

RESEARCH ARTICLE

Antimicrobial lotion containing red *Piper betle* leaf (*Piper crocatum* Ruiz and Pav) ethanolic extract for topical applicationSri Agung Fitri Kusuma¹, Soraya Ratnawulan Mita², Agusri Mutiara¹¹Department of Biology Pharmacy, Faculty of Pharmacy, Padjadjaran University, Bandung, Indonesia, ²Department of Pharmaceutic and Pharmaceutical Technology, Faculty of Pharmacy, Padjadjaran University, Bandung, Indonesia

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ABSTRACT

Background: Empirically red *Piper betle* leaf (*Piper crocatum* Ruiz and Pav) is used as a natural antiseptic, and it is proved that the ethanol extract of red *P. betle* leaf ethanol extract provides antimicrobial activity against the airborne pathogen. **Aims and Objectives:** To investigate and determine the most effective of the lotion formulated with red *P. betle* leaf ethanol extract against airborne pathogens. **Materials and Methods:** The plant material in this study was red *P. betle* leaves that obtained from Bogor Indonesia. The preliminary antimicrobial activity of the extracts at various concentrations was conducted to determine the minimum inhibitory concentration (MIC) of the extract against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. The MIC test was performed using an agar diffusion method with perforation technique. Then, antimicrobial lotions (F1–F3) were formulated in varied concentrations (5, 7.5 and 10% w/w) of red *P. betle* leaf extracts using different excipients and kept for 28 days at 8°C, 25°C, and 40°C. The physicochemical and microbiological parameters of the lotion formulated were evaluated using standard procedures. **Results:** MIC range for red *P. betle* leaf extract was found to be 2.5–5% b/v for all bacteria and 1.25–2.5% b/v for *C. albicans*. A formulation containing these extracts showed a nonsignificant zone of inhibition for 5%, 7.5%, and 10% of which 10% showed maximum zone of inhibition (ranging from 11.23 to 11.83 mm). All of the lotion formulated were stabil for the physicochemical parameters of evaluation. **Conclusion:** The F2 lotion formula had good pharmaceutical properties and potential antimicrobial activity.


KEY WORDS: Lotion; Antibacterial; *Piper crocatum* Ruiz and Pav; Extract; Airborne

INTRODUCTION

Microorganisms are closely related to human life, especially microorganisms that exist around us.^[1] These microorganisms can be normal and pathogenic to humans. Contamination can directly spread microorganisms from one human to another through direct skin contact with a source of transmission or

through the air and caused topical skin infection. Microbes such as bacteria and yeasts often cocontribute to these infections.^[2] Based on the results of the study, it found some contamination of microbes around us such as: *Candida albicans* (13%), *Staphylococcus aureus* (11%), *Escherichia coli* (9, 2%), and *Pseudomonas aeruginosa* (7, 1%).^[3] These data suggest that airborne contaminants and their spread through skin contact can be a significant source of topical skin infection.

An appealing way of treating superficial skin infections is through the use of topical antimicrobials.^[2] Various kinds of hygienic effort such as antiseptic use should be done to prevent microorganism contamination and prevention of infection.^[4] However, the long-term use of antiseptics has develop the

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microbial resistance to antiseptics.^[5] Several microbial resistance had been reported as follows: *Salmonella enterica* to benzalkonium chloride, *P. aeruginosa* to polyquaternium, chlorhexidine gluconate and benzalkonium, (methicillin-resistant *S. aureus*) against quaternary ammonium, and *C. albicans* against cetylpyridinium chloride.^[6-8] Therefore, the need of a new antiseptic candidate was become important to investigate. Such antiseptic candidates can be obtained from plant extracts that have been known to have antimicrobial activity.

