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Effects of Spatholobus Methanol Extract Hassk's Littoralis Against Morphology Candida Albicans and Serum IL-10 Levels in Rat Model Candidiasis Vulvovaginal

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ABSTRACT

Vulvovaginal candidiasis (VVC) is an infection of the lower female reproductive tract mucosa caused by the polymorphic opportunistic fungus Candida albicans. Colonization of C. Albicans in the vaginal lumen is generally asymptomatic. Vulvovaginal candidiasis (VVC) is a common infection of the genital tract that affects millions of women worldwide. This study aims to determine the effect of methanol extract of Bajakah (Spatholobus littoralis hassk) on the morphology of Candida albicans in the vagina of female rats (Rattus norvegicus) Vulvovaginal Candidiasis model. This study used a quantitative approach, namely a simple experimental design (actual trial) with a completely randomized design method, and the posttest-only control group design approach was chosen. Examination of the hyphae of the fungus Candida albicans was carried out using a qualitative descriptive design approach, which describes, describes, and describes the object under study. The results showed that there was an increase in serum levels of IL-10 in the blood of KVV model rats by administration of pirated extract in each treatment group (P1, P2, and P3) which showed the mean respectively: P1 136.609 pg/mL, P2 147.212 pg/ mL, and P3 148.270 pg/mL with a p-value of 0.021 and this study also showed that there was an effect of giving piracy extract in various concentrations on serum IL-10 levels, that is, the more concentrations used, the serum IL-10 levels increased. Administration of various doses of methanol extract of Bajakah (Spatholobus littoralis hassk) did not affect the decrease in the morphology of Candida albicans in the vagina of female rats (Rattus norvegicus) Vulvovaginal Candidiasis (KVV) model, in this case, the number of hyphae microscopically did not increase or decrease with increasing concentration of the extract.

1. Introduction

Candidiasis is a widespread fungal infection in Indonesia. Vulvovaginal candidiasis (VVC) is a common infection of the genital tract that affects millions of women worldwide. Candida is a commensal organism of the genitourinary tract with a colonization rate of 11.6%-17% [1]. It is estimated that 75% of all women will experience at least one episode of VVC in their lifetime. Between 40-50% of initially infected women will share a second episode, while 7%-8% will have VVC recurrences defined as at least four confirmed episodes per year [2], [3]. As many as three-quarters of women in their lifetime have experienced VVC, especially during their childbearing years, and 5% of women may experience VVC recurrence. Data in America states that 13 million cases of CVV per year are the disease with the second most recurrences after bacterial vaginosis. A retrospective study conducted by Harnindya (2016) at the Skin and Gender Health Outpatient Unit (URJ) Dr Soetomo Hospital Surabaya in 2012, found 102 cases of KVV. [4]. CVV symptoms result from an inflammatory response to Candida albicans in the vulvovaginal. There are many inflammatory mediators produced by vaginal epithelial cells that act in response to Candida albicans, such as interleukin-1α and Tumor Necrotizing Factor-a (TNF-a), Th-1 type cytokines, Th-2 type cytokines, chemokines monocyte chemoattractant protein 1, IL-8, IL-4, IL-5, IL-10, histamine and prostaglandin E2 (PGE2) [2], Ashraf et al., 2015 in Ariani, 2021) [5]. First-line treatment for VVC uses antifungals from the azole group, which can be administered orally or topically. Polyene antifungals, especially nystatin, are commonly used as topical treatments, and azoles, such as fluconazole, are used orally [6]. However, in recent decades, there has been an increase in both acquired and innate fungal resistance to antifungal drugs. Antifungal resistance is a reasonably significant problem in Candida fungal infections. About 7% of all Candida blood samples tested at the CDC are resistant to the antifungal drug fluconazole. Antifungal resistance, especially fluconazole resistance, has been reported as high as 12% -18% in several institutions in the United States [7]. Antifungal resistance varies widely between institutions, with some saying no azole resistance. In contrast, others have reported that fluconazole-susceptible, dose-dependent plus isolate resistance may be as high as 50% in the intensive care unit.

