

## SYZYGIUM POLYANTHUM [WIGHT.] WALP LEAVES EXTRACT AS THE ANTIBACTERIAL AGENT FOR STAPHYLOCOCCUS AUREUS

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### ABSTRACT

**Objective:** This study was designed to evaluate the antibacterial potency of *Syzygium polyanthum* [Wight.] Walp leaves extract against *Staphylococcus aureus*. **Methods:** The *Syzygium* leaves were extracted using a maceration method with a 70 % ethanol as the solvent. Then the macerates were evaporated in a rotary evaporator at 40-50 °C to obtain a thick extract and the extract was screened using Harborne's standard method to detect its secondary metabolite content. The extract at various concentrations of 20, 40, 60 and 80 % w/v were evaluated to determine its antibacterial potency against *S. aureus* using the agar diffusion method. **Results:** The selected extraction method was effectively applied to the dried leaves of *S. polyanthum*. The secondary metabolites content of the leave, before and after extraction, was not influenced by the extraction method and the solvent. Both in dried leaves and extracts, they contain the same secondary metabolites, i.e. tannins and flavonoids. The leaves extract of *S. polyanthum* provided higher antibacterial activity against *S. aureus* as the increasing of the used extract concentrations. **Conclusion:** The leaf extract of *S. polyanthum* has a great opportunity to become a potential natural inhibitor against *S. aureus*.

**KEYWORDS:** *Syzygium polyanthum* [Wight.] Walp, leaf, *Staphylococcus aureus*, antibacterial, potency.

## INTRODUCTION

*Staphylococcus aureus* is a normal flora bacterium that provides a significant impact on human health because of its involved in several human diseases. As a normal flora, *S. aureus* colonizes on skin, mucous membrane and skin glands, but the bacteria have the capability to adapt to their environment and causing several infections to human, such as: bacteriemia, lethal pneumonia, osteomyelitis, toxic shock syndrome, scalded skin, and endocarditis.<sup>[1-4]</sup>

Recently, the resistance of *S. aureus* has gradually grown worldwide because of the bacterial evolution and the antibiotic abuse. The main cause of staphylococcal disease, commonly caused by strains of methicillin resistant *S. aureus* (MRSA) and the infections have reached epidemic proportions globally.<sup>[4-7]</sup>

The bioactive compounds in leaves of *S. polyanthum* present a valuable metabolite for antibacterial agent. Recent research has highlighted that *S. polyanthum* has potential antibacterial activities due to the promising antibacterial metabolites, i.e. flavonoids and tannins.<sup>[8]</sup> This encourages interest in evaluating the antibacterial potential of the extract against *S. aureus*, thus, it can add information on the antibacterial spectrum of the *S. polyanthum* leaves.

## MATERIALS AND METHODS

### Materials

*S. polyanthum* fresh leaves were purchased from the Manoko Botanical Garden in Lembang, West Java, Indonesia. The age of the leaves was 2 weeks old and the color was green. The leaves of the plant were determined in School of Biological Sciences and Technology, ITB Bandung, Indonesia. The *S. aureus* was taken from bacterial culture collection of microbiology laboratory, Padjadjaran University, Indonesia.

### Simplicia Preparation

*S. polyanthum* leaves were washed with running tap water in the shortest possible time to avoid leaf damage which allows the early release of active substances. After that, the leaves were treated by the following steps: drained, cut, grounded and indirect drying. The drying process was run until the dried leaves got the constant weight.

### Extraction

The dried leaves of *S. polyanthum* were macerated in ethanol 70% for 3x24 h. The macerate was collected everyday as the new solvent addition into the macerator. All macerates fractions were collected, then evaporated in a rotary evaporator at 40-50 °C to yield the thick extract with the constant weight. The thick extract was prepared to make a serial dilution of testing concentrations as follows: 20%, 40%, 60%, and 80% w/v to be evaluated for the antibacterial activity test. The extract concentration of 80% w/v was made by diluting the extract in DMSO, meanwhile, the other concentrations were serially diluted using sterile distilled water.

### Phytochemical Screening

Phytochemical screening was conducted using a standard method to determine the class of secondary metabolite content in the simplicia and the extract of *S. polyanthum* leaf.<sup>[9]</sup> The detection included several secondary metabolites such as: alkaloids, quinones, tannins, flavonoids, saponins, steroids and triterpenoids.

### Preparation of the bacterial suspension

The turbidity of the bacterial suspension to be used was 0.5 McFarland standard. The 0.5 McFarland solution was consisted of a 0.05 ml of 1% BaCl<sub>2</sub> solution and 9.95 ml of 1% H<sub>2</sub>SO<sub>4</sub> solution. The absorbance of the standard was measured at 530 nm. The *S. aureus* colonies from the slant agar were taken and suspended in 0.95% sterile saline. The turbidity of the *S. aureus* suspension then measured and adjusted to obtain a turbidity that equal to the 0.5 McFarland standard's turbidity.

### Antibacterial activity test

The antibacterial activity test of *S. polyanthum* leaf extract was carried out using the agar diffusion method. A total of 20 µL *S. aureus* suspension was mixed with Mueller Hinton agar (MHA) media (45°C) and gently homogenized in a sterile petri dish. The media was allowed to solidify at room temperature and aseptically perforated. The extract with in each concentration was filled in the hole in a volume of 50 µL. Then, the plates were incubated for 18 h at 37°C. The inhibitory zone diameters were measured using a caliper.<sup>[10]</sup>

## RESULTS AND DISCUSSION

After the drying process, 1.1 Kg of the dried leaves was obtained from 4.5 Kg of the fresh leaves. The drying process was aimed to reduce the water content in the leaves. According to

the traditional medicine requirements, the ideal water content of the extract is less than 10%.<sup>[11]</sup> The extraction yield was 6.65%. The extract, then evaluated for its antibacterial potency against *S. aureus*, and the diameters of the inhibition were presented in Table 1. The *S. aureus* demonstrated high sensitivity response to the tested extract. The inhibition diameter of the extract gradually increased with the increasing of extract concentration. The antibacterial activity of the extract was characterized as a very active antibacterial agent, because the resulted inhibition diameter was in the range of 13-18 mm.<sup>[12]</sup> The *S. polyanthum* leaf extract provided strong antibacterial characters against *S. aureus* and it suggested that the extract was very potential to be a medicinal plant for infectious disease caused by *S. aureus*. The confirming of the antibacterial potency of the *S. polyanthum* leaf extract was due to the potential of secondary metabolites contained in the extract. It is known that bioactive compounds can be synthesized in plants as secondary metabolites that have antimicrobial activity to inhibit the pathogens. The flavonoids and tannins were detected in the extract of *S. polyanthum* leaf. Both metabolites were known as an antibacterial agent with different mechanism of action. The flavonoids are contributed as inhibitor in: the nucleic acid synthesis and the cytoplasmic membrane,s function.<sup>[13,14]</sup> Meanwhile, tannins are reported to have a bacteriostatic or bactericidal effect against *S. aureus*, by complexing the microbial enzymes.<sup>[15,16]</sup>

**Table 1: Antibacterial activity of *S. polyanthum* leaf extract**

Extract concentration (%w/v)	Diameter of inhibition (mm)
20	14.20 ± 0.0000
40	15.55 ± 0.0000
60	16.40 ± 0.0000
80	17.10 ± 0.0002

Notes: the diameter of the perforator = 6 mm

## CONCLUSION

Our finding suggested that the leaf extract of *S. polyanthum* significantly inhibited *S. aureus* and could be further study to be developed as natural medicine, mainly to treat the infection caused by *S. aureus*.

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