

## FORMULATION AND TESTING OF ANTIBACTERIAL ACTIVITY OF FACIAL TONER PREPARATIONS CHINESE BETEL LEAF EXTRACT (*Peperomia pellucida* L. Kunth) AGAINST THE BACTERIA *Propionibacterium acnes*

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**Abstract:** Chinese betel leaf (*Peperomia pellucida* L.Kunth) is a herbal plant that can be used to treat abscesses, acne, boils and skin inflammation. Chinese betel leaves contain chemical compounds including alkaloids, flavonoids, saponins, tannins and triterpenoids which can inhibit the growth of *Propionibacterium acnes* bacteria. The Chinese betel leaf extract content can be used as a refreshing toner, especially for dry and acne-prone skin. The aim of this research is to determine the results of physical quality tests and antibacterial activity of Chinese betel leaf extract facial toner formulations with an additional active ingredient concentration of 7.5%; 15% and 30%. This type of research is experimental research with a descriptive research design. The organoleptic test results of the base are clear and odorless, while the other 3 formulas are brownish green in color, have a distinctive aroma of Chinese betel leaf extract and are in liquid form. The homogeneity test results of the preparation are homogeneous. F0 pH test results 6.327; FI 5.344; FII 5,243 and FIII 5,067. Viscosity test results F0 5.32 cps FI 6.34 cps and FII 6.33 cps. The results of the antibacterial activity test F0, FI, FII, and FIII were respectively 0.00 mm, there was no inhibition zone, 7.37 mm, 13.35 mm, 16.24 mm and in the positive control clindamycin it was 21.17 mm. Based on the results of the physical quality test and antibacterial activity of the toner preparation, the toner preparation has met existing quality standards so it is safe to use and the toner preparation with the strongest antibacterial activity is found in FIII.

**Keywords :** antibacterial activity test, toner, physical quality test, Chinese betel leaf extract

## INTRODUCTION

Acne is a skin disease that most commonly occurs in adolescence to adulthood (12-44 years) who have just experienced puberty, where the production of androgen hormones increases drastically and results in increased sebum keratin secretion.(Winarno & Ahnan, 2014). Acne is a condition where the pores become blocked and cause the oil glands to become inflamed. Apart from that, a face that is not clean is susceptible to health problems, both caused by excess oil gland production, hormonal factors and daily activities inside and outside the home. A disorder that often appears on facial skin is acne(Widiawati, 2014). The negative impact of acne can cause black spots on the face which is considered very annoying for some people. To reduce these skin problems, especially acne, it is necessary to provide skincare that functions as an exfoliant.(Ahda et al., 2022).

Acne can affect a person's quality of life by having a bad psychological effect on the way a person assesses, views and responds to their condition and situation(Wahdaningsih et al., 2014). The main organism that contributes to the occurrence of acne is *Propionibacterium acnes*. *Propionibacterium acnes* is the main microorganism found in the infra infundibulum area and this bacterium can reach the skin surface by following the flow of sebum(Indarto et al., 2019).

*Propionibacterium acnes* bacteria play a major role in the formation of acne by producing lipase which breaks down free fatty acids from skin lipids, resulting in tissue inflammation when in contact with the immune system.(Afifah, 2021).

Acne treatment is carried out by correcting follicular abnormalities, reducing sebum production, reducing the number of *Propionibacterium acnes* colonies or their metabolic products and reducing inflammation in the skin. The population of *Propionibacterium acnes* bacteria can be reduced by administering antibiotics such as erythromycin, clindamycin and tetracycline.(Marselia et al., 2015). Antibiotics can cause side effects, one of which is irritation and resistance, so you need to pay

attention to their use. Another alternative used to solve the problem of using antibiotics is using herbal plants which can minimize the side effects of using drugs(Rachmawaty et al., 2018). One plant that can be used for treatment is Chinese betel leaf.

Chinese betel leaf (*Peperomia pellucida* L. Kunth) is a herbal plant that can be used to treat abscesses, acne, boils and skin inflammation. Chinese betel leaves contain chemical compounds including alkaloids, flavonoids, saponins, tannins and triterpenoids which can inhibit the growth of *Propionibacterium acnes* bacteria.(Mufidah, 2021). Based on research(Yufiradani et al., 2020)Chinese betel leaves (*Peperomia pellucida* L. Kunth) have antibacterial activity against *Propionibacterium acnes* bacteria with the strongest inhibitory zone diameter at a concentration of 25% with an inhibitory power of 14.56 mm. One plant that can be used for cosmetics is Chinese betel leaf (*Peperomia pellucida* L. Kunth) as a facial toner preparation.(Mufidah, 2021).

