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Antibacterial activity test of patchouli leaf essential oil facemist (*Pogostemon cablin* Benth) against C*utibacterium acnes* bacteria

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A B S T R A C T

Cutibacterium acnes bacteria are gram-positive anaerobic bacteria that play a role in the pathogenesis of *Acne vulgaris* (acne). The use of facemist containing natural active ingredients of patchouli leaf essential oil (*Pogostemon cablin* Benth) can be an alternative in inhibiting the growth of acne-causing bacteria. This study aims to determine the antibacterial activity of patchouli leaf essential oil facemist (*Pogostemon cablin* Benth) against *Cutibacterium acnes* bacteria. The method used in making essential oils by steam distillation, chemical compound analysis using *Gas Chromatography-Mass Pectrometry* (GC-MS) and antibacterial activity testing using the disc diffusion method then formulated into a facemist product and characterized. The results of the study showed that patchouli leaf essential oil had met the standard requirements and contained several of the highest chemical compound components, namely *Patchouli alcohol* (15.71% and 10.16%), *Delta-Guaiene* (*CAS*) (7.14%), *Azulene, 1,2,3,4,5,6,7,8-Octahydro-1* (6.48%), *2-Butynyl-5-Hydroxy-3-Oxo-4-Hexanoic Acid Delta-Lactone* (5.89%) and *1,1,4,7-Tetramethyldecahydro-1H-Cyclopropa[E] Azulene-4-Ol* (5.17%) and the presence of antibacterial activity against *Cutibacterium acnes* bacteria with concentrations of 10%, 20%, 30%, 40% and 50% with inhibition zone diameters of 11.18 mm, 17.66 mm, 22.36 mm, 23.10 mm and 24.76 mm and the diameter of the antibacterial facemist inhibition zone with concentrations of 20% and 30% obtained results of 20.93 mm and 23.76 mm, then the facemist preparation showed characterization that was in accordance with the product's standard requirements. Based on the test results, it is known that the antibacterial face mist made from patchouli leaf essential oil has the ability to inhibit *Cutibacterium acnes* bacteria.

Keywords: Acne; *Cutibacterium acnes*; Facemist; GC-MS; Patchouli Leaf Essential Oil

1. INTRODUCTION

The skin is the outermost part of the body and is in direct contact with the environment. The skin can be a protector from ultraviolet (UV) radiation, dehydration and microorganisms. Activities that require direct exposure to sunlight can cause various skin problems, especially on the skin of the face which causes a dull face and the emergence of acne (*Acne vulgaris*) (Sari *et al.,* 2023). According to the Global Burden of Disease research, around 85% of teenagers are infected with acne aged 12-25 years (Nurhaini *et al.,* 2023).

Cutibacterium acnes bacteria are normal flora on human skin and their presence on facial skin and scalp reaches 105 organisms per cm² (Dewi and Deasy, 2021). *Cutibacterium acnes* plays a role in the pathogenesis of acne vulgaris by breaking down the triglyceride components of sebum into free fatty acids. These bacteria will enter the skin through pores that are clogged by a buildup of fat mixed with sweat, dust, and dirt, thus causing chronic inflammation of the polysebaceous unit. (Harefa *et al.,* 2022).

Patchouli (*Pogostemon cablin* Benth) has the potential as a natural solution for several skin health problems that are optimized by the antioxidant and antibacterial properties of this plant. The efficacy of the patchouli plant is supported by the content of phytochemical compounds such as flavonoids, saponins, tannins, glycosides, terpenoids, steroids and essential oils (Fadhilah *et al.,* 2023). The chemical components of patchouli essential oil are patchouli alcohol, α-guaiene, seychellene, and α-patchoulene (Kurniawan *et al.,* 2020). According to research by Adhayani *et al.* (2021) patchouli essential oil is able to inhibit *Staphylococcus aureus* (MRSA). This shows that patchouli leaves have the potential as an alternative natural active ingredient for facial skin care by being made into skincare preparations such as facemist.

Facemist is a spray-based skincare product that works to increase hydration in the outermost layer of skin. This product contains moisturizers that are easily absorbed into the skin and can remove excess oil. The advantage of this product is that it is easy to apply by spraying directly onto the skin, thus reducing contamination. Commercial

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skincare products generally contain chemicals that, if used over a long period, can cause side effects, including skin irritation, hyperpigmentation, and acne. Therefore, natural alternatives are needed to reduce these risks (Aspia *et al.,* 2024).

One ingredient that can be used as a cosmetic preparation is patchouli leaves, which have potential as antioxidants and antibacterials. Although the bioactivity of patchouli oil is known, its use in face mist formulations has not been widely studied. Considering the problems and potential benefits described previously, this study was designed to develop and comprehensively evaluate a natural-based face mist formulation using patchouli leaf essential oil (*Pogostemon cablin* Benth). This study included identification of bioactive compounds, formulation development, and assessment of antibacterial activity against *Cutibacterium acnes* using standardized laboratory methods.

2. MATERIAL AND METHODS

2.1 Materials

The tools and materials used in this study were beaker glass, measuring cup, petri dish, test tube, tube rack, Erlenmeyer flask, Bunsen flask, analytical balance, vortex, hot plate, magnetic stirrer, incubator, autoclave, biosafety cabinet, ose needle, cotton swab, aluminum foil, pH meter, vernier caliper, pycnometer, dropper pipette, spray bottle, glass slide, mica plastic, ruler, vial bottle, a series of distillation tools, Gas Chromatography-Mass Spectrometry (GC-MS), Patchouli leaves (*Pogostemon cablin* Benth), *Cutibacterium acnes* bacterial culture, Nutrient Agar media (NA), Muller Hinton Agar media (MHA), Dimethyl Sulfoxide (DMSO), distilled water, glycerin, Polyvinylpropylidine (PVP) Dimethyloldimethyl (DMDM) hydantoin, tween 80, Sodium sulfate (N_2SO_4), ethanol 90%, NaCl 0.9%, Barium chloride (BaCl), clindamycin, commercial facemist and disc paper.

