



Best Formulation Antibacterial Cream From 96% Ethanol Extraction Green Betel (*Piper Betle Linn.*)

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Abstract

Cream is an emulsion preparation with a water content of at least 60% and used for external application. In the manufacture of cream, active ingredients from green betle leaves one of nature ingredient for making cream. Green betel leaves are reported to contain flavonoids that can inhibit bacterial activity. This study aims to identify the best formula for a cream containing 96% ethanol extract of green betel leaf as the active ingredient by varying the concentration of the active ingredient, applying several stages of the cream preparation process, followed by testing the preparation, namely organoleptic testing, homogeneity testing, pH testing, viscosity testing, spreadability testing, adhesion testing, and cream type testing using the colour and dilution methods. The research result showed that the best formulation was formulation 3 with an extract content of 9, because all tests on formula 3 meet the standard

Keywords: Green Betel Leaf (*Piper Betle Linn.*), Maceration, Cream

Introduction

In order to reduce the adverse effects of chemical active ingredients in creams is to use natural or plant-based active ingredients, as these are derived from a natural

or herbal sources is used in cream. Research conducted by Sabir (2005) suggests that the use of natural ingredients can minimise the negative effects of chemicals on the body. One natural ingredient with potential for development in this cream formulation is green betel leaf (*Piper betle L.*). Betel leaf contains several main components, such as phenols, tannins, and flavonoids, which have very dominant antioxidant, antibacterial, and anti-inflammatory properties (Nurdianti et al., 2022). The method for making green betel leaf extract is to use the maceration method with 96% ethanol solvent (Aini et al., 2024). Maceration is a simplisia extraction technique carried out for materials or simplisia that are not heat-resistant by soaking them in a certain solvent for a certain period of time. 96% ethanol solvent is a polar compound that is highly volatile, making it suitable for use as an extraction solvent (Aini et al., 2024). Betel leaf extract obtained using 96% ethanol solvent has shown significant antibacterial activity. Therefore, the formulation of green betel leaf extract into topical preparations such as creams can be a practical and effective solution to utilise its potential as an antibacterial agent (Budiman et al., 2018).

The formulation of a cream preparation from betel leaf extract with varying concentrations of active ingredients aims to determine the optimal concentration that can provide maximum antibacterial benefits without reducing the physical stability of the cream. Proper formulation is important to ensure that the resulting cream has stable physical characteristics, such as pH, viscosity, homogeneity, and good spreadability, so that it is safe for use on the skin (Zakeri et al., 2022).

Material and Methods

Tools And Materials

3 L maceration vessel, 500 mL and 250 mL glass beakers (*Pyrex*), analytical balance (*Centaurus scale*), blender (*Cosmos*), rotary evaporator (*IKA RV 3 V*), parchment paper, 50 g, 100 g, 150 g, 200 g, 250 g weighing pans, aluminium foil, Petri dishes, stirring rods, turtle spoons, mortars and pestles, droppers, water bath (*Thremost HH-6*), porcelain dish (*Pyrex*), universal pH indicator (*Mquantity*), flannel cloth, filter paper, spatula, horn spoon, cream pot, microscope (*Monocular xsp-12*), viscometer (*NDJ-01*), No. 45 mesh sieve. Fresh green betel leaves, 96% ethanol,

stearic acid, triethanolamine, cetyl alcohol, methyl paraben, propyl paraben, propylene glycol and distilled water.

Research Procedure

1. Sample Preparation

The sample used was betel leaf (*Piper betle* Linn.) obtained from Morocalan Village, Lamongan Regency. Three kilograms of green betel leaves were washed using running water, then drained. Next, the leaf was chopped into small pieces to reduce their size and facilitate the drying process. The leaves were then dried in an oven at 50°C (Mulangsri, 2018). The dried green betel leaves were then ground into powder using a blender, and the powder was sieved using a No. 45 mesh sieve (Opod et al., 2024).

2. Ekstrakt Production

Green betel leaf powder was weighed at 250 g and then extracted using the maceration method (Zahra et al., 2024). The solvent used was 96% ethanol with a ratio of 1:6 between the herb and the solvent. The maceration process was carried out for 3 days, with stirring for 5 minutes 3 times a day. Maceration took place at room temperature in a tightly sealed maceration vessel lined with aluminium foil. After 3 days, filtration was carried out using flannel cloth, then the resulting filtrate was evaporated using a rotary evaporator at a temperature of 50°C. The liquid extract was then evaporated in a water bath at a temperature of 50°C until a thick extract was formed (Kurniadi et al., 2024).

