

CHARACTERISTIC AND ANTIBACTERIAL ACTIVITY TEST OF RED BETEL LEAF ESSENTIAL OIL AGAINST PROPIONIBACTERIUM ACNE BACTERIA

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ABSTRACT

Many Indonesian plants have the potential to have anti-acne activity, one of which is the red betel plant. Red betel leaf (*Piper crocatum*) contains flavonoids, alkaloids, tannins, and essential oils which are thought to be used as antimicrobials. The purpose of this study was to test the quality characteristics and test the antibacterial activity of red betel leaf essential oil against *Propionibacterium acne* bacteria. This research was an experimental study by identifying the physical quality and testing the antibacterial activity of the essential oil. 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 was yellow with a little brownish, bitter taste, the pH obtained from the essential oil was 4.65, the specific gravity of red betel leaf essential oil was 0.739, the ethanol soluble test obtained from the essential oil completely dissolved and white clear in a quantity of alcoholic solution. Antibacterial activity test results with red betel leaf essential oil concentrations of 5%, 10%, and 20%, could inhibit antibacterial of 7.16 mm, 10.3 mm, and 11.6 mm.

Keywords: anti-bacterial; essential oil; propionibacterium acne; physical quality characteristics of the oil, red betel leaf

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 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 (Syafriana and Rusyita 2017). There are many ways to treat acne, but synthetic drugs are often used topically. Commonly used synthetic drugs can cause other side effects, such as skin irritation (Noor et al. 2023). Therefore it is necessary to do other ways to reduce the occurrence of side effects 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 to 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 eugenol, allypyrocatechin, cineole-methyl eugenol, caryophyllene (sesquiterpenes), chavicol, kavibecol, estragole, and terpinene (Kurniawan et al., 2021).

Red betel leaves (*Piper crocatum*) contain flavonoids, alkaloids, tannins, and essential oils which are thought to be used as antimicrobials (Nisa, Nugroho, and Hendrawan 2014). 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 (Syafriana and Rusyita 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 are growth bacteria, while the disc diffusion method yielded concentrations of 10%: 9.53mm, 15%: 10.36mm, 20%: 10.50mm, and 25%: 10.90 (Syafriana and Rusyita 2017). From the background above, the researcher intends to test the characteristics of essential oil from red betel leaves and test its antibacterial activity against *Propionibacterium acnes* bacteria.

METHOD

The materials used in this study were Red betel leaf (*Piper crocatum*), aqua dest, glycerin, propylene glycol, DMDM hydantoin, TEA, benzoyl peroxide, *propionibacterium acnes* bacteria, DMSO (Dimethyl Sulfoxide) Mueller Hinton Agar medium, McFarland 0.5, NaCl, ethanol, 90% alcohol, media Nutrient Agar. 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. 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).

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). 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). 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 and Yuli 2013). 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). Antibacterial activity test on essential oils was carried out by the method disc diffusion with disc paper on Mueller Hinton agar media. Media agar was placed in a petri dish and then given *propionibacterium bacteria acnes*, then essential oils with concentrations of 5%, 10%, and 20% absorbed on the disc paper and the disc paper is placed on the media, then incubated for 24 hours at 37°C until a zone appears inhibition on the disc paper area and measured with a calipers shove. Growth inhibition response rate. For diameters < 6 mm showed that there was no resistance reaction, at 6–10 mm in diameter shows a weak resistance response, at 11-20 mm diameter shows moderate resistance reaction and a diameter of 21-30 mm indicates a reaction strong inhibition (Prayoga 2013).

The tools to be used are sterilized carried out using an autoclave at 121°C for 15 minutes, the purpose of this sterilization is to remove microbes contained in the tool to be used (Khazanah, 2020). A total of 2.28 g of Mueller Hinton agar medium was dissolved in 60 ml Distilled water is heated until dissolved. The media is sterilized using an autoclave for 15 minutes at 121°C (Sunarto et al., 2019). then cooled down to $\pm 50^\circ\text{C}$, then placed in sterile Petri dishes and stored in the

