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ANTIBACTERIAL ACTIVITY OF *AVERRHOA BILIMBI LEAF EXTRACT AGAINST STAPHYLOCOCCUS EPIDERMIDIS AND STAPHYLOCOCCUS AUREUS*

Sri Agung Fitri Kusuma*[1], Eriek Gunawan[2] and Nyi Mekar Saptarini[3]

[1]Department of Biology Pharmacy, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java, Indonesia 45363.

[2]Departement of Pharmacy, Faculty of Mathematics and Natural Sciences, Al-Ghifari University, Bandung, West Java, Indonesia.

[3]Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java, Indonesia 45363.

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***Corresponding Author**
Sri Agung Fitri Kusuma
Department of Biology Pharmacy, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java, Indonesia 45363.

ABSTRACT

Objective: The objective of this study was to investigate the antibacterial activity of *Averrhoa bilimbi* leaf extract against *Staphylococcus epidermidis* and *S. aureus*. **Methods:** The *A. bilimbi* leaf extract was obtained from a maceration process using 70% ethanol as the solvent. The thick extract then analyzed to detect secondary metabolites using the standard method. Followed by the determination of tannins percentage in the extract, using the cowhide as the substrat. The capability of the extract to produce inhibition against the test bacteria, was conducted by the agar diffusion method. **Results:** The phytochemical analysis of the *A. bilimbi* leaf extract revealed that it contained of alkaloids, tannins, and steroids. Among those phytochemicals, tannins are found to be the strongest antibacterial agent. But in fact, the *A. bilimbi* leaf extract did not produce antibacterial activity against *S. epidermidis* and *S. aureus.* **Conclusion:** The *A. bilimbi* leaf extract has no potential effect as an antibacterial against *S. epidermidis* and *S. aureus.*

KEYWORDS: *Averrhoa bilimbi*, leaf, extract, antibacterial, *Staphylococcus epidermidis*, *Staphylococcus aureus.*

INTRODUCTION

The use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases with no rational rules, and the ability of bacteria to transmit their genetic resistance to the bacteria in the similar or different species, has encouraged the use of new therapeutic agents.[1] As the reported case of bacterial resistance which is normal floras on human skin and can be a pathogen for humans, however, it has been reported to be resistant to several antibiotics that have been used so far. *Staphylococcus aureus* and *S. epidermidis* are normal flora on the skin and mucous membranes of humans and animals.[2] S. *aureus* seems to be the more virulent than *S. epidermidis, but* however because of those bacteria are normal bacterial flora on human, it is difficult to distinguish whether the bacterial isolate is the cause of infection or is the result of sample contamination. As *S. epidermidis*, a bacterium which is colonize on the skin and found as a contaminant in the hospital environment.[3-6] *S. epidermidis* often found as a contaminant bacterium in related with infections due to the prosthetic device contamination and other medical devices.[7-9] Both of *S. aureus* and *S. epidermidis* contribute to the increasing of antimicrobial resistance, mainly to the antibiotic vancomycin. Therefore, the treating of infectious diseases caused by both bacteria become more difficult.

The utilizing of plants in the pharmaceutical field in the world, has gradually increased for maintaining human health due to their pharmacological activities.[10] The acceptance of traditional medicine as an alternative form for health care and the development of microbial resistance to the available antibiotics has led authors to determine the antibacterial properties of *A. aroma* and validate the popular use of this plant. The fact that plants are accepted as a source of new natural medicines has encouraged the author to investigate the antibacterial activity of *A. bilimbi* leaves to inhibit the growth of *S. aureus* and *S. epidermidis*. The basic reason for the purpose of this study was based on the existence of supporting empirical data which reports that the *A. bilimbi* leaf capable to treat syphilis.[11] Likewise, the results reported by other researchers who published the antibacterial activity of the *A. bilimbi* leaves against Gram negative and positive bacteria. The aqueous and chloroform extracts of *A. bilimbi*'s leaves are known to inhibit *S. aureus* and *S. epidermidis*. However, there are no studies that report the antibacterial activity of this *A. bilimbi* leaf ethanol extract against the both test bacteria used in this study. Therefore, in this study, the *A. bilimbi* leaf ethanol extract was tested against both test bacteria.

MATERIALS AND METHODS

Samples

A. bilimbi fresh leaves were harvested from the trees planted in Sukajaya district, Subang, West Java, Indonesia. The leaves and the fruits of *A. bilimbi* were authenticated at Department of Biology, Faculty of Mathematical and Natural Sciences, Padjadjaran University.

Extraction Method

The fresh leaves of *A. bilimbi* were washed using a running tap water, drained and cut into small size. The leaves then dried and converted into crude powder for the extraction using a maceration method. The leaves powder was soaked in 70% ethanol solution and being replaced every 24 h with a new 70% ethanol solvent. The macerate was accommodated every 24 h and collected after 3x24 h. The macerates were evaporated in a rotary evaporator at 40^0C until the macerates become the thick extract with a constant weight.

