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Formulation and Physical Stability Test Preparation of Anti-Acne Gel Extract of Tebu Leaves (Saccharum officinarum (Linn) as Antibacterial Against Staphylococcus Epidermis

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ABST RACT

Increased processing of Tebu as sugar causes the resulting waste, namely the shoots of Tebu leaves. The phenolic and flavonoid compounds in Tebu are reported to have strong antioxidant, anti-inflammatory, antimutation and tyrosine inhibitor activities. Infectious diseases are diseases caused by the presence of pathogenic microbes, one of which causes infection, namely bacteria. Utilization of Tebu waste is expected to produce alternative products in the form of antibacterial preparations, especially anti-acne. Staphylococcus epidermis is one of the bacteria that causes infection on skin with acne. This study aims to determine the physical stability of the preparation Tebu leaf extract anti acne gel and its antibacterial activity against Staphylococcus Epidermis bacteria. Methods: This research design is an experimental method, making Tebu leaves extract by weighing as much as 400 grams of fine powder of Tebu leaves then put in a maceration container, closed then added 70% 2 liters of ethanol until completely submerged and stored for 24 hours protected from direct sunlight while stirring occasionally. Then filtered, separated the dregs and filtrate. The dregs were re-extracted with the same amount of new 70% ethanol for 2 days. The obtained 70% ethanol filtrate was evaporated to obtain a thick extract using a Vacuum Rotary Evaporator. Tebu leaves extract gel was made at a concentration of 5%, 10%, 15% and an antibacterial test was carried out using the well diffusion method. Results: The results of the physical stability test of the acne extract cream showed that the results met the requirements both in terms of pH, dispersion test, homogeneity. The results of the antibacterial test of Tebu leaves extract showed the presence of a zone of bacterial inhibition which was indicated by the presence of a clear area around the well. Conclusion: The results of the evaluation showed that the Tebu leaves extract gel met the requirements for the physical quality of the gel preparation, both organoleptic, pH, viscosity, adhesion, and spreadability. Tebu leaves extract gel was able to inhibit the growth of S. epidermis bacteria with the best concentration in 3rd formula, consentration 15% Tebu leaves extract gel.

I. Introduction

Tebu (Saccharum officinarum (Linn.) is a plant that can be processed into sugar. Tebu leaves extract contains phenolic acid and flavonoid aglycone tricin (5,7,4-trihydroxy-3,5-dimethoxyflavone) with cytotoxic activity and potential to kill cancer cells in the body. (CLSI. 2012). Flavonoids have antibacterial activity of Staphylococcus aureus, E. coli, Styphimurium and bacillus cereus, the mechanism of action of antibacterial flavonoids is by inhibiting nucleic acid synthesis, disrupting cell membrane function. (Feng, D.,Z. et. al. 2014). The antibacterial

activity of Tebu leaves extract has shown the potential to inhibit Bacillus sublitils and Escherichia coli bacteria. (Balouri, M. 2016). Tebu (Saccharum officinarum (Linn.) is a plant that can be processed into sugar. Tebu leaves extract contains phenolic acid and flavonoid aglycone tricin (5,7,4-trihydroxy-3,5-dimethoxyflavone) with cytotoxic activity and potential to kill cancer cells in the body. (CLSI. 2012). Flavonoids have antibacterial activity of Staphylococcus aureus, E. coli, Styphimurium and bacillus cereus, the mechanism of action of antibacterial flavonoids is by inhibiting nucleic acid synthesis, disrupting cell membrane function. (Feng, D.,Z. et. al. 2014). The antibacterial activity of Tebu leaves extract has shown the potential to inhibit Bacillus sublitils and Escherichia coli bacteria. (Balouri, M. 2016).