2.2 Methods

This type of research is experimental, namely the manufacture of patchouli leaf essential oil (*Pogostemon cablin* Benth) by steam distillation, identification of essential oil components using GC-MS, making facemist formulations, then conducting antibacterial activity tests against *Cutibacterium acnes* bacteria using the disc diffusion method.

2.3 Study Area

This research was conducted in February 2025 in 4 different laboratories, namely the USU Medanense Herbarium Laboratory for plant identification, the Jember State Polytechnic Bioscience Laboratory for GC-MS analysis, the Medan Industrial Chemical Polytechnic Bioprocess Laboratory for the manufacture of essential oils and the State Islamic University of North Sumatra Microbiology Laboratory for the manufacture of facemist preparations, antibacterial activity tests and organoleptic tests.

2.4 Plant identification

Identification of patchouli plants (*Pogostemon cablin* Benth) was carried out at the Medanense Herbarium Laboratory, USU, Jalan Bioteknologi Medan. The identification process was carried out by observing the morphological characteristics of patchouli plants (*Pogostemon cablin* Benth) (Raharjeng and Anis. 2020).

2.5 Essential oil extraction

The process of making patchouli leaf essential oil (*Pogostemon cablin* Benth) is carried out using the steam-water distillation method using a series of steam distillation apparatus. Fresh patchouli leaves (*Pogostemon cablin* Benth) that have been cut into smaller parts are weighed as much as 3 kg and 30 grams of NaCl and the sample is put into a steam distillation kettle that has been filled with ±1500 ml of distilled water. The distillation process is carried out for 7 hours. The oil that comes out of the condenser is added with Na2SO4 to remove the water content, then the oil is left until it is clear or the Na_2SO_4 begins to settle and the oil is poured into a new test tube so that the oil is separated from the water and Na_2SO_4. The separated oil is stored in a vial bottle at a temperature of 4 ^{0}C before further testing (Variyana *et al.,* 2023).

2.6 Organoleptic test of essential oils

Organoleptic observation of patchouli leaf essential oil (*Pogostemon cablin* Benth) was carried out visually consisting of color, aroma and spots. Color observation was carried out by inserting 10 ml of essential

oil into a test tube then leaning the tube against a white background and observing it directly at a distance of 30 cm. Then the sample was observed directly using the sense of smell to determine the aroma of the essential oil (Sari *et al.,* 2023). The spot test is done by dripping oil on filter paper and leaving it for a few minutes. If there are no spots left on the filter paper, then the essential oil is pure (Regina and Rahma, 2017).

2.7 Determination of density

Determination of the density of essential oils is done by setting the temperature of the separated essential oils to 25 ^{0}C. Weigh the empty pycnometer to find out its mass. Then the essential oil is put into the pycnometer up to the limit mark and then weighed again (Fatimura and Reno, 2021). Density can be calculated using the following formula:

$$Density = \frac{M2-M1}{V} \quad (1)$$

Description:

M1 : Mass of empty pycnometer

M2 : Mass of pycnometer + essential oil

V : Volume of essential oil

2.8 Determination of solubility in ethanol

Determination of solubility in ethanol is done by inserting 1 ml of patchouli essential oil sample (*Pogostemon cablin* Benth) into a test tube, then adding 90% Ethanol drop by drop and shaking it, then recording the volume when the solution changes to clear. If there is no change up to a volume of 10 ml, a higher alcohol concentration can be used than before (Latifah *et al.,* 2023).

2.9 Analysis of chemical compound components of essential oils using GC-MS

This is done by turning on the helium carrier gas on the GC-MS device, turning on the GC and MS devices and turning on the computer as a device that displays identification data. Then set the GC-MS instrument format with a gas flow of 1 mL/minute, a split ratio of 2:1, an injection volume of 1 µL, an oven program of 50 ^{0}C for 3 minutes. The heating rate is 250oC for and the helium carrier gas is 280 ^{0}C. Furthermore, the data input of the sample name to be injected is carried out in the data information on the computer. Then the essential oil is injected into the GC-MS device. The process of identifying the chemical compounds of essential oils takes about 30-60 minutes. After the process is complete, the results can be seen on the computer in the form of chromatogram data (Maria *et al.,* 2023). Identification of essential oil compounds using the GC-MS method has the advantages of requiring a shorter time, good separation, high sensitivity and being effective for identifying volatile compounds (Fitri *et al.,* 2024).

2.10 Sterilization of tools

Glassware such as petri dishes are sterilized using an oven at a temperature of 165 ^{0}C for 60 minutes. The media used in this study were sterilized using an autoclave at a temperature of 121o with a pressure of 15 psi or 1 atm for 15-20 minutes. Tools such as loop needles are sterilized using direct flame heating (bunsen) (Andriani. 2016).

2.11 Media preparation

NA (*Nutrient Agar*) media as much as 2.3 g was dissolved with 100 ml of aquades using an Erlenmeyer flask and heated using a hot plate until dissolved. Then coated using aluminum foil and sterilized in an autoclave for 15 minutes at a temperature of 121 ^{0}C. After that, the media was removed and waited until it cooled, then the media was poured into a petri dish and waited until it solidified (Deswita *et al.,* 2021).The preparation of MHA media was carried out by weighing 2.28 grams of media, adding 60 ml of distilled water to an Erlenmeyer flask and then heating until dissolved. Furthermore, the media was coated using aluminum

foil and sterilized in an autoclave at 121 ^{0}C for 15 minutes. The cooled MHA medium was poured into a sterile petri dish of ±15 ml and left to solidify (Widyasanti *et al.,* 2024).

