

# Phytochemical Composition and Antibacterial Activities of *Syzygium polyanthum* Methanolic Leaves Extract

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## ABSTRACT

**Background:** Since a long time ago, *Syzygium polyanthum* has become traditional herb for health, and thus many studies done to confirm the medical effectiveness. **Objectives:** This present study was conducted to evaluate phytochemical compounds and antibacterial activities of methanolic extract of *S. polyanthum* leaves. **Materials and Methods:** In the present study, standard methods of determination were used to determine the phytochemical compounds of *S. polyanthum*. The methanol extract of *S. polyanthum* leaves were subjected for antimicrobial activity. Antibacterial activity was evaluated against five bacterial strains by determining minimum inhibitory concentration (MIC) and zone of inhibition. Diameters of the zone of inhibition were compared with standard antibiotics. **Results:** Phytochemical analyses qualitatively reveal the presence of alkaloids, saponin, terpenoids and steroid. The extract was found to exert antibacterial activity against four tested bacteria which are *S. aureus*, *S. pyogenes*, MRSA and *K. pneumoniae* except for *E. coli*. The zones of inhibition shown by the disc diffusion method for *S. aureus* were between 8.6 to 14.0 mm, *S. pyogenes* were between 8.4 to 12.0 mm, MRSA was between 10.0 to 13.2 mm and *K. pneumoniae* were between 8.0 to 10.6 mm. Meanwhile, there was no zone of inhibition was observed for *E. coli*. The MIC was determined ranging from 6.25 to 12.5 mg/ml against all the tested bacteria. The highest value of MIC showed by *S. aureus*, *S. pyogenes*, MRSA and *K. pneumoniae* which is 6.25 mg/mL. **Conclusion:** The tested leaves extract showed promising antibacterial activity against both Gram positive and Gram-negative bacteria. Phytochemical screening revealed the presence of alkaloids, saponin, terpenoids and steroid in methanolic leaves extract qualitatively and these compounds could be responsible for antibacterial properties of leaves extract of *S. polyanthum*.

**Key words:** *Syzygium polyanthum*; Phytochemical; Antibacterial.

## INTRODUCTION

Antibacterial resistance is a serious clinical challenge worldwide because some of the bacteria develop enzymes that are capable of digesting and destroying antibiotic molecules. Therefore, this emerging issue has urged the scientist to find a potential source of treatment to encounter that problem. There are numerous reports on the inhibitory effects of various plant extracts on the growth of many bacteria in culture.<sup>1-3</sup> *Syzygium polyanthum* (Wight) Walp ” which is also recognized as “salam”, “serai kayu” or “samak kelat” by Malay people is a tropical plant.<sup>4</sup> *S. polyanthum* leaves, fruits and barks have been used traditionally as medicine or therapeutic agents including efficiency against ulcer, hypertension, diabetes mellitus, hyperuricemia, diarrhea, gastritis, skin diseases and inflammation.<sup>5</sup>

Furthermore, various bacteria and fungi have been tested by extracts from leaves, fruits, as well as essential oil of *S. polyanthum*. Plant extracts are studied and found to have phytochemical constituents such as phenolic compounds, protein-like compounds, flavonoids, and tannins that possess antimicrobial activity. Extracts and fractions of *S. polyanthum* leaves had a low inhibition and/or can inhibit the bacteria *P. aeruginosa* and *B. subtilis*. The highest antibacterial activity against bacteria *S. aureus* and *E. coli* is shown using the n-hexane extracts.<sup>6</sup> This can be explained by the active compounds in the non-polar fractions in relatively higher concentration

which is responsible for the antibacterial activity of the extract. Both extracts were observed to inhibit Gram positive and Gram negative bacteria tested *in vitro* to suggest that *S. polyanthum* leaves extract and fractions has a broad-spectrum antibacterial activity.

Previous studies have discussed the phytochemical constituents of *S. polyanthum* especially that focused on the plant leaves. Gas Chromatography-Mass Spectrometry (GC-MS) analyses organic crude extracts from *S. polyanthum* leaves on (hexane, ethyl acetate and methanol) showed that each crude extract was composed of different phytochemical compositions.<sup>7</sup> Overall, the study conducted revealed that the crude extracts composed of hydrocarbons, aldehydes, terpenoids, phenolics, fatty acids, monoterpenes, diterpenes, triterpenes and sesquiterpenes. There are nine compounds present in all three extracts include nerolidol, caryophyllene oxide, farnesol, phytol, squalene,  $\beta$ -tocopherol,  $\gamma$ -tocopherol,  $\alpha$ -tocopherol and  $\beta$ -sitosterol. Cytotoxic compounds such as linalool and octanal,  $\alpha$ -humulene, and pyrogallol (1,2,3-benzenetriol) also have been discovered in that study. Thus, this study, was carried out to evaluate phytochemical constituent and antibacterial activity of *S. polyanthum* methanol leaves extract in search for active antimicrobial activity.

