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Development of Feminine Area Cleansers with Anti-fungal Properties Using Apple Rose Leaves as an Active Ingredient

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Abstract.
Fungal infection has a high prevalence rate in the feminine area. The use of natural anti-fungal, natural feminine cleansers can be a solution to this problem. Apple rose leaves are thought to be an alternative active ingredient to be developed into a natural feminine area cleanser. In this study, in vitro antifungal activity of guava leaves was observed on *Candidia albicans* and their development into a feminine area cleanser. The results based on the ethanol extract, n-hexane fraction, ethyl acetate fraction and water fraction showed that there were high anti-fungal properties. The highest anti-fungal effect was shown by the n-hexane fraction. A feminine area cleanser was developed with the n-hexane fraction of apple rose leaves as the active ingredient. Pharmaceutic evaluation showed that the formulation obtained was milky white, odorless, homogeneous, produced foam with a height of 0.5 cm and had a pH of 4.5. Based on the results of the study, it can be concluded that apple rose leaves have the potential to be developed into a feminine area cleanser.

Keywords: apple rose leaves, leukorrhea, natural anti-leukorrhea, feminine area cleansing preparation

1. INTRODUCTION

Fungal infection is a female reproductive health problem with a very high prevalence rate. One of the causes of this infection is *Candida albicans*. *Candida albicans* is actually a normal flora in the female reproductive tract. However, excessive growth of *Candida albicans* will cause abnormal vaginal discharge [1,2]. Feminine hygiene preparations are preparations for cleaning the external genital area in women. Preparations can be in the form of liquid, gel, spray, foam, cream or talc. What needs to be considered in

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this preparation is that it should not irritate and the pH of the preparation must be in accordance with the female genital area, namely 3.5-4.7 for the vagina and 3.8-4.4 for the vulva. In addition, the preparation should not disturb the balance of normal flora in the genital area. These preparations are often added with active ingredients that have antibacterial or antifungal properties and are used to treat infections of the genital organs [3]. Currently, many antifungal drugs have been used to overcome this problem. However, based on safety evaluations, these drugs cause adverse side effects and there have been many cases of resistance [4-6]. One of the efforts made to overcome these detrimental effects is to develop drugs from natural ingredients. Some research results indicate that apple rose [*Eugenia aqueum* (Burn. F) Alston] leaves contain flavonoid and tannin compounds. These two classes of compounds are known to have anti-infective activity [2,7]. Thus, apple

rose leaves are thought to have anti-fungal activity and can be developed into a preparation for cleaning the feminine area with anti-fungal properties.

2. METHODOLOGY

This research is an effort to prove the potential of apple rose leaves as an antifungal in vitro, especially in *Candida albicans*. The research stage included collecting apple rose leaves, making simplicia, making ethanol extract, making extract fractions, testing anti-fungal activity and developing cleaning preparations for the female area. The research was conducted at the Integrated Pharmacy Laboratory, Universitas Islam Bandung.

The manufacture of simplicia was done by drying it without direct sunlight [8]. The extract was made by maceration [9]. The extract fraction was prepared by using the liquid-liquid extraction method using three types of solvents, namely: n-hexane (non polar), ethyl acetate (semipolar) and water (polar) [10].

The antifungal activity test was carried out on *Candida albicans* in vitro. All stages were carried out by aseptic technique. The activity test was carried out by the agar diffusion method using the well technique with various test concentrations. The anti-fungal activity was expressed by the minimum inhibitory concentration (MIC). MIC was determined by determining the lowest concentration which still caused an inhibition zone [11,12]. After being proven to have activity on *Candida albicans,* a formula for cleaning the female area with apple rose leaves was developed as an active ingredient. Furthermore, the preparations that have been made are evaluated for the quality by pharmaceutical. The parameters observed included organoleptic characteristics, homogeneity, foam height and pH [13].

3. RESULTS AND DISCUSSION

The results of observations of the antifungal activity of apple rose leaves both ethanol extract, n-hexane fraction, ethyl acetate fraction and water fraction are shown in Table 1.

TABLE 1: The results of the apple rose leaves activity test on *Candida albicans*.

