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ORIGINAL ARTICLE

The Effect of Bajakah Tampala as a Therapy for Vulvovaginal Candidiasis in Rattus Norvegicus

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ABSTRACT
Vulvovaginal candidiasis (VVC) is the second most prevalent form of vaginitis and is caused by Candida species, particularly Candida albicans. Bajakah Tampala is now being developed as an herbal medication due to the belief that it may heal a variety of diseases. This study aimed to determine the effect of Bajakah Tampala extract on vulvovaginal candidiasis in experimental model. This study measured the number of Candida albicans colonies, the number of neutrophil cells in the vaginal lumen of rats, and the levels of proinflammatory cytokines (TNF-α, IL-6) in rat blood serum to assess the efficacy of antifungal medication. Rats were separated into negative control, 10mg/kgBW Fluconazole, 427.5 mg, 451.3 mg, and 475 mg methanol extract of Bajakah Tampala groups. Fluconazole and Bajakah Tampala were able to decrease the amount of Candida albicans colonies, neutrophils, and serum TNF-α and IL-6 levels. According to the results of this study, Bajakah Tampala have the same antifungal potency as fluconazole.
Keywords: Vulvovaginal candidiasis; Bajakah Tampala; fluconazole; Candida albicans; number of neutrophil colonies; TNF-α; IL-6

INTRODUCTION
Vaginitis is marked by discomfort, itching, burning, abnormal vaginal discharge, and odour. (1). Vulvovaginal candidiasis, often known as VVC, is the second most prevalent vaginal infection, affecting 20 to 25 percent of women. 70% of women have had VVC, whereas 8% have suffered recurring VVC (1,2).

Ninety percent of cases of VVC are caused by alterations or overgrowth of fungi in the vaginal and vulvar epithelium due to infection with Candida species, particularly Candida albicans (C. albicans) (3). Patients with VVC experience irritation, itching, dysuria, or inflammation, and vaginal fluid contains 10^3 CFU/ml of fungal colonies (4,5). VVC can impair economic sectors and raise the burden of yearly medical expenditures. (3).

Candida albicans superficially penetrating the lining of the vaginal mucosa and causing an inflammatory response constitutes the pathogenesis of VVC infection (4). C. albicans initiates the transition from blastopore to pseudo hyphae under conditions that induce morphogenesis, such as elevated oestrogen, elevated vaginal pH, and microbiome disruption. Pathogen associated molecular patterns (PAMPs) are recognised by PRR, such as Toll-like receptors (TLR), C-Type lectin receptors (CLRs), NOD-like receptors (NLRs), and RIG-I-like receptors (RLRs), when C. albicans is encountered (Netea et al., 2015). C. albicans will adhere to, assault, and harm epithelial cells (6).

Due to the interaction between the hyphae of C. albicans and vaginal epithelial cells, neutrophils are activated. In the pathogenesis of VVC, the presence of epithelial neutrophils indicates the activation and recruitment of immune cells into the mucous layer. In vitro, neutrophils demonstrate the ability to kill C. albicans yeast and hyphae via phagocytosis and NET (7,8). Failure to reduce immunopathological triggers contributes to symptomatic and immunopathological infections and causes the vaginal epithelium to continue to express congenital immune effects (3). This will result in the production and release of inflammatory cytokines, including IL-1, TNF-α, and IL-6 (7).

TNF-α is an essential mediator of cell death in acute inflammatory responses (9). Compared to controls, the vaginitis model demonstrated a twofold increase in TNF-α levels in animal studies (10). By regulating the production of chemokines and leukocyte apoptosis, the pro-inflammatory cytokine IL-6 also contributes to the recruitment of neutrophils during an inflammatory response (11). IL-6 cytokine levels increased during the inflammatory phase of the pathogenic phase observed in rats 28 days after C. albicans infection (12).

