Mechanism and Antifungal Activities Vulvovaginal Candidiasis Isolated from Patients Against Ethanol Extracts of Parameria laevigata (Juss.) Moldenke Stem Bark

Wirda Anggraini1,4, Djoko Agus Purwanto2*, Idha Kusumawati2, Isnaeni3

ABSTRACT

Introduction: Fungal diseases are not an exception to the current antibiotic resistance situation. Antimicrobial stewardship programs and high drug screening are two of the measures that are being used. Today, fungal infections are severe health problems. Utilizing plant-based natural compounds that are effective against various human pathogenic fungi is one method for preventing the issues associated with fungal infection. In this research, extracts are used as an option to treat patients with Candida albicans infection. The research aimed to examine the antifungal properties of an ethanol extract from Parameria laevigata (Juss.) Moldenke stem bark against C. albicans isolated from patients. Methods: This research used a microdilution method. C. albicans from patients diagnosed with vulvovaginal candidiasis. The test sample is 70% and 96% ethanol extracted from P. laevigata stem bark. Data analysis used One-way ANOVA with a P value of 0.000. Results: The result showed that PLE-70 can inhibit the growth of C. albicans with the highest %inhibition for ATCC 14053, CP-1, CP-2, CP-3, and CP-4, respectively 36.39%; 37.51%; 38.66%; 45.78%; 84.87%. PLE-96 can inhibit the growth of C. albicans with the highest %inhibition for ATCC 14053, CP-1, CP-2, CP-3, and CP-4 respectively 17.49%; 17.77%; 29.27%; 34.12%; 38.42%. Conclusion: It was concluded that the ethanol extract from P. laevigata stem bark can inhibit C. albicans isolated from vulvovaginal candidiasis patients. Key words: Candida albicans, Compound, Medicine, Microdilution method, Ultrasound Assisted Extraction.

INTRODUCTION

Broad-spectrum antibiotics, immunosuppressive medications, and medical implant devices have significantly increased the frequency of fungal infections during the past few decades. Fungal infections now pose a severe risk to human health and are estimated to cause at least 1.5 million deaths per year. At least 20–25% of people worldwide suffer from a fungal infection. The prevalence of fungal infections has increased since 1980. Fungi-related human infections are severe in Indonesia and other tropical and subtropical nations. Indonesia is one of the nations with a tropical climate that features elements like high temperatures and humidity. The climate’s qualities make the skin sweaty and wet. In the human digestive, genital, and oral mucosa, Candida is a commensal microorganism. Individuals with robust immune systems can generally prevent this opportunistic fungal from growing and spreading. However, it can result in a severe illness if the host becomes weak and immunocompromised. These infections can cause various clinical consequences, including brain abscesses, endocarditis, meningitis, arthritis, and pyelonephritis, or they can penetrate the circulation and spread to any place in the human host, which can result in superficial infections like thrush, vaginitis, or skin infections. C. albicans is primarily responsible for invasive fungal infections, either locally or systemically. Since these infections are linked to high death rates, an increased financial burden, and prolonged hospital stays, they induce a severe financial and medical threat to the public health sector.

Regarding pathogens acquired in hospitals, Candida species are the fifth most prevalent parasite disease-causing species worldwide. C. albicans cause Vulvovaginal Candidiasis (VVC), a fungal vaginal and/or vulval mucous membrane infection. VVC is the second most common disease after total vaginal infections. According to Harrindya’s research, VVC was discovered in the Infection Division Infectious Sexual Outpatient Unit Skin and Sexual Health Regional General Hospital Dr. Soetomo Surabaya between January 1, 2010, and December 31, 2012, with 325 new cases. Commonly, Candida albicans causes VVC, accounting for about 80-90% of cases, followed by Candida nonalbicans, such as Candida parapsilosis, Candida tropicalis, Candida krusei, and Candida glabrata, which all cause VVC and are resistant to standard treatment.

Antifungal drugs such as ketoconazole, miconazole, imidazole, nystatin, pimaricin, amphotericin B, terbinafine, and antimetabolites like 5-fluorocytosine are the standard therapies for fungal infections. However, inappropriate use of antibiotics for fungal species can lead to resistance drugs. Therefore, new antifungals derived from natural sources are required. Actinomycetes, honey, saliva, snake venom, plants, actinomycetes, honey, actinomycetes, and actinomycetes are all sources of biological antifungals. Plants have the potential to be a source in the process of searching for new medicinal compounds as antifungals.
A plant with potential as an antifungal is *Parameria laevigata* (Juss.) Moldenke. Many people often use it as a vaginitis treatment. *P. laevigata* can be used as a slimming agent; it is also used as a medicine for wounds, scabs, dysentery, and uterine pain after maternity. This plant can be used for analgesic and antibacterial properties.\textsuperscript{13,14} *P. laevigata* stem bark contains alkaloids, flavonoids, saponins, quinones, tannins, and steroids.\textsuperscript{15,16} This research will examine 70% and 96% *P. laevigata* ethanol extract as an antifungal using the microdilution method.