Plant extracts have been developed and proposed for antimicrobials preparation.^[9] So as the antimicrobial activity of red *Piper betle* leaf that has been investigated for many years. This extract is known to provide antimicrobial activity against common airborne contaminants such as *S. aureus*, *P. aeruginosa*, *E. coli*, and *C. albicans*.^[5] In the previous study, red betel leaf *Simplicia* contained alkaloids, polyphenolics, tannins, flavonoids, quinones, steroids, monoterpenoids, sesquiterpenoids, and saponins.^[10] Flavonoids and polyphenolic compounds had been reported as antiseptics.^[11] Thus, in this study, the extract was formulated as topical lotion, and the *in vitro* antimicrobial activity of all formula was evaluated against airborne pathogens. A lotion containing natural ingredients is an innovation that can be used as one of the antimicrobial products that are convenient to use, practical, has longer contact time on the surface of the body, and leaves no residue on the skin. The topical approaches may offer effective, localized, well-tolerated alternatives to the systemic regimen.^[12]

MATERIALS AND METHODS

Materials

The red *P. betle* leaves (*Piper crocatum* Ruiz and Pav) were obtained from plantation area of Bogor, West Java. The following chemicals were used: Ethanol 70%, dimethyl sulfoxide (DMSO), physiological NaCl, stearic acid, lanolin, 70% sorbitol solution, span 80, tween 60, glyceryl monostearate, cetyl alcohol, vaselin flavum, liquid paraffin, isopropyl palmitate, triethanolamine, glycerin, methyl paraben, propylparaben, and distilled water. *E. coli*, *P. aeruginosa*, *S. aureus*, and *C. albicans* were obtained from the microbiology laboratory, Faculty of Pharmacy, University of Padjadjaran, Jatinangor. The growth medium that was used in this study were Mueller-Hinton agar (MHA) for bacteria and Sabouraud dextrose agar (SDA) for the fungal.

Extraction

About 691, 84 g of dried powder of the leaves was extracted in 70% ethanol by maceration method for 3 × 24 h. The maceration was processed with the solvent replacement every 24 h. The macerates was collected every 24 h and evaporated in a water bath to obtain concentrated extracts with constant weight.

Phytochemical Screening

The phytochemical screening was carried out to detect the presence of secondary metabolites such as alkaloids, flavonoids, quinones, polyphenols, tannins, saponins, steroids, monoterpenoid, triterpenoid, and sesquiterpenoid were performed following the method described by Farnsworth.^[13] The color intensity of the precipitation formation were observed.

Minimum Inhibitory Concentration (MIC) Test

MIC test was done by the agar diffusion technique. The microbial suspension was diluted using a sterile physiological NaCl to reach an equivalent turbidity of 0.5 Mc Farland for bacteria and 0.3 Mc Farland for fungi. A total of 20 µl of microbial suspension was poured into an agar medium and allowed to solidify. The test medium was perforated to form a reservoir to store the extract. The extracts were serially diluted to obtain the concentration from 20% b/v to % using a DMSO solvent. A total of 50 µl of test extract with certain concentration was pipetted into the reservoir on the test medium. All plates containing tested bacteria were then incubated at 37°C for 24 h, meanwhile, for the fungal, it needs 48 h for the incubation period of time. The MIC is the concentration of the highest dilution concentration in which the inhibitory zone was absent, hence no diameter of the inhibitory zone was observed.

Formulation of Lotion

Selection of the base formula of lotion was performed on nine formulas of water in type emulsion lotion. The base of losio formula could be seen in Table 1. The preparation of lotion was carried out by mixing non-polar phase to the polar phase.^[14] The mixing was done with rapid stirring to avoid water and oil phase separation. First, all the nonpolar phases and polar phase were melted together at 75°C. After that, the nonpolar phase was added to the polar phase bit by bit. Then, the distilled water was added to the emulsion slowly. The bases of the lotion were then stored at room temperature for 7 days and observed its organoleptic stability, which includes discoloration, odor, and basic homogeneity. The best stability base formula was formulated with red *P. betle* leaf ethanol extract at various minimal effective concentrations (MEC). The formulation was performed using a variety of ethanol extract concentration of red betel leaves with a MEC range of 2–4× MIC. The antimicrobial lotion formula could be seen in Table 2.

