



Antibacterial Activity of Salam (*Syzygium polyanthum*) Leaves 70% Ethanolic Extract on *Staphylococcus aureus* and *Staphylococcus epidermidis*

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Abstract

Indonesia has a high diversity of potential medicinal plants, which are the second-largest number of indigenous medicinal plants in the world. *Syzygium polyanthum*, known as Indonesian Bay Leaf or Salam, easily found, widely used in Indonesia as a spice in cooking and traditional medicine. Salam contains secondary metabolites such as flavonoids, alkaloids, tannins, essential oils, sesquiterpenes, triterpenes, phenols, steroids, and saponins. *Staphylococcus aureus* and *Staphylococcus epidermidis* are the main bacteria that cause commensal infection and the most common nosocomial infections. This study aims to know the antibacterial activity of the Salam ethanolic extract against *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria. Salam leaves were extracted by 70% ethanol in the maceration method. Antibacterial activity was conducted by the disk diffusion method. The extract exhibits moderate activity (10.51 ± 0.3 mm) at 75% of concentration and low activity (3.69 ± 0.4 mm) at 100% of concentration against *Staphylococcus aureus* and *Staphylococcus epidermidis* respectively. The test showed that salam leaves extract had antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Keywords: Salam, *Syzygium polyanthum*, Antibacterial activity, *Staphylococcus aureus*, *Staphylococcus epidermidis*

Submitted: 03 June 2021

Accepted: 25 December 2021

DOI: <https://doi.org/10.25026/jtpc.v5i4.352>

1 Introduction

Indonesia has a high diversity of potential medicinal plants, which are the second-largest number of indigenous medicinal plants in the world [1]. Approximately 70,000 species of

plants in Indonesia are known to have potential in medicine and only 1000 to 1200 species are utilized [2,3]. Natural ingredients in various developed countries are increasing significantly. It is associated with longer life expectancy, the failure of modern medicine

usage for certain diseases, and wider access to information on herbal medicine worldwide [4].

Various parts of the plant can be used directly or extracted with optimization in such a way as to provide better capabilities [5]. In addition, the use of extraction methods in the exploration of plant-based therapeutic agents may overcome conventional drug discovery problems that slow, lengthy, and very expensive [6]. Treatment selectivity and ease of standardization of medicinal ingredients can be achieved by extracting active substances [3], followed by making phytopharmaca preparations or even purifying them to obtain pure substances [7]. The extraction process is influenced by various factors such as the selection of the correct method and solvent [8], sample particle size, extraction condition and time, comparison of solvent and sample, and natural condition of the compound [9]. These factors will affect the yield and content of secondary metabolites [10].

Syzygium polyanthum, known as Indonesian Bay Leaf or Salam, was easily found, widely used in Indonesia as a spice in cooking and traditional medicine [2,11]. *Syzygium* belongs to the Myrtaceae family, which is known to have a distinctive aroma, distributed throughout Java, Kalimantan, and Sumatera, Indonesia [10]. Various secondary metabolites of salam has synergistic effect resulting pharmacological activity in various diseases [3,12].

Salam contains secondary metabolites such as flavonoids, alkaloids, essential oils, tannins, sesquiterpenes, triterpenes, phenols, steroids, and saponins [9,13]. Dewijanti reported that 70 % ethanol solvent showed higher total phenol and total flavonoids contents than 96% ethanol [14]. Higher amounts of secondary metabolites enhanced extract activity. Furthermore, salam contains compounds such as squalene, phytol [15], quercetin, quercitrin, α -pinene [16], myricetin, eugenol, and orientin [13]. These compounds are found to have antibacterial, antifungal, antidiabetic, antioxidant [17], antihypertensive, and antidiabetic properties [18]. The 96% ethanol extract of the Salam stem bark at 50% concentration showed 13.67 mm inhibition of the growth of *Staphylococcus aureus* while the inhibition zone was not shown against *Escherichia coli* [19]. Research by Nordin on the

hydromethanolic extract of Salam leaves showed 13.5 \pm 0.2 mm inhibition zone of *S. aureus* [2].