The development of antifungal drug resistance continues to increase, and today herbal plants can be a choice for the treatment of fungal infections. The result of alternative therapies for treating VVC has been increasing, associated with the search for fewer side effects, better safety, and lower costs [8]. Several studies have tested the effectiveness of pirated roots, ranging from reducing fat in obesity, the point of wound healing to being anti-bacterial (Novanty et al., 2021; Ariesanti, 2021; Saputera M. M. A., 2021) [9]. Until now, no research has been found regarding the effects of the pirate plant as an antifungal, especially for the treatment of VVC. Therefore further research is needed regarding the extract of the root of the plant (Spatholobus littoralis hassk) in terms of its effect on fungal growth in the form of changes in hyphae morphology in Candida Albicans and IL-10 levels in serum. female rat model of vulvovaginal candidiasis [10].

Survey Point

In candida infection conditions, anti-inflammatory cytokines such as IL-10 play a role in inhibiting the activity of Th2 cells, NK cells and macrophages. IL-10 will be produced to reduce inflammation, minimizing pathological conditions due to excessive inflammation. Along with the development of antifungal drug resistance, which continues to increase, today's herbal plants can be a choice for treating fungal infections. Therefore this study wanted to find out whether the administration of methanol extract of Bajakah (Spatholobus littoralis hassk) can affect the morphology of Candida Albicans hyphae and IL-10 levels in the serum of female rats (Rattus norvegicus) Vulvovaginal Candidiasis model.

2. FINDINGS AND DISCUSSION

This study consisted of five treatment groups: a negative control group, a positive control group, and

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treatment groups 1, 2, and 3. The negative control group was a group of female rats that were not given any treatment. The positive control group was a group of female rats injected with estradiol valerate and then inoculated with Candida albicans intravaginally. This estradiol valerate injection aims to create a pseudoestrous atmosphere in rats so that the fungus can grow in the rat's vagina and cause vulvovaginal candidiasis infection. This treatment is generally chosen because the response of the vaginal epithelium and stroma of the rodent cervix to sexual steroids is similar to that of humans. Thus, changes occur in the vaginal epithelial cells depending on the phase of the estrus cycle.

Microscopically, the pro-estrous smear showed a predominance of nucleated epithelial cells, the estrous smear consisted of nucleated cornified cells, the metestrous smear had an equal proportion of leukocytes and nucleated and nucleated epithelial cells, and the diestrous smear showed a predominance of leukocytes. Therefore, in vivo experimental models of vulvovaginal candidiasis are very useful in identifying factors regarding hormonal influences on infection, yeast virulence, susceptibility and treatment of infection.

Vulvovaginal candidiasis (VVC) is an opportunistic mucosal infection caused by Candida albicans that affects many healthy women of childbearing age. Acute episodes of vulvovaginal candidiasis are common during pregnancy and during the luteal phase of the menstrual cycle when levels of progesterone and estrogen increase. In the absence of estrogen treatment, the duration of the infection is shorter, with lower amounts of yeast in the vagina. In general, it is thought that the transition of estrogen-dependent epithelial cells from columnar to stratified squamous makes the fungus more permissive to C. Albicans attachment and growth.

Sex hormones such as estrogen, testosterone, and progesterone regulate many immune system functions. Generally, males are more susceptible to infection than females because the overall immune response is lower in the male population. In addition to the influence of sex hormones on immune cell function, sex hormones also directly affect microbial pathogenicity by increasing microbial persistence, metabolism, and expression of virulence genes. Estrogen increases glycogen production in the vaginal mucosa, providing a nutrient-rich environment for the development of C. Albicans. In the VVC mouse model, when pseudoestrus was induced to maintain fungal colonization, vaginal epithelial cells had a reduced ability to control C. Albicans growth. In rats, estrogen decreases phagocyte infiltration into the vaginal cavity and suppresses cell-mediated immunity. In response to increased estrogen, C. Albicans undergoes several forms of adaptation that alter host-pathogen interactions. The reduction in C. albicans phagocytosis is due to the fungus adapting reversibly to estrogen, and this adaptation interferes with the elimination mechanisms of the fungus.

