

## ANTI-STAPHYLOCOCCAL COMPARATIVE STUDY OF SYZYGIUM POLYANTHUM [WIGHT.] WALP. LEAVES EXTRACT

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Article Received on  
25 May 2020,

Revised on 15 June 2020,  
Accepted on 05 July 2020

DOI: 10.20959/wjpr20208-18076

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### ABSTRACT

**Objective:** This aim of this study was to compare the anti-staphylococcal potency of *Syzygium polyanthum* [Wight.] Walp leaf ethanolic extract against neomycin sulfate as antibiotic standard.

**Methods:** The test was carried out by determining the potency of the extract and the neomycin sulfate to generate the same inhibition against *S. aureus*. The data of inhibition diameters were obtained from the result of the agar diffusion method. The obtained diameters were plotted against the log of the use concentration and inhibition curves of both tested agent were produced. The line equation from each curve was used to determine the concentration of each tested agent in resulting the same diameter of inhibition. Both concentrations then

used to calculate the comparative value by dividing the concentration of the extract with the neomycin sulfate. **Results:** The anti-staphylococcal potency of neomycin sulfate was stronger than the *S. polyanthum* leaf extract. The comparative value of the neomycin sulfate against the extract was 1: 32,474.92679. **Conclusion:** The leaf extract of *S. polyanthum* is a promising natural agent to be an anti-staphylococcal candidate.

**KEYWORDS:** comparative, *Syzygium polyanthum* [Wight.] Walp, *Staphylococcus aureus*, potency, neomycin sulfate.

### INTRODUCTION

The basic knowledge about the efficacy of various medicinal plants is an ancestral heritage that still proves to be useful, and known as safe drug based on empirical evidence.<sup>[1,2]</sup>

Indonesia is one of countries with abundant of plant biodiversity that potential to be used as medicinal plants. Indonesian people have been used many plants based on empirical and evidence based to cure their disease. Sometimes, they use the same plant to cure different disease, both infectious or non-infectious diseases. Among those plants, many Indonesians also trust in *Syzygium polyanthum* [Wight.] Walp or well known as bay leaves to treat diseases, mainly infectious diseases.

An infectious disease results due to the pathogen capability to invade and grow within a host. Actually, our bodies have immune mechanisms to inhibit infection, but if these defenses fail, then infection occurs. Some infectious agents are may come from normal flora in excessive numbers. *Staphylococcus aureus* is one of normal flora on human skin habitat and mucous membrane, but they are reported as causative agent for several infections such as skin infections, damaged, chronic and recurrent airway infections, osteomyelitis, and mastitis.<sup>[3-8]</sup> Our bodies are very potential host to be infected by *S. aureus*, because about 50-60% of individuals are periodically or permanently colonized with this bacterium.<sup>[9,10]</sup> *S. aureus* produce toxins and enzymes as the virulence factors.<sup>[11]</sup> In addition to its high prevalence, *S. aureus* is well known for its ability to get resistance to antibiotics. In particular, antibiotic resistance to *S. aureus* has occurred in epidemic waves.<sup>[12]</sup> An overcrowded population facilitates the development and spread of pathogen-resistant bacteria more quickly.<sup>[13]</sup> Therefore, new antibiotics for Staphylococcal disease, important to cure infections, mainly caused by *S. aureus*. As mentioned before, *S. polyanthum* leaf has been used to treat infection diseases, such as: dysentery and diarrhea.<sup>[14,15]</sup> In addition, the leaf extract does not toxic to cells.<sup>[16]</sup> Pharmacological effects of the *S. polyanthum* leaf extract are contributed from the the phytochemical content of the leaf extract. Therefore, the anti-staphylococcal potency of the leaf extract was compared with the neomycin sulfate as the comparison antibiotic.

## MATERIALS AND METHODS

### Plant Materials

The plant material used in the study was fresh leaves of *S. polyanthum*, obtained from the Manoko Botanical Garden in Lembang, West Java, Indonesia.

