Comparative study on antibacterial effects of ethanolic leaf extracts of guava in variants of red and white pulp \textit{(Psidium guajava)} against sensitive and chloramphenicol-resistant \textit{Staphylococcus aureus}

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\textbf{INTRODUCTION}

\textit{Staphylococcus aureus} is a flora normal, which associated with chronic wounds that could lead to chronic inflammation and high morbidity.\textsuperscript{[1]} Moreover, the multiresistance \textit{S. aureus} cases are increasing worldwide reopen interest to use plant products as new antimicrobial agents to control pathogen.\textsuperscript{[2]} \textit{Psidium guajava}, as one of the popular alternative antimicrobial medicines that have been used in long time history, can be used as an antibacterial alternative to infectious diseases caused by \textit{S. aureus}. The most popular guava plant variants are based on the color of the fruit pulp, which may be red or white. Both types are easily grown and could be harvested several times in 1 year with high productivity. This plant is widely distributed in Indonesia and the price is relatively affordable.\textsuperscript{[3]} \textit{P. guajava} is a well-known phytotherapeutic plant used to treat and manage various diseases such as diarrhea, dysentery, malaria, toothache, gastroenteritis, vomiting, ulcers, wounds, sore throat, coughs, and inflamed gums.\textsuperscript{[4-7]} Phytochemicals obtained from \textit{P. guajava} plants may play roles in the remedy of these diseases. Actually, the function of phytochemicals in plants is to protect themselves, but through recent researches, they protect found to provide potential against human diseases. The medicines from plants are greatly believed as safe medicine, in contrast to the expensive synthetic drugs that reported to have unexpected side effects besides its beneficial effects.\textsuperscript{[8]} This fact drives the need to evaluate medicinal plants as a plant-based medicine that is biodegradable, safe, and almost no side effects.\textsuperscript{[9]}

The many parts of \textit{P. guajava} plant have been used in traditional medicine because the phytochemical compounds presence is distributed in all parts of the plant. The bark and leaves of guava plant have been used as traditional medicine for a long time ago.\textsuperscript{[10]} The leaves and bark of this plant is used in the form of

\textbf{ABSTRACT}

\textbf{Aim:} This study aims to compare the antibacterial potential of red and white pulp guava \textit{(Psidium guajava)} leaf extracts against sensitive and chloramphenicol-resistant \textit{Staphylococcus aureus}. \textbf{Materials and Methods:} The antibacterial effect of each guava leaf extract was evaluated against sensitive and chloramphenicol-resistant \textit{S. aureus} using an agar well diffusion method. The minimum inhibitory concentration (MIC) value of the potential extract was determined using the macrodilution method. \textbf{Results and Discussion:} The antibacterial study showed that the red and white guava leaf extracts were potential to inhibit the sensitive \textit{S. aureus}, but not to the resistant one. According to the data of inhibitory diameters against sensitive \textit{S. aureus}, the ethanol extracts of the red guava leaves showed stronger inhibitory activity than the white guava. The MIC value of red guava leaf extracts was in the range of 0.195–0.391% w/v. \textbf{Conclusion:} The leaf extract of red pulp guava was found to be a potent antibacterial candidate for a natural anti-staphylococcal agent.

\textbf{KEY WORDS:} Chloramphenicol, Leaf extract, \textit{Psidium guajava}, Red guava, \textit{Staphylococcus aureus}, White guava
decoction to cure dysentery, diarrhea, sore throats, and vomiting and to regulate the cycles of menstruation. *P. guajava* leaves were reported to contain many chemical substances such as flavonoids, essential oils, coumarins, and triterpenes, which are revealed to have antimicrobial effect. The long history of *P. guajava* leaves use has led researchers to study *P. guajava* leaf extract. They reported that the guava leaf extracts exhibited antimicrobial activity in a wide spectrum against microorganisms such as *Escherichia coli*, *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Clostridium*, *Salmonella*, *Shigella*, and *Candida* species. Biswas et al. revealed that the ethanol and methanol extracts of the guava leaves could inhibit Gram-positive bacteria, whereas the Gram-negative bacteria were reported to be resistant to all extract. Another study found that the white pulp variant of guava leaf extract showed stronger antibacterial activity than that of the red one in the inhibition of *E. coli*, *Shigella dysenteriae*, *Shigella flexneri*, and *Salmonella typhi* growth. The different variants may provide phytochemical compound in different concentration. The total phenolics and flavonoids of the red guava were higher than the white pulp. Therefore, this study was undertaken to evaluate and compare the antibacterial potential of red and white guava (*P. guajava*) leaf extracts from Indonesia, against sensitive and chloramphenicol-resistant *S. aureus*.

**MATERIALS AND METHODS**

**Plant Materials**

The plants used in the study were guava plants in variants of white and red pulp, obtained from the guava plantation at Cikutra, West Java, Indonesia. Leaves were properly checked so that the leaves used were healthy leaves and not leaves that were attacked by insects. The parts of *P. guajava* plant were identified in Plant Taxonomy Laboratory of Biology Major, Faculty of Mathematics and Natural Science, Padjadjaran University.

**Bacterial Strains**

The antibacterial activity of red and white pulp of guava ethanol leaf extracts was tested against the sensitive and chloramphenicol-resistant *S. aureus* from a culture collection of Microbiology Laboratory of Faculty of Pharmacy, Padjadjaran University, Indonesia.

**Growth Medium**

The Mueller-Hinton Agar (MHA – Oxoid) and Mueller-Hinton Broth (MHB – Oxoid) were used as bacterial growth media.