Infection with Candida sp. It is an opportunistic infection. Although initially it was thought that yeast passively participates in the pathogenesis and formation of fungal infections, this concept has been modified, proposing the active participation of these microorganisms through the action of virulence factors. Factors contributing to the pathogenesis of C. albicans include morphogenesis (the transition between single-celled yeast cells to the filamentous growth form), secretion of enzymes such as aspartyl protease (SAP) and phospholipase, and host recognition biomolecules (adhesins), which lead to the process of biofilm formation.

The pathogenesis of C. Albicans infection involves initial attachment of the yeast to the vaginal mucosa, followed by asymptomatic colonization, from which the yeast can attain a state of the infectious agent (symptomatic vaginitis). It occurs when the host colonization site becomes favorable for yeast development, usually due to some predisposing factor, such as the reduced immunological response observed in

immunosuppressive disease, diabetes mellitus, pregnancy, or chronic corticosteroid use. Other contributing factors may include antibiotics, estrogen therapy, minor trauma such as sexual acts, habit of wearing tight or synthetic clothing, and diet. In the absence of these factors, clinical observations suggest that candidosis occurs mainly during the menstrual cycle's luteal phase when estrogen and progesterone levels are high.

Effect of Methanol Extract of Bajakah (Spatholobus littoralis hassk) on the Morphology of Candida albicans in the Vagina of Female Rats (Rattus norvegicus) Model KVV.

In this study, histological observations were made on the vaginal tissue of healthy and KVV model rats that had been given fluconazole and various doses of pirated extract using the Aomori staining method to see the density of Candida albicans hyphae. The results of observations of Candida albicans hyphae on vaginal histology are as follows:

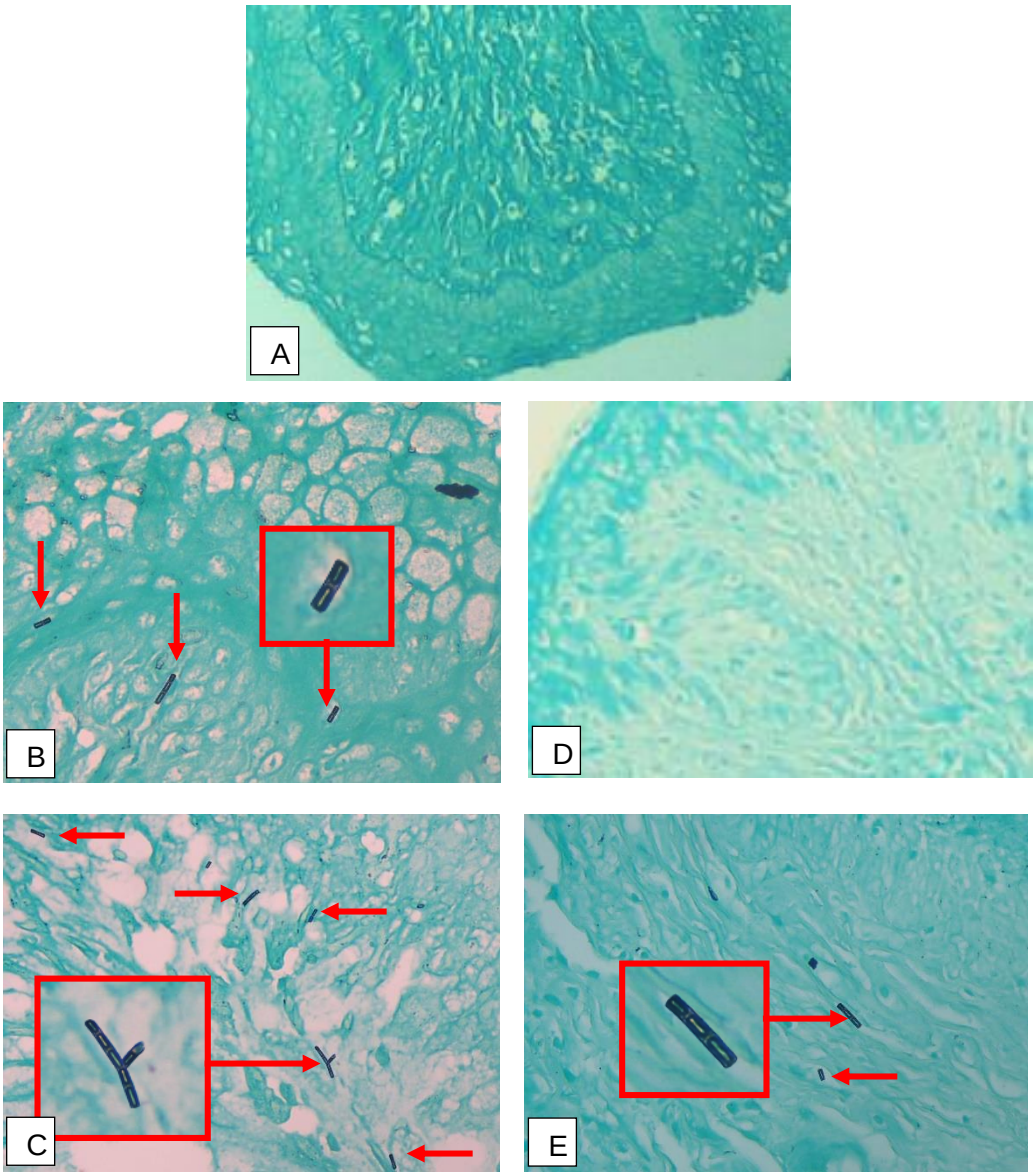

Figure 1. Candida albicans hyphae in rat vaginal histology

Figure 1 shows an overview of Candida albicans hyphae in each control and treatment group with 40x

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magnification microscope observations found hyphae on the mucosa of the lamina propria vaginal tissue of rats varied in several groups (indicated by red arrows), and no hyphae was found in the healthy rats and KVV rats which were given 47.5% bajakah extract.

In the normality analysis of the Shapiro-Wilk test, the description of the number of hyphae obtained p-value = 0.684 > 0.05. So the data has fulfilled the normality test with customarily distributed data results. The Levene test homogeneity analysis obtained the p-value = 0.332 > 0.05. So the data has fulfilled the homogeneity test with homogeneous data results. Furthermore, the data will be analyzed with parametric statistical tests to prove the research hypothesis that has been put forward.