### Bacteria test

The tested bacterium was *S. aureus*, taken from Laboratory of Microbiology, Faculty of Pharmacy, Padjadjaran University, Indonesia.

**Extract preparation**

The fresh leaves of *S. polyanthum* were washed using running water, drained, cut, indirect drying and powdered. The leaves were dried under indirect sun until the constant weight of the dried leaves was achieved. The dried materials were then powdered and weighed. The extraction process of *S. polyanthum* leaves were carried out using a maceration method and soaked with 70% ethanol as the solvent. The process needed solvent replacement every 24 h in 3x24 h. The macerates were accommodated every 24 h and collected in one plate before evaporated. The extract evaporation was run until the thick extract achieved a constant weight.

**Phytochemical screening**

The phytochemical components of *S. polyanthum simplicia* and leaf extracts were performed using the standard method to ensure the phytochemical substances, such as: flavonoids, quinones, alkaloids, tannins, steroids, saponins, and triterpenoids.<sup>[17]</sup>

**Inoculum preparation**

*S. aureus* colonies were taken from slant agar using Ose and put into a sterile tube containing 0.95% sterile normal saline. The turbidity of *S. aureus* suspension was adjusted to reach the equal turbidity level as the 0.5 McFarland standard.

**Comparison analysis of anti-staphylococcal activity**

The analysis of the comparison test was conducted using the agar diffusion method. The tested extract and neomycin sulfate were challenged in the same plate. The concentration of neomycin sulfate was arranged as follows: 20; 40 and 60 µg/ml, made by serially diluted using sterile distilled water. The extract concentrations were 200.000; 400.000 and 600.000µg/ml. Then the test medium was prepared by inoculating a 20 µL of *S. aureus* suspension in 20 mL MHA (40-45 °C). The mixture was gently homogenized and allowed to solidify. The solid medium was perforated to make the storage of the extract and neomycin sulfate in the same plate. A volume of 50 µL of the tested concentration was poured into the hole and the media was incubated for 18-24 h at 37 °C. The inhibitory diameters were measured and compared to be plotted to produce inhibitory curve.<sup>[18]</sup>

**RESULTS AND DISCUSSION**

The phytochemical screening result of *S. polyanthum simplicia* and leaf extracts showed that the extraction process did not damage the phytochemical content. Both *simplicia* and the

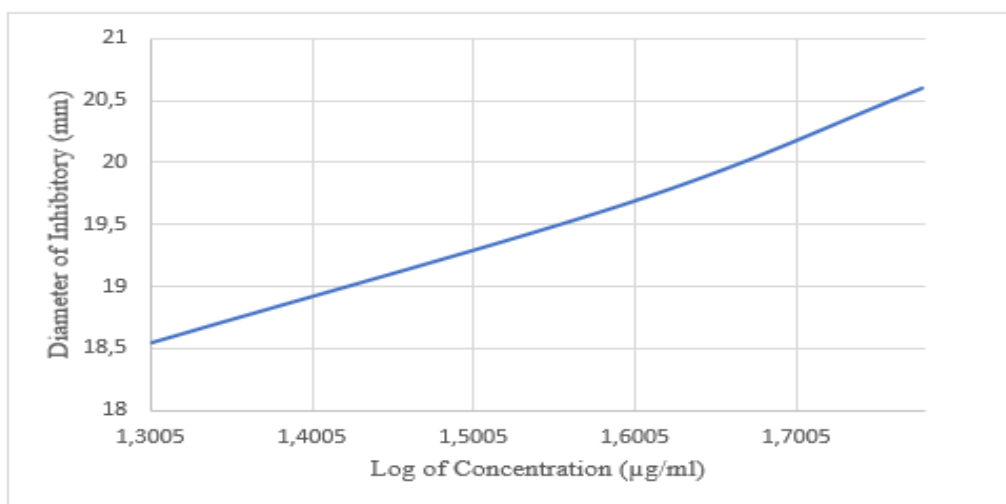
extract produced the same result, i.e. flavonoids and tannins. The phytochemical data are important to determine the antibacterial potency of the *S. polyanthum* leaf extract. The flavonoids have been studied as potent antibacterial agents by inhibiting the nucleic acid synthesis and the disrupting the function of the cytoplasmic membrane.<sup>[19,20]</sup> Meanwhile, tannins work by complexing the bacterial enzymes.<sup>[21,22]</sup>