2.12 Bacterial rejuvenation

This is done by taking a culture of *Cutibacterium acnes* bacteria using a sterile ose needle, then scratching it on NA (*Nutrient Agar*) media and then storing it in an incubator at 37 ^{0}C for 24 hours (Sari *et al.,* 2023).

2.13 Gram stain

Gram staining of *Cutibacterium acnes* bacteria is done by taking 1 loop of bacteria and then scratching it on a glass object in a circle with a diameter of 2-3 cm and fixing it over a Bunsen burner until dry. The preparation is dripped with gentian violet for 1 minute and then rinsed with distilled water. Next, it is dripped with lugol for 1 minute and rinsed with distilled water. After that, it is dripped with 70% alcohol until clear. Next, safranin is dripped for 1 minute and rinsed again with distilled water. Then it is dried and observed using a microscope with a magnification of 100× (Hikma *et al.,* 2023).

2.14 Preparation of Mc Farland 0.5 standard solution

The preparation of 0.5 McFarland solution is done by taking 0.05 ml of BaCl solution and putting it into a test tube, then pipetting 9.95 ml of H_2SO_4 solution into the test tube and vortexing until homogeneous/cloudy (Rosmania and Fitri. 2020).

2.15 Bacterial suspension preparation

The preparation of *Cutibacterium acnes* bacterial suspension is done by taking a *Cutibacterium acnes* culture using a seril loop needle, then suspending it in a test tube containing 10 ml of 0.9% NaCl until the same turbidity is obtained as the Mc. Farland turbidity standard (Sari *et al.,* 2023).

2.16 Antibacterial activity test

Scratch a sterile cotton swab that has been dipped in *Cutibacterium acnes* suspension on the entire surface of the MHA medium until evenly distributed and let stand for 5-15 minutes. Dip the 6 mm diameter disc paper into essential oils with concentrations of 10%, 20%, 30%, 40%, 50%, clindamycin positive control and DMSO negative control, after which the disc paper is placed on the surface of the MHA media. Then incubated at 37 ^{0}C for 1x24 hours. After incubation, the inhibition zone is measured using a caliper (Widyasanti *et al.,* 2024). DMSO was used as a negative control because this solution does not have antibacterial properties, while Clindamycin was used as a positive control because this antibiotic has a broad antibacterial spectrum and is effective against various gram-positive and gram-negative bacteria. The inhibition zone according to Davis and Stout (1971) is categorized into several parts, namely <5 mm (weak category), 5-10 mm (moderate category), 10-20 mm (strong category),> 20 mm (very strong category) (Pananginan *et al.,* 2020). Based on the Pharmacopoeia IV edition (1995), the inhibition zone is said to be effective if it has a diameter of 14 mm-16 mm (Shafira *et al.,* 2023).

2.17 Facemist preparation

The manufacture of facemist products uses the 2 most effective concentrations in the previous antibacterial activity test of essential oils and as a negative control without the addition of essential oils. The preparation of patchouli essential oil (*Pogostemon cablin* Benth) antibacterial facemist is done by dissolving PVP as an additional ingredient that binds oil with water as much as 1.2 grams into a beaker with warm water and stirred until homogeneous. Next, 6 ml of glycerin as a moisturizer and softener of the formulation and 0.18 grams of DMD Hydantion as a product preservative are added to the beaker containing the previous PVP solution and stirred until homogeneous. Then the formulation is put into a spray bottle and added essential oil as an active substance with a predetermined concentration. Then each formula is added with tween 80 as a solvent surfactant and formulation stabilizer and then shaken vigorously for ± 5 minutes until homogeneous.

The final stage is to add 30 ml of distilled water as a product base to each formula and shaken until evenly mixed (Sari *et al.,* 2023).

Table 1. Formulation of antibacterial facemist preparation of patchouli essential oil (*Pogostemon cablin* Benth)

Ingredient	Usage	Formula Concentration		
		F1	F2	K-
Patchouli leaf essential oil	Active substance	X	X	0%
Glycerin	Moisturizer and Emollient	6 ml	6 ml	6 ml
PVP (Polivinilpirolidin)	Supplements	1.2 gr	1.2 gr	1.2 gr
DMDM Hydantion	Preservative	0.18 gr	0.18 gr	0.18 gr
Tween 80	Surfactan	3 ml	3 ml	3 ml
Aquadest	Basis	Ad 30	Ad 30	Ad 30

2.18 Organoleptic test of facemist preparation

Organoleptic test is conducted by observing the facemist preparation visually to see the physical appearance of the preparation by observing the texture, color and smell of the preparation that has been made.

2.19 Homogeneity test

Homogeneity test can be done by spraying the facemist preparation on a transparent glass slide. The preparation can be said to be homogeneous if there are no coarse particles and shows the same arrangement (Andalia *et al.,* 2024).

2.20 pH test

Determination of pH value is done using a pH meter, inserted into the sample, left to stabilize and recorded the pH listed. Facemist preparations must meet the skin pH criteria according to SNI 16-4399-1996 standards, namely 4.5-7.5. A pH that is too high can cause dry and itchy skin while a pH that is too low can cause skin irritation (Aspia *et al.,* 2024).

2.21 Spray spread power test

The spray spread power test was carried out by spraying the facemist preparation on mica plastic at a distance of 5 cm. Then the spray diameter was measured using a ruler. Good spray spread power is 5-7 cm (Tricamila *et al.,* 2024).

2.22 Dry time test

The facemist preparation is sprayed on the lower wrist to determine skin absorption for 5 minutes, then the time required for the liquid to dry is calculated. The preparation can be said to be good if it meets the drying time standard, which is less than 5 minutes (Rini *et al.,* 2024).

