

FORMULATION AND ACTIVITY TESTS OF ANTI-ACNE TONER ESSENTIAL OIL OF RED BETEL LEAF (*Piper crocatum*)

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ABSTRACT

Red betel leaf (Piper crocatum) is a plant that can be used as an antibacterial. Red betel leaves contain flavonoids, alkaloids, tannins, and essential oils which are thought to be used as antimicrobials. The purpose of this study was to determine the antibacterial activity of red betel leaf (Piper crocatum) essential oil facial toner as an anti-acne toner against Propionibacterium acnes bacteria. Red betel leaf is distilled to obtain essential oil, then tested for organoleptic, specific gravity, pH, viscosity, solubility in alcohol. Making facial toner preparations using concentrations of 10%, 20% and 30%. The results of the formula were then subjected to physical quality tests including organoleptic tests, pH tests, viscosity, anti-irritation tests, hedonic tests and activity tests against Propionibacterium acnes bacteria. Toner preparation activity test results obtained concentrations of 10% 9.2 mm, 20% 11.26 mm, 30% 13.46 mm. Red betel leaf essential oil toner can inhibit the growth of Propionibacterium acnes bacteria with the highest concentration of 30% obtained inhibition of 13.46 mm in the strong category.

KEYWORDS

Piper crocatum, antiacne, formulation, Propionibacterium acnes



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INTRODUCTION

Acne is a disease of the surface of the skin on the face, neck, chest and back that appears in a short time. Oil glands in the skin that produce a lot of oil to keep skin pores from becoming clogged with excess fat deposits. When the pile mixes with sweat, dust and other contaminants, it causes a pile called a blackhead with black spots on it. If a comedo has a bacterial infection, then inflammation occurs, it is known as acne (Oktaviani et al., 2022).

There are many ways to treat acne, but synthetic drugs are often used topically. Commonly used topical drugs are usually antibiotics and retinoids such as benzoyl

peroxide, one of them. However, the use of these drugs can cause other side effects, such as skin irritation. Another effect of using antibiotics is drug resistance, which means drugs can no longer kill bacteria and the ability of antibiotics to kill acne-causing bacteria is reduced (Noor et al., 2023). Therefore it is necessary to do other ways to reduce the occurrence of resistance and reduce drug sensitivity to acne-causing bacteria, one of which is by using natural ingredients (Indarto et al., 2019). One of the natural ingredients that has the ability as an antibacterial is betel leaf.

Red betel leaf (*Piper crocatum*) contains 4.2% essential oil which mainly consists of betephenol which is an isomer of euganol, allypyrocatechin, cineole-methyleuganol, caryophyllene (sisquiterpenes), kavicol, kavibecol, estragole, and terpinene (Andayani et al., 2014). Red betel leaves (*Piper crocatum*) contain flavonoids, alkaloids, tannins and essential oils which are thought to be used as antimicrobials (Aisyiyah et al., 2021)

Betel leaves have antibacterial activity against *Propionibacterium acnes* at concentrations of 10%, 15%, 20% and 25%. Red betel leaves have antibacterial activity against *Propionibacterium acnes* at the minimum inhibitory concentration value of the ethanol extract of red betel leaves at a concentration of 10%. This is evidenced by the research of Winda et al., (2017) using the solid dilution method. The results of the minimum inhibitory concentration test of betel leaf ethanol extract against *Propionibacterium acnes* at a concentration of 1-9% in media planted with bacteria still contained bacterial growth, while at a concentration of 10% it was not. There was bacterial growth, while the disc diffusion method yielded concentrations of 10%: 9.53 mm, 15%: 10.36 mm, 20%: 10.50 mm, 25%: 10.90 (Winda et al., 2022.)