*Toneris* a liquid preparation used as a facial cleanser and facial freshener. Toner is applied after using cleansing cream. Toner is used to perfect facial cleanliness(Khansa & Mardhiyah, 2017). There are three types of toner which are differentiated based on their alcohol content, namely astringent which is used for oily or acne-prone skin because it contains alcohol, face tonic which is used for normal skin types and freshener which does not contain any alcohol at all which is intended for dry and sensitive skin.(Muliawan, 2013). Facial toner can not only remove dirt or clean facial skin but can also remove excess oil on the face without drying out sensitive skin(Alviniari, 2019). There is a special use for facial toner, namely protecting the skin after the cleansing process because it is able to return the skin's pH to its natural position(Alviniari, 2019).

Based on the above background, researchers are interested in researching the formulation and testing of the antibacterial activity of Chinese betel leaf extract (*Peperomia pellucida* L. Kunth) facial toner preparations against *Propionibacterium acnes*

bacteria.

**METHOD**

The type of research used is laboratory experimental research. Laboratory experimental research is research that is used to find the relationship between the cause and effect of an independent variable and a dependent variable by implementing strict control over other variables that are likely to pollute or disrupt the relationship.(Sugiyono, 2016).

This research design was carried out by describing the collected data. Descriptive studies are the ability to describe or provide a picture of an object being studied using sample or population data without analyzing and drawing general conclusions. This research design was carried out using the disc diffusion method.

The materials used in this research include Chinese betel leaves, paper discs, black plastic ethanol 70%, H2SO4 1%, BaCl2 1.175%, polysorbate 80, glycerin, Na CMC, propylene glycol, distilled water, Nutrient Agar, NaCl 0.9% , buffer, Propionibacterium acnes bacteria.

The tools used in this research include a maceration container, rotary evaporator, porcelain cup, oven, dropper pipette, horn spoon, water bath, analytical balance, mortar and stamper, stirring rod, vernier caliper, toner container, autoclave, petri dish, Erlenmeyer , measuring cup, beaker, incubator, tweezers, micro pipette, tube rack, test tube, Laminar Air Flow (LAF), Bunsen burner, Ose, pH meter, viscometer.

a. Raw Material Preparation

- 1) Chinese betel leaves were obtained from the yard of a house in Sokokidul Village, Demak, Central Java. The Chinese betel leaves that have been obtained are dried using an oven at a temperature of 50°C. The simplicia that has been obtained is then dry sorted and blended to obtain simplicia powder(Utami & Andriani, 2020).
- 2) The extraction process was carried out by weighing 1 kg of dried Chinese betel leaf powder, then soaking it in a

maceration container then covering it with black plastic with 10 liters of 70% ethanol solvent for 4 days and filtering every 24 hours. The filtrate is filtered then thickened with a rotary evaporator and followed by a water bath at 50°C until a thick extract is obtained. The percent yield obtained is calculated.

$$\% \text{ Rendemen} = \frac{\text{Berat Ekstrak Kental}}{\text{Berat Serbuk Simplicia}} 100\%$$

b. Making Chinese Betel Leaf Facial Toner Preparations

**Table 3.1.**Formulation of Chinese Betel Leaf Facial Toner (Peperomia pellucida L. Kunth

Source: Modification from mayefis et al., 2020 and Khansa, 2017.

The ingredients used in the formulation are prepared first. The extract used in this research is a thick extract of Chinese betel leaves with variations in the addition of extracts of 7.5%, 15%, 30% which is based on the modification of Mayefis et al., (2020), namely a concentration of 15% Chinese betel leaf extract which has antibacterial

No	Bahan	Formulasi				Fungsi
		F0	FI	FII	FIII	
1	Ekstrak Daun Sirih Cina	-	7,5 g	15 g	30 g	Menghambat bakteri <i>Propionibacterium acnes</i>
2	Polisorbat 80	0,15 ml	0,15 ml	0,15 ml	0,15 ml	Meningkatkan daya lekat, surfaktan dan kestabilan produk
3	Gliserin	5 ml	5 ml	5 ml	5 ml	Menjaga kelembapan kulit wajah
4	Na CMC	0,25 g	0,25 g	0,25 g	0,25 g	Penstabil, pengental, dan bahan pengikat
5	Propilen glikol	4 ml	4 ml	4 ml	4 ml	Pengawet
6	Aquadest	ad 100 ml	ad 100 ml	ad 100 ml	ad 100 ml	Mengencerkan suatu zat

activity in strong, 20% strong, and 25% strong categories. So the concentration taken starting from 15% is declared

strong at 11.91 mm in inhibiting the *Propionibacterium acnes* bacteria.