2.23 Irritation test

The irritation test is conducted by means of an open patch test by spraying the facemist preparation on the back of the ear, leaving it open and observing it. A positive irritation reaction is indicated by redness, itching or swelling in the part of the ear that was treated (Oktarina *et al.,* 2023).

2.24 Antibacterial activity test of facemist

A suspension of *Cutibacterium acnes* bacteria was taken using a sterile cotton swab, scratched onto the surface of the MHA media until evenly distributed and left for 5-15 minutes. Then the paper disc was soaked for 10 minutes in the facemist preparation with 2 of the best essential oil concentrations and a positive control using a commercial anti-acne facemist and a negative control using a preparation without essential oil. After that, it was placed on the surface of the MHA medium. Then incubated at 37oC for 1×24 hours. This test was repeated 4 times. The presence of antibacterial activity is indicated by the presence of a clear zone around the disc (Sari *et al.,* 2023).

2.25 Data analysis

The data from the antibacterial activity test of patchouli leaf essential oil (*Pogostemon cablin* Benth) antibacterial facemist against *Cutibacterium acnes* bacteria were analyzed statistically using the *One Way Anova* method (one-way analysis of variance) with the *Statistical Product Services Solution* (SPSS) program with a confidence level of 95% or $\alpha = 0.05$. The analysis includes normality tests, homogeneity tests, ANOVA tests and further tests are carried out if the results are significant.

3. RESULTS AND DISCUSSION

3.1 Identification of patchouli leaces (*Pogostemon cablin* Benth)

Plant identification was conducted at the Medanense Herbarium Laboratory (MEDA), Jl. Biotechnology, No.1, University of North Sumatra, Medan. The test results are stated in letter No. 504/MEDA/2025. The plant used is patchouli leaves (*Pogostemon cablin* (Blanco.) Benth.). This shows that the plants used in this test are genuine patchouli leaves, so they are suitable for the purpose of analyzing the potential of patchouli leaf essential oil.

3.2 Organoleptic test results of patchouli leaf essential oil

The distillation results of making patchouli leaf essential oil (*Pogostemon cablin* Benth) with a leaf sample of 3 kg produced 150 mL of oil with a reddish brown color. Characterization of patchouli leaf essential oil (*Pogostemon cablin* Benth) includes organoleptic observations, determination of density, determination of solubility in ethanol and GC-MS (*Gas Chromatography-Mass pectrometry*) analysis. This aims to determine the quality of the essential oil produced. The results of the organoleptic test can be seen in the following table.

Table 2. Organoleptic test results of patchouli leaf essential oil (*Pogostemon cablin* Benth)

Parameter Test	Result
Colour	Reddish Brown
Aroma	Typical of the essential oil of patchouli leaves (*Pogostemon cablin* Benth) which is strong and not pungent
Spotting	Positive (leaves no spots)

Based on the data in table 2, it shows that patchouli leaf essential oil has a reddish brown color, the aroma produced is typical of strong patchouli leaves and spots that do not leave marks on the filter paper. Based on these results, it is in line with research conducted by Kautsarah *et al* (2023) that in accordance with the Indonesian National Standard which states that patchouli leaf essential oil has a light yellow to reddish brown color (SNI 06-2385-2006). The distinctive aroma of patchouli leaves and oil blends with the filter paper without leaving spots indicating that the patchouli leaf essential oil (*Pogostemon cablin* Benth) obtained has volatile and pure properties. This is in line with research conducted by Regina and Rahma (2017) which stated that one of the properties of essential oils is that they evaporate easily.

3.3 Determination of density

The determination of the density of patchouli leaf essential oil (*Pogostemon cablin* Benth) which has been calculated using a pycnometer that has been filled with essential oil that has been weighed is worth 20.5009 gr minus the weight of the empty pycnometer which is 15.6994 gr produces a density of patchouli leaf essential oil (*Pogostemon cablin* Benth) of 0.9603 gr/ml. The density value of good essential oils generally does not exceed 1,000 grams/mL. According to the SNI No.06-2385-2006 standard, patchouli essential oil has a density of 0.952-0.975 grams/mL (Yuliana *et al.,* 2020). The results of the analysis of the density of essential oils from patchouli leaves show that patchouli leaf essential oil is classified as pure.

3.4 Solubility in ethanol

The results of the solubility test of patchouli leaf essential oil (*Pogostemon cablin* Benth) in 90% ethanol showed that the patchouli leaf essential oil (*Pogostemon cablin* Benth) was able to dissolve and was clear with a ratio of 1:9, namely 1 ml of essential oil with 9 ml of 90% ethanol. Based on the solubility value in ethanol, the patchouli leaf essential oil has met the SNI requirements. In accordance with the SNI 06-2385-2006

standard which stipulates the solubility of patchouli leaves in 90% ethanol, namely 1:10. Based on research by Amaliah *et al* (2022), it was stated that the quality of patchouli leaf essential oil can be seen from its solubility level in ethanol.

3.5 Chemical compound analysis using GC-MS *(Gas Chromatography-Mass Spectrometry)*

Based on the GC-MS (Gas Chromatography-Mass Spectrometry) test at the Bioscience Laboratory of Jember State Polytechnic, the results of the analysis of patchouli leaf essential oil samples (Pogostemon cablin Benth) found 35 chemical compound components with the five highest compounds with concentrations above 5% as seen in the following table.

