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Formulation and activity test of foot spray from sungkai leaf extract (*Peronema canescens* Jack) as an antibacterial that causes foot odor

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Abstract

Background: Sungkai is one of the medicinal plants used by the Suku Anak Dalam Tribe in Jambi to treat infectious diseases. Sungkai leaves contain secondary metabolites, including alkaloids, flavonoids, terpenoids, steroids, and tannins. Tannins and terpenoids are metabolites that have potential as antifungal, antibacterial, and antiviral, so they can be used in the cosmetics and pharmaceutical industries. Some bacterial infections can cause foot odor, which can reduce a person's self-confidence. This research aimed to test Sungkai leaf extract, formulated in foot spray dosage form to treat bacteria that cause foot odor, namely *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*.

Method: This research begins with the extraction stage of Sungkai leaves using the maceration method using 96% ethanol. The result extract is continued with phytochemical screening testing, formulating the foot spray, and testing the antibacterial activity of foot spray. Antibacterial testing using the disc diffusion method, preparation of foot spray formulation using several series concentrations of Sungkai leaf extract are 15% (F1), 20% (F2), and 25% (F3), and followed by evaluation of foot spray formula includes organoleptic tests, pH tests, stability tests, irritation tests and liking tests.

Results: Antibacterial activity of foot spray for both bacteria is in the medium category with average values of 7.29 and 7.11. The statistical test results at a p-value 0.05 show a significant difference between F0, FP, and F1.

Conclusion: Based on the research that has been carried out, it can be concluded that foot preparation spray from Sungkai leaf extract is effective as an antibacterial, especially for (F3), which contains an extract concentration of 25%.

Keywords: Extract; foot spray; *Peronema canescens* Jack; *Pseudomonas aeruginosa* and *Staphylococcus epidermidis.*

INTRODUCTION

Every person has various kinds of activities, both light and heavy, which trigger sweat secretion in the body. One part of the body that produces more sweat is the feet. This is because this part is often covered with socks and shoes. If your feet are often closed, they can cause your feet to become sweaty and damp, causing odor (1). Foot odor really interferes with activities and is quite an important problem. When feet sweat, they become damp and cause an odor that can make a person feel less confident. This problem is a disorder of the apocrine sweat with glands the term Bromhidrosis. Bromhidrosis is a condition where a person's body odor is More excessive due to the secretion of apocrine sweat glands located in

the armpits, scalp, soles of the feet, between the fingers and genitals. In this condition, the skin becomes wet and sticky and produces an unpleasant odor as a result of degradation of apocrine gland products by skin microbes (2). Some bacteria that cause infections that play a role in the process of rotting the apocrine glands resulting in smelly feet include Staphylococcus epidermidis, Corynebacterium Pseudomonas acne, aeruginosa, Staphylococcus aureus and Streptococcus phyogenesis (1). These bacteria are gram-positive and gramnegative which can be found in damp areas such as water, soil and human skin (3). The bacteria that cause this infection can be overcome using one of the Sungkai leaves (4).

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Sungkai plants contain secondary metabolites including alkaloids, flavonoids, terpenoids - steroids, and tannins. Tannin is a group of terpenoids which have the potential to be antifungal, antibacterial and antiviral so they can be used in the cosmetics and pharmaceutical industries (3). Using sungkai as an antibacterial in controlling foot odor can be processed in several forms of pharmaceutical preparations, one of which is foot products spray. The spray form was chosen based on the nature of the spray which can provide a concentrated content but at the same time has a fastdrying profile making it easy to use for users (5). Seeing the problems above, an idea emerged to use sungkai leaves as an active substance to make foot preparations sprayas antibacterial Staphylococcus epidermidis and Pseudomonas aeruginosa causes foot odor.

METHOD

This research was conducted at the Research Laboratory, Biology Laboratory, Pharmaceutical Technology Laboratory and Microbiology Laboratory, Harapan Ibu Jambi College of Health Sciences.