## MATERIALS AND METHODS

### Plant materials and extraction

The fresh plant of *S. polyanthum* was collected from the state of Terengganu, Malaysia. The plants

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authentication was performed by competent botanist from Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin. The plants were cleaned with tap water to remove dirt and oven-dried at 60°C before homogenized into a fine powder. Leaves powder was weighed into 200 g then was immersed in methanol (1500 mL) for three days at room temperature. After that, the mixture was filtered to make sure that all the mixture was filtered completely. The filtrate was concentrated in a rotary evaporator (Eyela N-1110, Japan) under a controlled vacuum. The extract was stored in the freezer till the use.<sup>8</sup>

### Phytochemical screening

Phytochemical qualitative screening was carried out for *S. polyanthum* extract according to standard procedures used to test for the presence of alkaloids, saponins, terpenoid and steroids.<sup>9</sup>

### Determination of alkaloid

10 mg/mL of extract was diluted in 2 mL of 25% ammonia for several minutes. After that, 5 mL of chloroform was added and shaken gently to extract the alkaloidal base. Mayer's reagent was added. The formation of a cream with Mayer's reagent was observed indicating the presence of alkaloids.

### Determination of saponin

2 mL of distilled water was added to extract suspended in ethanol and was shaken vigorously. The formation of copious foam layer indicates the presence of saponins.

### Determination of terpenoid

2 mL of chloroform was added to 10 mg of the extract. After that, 3 mL of concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

### Determination of steroid

2 mL of chloroform and 2 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was added to 10 mg/mL of extract and shaken well. Chloroform layer appeared red. This confirm the presence of sterols steroid.

### Bacteria cultures

Two pathogenic Gram positive bacteria, *Staphylococcus aureus* (ATCC 11632), clinical isolate methicillin-resistant *Staphylococcus aureus* (MRSA) and three pathogenic Gram negative bacteria *Streptococcus pyogenes* (ATCC 12344), *Klebsiella pneumoniae* (ATCC 10031) and *Escherichia coli* (ATCC 10536) were used. Bacterial strains were grown and maintained on Mueller-Hinton agar (MHA).

### Disk diffusion test

*S. polyanthum* extract was tested for antimicrobial activity using the disk diffusion method. Overnight cultures (10<sup>7</sup>-10<sup>9</sup> CFU mL<sup>-1</sup>) of bacteria strains were spread on Mueller Hinton agar (MHA) with a sterile cotton swab. Sterile filter paper discs with 6 mm diameter were placed on top of the culture and 20 µL of 125 mg/mL, 250 mg/mL and 500 mg/mL (w/v) of *S. polyanthum* extract were loaded on the paper discs. The 10% methanol-loaded disc was used as the negative control. All impregnated discs were fully dried at room temperature in a laminar flow hood before the application on the bacterial lawn. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone (in mm) produce by the leaf extract around the discs.<sup>10</sup>

### Determination of minimum inhibitory concentrations (MIC)

MIC was performed to determine the lowest concentration of the plant extract that inhibits the growth of a microorganism.<sup>11</sup> Two

sets of standardized microtiter plates with shallow wells that contain increasing dilutions (decreasing concentration) of plant extract was inoculated with bacterial suspensions. The plates were incubated at 37 °C for 24 h. The lowest concentration of the extract in the well of the microtiter plate that showed no turbidity after incubation are MIC. Bacterial suspensions were used as negative control, while broth containing standard drug was used as positive control.

## RESULTS

### Phytochemical analysis

Phytochemical analysis shown in Table 1 indicates the methanolic extract of *S. polyanthum* leaves contained a few secondary metabolites such as alkaloids, saponin, terpenoids and steroid.

### Disk diffusion test

The inhibition zone of *S. polyanthum* methanolic leaves extract against Gram positive and Gram negative bacteria is shown in Table 2. Results obtained in the present study revealed that the methanolic extract of *S. polyanthum* leaves exhibits antibacterial activity against *S. aureus*, MRSA, *S. pyogenes* and *K. pneumoniae* but not *E. coli*. The inhibition zones were between 8.0 and 14.0 mm. The larger inhibition zone gave the meaning of higher antibacterial activity of the extract on the tested microbial species.