Table 3. GC-MS Analysis data of chemical compounds of patchouli leaf essential oil (*Pogostemon cablin* Benth)

Peak#	R. Time	Area%	Name
1	2.248	6.48	Azulene, 1,2,3,4,5,6,7,8-Octahydro-1
2	2.643	7.14	Delta-Guaiene (CAS)
3	4.022	5.17	1,1,4,7-Tetramethyldecahydro-1H-Cyclopropa[E] Azulene-4-Ol
4	4.733	10.16	Patchouli alcohol
5	5.374	15.71	Patchouli alcohol
6	6.197	5.89	2-Butynyl-5-Hydroxy-3-Oxo-4-Hexanoic Acid Delta-Lactone

The compounds shown in table 3 start from the highest, namely *Patchouli alcohol* (15.71% and 10.16%), *Delta-Guaiene* (*CAS*) (7.14%), *Azulene, 1,2,3,4,5,6,7,8-Octahydro-1* (6.48%), *2-Butynyl-5-Hydroxy-3-Oxo-4-Hexanoic Acid Delta-Lactone* (5.89%) and *1,1,4,7-Tetramethyldecahydro-1H-Cyclopropa[E] Azulene-4-Ol* (5.17%). These five compounds are included in sesquiterpene compounds. Sesquiterpene compounds have antibacterial, anti-inflammatory and anti-bacterial properties (Widyaningrum *et al.,* 2020). *Patchouli alcohol* is the main component of patchouli essential oil and determines the aroma of patchouli essential oil. The content of *Patchouli alcohol* in patchouli leaf essential oil can function as an antioxidant, antibacterial and antifungal. This is in line with the research of Nurjanah *et al* (2019) which states that the Patchouli alcohol compound is able to inhibit the growth of *P. acnes, S. aureus, S. epidermidis* and *Micrococcus luteus* bacteria. *Delta-Guaiene* has the same antibacterial activity as the *Patchouli alcohol* compound because it is a sesquiterpene compound. The *Delta-Guaiene* compound of patchouli oil has been tested against S. aureus and *S. epidermidis* bacteria and has antibacterial activity with an average diameter of the inhibition zone formed in *S. aureus* bacteria of 11.2 mm and in *S. epidermidis* bacteria of 5.16 mm (Kurniawan *et al.,* 2020). *Azulene compound*, *1,2,3,4,5,6,7,8-Octahydro-1* is an aromatic hydrocarbon. This compound has potential in the field of medicine, namely as an anti-inflammatory, anti-allergic, antifungal and antimicrobial (Bakun *et al.,* 2021). This substance is able to damage the fungal cell membrane. The antimicrobial activity of this compound has been studied and is able to inhibit the growth of *E. coli* and *P. aeruginosa* bacteria (Alhafidz *et al.,* 2022).

2-Butynyl-5-Hydroxy-3-Oxo-4-Hexanoic Acid Delta-Lactone including sesquiterpene compounds and can be classified as intramolecular esters and have cytotoxic, anti-inflammatory, antiplasmodial, antiviral and antibacterial properties (Mazur and Dorota. 2022). This compound is an organic substance that contributes to a specific taste and odor. Several studies have shown that the *Delta-lactone* structure has antibacterial activity against various types of bacteria, including strains of *E. coli* and *Bacillus subtilis*. This activity depends on the specific structure of the *Delta-lactone* and the length of the polysaccharide lipid (LPS) chain in the bacteria (Kowalczyk *et al*., 2021). *1,1,4,7-Tetramethyldecahydro-1H-Cyclopropa[E] Azulene-4-Ol* (also known as *globulol*) and belongs to the sesquiterpene family, known to have antibacterial activity. Most of its derivatives are also sesquiterpenes, such as *alpha-caryophyllene (alpha-humulene)* and *alpha-calacorene* (Hasim *et al.,* 2014). This is in line with research by Astiani *et al* (2014) which stated that the *1,1,4,7-Tetramethyldecahydro-1H-Cyclopropa[E] Azulene-4-Ol* (*globulol*) content in *Eucalyptus Pellita* essential oil has the potential to

inhibit *E. coli* and *S. aureus* bacteria. Based on research by Fauzi *et al* (2021) it was stated that the globulol compound has the potential to effectively inhibit viruses, with a free energy of -7.23 kcal/mol.

3.6 Gram stain

Before the antibacterial activity test, gram staining of bacteria was performed and the following results were obtained:

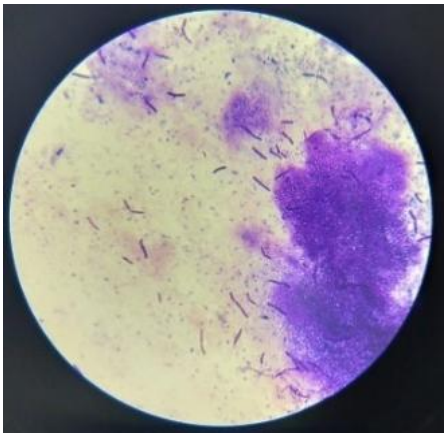

Figure 1. Gram staining of *Cutibacterium acnes* bacteria 100x magnification

Based on the results of gram staining in Figure 1 *Cutibacterium acnes* bacteria have a characteristic purple color which indicates that *Cutibacterium acnes* bacteria are gram-positive bacteria with a bacillus (rod) shape and a spread-out bacterial arrangement (Nurhaini *et al.,* 2023). Gram-positive bacteria will appear purple when observed under a microscope after Gram staining. This is because the cell walls of gram-positive bacteria are composed of thicker peptidoglycan than those of gram-negative bacteria. This thicker peptidoglycan allows the crystal violet dye to be retained even after bleaching (Hamidah *et al.,* 2019).