The azole class of drugs can be used to treat VVC. These medications are generally effective, but drug resistance is possible (10). Herbal plants are believed to have the potential to become alternative medicines that have been employed in treatment. The Dayak have used the Bajakah Tampala plant (Spatholobus littoralis Hassk), which is indigenous to Indonesian Borneo, to treat a variety of diseases (13). It contains flavonoids, phenolics, tannins, and saponins (14). This compound is known to inhibit the growth of microorganisms and fungi (15). However, studies on Bajakah Tampala's potential to combat C. albicans in vivo have been limited, so the authors are interested in conducting this research.

MATERIALS AND METHODS
Study Design: This study is true experimental. 30 rats were separated into five groups: negative control, fluconazole 10 mg/KgBB, and Bajakah Tampala methanol extract doses of 45, 47.5, and 50%. As research subjects, healthy female Wistar rats aged eight to ten weeks were involved. The samples will be excluded if the mice die and C. albicans does not develop.
Materials: Bajakah Tampala stems may be acquired from Pontianak West Kalimantan Indonesia. Kimia Farma was the source of 150 mg of Fluconazole. Estradiol benzoate was manufactured by Bayer Zydus Pharma Pvt. Candida albicans isolates were donated by the Microbiology Laboratory of Brawijaya University. Rat Interleukin 6 ELISA kit (Cat. No. E0135Ra) and Rat Tumor necrosis factor alpha (Cat. No. E0764Ra) were purchased from BT Lab China. Faculty of Medicine Brawijaya University gives access to extra resources.
Extraction Technique: 2000 grammes of Bajakah Tampala stems were dried at 50^{o}C for twenty-four hours. 1960 grammes of dried stems are processed into powder to create simplicia. Three times over twenty-four hours, 500 grammes of powdered simplicia was macerated in 1:4 methanol. Following filtration and evaporation until no solvent remained, 67 ml of a viscous extract was produced.
Suspension of Candida albicans Production: Pure C. albicans colony isolate was isolated using sterile ose and combined with 5 ml of 0.9% NaCl. The absorbance of the suspension was determined to be 0.619 nm using spectrophotometry with a wavelength of 520 nm. Mixing 4.1 ml of C. albicans solution with 5.9 ml of sterile PBS yielded a suspension containing $2.5x10^6$ CFU/ml.
VVC Model Experiment Animals: Wistar rats (Rattus norvegicus) aged 8 to 10 weeks were obtained from Wistar Farm "Purnomo"

and confirmed healthy by the Veterinary Laboratory of the Faculty of Medicine, Universitas Brawijaya. Standard laboratory food and water were used to acclimate mice for seven days. Protocol for Animal Models of Candidiasis was followed for creating VVC model mice (16,17). Rats were put into the following five groups: negative control, fluconazole 10 mg/kgBW/rat, and pirate extract 427.5 mg, 451.3 mg, and 475 mg/day/rat. Mice were slaughtered and dissected following a three-day treatment.

Colony Growth of Candida albicans in the Rat Vagina: The growth of C. albicans fungus colonies in rat vaginas were measured with colony-forming units (CFU) twice, once before treatment (on the second day after C. albicans inoculation) and once after three days of treatment in mice. The vaginal rinse is diluted by a factor of 10 and then inoculated into an SDA medium. After 48 hours of incubation at 30ºC, the colony is visually determined.

Neutrophil Cells in Rat Vaginal Lumen: Histological preparations of vaginal tissue were stained with hematoxylin and eosin. Using a microscope with a 400x magnification, eight to ten fields of vision were examined. Neutrophil cells were counted in the vaginal lumen.

Cytokine Evaluation: Blood from the heart of rats was drawn and left to stand for 10 to 20 minutes at room temperature. Blood serum was produced by centrifuging cardiac blood at 4oC for 15 minutes at a speed of 5000 revolutions per minute. TNF-a and IL-6 concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) kit manufactured by BT-LAB, China.

Ethics: The Faculty of Medicine at Brawijaya University has deemed this research ethically permissible (No. 63 /EC/KEPK-S2/03/2022; March 25, 2022).

Analytical Statistics: The data were analysed with the One-Way ANOVA test when the data distribution was normal (p>0.05) and homogeneous (p>0.05) using SPSS for Windows 25 and a significance level of p 0.05. If a significant difference exists, continue with the Tukey HSD test (p 0.05).