Tool

The tools used are analytical scales, rotary evaporator, maceration bottle, evaporating cup, spatula, stirring rod, water bath, autoclave, incubator, container for storing feet. spray, mortar, stamper, LAF (laminar air flow), homogenizer, dropper pipette, spatula, funnel, pH meter, hot plate, magnetic stirrer, test tube, tube rack, dropper pipette, Bunsen lamp, tweezers, sterile tube, sterile swab, petri dishes, spray bottles, ovens, refrigerators, and glass equipment found in the laboratory.

Material

The material used is Sungkai leaves (*Peronemacanescens* Jack), ethanol 96%, Ascorbic acid, glycerin, isopropyl alcohol, propylene glycol (PEG), carbopol 940, NaOH, tween 80, distilled water, Mayer's reagent, Wagner, Dragendorff, Liebermann-Burchard, Nutrient Agar (NA), Mueller Hinton

Agar (MHA), Staphylococcus epidermidis and Pseudomonas aeruginosa.

Sample Collection and Determination

Sungkai plants were obtained from Tebo Regency, Jambi Province, then determined at the UNPAD Plant Taxonomy Laboratory to ensure the plants accuracy. Determination of sungkai plants is determined by comparing the morphological characteristics of the plant with library data.

Making Extracts

Simplicia powder is extracted by maceration using ethanol solvent. According to the Indonesian Pharmacopoeia Edition VI (2020), the method is as follows: A total of 500 g of simplicia powder is put into a vessel, poured with 75 parts (3750 ml) of 80% ethanol, covered with aluminum foil, left for 5 days protected from light, occasionally stirred, then squeezed. The dregs were macerated with 80% ethanol, 25 parts (1250 ml) left at room temperature protected from light for 2 days, filtered with filter paper. Concentration of the extract was carried out using a Rotary evaporator (6).

Phytochemical Screening Test

- a. Alkaloids
 - Several milligrams of each extract were dissolved individually in dilute HCL and filtered. Then, the filtrate was treated separately with Mayer's, Wagner's, and Dragendorff's reagents. Positive results are turbidity or cream precipitate in the Mayer test, yellow-brown precipitate in the Wagner test, and turbidity or orangered precipitate in the Dragendorff test (7).
- b. Flavonoids
 - In the Alkali test, approximately 2 mL of 20% NaOH solution is added to 1 mL of alcohol solution of each plant extract individually. Positive results of intensive observation are yellow. In the Zn / HCl test, a pinch of zinc dust is added to 2 mL of the sample alcohol solution. Then, a few drops of concentrated HCL are added slowly. Positive results are pink to red (7).
- c. Tannin

A total of 10 mg of extract was boiled in

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10 mL of water in a test tube and then filtered. Then, a few drops of 1% ferric chloride were added to the filtrate. Positive results Hydrolyzed tannin gives a bluish black color, while condensed tannin gives a brownish green color (7).

d. Saponin

Several milligrams of each extract were mixed with 5 mL of distilled water and mixed vigorously. Continuous positive foam results (7).

e. Steroids

In the Salkowski test, a few milligrams of the sample are treated with chloroform and filtered. The filtrate is then reacted with several drops of concentrated sulfuric acid. A positive result with a greenish yellow color indicates the presence of steroids. In the Liebermann - Burchard test, approximately 2 mg of each extract is dissolved in acetic anhydride, heated, and cooled before adding 1 mL of concentrated sulfuric acid along the side of the test tube. A positive green result indicates the presence of steroids (7).

f. Triterpenoids

Approximately 1 mL of each of the four extracts was added to 1 mL of chloroform and filtered to clarify the solution, followed by adding a few drops of concentrated sulfuric acid to the side walls of the test tube. Positive results are reddish brown (7).