### Determination of Minimum Inhibitory Concentration (MIC)

From the result shown in Table 3, *S. polyanthum* methanolic leaves extract demonstrated broad-spectrum activity against four selected

**Table 1: Phytochemical analysis of *S. polyanthum* methanolic leaves extract.**

| Phytochemical substances | +: Present; -: Absent |
|--------------------------|-----------------------|
| Alkaloid                 | +                     |
| Saponin                  | +                     |
| Terpenoids               | +                     |
| Steroid                  | +                     |

**Table 2: Zone of inhibition for disc diffusion method of *S. polyanthum* methanolic leaves extract against selected bacteria.**

| Concentration of extract (mg/mL)   | Zone of inhibition (mm) |                    |      |                |                      |
|------------------------------------|-------------------------|--------------------|------|----------------|----------------------|
|                                    | <i>S. aureus</i>        | <i>S. pyogenes</i> | MRSA | <i>E. coli</i> | <i>K. pneumoniae</i> |
| 125                                | 8.6                     | 8.4                | 10.0 | -              | 8.0                  |
| 250                                | 10.6                    | 10.0               | 12.0 | -              | 8.0                  |
| 500                                | 14.0                    | 12.0               | 13.2 | -              | 10.6                 |
| 10% methanol (negative control)    | -                       | -                  | -    | -              | -                    |
| Chloramphenicol (positive control) | 28.0                    | 30.0               | 22.0 | 24.0           | 26.0                 |

-: No inhibition activity

Zone of inhibition (mm) are average of triplicate experiments. Disc diameter = 6 mm.

**Table 3: MIC value of *S. polyanthum* methanolic leaves extract.**

| Bacterial Strains Tested | MIC (mg/mL) |
|--------------------------|-------------|
| <i>S. aureus</i>         | 6.25        |
| <i>S. pyogenes</i>       | 6.25        |
| MRSA                     | 6.25        |
| <i>K. pneumoniae</i>     | 6.25        |
| <i>E. coli</i>           | 12.5        |

The MIC values (mg/mL) are average of triplicate experiments.

bacteria with the MIC values ranging from 6.25 to 12.5 mg/mL. Results showed that all of the tested bacteria were susceptible pathogens with the MIC value of 6.25 mg/mL except *E. coli* with MIC value of 12.5 mg/mL

## DISCUSSION

Medicinal plants have long been deployed as the basis of treatment of various diseases in traditional medicine such as in Asian and also as worldwide ethnomedicine.<sup>12,13</sup> The ethnomedicinal plant is one of the important sources for drug discovery and *Syzygium polyanthum* (Wight) Walp is one of the ethnomedicinal plants. *S. polyanthum* is currently become popular because of its various pharmacological potentials. Many natural products are known with their different secondary metabolites and part of them has been already used for the treatment of various chronic diseases in human.<sup>14</sup> Interestingly, different secondary plant metabolites such as flavonoids, saponins, lignans, tannins, alkaloids and thiophenes, phenolic acids were found to have significant antibacterial activity against variety of bacteria.<sup>15-17</sup> Based on phytochemical analyses, *S. polyanthum* methanolic extract has been proven to be rich in secondary metabolites such as alkaloids, saponin, terpenoids and steroid.

The results revealed that *S. polyanthum* methanolic extract was potentially effective in suppressing bacterial growth with variable potency. Gram positive *S. aureus* was more sensitive towards the plant extract at the highest concentration. In addition, *S. polyanthum* also exhibited an inhibitory effect against MRSA. MRSA is difficult to treat bacterial because it already develops resistance to some commonly used antibiotics. Thus, *S. polyanthum* has the potential to develop into an antibiotic against MRSA. The growth of *K. pneumoniae* also was inhibited by *S. polyanthum*. In this study, the findings showed that the inhibition zone was directly proportional to concentration. The zone of inhibition shown an increment as the concentration of extract increases. Apart from that, this study also suggested that *E. coli* was the most resistant strain to *S. polyanthum* because there is no inhibitory effect exhibited by the bacteria at all concentrations (125-500 mg/mL). This result showed that Gram positive bacteria were easier to inhibit compared to Gram negative ones. Another study reported the methanol extract and n-hexane fraction showed higher antibacterial activity against *S. aureus* as compared to *E. coli*.<sup>18</sup> The resistance level of Gram negative bacteria to herbal extract is consistent with that reported in the previous study confirming the validity of the assay. Gram negative bacteria are more resistant to herbal extracts than Gram positive bacteria may be due to their distinctive structure. Moreover, it also may be because of the inherent tolerance of Gram negative bacteria and the nature and composition of herbs.<sup>19</sup> This previous study also reported that the lipopolysaccharides layer and periplasmic space of Gram negative bacteria are the reasons for their relative resistance. Their outer membranes serve as a permeability barrier that allows only small hydrophilic molecules to pass through into the cell, restricting their rate of penetration for certain antimicrobial compounds and excluding larger molecules.<sup>20</sup> Besides, these bacteria possess multidrug-resistant pumps which are effective as permeability barriers could restrict the penetration of the antibacterial compounds. Gram negative bacteria are more tolerant to any foreign compounds intake with these special buildings. Furthermore, Gram positive bacteria are more accessible to permeation by the extracts because of having a mesh-like peptidoglycan layer.<sup>21</sup>