II. Methods

Materials

Tebu leaves, 70% alcohol, Carbopol 940, Propilenglycol, Trietanolamin, Propil Paraben, Methyl Paraben, aquadest. Nutrient Agar (NA) media, Nutrient broth Agar (NB), Staphylococcus epidermis (S.epidermis) bacteria obtained from the Microbiology Laboratory of IIK STRADA Indonesia Kediri, Clindamycine Gel, H2SO4 0.36 N, BaCl2 .2H2O 1.175%, Nacl 0.9%, filter paper, label paper, aluminum foil, TLC.

Setup Simplicity

Maceration method, is a simple filtration by immersing simplicia powder in a liquid filter that will penetrate the cell wall and enter the cell cavity containing the active substance. The active substance will dissolve because of the difference in concentration between the solution and the active substance inside the cell and outside the cell, so the concentrated solution is pushed out. This event can be done repeatedly until the concentration balance between the solution outside the cell and inside the cell. (Hasanuddin, H., & Purba, E. 2016).

Making Extract

Making Tebu leaves extract by weighing as much as 400 grams of fine powder of sugarcane leaves then put in a maceration container, closed then added 70% 2 liters of ethanol until completely submerged and stored for 24 hours protected from direct sunlight while stirring occasionally. Then filtered, separated the dregs and filtrate. The dregs were re-extracted with the same amount of new 70% ethanol for 2 days. The obtained 70% ethanol filtrate was evaporated to obtain a thick extract using a vaccum rotary evaporator. Tebu leaves thick extract is then formulated into gel preparations according to the formulations listed in the table. Tebu leaves extract cream was made with 3 formulas with a gel weight of 100g. Formulas I, II, and III with various extract concentrations of 5%, 10%, and 15%.

Preparation of Test Bacterial Suspension

The equipment used in antibacterial test study was sterilized first. Glass utensils were sterilized in an oven at 170^{o}C for 24 hours, ose needles and tweezers were sterilized by burning directly using a spirit bender fire, while the media were sterilized using an autoclave at 121^{o}C for 15 minutes. Basic medium was made by weighing 6.50 grams of NA, dissolved in 500 ml of distilled water, then mixed until homogeneous and heated to boiling. After dissolved and homogeneous, the media was sterilized by autoclaving at 121^{o}C for 15 minutes, then cooled. Preparation of a test bacterial suspension by taking 1 sterile ose of bacterial colonies mixed with NaCL solution in a test tube and homogenized with a vortex mixer then compared with a 0.5 Mc.Farland solution which is equal to 1.5 x 108 bacteria.

Formulation of Tebu Leaves Extract Gels

Tebu leaves extract gel was made with 3 formulas with a gel weight of 100 grams. Formulas I, II, and III with various extract concentrations of 5%, 10%, and 15%.

Table 1st. Formulation of Tebu leaf extract gel

Material Name	**Quantity of ingredients (%)**		
	F1	**F2**	**F3**
Tebu leaves extract	5	10	15
Carbopol 940	0.5	0.75	1
Propilenglycol	10	10	10
Trietanolamin	2	2	2
Propil Paraben	0,02	0,02	0,02
Metil Paraben	0,075	0,075	0,075
Aquadest (ad)	100	100	100

Carbopol 940 was developed with 70°C distilled water, stirred until swelled. then Trietanolamin is mixed in the base and then homogenized. Methyl paraben and propyl paraben were added which had previously been dissolved in 3 ml of distilled water at 90°C, homogenized. Added ethanol extract of Tebu leaves (Saccharum officinarum L.) to propylene glycol, then put into the base little by little until the remaining water is used up and homogeneous.