RESULT

Effect of Bajakah Tampala Methanol Extract (Spatholobus littoralis Hassk) on the Growth of Candida albicans Colonies: The number of C. albicans colonies is distributed normally and consistently (p=0.696; p=0.644). The number of C. albicans colonies decreased significantly after administration of fluconazole and methanol extract of Bajakah Tampala compared to the number of colonies before treatment (p= 0.00). After administration of Bajakah Tampala, there was no significant difference between the treatment and control groups in the number of C. albicans colonies (p=0.266). These results indicate that the methanol extract of Bajakah Tampala inhibits the growth of C. albicans colonies similarly to fluconazole (Figure 1).

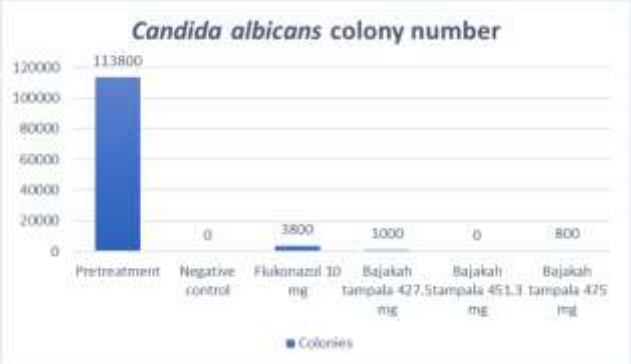

Figure 1: Candida albicans colony growth in rats with vulvovaginal candidiasis before and after therapy

Effect of Methanol Extract of Tampala Bajakah (Hassk Spatholobus littoralis) on Neutrophil Count in VVC Rats: The number of neutrophils with normal and homogeneous distribution (p-value=0.587; p-value=0.198). The One-Way Anova test revealed no significant differences in neutrophil cell counts between the control and treatment groups (p=0.545). These results demonstrated that the administration of Bajakah Tampala methanol extract (Spatholobus littoralis Hassk) resulted in an identical number of neutrophil cells in the vagina between VVC rats given Bajakah Tampala extracts of 427.5 mg, 451.3 mg, and 475 mg, and negative controls (Figure 2).

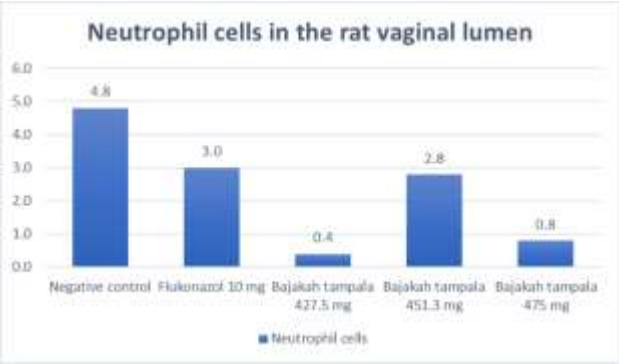

Figure 2. The number of neutrophil cells in the rat vaginal lumen.

Bajakah Tampala Methanol Extract (Spatholobus littoralis Hassk) and TNF-a Serum Levels: Data on serum TNF-α levels were distributed normally and homogenous (p=0.091; p=0.517). The average TNF-α levels in the five samples did not differ significantly based on the results of a one-way ANOVA (p=0.285). This demonstrated that there was no difference in TNF-α levels between the groups administered fluconazole or Bajakah Tampala extract. So, it can be concluded that Bajakah Tampala extract has the same effect as fluconazole in reducing TNF-α levels (Figure 3).