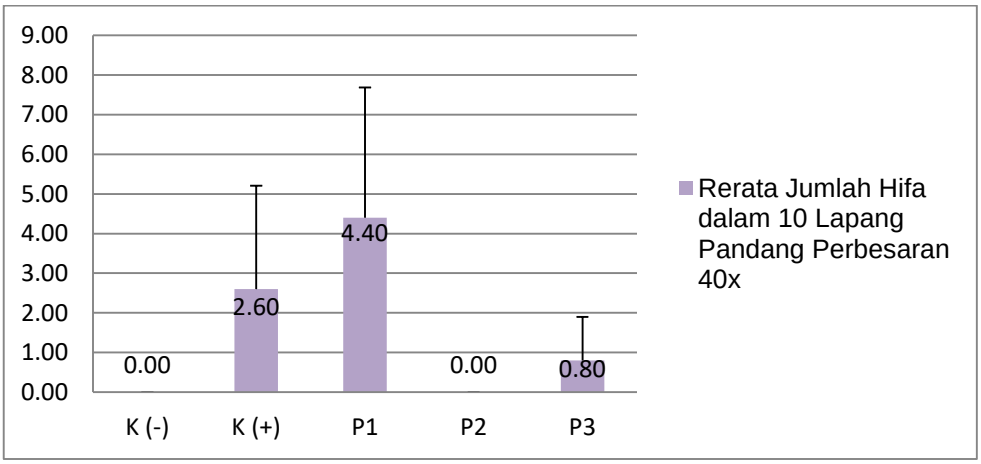

Figure 2 Average Density of Candida albicans Hyphae in KVV Model Mice

Figure 2 shows the average number of Candida albicans hyphae in 10 fields of view under a microscope with 40x magnification. It can be seen that the number of hyphae in P1 (45% pirate extract) is ±4.40; K (+) (fluconazole 10 mg), namely ±2.60; and P3 (50% pirated extract) which is ±0.80. Based on Figure 1 and Figure 2 show that in the K (-) and P2 groups, there was no hyphae number in the histology of the vaginal tissue of KVV mice. The results of this study indicate that the administration of extracts of bajakah affects the number of C. Albicans hyphae in the vaginal histological tissue of KVV rats.

The morphological description of C. Albicans (hyphae) under 40x magnification microscopy showed that there were hyphae in the treatment group, including the KVV rat group that was given 45% and 50% bajakah extract. No hyphae were found in the KVV rat group that was given 47.5% bajakah extract. The live/dead ratio of C. Albicans exposed to treatment will decrease depending on the dose of treatment with extracts containing flavonoids. The flavonoids present in this extract interfered with the permeability of the C. albicans biofilm membrane, inhibiting hyphae formation, which was reduced by more than 70% when served at 1x or 2x MIC concentrations. The inhibitory effect of biofilm formation and hyphal growth was more significant with increasing doses of extracts containing flavonoids to greater than 80% at concentrations between 1250 and 312.5 g/mL.

Bajakah is rich in active ingredients such as flavonoids, phenolics, tannins, and saponins. In addition, many people believe this plant has many benefits as a medicinal ingredient for various diseases using traditional medicine methods. The biofilms' formation in fungi is generally characterized by three stages: attachment, maturation, and dispersion. Flavonoids can interfere with and suppress biofilm formation during these three stages. Flavonoids cause inhibition of biofilm formation or cellular membrane function, which contributes to reducing fungal proliferation so that the density of fungi will also be reduced.

Flavonoids contribute to the disruption of antibiofilm activity and the growth of Candida hyphae. These flavonoids suppress the secretion of proteases and phospholipases by reducing the expression of protease-encoding genes secreted by aspartyl protease (SAP)1 and SAP2, as well as phospholipase B1. The antifungal activity of phloretin as a flavonoid was proven by the improvement of inflammation and increased number of colony-forming units caused by C. Albicans in candidiasis rats. Thus, there was a decrease in hyphal colonies.

Based on their target of the action, antifungal drugs can be divided into three significant groups: azoles, which target ergosterol biosynthesis, and echinocandins, which inhibit fungal cell wall biosynthesis. Moreover, polyenes bind ergosterol in the fungal cell membrane causing cell lysis. Many things can influence the action of antifungals, and what is detrimental can cause drug resistance so that the sensitivity to antifungals is reduced compared to normal conditions. The cause could be impaired immune function, poor drug administration bioavailability, or increased drug metabolism. Factors that affect the action of antifungal drugs themselves depend on the host, the drug, and the fungus.

The drug factor, fungistatic drugs, will more quickly develop resistance than fungicidal drugs. The dosage of the drug can also affect the action of antifungals. Combining antifungals with other drugs can also change the effectiveness of antifungal drugs. In addition, delay in starting adequate antifungal doses increases the likelihood of treatment failure. Drug selection factors such as fluconazole have better cerebrospinal fluid (CSF) penetration than itraconazole, making it a better choice in treating fungal meningitis. The factor of the fungus itself is the type of species or strain and cell type that can change the effectiveness of therapy. Some fungi, including Candida albicans and Candida glabrata, exhibit a phenotype switch mechanism so that they have several morphologies that can change depending on the location of the infection, which can increase their adaptability to the host environment. Some fungi also have biofilms which can make them less susceptible to antifungal drugs.