Formulation of Foot Spray

Making supplies foot spray using the formula in table 1 below:

Table 1. Foot Spray Formula

No	Material		Formula %					
NO	Materiai	F0	F1	F2	F3			
1	Sungkai Leaf extract	-	15	20	25			
2	Ascorbic Acid	0.2	0.2	0.2	0.2			
3	Glycerine	0.2	0.2	0.2	0.2			
4	Isopropyl alcohol	25	25	25	25			
5	Propylene glycol	5	5	5	5			
6	Carbopol	0.06	0.06	0.06	0.06			
7	NaOH	0.02 4	0.024	0.024	0.024			
8	Tween 80	4.3	4.3	4.3	4.3			
9	Aquades	add 100	add 100	add 100	add 100			

How to Make Foot Spray

Carbopol 940 was homogenized in a certain amount of water using a magnetic stirrer. In a separate container, NaOH is dissolved in water. In the next stage, the previously formed carbopol 940 and water is mixed with NaOH. Next, into this solution, propylene glycol was added while stirring until homogeneous. Then, ascorbic acid was added and stirred until homogeneous (mixture A). In a separate container, dissolve enough sungkai leaf extract into its isopropyl alcohol. After the sungkai leaf extract has dissolved, then 0.2 ml of glycerin is added and homogenized until mixed (mixture B). Mixture B is added to mixture A, the two are homogenized until completely mixed. Next, solubilizing is an added agent, namely Tween 80 (8).

Evaluation of Foot Spray

Organoleptic Test
 Observations were made on the foot preparation spray's color, odor and form

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2. Test the Degree of pH Acidity

Determination the рH of the preparation is carried out using a pH meter. The instrument is first calibrated using a standard buffer solution of neutral pH (pH 7) and buffer solution Acidic pH (pH 4) until the tool shows the pH value. Then the electrode was washed with distilled water, then dried with a tissue. Then the electrode is dipped in the preparation solution until the device shows a constant pH value, the number shown by the pH meter is the pH value of the preparation (8).

3. Stability Test

Stability testing is the ability of a product maintain its properties characteristics to be the same as those when it was made within specified limits throughout the period of storage and use. Stability observations are carried out when the preparation has been completed. Storage was carried out for 4 weeks at room temperature with weekly observations. The parameters observed in this physical stability test include changes in shape, color and odor of the preparation (9).

4. Irritation Test

The irritation test was carried out on 6 volunteers who were previously given a statement letter stating that they were willing to volunteer and were also given information regarding the irritation test and how to determine if irritation was present (8).

5. Test Likeability

Test the preferences for the foot preparation spray carried out to determine consumer preference for aroma/smell, color, softness and ease of spray. This test uses 10 volunteers (panelists) with a rating scale (8).

Foot Spray Antibacterial Activity Test

The test method used is the diffusion method with paper discs against Staphylococcus epidermidis and Pseudomonas aeruginosa. The first is done by: suspension of bacteria Staphylococcus epidermidis and Pseudomonas aeruginosa

was taken using a sterile swab then smeared on Mueller's medium Hinton Agar (MHA) evenly over the entire surface and left for 5 minutes so that the suspension is absorbed into the media. 5 mm paper discs were inserted into a spray formula containing sungkai leaf extract in various concentrations (15%, 20% and 25%). Then the paper is attached to the surface of the MHA media. All media were incubated at 37°C for 24 hours. Then observe and measure the diameter of the clear zone formed using a caliper. Each

Data Analysis

test was carried out 3 times (8).

Data obtained from extract yield results, phytochemical screening and preparation evaluation data were processed using descriptive analysis. Data obtained from the inhibition zone results were statistically analyzed for homogeneity, normality and difference tests using one-way ANOVA. Next, further tests were carried out with Duncan on the SPSS application.

RESULTS

Plant Determination and Ethical Clearance

Determination of sungkai leaves (*Peronema canescens* Jack.) carried out at the Jatinangor Herbarium, Plant Taxonomy Laboratory, Department of Biology, FMIPA, Padjadjaran University, West Java. Shows that the plant used as the active ingredient is sungkai (*Peronema canescens* Jack) which was obtained from the deep forest of Jambi Province, Muara Kilis Village, Tengah Ilir District, Tebo Regency.

Ethical Clearance Obtained from Health Research Ethics Committee Poltekkes Kemenkes Jambi No. LB.02.06/2/642/2023.

