Original Research

Comparison of Phytochemical Content and Antifungal Activity of Bajakah Tampala Stem (Spatholobus Littoralis Hassk.) Methanol and Ethanol Extracts Against Candida Albicans

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ARTICLE HISTORY
Received: 27 June 2022
Accepted: 26 July 2022

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KEYWORDS
Bajakah Tampala; phytochemicals; antifungal activity of Candida albicans

ABSTRACT
Introduction: Candida albicans causes the second most frequent vaginal infection, vulvovaginal candidiasis (VVC). Bajakah Tampala is a natural plant of Kalimantan that has the potential to be used as a traditional remedy for various diseases. This study aims to determine the phytochemical content and antifungal potential of methanol and ethanol Bajakah Tampala stem extract against the growth of Candida albicans.

Methods: Flavonoids, tannin, phenol, and saponin were identified through qualitative analysis of the phytochemical content. Different concentration of Bajakah Tampala methanol and ethanol extract (1.56%, 3.175%, 6.25%, 12.5%, 25%, 50%, and 100%) was tested for antifungal potency using dilution method. The extract that could inhibit the growth of Candida albicans colonies was continued with the concentration density (35%, 75%, 40%, 25%, 45%, 47.5%, and 50%) in order to determine the Minimum Inhibitory Concentration (MIC) and Minimum Killing Concentration (MKC) by counting the number of colonies.

Results: The methanol extract of Bajakah Tampala contains flavonoids, tannins, phenol, and saponin. In contrast, no flavonoids were detected in the ethanol extract. Candida albicans colonies were not inhibited by concentrations of 1.56%, 3.125%, 6.25%, 12.5%, 25% in ethanol and methanol extracts, respectively. At concentrations of 50% and 100%, methanol extract inhibits colony growth, but ethanol extract is still growing. The antifungal activity of methanol extract showed a MIC of 35% and a MKC of 47.5%, as measured by its concentration density.

Conclusion: Flavonoids are present in the Bajakah Tampala methanol extract, which inhibits and kills the growth of Candida albicans but not ethanol.

INTRODUCTION

Vulvovaginal candidiasis (VVC) is the second most frequent vaginal infectious illness in women of reproductive age and causes vulvovaginal diseases [1,2]. Seventy to seventy-five percent of women of childbearing age will encounter VVC at least once in their lifetime, and forty to fifty percent will experience a recurrence. Candida albicans (C. albicans) is the primary cause of vaginal candidiasis (80-90%) [3,4]. VVC is typically treated withazole antifungal medications. These medications are generally successful. However, drug resistance is possible [5,6]. Many researchers develop herbal medicines because they have nearly identical effects to the original medication.
**Bajakah Tampala** (Spatholobus littoralis Hassk.) is one of the plants with potential for traditional medicine [7–9]. Increasing numbers of people are utilizing Bajakah Tampala since it is believed to have a wide variety of disease-curing capabilities. It is believed that Bajakah Tampala contains antifungal components, including phenols, flavonoids, tannins, and saponins [10–12]. The phytochemical is extracted using ethanol, methanol, and petroleum ether [13,14]. Methanol and ethanol are polar compounds because they have a hydroxyl group (-OH), and methanol is also non-polar because it has a methyl group (-CH3) [15]. This molecule will attract active components, including flavonoids, saponins, tannins, and phenols. The solvent's polarity significantly affects the total phenol and total flavonoid extracted [16,17].

There is currently no scientific evidence about the antifungal potential of Bajakah Tampala, especially against *C. albicans*. This study aims to determine the phytochemical component and antifungal efficacy of methanol and ethanol Bajakah Tampala extracts against the in vitro development of *C. albicans*.

