total flavonoid and phenolic contents of n-butanol extract of *Samanea saman* leaf and to study the antibacterial activity. Antibacterial activity was assessed in vitro against two bacterial: *Escherichia coli* and *Staphylococcus aureus*.

2. MATERIAL AND METHODS

Plant Material

Materials used in this study was rain tree leaf collected around Denpasar Bali. Fresh leaf was washed with tap water, then dried at room temperature for 15 days, powdered, and used for extraction.

Bacterial Agents

Pure cultures of two species of bacterial namely *Escherichia coli* and *Staphylococcus aureus* were obtained from the Laboratory of Microbiology of Faculty of Medicine, University of Udayana. The isolates were purified and maintained at 4 °C until use.

Extraction

Around 1 kg of rain tree leaf powder was extracted with 10 L of ethanol 70% for 24 h at room temperature (25 °C). Ethanol extract was evaporated in vacuum and fractionated with solvents of increasing polarity, hexane, ethyl acetate, and n-butanol. Phytochemical test for flavonoids and phenolic compounds were applied to all extracts. The extracts were kept for 24 h at 4 °C, filtered through Whatman No. 4 filter paper, evaporated to dryness under vacuum and stored at 4 °C until analysis.

Total Flavonoid content

Total flavonoids were measured by an aluminum chloride method according to Khatiwora et al. [10] with modification. Aliquots of extract solutions were taken and made up the volume 2 ml with ethanol. Then 0.1ml AlCl3 (10%), 0.1ml Na-K tartarate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance at 415 nm was recorded after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /100g of sample. Total phenols can be calculated by the following formula:

\[
F_1 = \frac{C \times V \times F \times 10^{-6}}{m} \times 100\%
\]

where: \( F_1 \) = total flavonoids , \( C \) = Equality of quercetin (g/mL), \( V \) = total volume of extract (mL), \( F= \) the dilution factor (10), \( m \) = weight of sample (g)

Total Phenolic Content

Total phenols were assayed according to Medini et al. [10] with modification. As many as 1 g extract (1 g) was dissolved into 5 ml ethanol 85%, homogenized, centrifuged at 3000 rpm for 15 minutes, and filtered obtain a filtrate. The filtrate was diluted to a volume of 5 mL. 0.4 mL of Filtrate was placed in a test tube, added 0.4 mL of Folin-Ciocalteu reagent, homogenized and added 4.2 mL of sodium carbonate 5%. The mixture was allowed to stand 90 minutes at room temperature. Absorbance at 760 nm was read versus a prepared blank. The total phenol content of n-
butanol extract was expressed as milligrams of gallic acid equivalents per 100 gram of sample calibration curve with gallic acid with various concentrations of 10-100 mg/L. Total phenols can be calculated by the following formula:

$$F_2 = \frac{C \cdot V \cdot F \cdot 10^{-6}}{m} \times 100\%$$  \hspace{1cm} (2)

where: F2 = total phenol, C = Equality of gallic acid (g/mL), V = total volume of extract (mL), F= the dilution factor (10), m = weight of sample (g).

**Antimicrobial activity**

**Preparation of agar medium**

Medium was made from 20.4 grams of powder Mueller Hinton Agar (MHA), which was dissolved in 600 mL of distilled water in erlenmeyer, then heated to dissolve, plugged with cotton and, covered with aluminum foil. Furthermore agar medium sterilized by autoclaving at 121 °C for 15 minutes.

**Preparation of the bacterial suspension test**

One to three loops of *Escherichia coli* colonies were taken and suspended in a tube containing 5 mL of physiological saline solution. The turbidity of suspension was compared with a standard of Mc Farland 0.5%.

**Disc diffusion assay**

Antibacterial activity test of the rain tree leaf extract was performed at concentrations of 0, 2.0, 4.0, and 8.0 % (w/v). Each blank discs soaked with 20 mL extract, negative control, and antibiotics (meropenem 10) for 2 hours. MHA then placed on the media that have been implanted bacterial suspension and incubated at 37°C for 24 hours in an inverted position. Diameter of inhibition was observed after incubation period. The diameter of the inhibition was evaluated in milli metres. Each assay was repeated in triplicate. Statistical analysis was then performed using ANOVA test Duncan's Multiple Range Test.

**RESULT AND DISCUSSION**

Extraction of 1000 g of *Samanea saman* leaf was obtained 80 g of ethanol extracts, fractionation of the extract was obtained 8.02 g of n-hexane extracts, 7.11 g of ethyl acetate extracts, 13.5 g of n-butanol extracts, and 14.16 g of aqueous extracts. Phytochemical screening of the extracts showed that n-butanol extracts contained flavonoid and phenolic compounds.

The calibration plot for the determination of phenols and flavonoids were presented at Figure 1 and Figure 2, respectively.