3.7 Antibacterial Activity Test of Patchouli Leaf Essential Oil (*Pogostemon cablin* Benth)

The antibacterial activity test of patchouli leaf essential oil is an initial test to determine the concentration that has the activity to inhibit bacteria. Based on the results of the antibacterial activity test of patchouli leaf essential oil (*Pogostemon cablin* Benth) against *Cutibacterium acnes* bacteria, the results of the inhibition zone diameter are as follows:

Table 4. Data from the antibacterial activity test of patchouli leaf essential oil (*Pogostemon cablin* Benth) against *Cutibacterium acnes* bacteria

Concentration (%)	Bacterial Inhibition Zone *Cutibacterium acnes* (mm)				Average Inhibition Zone (mm)	Criteria
	U1	U2	U3	U4		
K (+)	38.35	42.45	32.85	37.8	37.86	Very Strong
K (-)	0	0	0	0	0	No Activity
10	11.5	10.6	11.15	11.5	11.18	Strong
20	17.75	18.2	17.1	17.6	17.66	Strong
30	23.65	19.45	24.15	22.2	22.36	Very Strong
40	21.5	21.8	24.25	24.85	23.1	Very Strong
50	25.75	24.65	24.45	24.2	24.76	Very Strong

The results of the antibacterial activity test of patchouli leaf essential oil in table 4 show that the positive control produces an inhibition zone of 37.86 mm. Clindamycin produces a very strong zone because this antibiotic is a derivative of lincomycin which has a broad spectrum, the mechanism of action of this antibiotic is by inhibiting bacterial protein synthesis (Emelda *et al.,* 2021). The negative control produces an inhibition zone of 0 mm which means it does not show the ability to inhibit bacterial growth. The average inhibitory power values at concentrations of 10%, 20%, 30%, 40%, and 50% are 11.18 mm, 17.66 mm, 22.36 mm, 23.1 mm and 24.76. This inhibitory ability is due to the presence of several chemical compounds in it, including *Patchouli alcohol, Delta-Guaiene*, *Azulene, 1,2,3,4,5,6,7,8-Octahydro-1*, *2-Butynyl-5-Hydroxy-3-Oxo-4-*

Hexanoic Acid Delta-Lactone and *1,1,4,7-Tetramethyldecahydro-1H-Cyclopropa[E] Azulene-4-Ol* which are known to be included in sesquiterpene compounds which have the property of damaging bacterial cell membranes by binding to enzyme proteins so that they are able to inhibit bacterial cell growth (Kurniawan *et al.,* 2020).

Based on these data, it can be stated that the higher the concentration of patchouli leaf essential oil (*Pogostemon cablin* Benth), the greater its ability to inhibit the growth of *Cutibacterium acnes* bacteria and the inhibition zone formed. This can be seen in the graph below.

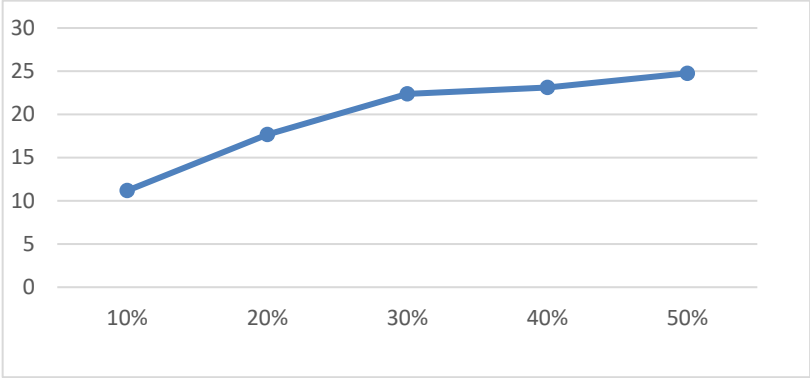

Figure 2. Antibacterial activity test graph of patchouli leaf essential oil (*Pogostemon cablin* Benth) against *C. acnes* bacteria

The inhibition zone diameter data obtained were then analyzed using the ANOVA table after passing the normality test (Shapiro-Wilk) and homogeneity test (Levene's) and significance so that a further Duncan test was carried out with the following results:

Table 5. Diameter of inhibition zone of patchouli leaf essential oil (*Pogostemon cablin* Benth) against *Cutibacterium acnes* bacteria

Treatment	Average
Negative Control	0.0 ± 0.0 ^{e}
10%	11.18 ± 0.42 ^{d}
20%	17.66 ± 0.45 ^{c}
30%	22.36 ± 2.11 ^{b}
40%	23.10 ± 1.69 ^{b}
50%	24.76 ± 0.68 ^{b}
Positive Control	37.86 ± 3.93 ^{a}

The data in table 5 are the results of the Least Significant Difference (LSD) test, which was conducted to determine which treatment pairs were significantly different. Data followed by different notations indicate a significant difference ($P > 0.05$), and the ± symbol indicates the standard deviation from the mean.

The results of the Duncan test showed that the concentrations of 10%, 20%, 30%, 40% and 50% were significantly different from the negative control treatment, because the negative control did not show any antibacterial activity. The concentration treatments of 10% and 20% were significantly different from the concentrations of 30%, 40% and 50%. This can occur because there is an additional concentration of patchouli leaf essential oil, where the higher the concentration of oil used, the greater the data produced.

3.8 Facemist organoleptic test

Based on the diameter of the inhibition zone that has been obtained, the manufacture of facemist products with 2 best concentrations was carried out, then the characterization of the preparation was carried out which included organoleptic tests, homogeneity tests, pH tests, spray spread tests, drying time tests and irritation tests. Organoleptic tests include aroma, color and texture which can be seen in Table 6.

Table 6. Organoleptic data of antibacterial facemist from patchouli leaf essential oil (*Pogostemon cablin* Benth)

Concentration	Aroma	Color	Texture
0%	Unscented	Clear	Liquid
20%	Special aroma of patchouli leaves	Yellow	Liquid
30%	Special aroma of patchouli leaves	Dark Yellow	Liquid

The shape and appearance of the antibacterial facemist for patchouli leaf essential oil (*Pogostemon cablin* Benth) can be seen in Figure 3.