**MATERIAL AND METHODS**

**Study Design**

This research is an experimental study with a qualitative test method to observe the phytochemical content in Materia Medica Laboratory Batu. The antifungal activity test was carried out in vitro with the agar dilution method in the Microbiology laboratory of the Faculty of Medicine, Universitas Brawijaya. The study was conducted from February to March 2022. Sample needed for this research is Bajakah Tampala stems from Pontianak, West Kalimantan. Pure isolates of *C. albicans* were obtained from the Microbiology Laboratory, Faculty of Medicine, Universitas Brawijaya. The total repetition for this research is three times.

**Experimental Procedures**

**Extraction Process**

The stems of Bajakah Tampala have been identified by the Materia Medica Laboratory Batu (No. letter 074/654/102.7-A/2021). The Materia Medica Laboratory Batu was responsible for the production of simplicia, Bajakah Tampala methanol and ethanol extracts and the examination of the phytochemical composition of Bajakah Tampala extract. One day of dehydrating 140 grams of Bajakah Tampala stems at 50 degrees Celsius resulted in 110 grams of simplicia powder. The simplicia powder was split into two and macerated in a mixture of methanol and 96% ethanol at a ratio of 1:4 for 3x24 hours. The results of immersion were filtered and evaporated to obtain a 12 ml methanol and 8 ml ethanol extract.

**Phytochemical Active Content Analysis**

**Identification of Flavonoid**

The phytochemical content was examined using qualitative techniques. Two milliliters of methanol and ethanol extract of Bajakah Tampala were mixed with 0.5 milliliters of concentrated HCL and three to four bands of Mg metal. The presence of flavonoids is indicated by color changes to orange, brick red, and dark red [18].

**Identification of Tannin/Phenols**

Two milliliters of ethanol and methanol extract were added to two milliliters of distilled water for the tannin and phenol screening. One or two drops of 1% FeCl3 are dripped into the extract solution. A blackish-brown or blue-black color signifies the presence of tannins and phenols [18].

**Identification of Saponin**

The saponin test was conducted by shaking a mixture of extract, aquadest, and 2N HCL. Saponins can produce foam due to their soap-like characteristics [18].

**Antifungi Bajakah Tampala Potential Test**

Pure isolates of *C. albicans* from the Microbiology laboratory of the Faculty of Medicine, Universitas Brawijaya. Examination of antifungal potential was conducted using the dilution method with a concentration of 1.56%, 3.125%, 6.25%, 12.5%, 25%, 50%, and 100%. A total of 1 ml of *C. albicans* suspension 10^4 CFU/ml was mixed with 1 ml of extract of various concentrations and then incubated for 24 hours in an incubator with a temperature of 35°C. After incubation, inoculation of 10 μl of *C. albicans* suspension on the Sabouraud Dextrose Agar (SDA) scratches was performed and re-incubated for 24 hours. The number of *C. albicans* colonies that developed was determined by counting CFU/ml units.

The extract that could inhibit the growth of *C. albicans* colonies was continued with the concentration density to determine the MIC and MKC values through the dilution method. The density concentration used is 35%, 37.5%, 40%, 42.5%, 45%, 47.5%, 50%. The number of *C. albicans* colonies that developed at the concentration of the extract was determined using a colony counter and compared to the concentration of 0%. MIC was the lowest concentration that may inhibit *C. albicans* colonies. The MKC value was calculated using the lowest concentration capable of killing *C. albicans* colonies.

**Ethics**

This study received ethical approval from the Health Research Ethics Committee Faculty of Medicine, Brawijaya University No.63/EC/KEPK-S2/03/2022.
Statistical Analysis
The Shapiro-Wilk and Levene tests utilized the extract density concentration data as a requirement for the subsequent parametric test (p≥0.05). If the requirements for the parametric test are met, the One-Way ANOVA test is conducted to see whether there is a difference in the mean colony growth (significance p≤0.05); if there is a difference, the Tukey HSD test is performed (significance p≤0.05). Utilizing SPSS for Windows 25 to calculate data analysis.