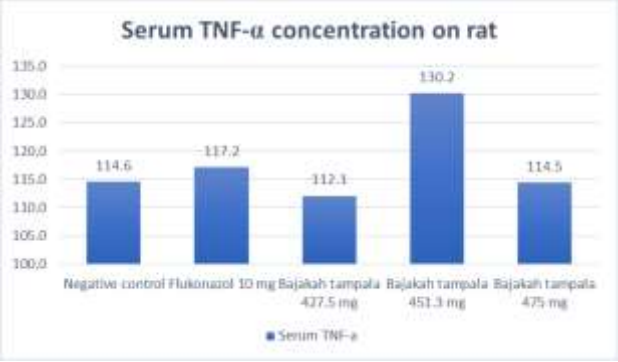

Figure 3: Variations in serum TNF-α levels following administration of Bajakah Tampala methanol extract to rats with vulvovaginal candidiasis

Bajakah Tampala Methanol Extract (Spatholobus littoralis Hassk) and IL-6 Serum Levels: The IL-6 serum level has normal and homogeneous distribution (p-value=0.308; p-value=0.376). The results of the One-Way Anova test indicated that serum levels of IL-6 differed significantly (p-value 0.024). The results of Tukey HSD on the effect of administering different concentrations of Bajakah Tampala (Spatholobus Littoralis Hassk) methanol extract are identical for the fluconazole group and negative control groups (p-value = 0.92). These results demonstrated that administration of Bajakah Tampala methanol extract (Spatholobus littoralis Hassk) resulted in serum IL-6 levels identical to those of VVC rats given 10 mg/KgBB fluconazole and healthy mice.

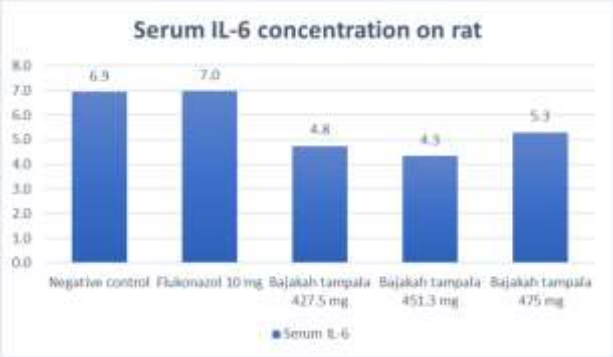

Figure 3: variations in serum IL-6 levels following Bajakah Tampala methanol extract administration in rats with vulvovaginal candidiasis.

DISCUSSION

Vulvovaginal candidiasis is an inflammation of the vagina and vulva brought on by Candida species, particularly C. albicans (5). This infection is extremely prevalent in the female lower reproductive system (3). VVC occurs when the environment supports the growth of Candida albicans, including pregnancy hormone fluctuations, luteal phases of the menstrual cycle, oral contraceptive use, hormone replacement therapy, genetics, biology, behaviour, antibiotic use, and uncontrolled diabetes (5). Epithelial cells respond to Candida colonisation via a TLR-4-dependent mechanism that induces NF-kB and AP-1 activation and the production of pro-inflammatory cytokines such as IL-1, TNF, and IL-6. TLR9 and nucleotide-binding oligomerization domain-containing protein 2 (NOD2) will recognise chitin (12,18).

Macrophages, neutrophils, and dendritic cells will also phagocytose C. albicans and produce pro-inflammatory cytokines. Neutrophils are an essential component of the body's first line of defence, which modulates the repair process following tissue damage or infection (19). Neutrophils are the most abundant leukocytes in the bloodstream. Plays a role in inflammatory mediators and in capturing and destroying microorganisms that attack the host (20).

In this study, we determined the efficacy of Bajakah Tampala against C. albicans-induced vaginal inflammation and the mechanism by which it inhibits this inflammation. Spatholobus littoralis Hassk., also known as Bajakah Tampala, is a member of the Fabaceae family. The inhibition of C. albicans growth in various concentrations of Bajakah Tampala methanol extract is dependent on the activity of the extract's active components. There are flavonoids, phenols, tannins, and saponins in Bajakah Tampala (14,21).