The two variations of mixing method were undertaken to optimize the lotion stability. The first method (A1), each of the nonpolar phase and the polar phase were melted at 75°C. The nonpolar phase was allowed to cool to a temperature of 40°C. The extract was added into the nonpolar phase. This mixture was then poured slowly into the polar phase at a

Table 1: Base lotion formulas

Composition	Formula (% w/v)								
	B1	B2	B3	B4	B5	B6	B7	B8	B9
Stearic acid	3.5	5	7	2	2.5	3	4	4.5	5
Lanolin	0.5	0.5	0.5	-	-	-	-	-	-
Tween 60	7.4	7.4	7.4	-	-	-	-	-	-
Span 80	2.12	2.12	2.12	-	-	-	-	-	-
Sorbitol solution 70%	10	5	10	-	-	-	-	-	-
Glyceryl monostearate	-	-	-	1	1	1	1	1	1
Cetyl alcohol	-	-	-	1	1	1	1	1	1
Vaselin flavum	-	-	-	1	1	1	1	1	1
Liquid paraffin	-	-	-	2	2	2	2	2	2
Isopropyl palmitate	-	-	-	2	2	2	2	2	2
Triethanolamine	-	-	-	1	1	1	1	1	1
Glycerin	-	-	-	5	5	5	5	5	5
Methyl paraben	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Propyl paraben	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Distilled water	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100

Table 2: Antimicrobial lotion formulas

Composition	Formula (% w/v)			
	F0	F1	F2	F3
Extract	0	5	7.5	10
Stearic acid	5	5	5	5
Lanolin	0.5	0.5	0.5	0.5
Sorbitol solution 70%	5	5	5	5
Span 80	2.12	2.12	2.12	2.12
Tween 60	7.4	7.4	7.4	7.4
Methyl paraben	0.25	0.25	0.25	0.25
Propyl paraben	0.5	0.5	0.5	0.5
Distilled water	ad 100	ad 100	ad 100	ad 100

temperature of 40°C. Finally, the distilled water was added into the emulsion up to 100%. Meanwhile, for the second method, each of the nonpolar phase and the polar phase were melted at 75°C. The nonpolar phase was added slowly into the polar phase at 75°C. Then, the distilled water was added into the emulsion. Finally, the crushed extract was introduced into the base emulsion.

Physical Evaluation

The physical parameter evaluation was carried out by observing the changes of lotion in shape, consistency, color, odor, homogeneity, and viscosity during storage at three different storage temperatures, i.e., 8°C, 25°C, and 40°C.^[15] The size changes of the globule losio were measured using the freeze-thaw method. Storage in the freeze-thaw cycle was performed to observe the physical stability and globule size after being kept at least six cycles at different temperatures of 4°C and 45°C. Storage was carried out in six cycles where

each cycle lasts for 48 h at each temperature. Lotion was put in a vial and stored in the refrigerator (4°C) for 48 h, followed by storing the preparation in the oven (45°C temperature) at the same time.^[16] Each preparation was observed to measure the phase globules dispersed under a microscope using the micrometer method. The phase separation of antimicrobial lotion emulsion was done by centrifugation method. The centrifugation method was performed after the preparation of lotion was stored for 3 weeks at room temperature, weighed as much as 1 g and centrifuged with centrifugation at 2500, 3000, and 3750 rpm for 5 h. The globule size changes were observed using a microscope.

Chemical Evaluation

The preparation was chemically observed to change pH during storage time. The pH measurements were performed using a calibrated digital spear pH. The measurements were made during 28 days storage at three different storage temperatures, i.e., 8°C, 25°C, and 40°C.

Microbiological Evaluation

Microbiological evaluation of the lotion was investigated as follows: Microbial contamination, antimicrobial activity at the beginning and the end of storage period, as well as testing the effectiveness of the antimicrobial lotion. Testing of microbial contamination of the lotion preparation was done using pour plate method. The lotion was diluted to obtain concentrations of 10, 1 and 0.1% w/v in sterile physiological NaCl. A volume of 1 mL was taken from each dilution then pour it in a sterile Petri dish and the MHA was poured into it, then allowed to solidify. Thereafter, the test medium was incubated at 37°C for 20 h. The same method was performed

to detect fungus contamination with different medium and incubation condition. The fungal medium was SDA and incubated at 25°C for 2–3 days.