To make the toner preparation, first add 5 ml of warm water to the mortar and stamper, then add 0.25 grams of Na CMC, then wait until it expands and stir until homogeneous. Next, add 5 ml of glycerin, 4 ml of propylene glycol, 0.15 ml of polysorbate 80, add it to the mixture, then add the thick extract of Chinese betel leaves according to the extract variations, 7.5 grams, 15 grams, 30 grams, then add the remaining distilled water ad 100 ml dissolve until homogeneous. Put it in the toner container.

c. Physical Quality Test of Chinese Betel Leaf Extract Facial Toner Preparations (*Peperomia pellucida* L. Kunth)

1) Organoleptic Test

Put 10 ml of facial toner into a beaker. Then the preparation is observed for color, smell and shape using the five senses (BSN, 2006). The test was carried out for each formulation with three replications.

2) Homogeneity Test

The homogeneity test is carried out by observing a 100 ml toner preparation to see whether there are particles or precipitates in the preparation. It is said to be homogeneous if the preparation does not have lumps or sediment in the solution (Yanuarto *et al.*, 2022). The test was carried out for each formulation with three replications.

3) Test pH

Put 20 ml of facial toner into a beaker. The pH test in this research uses a pH meter which has previously been calibrated using a standard buffer solution at pH 4 and 7. This treatment is carried out by turning on the on button on the pH meter, then dipping the pH meter electrode which has been rinsed with distilled water into the preparation to be tested. , then wait

for the numbers on the pH meter monitor to stabilize (Yanuarto *et al.*, 2022). The test was carried out for each formulation with three replications.

4) Viscosity Test

Put 100 ml of facial toner into a beaker. Viscosity measurement using a Brookfield viscometer. The spindle used is spindle number 2. The spindle is dipped into 100 ml of the preparation at a speed of 60 rpm. Let the spindle rotate and the number obtained is the viscosity of the facial toner preparation (Nuraeni, 2019). The test was carried out for each formulation with three replications.

d. Antibacterial Activity Test using the Disc Diffusion Method

1) Sterilization of Tools and Materials

The glassware in this study was washed with soap, then the glassware was wrapped in paper, for scaled glassware such as Erlenmeyer, measuring cups, chemical glasses, test tubes it was sterilized by autoclaving at a temperature of 121°C for 15 minutes and for glassware that was not scales such as petri dishes, stir sticks and tweezers are sterilized in an oven at 160 - 180°C for 2 hours. The ose needle is ignited using a Bunsen flame.

2) Making Nutrient Agar (NA)

Weighed 4 grams of Nutrient Agar (NA) then dissolved it in 200 ml of distilled water. Then, homogenized and sterilized in an autoclave at 121°C for 15 minutes (Ninsih *et al.*, 2020).

3) Bacterial Rejuvenation

The slanted agar medium is made first before rejuvenating the bacteria by pouring the NA medium into a test tube and then leaving it until the medium solidifies at a 30° slope. then incubated in an incubator at

37°C for 1 x 24 hours(Ninsih et al., 2020).

4) Making Bacterial Suspensions

For the McFarland turbidity standard, 9.9 ml of 1% H<sub>2</sub>SO<sub>4</sub> solution was taken, mixed with 0.1 ml of 1.175% BaCl<sub>2</sub> solution in an Erlenmeyer flask then shaken until a turbid solution was formed (Ninsih et al., 2020).

The test bacteria on the agar slant medium were taken with a sterile tube needle, suspended in a tube containing 2 ml of 0.9% NaCl solution and then vortexed until turbidity was obtained. After that, the turbidity was tested with Mc solution. Farland 0.5 (equivalent to (108 CFU/ml). If the bacterial suspension is too turbid, then add NaCl, if it is not turbid enough then add bacteria (Ninsih et al., 2020).