One effort to reduce or prevent acne is to keep your face clean by using a toner to further clean your face. Toner is a cosmetic preparation used as a complement in facial cleansing. Toner is a liquid cosmetic that is formulated as a substitute for facial cleansing or a complement to facial cleansing and also as a moisturizer to control sebum production and can help percutaneous absorption which acts as a barrier so as to increase skin hydration. Apart from being a facial cleanser, toner can also be added with active substances such as anti-acne or anti-acne, one of which is red betel leaf extract (*Piper crocatum*) (Noor et al., 2023)

RESEARCH METHOD

Materials and Equipment

The research method used in this study was an experimental method with several stages, namely determination, collection of materials, manufacture of essential oils, testing of anti-bacterial essential oils, formulation of preparations, preparation of preparations, testing of anti-acne activity against *Propionibacterium acnes* bacteria. Research has been carried out at the Microbiology Laboratory of Duta Bangsa University Surakarta in 2023. This research was conducted in April-May 2023

The materials used in this study were Red betel leaf (*Piper crocatum*), McFarland 0.5, NaCl, ethanol, 90% alcohol, media Nutrient Agar aqua dest, glycerin, propylene glycol, DMDM hydantoin, TEA, benzoyl peroxide, *propionibacterium acnes* bacteria, DMSO (Dimethyl Sulfoxide) Mueller Hinton Agar medium. The tools used in this study included a beaker glass, thermometer, test tube, measuring cup, petri dish, spatula, scissors, pipette, pH paper, stir bar, filter paper, digital scales, porcelain cup, packing bottle, watch glass, tool distillation, disc paper, autoclave, pycnometer, viscometer, ose needle.

Research procedure

(1) Making Red Betel Leaf Essential Oil

Essential oils are made by simple distillation, by using a distillation apparatus, the material used is betel leaf red that has been withered or aerated and chopped to make it easier release of essential oils, then put into the distillation flask with 27 water has been added to the bottom and heated to a temperature until evaporation occurs, so that the steam enters the condenser which there is a cooler around it so that the steam will produce oil in the condenser (Andayani et al., 2014).

(2) Physical Quality of Essential Oils

(a) Organoleptic The organoleptic test is carried out by conducting directly through human senses in the form of smell, color, taste, and solubility soluble in ethanol (Damayanti et al., 2015). **(b) pH** Checking the pH is done using pH paper. pH of the expected essential oil ranges from 3.9 to 5.3.(Saraswati et al., 2018) **(c) Specific Weight** Measurement of specific gravity using a pycnometer, by the way weigh the empty pycnometer, the pycnometer filled with water, and the pycnometer containing essential oils three times repetition was weighed with a balance analytic. The expected specific gravity ranges from 0.696 to 1.188 (Nurhayati & Yuli, 2013) **(d) Solubility In Alcohol** Solubility in alcohol is carried out by as much as 1 ml essential oil is measured in a measuring cup then added 90% alcohol little by little while shaking until a solution is obtained clear at 20°C. If the solution is not clear, then do it comparison with the reference solution through the solution of the same thickness ((Wibowo et al., 2016). **(e) Toner Formulation** Anti-acne toner formulation from red betel leaf essential oil using glycerin, propylene glycol, TEA, DMDM hydantoin, and distilled water. Red betel leaf (*Piper crocatum*) has antibacterial activity against *Propiobacterium acnes* bacteria. Glycerin and propylene glycol can be used as humectants so that they can keep the user's skin moist. Glycerin is one of the most widely used humectants because glycerin can reduce the amount of water that comes out of the skin in high humidity (Muhsinin et al., 2023). DMDM hydantoin is a preservative commonly used in cosmetic preparations, because it has properties that dissolve easily in water, works as a broad-spectrum antimicrobial that is effective on gram-positive and gram-negative bacteria (Istiqomah & Akuba, 2021). The concentration of DMDM Hydantoin that is safe for use in cosmetics is 0.1-1%. TEA (Triethanolamine) functions as a pH neutralizer (Muhsinin et al., 2023)

Table 1. Anti-acne Toner Modification Formulasi Red Betel Leaf Essential Oil (*Piper crocatum*)

Material	FI%	FII%	FIII%	Function
Gliserin	2	2	2	Humektan
<i>Propilen glikol</i>	3	3	3	Humektan
DMDM <i>hydantoin</i>	0,6	0,6	0,6	Preservative
<i>TEA</i>	0,1	0,1	0,1	Pengatur pH
Red betel leaf essential oil	10%	20%	30%	Active agent
Aquadest	Ad 30ml	Ad 30ml	Ad 30ml	Solvent

(b) Manufacturing Toner Preparations

The toner manufacturing process is carried out by weighing all the ingredients needed such as glycerin, propylene glycol, DMDM hydantoin, TEA and essential oils. Mixing is done by adding glycerin and propylene glycol and adding DMDM hydantoin and essential oils, mixing until homogeneous, then adding TEA as a pH neutralizer to get a facial skin pH of 5.5-6.0, add distilled water to the desired volume mark, which is 30 ml.