Antimicrobial Activity Test

The antimicrobial activity test was performed using the agar diffusion method. The test medium was prepared by means of 20 µL of bacterial suspension and 20 mL of MHA for bacteria and SDA for fungi, inserted into sterile Petri dishes aseptically then allowed to solidify. Then, the test medium was perforated with a sterile perforator in a marked position. The test material (antimicrobial lotion) which has been dissolved in DMSO was inserted into the hole aseptically. All test media containing bacteria were incubated at 37°C for 24 h and at 25°C for 2–3 days for the fungal.

Antimicrobial Effectivity Test

The effectiveness of antimicrobial lotion was done by replication method and tested by decreasing the amount of bacteria and fungus on the volunteer's fingers before and after using the lotion, which was attached to media for MHA and SDA. Volunteer finger palms were marked with a size of 1.5 × 1 cm. The first finger was not lubricated and the second finger was lubricated with 0.5 g of lotion. After 1 min, a fingerprint contact was made on the media in a Petri dish. Petri dishes with MHA media were incubated at 37°C for 18–24 h, while Petri dishes with SDA media were incubated at 25°C for 2–3 days. The number of bacteria from both areas of the back of the volunteer hand were compared and the number of declines counted. The test was conducted on 10 volunteers.

Statistical Analysis

Data analysis was performed by statistics method of complete square design (RBSL) and group random design random acts of kindness with 95% ($\alpha = 0,05$) confidence level and Duncan advanced test method using SAS software version 9.1.3. Data entered on SAS input then data were processed. The result of ANOVA and further test were analyzed.

RESULTS

Extraction Results

The extraction was done by maceration or immersion method. This method was chosen to prevent damage to extract's compounds by high temperatures. Based on the formula, the obtained rendement of red betle leaf extract was 16.65%.

Phytochemical Screening Results

Based on the results of the phytochemical screening, in red betle leaf extract contained several classes of compounds

such as saponins, polyphenols, quinones, tannins, steroids, monoterpene, sesquiterpenoids, and flavonoids.

MIC Test Result

The result of MIC test value of red *P. betle* leaf ethanol extract against test microbes could be seen in Table 3.

Formulation of Lotion Results

Selection of the nine formulas lotion base was done with different variations of the nonpolar phase components, could be seen in Table 4.

Physical Evaluation Results

The organoleptic observation results indicated that all of the lotion formula had not changed on its shape, color, and odor. The viscosity measurements were performed to observe whether there was a change in consistency of the viscosity of the antimicrobial lotion. The evaluation results of storage temperature for 28 days, could be seen in Figure 1. The evaluation of the globul size of the losio was performed to determine the physical quality of the lotion and the stability of the lotion based on the globul size of the lotion during the freeze-thaw cycle. The results of globule lotion measurement during the freeze-thaw cycle could be seen in Table 5.

Chemical Evaluation Results

An antimicrobial lotion preparation was chemically observed for changes in pH during storage time. The changes in the pH value of the preparation during storage time were made to ensure that the pH of the preparation was stable during storage and entered the appropriate pH value range for a lotion preparation. For 28 days of storage time, the pH values of all formulas were relatively stable at their respective storage temperatures, ranging between 6.3 and 6.72. The pH value of this preparation was in the range of normal physiological pH values, i.e., 4.5–7.0.^[17] SNI 16-4952-1998 stated that good pH value of the preparation of lotio is 4.0–7.5. From the literature, it could be seen that the preparation of these lotion was a good preparation of lotion. The pH values of all formulas in storage condition were performed in Figure 2.

Microbiological Evaluation Results

The observation of microbial contamination was done to investigate the microbial contamination presence on antimicrobial lotion preparation of red *P. betle* leaf ethanolic extract. The results could be seen in Table 6, showed that all formulas were sterile. This could be due to the antimicrobial effects produced by the extract.