The number of C. albicans colonies decreased significantly after treatment compared to before treatment (p=0.00). This indicates that administration of fluconazole and Bajakah Tampala methanol extract has an effect on the number of C. albicans colonies in VVC rats. The number of C. albicans colonies following administration of fluconazole and Bajakah Tampala did not differ significantly (p=0.266). There was no statistically significant difference in the number of neutrophils between the study groups (p=0.545). Serum TNF-α levels did not differ significantly between treatment groups (p=0.285). There were significant differences in overall serum IL-6 levels (p=0.024), but different tests revealed that there was no discernible difference between the study groups (p=0.92). The influence of Bajakah Tampala on the number of C. albicans colonies, neutrophils, and pro-inflammatory cytokines (TNF-α and IL-6) is comparable to that of the antifungal drug fluconazole.

Azole inhibits 14-demethylase (Erg11p), a crucial enzyme in the biosynthesis of ergosterol. Ergosterol is the most abundant sterol found in fungal cell membranes, including plasma and mitochondrial membranes (22). Flavonoids, tannins, saponins, and phenols can inhibit the growth of fungi through a variety of mechanisms, including damaging plasma membranes, particularly ergosterol, affecting mitochondrial dysfunction, inhibiting cell wall formation, and inhibiting microbial adhesion. In addition, the phytochemical content of Bajakah Tampala can inhibit the growth of mycelium, cell division, synthesis of RNA and proteins, efflux pumps (transporter that serves to remove toxic substances from the body of fungi), the development of hyphae through targets in specific genes, disturbances in metabolic pathways, and/or induce apoptosis by interfering with redox homeostasis (23–27).

The activation of phosphatidylserine by flavonoids can inhibit the synthesis of fatty acids. In addition to increasing intracellular ROS production, flavonoids can alter the structure of fungi, induce apoptosis, mitochondrial depolarization, and cause DNA fragmentation in fungi (28,29). Flavonols and chalcones appear to possess the greatest antifungal activity. Both flavonoid compounds are effective at eradicating fungi via biofilm inhibition for each of their subclasses by inducing membrane damage, resulting in cell size reduction and intracellular component leakage (30,31).

Flavonoid, saponins, tannins, and phenols compounds can inhibit the NF-kB pathway that regulates the expression of the endothelial cell activating enzyme cyclooxygenase 2 (COX2) and cytokines. Neutrophils that release prostaglandin E2 (PGE2) using the enzymes COX1 or COX2, cytokines, reactive oxygen species, and histamine can be attracted by cascade signalling. According to reports, flavonoids' quercetin inhibits neutrophil recruitment and modulates actin polymerization in neutrophils. Additionally, flavonoids can inhibit the production of neutrophil-producing elstase enzymes (32–36).

TNF-α, IL-1, IL-6, IL-17, and IFN-γ are examples of cytokines inhibited via NF-kB signalling pathways (Al-Khayri et al., 2022). In cells induced by LPS or IF-γ, flavonoids reprogram the pro-inflammatory response into an anti-inflammatory one. Flavonoids also enhance the activity of natural killer cells (NK) and cytotoxic T cells. To reduce cytokine secretion, flavonoids inhibit MAPK phosphorylation and NF-kB translocation by inhibiting IB kinase activity in DC. Additionally, flavonoids inhibit the release of arachidonic acid from inflammatory cells, resulting in a lack of arachidonic substrates for cyclooxygenase and lipoxygenase pathways. Consequently, the number of prostaglandins, prostacyclin, endoperoxides, and thromboxane on one side and hydroperoxide acids, eicosatetraenoic hydroxy acids, and leukotrienes on the other side will be reduced (37). The flavonoid structure has a planar ring structure with C2-C3 unsaturation. Without the hydroxyl groups at positions 3' and 4' on ring B, the anti-inflammatory activity of the compound will be lost (38). Flavonoids inhibit the synthesis of inflammatory mediators such as IL-1, TNF-, NO, and COX-2, thereby inhibiting the expression of VEGF and ICAM-1 and activating the inflammasome pathways STAT3, NF-kB, NLRP3, and MAP kinase (39).

CONCLUSION

In VVC rats, the administration of Bajakah Tampala methanol extract can reduce the number of colonies, the number of neutrophils, and the serum levels of TNF-α and IL-6. Bajakah Tampala has the potential to possess the same efficacy as fluconazole. Further research is required to determine the toxicity of Bajakah Tampala at these concentrations.

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