**Syzygium polyanthum (Wight) Walp. leaves extract as the antifungal agent for oral candidiasis**

Sri Agung Fitri Kusuma*, Evi Purnamasari², Irma Erika Herawati²

**ABSTRACT**

**Aim:** The aim of this study was purposed to investigate the antifungal activity of *Syzygium polyanthum* (Wight) Walp. leaves extract against *Candida albicans* as natural antioral candidiasis (OC) candidate. **Materials and Methods:** The secondary metabolites content of *S. polyanthum* was detected by Harborne standard method. Ethanolic leaves extract at concentrations of 20, 40, 60, and 80% w/v was tested for the antifungal activity using the agar diffusion method. The minimum inhibitory concentration (MIC) value was determined as the lowest concentration that completely inhibited fungal growth using macrodilution method. For minimum fungicidal concentration (MFC) determination, a volume of liquid was taken from each tube that showed no fungal growth, then subcultured onto the surface of Sabouraud dextrose agar (SDA) medium. **Results and Discussion:** The phytochemical analysis of *S. polyanthum* exhibited the presence of total flavonoids and tannins. The antifungal activity of the extract exhibited a significant increasing of inhibitory diameters as the increasing of extract concentration. This statement correlated with the finding of MIC and MBC value of the extract in the low concentration range that of 0.1562–0.3125% w/v. **Conclusion:** The antifungal potent of *S. polyanthum* (Wight) Walp. leaves extract could give an important contribution in the treatment of OC caused by *C. albicans*.

**KEY WORDS:** Oral candidiasis, Candida albicans, Extract, Leaves, Syzygium polyanthum (Wight) Walp

**INTRODUCTION**

Fungal infections are common diseases throughout much of the world. In humans, these infections occur when an invading fungus colonizes an area of the body and overgrowth so the immune system cannot handle it. There are millions of fungal with different species on earth and about 300 of those are reported cause human diseases. In human, *Candida albicans* is the most prevalent fungal species that is asymptomatically colonizes many areas of the body, particularly the genitourinary and gastrointestinal tracts of healthy individuals. However, *C. albicans* can be an overgrowth and lead to be harmful when host immunity was altered, resident microbiota presence, stress induction, and other factors can lead to *C. albicans* infections. Two types of human infections caused by *C. albicans* are superficial infections such as oral candidiasis (OC) or vaginal (vulvovaginal candidiasis) candidiasis and systemic infections. *C. albicans* and other *Candida* species are reported to be found for up to 75% of the population in the oral cavity. However, *C. albicans* predominantly can affect the oropharynx and the esophagus of persons with adaptive immune system dysfunctions. Therefore, HIV patients have major risk in developing OC. For immunocompromised patients, fungal infections have become one of the major factors of morbidity and mortality. Further, risk factors for developing OC include the dentures wearing and age extremes.

The development of antifungal drugs is purposed to save human life from fungal infections. Nystatin B and amphotericin B are polyenes group and targeted for ergosterol synthesis inhibition. However, these compounds were reported to cause high toxicity for humans. Two decades later, azoles were found and targeted on the biosynthetic pathway of ergosterol by inhibiting an early-phase enzyme called lanosterol 14α-demethylase encoded by ERG11. Despite the availability of antifungal drugs, the incidence of fungal infections remains increased significantly. These are
caused by an increase in antifungal drug resistance. Resistance to azole was reported to be continuing as a significant problem in the fungal infections cause by \( C. albicans \).[11] Therefore, the need for new antifungal drugs with improved efficacy against \( C. albicans \) and safe is an urgent need to break the barrier of Candida infections, especially for OC. Drugs derived from plants commonly used as a spice added to dishes can be hypothesized as a safe antifungal candidate.