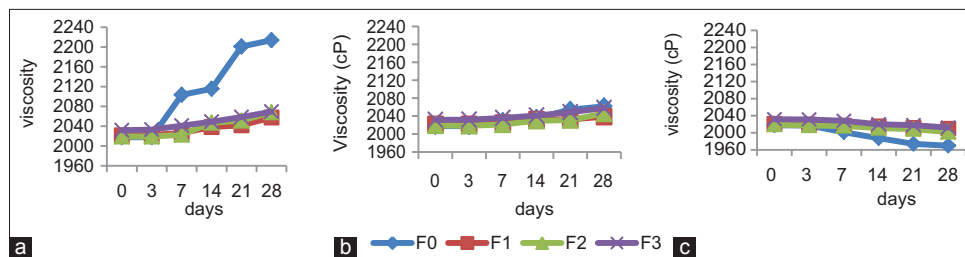


Figure 1: Graph of viscosity formula lotion on three different temperature for 28 days of storage period; (a) (8°C), (b) (25°C), (c) (40°C)

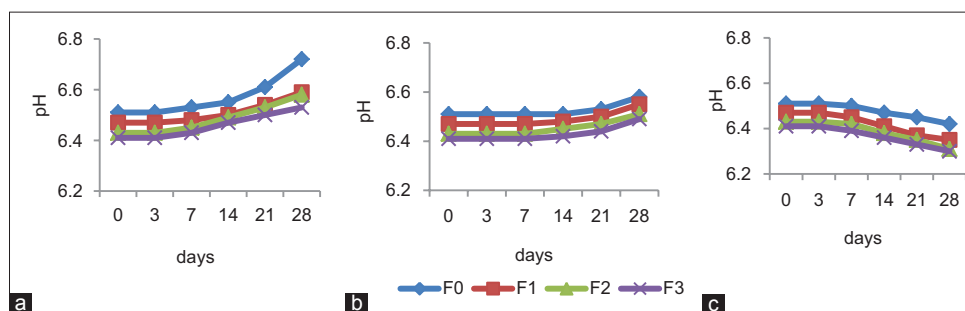


Figure 2: Graph of losio pH value at different temperature; (a) (8°C), (b) (25°C), (c) (40°C)

Table 3: MIC value of the extract for each microbe

Extract concentration (% w/v)	Microbes growth			
	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
20	-	-	-	-
10	-	-	-	-
5	-	-	-	-
2.5	+	+	+	-
1.25	+	+	+	+

(+): Presence, (-): Absence. MIC: Minimum inhibitory concentration, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *C. albicans*: *Candida albicans*

Table 4: Physicochemical evaluation of base lotion formulas

Formula	Shape	Color	Odor	pH	Viscosity (cP)
B1	Liquid	White	No odor	7.96	167.2
B2	Liquid	White	No odor	8.18	132.2
B3	Liquid	White	No odor	8.32	254.5
B4	Solid	White	No odor	4.81	128.7
B5	Semi liquid	White	No odor	5.15	112.3
B6	Thick	White	No odor	6.53	152.7
B7	Thick	White	No odor	7.53	165.7
B8	Semi liquid	White	No odor	6-7	78.2
B9	Semi liquid	White	No odor	7-8	132.6

Antimicrobial Activity Result

The results of lotion antimicrobial activity during storage period could be seen in Table 7. Based on the data in the table, the higher concentration of extracts added to the lotion

formula, the greater the diameter of the inhibit zone produced. However, after 28 days of storage, the antimicrobial activity of the preparations was decreased. This was thought to be due to the interaction between excipients and active compounds from red *P. betle* leaf ethanolic extract, and storage time that could decreased the activity of extracts present in the preparation.

Efficacy of Antimicrobial Result

The effectiveness of antimicrobial lotion test was done to determine the microbial decreased on the volunteer fingers before and after using lotion. The value of decreasing colony on each formula could be seen in Tables 8 and 9.