Making Extracts

Making the extract begins by washing the sungkai leaves and chopping them, then drying them to obtain a weight of 1.5 kg. Next, extraction was carried out using methanol solvent and allowed to stand, stirring occasionally, for 3x24 hours. After obtaining the liquid extract, it evaporated until the thick

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extract weighed 213.9 grams. The yield obtained was 14.26%. The extract is blackish green in color.

Phytochemical Screening

extract sungkai (Peronema Leaf canescens Jack) it is known that this extract contains several secondary metabolites, namely Alkaloids, Flavonoids, Terpenoids, Steroids, Tannins and Saponins. The results of the metabolite examination can be seen in table 2 below:

Table 2 Phytochemical Screening of Extracts

Table 2. Phytochemical Screening of Extracts							
Secondary Metabolite Group	Reagent	Literature Results	Observa tion result				
	Mayer	A white precipitate is formed	+				
Alkaloids	Dragendorff	An orange precipitate is formed	+				
	Wagner	A brown precipitate is formed	+				
Flavonoids	Alkali	An orange layer is formed	+				
Saponin	HCI	Stable foam ± 5 minutes	+				
Tannin	FeCl3	Blackish green color	+				
Steroids	Liebermann Bourchardat	A Green Ring is formed	+				
Triterpenoids	Liebermann Bourchardat	A Violet Ring is formed	+				

Evaluation of Foot Spray

1. Organoleptic Test

From the results of the organoleptic examination that was carried out, the results were obtained as shown in the table below:

Table 3. Organoleptic Examination Results

Obse			Formulas	ormulas				
rvati on	F0	F1	F2	F3	FP			
Form	Liquid	Liquid	Liquid	Liquid	Liquid			
Smell	Weak Smell	Charac teristic Extract Smell	Charac teristic Extract Smell	Charac teristic Extract Smell	Mint Smell			
Color	Clear White	Deep Green	Deep Green	Deep Green	Clear Blue			
Hom ogen eity	Homog eneous	Homog eneous	Homog eneous	Homog eneous	Homog eneous			

Information:

F0 : Blank

F1 : Formula foot spray contains 15% sungkai

leaf extract

F2 : Formula foot spray contains 20% sungkai

leaf extract

F3 : Formula foot spray contains 25% sungkai leaf extract

: Comparative formula for foot preparations FΡ

spray which is on the market

2. Test the Degree of pH Acidity

From the results examination that has been carried out, the results obtained are as shown in the table below:

Table 4. Examination Results pH

Formulas	•	est Res peatab	Mean ± SD	
	1	2	3	
F0	4.7	4.6	4.6	4.63 ± 0.0577
F1	5	5	5	5 ± 0
F2	4.8	4.8	4.8	4.8 ± 0
F3	4.8	4.8	4.9	4.83 ± 0.0577
FP	6	6	6	6 ± 0

3. Stability Test

From the results of the stability tests that have been carried out, the results as shown below are obtained:

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Table 5. Stability Test Results

Formula	Duration of Observation (Week)								
	Obser vation	Week I	Week II	Week III	Week IV				
	Form	L	L	L	L				
F0	Color	WC	WC	WC	WC				
	Smell	NS	NS	NS	NS				
- 4	Form	L	L	L	L				
F1	Color	SG	SG	SG	SG				
	Smell	Т	Т	Т	Т				
	Form	L	L	L	L				
F2	Color	SG	SG	SG	SG				
	Smell	Т	Т	Т	Т				
F3	Form	L	L	L	L				
	Color	HP	HP	HP	HP				
	Smell	Т	Т	Т	Т				
- FD	Form	L	L	L	L				
FP	Color	ВС	ВС	ВС	ВС				
	Smell	MT	MT	MT	MT				

Description:

L : Liquid
WC : White Clear
NS : No Smells
SG : Solid Green

T : Typical BC : Blue Clear MT : Mint

4. Irritation Test

From the results of the irritation examination that was carried out, the results were as shown below:

Table 6. Examination Results Irritation Test

No	Irritation Test	Volunteer						
NO	no irritation rest — 1		2	3	4	5	6	
1	Redness of the Skin	-	-	-	-	-	-	
2	Itchy Skin	-	-	-	-	-	-	
3	Skin Becomes Rough	-	-	-	-	-	-	