Leaves of \textit{Syzygium polyanthum} (Wight) Walp. are used as one of the additive ingredients in almost every dish and traditionally consumed to treat various such as hypertension, diabetes mellitus, ulcers, gastritis, skin diseases, diarrhea, and other infections.[12] \( S. polyanthum \) is a well-known plant in Indonesia, is commonly recognized as manting, meselengan, ubar serai, and Indonesian bay leaf.[13] The phytochemical components of \( S. polyanthum \) leaf part have revealed in several studies. Kusuma et al. found that in the ethanolic extract of \( S. polyanthum \) leaves contain flavonoids and tannins.[14] Among those secondary metabolites, the flavonoid is an antifungal agent with the mechanism of action that has been studied. The antifungal activity of flavonoids potent compounds against \( C. albicans \) in vitro such as catechins (flavanols), quercetin and myricetin (flavonols), baicalein (flavones), and carvacrol (chalcones) was reported. The action mechanisms of flavonoids as an antifungal agent included cell wall damage (catechins), efflux pump inhibition and apoptosis induction (baicalein and sedonan A), and disruption of cytoplasmic membrane (carvacrol).[15] Thus, the effect of \( S. polyanthum \) leaf extracts against \( C. albicans \) was investigated.

**MATERIALS AND METHODS**

**Materials**

Fresh leaves of \( S. polyanthum \) were collected from the Manoko Botanical Garden in Lembang, West Java, Indonesia. The leaves are around 2 weeks old with the leaf width between 2 and 3 cm. The plant was identified in school of biological sciences and technology, ITB, Bandung, Indonesia. \( C. albicans \) as tested fungus used in this study was obtained from the microbiology laboratory of Faculty of Pharmacy, Padjadjaran University, Indonesia. The growth media that were used are Sabouraud dextrose agar (SDA-Oxoid) and Sabouraud dextrose broth (SDB-Oxoid). The chemicals used were (CV. Agung Menara Abadi), dimethylsulfoxide (DMSO-Merck), hydrochloric acid (Merck), amyl alcohol (Merck), acetic acid (Merck), sulfuric acid (Merck), (PT. Widatra Bhakti), methanol (Merck), chloroform (Merck), iron (III) chloride (Merck), ether (Merck), Mayer reagents (Merck), Dragendorff reagents (Merck), metal powder (CV. Agung Menara Abadi), 1% gelatin solution (CV. Medilabs), Liebermann–Burchard reagents (Merck), ammonia solution (Merck), vanillin (Merck), sodium hydroxide (PT. Brataco), and distilled water (Chemistry Dept., UNPAD).

**Preparation of Simplicia**

Fresh leaves of \( S. polyanthum \) were washed with clean water to remove the dirt and reduced the microbes attached to the material. The washing process must be carried out in the shortest possible time to avoid substances dissolving and wasting that contained in the material. Then, the leaves were drained and cut into small pieces about 1 cm in length. This process was done to speed up the drying process; then, it put on the ground and aerated, not exposed to direct sunlight. Simplicia drying was carried out until the weight of the simplicia was constant. According to the requirements of traditional medicine, the drying process must carry out until the water content is <10%.[16]

**Extraction**

The simplicia of \( S. polyanthum \) leaves was macerated using ethanol 70% as the solvent for 3 h × 24 h. The process was employed by changing the solvents every 24 h. Then, the collected extracts were evaporated in a rotary evaporator at 40–50°C the extracts achieved its constant weight.[17] Then, the extract was stored at room temperature and weighed gravimetrically to obtain the extract yields. The extract then prepared to make various dilutions of testing concentrations, those of 20%, 40%, 60%, and 80% w/v for the antifungal activity method. The extract in a concentration of 80% w/v was diluted using DMSO; then, the concentration was diluted gradually using sterile distilled water.

**Phytochemical Screening**

The preliminary phytochemical screening was purposed to detect various metabolites such as alkaloids, tannins, flavonoids, quinones, steroids, triterpenoids, and saponins. The phytochemical analysis was conducted using a standard method.[18]
Antifungal Activity

The antifungal activity of *S. polyanthum* leaf extracts was done using the agar diffusion method. A volume of 20 μL *Candida* suspension was poured and homogenized uniformly in the Petri dish sterile, containing 20 mL culture media (SDA). After the medium was solidified, the medium was perforated using a sterile perforator to make the holes as extract storage. A volume of 50 μL of each extract concentration was pipetted into the hole in the medium. Then, the medium was incubated at 37°C for 18–24 h. The inhibition of diameter zones was observed and measured using a caliper.