DISCUSSION

Screening Phytochemical

The screening results were consistent results, compared with the previous study. Allegedly among the groups of these compounds had antimicrobial activity against microbes. The

Table 5: Globul size value

Formula	Globul size (µm) per cycle						
	0	1	2	3	4	5	6
F0	26.50	27.23	27.76	28.64	29.05	28.69	27.85
F1	37.53	38.21	37.74	38.00	38.19	38.53	38.04
F2	38.66	40.84	41.44	41.91	42.24	42.30	42.32
F3	37.70	39.64	40.28	40.83	40.88	40.69	41.83

Table 6: Microbial contamination on lotion

Formula	Bacterial contamination (cfu/g)			Fungal contamination (cfu/g)		
	K1	K2	K3	K1	K2	K3
	F0	-	-	-	-	-
F1	-	-	-	-	-	-
F2	-	-	-	-	-	-
F3	-	-	-	-	-	-

K1: Sample concentration: 10%, K2: Sample concentration: 1%,
K3: Sample concentration: 0.1%

alkaloids possess the ability to act as antibacterial by interfering with the peptidoglycan component of the bacterial cell so that the cell wall layer is not completely formed and causes the cell's death.^[18] According to another study, alkaloids were compounds that have antimicrobial activity, which inhibits esterase, DNA and RNA polymerase, and also inhibit cell respiration and play a role in DNA intercalation.^[19] Alkaloids also reported as an active substances from plants that serve as powerful drugs and activators for immune cells that destroy bacteria and fungi.^[20] Meanwhile, phenol derivatives interact with bacterial cells through an adsorption process involving hydrogen bonds. At low levels form a complex of phenol proteins with weak bonds and immediately decomposes, followed by penetration of phenol into cells and causes precipitation and protein denaturation. High levels of phenol cause coagulation of proteins and membrane cells undergoing lysis.^[21] Flavonoids act as antibacterial by forming complex compounds against extracellular proteins that interfere with the integrity of bacterial cell membranes.^[22] The antibacterial effects of tannins are, among others, through reaction with cell membranes, enzyme inactivation, and destruction or inactivation of genetic material functions. Essential oils act as an antibacterial by interfering with the process of membrane formation or cell wall, so it is not formed or formed imperfectly.^[23]

MIC Test

The MIC value of the extract against tested microbes were as follows: *S. aureus* (2.5–5% w/v), *E. coli* (2.5–5% w/v), *P. aeruginosa* (2.5–5% w/v), and *C. albicans* (1.25–2.5% w/v). Based on those data, it was concluded that 2.5% w/v could be used as MIC concentration for reference dose in antimicrobial lotion of the extract.

Formulation of Lotion

Based on the observations, the B6 and B7 bases had a thick form in accordance with the requirements of the lotion and had a pH suitable for the skin. Therefore, both lotion base must be selected to obtain the best formula. The two bases were then tested for the separation of the emulsion phase using centrifugation method at 3750 rpm for 5 h. Based on the centrifugation test results, the B6 losio base did not separate while the B7 base separated. Hence, it could be concluded that the B6 base formula had the best homogeneity among other base formulas and could be used in an antimicrobial lotion formulation. The formulation was carried out using the B6 base formula with the addition of three variations of ethanol extract concentration as follows: 5%, 7.5%, and 10% w/v. The optimization result of the mixing method indicated that the method of A2 yielded an unbiased consistency of antiseptic losio compared to the result of method A1. Thus, the formulation of the three antiseptic lotion formulas based on active red *P. betle* leaf extract was done using A2 mixing method.

Physical Evaluation

Based on organoleptic results in terms of color, the formulas F1 and F2 did not give a color that was too thick compared to F3. Thus for its use as a skin lotion, the formula F1 and F2 did not cause skin discoloration too much. The addition of white dye was one of the solutions to produce an attractive antimicrobial lotion preparations. Thus, it was necessary to optimize the variation and concentration of the white dyestuff effectively coloring but not to decrease the effectiveness of the antimicrobial lotion preparation. While in terms of odor, the three formulas provide a distinctive odor of red betel leaf. For more optimal results, it was also necessary to optimize the variation of fragrance concentrations that could cover up the natural scent of the extract. When viewed in terms of shape, F1 and F2 produced an adequate consistency in the ease of dispersion of the active ingredient on the skin.