refrigerator (Sandy, 2021). Bacteria obtained from the Microbiology laboratory Duta Bangsa University was cultured for 24 hours at 37°C in the media so. *Propionibacterium acnes* bacteria were suspended in 29 NaCl solution 0.9%, the turbidity is equivalent to 0.5 McFarland's standard solution (Sunarto et al., 2019). The concentration of red betel leaf (*Piper crocatum*) essential oil to be tested are 5%, 10%, and 20%. Solution making Various concentrations were carried out using 5 ml of essential oil dissolved in 95 ml ethanol for a concentration of 5%. The concentration of 10% take Put 10 ml of essential oil in a volumetric flask and dilute with ethanol 90ml. To make a concentration of 20%, 20ml of oil is taken volatiles were put in a volumetric flask and diluted with 80 ml of ethanol (Simanjuntak, 2014).

Dip a sterile cotton swab into the bacterial suspension that has been made. Then inoculated on Mueller Hinton Agar medium alignment method. The petri dish is divided into 5 parts using a marker on the back of the cup to provide insulation between samples. Medium Allow 10 minutes for the bacteria to diffuse in the media. Disc paper in soak for 15 minutes in the sample of each concentration, control DMSO negative and Benzoyl Peroxide positive control. Then paper Discs that have been soaked are inserted into the petri dish filled with media and culture. After that, incubation was carried out 24 hours at 37°C. The results obtained were observed and then carried out Measurement of the zone of inhibition in the clear area around the paper disc (Sandy, 2021).

RESULTS 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.

Table 1.
The wet weight of red betel leaf essential oil

Wet Weight (kg)	essential oil (ml)	Persentase (%)
8 (kg)	24,3 (ml)	0,30

Table 1 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.

Table 2.
 Results for Specific Gravity of Red Betel Leaf Essential Oil

Pycnometer Blank	Piknometer + Aquadest	Piknometer + essential oil	Specific gravity
20,24	45,13	39,00	0,755
20,24	45,14	38,99	0,753
20,23	45,13	39,00	0,7538

Table 2 The activity of essential oils using the diffusion method to determine the Minimum Inhibitory Concentration (MIC). The medium used was Mueller Hinton Agar (MHA). The bacterial suspension was smeared on the media, then left for 10 minutes so that the bacterial suspension diffused in the MHA media. Paper discs were soaked for 15 minutes in essential oils of various concentrations, namely concentrations of 5%, 10%, and 20%, and positive controls, namely 2.5% benzoyl peroxide and 1% DMSO (Dimethyl Sulfoxide) as negative controls. The area where bacteria are not overgrown around the disc paper indicates that the essential oil of red betel leaf (*Piper crocatum*) has an inhibitory effect on *Propionibacterium acnes* bacteria. Antibacterial testing showed that red betel leaf essential oil inhibited the growth of *Propionibacterium acnes* bacteria by showing inhibition around the disc.

Table 3.
 Results of testing the antibacterial activity of essential oils by diffusion against
Propionibacterium acnes bacteria

material	concentrations	Inhibitory (mm)			Average (mm)
		I	II	III	
essential oil	5%	6,6	7,7	7,2	7,16
	10%	10,7	9,9	10,3	10,3
	20%	11,8	12,2	10,8	11,6
Benzoil Peroksida	2,5%	3,6	3,9	4,3	3,93
DMSO	1%	0	0	0	0

Based on the results in Table 3, it can be seen that the greater the concentration, the greater the inhibition of *Propionibacterium acnes* bacteria. The inhibitory concentration of red betel leaf essential oil obtained an average result at a concentration of 5%, namely 7.16 mm, which means that the diameter of the bright zone is less than 10 mm, which means there is no response to inhibition of bacterial growth.

CONCLUSION

The results of the characteristic test of red betel leaf essential oil showed that the oil had entered the good essential oil category and was by the existing literature which included organoleptic, pH testing, specific gravity, and soluble in ethanol. The results of bacterial inhibition testing of red betel leaf essential oil at a concentration of 5% are 7.16 mm which can be interpreted that the diameter of the bright zone is less than 10 mm, which means there is no response to inhibition of bacterial growth (Suhaimi, 2019). Red betel leaf essential oil at a concentration of 10%, 20% can inhibit 10.3 mm and 11.9 mm can mean that the diameter of the bright zone is more than 10 mm which means it can inhibit bacterial growth with weak criteria (Rachmawaty et al. 2009).

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