**MATERIALS AND METHODS**

**Extraction of *Parameria laevigata* (Juss.) Moldenke**

*Parameria laevigata* (Juss.) Moldenke was collected from CV. Merapi Farma Herbal Yogyakarta. The dried powder of the plant material was extracted using Ultrasound-Assisted Extraction (UAE) (Sonica). 500 g of the dried plant powder was extracted with 1000 ml of 70% and 96% ethanol (Merck) for 10 min. This extraction process was repeated three times. The filtrates were then concentrated using a rotary evaporator (Heidolph) and put in an oven (Memmert) until dry.

**Fungal collection**

*Candida albicans* were obtained from vaginal swabs isolated from patients in the dermatology, venereology, obstetrics, and gynecology departments at a hospital in Malang with the ethical approval number 072.1/EA.KEPK-019/35.07.208/2021. The characteristics of the patients can be seen in Table 1.

A swab from patients was cultured on specific media on a Corn Meal Agar (CMA) medium (Himedia) and incubated at 37°C for 24 h. Each strain was sub-cultured on Potato Dextrose Agar (PDA) medium (Himedia) and incubated at 37°C for 24 h.\textsuperscript{17}

**Preparation of suspension Candida albicans**

*Candida albicans* ATCC 14053 and vaginal swabs culture were rehydrated using 10 ml of Potato Dextrose Broth (PDB) medium (Himedia) and then homogenized using a vortex. Next, using a UV-VIS spectrophotometer (Genesys IOS), with a 625 nm wavelength, the absorbance score was read with a range of 0.08-0.13 (1x10^7 CFU/ml) equivalent to 0.5 McFarland. The suspension was diluted using a 1:10 dilution ratio to obtain a 1x10^7 CFU/ml biomass cell count.\textsuperscript{18}

**Antifungal testing**

The microdilution method was used to conduct the antifungal test. The sample test using 70% *P. laevigata* stem bark ethanol extract (PLE-70) and 96% *P. laevigata* stem bark ethanol extract (PLE-96) of 3.75 µg/ml, 12.5 µg/ml, 25 µg/ml, and 50 µg/ml were tested in 96 wells of microtiter plates. *Nystatin* 12.5 µg/ml, 25 µg/ml, and 50 µg/ml, 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, and 50 µg/ml were tested in 96 wells of microtiter plates. *PLE-96* was utilized as a drug reference, which was a positive control. The solvent control was set to the solvent of the test substance, and the negative control was a microbiological suspension. Each microplate well was filled with PDB medium and then incubated for 72 h at 37°C using a microplate reader (Bio-Rad iMark) to measure the absorbance of a plate at a 595 nm wavelength.\textsuperscript{19}

**Table 1: Demography of patients.**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>History of Disease</th>
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<tbody>
<tr>
<td>CP-1</td>
<td>Diabetes Mellitus Type 2, Hypertension and Heart Failure</td>
</tr>
<tr>
<td>CP-2</td>
<td>Pregnancy 7 months</td>
</tr>
<tr>
<td>CP-3</td>
<td></td>
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<tr>
<td>CP-4</td>
<td></td>
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</tbody>
</table>

Description: CP: Candidiasis Patient

**Data analysis**

Data analysis used One-way ANOVA. Data analysis was done using SPSS software version 26.

**RESULTS**

**Extraction of the stem bark of *Parameria laevigata***

The sample used *Parameria laevigata* (Juss.) Moldenke stem bark (PLSB). The yield value of PLSB showed 9.86% for PLE-70 and 6.69% for PLE-96.

**Antifungal activity of *Parameria laevigata* steam bark ethanol extract**

The N-50, PLE-70, and PLE-96 antifungal activities were assayed in vitro by microdilution method against *C. albicans* isolated from patients with vulvovaginal candidiasis, as presented in Table 2. Table 2 shows that PLE-70 can inhibit the growth of *C. albicans* in patients isolated from vulvovaginal candidiasis. *C. albicans* ATCC 14053 showed the highest inhibition in 50 µg/ml concentration. CP-1 showed the highest inhibition in 50 µg/ml concentration. CP-2 showed the highest inhibition in 25 µg/ml concentration. CP-3 showed the highest inhibition in 50 µg/ml concentration. CP-4 showed the highest inhibition in 12.5 g/ml concentration. CP-2 showed the highest inhibition in 12.5 g/ml concentration. CP-3 showed the highest inhibition in 25 µg/ml concentration.

