ANTIBACTERIAL ACTIVITY TEST OF TEA TREE AND LEMON OIL COMBINATION AND ITS FORMULATION IN CREAM PREPARATION

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ABSTRACT

Tea tree oil contains hydrocarbon compounds, namely terpenes, especially monoterpenes, sesquiterpenes, and alcohols. Terpinen-4-ol is the main component of tea tree oil, which has antimicrobial activity. Lemon oil is useful as an antibacterial because it contains flavonoids that prevent attacks from pathogenic bacteria. This study aims to determine whether tea tree and lemon oil have antibacterial activity against *Propionibacterium acnes* bacteria and can be formulated into cream preparations. The antibacterial activity test of the combination of tea tree oil and lemon oil in the 1: 1, 1: 2, and 2: 1 ratio was carried out in vitro against *Propionibacterium acnes* bacteria by disc diffusion method and using virile gel as a positive control. The cream stability test is replicated using the cycling test method with organoleptic test parameters, homogeneity, pH, dispersion, cream type testing, viscosity, and flow properties. The stability test results with the cycling test method showed good stability results, including organoleptic tests, homogeneity, spreadability, pH, emulsion type, viscosity, and flow properties. The combination of tea tree and lemon essential oils in a 1:1, 1:2, 2:1 ratio has antibacterial activity against *Propionibacterium acnes* bacteria with inhibition zones diameter ±1,23 cm, ±1,12 cm, and ±1,12 cm and control has ±1,53 cm. Combining tea tree and lemon essential oil in a 1:1, 1:2, and 2:1 ratio has antibacterial activity against *Propionibacterium acnes* bacteria and can be formulated into cream preparations.

Keywords: tea tree oil, lemon oil, antibacterial, *Propionibacterium acnes*, cream.

INTRODUCTION

Acne vulgaris is a fascinating disorder of the pilosebaceous unit that most commonly affects adolescents. It presents with a pleomorphic array of lesions, including comedones, papules, pustules, and nodules of varying severity (Zaenglein *et al*., 2016). The occurrence of oil buildup in the oil glands of human skin is the main factor that causes acne bacteria to grow and trigger inflammation (Hafsari, 2015). Excessive fat deposits on the surface area of the skin such as on the back, neck, chest, and face, can make clogged pores and cause acne (Yulianti *et al*., 2015). *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* are bacteria that can cause acne (Olufemi *et al*., 2013).

Propionibacterium acnes is a class of gram-positive bacteria that cause opportunistic infections by making lipase support the formation of acne (Warren Levinson, 2004). Acne generally occurs in adolescents, with an incidence of about 85%. The highest prevalence of 14-17 years old in women is 83-85%, and 16-19 years in men 95-100% (Sifatullah, 2021).

Essential oils (EOs) are important aromatic components of herbs and spices and their biological activities have been known and utilized since ancient times in perfumery, food preservation, flavoring, and medicine. The antimicrobial activities of essential oils clearly indicates that, they are more acceptable because of their unique antibacterial, antifungal and antiviral properties (Herman *et al*., 2019).

Tea tree oil is one of plant that have essential oils. It is the result of the distillation of the Melaleuca alternifolia leaves steam distillation method and is useful as an antimicrobial (Nugraheni *et al*., 2019). Tea tree oil contains hydrocarbon compounds namely terpenes, monoterpenes, sesquiterpenes, and alcohol. Tea tree oil has a 40% terpinen-4-ol compound with antibacterial and anti-inflammatory activity (Li *et al*., 2016). The anti-inflammatory properties of tea tree oil can reduce the production of superoxide and proinflammatory cytokines shown by a decrease in inflammation (Nugraheni *et al*., 2019). Tea Tree oil has antibacterial properties on Propionibacterium acnes with a MIC value of 0.05% - 0.63% (Carson *et al*., 2006). Lemon has benefits as an antibacterial, antidiabetic, anticancer, and antiviral (Hindi et al, 2013). In lemons, some flavonoids are useful in preventing the entry of pathogens including bacteria, viruses, and fungi (Mierziak et al., 2014).