During the storage period of time, viscosities of all formulas were tend to increase at 8°C and 25°C of storage temperatures. Losio F0 had highest increased on viscosity during storage at 8°C. While during storage at 40°C, the viscosity of the four losio was tend to decrease. The differences of viscosity value were probably thought to be caused by the influence

Table 7: Diameters of inhibitory zone

Formula	Day	Diameters of inhibitory zone (mm) against tested microbial			
		<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
F0	1	0	0	0	0
	28	0	0	0	0
F1	1	11.18	12.20	11.37	11.80
	28	11.15	12.17	11.32	11.75
F2	1	11.20	12.37	11.40	11.80
	28	11.18	12.35	11.37	11.78
F3	1	11.24	12.44	11.43	11.84
	28	11.23	12.42	11.40	11.83

E. coli: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *C. albicans*: *Candida albicans*

Table 8: The results of efficacy test of antimicrobial lotion against bacteria

Volunteer	F0			F1			F2			F3		
	A	B	C	A	B	C	A	B	C	A	B	C
1	5	5	0	2	1	1	23	3	20	3	2	1
2	6	6	0	1	1	0	17	3	14	24	12	12
3	8	13	-5	13	7	6	5	2	3	10	2	8
4	5	17	-12	8	5	3	25	7	18	19	2	17
5	6	10	-4	6	1	5	8	3	5	12	11	1
6	6	4	2	4	1	3	89	38	51	16	1	15
7	1	3	-2	2	1	1	8	2	6	12	1	11
8	18	1	17	3	1	2	10	2	8	16	4	12
9	11	8	3	3	1	2	25	2	23	10	0	10
10	5	1	4	7	5	2	36	1	35	7	5	2

A: Before the use of losio, B: After the use of losio, C: Decrease effect

Table 9: The results of efficacy test of antimicrobial lotion against fungal

Volunteer	F0			F1			F2			F3		
	A	B	C	A	B	C	A	B	C	A	B	C
1	1	1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	1	0	1	0	0	0	0	0	0
3	1	2	-1	0	0	0	1	0	1	1	0	1
4	0	0	0	0	0	0	0	0	0	2	1	1
5	0	0	0	0	0	0	0	0	0	1	0	1
6	1	4	-3	1	0	1	0	0	0	0	0	0
7	0	0	0	1	0	1	1	0	1	0	0	0
8	3	2	1	0	0	0	0	0	0	0	0	0
9	0	0	0	2	1	1	4	0	4	1	0	1
10	0	0	0	1	0	1	1	0	1	0	0	0

A: Before the use of losio, B: After the use of losio, C: Decrease effect

of temperature, pH, and globul. Differences of the viscosity value of each losio formula were calculated statistically with the complete design rundown factorial design at the 0.05 ($\alpha = 0.05$) confidence level, the first hypothesis 0 (H_0) was assumed that there was no difference in the viscosity value the real between the formula one with the other. The result of statistical analysis could be seen in Table 4. The second

null hypothesis (H_0) assumed that there was no viscosity change visible during storage time. The third null hypothesis (H_0) assumes that there is no apparent change in viscosity value between the storage temperature of one another. As a result, the first and second H_0 was accepted because the significance value of the effect of the formula and the length of storage on the value of the viscosity of the preparation

was >0.05 with a 95% confidence level. While the third H_0 was rejected because the significance value of the effect of storage temperature on the viscosity of the dosage was <0.05 with 95% confidence level. This shows that there was no significant difference in viscosity value between the formula one to the other during storage, but there was a significant change in viscosity value between the storage temperature one with the other. Furthermore, the effect of storage temperature was tested further using Duncan's advanced test with 95% confidence level to know whether or not there was any similarity between test treatments in more detail. Based on Duncan's advanced test, each storage temperature gave a different effect on the viscosity value of the preparation. Good lotion had viscosity with a range of 500–5000 cP. [24] Thus, in spite of the change in viscosity value in the antiseptic lotion preparation, however, the viscosity value of the preparation during storage at three different temperatures still falls into the required range of viscosity lotion.