![Figure 1. Calibration plot for flavonoid determination (quercetin)](image)
Based on the calibration plot equation of quercetin (Figure 1), \( y = 0.052x - 0.003 \) and the calibration plot equation of gallic acid (Figure 2), \( Y = 0.181x - 0.190 \), total flavonoids and phenols in n-butanol extract of rain tree leaves respectively could be determined (Table 1).

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Sample Weight (g)</th>
<th>Abs (Y)</th>
<th>Cons (x)</th>
<th>Volume (mL)</th>
<th>Dilution</th>
<th>Contents</th>
<th>%</th>
<th>mg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>0.1277</td>
<td>0.287</td>
<td>5.5651</td>
<td>1.00</td>
<td>10</td>
<td>0.0436</td>
<td>43.5798</td>
<td></td>
</tr>
<tr>
<td>Phenols</td>
<td>0.1026</td>
<td>0.317</td>
<td>2.7922</td>
<td>1.25</td>
<td>10</td>
<td>0.0340</td>
<td>34.0180</td>
<td></td>
</tr>
</tbody>
</table>

Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenols. From Table 1, it can be shown that total flavonoid was higher than total phenol, this is likely due to not all flavonoids in rain tree leaves contain phenol groups. Total phenol content in the butanol extract relatively low, because according Puspita et al [11], the best solvent to extract phenols was acetone.

Antibacterial activity test against E. coli and S. aureus of rain tree leaf butanol extract was conducted at various concentrations of 0, 2, 4, 6, and 8% with a positive control of meropenem. Antibacterial test results are shown in Table 2 and Figure 3.
* values followed by the same letters are not significantly different (P>0.05) according to the Duncan’s Multiple Range Test at 5% level.

Table 2 and Figure 3 showed that treatment with 2% of extract was not significantly different (P>0.05) with the control towards E-coli, but significantly inhibited the growth of S.aureus. The inhibition of E-coli increased with increasing concentrations of the extract. The inhibition of S.aureus rose significantly until 4% extract treatment, but the treatment over 4% was not significantly increase. It can be said that the concentration of 4% was the optimum concentration to inhibit the growth of S.aureus.

Inhibition of butanol extract of rain tree leaf against E. coli and S. aureus at concentrations of 8% were respectively 5.34 and 12.00 mm. According to Ardiansyah [12], if zone of inhibition ≥ 20 mm: the inhibition was very strong; 10-20 mm: strong; 5-10 mm: moderate; and <5 mm: the inhibition is low. Base on the result, it can be concluded that butanol extract of rain tree leaf led to a low inhibition on the growth of E. coli and strong inhibition on the growth of S. aureus.

Cushnie and Lamb [13] reported that there was a different mechanism for a variety of flavonoids, such mechanisms include inhibiting the synthesis of nucleic acids, cytoplasmic membrane function, and energy metabolism. The mechanism involves the interaction between the enzyme in bacterial with flavonoids. Enzymes are active proteins consisting of various kinds of amino acids, resulting in the formation of hydrogen bonds between flavonoids with amino acids causes the enzyme to be disturbed.

According to Kumar and Pandey [14], Antibacterial flavonoids might be having multiple cellular targets, rather than one specific site of action. One of their molecular actions is to form complex with proteins through nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation. Thus, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, and so forth. Lipophilic flavonoids may also disrupt microbial membranes.

According to Singh [15], phenolic compounds have the mechanism of action in inhibiting the growth of bacteria by inactivation of the protein (enzyme) on the cell membrane. According Susanti [16], phenols bind to the protein through hydrogen bonding resulting protein structure become damaged. Where most of the structure of the cell wall and bacterial cytoplasmic membrane protein and fat. Instability in the cell wall and the cytoplasmic membrane of bacteria causing the function of selective permeability, active transport function, controlling the composition of proteins from bacterial cells to be disrupted, which will result in escape macromolecules, and ions from the cell. So the bacterial cells becomes lost its shape, and there is a lysis.

Mailoa et al. [17] reported the antimicrobial activity of phenolic compounds (tannin) extract of guava leaves against microbial pathogens. The results showed that the inhibitory activity of compounds on five microbial pathogens was different. This was because the composition of the microbial cell wall five microbes was different. The results showed that the tannin extract can inhibit the growth of Escherichia coli, Pseudomonas aureginosa, Staphilococcus aureus, Aspergillus niger and Candida albicans.

**CONCLUSION**

Based on the results of research and discussion, it can be concluded as follows:
• Total flavonoid and phenolic contents of rain tree leaf butanol extract were successively 43.5798 mg QE/100g and 34.0180 mg GAE/100g.
• The butanol extract of rain tree leaf led to a low inhibition on the growth of E. coli and strong inhibition on the growth of S. aureus.

ACKNOWLEDGEMENTS

This work was supported by a grant from DP2M Ditjen Dikti Indonesia (Competitive research Grand). We wish to express our gratitude to head of Research and Community Institutions of Udayana University facilitating all the needs in the disbursement of research funds.

REFERENCES