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The Effect of Ethanol Extract Concentration of Rapat Bark (*Parameria laevigata* (Juss.) Moldenke) in Feminine Hygiene Preparations on Antimicrobial Activity

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Abstract

Rapat bark (*Parameria laevigata* (Juss.) Moldenke) is a natural material containing secondary metabolites that can be utilized as antimicrobial agents. This study aims to determine the effect of ethanol extract concentration of rapet bark in feminine hygiene formulations on the growth of *Candida albicans* and *Staphylococcus aureus*. The research employed an experimental method in the preparation of feminine hygiene products using ethanol extract of rapet bark at concentrations of FI (5%), FII (10%), and FIII (15%). The soap evaluation parameters included cycling test, organoleptic test, pH test, foam height test, irritation test, and antimicrobial activity test using the cylinder cup method with pour plate inoculation technique. The feminine hygiene formulations met the soap evaluation requirements, with antimicrobial activity against fungi of 11.53 ± 0.0354 (FII) and 12.55 ± 0.037 (FIII), and against bacteria of 11.29 ± 0.023 (FI), 12.56 ± 0.0444 (FII), and 15.11 ± 0.018 (FIII). Data analysis using one-way ANOVA showed a significance value ($p < 0.05$), indicating that each extract concentration differed significantly, thereby affecting the inhibition zone against fungi and bacteria.

Keywords: Rapet bark, Feminine hygiene, Antimicrobial.

1. INTRODUCTION

Maintaining feminine hygiene is a crucial factor for women cause it helps prevent infections and diseases transmitted through the female reproductive organs, which can be caused by fungi, bacteria, parasites, and viruses (Ningsih et al., 2019). Urinary Tract Infection is a disease of the female reproduction organ that can be caused by *S. aureus* and *C.albicans*. *S. aureus* is a normal flora of the skin and mucous membranes, however, this bacterium can become pathogenic by infecting the skin (Elizabeth et al., 2020). *C. albicans* can cause progressive diseases when the immune cells are weakened, such as acute and subacute candidiasis (Rodiah et al., 2022). *Feminine hygiene* products containing natural ingredients are one of the solutions for preventing feminine infections (Rahmi et al., 2017).

Parameria laevigata (Juss.) Moldenke or *P. laevigata* in Indonesian known as Rapat, is a plant that has been empirically used by the community to support health, as it contains active compounds with pharmacological and biological activities (Barus et al., 2019). Phenolic, Flavonoids, Tannins, Alkaloids, Triterpenoid as a secondary metabolite compounds in *P.*

laevigata that have a various roles in pharmacological activities, such as antibacterial, antifungi, antioxidant, antibiofilm, antiinflammatory, etc. An antifungal activity research has been carried out on ethanol extract of rapat bark against *C. albicans* has been conducted, showing that concentrations of 0.375%, 0.625%, and 1.25% were able to inhibit fungal growth (Anggraini et al., 2024). Therefore, based on the research that has been conducted, the researcher is interested in developing an ethanol extract of *P. laevigata* into a feminine hygiene formulation to determine the antimicrobial activity of the sample against *S. aureus* and *C. albicans*.

2. METHOD

Research Materials

The sample used in this study was rapat bark obtained from Kalikuto, Grabag, Magelang. Materials for screening phytochemical compounds used ethanol 96%, FeCl□, Mg, rutin, amyl alcohol, gelatin, acetic anhydride, ethyl acetate, toluene, acetone, H□SO□, 2N HCl, Mayer's, Bouchardat's, Dragendorff's, ethanol, distilled water. Materials for formulations used sodium lauryl sulfate, NaCl, propylene glycol, citric acid, *Ol. Rosae*, and EDTA, meanwhile material for antimicrobial test used ½ Mc.Farland solution, MSA, EMBA, NB, NA, and Feminime Wash (Lactacyd All Day Care).

Research Instruments

The instruments used in this study included glassware, petri dishes, test tubes, tweezers, micropipettes, round and straight inoculating loops, cylinder cups, incubator, UV-Vis Spectrophotometer (*Shimadzu UV-1700 PharmaSpec*), vernier caliper, pH meter, autoclave, and thermal cycling chamber.

Extraction and Formulation

The extraction using remaceration method, was carried out by 100 grams sample and 1000 mL ethanol 96% in a glass vessel for 3 × 24 hours, and stir onca a while.