RESULTS
Phytochemical Active Content Analysis Results
The result of the phytochemical active content test between methanol and ethanol extract of Bajakah Tampala is presented in Table 1. The Bajakah Tampala methanol extract contains flavonoids, tannins, phenol, and saponin. However, only tannin, phenol, and saponin are present in Bajakah Tampala ethanol extract.

Bajakah Tampala’s Effect as Antifungal
Concentrations of 1.56%, 3.125%, 6.25%, 12.5%, and 25% methanol extract and ethanol extract of Bajakah Tampala were ineffective at inhibiting the growth of C. albicans colonies (Fig. 1). Differences in C. albicans colony growth were observed at 50% and 100% concentrations, as shown in Figure 2. There was no growth of C. albicans colonies when 50% and 100% Bajakah Tampala methanol extract were administered (Fig. 2A and Fig. 2B). The administration of 50% and 100% of Bajakah Tampala ethanol extract show the colony growth of C. albicans, characterized by round, convex, yellowish-white, and shiny colonies (Fig. 2C and Fig. 2D). The absence of colony growth at concentrations of 50% and 100% indicates that Bajakah

Table 1. Active phytochemical content of Bajakah Tampala

<table>
<thead>
<tr>
<th>Compound Identification</th>
<th>Flavonoid</th>
<th>Tannin</th>
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Fig. 1. Candida albicans colony growth in different concentration of Bajakah Tampala. (A) methanol extract; (B) ethanol extract. Concentrations: 1.56%, 3.125%, 6.25%, 12.5%, 25%, 50%, 100%.
Fig. 2. *Candida albicans* colony growth on methanol and ethanol extract of Bajakah Tampala. (A) Growth of colonies of *C. albicans* at 50% concentration of Bajakah Tampala methanol extract; (B) Growth of colonies of *C. albicans* at 100% concentration of Bajakah Tampala methanol extract; (C) Growth of colonies of *C. albicans* at bajakah Tampala ethanol extract concentration of 50%; (D) Growth of colonies of *C. albicans* at bajakah Tampala ethanol extract concentration of 100%. NCA is a non-*C. albicans* colony, and CA is a *C. albicans* colony characterized by round, convex, yellowish-white, and shiny colonies.

![Candida Albicans Colony Growth](image)

**Fig. 3.** Bajakah Tampala methanol extract against *Candida albicans* colony growth. Colony growth of *C. albicans* at a concentration of 0% of 19,233 CFU/ml; concentration of 35% of 17,300 CFU/ml; concentration of 37.5% of 2,333 CFU/ml; concentration of 40% of 767 CFU/ml; concentration of 42.5% of 600 CFU/ml; concentration of 45% of 433 CFU/ml; concentration of 47.5% of 0 CFU/ml; concentration of 50% of 0 CFU/ml.

**DISCUSSION**

**Phytochemical Active Content Analysis Results**

The skin and stems of *Bajakah Tampala* plants contain phytochemicals, including *phenol*, *tannins*, and *flavonoids*, and have antioxidant activity (IC50). However, further research on the extraction method procedure using multiple solvent types is still required [10]. The Bajakah Tampala methanol extract contains flavonoids, tannin, phenol, and saponin in this study. The flavonoids are brick-red in color, the tannins/phenols are blackish-brown, and the saponins have a persistent froth. According to earlier research [10–12], *Bajakah Tampala* contains phenols, flavonoids, tannins, and saponins. However, the ethanol extract of *Bajakah Tampala* only contains tannin, phenol, and saponin. This result contradicts Saputera (2019), which reports that the ethanol extract of *Bajakah Tampala* contains flavonoids, tannins, phenols, and saponins.

*Flavonoids* are polar compounds that can dissolve into other polar solvents such as ethanol, methanol, butanol, acetone, dimethylformamide, and others [19]. In some studies, organic solvents such as methanol were
shown to dissolve flavonoids more effectively than other solvents [20,21]. Another study revealed that the concentration of flavonoids in methanol extract was the highest compared to distilled water, ethanol, acetone, chloroform, and dioxanemone [22]. Therefore, flavonoids are present in the methanol extract of Bajakah Tampala, whereas they are absent from the ethanol extract.