The fungus's ability can also reduce the accumulation of drugs in the fungus. As is the case with the resistance of azole group drugs in the presence of the ATP-binding cassette (ABC) superfamily and the major facilitator superfamily in the mushroom body, the levels of drugs that attack the fungus can be lowered. In addition, fungi also can mutate, which has the effect of reducing the affinity of drugs to their targets and can also modify the ergosterol biosynthetic pathway (in C.albicans). Due to these factors, several studies have investigated the use of herbal plants as antifungal agents. Effective herbs can be used to treat infections at a meager cost by using practical herbal components in toothpaste, oral gels, ointments, etc., as these products completely remove fungal colonies from the oral cavity without causing side effects or toxicity as drugs.

Effect of Methanol Extract of Bajakah (Spatholobus littoralis hassk) on IL-10 Levels in KVV Female Rats (Rattus norvegicus) using the ELISA Kit. In this study, the results of data analysis in the normality test were carried out using the Shapiro-Wilk test because the sample is less than 50. The data is usually distributed if the p-value (sig) is more significant than the significance value $\alpha = 0.05$. The data is not generally distributed if the p-value is (sig) smaller than the significance value $\alpha=0.05$.

In the Shapiro-Wilk test analysis, the p-value = 0.201 is greater than the significance value $\alpha = 0.05$. So the data has fulfilled the normality test with customarily distributed data results. Furthermore, the data were tested for homogeneity using the Levene test with the criteria that if the p-value is more than 0.05 then the data can be said to be homogeneous. If the p-value is less than 0.05 then the data can be said to be non-homogeneous.

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In the Levene test analysis, the p-value = 0.329 is more significant than the significance value α = 0.05. So the data has fulfilled the homogeneity test with homogeneous data results. So that further data can be analyzed further with parametric statistical tests to prove the research hypothesis that has been put forward.

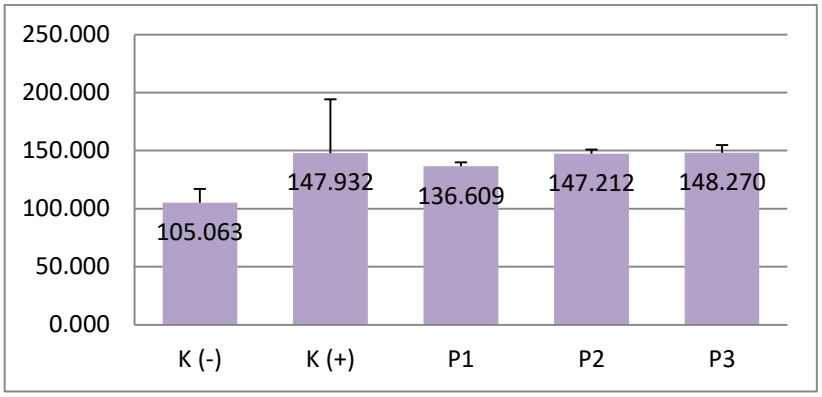

Figure 3 Mean IL-10 Serum Levels in KVV Model Mice

Based on Figure 3, shows that there is a difference in the average effect of giving Bajakah extract on IL-10 serum levels in each treatment group (P1, P2, and P3) which shows a successive increase, namely P1 = 136.609 pg/mL, P2 = 147.212 pg/mL, and P3 = 148.270 pg/mL with a p-value = 0.021. At the three concentrations of piracy extract in KVV model rats, the serum IL-10 level at a concentration of 45% was the lowest. At a concentration of 50%, the increase in serum IL-10 level was the highest. Picture 3 also shows that the group receiving the extract with a concentration of 47.5% was able to increase serum levels of IL-10 as well as the administration of 10 mg of fluconazole.

The results of this study indicated that there was an increase in serum levels of IL-10 in the blood of KVV model rats by administration of pirated extract in each treatment group (P1, P2, and P3), which showed the average respectively: P1 = 136.609 pg/mL, P2 = 147.212 pg/mL, and P3 = 148.270 pg/mL with a p-value = 0.021. The results of this study also showed the effect of the administration of pirated extract in various concentrations on serum IL-10 levels. The more concentrations used, the more serum IL-10 levels increased. Under infectious conditions, treatment with polyphenol and flavonoid substrates 5.0 mg/mL or 10.0 mg/mL in the infected group resulted in markedly lower IL-10 levels.