5) Preparation Activity Test

Microbiological tests to determine the antibacterial activity of Chinese betel leaf facial toner preparations were carried out using the disc diffusion method by measuring the diameter of bacterial growth barriers against *Propionibacterium acnes* bacteria. Treatment varied concentrations of 7.5%, 15%, 30%, clindamycin as positive control and toner base as negative control. The first step, clean both hands using 70% alcohol then prepare 12 petri dishes and each petri dish is labeled for each treatment. Next, sterilize the mouth of the petri dish using a spirit lamp, then pipet ± 2 ml of Nutrient Agar (NA) into the petri dish and leave it until it solidifies. Dip a sterile hose needle into the *Propionobacterium acnes* suspension, then rub it on the surface of the solidified agar medium, then leave it for 1-5 minutes so that the suspension enters the agar.

Next, the paper discs were soaked in Chinese betel leaf facial toner preparations with varying concentrations of 7.5%, 15%, 30% to be tested. Then dip the paper disc in the positive control and negative control. Remove the disc paper using sterile tweezers then wait until the Chinese betel leaf facial toner preparation, positive control and negative control no longer drip from the disc paper. Then the disc paper was placed on Nutrient Agar (NA) media, incubated at 37°C for 24 hours and the inhibitory power was measured in the form of a clear zone using a caliper (mm). Replication was carried out three times for each concentration(Yufiradani et al., 2020).

**RESULTS AND DISCUSSION**

1) Macerated Extraction of Chinese Betel Leaves

Samples of Chinese betel leaves were obtained in Sokokidul Village, Kebonagung District, Demak Regency. The Chinese betel leaves are sorted to select the good leaves, then the Chinese betel leaves are washed until clean, then placed in the oven. Chinese betel powder was macerated with 70% ethanol solvent, concentrated using a rotary evaporator using a temperature of 50°C and continued with evaporation using a water bath for 2 days at a temperature of 50°C. The results obtained are as shown in table 4.1.

**Table 4.1** Extraction Results of Chinese Betel Leaves

Fresh Chinese Betel Leaves (gram)	Weight powder material (gram)	Viscous Extraction (gram)	Rendement (%)
17000	800	82,47	10,308

1. Formulation of Chinese Betel Leaf Extract Facial Toner



The Chinese betel leaf extract that has been obtained is then used to make facial toner with different concentrations. Formula F0 is a facial toner base without the addition of Chinese betel leaf extract, FI uses 7.5% Chinese betel leaf extract, FII uses 15% Chinese betel leaf extract, FIII uses 30% Chinese betel leaf extract. Each formula is made 100 mL

2. Physical Quality Evaluation

a. Organoleptic Test

Organoleptic tests are carried out to see the physical appearance of facial toner preparations includes parameters of color, smell and shape. Organoleptic test results can be seen in table 4.2.

**Table 4.2** Organoleptic Test Results

Parameter	F0	FI	FII	FIII
Color	Clear	Brownish green	Brownish green	Brownish green
Smell	Odorless	Typical chinese betel leaf	Typical chinese betel leaf	Typical chinese betel leaf
Form	Liquid	Liquid	Liquid	Liquid

Information :

F0: Formulation without Chinese betel leaf extract

FI: Formulation with 7.5% Chinese betel leaf extract

FII: Formulation with 15% Chinese betel leaf extract

FIII: Formulation with 30% Chinese betel leaf extract

Based on table 4.2, after the organoleptic test was carried out, the results showed that the facial toner was in liquid form with a brownish green color and the characteristic smell of Chinese betel leaves.

b. Homogeneity Test

Homogeneity test to determine whether the facial toner preparation is homogeneous or not, if it is not homogeneous then the preparation will contain coarse particles. The homogeneity test results can be seen in table 4.3.

**Table 4.2** Homogeneity Test Results

Formula	Results	Requirements test	Interpretation
F0	There are no small particles	There are no small particles	Homogen
FI	There are no small particles	There are no small particles	Homogen
FII	There are no small particles	There are no small particles	Homogen
FIII	There are no small particles	There are no small particles	Homogen

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Based on table 4.3, after the homogeneity test was carried out, the results showed that the preparation made met the homogeneity requirements because there were no particles or lumps in the preparation.

c. Test pH

The pH test is carried out to see the acidity or alkalinity level of the facial toner preparation.

The pH test results can be seen in table 4.4.