(c) Physical Quality Test of Toner Preparations

(1) Organoleptic Test, Tests were carried out by physical observations including color, shape, aroma. (2) pH test, The degree of acidity (pH) test was measured using a pH meter. A good pH value for toner preparations is 4.5-6.5 (Noor et al., 2023) (3) Homogeneity Test (4) The homogeneity test is carried out by observing whether there are materials or ingredients used that are not mixed evenly or there are residues that have not dissolved. (5) Viscosity Test Testing the viscosity of the toner preparation was carried out using a viscometer with spindle number 1 at a speed of 60 rpm. The toner preparation is put into a chemical glass. The spindle that has been attached is then lowered until it is submerged in the preparation and starts spinning until the spindle stops and results are obtained (Noor et al., 2023).

(d) Toner Preparation Anti-Bacterial Activity Test

Dip the sterile cotton swab into the bacterial suspension that has been made. Then inoculation was carried out on Mueller Hinton Agar media with the smoothing method. The petri dish is marked and divided into 5 parts using a marker on the back of the cup to provide a partition or boundary between samples. The medium was left for 10 minutes so that the bacteria diffused in the media. Paper discs were soaked for 15 minutes in samples of each concentration, DMSO negative control and positive control antibacterial toner preparations containing salicylic acid, then disc papers that had been soaked were put into a petri dish filled with media and culture. After that, incubation was carried out for 24 hours at 37°C. The results obtained were observed and then measurements of the inhibition zone were carried out in the clear area around the disc paper (Sandy et al., 2021).

RESULT AND DISCUSSION

The essential oil obtained from this study is a clear yellow liquid with a characteristic odor of red betel leaves. A total of 6.5 kg of red betel leaves were distilled using steam and water distillation to produce 14 mL of essential oil. The yield of red betel leaf essential oil obtained was 0.30% with a specific gravity of 0.7539, this is by what is desired, namely the specific gravity of essential oils ranging from 0.696 to 1.188.

Physical Quality Test Results of Essential Oils, Organoleptic tests include smell, taste, and color, this test is carried out through the human senses with the results of the smell or aroma obtained, namely the strong or stinging characteristic odor of red betel. The color of the essential oil obtained is yellow with a little brownish, and the taste of red betel leaf essential oil is bitter. The pH test was carried out using a pH meter, the pH obtained from essential oils was 4.65, which met the requirements for a good pH for essential oils, namely 3.9-5.3. The solubility test in alcohol was carried out using 90% alcohol by dissolving 1 ml of essential oil in 90% alcohol and then shaking it until dissolved and clear. The results of this test showed that essential oils were perfectly dissolved and clear white in the amount of 25 ml of alcohol solution used so that they were by existing standards

the calculation of the specific gravity of red betel leaf essential oil with three repetitions is calculated by means of the weight of the pycnometer containing essential oil minus the weight of the empty pycnometer reduced by dividing it by the result of reducing the weight of the pycnometer filled with water minus the weight of the empty pycnometer after the calculations were carried out, the results of the calculation of the first replication were 0.755, the second replication was 0.753 and the third replication was 0.7538, so that an average of 0.739 was obtained, this is in accordance with the literature (Andayani et al., 2014) in general, the specific gravity value of essential oils ranges from 0.696 to 1.188. Based on this statement, the results of the specific gravity of the essential oil produced meet

the range of good essential oil density. Rismawati,(2011) said that the yield of fresh red betel leaf essential oil was 0.883, which means that it was not much different from the results obtained in this study.