The content of alkaloids, essential oils, sesquiterpenes, and other terpene compounds in lemon serves antibacterial and fungal functions (Ghosh *et al*., 2011). The mechanism of action of alkaloids as antibacterial is to inhibit cell wall synthesis and trigger lysis in cells so that cells will die and cannot multiply (Erlinda, T., 2012). Flavonoids represent a significant class of secondary metabolites that are widely distributed in various plants. Many of these compounds exhibit varying degrees of inhibitory activity against a multitude of pathogenic bacteria. Of particular note is their capacity to enhance the antimicrobial activities of antimicrobial agents and, in some cases, even reverse antimicrobial resistance (AMR). Various antibacterial mechanisms have been documented for plant flavonoids, including the inhibition of DNA, proteins, and cell envelope biosynthesis, as well as the damage to the cell membrane (Gorniak, 2019; Farhadi *et al*., 2019). The main compound terpinene-4-ol in tea tree oil as an antibacterial causes an increase in cell membrane permeability which can interfere with bacterial cell growth (Karimah *et al*., 2021). Extracted essential oils from tea tree plants were shown to show the most effective inhibition against *P. acnes* and *S. epidermidis*, the minimum inhibitory concentrations (MICs) value of *P. acnes* 0.128%, and *S. epidermidis* 0.127% using the disc diffusion method and MIC test (Karimah *et al*., 2021).

Based on research (Ji Hye et al., 2011) on antioxidant and antimicrobial effects of lemon and eucalyptus essential oils against skin floras, lemon essential oil is effective against acne-causing bacteria and antioxidants with inhibitory measures in the clear zone of 13.02±0.08 mm against *P. acnes* and 10.07±0.06 mm against *S. aureus*. At 4µL / mL lemon oil has a diameter of antibacterial activity on paper disk with a diffusion disk agar method of 23.0 ± 0.25 mm (Ulfa, 2014). The disc paper diffusion method was chosen because it tests faster in disc preparation (Nurhayati *et al*., 2020). In this study, an oil-in-water type cream base (m/a) was chosen because the oil-in-water type (m/a) is easy to use on the skin and leaves a sense of comfort compared to the water-in-oil cream type (a/m). The kind of oil in water (m/a) is also easily rinsed using water, when applied to the skin type (m/a) will experience evaporation and cause an increase in the concentration of water-soluble drugs therefore stimulating absorption into the skin tissue.

This study aims to determine whether tea tree and lemon oil have antibacterial activity against *Propionibacterium acnes* bacteria and can be formulated into cream preparations. The novelty of this research is to combine 2 essential oils (tea tree essential oil and lemon oil) in the form of a cream where no previous research has been done.

RESEARCH METHODS

Equipment and Materials

The tools used in this study include analytical balances (Ohaus Corporation Model CP214), glassware from caliper Pyrex (Krisbrow), autoclave (All American 25x), incubator (Memmert), cream pot, pH meter (Mettler Toledo), Brookfield RV viscometer. The

ingredients used include Tea tree and lemon oil (Rumah Atsiri Indonesia Jl. Watusambang, Plumbon, Kec. Tawangmangu, Karanganyar Regency, Central Java 57792), Stearic Acid (CV. Mustika Lab), Ethyl Alcohol (CV. Mustika Lab), Propylenglikol (CV. Mustika Lab), Triethanolamine (CV. Mustika Lab), Glycerin (CV. Mustika Lab), Verile acne gel (Medikon Prima Laboratories), Methylparaben (CV. Mustika Lab), Sodium metabisulfite (CV. Mustika Lab), Propyl parabens (CV. Mustika Lab), Sterile Aquadest (PT Brataco Indonesia), Medium Nutrient Agar (NA), and *Propionibacterium acnes* (ATTCC 6919).

Research Procedure

1. Research Procedure Material Collection

   The tea tree and lemon oil studied were purchased from Rumah Atsiri Indonesia Jl. Watusambang, Plumbon, Tawangmangu District, Karanganyar Regency, Central Java 57792.