**Table 4.3** pH Test Results

Sample	pH Mean ± SD	Requirements (Khanza, 2017)	Interpretation
F0	6,327 ± 0,005	4,5-6,5	Fulfil
FI	5,344 ± 0,004	4,5-6,5	Fulfil
FII	5,243 ± 0,003	4,5-6,5	Fulfil
FIII	5,067 ± 0,007	4,5-6,5	Fulfil

Based on table 4.4, after carrying out the pH test, the average results of the 4 formulas were obtained, which met the requirements because they were included in the skin pH range, namely 4.5-6.5.

d. Viscosity Test

The viscosity test is carried out to measure the viscosity level of a preparation. The viscosity test results can be seen in table 4.5.

**Table 4.4** Viscosity Test Results

Sample	Viscosity Average ± SD (Cps)	Reference Ani et al., 2019	Interpretation
F0	6,437 ± 0,046	5,67-6,44	Fulfil
F1	6,180 ± 0,020		Fulfil
F2	6,110 ± 0,010		Fulfil
F3	6,190 ± 0,026		Fulfil

Based on table 4.5, after conducting a viscosity test on the facial toner preparation that has been made it meets the viscosity range of 5.67-6.44 cps but there is no viscosity requirement range so it refers to previous research by Ani et al., (2019).

3. Antibacterial Activity Test Against Propionibacterium Acnes Bacteria

The antibacterial activity test was carried out using Mueller Hilton Blood (MHD) media and replicated 3 times. Then determine the bacterial inhibition zone using a caliper. The results of the inhibition zone can be seen in table 4.6

**Table 4.5** Antibacterial Activity Test Results

Formula	Obstacles Zone (mm) ± SD	Inhibitory Power Category (Hapsari, 2015)
Positive control	21,17 ± 0,042	Very Strong
F0	0,00 ± 0,00	Weak
FI	7,37 ± 0,074	Currently
FII	13,35 ± 0,057	Strong
FIII	16,24 ± 0,050	Strong

The results of the antibacterial test showed that the positive control had a very strong inhibition zone category, F0 0.00 mm no inhibition zone, FI a moderate inhibition zone, FII, FIII strong inhibition zone categories.

This research used samples of Chinese betel leaves (*Peperomia pellucida* L. Kunth) obtained in Sokokidul Village, Kebonagung District, Demak Regency. 17 kg of fresh Chinese betel leaves (*Peperomia pellucida* L.Kunth) were cleaned with running water and cut into small pieces to make the drying process easier, then the Chinese betel leaves were placed in the oven at a temperature of 50 °C. Chinese betel leaves (*Peperomia pellucida* L.Kunth) which have become simplicia, are then extracted using the maceration method because the procedures and equipment used are simple and are not heated so that the natural material does not decompose (Puspitasari, 2017). The Chinese betel leaves used are harvested before 12.00 WIB. The harvest time is in the morning to avoid the process of evaporation of plants during the day, supported by research by Leba, (2017) that the best harvest time is in the morning because some of the harvested plants are fermented and their metabolites are damaged if exposed to excessive heat so that the chemical quality is not good.

The maceration process was carried out with 800 grams of Chinese betel leaf simplicia powder soaked in 70% ethanol solvent. 70% ethanol can attract more active compounds

compared to other types of organic solvents. 70% ethanol has a low boiling point, namely 79°C, so it requires less heat for the concentration process (Winangsih, 2013). Supported by research by Hasanah *et al.*, (2020) because 70% ethanol has non-toxic properties, is safe and is able to attract more compounds in simplicia.

Apart from that, 70% ethanol is used in making extracts because mold and germs are difficult to grow and the absorbance is good (Sundari, 2019). Then stir every 6 hours. Stirring is needed to even out the concentration of the solution outside the simplicia powder grains, supported by Sundari's research, (2019) so that with this mixing, there is still a degree of concentration difference between the smallest and smallest between the solution inside and outside the cell, left for 3 x 24 hours, this is This is done so that the compounds contained in the plant are attracted, after the maceration process it is then filtered using filter paper to obtain a greenish brown filtrate and dregs. The filtrate was obtained and then thickened using a rotary evaporator at a temperature of 50 °C, then continued with evaporation using a water bath at a temperature of 50 °C. The results of extraction using simplicia were 800 grams macerated with 8 liters of 70% ethanol, resulting in a thick extract of 82.47 grams with a yield of 10.308%.