Organoleptic testing is carried out to see the feasibility of the preparation based on the physical appearance of the toner preparation by observing the color and aroma through the human senses. The color of the toner preparation obtained is white. The form of the toner preparation produced is liquid so it is easy to apply, (Noor et al., 2023) reported that a toner preparation that has a high viscosity can make the skin feel sticky to the skin, making it uncomfortable when using the toner. The aroma of the toner is the distinctive smell of red betel which is quite strong, it is necessary to add the right fragrance to avoid difficulties with the strong aroma of red betel. (Utami, 2017) said the red betel plant has a more fragrant aroma compared to green betel, this is also likely from the aroma of essential oil from red betel leaves which is more fragrant or stronger than green betel.

Homogeneity testing of the toner preparations is carried out to see whether the particles are mixed or not mixed in the toner preparations that are made. Good preparations can be seen by producing preparations that are homogeneous or evenly mixed, this is in accordance with research conducted by Abidin, (2021) homogeneous facial toner preparations are characterized by all the ingredients used in making the preparations mixed or none of the mixed ingredients. clot. The test was carried out by pouring the toner preparation into a glass beaker and then observing, the results of the preparation obtained were that there was a slight separation of the essential oil from the toner preparation. Separation that occurs due to the addition of aquadest used in the solvent. Before using or applying the toner, it is necessary to shake it first so that the results are evenly mixed or there are no separations.

Testing the pH or degree of acidity is carried out using a calibrated pH meter. Checking the pH or degree of acidity is carried out with the aim of seeing or ensuring the safety of preparations used on the skin. The pH of a good facial toner is around 4.5-6.5. After testing the pH at each concentration, the results obtained were 10% preparation with an average yield of 4.8, 20% preparation 4.5, 30% preparation 4.6. Based on these results it can be concluded that the pH of all concentrations meets the requirements for a good toner preparation, research conducted by (Noor et al., 2023) reports that the pH of a toner that complies with the standard is 4.5-6.5 pH which is too low from the standard. cause irritation to the skin while a pH that is too high from the standard can cause dry and irritated skin or an itchy sensation.

Table 2. Results of Measurement of Degree of Acidity (pH)

Concentration	Replication			Average
	I	II	III	
10%	4,85	4,87	4,85	4,8
20%	4,56	4,56	4,55	4,5
30%	4,65	4,66	4,66	4,6

The results of the average of each formulation all results meet good pH standards. Iswandana et al, (2017) explained that betel leaf showed the best antibacterial activity at a neutral pH, namely pH 7. An increase in the pH value indicated a decrease in antibacterial activity against pathogens. Antibacterial activity was shown starting from pH 5, and continued to increase up to pH 7, before then dropping to pH 9 to 10. One of the things that can affect the decrease in pH is the decomposition of substances that can occur in the preparation. The content that can undergo decomposition is tween 80. The pH value is not too acidic and not too alkaline, so it is not worrying when using it, because it is considered not to cause skin irritation due to the large difference in pH

Viscosity testing was carried out using the NDJ-8S spindle number 1 viscometer at a speed of 60 rpm. Tests are carried out to determine the thickness level of the toner preparations and the feasibility of the toner preparations whether they are in accordance with the good viscosity standards of the toner preparations or not. The test was replicated three times at each concentration, the results of the three repetitions then obtained the average viscosity of each concentration. The results of the three times replication test are as follows

Table 3. Toner Preparation Viscosity Test Results

Concentration	Replication (cPs)			Average (cPs)
	I	II	III	
10%	2,8	2,8	2,9	2,83
20%	3,3	3,1	3,3	3,2
30%	4,1	4,5	5,0	4,5

Based on the results obtained by testing three replications, the average viscosity for toner preparations with a concentration of 10% obtained a result of 2.83 cPs, preparations with a concentration of 20% an average yield of 3.2 cPs, preparations with a concentration of 30% an average yield -average 4.5 cPs. Based on the results of table 11. It can be said that the higher the concentration, the higher the viscosity of the toner preparation. The results obtained meet the specifications for toner preparations. (Aisyiyah et al., 2021) reported that a good standard of viscosity for facial toner is <5 cPs. Viscosity measurement using a viscometer using spindle number 1 with a speed of 60 rpm

Testing the activity of anti-acne toner preparations of red betel leaf essential oil on the growth of *Propionibacterium acnes* bacteria using the disk diffusion method which aims to determine the Minimum Inhibitory Concentration (MIC) using blood Mueller Hinton Agar (MHA) media. The medium used was added with 3 drops of human blood to increase the activity of the bacteria. The test was carried out by smearing the bacterial suspension on the solidified media and then letting it stand for 10 minutes so that the bacteria diffused in the media. Paper disks were soaked in samples with concentrations of 10%, 20% and 30%, respectively, as well as a positive control for a toner preparation which had anti-acne properties containing salicylic acid and 1% DMSO. Paper discs are placed on the media that has been marked, then incubated for 24 hours and the test results will be obtained.