**Bajakah Tampala’s Effect as Antifungal**

Bajakah Tampala methanol extract is more effective than ethanol extract in reducing the number of *C. albicans* colonies because there is no colony growth at concentration of 50% and 100%. This colony decrease is due to the solvent properties of methanol, which can attract compounds effectively flavonoids, saponins, tannins, and phenols in Bajakah Tampala methanol extract. However, the Bajakah Tampala ethanol extract contains only saponin, tannin, and phenol compounds. According to Hakim and Saputri (2020), methanol is both polar and non-polar, whereas ethanol is only polar. Therefore, methanol is more effective than ethanol at attracting phytochemical compounds from natural materials. The presence of flavonoids is essential as an anti-*C. albicans* product because flavonoids damage cell membranes, inhibiting fungal adherence, fungal growth, *C. albicans* proliferation, changes in yeast to hyphal forms, and biofilm formation [23,24].

Further investigation was conducted to determine the concentration that can effectively inhibit and kill *C. albicans*. The administration of Bajakah Tampala methanol extracts at a concentration of 35% did not significantly reduce the number of *C. albicans* colonies (p=0.219). Meanwhile, 37.5%, 40%, 42.5%, and 45.5% reduced the number of *C. albicans* colonies significantly (p=0.00). *C. albicans* did not grow at 47.5% and 50% (Fig. 3). Therefore, the MIC value of Bajakah Tampala methanol extract is 35% because the number of candida albicans colonies decreased compared to a concentration of 0%, and the MKC value of Bajakah Tampala methanol extract is 47.5%.

The One-Way ANOVA test of Bajakah Tampala methanol extract with concentrations of 35%, 37.5%, 40%, 42.5%, 45%, 47.5%, and 50% showed significant differences in inhibiting the growth of *C. albicans* colonies (p=0.00, p<0.05). The inhibition of *C. albicans* growth at various concentrations of Bajakah Tampala methanol extract is dependent on the activity of the extract’s active components.

Flavonoids inhibit the growth of fungi by various mechanisms such as damaging plasma membranes, affecting mitochondrial dysfunction, and inhibiting cell wall formation, cell division, RNA synthesis, and proteins, as well as inhibiting efflux pumps (transporters that serve to remove toxic substances from the body of fungi) [25,26].

Saponins can damage the structure of cell membranes, leading to cell content leakage and the inhibition of mycelium growth, cell adhesion and aggregation, and the formation of biofilm *C. albicans* [27,28]. Phenols can damage cell membranes, inhibit cell division and hyphae development via specific gene targets, and disturb metabolic pathways or induce apoptosis by interfering with redox homeostasis [25,29,30].

Tannins have antimicrobial activities against fungi, yeast, and bacteria. Tannins are antimicrobial agents that inhibit microbial adhesion and cell shear transport proteins. As antifungals, the primary function of tannins is to damage cell walls and plasma membranes so that vital metabolites are lost [3].

**CONCLUSION**

Bajakah Tampala methanol extract contains flavonoids, phenols, tannins, and saponins, whereas the ethanol extract only contains phenols, tannins, and saponin. Bajakah Tampala methanol extract inhibits and kills *C. albicans* colony growth more effectively than Bajakah Tampala ethanol extract. Methanol extract of Bajakah Tampala showed antifungal activity with MIC 35% and MKC 47.5%. The limitation of this study is the presence of contamination during the dilution process, even though there is no contamination in the Bajakah Tampala extract, despite the absence of contamination in the Bajakah Tampala extract and the sterile performance of the procedure.

**ACKNOWLEDGMENT**

The authors thank all who supported and collected this study’s data.

**CONFLICT OF INTEREST**

The authors declare there are no conflicts of interest in this study.

**REFERENCES**