Information:

- : No irritation occurs + : Reddish skin ++ : Itchy skin +++ : Swollen skin Formulation and activity test of foot spray from sungkai leaf extract (Peronema canescens Jack) as an antibacterial that causes foot odor

5. Test Likeability

From the results of the preference tests that have been carried out, the results obtained are as shown below:

Table 7. Likeability Test Result

	Like				No Like					
Indicator	F 0	F 1	F 2	F 3	FP	F 0	F 1	F 2	F 3	FP
Color	2	8	6	4	10	8	2	4	6	0
Smell	7	8	5	3	9	3	2	5	7	1
Flavor	2	5	4	4	9	8	5	6	6	1
Texture	6	7	5	4	9	4	3	5	6	1
Ease n Use	6	9	8	7	10	4	1	2	3	0

Information:

F0 : Blank

F1 : Formula foot spray contains 15% sungkai leaf extract

F2 : Formula foot spray contains 20% sungkai leaf extract

F3 : Formula foot spray contains 25% sungkai leaf extract

FP : Comparative formula for foot preparations spray which is on the market

Antibacterial Activity Test

From the results of the antibacterial activity test that was carried out, the results as shown below were obtained:

Table 8. Test Results Activity Antibacterial Staphylococcus epidermidis

Formulas	Inhi Dia	Mean (mm) ± SD		
	1	2	3	
F0	3.5	3.3	3,4	3.40 ± 0.1
F1	6.23	6.51	7.1	6.61 ± 0.44
F2	7.21	7.42	7.68	7.43 ± 0.23
F3	7.66	8.1	7.8	7.85 ± 0.22
FP	4.8	5.35	5.12	5.09 ± 0.27

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Table 9. Test Results Activity Antibacterial Pseudomonas aeruginosa

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Formulas		ibition Zo meter (n	Mean				
	1	2	3	(mm) ± SD			
F0	3.55	2.25	3.35	3.05 ± 0.70			
F1	7	5.85	6.05	6.30 ± 0.61			
F2	7.4	7.55	7.1	7.35 ± 0.23			
F3	7.85	8	7.25	7.70 ± 0.39			
FP	5	5.35	4.9	5.08 ± 0.23			

Information:

F0 : Blank

F1 : Formula foot spray Contains 15% sungkai leaf extract

F2 : Formula foot spray contains 20% sungkai leaf extract

F3 : Formula foot spray Contains 25% sungkai leaf extract

FP : Comparative formula for foot preparations spray which is on the market

Data Analysis

Data on MIC values from antibacterial activity testing produced in each test group for each Bacteria were analyzed using oneway ANOVA, and further tests were carried out using Duncan data can be seen in tables 10 and 11 below:

Table 10. Duncan Continued Testing Foot Spray Against *Staphylococcus epidermidis*

Formulas	N	Subsets for alpha = 0.05					
FOIIIIIIIas	IN	1	2	3	4		
F0	3	3.3833					
FP	3		5.0833				
F1	3			6,3000			
F2	3				7.3500		
F3	3				7.7000		
Sig		1,000	1,000	1,000	0.268		

Table 11. Duncan Continued Testing Foot Spray Against *Pseudomonas aeruginosa*

Formulas	N	Subsets for alpha = 0.05				
Formulas	IN	1	2	3	4	
F0	3	3,4000				
FP	3		5.0900			
F1	3			6.6133		
F2	3				7.4367	
F3	3				7.8533	
Sig		1,000	1,000	1,000	0.097	

In the data from further test results against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa, there are* significant differences between F0, FP, and F1, while for F2 and F3 there is no significant difference.

DISCUSSION

This research uses 3 variations of the formula with blanks. The aim of using variations in the concentration of sungkai leaf extract is to determine the effectiveness of the sungkai leaf extract content on antibacterial activity. Blanko does not contain sungkai leaf extract. Formula 1 contains sungkai leaf extract with a concentration of 15%, formula 2 contains sungkai leaf extract with a concentration of 20%, and formula 3 contains sungkai leaf extract with a concentration of 25%.