**DISCUSSION**

*Candida albicans* is the most common fungal that causes health problems for all *Candida* species. Vulvovaginal Candidiasis (VVC) is the second most common disease among all vaginal infections. In cases of severe hyperglycemia, fungal vaginitis is the most disturbing of the illnesses because it causes vaginal inflammation or infection. The yeast (fungal) organism, typically *Candida*, is the most frequent etiological agent for this infection. Between 7% to over 50% of cases were reported, of which *Candida albicans* is the majority. Women who have uncontrollable blood sugar levels have a major problem with vulvovaginitis. Controlling vaginal *Candida* infection in diabetic women relies heavily on maintaining healthy blood glucose levels and using appropriate antifungal drugs. It was suggested that glucose levels be maintained below 200 mg/dl to avoid dehydration, caloric loss, and glycosuria and to reduce the risk of infection.\textsuperscript{20}

Uncontrolled blood glucose levels cause several metabolic changes, including increased glycogen levels. Increased glycogen levels cause a decrease in vaginal pH, which increases the environmental susceptibility to the development of a VVC community.\textsuperscript{21} This can result in a marked increase in the colonizing and pathogenic vigor of *Candida* species. During their reproductive years, 75% of women experience at least one episode of VVC. and 50% experience two or more episodes of VVC.\textsuperscript{22} In research involving 101 DM subjects, De Leon et al. showed that the frequency of *Candida* colonization was three times higher in type 1 DM patients compared to type 2 DM patients. In the same research, the most prevalent colonies were *Candida albicans*, isolated from 56% of type 1 DM subjects. In contrast, *Candida glabrata* had a 54% prevalence rate of colonies in type 2 DM patients.\textsuperscript{23} Pregnant women are more infected than other women, with more than 40% of affected women experiencing two or more VVC episodes. The probability

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of a woman developing VVC is thought to increase during pregnancy due to higher estrogen levels and higher glycogen content in vaginal fluids.27

Given the significant and widespread levels of antibiotic resistance among fungal pathogenic, the search for antifungal drugs made from natural substances has become a priority. As a result, there is an increasing global need for research into natural plant-derived remedies that are effective against microbial pathogens.28 This research emphasized the use of *Parameria laevigata* (Juss.) Moldenke, which is widely accessible and affordable. This extraction process uses ethanol, including 70% and 96% ethanol. The aim of using this solvent was to extract all the compounds contained. The yield of the 70% ethanol extract was greater than that of the 96% ethanol extract. This is because the difference between 70% and 96% ethanol lies in polarity, where 70% ethanol has a higher degree of polarity when compared to 96% ethanol.29

Ethanol extract of the stem bark of *P. laevigata* can inhibit the growth of *C. albicans* in patients isolated from vulvovaginal candidiasis. *P. laevigata* stem bark contains alkaloids, flavonoids, saponins, quinones, tannins, and steroids.14 Polyphenols such as tannins, terpenoids, saponins, and phenols like gallic acid, thymol, and flavonoids stand out for their strong antifungal activity.30 Most vegetables, especially green and red vegetables, contain flavonoids, which are common antioxidants. According to research, the fungicidal action that causes proteins to become inactive and lose their functionality is antifungal effects through several mechanisms, one is their ability to interact with the fungal cell walls, causing disruption and damage. Tannins can bind to proteins and enzymes in the cell wall, causing denaturation and inhibition of important cellular processes.31,42 Another mechanism that causes proteins to become inactive and lose their functionality is the complex synthesis of tannins, flavonoids, and nucleophilic amino acids in proteins. Tannins are found to be present and have antifungal properties in the herbal extract.6,14

Based on the result of the %inhibitory of PLE-70 and PLE-96 on the growth of *C. albicans* in vulvovaginal candidiasis patients. It showed an increase in %inhibition of *C. albicans* growth when given PLE-70 and PLE-96. However, there was also a decrease in %inhibition of growth of *C. albicans* at several concentrations because low extract concentrations can trigger the formation of biofilms; besides that, each concentration can have a different content, and more chemical compounds are contained in the extract. This means that the higher the concentration at a certain limit, the higher the antimicrobial effect provided. However, if the dissolved compound content in the extract is too high, meaning the concentration is too high, it can cause saturation, thus affecting the antimicrobial properties of the extract.

**CONCLUSION**

The research showed that PLE-70 can inhibit the growth of *C. albicans* with the highest %inhibition for ATCC 14053, CP-1, CP-2, CP-3, and CP-4 respectively 36.39%; 37.51%; 38.66%; 45.78%; 84.87%. PLE-96 can inhibit the growth of *C. albicans* with the highest %inhibition for ATCC 14053, CP-1, CP-2, CP-3, and CP-4 respectively 17.49%; 17.77%; 29.27%; 34.12%; 38.42%.

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**CONFLICTS OF INTEREST**

The authors reported no potential conflict of interest.

**REFERENCES**

Anggraini W, et al. Mechanism and Antifungal Activities Vulvovaginal Candidiasis Isolated from Patients Against Ethanol Extracts of Parameria laevigata (Juss.) Moldenke Stem Bark