Comparative test was conducted to determine the antibacterial comparative value of *S. polyanthum* leaves extract to neomycin sulfate by comparing the concentration level in generating the same inhibition diameter against the tested bacteria. The inhibitory diameters are presented in Table 1. Each of diameters was plotted against the log of concentration to produce a curve and the equation using linear regression methods. The relationship of log concentration and inhibition diameter on the neomycin sulfate and *S. polyanthum* leaf extract can be seen in figure 1 and 2. The line equation of neomycin sulfate against *S. aureus* was  $y = 4.2966x + 12.9599$ ; as for the *S. polyanthum* leaves extract was  $y = 5.6589x - 15.2982$ . It was found that the neomycin sulfate in a concentration of 100 µg/mL would produce diameter inhibition in 21.55 mm. For the extract, to produce the same diameter needed a concentration of 3,247,492.679 µg/mL. Therefore, to produce the same inhibition zone, neomycin sulfate needed 1 unit and the *S. polyanthum* leaves extract needed 32,474.92679 units.

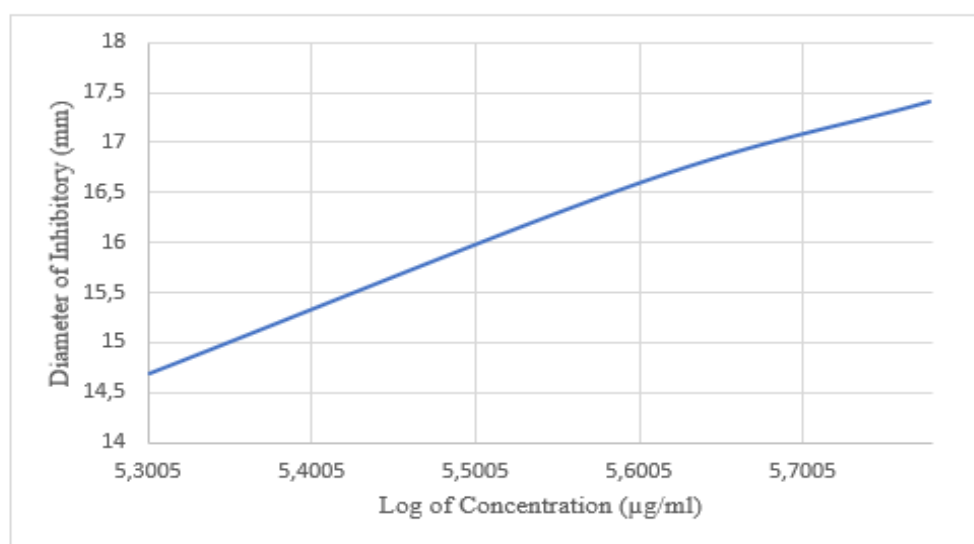
**Table 1: Comparison of inhibition zone diameter of *S. polyanthum* leaf extract to neomycin sulfate.**

Materials	Concentration (µg/ml)	Inhibitory Diameter (mm)
Extract	200.000	14.70±0.0001
	400.000	16.60±0.0000
	600.000	17.40±0.0000
Neomycin sulfate	20	18.55±0.0000
	40	19.70±0.0000
	60	20.60±0.0001

Note: Perforator diameter = 6 mm



**Figure 1: Relationship of log concentration and inhibition diameter on the neomycin sulfate.**



**Figure 2: Relationship of log concentration and inhibition diameter on the S. polyanthum leaf extract**

## CONCLUSION

In conclusion, the leaf extract of *S. polyanthum* is a promising natural agent to be an anti-staphylococcal candidate, even though the anti-staphylococcal of the extract was lower than the neomycin sulfate.

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