The results of the test are areas where there is no bacterial growth around the discs indicating antibacterial activity or inhibition of *Propionibacterium acnes* bacteria. Antibacterial testing was carried out by measuring the diameter of the bright zone on a sample of red betel leaf essential oil facial toner, 1% DMSO as a negative control and an antibacterial toner containing salicylic acid which has anti-acne properties as a positive control. Antibacterial activity testing showed that the anti-acne toner preparation, essential oil from red betel leaves could inhibit the growth of *Propionibacterium acnes* bacteria from the lowest concentration of 10% to the highest concentration of 30%. A concentration of 10% obtained an average result of 9.2 mm in the medium category inhibiting bacterial growth, a concentration of 20% obtained an average result of 10.7 mm in a strong category inhibiting bacterial growth, a concentration of 30% obtained an average result of 13.46 mm in a strong category inhibits the growth of *Propionibacterium acnes* bacteria. This study is similar to research conducted by (Hasanah & Novian, 2020) the results of measuring the inhibition zone of *Propionibacterium acnes* bacteria in the facial toner formulation of belimbing wuluh leaf extract using the disc method showed that the facial toner formulation showed inhibition of *Propionibacterium acnes* bacteria.

Table 4. Test Results for Antibacterial Activity of Toner Preparations By Diffusion Against *Propionibacterium acnes* Bacteria

Material Test	Formula (Concentration)	Inhibitory (mm)			Average (mm)	Category
		I	II	III		
Red betel leaf	1 (10%)	9,6	9,4	8,6	9,2	Currently
essential oil	2 (20%)	11,8	11,3	10,7	11,26	strong
toner	3 (30%)	13,7	14,9	11,8	13,46	strong
Toner	2,5%	18,5	19,6	18,5	18,86	strong
Antibacteri						
DMSO	1%	0	0	0	0	-

Based on the results obtained after three replications, it was found that the toner preparation with an essential oil concentration of 10% was able to inhibit the growth of *Propionibacterium acnes* bacteria. In the third replication, the results were not good. The clear zone results obtained were measured with a caliper. Rastina et al., (2015) reported that antibacterial activity in inhibition was influenced by the concentration and type of bacteria. The higher the concentration used, the greater the inhibition zone that is formed, the higher the concentration of the active substance used, the more active substance is contained so that the ability to inhibit bacterial growth is higher and the clear zone or inhibition zone that is formed is wider. The presence of inhibition against *Propionibacterium acnes* bacteria is influenced by the presence of compounds that can be efficacious as antibacterial in red betel leaf (*Piper crocatum*) essential oil. The results obtained are in accordance with research conducted by Andayani et al., (2014) an inhibition zone of 5 mm or less has weak category, 5-10 mm inhibition zone has moderate category, 10-20 mm inhibition zone has strong category and 20 mm or more inhibition zone is said to be very strong

CONCLUSION

The conclusions that can be drawn based on the research results of the formulation and activity test of anti-acne toner preparations of red betel leaf (*Piper crocatum*) essential oil against *Propionibacterium acnes* bacteria can be concluded that:

- (1) Anti-acne toner preparations red betel leaf essential oil can inhibit the activity of *Propionibacterium acnes* bacteria with the highest average inhibition value of 13.46 mm in formula 3 with an essential oil concentration of 30%, the inhibitory response is still in the strong response group.
- (2) The red betel leaf (*Piper crocatum*) essential oil toner meets good physical quality including organoleptic, pH, homogeneity, viscosity, anti-irritation and hedonic tests according to the physical quality standards of good toner preparations.

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