3. Cream Formulation

The cream was made in 3 variation concentration of betel leaf as show in tabel 1 formulas differentiated by the concentration of betel leaf extract (*Piper betle* Linn.): F1 with a concentration of 3%, F2 with a concentration of 6% and F3 with a concentration of 9%. Each preparation was made in 30 gram batches with the same base (Vifta et al., 2017).

Table 1. Green Betel Leaf Extract Cream Formula (*Piper Betle L.*)

Materials	Function	Formula (%)		
		Formula 1	Formula 2	Formula 3
Ethanol extract of green betel leaves	Active ingredient	3%	6%	9%
Stearic acid	Emulsifier	16%	16%	16%
Triethanolamine	Emulsifier	2%	2%	2%
Cetyl alcohol	Thickening agent	4%	4%	4%
Metyl paraben	Preservative	0,18%	0,18%	0,18%
Propyl paraben	Preservative	0,02%	0,02%	0,02%
Propilene glikol	Humectant	25%	25%	25%
Distilled water	Solvent	Ad 100 mL	Ad 100 mL	Ad 100 mL

Cream production

The cream production process begins with making a cream-type base (M/A). The prepared base consists of two phases, namely the oil phase and the water phase. The oil phase, consisting of stearic acid and cetyl alcohol, is placed in a porcelain bowl, propyl paraben is added, and then melted in a water bath at 70°C. The water phase, consisting of TEA, propylene glycol, methyl paraben, and distilled water, is placed in a glass beaker and heated to 70°C. The melted oil phase is poured into a warm mortar and stirred until homogeneous. The water phase is added little by little while stirring slowly until a cream mass is formed. The thick extract of green betel leaves is added to the cream mass little by little and stirred until homogeneous. Once the cream is homogeneous, it is then placed in a cream box.

Physical Quality Evaluation of Cream Preparations

The evaluation test were conducted from the first day to the seventh day. The evaluation of the physical quality of the cream preparation included organoleptic testing, homogeneity, pH, viscosity, spreadability, adhesiveness and cream type testing using dilution and colour methods.

Results and Discussion

Sample Preparation

The green betel leaves used come from Morocalan Village, Glagah Subdistrict, Lamongan Regency. The wet weight of the green betel leaves is 3 kg, and after drying in an oven, the weight of the green betel leaves is 255 grams. The purpose of drying is to remove moisture content that can cause bacterial growth or decay during storage (Haryanti et al, 2020). The green betel leaves are extracted using the maceration method with 96% ethanol solvent.



Figure 1. Sample Preparation 1. Green betel leaves, 2. Oven drying, 3. Green betel leaf powder

Extraction

The extraction process used in this study employed the maceration method. The solvent used was 2.5 L of 96% ethanol, which is polar in nature. The maceration process was carried out for 3 days with stirring for 5 minutes 3 times a day. The purpose of stirring is to achieve a faster equilibrium in the concentration of the extracted material in the solvent (Tutik et al., 2022). Maceration took place done at room temperature in a tightly sealed maceration vessel lined with aluminium foil to prevent the sample from coming into contact with light (Candra et al., 2023). After 3 days, filtration is carried out using flannel cloth. This is done to separate the pulp from the macerate (Kurniadi et al., 2024).

The next stage is the evaporation process, which aims to evaporate the solvent contained in the extract (Siskawati et al., 2023) using a rotary evaporator and water bath. From the results of the rotary evaporator at a temperature of 50°C with 40 rpm and an evaporation time of 1 hour, 20 mL of liquid extract was obtained. It was then evaporated again using a water bath at a temperature of 50°C, yielding 5.4 grams of thick extract with a yield of 2.16%. Organoleptically, the

result result was a thick extract, blackish green in colour, and with the distinctive smell of green betel leaves



Figure 2. Maceration process until a thick extract is obtained

Results of the Evaluation of 96% Ethanol Extract Cream Preparation from Green Betel Leaves

Evaluation of green betel leaf extract cream was conducted to compare three formulations with different concentrations of 96% ethanol extract, namely F1 (3%), F2 (6%), and F3 (9%). The tests conducted included organoleptic tests, homogeneity, ph, viscosity, adhesive strength, spreadability, and cream type through colour and dilution methods. The cream tests were conducted on day 0 and day 7 to assess the stability and physical changes in the cream preparations before and after storage (Indriani & Tari, 2023).

1. Organoleptic Test

Organoleptic testing was conducted by observing the shape, colour, and smell of the green betel leaf extract cream on day 0 and day 7. The results showed that all cream formulations had the characteristic smell of betel leaves and a semi-solid shape. The addition of betel leaf extract at different concentrations affected the colour and aroma of the cream; the higher the extract concentration, the more intense the colour of the cream (Indriani & Tari, 2023). However, during storage for 7 days, the colour changed from green to brownish due to oxidation. This change was caused by the absence of antioxidant excipients in the formulation to prevent damage from free radicals (Purwaningsih *et al* 2014).