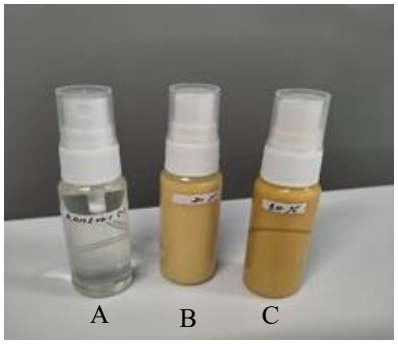

Figure 3. Facemist preparation (A. 0%, B.20%, C.30%)

Based on the data in table 6, it can be seen that the aroma of the facemist preparation is typical of patchouli leaves. In the color test, there are differences in color at each concentration of the formulation, namely, clear, light yellow and dark yellow. This shows that the higher the concentration of essential oil, the more concentrated the color of the preparation. The results of the texture test on the patchouli leaf essential oil facemist preparation (*Pogostemon cablin* Benth) at concentrations of 0%, 20% and 30% have a liquid texture. At concentrations of 20% and 30% it appears thicker due to the addition of patchouli leaf essential oil. In the opinion of Wulandari and Ringga (2023) facemist is a cosmetic preparation that is liquid and contains natural ingredients that are beneficial for skin health.

3.9 Homogeneity Test

The homogeneity test aims to ensure that all ingredients used in the facemist preparation can be mixed evenly without any particles and coarse grains that are felt when touched. The test results can be seen in Table 7.

Table 7. Homogeneity test data of antibacterial facemist of patchouli leaf essential oil (*Pogostemon cablin* Benth)

Concentration	Homogeneity
0%	No coarse particles
20%	No coarse particles and separation of the preparation occurs
30%	No coarse particles and separation of the preparation occurs

The results of the homogeneity test in Table 6 show that all face mists do not contain coarse grains. However, after the product was left for ± 3 hours at room temperature, there was a separation of the preparation, where the active ingredient of the essential oil used was separated from the aquades-based formulation. Patchouli leaf essential oil contains sesquiterpene compounds which are hydrocarbon compounds that are hydrophobic (insoluble in water) (Angin, 2017). According to Maulana *et al.* (2017), the criteria for a good product are if there are no solid particles that clump together or separate, then the preparation is considered homogeneous. This separation indicates physical instability of the formulation, so that the face mist product that has been made does not meet the homogeneity requirements.

3.10 pH test

pH test on the facemist preparation was carried out using a pH meter. The results of the pH test of the essential oil facemist can be seen in the following Table 8.

Table 8. Data on the results of the ph test of the antibacterial facemist of patchouli leaf essential oil (*Pogostemon cablin* Benth)

Concentration	pH
0%	6. 19
20%	4. 81
30%	4. 88

Based on the data in table 8, it shows that the pH of the 0%, 20% and 30% concentration preparations are respectively, 6.19; 4.81 and 4.88. If a preparation has a pH that is too acidic, it can cause the skin to feel sore due to inflammation, while a pH that is too alkaline can cause the skin to become scaly and sensitive. The skin pH standard according to SNI 16-4399-1996 is 4.5-7.5 (Aspia *et al.,* 2024). Based on the data from the pH test results, the face mist preparation has met the SNI standard so that it is safe for facial skin.

3.11 Spray spread power test

The purpose of the spray spreadability test is to determine the spread of the facemist area when used. The results of the spray spreadability test of patchouli leaf essential oil facemist can be seen in table 9.

Table 9. Data on the results of the spray spreadability test of patchouli leaf essential oil antibacterial facemist (*Pogostemon cablin* Benth)

Concentration	Spray spread power
0%	6 cm
20%	5.8 cm
30%	5 cm

The spray spread of the formulation in Table 8 with concentrations of 0%, 20% and 30% obtained a spread of 6 cm, 5.8 cm and 5 cm. The diameter of the spreading power at 20% and 30% concentrations is smaller due to the addition of essential oils which makes the formulation thicker than the 0% concentration. This has met the spray spread standard for topical preparations, which is 5-7 cm. This is in line with the research of Sari *et al* (2023) which states that the higher the concentration of essential oil used in the formulation, the smaller the diameter of the spray spread.

3.12 Dry time test

Drying time testing was conducted to determine how long it takes for the facemist preparation to dry after being applied to the skin. The test results can be seen in table 10.

Table 10. Drying time test data for antibacterial facemist with patchouli leaf essential oil (*Pogostemon cablin* Benth)

Concentration	Drying time of facemist preparation
0%	3:20
20%	4:13
30%	4:25

Based on the results of the drying power test listed in table 10, the 0%, 20% and 30% formulations had a drying time that did not exceed the optimal time, namely 5 minutes. Based on research Ahmad dan Nur (2025) it was stated that if the drying time is less than 5 minutes, it can minimize the growth of microorganisms and if it exceeds 5 minutes, it is possible for microorganisms to grow because the skin is still wet. The difference in drying time between each formulation is influenced by the concentration of essential oil added. The higher

the concentration of essential oil added, the longer the drying time. This is because oil is absorbed more slowly by the skin than water (Andalia *et al.,* 2024).

3.13 Irritation test

Irritation testing is carried out to determine the potential of the preparation to cause skin irritation and to ensure product safety. The results of the patchouli leaf essential oil facemist irritation test can be seen in Table 11.

Table 11. Data on the results of the patchouli leaf essential oil antibacterial facemist irritation test (*Pogostemon cablin* Benth)

Concentration	Irritation test assessment
0%	Not irritating
20%	Not irritating
30%	Not irritating

Based on the results of the irritation test in table 11, the results of the irritation test by spraying the preparation on the back of the panelist's ear with a product concentration of 0%, 20% and 30% showed that all preparations did not cause irritation, because there were no symptoms of irritation such as redness, itching or swelling. So it can be concluded that the essential oil facemist preparation made is safe to apply to the skin.

