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Potential of Chinese Petai Leaf Extract (*Leucaena Leucocephala*) in Inhibiting the Growth of *Candida Albicans* (Laboratory Study on *Leucorrhoea Culture*)

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ABSTRACT

Background

Leukore is an illustration of excess secretion from intercourse and the problem most often complained of by women at reproductive age. If not treated at an early stage it can become chronic and lead to PID (Pelvic Inflammatory Diseases) which can cause difficulties in obtaining offspring. Leukore caused by *Candida albicans* and experienced by 75% of women of reproductive age experience vulvovaginal candidiasis. Flukonazole antifungi from the azole group, which inhibit 14- α -sterol demethylase, but *C. Albicans* is resistant to azole, needs another alternative. Chinese petai leaves (*Leucaena Leucocephala*) are plants that overcome microbial diseases, which contain alkaloids, tannins, saponins, phenols, flavonoids which have the potential to be anti-fungal bacteria.

Objective

To analyze the compounds of Chinese petai leaf extract (*Leucaena leucocephala*) of various concentration variants in inhibiting the growth of *Candida albicans*.

Methods

True experiment with post test only control group design, using a completely randomized design.

Results

The flavonoid compounds of Chinese petai leaf extract concentration of 20%, 60%, and 100% have an average inhibitory power to the growth of *albicans candida* is 8.7 mm, 12.26 mm, 16.06 mm. The results of the post test showed a significant difference between 20% flavonoids with 60% flavonoid and 100% flavonoids and 60% flavonoids with 100%, there were significant differences ($P < 0.05$).

Conclusions and recommendations

Flavonoid compounds from Chinese petai leaf extract have the potential as an alternative treatment for vaginal discharge caused by *Candida albicans*.

Keywords: Chinese petai, *Candida Albicans*, Leukorea

INTRODUCTION

Whitish (leucorrhoea) is a general term commonly used to describe excessive secretion expenditure from intercourse and one of the problems most often complained of by women at reproductive age and / or sexually active [1][2].

When the condition changes to pathology, vaginal secretions can be accompanied by a rash around the genital area, burning sensations, itching, low-back pain, unpleasant odors and discoloration of the discharge into yellowish, greenish, or mixed with blood [1][3][4]. Pathological leucorrhoea usually signifies ongoing reproductive tract infections[1][5]. Abnormal conditions in the female reproductive organs if not treated at an early stage can become chronic and lead to PID (Pelvic Inflammatory Diseases), which can cause difficulties in obtaining offspring[2][3]. The prevalence of infertility in Indonesia is more than 20%, where 40% of these figures are cases of infertility in women, one of which is caused by PID due to untreated pathological leucorrhoea[6].

The three most common diseases associated with leucorrhoea are bacterial vaginosis (replacement of normal vaginal flora with overgrowth of anaerobic, myco-plasma, and *Gardnerella vaginalis*), Trichomoniasis (caused by *Trichomonas vaginalis*) and Candidiasis (usually caused by *Candida albicans*)[7]. *Candida albicans* is one of the four main pathogenic fungi that causes most cases of severe fungal disease and is the main agent that causes mucosal infections[8][9].

Although the medical world continues to progress and modernize, the incidence of fungal infections has actually increased, especially infections caused by *Candida* species. 75% of reproductive-age women experience Vulvovaginal candidiasis (VVC) at least once during their lifetime, almost half of them will experience recurrence, and 5-8% have several episodes each year[7].

The most commonly used antifungi to treat most *Candida albicans* infections is fluconazole, an antifungal of the azole group. Azole inhibits 14- α -sterol demethylase which is an enzyme involved in biosynthesis of ergosterol sterol membranes and disrupts the integrity of the cell membrane of *Candida albicans*. Fluconazole is preferred because of its low toxicity and is available in oral

preparations. However, this therapy is undergoing challenges because now *Candida albicans* is starting to be resistant to fluconazole[10][11].

In another study, Nasrollahi et al (2015) examined women who experienced recurrent VVC. The results show that 94% of recurrent VVC events are caused by *Candida albicans* which is resistant to fluconazole. The results of these studies recommend the need to seek other therapeutic alternatives to overcome VVC problems[12][13][14].

About 80% of the world's population in developing countries to treat primary health depends on traditional medicines. While in modern medicine, today nearly 25% are based on medicines derived from plants[15]. Chinese petai plants (*Leucaena leucocephala*) are one of the plants commonly used to treat microbial diseases[16][17]. From the phytochemical test results, Chinese petai leaves are known to contain carbohydrates, tannins, saponins, flavonoids, quinones, glycosides, terpenoids, phenols, coumarins, proteins, phytosteroids, alkaloids, oils and fats, and resins[18][19].