Test material	MIC (%)	Inhibition zone diameter (mm)
Ethanol extract	1.00	11.40 ± 0,02
N-hexane fraction	0.13	12.20 ± 0,09
Ethyl acetate fraction	0.13	10.10 ± 0,02
Water fraction	16.00	11.00 ± 0,00

Based on table 1 it can be seen that all the test materials showed activity on *Candida albicans*. Based on the category of strength of fungal growth inhibition activity, all test materials can be classified into the category of having high activity because they produce an inhibition zone diameter of more than 6 mm [14], as listed in table 2. According to the four test materials, fraction n- hexane and ethyl acetate fraction had the lowest MIC of 0.13%. Even though the MIC values of the two fractions were the same, the n-hexane fraction produced a longer diameter of the zone of fungus growth inhibition than the ethyl acetate fraction. Therefore, the n-hexane fraction was determined as the test material that had the best activity and was used as an active ingredient in the feminine area cleansing preparations developed in this study.

TABLE 2: Fungal growth inhibitory activity category.

Diameter of the growth inhibition zone (mm)	Growth inhibitory response
0-3	Low
3-6	Medium
More than 6	High

The anti-fungal activity of apple rose leaves is due to the flavonoid content. The effect of flavonoids is to inhibit the formation of cell biofilm synthesis. Cell biofilms are protective for fungal cells against toxic materials from outside [15]. Flavonoids dissolve in hot water and alcohol. Several types of compounds in the flavonoid class are insoluble in water [9]. Thus the composition of the flavonoid group compounds is more likely to be in high concentrations in the n-hexane fraction which is a semi-polar fraction. Therefore the n-hexane fraction produced a stronger effect than the

ethanol extract and the polar fraction (water). The MIC value for *Candida albicans* from the n-hexane fraction was the same as the semi-polar fraction (ethyl acetate).

This is because it is possible for flavonoids to dissolve in semi- polar compounds [9]. The presence of growth inhibition activity in the strong category of *Candida albicans* indicates that apple rose leaves have a high potential to be developed as a preparation for cleaning the feminine area with anti- fungal properties.

The formula for the preparation was made with the n-hexane fraction as the active ingredient as listed in Table 3.

After the formula had been determined, then a cleaning preparation for the feminine area was carried out.

Table 3: Feminine area cleanser preparations formula with apple rose leaves as an active ingredient.

Component	Function	Composition
N-hexane fraction	Active ingredients	0.13%
Stearic acid	Emulsifier	1%
Adeps lanae	Soap-forming	1%
Triethanolamine	Emulsifier	2%
glycerol	Humectant	5%
Citrate buffer	pH regulator	q.s.
Lactic acid	Maintain Vaginal acidity	q.s.
Butylhydroxybenzoate	preservative	0,5%
Aquadest	sovent	ad 100%

From the process of making preparations based on the formulas in table 3, liquid preparations are produced. The preparations that have been made are then evaluated. The evaluation was carried out in the form of organoleptic observations, pH, homogeneity and foam height.

Table 4: Results of feminine area cleanser pharmaceutical evaluation.

Parameter	Observation Results
Organoleptic	Thick liquid milky white and odorless
Homogeneity	Homogenous, no coarse grain
Foam height	0.5 cm
pH	4.5

Based on the pharmaceutical evaluation (Table 4), the preparation showed the characteristics of a milky white color, odorless and in the form of a thick liquid. The physical form as above is a common form in feminine area cleansing preparations. Based on the homogeneity evaluation, the preparation is homogeneous and there are no coarse grains. Based on the evaluation of the foam height, the foam showed a foam height of 0.5 cm. The pH evaluation results show that the pH of the preparation is in accordance with the normal pH range in the female area [3].

4. CONCLUSION

Apple rose leaves have high activity in inhibiting the growth of *Candidia albicans*. The best activity was shown by the n-hexane fraction (non polar). The n-hexane fraction of apple rose leaves had been successfully developed into a feminine area cleanser preparation, which was a thick and homogeneous milky white liquid preparation, had a foam height of 0.5 cm and a pH value corresponding to the range of normal female areas, namely 4.5. Apple rose leaves had the potential to be developed into a femanine area cleanser preparation with antifungal properties.

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