**Chemical Materials**

The chemicals used were 95% ethanol, chloramphenicol (PT. Kimia Farma), normal saline solution, Dragendorff reagents, Lieberman–Burchard reagent, Mayer reagent, barium chloride solution (Merck), sulfuric acid solution (Merck), n-butanol, ferric chloride reagent (Merck), technical toluene (Brataco), vanillin (Merck), and distilled water.

**Preparation of Extracts**

*P. guajava* leaves were washed using tap water and then dried in the open air that was not exposed to direct sunlight. The dried leaves were then ground into powder and used for the extract preparation using 95% ethanol as the solvent.

**Extraction**

The powder of each guava leaf variants was put into a macerator, which has a capacity of 5 L and 95% ethanol was added 10 times as much as the dried leaves or until all the leaves were soaked. The extract was stirred for a while, then left for 1 × 24 h. The macerate was collected every 24 h for 3 days. Then, the collected macerates were evaporated using a rotary evaporator to get the thick extract with a constant weight.

**Preliminary Phytochemical Screening**

The preliminary phytochemical analysis of both extracts was performed to detect the presence of alkaloids, tannins, flavonoids, polyphenols, and saponins using a standard method.

**Inoculum Preparation**

Chloramphenicol-sensitive *S. aureus* was cultured in the MHA medium and incubated at 37°C for 18–24 h. In contrast, the resistant *S. aureus* must be inoculated in the MHA medium containing 100 µg/mL chloramphenicol. Each colony of the tested bacteria was taken from the agar plate and then suspended in 0.95% sterile saline. The turbidity of the suspension was observed and adjusted as the turbidity of the 0.5 McFarland standard (equal to 1.5 × 10^8 CFU/ml). This standard was composed of 9.95 ml of 1% H_2SO_4 and 0.05 ml of 1% BaCl_2 and was measured for its turbidity at a wavelength of 530 nm.

**Antibacterial Assay**

The both of leaf extracts were tested on MHA medium to compare their antibacterial activity against chloramphenicol-sensitive and chloramphenicol-resistant *S. aureus* using well-diffusion agar. The total extracts of the guava leaves were diluted with a serial dilution using DMSO as the solvent to achieve the test concentration of 100, 50, 25, 12.5, and 6.25%w/v. A volume of 20 µL bacterial suspension was suspended into 20 mL liquid MHA and the medium was allowed to solidify. After that, the medium was perforated to form wells of 6 mm in diameter and approximately 50 µL for each test concentration of the extract was dripped into each well. The plates were allowed for pre-diffusion in 1 h at room temperature and then incubated at 37°C for


Determination of Minimum Inhibitory Concentration (MIC) and MBC Value

The MIC value was determined for the extract with the stronger antibacterial activity using macrodilution broth. The extract concentration was prepared as follows: 3.125, 1.562, 0.781, 0.391, and 0.195% w/v. The sterile tubes were filled with 1 mL of MHB sterile and suspended with the 1 mL of the extract in a concentration of 6.25% w/v to achieve a concentration of 3.125 w/v, likewise the subsequent dilution of the other extracts concentration. Then, a loopful use of S. aureus suspension was put into every tube and incubated at 37°C for 18–24 h. The MIC value of the extract was defined as the lowest concentration of extract that can inhibit the bacterial growth, indicated as the clear solution formation.

RESULTS AND DISCUSSION

Extraction Result

Of 1 Kg fresh guava leaves, the dried leaves were gained as much as 275 g and the extract yield of each leaf guava was approximately 33 g. The yield extract of both guava leaves was 12%.

Phytochemical Screening Result

The phytochemical analysis of both extracts performed the presence of polyphenols, tannins, and flavonoid. The presence of these metabolites has been reported to have antibacterial activities.[17,18] Polyphenols and flavonoids could increase the production of reactive oxygen species (ROS) and hydrogen peroxide (H₂O₂) which may lead to bacterial lysis.[19] The respiration of bacterial aerobic such as S. aureus produces oxygen (O₂) that is required for energy production in cellular process.[20] However, if the O₂ reduction by aerobe bacteria is incomplete during respiration, ROS will be generated, including H₂O₂ and the hydroxyl radical (OH⁻).[21] Meanwhile, tannins are water-soluble polyphenols that are reported to have a bacteriostatic or bactericidal effect against S. aureus.[22] The astringent components of the tannin could induce complexation with microbial enzymes. Besides that, other mechanisms are tannin may act on the microbial membranes and tannin may induce complexation with metal ions.[23]

Antibacterial Activity Result

All extracts demonstrated that the leaf extracts of both guava variants provided an inhibitory effect against sensitive S. aureus, but not to the resistant. Based on the data of inhibition diameter, leaf extracts of red pulp guava variant gave stronger antibacterial effect against sensitive S. aureus than that of the white pulp. The diameters were summarized in Table 1. The antibacterial activity of guava leaf extracts might be supported by the presence of antibacterial compounds in the extract that possesses bioactive activity.

MIC and MBC Determination Result

The MIC test demonstrated that the minimal concentration of red guava leaf extract to inhibit the growth of sensitive S. aureus was in the range of 0.195–0.391% w/v, presented in Table 2. The low-value MIC of this extract showed the significant potential of red guava leaf extracts to be further studied and developed to produce natural antibacterial for S. aureus infection diseases.

CONCLUSION

Our finding showed that total extracts of red and white pulp P. guajava leaf extracts significantly contribute its antibacterial activity against S. aureus and could be further study and develop to be formulated as natural medicine, with an affordable price due to the guava leaf available in abundance and its low concentration needed.

REFERENCES


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