Table 1. Feminime Hygiene Formulation

Ingredients	Formula (%)			
	F0	FI	FII	FIII
***P. laevigata* bark xtract**	-	5	10	15
Na. Lauril Sulfat	1	1	1	1
NaCl	3	3	3	3
Propylene glycol	1	1	1	1
Citric acid	0,5	0,5	0,5	0,5
Ol. citrus (guttae)	2	2	2	2
EDTA	0,1	0,1	0,1	0,1
Destilled Water	ad 100 mL			

In the first stage, the extract was dissolved in a beaker glass containing distilled water. Propylene glycol, sodium lauryl sulfate, and hot water were then added and stirred until homogeneous. In the second stage, NaCl, citric acid, EDTA, and *Ol. Citrus* were added, and the volume was adjusted to 100 mL using distilled water. The mixture was stirred until homogeneous and then transferred into containers. For the F0 treatment, the same procedure was followed without the addition of the extract.

Evaluation of Feminine Hygiene Characteristics

Various test were conducted to evaluate quality and stability of feminime hygiene *P. laevigata*, including an organoleptic test to observe of the odor, color, and texture of the formulation (Lolok et al., 2020), pH Test by disolving 1 gram of the soap formulation in 10 mL of distilled water and measuring the pH using a pH meter. The pH requirement for liquid soap formulations is between 3.5 and 4.5 (Jumain & Asmawati, 2021). Foam heigh test by shaking the soap and compared with the SNI (Indonesian National Standard) requirement of 13–220 mm (Hutauruk, 2020). Homogeneity test by visually inspecting soap on a watch glass and to identify whether any coarse particles were present (Lolok et al., 2020), irritation test using patch test method where 0.5 grams formulations was applied on the 2.5 × 2.5 cm lower arm area of 10 respondents for 15 minutes to observe any adverse skin reactions (Pratiwi, 2022), and a cycling test where the formulation was stored at a cold temperature (4 ± 2 °C) for 24 hours and heated conditions in oven at 40 ± 2 °C for another 24 hours for six cycles to monitor any physical changes in the liquid soap (Ningsih et al., 2019).

Antimicrobial Activity

The antimicrobial activity test in this study was carried out using the well diffusion method. Saboraud Dextrose Agar (SDA) medium was used for fungi and Manitol Salt Agar (MSA) medium for bacteria, each measured at 10 mL and aseptically poured into petri dish, then allowed to solidify this was referred to as the first layer. After the first layer solidified, five wells were made in each petri dish using a cylinder cup on the medium. A suspension of *S.aureus* or *C.albicans* (50 μL) was then measured and added to a measuring glass containing 15 mL of Saboraud Dextrose Agar (SDA) or Manitol Salt Agar (MSA) medium, and homogenized. This mixture was referred to as the second layer.

The second layer was poured using the pour plate method, spread evenly in a figure-eight motion, and allowed to solidify. Once the medium solidified, the cylinder cups were removed, and the wells formed were filled with 50 μL each of formula 0, 1, 2, 3, and the positive control, with five replications for each. The final step was incubating the media containing the samples and microorganisms at 24 °C for 3 × 24 hours for fungi, and at 37 °C for 1 × 24 hours for bacteria.

Data Analysis

The data were statistically analyzed using a one-way ANOVA test with IBM SPSS Statistics Version 23.

3. RESULTS AND DISCUSSION

In this study, remaceration extraction was carried out, in which the solvent was replaced every 24 hours and occasional stirring was performed to ensure equilibrium between the sample and the solvent, thereby maximizing the extraction process. (Tutik et al., 2022). The obtained filtrate was then concentrated using a rotary evaporator and a water bath to evaporate any remaining solvent, resulting in a thick extract with a yield of 16.63%. This yield is considered good, as it exceeds the required minimum of 10% (Subaryanti et al., 2022).

The preliminary test using colour reaction was the initial step to identify secondary metabolites, and the results showed that *P. laevigata* contains phenolic compounds, flavonoids, tannins, triterpenoids, and saponins as presented in Table 2 below. The presence of these secondary metabolites confirms that *P. laevigata* possesses both biological and

pharmacological activities (Julianto, 2019). The ethanol-free test results showed that the extract was free of ethanol, indicating that the sample did not contain ethanol and was thus protected from false positive results, as ethanol itself has antibacterial activity (Sukadiasa et al., 2023).

Table 2. Phytochemical Test Result Data

Group	Result	Group	Result
Ethanol free	Negative	**Alkaloid**	Positive
Fenolik	Positive	**Triterpenoids**	Positive
Flavonoid	Positive	**Saponin**	Positive

The ethanol extract of *P. laevigata* obtained was then formulated into a feminine hygiene preparation as shown in Table 1, and its characteristics were evaluated based on the cycling test parameter to determine the stability of the formulation over a specific storage period (Ningsih et al., 2019). The results shown in 3 indicate that there were no differences in the formulation before and after undergoing the cycling test.