The innate immune system provides the first barrier against vulvovaginal Candida infection. Pattern recognition receptors (PRRs) in innate immune cells sense molecular sites on the surface of microorganisms and induce intracellular signals that stimulate the production of effector molecules such as cytokines or defensins. Two classes of PRRs have been reported to be significant recognition receptors for C. Albicans: Toll-like receptors (TLRs) and C-type lectin receptors. TLR4 recognizes fungal cell wall mannans, whereas TLR2/TLR1 and TLR2/TLR6 heterodimers recognize Candida phospholipomannan. In addition, TLR2 synergizes with DECTIN-1, the receptor for -glucan, for the induction of proinflammatory cytokines. DECTIN-1 can also induce TLR-independent signals for IL-17, IL-6, and IL-10 production via a Syk/CARD9-dependent pathway.

Th2-derived anti-inflammatory cytokines such as IL-10 also play an essential role in host defense against candidiasis. IL-10 cytokine is an anti-inflammatory cytokine. These cytokines inhibit the activity of Th2 cells, NK cells, and macrophages. Increased IL-10 production, modulated via differential TLRs and dectin-1, shifts the balance towards an anti-inflammatory cytokine response. When it can withstand destruction through standard immune mechanisms, IL-10 will be produced to reduce inflammation, leading to

pathological conditions due to excessive inflammation. Anti-inflammatory cytokines consist of interleukin-4 and interleukin-10, which regulate proinflammatory cytokine secretion. Flavonoid compounds in Spatholobus sp. plays a role in inhibiting mRNA expression of several proinflammatory cytokines such as interleukin 1β (Il-1β), Tumor Necrosis Factor (TNF-α), nitric oxide synthase (iNOS) and cyclooxygenase (COX -2) enzymes.

Flavonoid compounds also increase the secretion of IL-10 as an anti-inflammatory cytokine that can direct the T-helper immune reaction. IL-10 is also known for its ability to inhibit the differentiation of monocytes into antigen-presenting cells. It also suppresses the expression of most inducible chemokines in the inflammatory process. IL-10 can suppress cyclooxygenase-2-dependent prostaglandin E2 synthesis and increase the production of anti-inflammatory mediators.

The dose of the extract on serum IL-10 levels in this study had an effect, where the higher the concentration used, the more serum IL-10 levels increased. IL-10 serum levels affect the extract because IL-10 is essential in inhibiting candida Albicans. The extract can affect the cytokine profile of IL-10 cells because of the interaction between candida Albicans and different combinations of adapter proteins resulting from interactions with the extract. It will activate various transcription factors, so the extract is needed to increase the optimal immune response against candida Albicans.

3. Conclusion

Administration of various doses of methanol extract of Bajakah (Spatholobus littoralis hassk) did not affect the decrease in the morphology of Candida albicans in the vagina of female rats (Rattus norvegicus) Vulvovaginal Candidiasis (KVV) model, in this case, the number of hyphae microscopically did not increase or decrease with increasing concentration of the extract. Administration of various doses of methanol extract of Bajakah (Spatholobus littoralis hassk) can affect the increase in serum IL-10 levels in female rats (Rattus norvegicus) Vulvovaginal Candidiasis model, IL-10 levels in the blood serum of rats in each treatment group increased successively with increasing concentrations extract with an average P1 = 136.609 pg/mL, P2 = 147.212 pg/mL, and P3 = 148.270 pg/mL. Giving various doses of methanol extract of Bajakah (Spatholobus littoralis hassk) was not effective in reducing the morphology of Candida albicans in KVV model rats. In contrast, the administration of methanol extract of bajakah (Spatholobus littoralis hassk) in KVV rats at a dose of 47.5% increased anti-inflammatory cytokine (IL-10) as in the administration of fluconazole 10 mg.

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