Mohammed et al (2015) examined the potential antifungal compounds of flavonoid glycoside in Chinese petai leaves. The results of the study showed that Chinese petai leaf extract with the main compounds of flavonoid glycosides had moderate inhibition on the growth of *Candida albicans*[20].

Flavonoids, phenols, alkaloids, saponins and tannins are compounds that are thought to have the potential as antifungi, among compounds contained in Chinese petai leaves[21]. Flavonoids have antifungal potential because they can interfere with cell metabolism by inhibiting nutrient transport, forming complex compounds against extracellular protein compounds that disrupt membrane integrity and cell walls. Meanwhile, which can denature protein bonds in cell membranes so that the cell membrane becomes lysis and can penetrate into the cell nucleus is the way the phenol compounds work[22].

The workings of these compounds are similar to the way fluconazole works where the growth of *Candida albicans* is inhibited by disturbing the permeability of its cell wall[21][22]. Therefore, researchers were interested in knowing the

potential of Chinese petai leaf extract in inhibiting the growth of *Candida albicans*.

Study Objectives

Analyzing the flavonoid content of Chinese petai leaf extract (*Leucaena leucocephala*) with a concentration of 100%, 60%, 20% in inhibiting the growth of *Candida albicans*

METHODS

This study is a true experiment with post test only control group design using a completely randomized design (CRD).

Data Analysis

The data analysis used is the normality test using Shapiro Wilk to see whether the data obtained is normally distributed with a value of $p > 0.05$. The data obtained are normally distributed so

that the One Way ANOVA test is performed to see whether there is significance or difference between treatment groups. Furthermore, a follow-up test was carried out in the form of a Post Hoc test to find out which treatment groups gave different effects.

RESULT

The inhibition of Chinese petai leaves (Flavonoids) with various concentrations on the growth of *Candida Albicans*.

The research results of the potential of Chinese petai leaf extract (Flavonoids, Phenol, Saponin, Alkaloid and Tanin) on the growth inhibition of *Candida albicans* with various concentrations obtained the following results:

Table 1 Diameter of inhibition zone

Treatment	Obstacles zone (mm)			Mean
	Replica			
	1	2	3	
Flavonoid compounds 20%	8,9	8,5	9,0	8,7
Flavonoid compounds 60%	11,9	12,4	12,5	12,26
Flavonoid compounds 100%	16,2	15,9	16,1	16,06

From table 1 shows that

1. The inhibition of flavonoid compounds with a concentration of 20% to the growth of *Candida albicans*
The inhibition zone of the flavonoid compound with a concentration of 20% on the growth of the first *Candida albicans* measurement of 8.9 mm was replicated to the second 8.5 mm and the third replication was 9.0 mm and the average inhibition zone was 8.7 mm. This shows that the flavonoid compound with a concentration of 20% is not able to inhibit the growth of *Candida albicans*
2. The inhibition of flavonoid compounds with a concentration of 60% to the growth of *Candida albicans*
The inhibition zone of the flavonoid compound with a concentration of 60% on the growth of the first *Candida albicans* measurement 11.9 mm was second replicated

12.4 mm and the third replication 12.5 mm was obtained by the inhibition zone average of 12.26 mm. This shows that the flavonoid compound with a concentration of 60% can inhibit the growth of *Candida albicans* in the weak category

3. The inhibition of flavonoid compounds with a concentration of 100% to the growth of *Candida albicans*
The inhibition zone of the flavonoid compound with a concentration of 100% on the growth of the *Candida albicans*, the first measurement of 16.2 mm was replicated at the second 15.9 mm and the third replication was 16.1 mm, the average inhibition zone was 16.06 mm This shows that flavonoid compounds with a concentration of 100% can inhibit the growth of *Candida albicans* in the medium category

The inhibition of albicans candida growth between groups

Table 2 One Way ANOVA Test Results Power of inhibiting the growth of Candida Albicans

	Average \pm SD	P
Flavonoid 20%	8,76 \pm 0,321	0,000
Flavonoid 60%	12,10 \pm 0,608	
Flavonoid 100%	16,06 \pm 0,152	

Table 2 shows that all compounds have a significant difference in the inhibitory growth of candida albicans with (p <0.05).