Based on the data in Table 6, it was known that the addition of ethanol extract of red *P. betle* leaves in different concentration on the lotion formula caused globul size increasing. The presence of a significant increased in temperature and a significant decreased in temperature during the storage cycle caused the bonding between the particles to stretch and tends to form larger sizes. Although the size of the globule in the freeze-thaw cycle was increased, the physical quality of the losio was still good because the size of the globule formed was still within the emulsion size range, which was 1–100 μm . [25] The change of globul size value of each losio formula was calculated statistically with randomized block design at 95% confidence level ($\alpha = 0,05$). The first hypothesis (H_0) assuming there was no significant difference in globule size values between the formula one to another and the second H_0 hypothesis assuming there was no change in real globule size values during the storage cycle. The result of the first and second H_0 was accepted because the significance value of the effect of the formula on the globular size was >0.05 with a 95% confidence level. This showed that there was no significant difference in globule size values between the formula one to another and during the freeze-thaw storage cycle. A stable lotion should withstand at least six freeze-thaw cycles. Based on statistical analysis, all four antiseptic lotions could be said to be stable because they have globule size values that were not significantly different for 6 storage cycles. [16]

In addition, the observation of phase separation of losio was done to evaluate the stability of lotion by centrifugation method at different centrifugation speed. The results indicated that the centrifugation with different speed did not cause the separation of phase lotion. The use of the centrifugation method in viewing the emulsion phase separation was intended to predict the shelf life of the preparation. A stable emulsion system showed no phase separation by centrifugation at a speed of 2000–3000 rpm. No 5-h phase separation at a speed

of 3750 rpm indicated that the preparation was stable for a year at room temperature storage. [16]

Chemical Evaluation

The resulting lotion was tend to be slightly acidic neutral. The addition of red betle leaf ethanol extract was decreasing the pH value of lotion. This was because the pH of the red betel leaf ethanol extract was slightly acidic, between 5 and 6. From the figure, it could also be seen that pH changes tend to increase over a 28-days storage time at 8°C and 25°C storage temperatures, but tend to decrease over time 28 days storage at 40°C storage temperature. Based on the result of statistical analysis with RBSL at $\alpha = 0,05$, showed that there was no significant difference in pH values during storage time, but there was a significant difference in pH values between the formula one to another and the storage temperature of one another. Based on Duncan's analysis as further test, it could be concluded that all storage temperatures had different effects on the pH value of the lotion. The pH value of F3 was not significantly different from the pH value of F2, but it was significantly different from F0 and F1. The pH value of F1 was significantly different from the pH values of the other formulas. Likewise, the pH value of F0 was significantly different from the pH value of other formulas. Although the pH value of the lotion preparation tends to change, the pH value of the lotion preparation still falls within the required range of pH lotion.

Antimicrobial Efficacy Test

The whole formula showed the highest antibacterial efficacy against *S. aureus*. It is known that *S. aureus* is a Gram-positive bacteria which has thick cell walls; therefore, *S. aureus* will be more resistance than Gram-negative. However, in this study, the lotion containing red *P. betle* leaves extract, instead, produced a stronger antibacterial power against *S. aureus*, compared with another tested bacteria which was Gram-negative bacteria. Even these lotions were able to inhibit the growth of *C. albicans* stronger than the inhibitory effect to tested bacteria. The possess of antimicrobial activity of the lotions were suggested due to the phytochemical content of red *P. betle* leaves. The same secondary metabolites in the red *P. betle* extract were found the same metabolites content in methanol and n-butanol extracts of *C. dactylon* leaves which reported have potential antibacterial and antifungal activity, particularly against the same tested microorganism such as *Staphylococcus*, *E. coli*, *Pseudomonas*, and, *Candida* sp. [26]

Based on statistical analysis of Anova test, the value of significance of formula = 0.00 with significance value <0.05 , it was concluded that there was a difference of the amount of significant microbe decrease between formula one with another. As further analysis, Duncan test was done to determine whether there was equality between test treatments in more detail (to determine the effect of the formula on the

amount of microbial decline in further). Based on further tests of Duncan, it was known that lotion with the formula F0, F1, and F3 were not significantly different, but significantly different from F2. Based on further tests Duncan also known that F0 did not give effect to the amount of microbial decline and F2 gave the higher effect on the microbial decline. Therefore, it could be concluded that the lotion that gave the best antimicrobial effectiveness was formula F2.

CONCLUSION

The F2 lotion formula had good pharmaceutical properties and potential antimicrobial activity.

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