Determination of Minimum Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC) Value

The tube macrodilution method was employed for the determination of MIC and MFC. Serial dilutions are done for the extract using the SDB medium to obtain several test concentrations as follows: 10%, 5%, 2.5%, 1.25%, 0.625%, 0.3125%, 0.1562%, and 0.0781% w/v. *C. albicans* suspension was then added to the dilutions of the extract, incubated at 37°C for 18–24 h, and analyzed the fungal growth by observing the turbidity. For MFC determination, a volume of 10 μL was taken from the MIC incubation results that showed no fungal growth, then subcultured onto the surface of SDA medium and incubated at 37°C for 18–24 h.

RESULTS AND DISCUSSION

Extraction Result

From 5 kg of fresh *S. polyanthum* leaves were gained 1.2 kg of dried *S. polyanthum* leaves simplicia and the extract yielded was 42.4 g total extract; thus, the rendement was 8.48%. Based on the water content value, the extract had good quality standard with water content of 6%, not exceed than 10%.[16]

Phytochemical Screening Result

Phytochemicals such as tannins and flavonoids were all detected in *S. polyanthum* methanol extract. These phytochemicals are important starting materials for plants medicinal properties, especially for the invention of new antimicrobial drug candidates today from nature.

Antifungal Activity Result

*C. albicans* employed in this study was susceptible to *S. polyanthum* leaves extracts used. The activity trend was observed by as raising the extract concentrations to 80% w/v; then, the diameter of inhibition zones provided the increasing values, presented in Table 1. The high antifungal activity in the ethanolic extract may be due to the presence of tannins and flavonoids.

<table>
<thead>
<tr>
<th>Extract concentration (%w/v)</th>
<th>Diameter of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>11.85±0.0000</td>
</tr>
<tr>
<td>40</td>
<td>11.90±0.0000</td>
</tr>
<tr>
<td>60</td>
<td>12.06±0.0001</td>
</tr>
<tr>
<td>80</td>
<td>12.17±0.0004</td>
</tr>
</tbody>
</table>

Perforator diameter=6 mm

Table 1: Antifungal activity result

<table>
<thead>
<tr>
<th>Extract concentration (%w/v)</th>
<th>MIC result</th>
<th>Fungal growth</th>
<th>MFC result</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>1.25</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>0.625</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>0.3125</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>0.1562</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.0781</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

MIC: Minimum inhibitory concentration, MFC: Minimal fungicidal concentration

The antimicrobial mechanisms of tannins and flavonoids are by forming complex with nucleophilic amino acids in protein, which lead to the protein inactivation, thus causing loss of protein function. The antimicrobial effect is supported as their microbial target on surface-exposed adhesins, membrane-bound enzymes, and cell wall polypeptides.[19] The extract was found to be effective against *C. albicans* used in this study, which highlights the potential of *S. polyanthum* leaves as herbal drugs and their opportunity use as local medicine.

MIC and MFC Determination Result

MIC value refers to the lowest concentration of an antifungal that inhibits the visible growth of fungal cell. The MFC is the lowest broth dilution of overnight results that prevent growth of the fungi on the agar plate. The result of MIC and MFC determination showed the similar result presented in Table 2. *C. albicans* performed its sensitivity against *S. polyanthum* leaves extracts. The lowest concentration of the extract (3.125% w/v) could eliminate 100% of *C. albicans* growth. Thus, the extracts demonstrated significantly high inhibitory activity against *C. albicans* with MFC ranging from 0.1562 to 3.125% w/v.

CONCLUSION

This research gives a scientific improvement from the traditional use of *S. polyanthum* leaves as herbal drug to a highly promising antifungal for OC caused by *C. albicans*.

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