The genus *Staphylococcus* is known to cause skin infections and is a pathogen in a wide range of diseases. *Staphylococcus aureus* and *Staphylococcus epidermidis* are the main bacteria that cause commensal infection and the most common nosocomial infections [20,21]. These two bacteria are included in important opportunistic pathogens colonized on the skin [21,22,23]. Increasing bacterial resistance to antibiotics has led to the discovery of alternative antibacterial agents as plant-derived therapeutic agents [24,25]. This study aims to know the antibacterial activity of the Salam 70% ethanolic extract against *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria.

2 Materials and Methods

2.1 Plant material

Leaves were collected from Sukabumi, West Java, Indonesia. The plants were determined at School of Life Sciences and Technology (SITH), Institut Teknologi Bandung, Indonesia with identification number 494/11.002.2/PL/2020 as *Syzygium polyanthum* (Wight) Walp.

2.2 Extraction

Syzygium polyanthum leaves were washed and dried after sorting, at room temperature without direct sunlight. Dry leaves, easily crushed by hand to small pieces. Dried leaves were ground to powder with blender and sieved at 60 mesh. Salam leaves were extracted by 70% ethanol on the maceration method. Five hundred grams powdered simplicia soaked in five liters ethanol (1:10) for 24 hours at room temperature, stirring occasionally. This step was repeated five times. Following filtration of the suspension through filter paper, Salam leaves extract was evaporated at 50 °C using rotary vacuum evaporator (Buchi R-100) and waterbath (CAPP CRWB-30). Yield extract percentage counted by comparing before and after extraction. The crude extract was stored at 4 °C until assayed.

2.3 Phytochemical analysis

Compounds were identified by qualitative analysis. Extract was qualitatively tested to determine their content, including flavonoids, alkaloids, saponins, tannin, steroids, and terpenoids following standard methods [26].

2.4 Bacterial reculture

Staphylococcus aureus ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228 isolates were obtained from Unisba, Indonesia. The bacteria were recultured by streaking a loop of bacteria in the blood agar medium and incubated at 37 °C for 18-24 hours.

2.5 Antibacterial activity

Bacterial suspension in 0.9% physiological NaCl was evaluated using the McFarland 0.5 standard, the turbidity equivalent to 1.5×10^8 CFU/mL. The antibacterial activity has been tested using a disk diffusion method. The bacteria were planted in the solidified Mueller Hinton Agar (MHA) media using the spread-plate method. The bacterial suspension was taken by a 50 μ L micropipette and then dropped to the surface of the media. The droplets were flattened using a sterile L-rod, and the petri dish was rotated occasionally so that the petri dish would spread evenly.

Six mm disks were immersed in a variety of test solutions for about 5 minutes and made 4 repetitions. Solvent as negative control, clindamycin as positive control of disk, and growth control by suspension only. A test solution and control disks were provided to the MHA solid media that already contained the bacterial suspension. Petri dishes were incubated at 37 °C in the inverted position for 24 hours. The inhibition zone is calculated using a caliper minus the disk diameter.

2.6 Statistical analysis

The zone of inhibition values was expressed as mean (n=3) per plate of four repetitions \pm standard deviation (SD) and was analyzed by IBM SPSS Statistic 25 for Windows. Statistical analysis performed by *Analysis of Variance* (ANOVA) and Kruskal-Wallis, followed by Post-Hoc analysis. $p < 0.05$ was considered as significantly different.

3 Results and Discussion

The extract produced from salam leaves *simplicia* has a weak aromatic odour, blackish-brown colour, and bitter taste. The yield extract was 30.25%. This value was associated with the number of secondary metabolites that had been extracted. *Simplicia* sorted, washed, and dried. The drying of the sample will inhibit microbial growth and inhibit biochemical changes in the sample while at the same time removing some of the contents of compounds such as volatile compounds [27] so that the drying process used is chosen with the lowest loss [28]. Reducing the size of the *simplicia* after drying is intended to enhance the penetration of solvents to *simplicia* cells and allow the compounds to be extracted optimally in the extraction process [9,28]. The extraction was carried out using 70% ethanol. Tiwari reported that higher amounts of flavonoid active compounds were detected in 70% ethanol than 96% [29].