In this study, the feminine hygiene formulation showed that the extract particles were evenly distributed or homogeneous, and it did not cause skin irritation, as indicated by the absence of edema, itching, and redness (Sari & Triski, 2023). The pH test aims to determine the safety of the formulation's acidity level to ensure it does not disrupt the normal vaginal flora (Sari & Triski, 2023) and the results showed that the formulation meets the required pH range for women, which is between 3.5 and 4.5 (Jumain & Asmawati, 2021). The formulation exhibited foam height that meets the SNI (Indonesian National Standard) requirement, ranging from 13 to 220 mm (Hutauruk, 2020).

Table 3. Result Test Formulation

Parameter Test	Formula	Result Test	
		Before	**After**
Organoleptic	F0	It has a characteristic rose scent and clean, also has a liquid form	
	FI	It has a characteristic rose scent, is brown in color, and has a liquid form.	
	FII		
	FIII		
pH	F0	3,80	3,65
	FI	3,85	3,70
	FII	3,90	3,75
	FIII	3,85	3,70
Foam Height	F0	60 mm	60 mm
	FI	60 mm	60 mm
	FII	60 mm	60 mm
	FIII	60 mm	60 mm
Homogenity	F0	Homogeneous	Homogeneous
	FI	Homogeneous	Homogeneous
	FII	Homogeneous	Homogeneous
	FIII	Homogeneous	Homogeneous
Irritation	F0	Non-irritating	Non-irritating
	FI	Non-irritating	Non-irritating
	FII	Non-irritating	Non-irritating
	FIII	Non-irritating	Non-irritating

The formulated feminine hygiene products were then tested for antimicrobial activity against test microbes using the well diffusion method. This method is based on creating wells in agar inoculated with bacteria, into which the sample is placed. The presence of a clear zone around the well indicates that the sample has inhibitory activity. The well diffusion method was chosen because it allows for easier measurement of the inhibition zone's size, as the sample diffuses downward through the agar layers, however, a drawback is that the medium is more susceptible to contamination (Sari & Triski, 2023).

Feminime Wash (Lactacyd All Day Care) was used in this study as a positive control because it is a commercially available product widely used by the public as a feminine wash. The test results, shown in Table 8, indicate that the feminine hygiene formulation containing ethanol extract of *P. laevigata* exhibited antimicrobial activity comparable to Lactacyd, as evidenced by the presence of clear inhibition zones ranging from 10 to 20 mm. The formed clear zones were classified based on the following criteria (Davis & Stout, 1971) it is classified as having strong antimicrobial activity.

The presence of clear zones in the study is attributed to the secondary metabolites in *P. laevigata* bark, which exhibit both antibacterial and antifungal properties. The mechanism of these secondary metabolites includes saponins, which form complex compounds with cell membranes through hydrogen bonding, thereby disrupting membrane permeability, and phenols, which cause protein denaturation in bacterial cells (Haryati et al., 2015), flavonoids act by inhibiting nucleic acid synthesis, disrupting membrane function, and interfering with energy metabolism (Pendit et al., 2016), alkaloids work by damaging the components that make up bacterial peptidoglycan (Purwantiningsih et al., 2014), tannins cause bacterial cell lysis and microbial cell inactivation (Nabil & Kafesa, 2024).

Figure 1. Antimicrobial Test Result Data

Average Inhibition Zone of Feminine Hygiene Formulation with P.laevigata Extract					
	F0	**FI**	**FII**	**FIII**	**K+**
S.aureus	10.15±0.037	11.29±0.023	12.56±0.044	15.11±0.018	16.30±0.097
C.albicans	9.650±0.046	0.0000±0.000	11.53±0.035	12.55±0.037	12.82±0.021

Description:
F0 : Negative control (base formulation)
FI : Feminine hygiene with 5% *P. laevigata* extract concentration
FII : Feminine hygiene with 10% *P. laevigata* extract concentration
FIII : Feminine hygiene with 15% *P. laevigata* extract concentration
K+ : Positive control (Lactacyd Feminine Hygiene)

The results of the antimicrobial tests of the feminine hygiene formulations against *S.aureus* and *C.albicans* were then analyzed statistically using IBM SPSS Statistics software. The data showed significance values of $p > 0.05$, indicating that the data were normally distributed and homogeneous. The one-way ANOVA test yielded a significance value of $p < 0.05$, indicating that there were differences among the groups. This was followed by a post hoc

test, which also showed significance values of $p < 0.05$, leading to the conclusion that there are significant differences in antimicrobial effects between the different formulations. Based on the research that has been conducted by author, in the future research can be isolated from the phytochemical compound *P. laevigata* which can play a specific role as an antibacterial agents.

4. CONCLUSION

Based on the one-way ANOVA statistical test, a value of ($p < 0.05$) was obtained, indicating a significant difference in the effects among the formulations. The feminine hygiene formulations containing *P. laevigata* extract exhibited strong antimicrobial activity against *S. aureus* at concentrations of 5%, 10%, and 15%, and against *C. albicans* at concentrations of 10% and 15%.

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