Table 3 Data Comparison Results Between Treatment Groups

	Mean difference	IK 95 %		P
		Min	Max	
Flavonoid 20 VS Flavonoid 60%	-3,333	-4,585	-2,081	0,000
Flavonoid 20% VS Flavonoid 100%	-7,300	-8,552	-6,047	0,000
flavonoid 60% VS flavonoid 100%	-3,966	-5,219	-2,714	0,000

From table 3, the comparison of candida albicans growth inhibition power in each group can be seen that there is a significant difference between 20% flavonoids and 60% flavonoid and 100% flavonoids and 60% flavonoids with 100%. showed that there were significant differences (P <0.05)

DISCUSSION

Mechanism of flavonoid content of Chinese petai leaf extract (*Leucaena leucocephala*) with a concentration of 20%, 60%, 100% in the process of inhibiting the growth of Candida Albicans.

The results showed that after exposure to Chinese petai leaves (*Leucaena leucocephala*) flavonoids for 24 hours which were divided into 3 concentrations namely 20% did not have inhibition while the concentration of 60% had a weak inhibition and 100% concentration had moderate inhibition of the growth of Candida Albicans. Table 4.2 shows the concentration of 20% has an average inhibition zone of 8.7 mm, a concentration of 60% has an average inhibition zone of 12.6 mm and a concentration of 100% has an average inhibition zone of 16.06 mm.

Theoretically, Chinese petai leaf extract (*Leucaena leucocephala*) begins to provide inhibitory effects at concentrations of 60% and 100% through the mechanism of action to inhibit the function of the cytoplasmic membrane and

energy metabolism of albicans candida mushroom cells.

Previous research stated that the flavonoid glycoside compound in Chinese petai leaves has the potential as an antifungal. The results of his research showed that Chinese petai leaf extract with the main compounds of flavonoid glycosides had moderate inhibition on the growth of Candida albicans[20]. In other studies, flavonoid compounds in plant extracts have a inhibitory effect on the growth of *C. albicans*[23][24].

Flavonoids have a broad spectrum in inhibiting the growth of *C. albicans*. Although the inhibitory strength of the flavonoid compounds in Chinese petai leaves has antifungal potential because it can interfere with cell metabolism by inhibiting nutrient transport, forming complex compounds against extracellular protein compounds that interfere with the integrity of membranes and cell walls with high concentration and as long as possible work of flavonoid compounds which are groups of compounds that have a basic skeletal structure C6-C3-C6. Each part of C6 is a zone ring used with C3 atoms. Flavonoids are found in plants and products related to propolis and honey. In the leaf section, flavonoids are useful as a physiological function of plants, which is to guard against fungi and radiation. Flavonoids are useful in photosynthesis, energy transfer, performance of growth hormones, control of respiration, and morphogenesis. Flavonoids are divided into 14 classes that are distinguished by their basic forms, such as flavones, isoflavones, and flavonols. The potential of flavonoids is thought to be a treatment

of infection and bacteria, toxic and diuretic. Flavonoids are the largest group of phenols which can denature proteins and function as antibacterial and antifungal genes [25][26]. Flavonoids can cause damage to the cytoplasmic membrane by reducing the fluidity of the membrane, causing leakage, and producing hydrogen peroxide. The working system of flavonoids in inhibiting nucleic acid synthesis is by inhibiting topoisomerase. The antibacterial mechanism is inhibited by inhibiting ATP synthesis. Flavonoids are effective in inhibiting the growth process of candida albicans by damaging the fungal cell walls. Flavonoids complex with fungal cell walls can cause damage to hydrogen bonds in cell wall proteins[26]. Based on previous research, flavonoid glyco-side extract from Chinese 7-tai leaves (*Leucaena leucocephala*) showed significant antimicrobial activity against Gram negative bacteria *E. Coli*, *Salmonella typhimurium* and *Pseudomonas*, moderate activity against gram-positive bacteria and *C. albicans*, and weak activity

as antifungal against *Mucor miehei* and negative activity against other fungi tested[20].

CONCLUSIONS

Flavonoid compounds in petai leaf extract of China (*Leucaena Leucocephala*) concentration of 20% did not have inhibitory power, concentration of 60% had a weak inhibition, and a concentration of 100% had a moderate inhibitory power. The increase in concentration from 60% to 100% is more effective 51.6% (2-fold) because the content of the bioactive flavonoid compounds is getting bigger. Flavonoid compounds in Chinese petai leaf extract (*Leucaena Leucocephala*) inhibit the growth of candida albicans adhesion stage by inhibiting protein proliferation and fungal respiration (inhibiting cyclooxygenase and lipooxygenase).

Recommendation Future

Further research is needed, namely Chinese petai leaf extract in inhibiting candida albicans cells using a microscope.

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