2. Antibacterial Activity Testing of Tea Tree and Lemon Oil Combination

   Testing the antibacterial activity of a combination of tea tree and lemon oil using agar diffusion method with disc technique. Antibacterial activity tests are carried out by placing disc paper that has been saturated with test samples on the surface of agar media that has been inoculated with test microbial cultures, then incubated for 18-24 hours at a temperature of 35°C for 24 hours. Verile gel was used as a positive control. Verile contains the active ingredients salicylic acid, allantonin, vitamin B_3, and triclosan. The combination of ingredients in Verile Acne Gel works by fighting acne-causing bacteria. while a negative control used aquadest. After incubation, the inhibitory zone measurement was carried out using a caliper (Indarto *et al*., 2019).

3. Formulation Cream

   Melt the oil phase (stearic acid, cetyl alcohol, propylparaben, and propyleneglycol) in a water bath until melted and mixed. The water phase (triethanolamine, glycerin, methylparaben, sodium metabisulfite, and aquadest) is also heated over a water bath like the oil phase, both phases are heated for ± 40 minutes. After heating the two phases are mixed until a cream base is formed, add a combination of tea tree and lemon oil at the last moment, and the cream is ready to be put into the pot (Kusumastuti et al., 2019). The formulation of the cream can be seen in **Table I.**

Table I. Tea Tree and Lemon Oil Combination Cream Formulation

Composition	Concentration % (b/b)		
	F1	F2	F3
Tea Tree Oil	1	1	2
Lemon Oil	1	2	1
Asam stearat	10	10	10
Setil alkohol	1	1	1
Propylenglikol	5	5	5
Triethanolamine (TEA)	0,5	0,5	0,5
Gliserin	5	5	5
Metil paraben	0,18	0,18	0,18
Natrium metabisulfit	0,1	0,1	0,1
Propil paraben	0,02	0,02	0,02
Aquadest ad	100	100	100

F1: Formulation I F2: Formulation II F3: Formulation III

4. Stability test

   The cream stability test was used the cycling test method with organoleptic test parameters, homogeneity, pH, dispersion, cream type testing, viscosity, and flow properties (Kurniasih, 2016).

RESULTS AND DISCUSSION

Antibacterial Activity of Tea Tree and Lemon Oil Combination can be seen in **Table II**.

Table II. Results the diameter of the inhibitory zone of the combination of tea tree and lemon oil

Replication	Control (+) Verile Gel (mm)	Control (-) Aquadest Steril (mm)	Diameter of inhibition zone (mm)		
			Combination of tea tree and lemon oil 1:1	Combination of tea tree and lemon oil 1:2	Combination of tea tree and lemon oil 2:1
1	14,4	-	12,5	12,1	11,7
2	15,8	-	12,3	11,2	11,2
3	15,9	-	12,1	10,5	10,9
Sum	46,1	-	36,9	33,8	33,8
Average	15,3	-	12,3	11,2	11,2
SD	0.83	-	0.02	0.80	0.40

Remarks :(-) = Does not have an inhibitory zone

The inhibitory zone of the combination of tea tree and lemon oil was analyzed using IBM SPSS Version 22 statistics, the analysis carried out was a normality test, a homogeneity test if the data was normally distributed and homogeneous followed by the analysis of the One Way ANOVA test. If the data is not normally distributed, a Kruskal-Wallis analysis is performed. The results of the Mann-Whitney test analysis between negative controls (aquadest) with tea tree and lemon oil all comparisons showed a significance value of 0.037 ($p < 0.050$) which means there is a significant difference between the diameter of the resistance produced by the two. This means that tea tree and lemon oil have antibacterial inhibiting activity. The results of the Mann-Whitney test analysis between negative controls with tea tree and lemon oil all comparisons showed a significance value of 0.037 ($p < 0.050$) which means there is a significant difference between the diameter of the resistance produced by the two. This means that tea tree and lemon oil have antibacterial inhibiting activity.

The antibacterial test of each sample, the ratio of the combination of tea tree and lemon oil 1: 1, 1: 2, and 2: 1 has inhibitory power against *Propionibacterium acnes*. This can be seen from the presence of a clear zone formed around the disc paper. While in the negative control, aquadest does not have a clear zone which means there is no inhibition zone against *Propionibacterium bacteria acnes.*The results of the inhibitory power test of the three formulas are presented in **Figure 1**.