## CONCLUSION

The findings indicated that the methanolic extract prepared from *S. polyanthum* leaves has good potential for prospective nature-based antimicrobial drug. Further studies are needed to investigate the exact mechanism of their antimicrobial action, purification and characterization of their bioactive contents.

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## CONFLICTS OF INTEREST

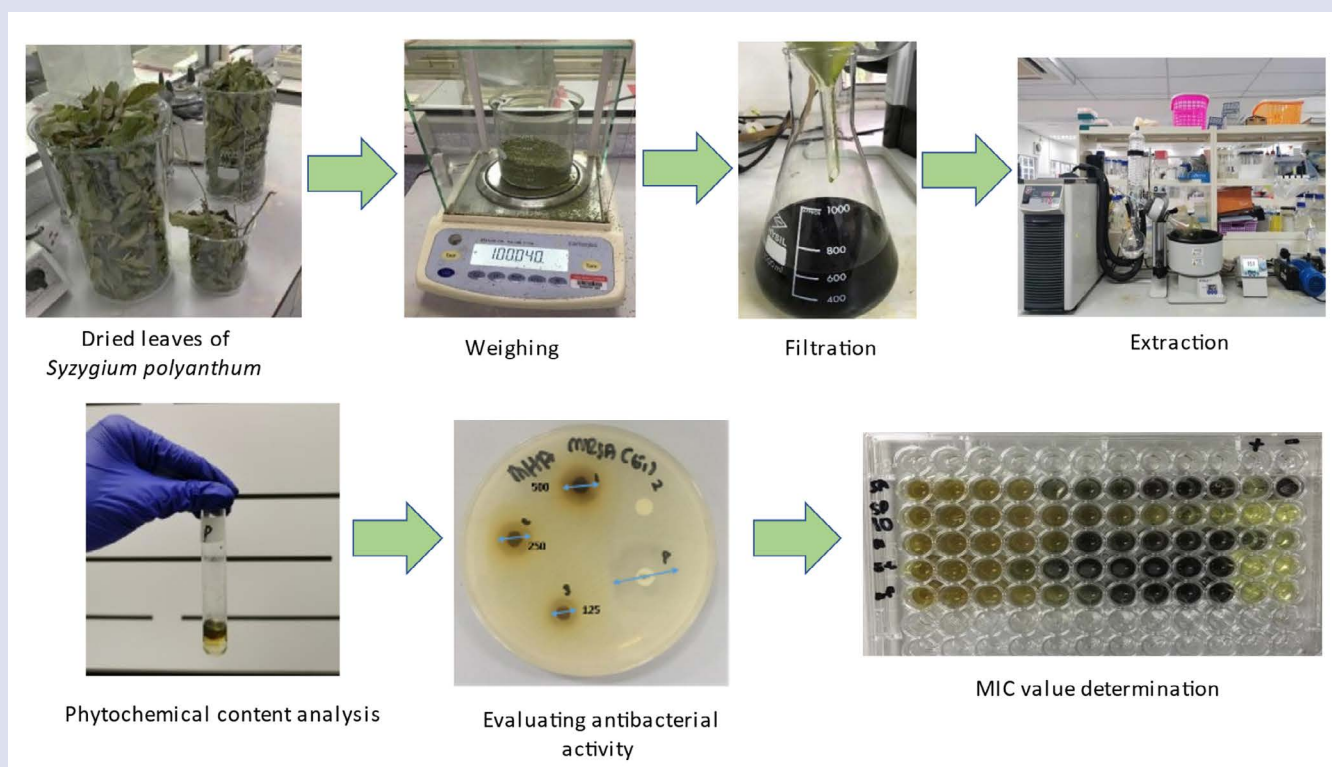
None.

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## GRAPHICAL ABSTRACT



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