3.14 Antibacterial activity test of patchouli leaf essential oil facemist (*Pogostemon cablin* Benth)

This antibacterial activity test was conducted using the disc diffusion method with 2 concentrations of 20% and 30%, negative control using facemist without essential oils and positive control using commercial facemist. The test results can be seen in Table 12.

Table 12. antibacterial activity test results of patchouli leaf essential oil facemist (*Pogostemon cablin* Benth) against *Cutibacterium acnes* bacteria

Concentration (%)	Bacterial Inhibition Zone *Cutibacterium acnes* (mm)				Average Inhibition Zone (mm)	Criteria
	U1	U2	U3	U4		
K (+)	15.8	16.05	13.9	13.4	14.78	Strong
K (-)	10.45	11.1	9.9	11.25	10.67	Strong
20	21.45	20.15	21.25	20.9	20.93	Very Strong
30	23.55	22.4	24.4	24.7	23.76	Very Strong

Based on the data, it shows that the higher the concentration of patchouli leaf essential oil (*Pogostemon cablin* Benth) in the preparation, the greater its ability to inhibit *Cutibacterium acnes* bacteria. This can be seen in the diagram below.

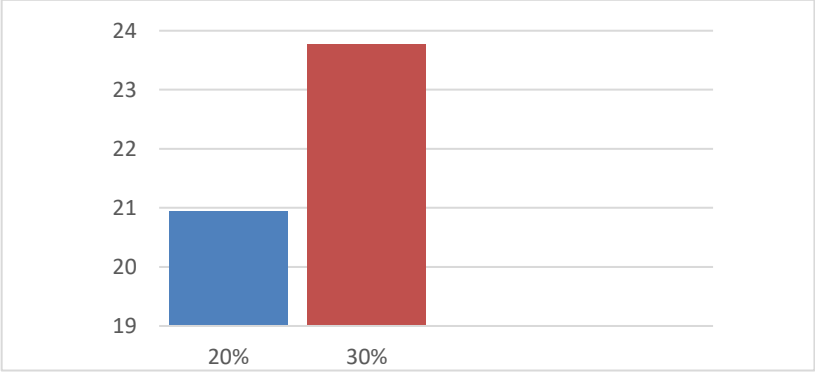

Figure 4. Antibacterial activity test diagram of patchouli leaf essential oil facemist (*Pogostemon Cablin* Benth) against *C. acnes* bacteria

Table 11 shows the results of the bacterial inhibition zone of the facemist preparation with a concentration of 20% and 30% showing an average result of 20.93 mm and 23.76 mm. The positive control produced a smaller inhibition zone, namely 14.78 mm. The clear zone in the positive control using commercial products produced a smaller inhibition zone compared to all test preparations because the positive control contained limited antibacterial compounds, while the product preparations used active essential oils. The negative control also had an inhibition power of 10.67 mm. This can occur allegedly due to the presence of a formulation ingredient that can inhibit and kill bacteria, namely DMDM hydantoin. According to Saputra *et al* (2023) DMDM hydantoin is a preservative that releases a little formaldehyde which can kill microorganisms, this ingredient can prevent the growth of microorganisms in skin care products or cosmetics.

The large clear zone formed at a concentration of 20% and 30% is due to the addition of patchouli leaf essential oil (*Pogostemon cablin* Benth) which contains active compounds that inhibit bacteria by damaging cell walls, inhibiting bacterial folic acid metabolism, inhibiting bacterial protein biosynthesis and inhibiting bacterial DNA replication. These compounds, such as *Patchouli alcohol* and other compounds in patchouli essential oil, which have the ability to inhibit bacteria are *α-Guaiene* and *α-Selinene* (Adhayani *et al.,* 2021). According to Alouw *et al* (2022) the higher the concentration of the active ingredient used, the greater the active compound content.

The inhibition zone diameter data obtained were then analyzed using the ANOVA table after passing the normality test (Shapiro-Wilk) and homogeneity test (Levene's) and significance so that a further Duncan test was carried out with the following results:

Table 13. Inhibition zone diameter of antibacterial facemist patchouli leaf essential oil (*Pogostemon cablin* Benth) against *Cutibacterium acnes* bacteria.

Treatment	Average
Negative Control	10.67 ± 0.62 ^{d}
Positive Control	14.78 ± 1.33 ^{c}
20%	20.93 ± 0.57 ^{b}
30%	23.76 ± 1.03 ^{a}

The data in table 13 are the results of the Least Significant Difference (LSD) test, which was conducted to determine which treatment pairs were significantly different. Data followed by different notations indicate a significant difference ($P > 0.05$), and the ± symbol indicates the standard deviation from the mean. Duncan's test results showed that the 20% and 30% concentration treatments were significantly different from the negative control treatment. Then the 20% and 30% treatments also showed significantly different results.

4. CONCLUSION

Patchouli leaf essential oil (*Pogostemon cablin* Benth) is able to inhibit the growth of *Cutibacterium acnes* bacteria. According to the Davis and Stout (1971) standards, the test results of patchouli leaf essential oil at a concentration of 10%-20% are classified as strong and at a concentration of 30%-50% are classified as very strong. The characteristics of the antibacterial *facemist* from patchouli leaf essential oil (*Pogostemon cablin* Benth) which include organoleptic (aroma, color and texture), homogeneity, pH, spray spread, drying time and irritation test have met the quality standard requirements for *facemist* preparations. The *facemist* preparation from patchouli leaf essential oil (*Pogostemon cablin* Benth) is also able to inhibit the growth of *Cutibacterium acnes* bacteria at concentrations of 20% and 30% with an inhibition zone diameter of 20.93 mm and 23.76 mm, according to the Davis and Stout (1971) standards, both concentrations are included in the very strong category.

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