Phytochemical Screening

The systematic screening of *A. bilimbi* leaf extract was analyzed to determine bioactive substances for antibacterial agents against *S. aureus* and *S. epidermidis*. The alkaloids, saponin, flavonoid, quinones, tannin, and steroid/ triterpenoid were detected as the described method in Farnsworth study.[12]

Preparation of Bacterial Suspension

S. aureus and *S. epidermidis* used in this antibacterial activity test were cultured in a slant agar at Laboratory of Microbiology, Faculty of Pharmacy, Padjadjaran University, Indonesia. A loopful colonies of each bacteria were swabbed and suspended in a sterile physiological NaCl. The turbidity of the bacterial suspension then compared with a 0.5 Mc Farland standard and adjusted to be equal as the standard turbidity. The 0.5 McFarland solution is composed of 0.05 ml of 1% $BaCl_2$ and 9.95 ml of 1% H_2SO_4.[13]

Antibacterial Activity Test

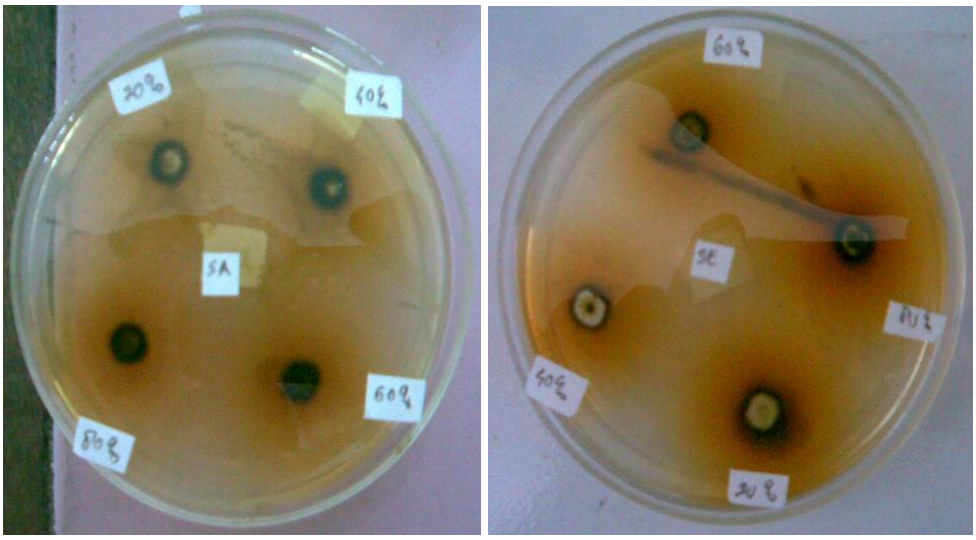
The antibacterial activity test of the *A. bilimbi* leaf extract was employed using the agar diffusion method. Each bacterial suspension in a volume of 0.02 ml bacterial suspension was poured into a sterile petri disk containing 20 ml of liquid MHA (at a temperature of 40^0C) and homogenized. The medium then allowed to solidify, then the medium were perforated. Each well was filled with the leaf extract of *A. bilimbi* in different concentration at fixed

volume (50 µL). The test concentration was diluted by two-fold dilution method to produce following concentration: 20, 40, 60 and 80%w/v. All plates were incubated at 37ºC for 24 h and the inhibitory diameters were measured.

RESULTS AND DISCUSSION

Averrhoa bilimbi Linn. most often found as garden plants and rarely planted for commercial purposes. It is mainly cultivated for folk medicinal purposes in many countries of the world, to treat diabetes mellitus, infectious disease and hypertension.[14] The *A. bilimbi* leaf in infusions and decoctions are used as an antibacterial, astringent, antiscorbutic, postpartum protective medicine, and treatment for fever, rectum inflammation and diabetes.[11,15] Meanwhile, the paste of the *A. bilimbi* leaves is utilized in the treatment of syphilis skin eruptions, itches, rheumatism, cough, cold, mumps, bites of poisonous creatures, and boils.[16,17] The ethanolic extract of the leaf was reported to demonstrate the valuable antimicrobial activity against *Bacillus cereus, B. megaterium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus ochraceous* and *Cryptococcus neoformans*.[18] In addition, another study reported that the aqueous and chloroform extracts of *A. bilimbi*'s leaves and fruits possess antibacterial activity against *Staphylococcus aureus*, *S. epidermis*, *Salmonella typhi*, *Bacillus cereus*, *Citrobacter freundii*, *Proteus vulgaris*, *Aeromonas hydrophila*, and *Kocuria rhizophila*.[19] From those evidence base, *A. bilimbi* leaves are very prospective to improve as a natural antibacterial agent. However, the antibacterial activity of *A. bilimbi* leaf ethanolic extract against *S. aureus* and *S. epidermidis* has not been study yet. We hypothesized that the leaf ethanolic extract would give the potent inhibition against both test bacteria, considering the antibacterial spectrum of the leaf that has been studied. This assumption was also supported by the result of phytochemical analysis of the leaf extract, which found alkaloids, tannins, and steroids containing in the ethanolic extract. In this regard, the preliminary phytochemical screening of the *A. bilimbi* leaves extracts from Subang, revealed the same metabolites as the ethanolic extract reported by another study.[20,21] Those bioactive substances are known to have antibacterial mechanism. Several studies reported that flavonoids, tannins, and steroid of their extract are the responsible metabolites for the anti-staphylococcal activities.[22-27] However, our result study showed the negative result for the antibacterial inhibition against both test bacteria, presented in figure 1. It is not easy to compare the results of this study with other different studies due to the possibility of differences in analysis and workmanship, the quality of the material used, the manufacturers, the microbial strains used, and the extraction technique used. In addition, some active

substances of the extracts might be inactivated or their concentrations might be different in terms of the differences on geographical location, seasons, and cultivation processes.[28] Those parameters known to be affected the activities of *A. bilimbi* leaf extract as antibacterial agent.



A **B**

Figure 1: Antibacterial activity test of *A. bilimbi* leaf ethanolic extract.

Notes: Antibacterial activity against *S. aureus* (A) and *S. epidermidis* (B)

CONCLUSION

The *A. bilimbi* leaf extract has no potential effect as an antibacterial against *S. epidermidis* and *S. aureus.*

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