Antibacterial Activity Test

The antibacterial activity was tested by inserting 15 ml of NA in a petri dish and then adding 0.5 ml of suspension of S. aureus and S. epidermis bacteria inside and waiting until solid. After the solid media, bore holes according to the number of test preparations, then the test preparations are placed according to the marks on the wells and incubated at 37^{o}C for 24 hours and observed. Observations were made after 1x24 hours of bacterial incubation, the results of antibacterial activity were based on the measurement of the Diameter of the Inhibitory Area (DDH) of bacterial growth formed around the hole or well, the measurement was made from the bottom of the petri dish with a caliper in millimeters (mms)

Data Analysis

The results of the antibacterial test of Tebu leaves extract gels on the diameter of inhibition zone for the growth of S. epidermis bacteria were analyzed using the One way ANOVA method with Statistical Services Solution (SPSS 24). Carried out by the Tukey method. The test aims to determine the difference between the gels formulations (F1, F2, F3) and the positive control. The existence of a significant difference in the test was marked by the value of $p<0.05$.

III. Results and Discussion

The use of 70% ethanol containing water in the maceration process can help the process of wetting and penetration of the solvent in the simplicia, where the cells in the Tebu leaves simplicia will expand making it easier for the liquid to bind the compounds contained therein. The maceration results were separated and put in a rotary evaporator until a thick extract of 71.24 grams was obtained. The results of organoleptic examination of the thick extract of katuk leaves were greenish brown. After conducting preliminary tests of secondary metabolites from Tebu leaves extract, it is known that Tebu leaves extract contains flavonoid, phenolic, alkaloid and saponin compounds. The organoleptic results of Tebu leaves extract gel from the three formulas were odorless, soft semi solid, brownish in color. Tebu leaves extract gel preparations produced homogeneous preparations and the preparations obtained were somewhat transparent. The results of the evaluation of pH, viscosity, spreadability, adhesion and stability of the cream can be seen in table 2.

Table 2nd. Evaluation Results of Tebu Leaves Extract gels

Evaluation	Average Evaluation Results		
	F1	**F2**	**F3**
pH	4.8	5.9	6.4
Viscosity (cP)	4.5	4.3	4.4
Spreadability (cm)	6.5	6.3	5.9
Adhesion (s)	3.6	3.7	4.0

Antibacterial testing used a negative control using a gel base without Tebu leaves extract and a positive control, namely Clindamycin gel which had antibacterial activities. The results of the measurement of the diameter of the inhibition zone of sugarcane leaves extract gels against S. epidermis can be seen in table 3.

Table 3rd. Results of Measurement of Inhibitory Zone Diameter of Tebu LeavesExtract gels.

Concentrations	Diameter of Bacterial Growth Inhibitory Zone (mm) *Stapylococcus epidermis*			
	I	II	III	Average
5%	1.41	1.12	1.44	1.32
10%	1.43	1.64	1.50	1.52
15%	2.54	2.48	2.72	2.58
Positive Control	12.16	11.87	9.2	11.08
Negative Control	0	0	0	0

The results of diameter bacterial inhibition zone in three Tebu leaves extract gel formulations compared to the positive control illustrates the presence of antibacterial activity but the results of the antibacterial activity test are still lower. The three Tebu leaves extract gel formulas were compared with negative controls that did not contain antibacterial active ingredients, indicating that the three Tebu leaves extract gel formulas showed anti-bacterial activity against S. epidermis. The antibacterial activity test of the three formulations showed that the highest antibacterial activity was the third formulation with a concentration of 15% Tebu leaves extract.

The results analysis of normality test showed that data were not normally, distributed ($p<0.05$), followed by the homogeneity test and the significant results showed that it was not homogeneous ($p<0.05$) while the significant results showed that there were significant differences in the treatment given. distributed ($p<0.05$), followed by the homogeneity test and the significant results showed that it was not homogeneous ($p<0.05$) while the significant results showed that there were significant differences in the treatment given.

IV. Conclusion

1. The results of the research on the formulation of an anti-acne gel, Tebu leaves extract (Saccharum officinarum (Linn) as an antibacterial for Staphylococcus epidermis, it can be concluded that: quality requirements of the gels preparation, both organoleptic, pH, viscosity, adhesion, and spreadability.
2. Tebu leaves extract gels has antibacterial activity against S. epidermis with the best concentration in the third formulation with a Tebu leaf extract concentration of 15%.

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