Table 2. Organoleptic Test Results for Green Betel Leaf Extract Cream (*Piper Betle Linn.*)

Day	Formula	Colour	Smell	Texture
0	FI	Bright green	Characteristic of betel leaf	Soft
	FII	Pale green	Characteristic of betel leaf	Soft
	FIII	Brownish green	Characteristic of betel leaf	Soft
7	FI	Bright brown	Characteristic of betel leaf	Soft
	FII	Pale brown	Characteristic of betel leaf	Soft
	FIII	Greenish brown	Characteristic of betel leaf	Soft

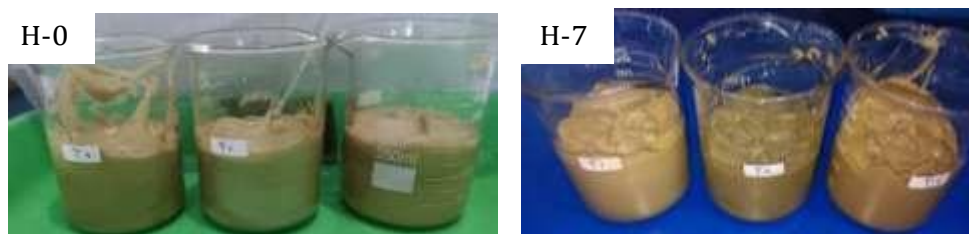


Figure 3. Results of cream preparation on day 1(1) and day 7(7)

2. Homogeneity Test

Homogeneity testing was conducted to ensure that all components of the cream, including the active ingredients, oil phase, and water phase, were evenly mixed (Indriani & Tari, 2023).

Based on the homogeneity test results, cream preparations F1, F2, and F3 were declared homogeneous if no fine particles were found, they had a uniform colour, and they were free from black spots when observed under a microscope (Sugiyono et al., 2012 in Istiqomah & Azzahra, 2020). The examination results showed that all three cream formulas met the requirements for good homogeneity. If the cream is not homogeneous, it means that the formula is not evenly distributed, so the therapeutic effect is not optimal and may cause coarse particles in the preparation (Indriani & Tari, 2023).

Table 3. Results of homogeneity testing of green betel leaf ethanol extract cream (*Piper Betle Linn.*)

Day-	Formula	Conclusion
0	1	homogeneous
	2	homogeneous
	3	homogeneous
7	1	homogeneous
	2	homogeneous
	3	homogeneous

3. pH Test

pH testing is an important parameter in topical preparations because it is related to effectiveness and comfort when used on the skin (Indriani & Tari, 2023).

The pH test results for all three formulas, both on day 0 and day 7, showed the same pH range and no changes. All cream preparations met the skin pH standard of 4.5–6.5. In topical preparations, the pH should not be too acidic as this can because it cause irritation, nor should it be too alkaline as this can cause the skin to become scaly (Indriani & Tari, 2023).

Table 4. Results of pH testing of green betel leaf ethanol extract cream (*Piper Betle Linn.*)

Formula	pH requirements	pH on day-0	pH on day-7
I	4,5-6,5	6	6
II	(Deniansyah & Pujiastuti, 2022).	6	6
III		6	6

4. Viscosity Test

Viscosity testing using an NDJ viscometer aims to determine the thickness of the cream so that it is easy to apply and remove from the container. The ideal viscosity value for cream ranges from 2,000 to 50,000 cPs (Deniansyah & Pujiastuti, 2022).

Based on the results, all three cream formulas tested had viscosities within this range, namely formula 1 at 4,600 cPs, formula 2 at 4,700 cPs, and formula 3 at 4,750 cPs. The viscosity increased with the concentration of betel leaf extract, causing the cream to become thicker and more adhesive (Indriani & Tari, 2023). In addition, the pH of the preparation also affected the viscosity; a higher pH tended to increase the thickness of the cream. Generally, viscosity and spreadability are inversely proportional (Muthoharah & Rianti, 2020). However, this study found a proportional relationship between viscosity and spreadability, possibly due to the difference in the timing of viscosity testing (day 0) and spreadability testing (days 0 and 7).

Table 5. Viscosity test results of green betel leaf ethanol extract cream (*Piper Betle Linn.*)

Formula (day on-0)	Viscosity requirements	Rot or	Spin dle	Coefficient	Reading result	Viscosity
I	2.000-50.000 cPs	60	4	100	46	4.600 cPs
II	(Deniansyah &	60	4	100	47	4.700 cPs
III	Pujiastuti, 2022).	60	4	100	47,5	4.750 cPs

5. Spread Test

Spreadability test was conducted to assess the softness of the cream mass so that its ease of application on the skin could be determined. A total of 0.5 g of cream was weighed and placed in the centre of an inverted Petri dish. A petri dish lid was placed on top, then gradually weighted using a weighing scale until it reached 50 g, 100 g, 150 g, 200 g, and 250 g. After being left for 1 minute, the diameter of the cream spread was measured constantly using a ruler (Deniansyah & Pujiastuti, 2022).