Table 1. Phytochemical screening of 70% ethanol extract of salam leaves (*Syzygium polyanthum*)

Compounds	Results
Flavonoid	+
Alkaloid	+
Tannin	+
Terpenoid	+
Saponin	+

*(+) presence; (-) absence

Qualitative phytochemical screening to identify secondary metabolites contained in the extract, which is responsible for the biological response of the extract. Secondary metabolites are tested by specific reactions and identified based on the color or sediment produced. The test results were shown in Table 1, salam leaves extracts contain flavonoid, alkaloid, tannins, terpenoid, and saponins. Salam extract produced a stable foam with a height of 1 cm, indicating the presence of saponin. Liebermann-Burchard and Wilstater reagents displayed red colour in terpenoid and flavonoid testing. The extract produced a white precipitate when tested with Mayer reagent for alkaloid and gelatin for tannin, showing that it contains both alkaloid and tannin. According to research Hartanti, salam leaves 96% ethanolic extract contains flavonoids, alkaloids, tannins, saponins

and steroids [30]. Research by Hassan showed that aqueous extract of salam leaves contains flavonoids, phenols, tannin and saponin at great amount. The different metabolite content could be caused by using a different solvent [31].

Flavonoid compounds in salam extract that have antibacterial activity are quercetin [14]. The antibacterial mechanism for quercetin in *Staphylococcus aureus* was tested by Wang, decreased bacterial protein synthesis, affected protein expression in cells, and ultimately resulted in cell lyses and death [32]. The positive control used was clindamycin, which has sensitivity to *Staphylococcus aureus* and *Staphylococcus epidermidis*. In line with quercetin, clindamycin has a mechanism to inhibit bacterial protein synthesis by binding to the bacterial 50S ribosome sub-unit [33].

Tabel 2. Antibacterial activity of salam leaves ethanolic extract

Sample		Inhibition zone (mm)	
%	mg/mL	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>
25	250	8.94±2	1.07±0.5
50	500	9.55±0.3	1.16±0.3
75	750	10.51±0.3	1.32±0.3
100	1000	10.12±0.8	3.69±0.4
Positive control (Clindamycin)		18.75±1.3	36.68±1.02
Negative control (10% DMSO)		-	-

Salam extract at each concentration has ability to inhibit both bacteria as seen from inhibition zone after incubation (Table 2). Highest antibacterial activity of salam extract against *Staphylococcus aureus* and *Staphylococcus epidermidis*, respectively 10.51±0.3 mm and 3.69±0.4 mm at a concentration of 75% and 100%. The results showed that the extract was more effective at *Staphylococcus aureus*. The extract had low (*Staphylococcus epidermidis*) and moderate (*Staphylococcus aureus*) activity when compared to the positive control. The zone of inhibition below 8 mm was considered low; 8-14 moderate; 15-19 mm strong and > 20 mm very strong [34]. Statistical analysis shows that the data are normally distributed but not homogeneous. The concentration test sample of 25 to 100% was significantly different from the negative control, however, the inhibition zone was not comparable to the positive control

($p < 0.05$). The highest inhibition zone of various ethanol extract to *Staphylococcus aureus* showed at 75% concentration but there was not significantly different to others concentration. While inhibition zone of 100% ethanol extract to *Staphylococcus epidermidis* significantly different.

Other studies have observed antimicrobial activity of the decoction salam leaves at 100% gave an inhibition zone of 11.50±0.3 mm against *Staphylococcus aureus* [35] and 96% ethanol extract gave an inhibition zone of 9.73 in the same concentration and bacteria. However, in the same solvent, 96% ethanol was used by Tammi showed an inhibition zone of 22.75 mm at 100% [36].

4 Conclusions

The results showed that the 70% ethanol extract of Salam leaves had antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. The 75% concentration resulted in the best zone of inhibition in the *Staphylococcus aureus* and 100% in the *Staphylococcus epidermidis* of 10.51±0.3 mm and 3.69±0.4 mm, respectively.

5 Author Contributions

The first author contributed data or analysis, and wrote the paper. The first and third author conceived and designed the analysis. The antibacterial analysis was performed by the second author, and the sample preparation was done by the third author.

6 Conflicts of Interest

The authors declare no conflict of interest.

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