The color organoleptic test results in this study were that formula 0 which was used as a control base was clear in color while formulas I, II and III were brownish green in color, this was because Chinese betel leaf extract had been added at FI 7.5%, FII 15%, FIII 30%. This statement is supported by Khansa *et al.*, (2017) that the color formed in toner preparations is influenced by the constituent ingredients, the greater the concentration of the extract used can produce a more intense color. The form of the toner dosage form that has been made is in liquid form. This is in accordance with Rohma's statement, (2022) because the additional ingredients in making the preparation are in liquid form, such as distilled water, polysorbate 80, glycerin and propylene glycol.

The homogeneity test is carried out to determine whether or not there are small particles caused by the ingredients not being completely mixed. Based on observations made on the

preparation that had been made, there were no small particles between the preparation base and Chinese betel leaf extract. This shows that the base can support Chinese betel leaf extract to be made into an active ingredient in toner preparations. This is in accordance with research conducted by Yuliana, (2015), an emulsion preparation made using the active ingredient homogeneous Chinese betel leaf extract.

The pH test results obtained for each variation in concentration of the toner preparation in formula 0; I, II, and III respectively amounted to 6,327; 5,344; 5,243; and 5,067. The results obtained are in accordance with research by Khansa, (2017) that the pH requirements for topical preparations are 4.6 – 6.5. The pH test results stated that the four formulations had different pH values. This is influenced by differences in the concentration of Chinese betel leaf extract used. The greater the concentration of the extract, the lower the pH of the toner preparation. This is in accordance with research by Arbie *et al.*, (2020), the more extract added, the lower the pH produced.

The viscosity test results obtained for each variation in the concentration of the toner preparation in formula 0; I; II; and III respectively at 6,437 cps; 6, 180 cps; 6,110 cps and 6,190 cps. There are no viscosity requirements for toner preparations, so for viscosity we refer to previous research by Ani *et al.*, (2019) on toners where the viscosity obtained was 5.67-6.44 cps. The viscosity of the toner preparation in this study is in this range so it is in accordance with research by Ani *et al.*, (2019),

The antibacterial activity test in this study aims to determine the antibacterial activity of Chinese betel leaf extract against *Propionibacterium acnes* bacteria in facial toner preparations. The results of the antibacterial activity test on the positive control were 21.17 mm in the very strong category, F0 0.00 mm in the weak category, FI 7.37 mm in the medium category, FII 13.35 mm in the strong category, and FIII 16.24 mm in the strong category. This antibacterial test shows that the higher the extract concentration, the larger the diameter of the inhibition zone produced. This is supported by research by Mayefis *et al.*, (2020) The inhibition

zone for bacterial growth will be larger the higher the concentration added.

Positive control testing uses clindamycin. Clindamycin is used to treat anaerobic bacterial infections. *Propionibacterium acnes* bacteria are purple gram-positive anaerobic bacteria that are tolerant of air (Mayefis *et al.*, (2020). Side effects of using clindamycin include blistering of the skin, a skin disorder characterized by circular lesions, the appearance of fluid-filled blisters on the skin that feel itchy. (Damayanti, 2014).

The results of the positive control test, which formed an inhibition zone of 21.17 mm, were declared in the very strong category, so that the results of the antibacterial activity in the Chinese betel leaf extract facial toner preparation were smaller than clindamycin. But Chinese betel leaf extract facial toner contains natural ingredients that are not harmful to human health. This is supported by research by Ninsih, (2022), that antibacterial activity can be produced from plants that contain active compounds in the form of secondary metabolites such as alkaloids, flavonoids, saponins, tannins and triterpenoids. These compounds generally have bioactivity capabilities, where bioactive compounds have various functions for human health, one of which is anti-bacterial activity.

## CONCLUSION

From the research results it can be concluded that:

1. The results of the physical quality test of the Chinese betel leaf extract facial toner preparation met the requirements obtained from the organoleptic test of the toner preparation base which was clear in color, had no aroma and was in liquid form, while the 3 formulas were brownish green in color, had a distinctive odor of Chinese betel leaf extract and were in liquid form. The homogeneity test results of the three formulas obtained homogeneous results. The pH test results for the F0 toner preparation were 6.327; FI 5.344; FII 5,243; FIII 5,067. F0 viscosity test results 6.437; FI 6.180; FII 6,110; FIII 6,190.
2. The antibacterial activity results of the positive control were 21.17 mm in the very strong category, F0 0.00 mm in the weak category, FI 7.37 mm in the strong category, FII 13.35 mm



in the strong category and FIII 16.24 mm in the strong category.

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