Spray preparation is made slightly thick to maintain the stability of the contents in the preparation. Therefore, carbopol is added so that the spray preparation is slightly thick but can flow. The carbopol material was chosen because it has good compatibility with other ingredients in the formulation. When used in cosmetics, carbopol does not leave traces when used (10).

Organoleptic examination includes shape, smell and color. In formula 0 or blank it has a liquid form, a weak or no odor, and a clear white color, in formula 1 it has a liquid form, a typical extract odor, and a thick green color, in formula 2 it has a liquid form, a typical extract odor, and color dark green, and in the comparison formula it has a liquid form, a mint smell, and a clear blue color.

pH check is carried out to determine the pH value, whether it is safe for use on the skin. Based on parameters, pH requirements for foot preparations A good spray according to skin pH in general is 4-7 (11). Measuring the pH of foot preparations Sungkai leaf extract spray is carried out using a pH meter. The results of the examination showed that the pH of the foot preparation spray that has been made has a pH of 4-5 and foot spray on the market has a pH of 6.0. This shows that the entire foot formula Sungkai leaf extract spray has the same pH as the skin's pH

because it is in the pH range 4-7. If the preparation has a pH that is too acidic it can cause skin irritation (12).

Stability tests were carried out to determine the stability of the preparation during 4 weeks of storage. Foot spray preparation stability test results show that all preparations made remain stable during storage at room temperature. The parameters observed in this physical stability test include changes in shape, color and odor (9). From the results of shape observations, it was found that all the foot spray preparations made did not change shape until the 4th week of room temperature storage. From the results of color observations, at week 4 the color of the preparation remained stable. Meanwhile, the odor produced from all foot spray preparations was the characteristic odor of the preparation. The odor of the preparation remained stable after 4 weeks of observation storage at room temperature.

The aim of the irritation test is to see whether the preparation can cause irritation or sensitivity to the skin. Foot preparation irritation test results spray that was carried out on 10 volunteers showed that foot preparation The spray does not cause an irritation reaction when sprayed on the feet. This shows that the foot preparation spray is a safe preparation to use.

The preference test aims to determine the respondent's level of preference for the preparation that has been made based on color, smell, taste, texture and ease of use. From the results of the preference test which was carried out by distributing assessment sheets to 10 respondents to ask for opinions on the color, smell, taste, texture and ease of use of the foot preparation. spray that has been made, the results obtained are that the formulas most liked by respondents are FP and F1. The results obtained were based on color, there were 10 respondents who liked FP and 8 respondents liked F1, based on smell there were 9 respondents who liked FP and 8 people who liked F1, based on taste there were 9 respondents who liked FP and 5 people liked F1, based on texture, there are 9 respondents who like FP and 7 respondents who like F1, based on ease of use, there are

10 respondents who like FP and 9 respondents who like F1.

Based on the results of the antibacterial activity test, it can be seen that formula 3 has the largest inhibition zone compared to formula 1 and formula 2. This is because in formula 3 the most extract is added, namely 25% w/v so that the flavonoid content contained in the extract preparation is also the greater it is. The results of determining the bacterial inhibition zone show that 25% w/v of sungkai leaf extract is needed to form a minimum inhibition zone for *Staphylococcus*. *epidermidis* and *Pseudomonas aeruginosa*.

The Chemical ingredients that play a role in antibacterial activity are known from polyphenolic secondary metabolites such as Tannin and Flavonoids (13). Sungkai leaf extract contains Flavonoid and Tannins. Another compound also has antibacterial activity such as Alkaloid, Saponin, and Triterpenoid (14). So many kinds of plant medicine from Suku Anak Dalam Jambi, one of them Sungkai which has been proven to treat infection (15).

CONCLUSIONS

Based on the research that has been carried out, it can be concluded that foot preparation spray from sungkai leaf extract is effective as an antibacterial where a good concentration is found in formula 3 with a concentration of 25%.

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