The spreadability test results showed that the F1, F2, and F3 formulations experienced a decrease in value from day 0 to day 7. However, all three cream formulations were still within the acceptable spread diameter range of 5–7 cm, making them easy to apply to the skin (Deniansyah & Pujiastuti, 2022). Low spreadability values can be influenced by high viscosity values (Indriani & Tari, 2023). Spreadability and viscosity have an inverse relationship: the lower the viscosity, the less resistance to spreading, thereby increasing spreadability, whereas the higher the viscosity, the greater the resistance, thereby decreasing spreadability and making the cream thicker (Suryani et al., 2019).

Table 6. Results of the spreadability test of green betel leaf ethanol extract cream (*Piper Betle Linn.*)

Formula	Spreadability requirements	Day on-	
		0	7
I	5-7 cm (Deniansyah & Pujiastuti, 2022).	5,68 cm	5,52 cm
II		5,76 cm	5,68 cm
III		5,78 cm	5,76 cm

6. Adhesion Test

This adhesion test aims to determine the time required for the cream to adhere to the skin. Good adhesion allows the cream to remain on the skin longer and prevents it from coming off easily, thereby producing the desired effect (Indriani & Tari, 2023).

The adhesion test results show that F1 has an adhesion time of 6.20 seconds, F2 has 8.62 seconds, and F3 has 10.56 seconds. From this data, it can be concluded that F3 has the longest adhesion time compared to F2 and F1. This is because adhesion is directly proportional to viscosity, where the higher the viscosity, the longer the cream adheres. All three formulas still meet the requirements for good adhesion, as all of them show a time of more than 4 seconds according to the literature (Endriyatno & Puspitasari, 2023).

Table 7. Adhesion test results of green betel leaf ethanol extract cream (*Piper Betle Linn.*)

Formula	Adhesion requirements	Observation results
I	>4 detik	06,20 second
II	(Endriyatno & Puspitasari, 2023).	08,62 second
III		10,56 second

7. Testing Cream Types Using The Colour Method

This test aims to determine the type of cream, whether it is oil-in-water (O/W) or water-in-oil (W/O), using methylene blue dye. If the methylene blue is evenly mixed, then the cream is classified as W/O type because the dye dissolves in water (Endriyatno & Puspitasari, 2023).

The test results show that all three formulations are O/W type creams. Under microscopic observation, methylene blue dissolves in the aqueous phase, and visually, the cream also appears to be evenly mixed with the methylene blue colour. This condition occurs because the volume of the dispersed phase (oil) is smaller than the dispersing phase (water), so that the oil globules are dispersed in the aqueous phase and form an M/A type emulsion (Maleh et al, 2024).

Table 8. Results of cream type testing using the colour method of green betel leaf ethanol extract cream (*Piper Betle Linn.*)

Formula	Conclusion
I	M/A
II	M/A
III	M/A

8. Testing Cream Types Using the Dilution Method

The method used was the dilution method, based on the principle that emulsions will mix with their external phase. If the cream cannot be diluted with water, it is classified as an oil-in-water (O/W) type, whereas if it can be diluted with water, it is classified as a water-in-oil (W/O) type (Mutaharah et al, 2024). The dilution test results for the three green betel leaf extract cream formulas showed that all preparations dissolved in 10 mL of distilled water, so it can be concluded that formulas I, II, and III are oil-in-water (O/W) emulsions. In

addition, in the washability test, all three formulas were easily and thoroughly cleaned. These findings are in line with the initial formulation objective, which was to produce a cream with an O/W emulsion type, which has advantages such as being easy to spread, easy to wash off, practical, and non-sticky (Idawati et al., 2024).

Table 9. Results of cream type testing using the dilution method of green betel leaf ethanol extract cream (*Piper Betle Linn.*)

Formula	Conclusion
I	Soluble in water
II	Soluble in water
III	Soluble in water

Conclusion

Based on the results of research into the manufacture of cream preparations from green betel leaf extract (*Piper Betle Linn.*), the best formula obtained was formula 3 with an extract concentration of 9%, which met several requirements of the preparation evaluation results. In formula 3, the results of the organoleptic test on the 7th day showed a greenish-brown colour, a distinctive betel leaf aroma, a homogeneous preparation, a pH of 6, and good spreadability and adhesion compared to formula 1 and formula 2.

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