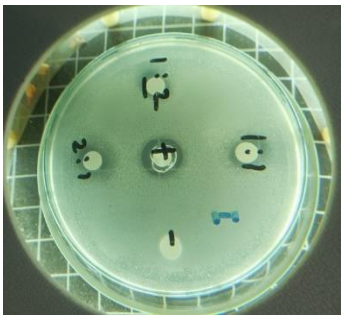
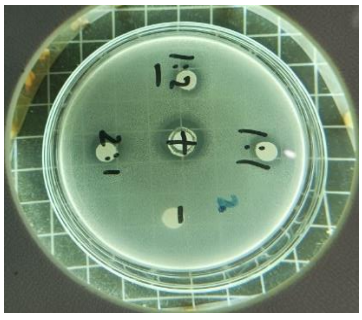
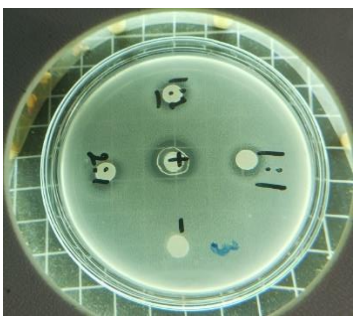
Replication 1	Replication2	Replication 3
		

Figure 1. The inhibitory zone of the combination of tea tree and lemon oil

The results of the stability test can be seen in the **Table III**.

Table III. Stability test of cream preparations after six cycles

Cream preparations	Organoleptic			Homogeneity	pH	spreadability	Emulsion type
	Colour	Smell	Tekstur				
Base	White	Odorless	L	H	7,17	6,05	M/A
Formula 1	White	Weak (typical lemon)	L, M	H	6,67	6,49	M/A
Formula 2	White	Strong (Typical Lemon)	L, M	H	6,73	6,57	M/A
Formula 3	White	Medium (Typical Lemon)	L, M	H	6,76	6,51	M/A

L: Soft
M: Shiny
H: Homogeneous
M/A: Oil in water

Organoleptic test are based on the characteristics of smell, texture, and color. From the results of organoleptic observations, cream preparations are white and soft shiny textured in formulas I, II, and III. The distinctive smell of lemon oil is stronger than the smell of tea tree oil. The homogeneity test is carried out to determine the presence of coarse particles that are clumped or inhomogeneous under light (Pratasik *et al*., 2019).

The pH of a good topical preparation is between 4.5 and 8.0 (Prolapita *et al*., 2021). Based on the test results, the cream preparation in each formula is in the pH range of 6-7 so that it meets the physiological pH requirements of the skin. Formulas I, II, and III are safe to use because they have a safe pH value for topical preparations. The pH of the preparation must be neither acidic nor alkaline. If it is acidic, it will irritate the skin. If it is alkaline, it will result in dry and flaky skin (Titaley *et al*., 2014).

The cream spreadability test show that the cream preparation has good spreadability because of the requirements of good cream spreadability, which is 5-7 cm (Jaya *et al*., 2016). Good spreadability is essential for the distribution of a product when applied to the skin. According to Saryanti and Ni'mah Zulfa (2017).

The type of emulsion can be determined by using the dilution method. If the cream can be diluted, the cream emulsion type is type M/A, otherwise, if it cannot be diluted, it is the A/M emulsion type (Pratasik *et al*., 2019). Based on the results of observations on cream preparations, namely the M/A emulsion type.

Viscosity testing is carried out to determine the viscosity of a preparation. Viscosity affects the ability of semisolid preparations to flow and spread. The viscosity test was performed with the Brookfield RV Viscometer. Based on the viscosity test results of cream preparations in each formula and base, viscosity values decreased from cycle 0 and cycle 6. This shows that the viscosity of the preparation is unstable, caused by several factors including pressure, storage temperature, and solution concentration. Viscosity results obtained are 36400–11900 cps where the viscosity range is included in a good viscosity range in cream preparations, according to SNI the viscosity value of good cream preparations is 2000-50,000 cPs (Mailana, 2016). The results of viscosity test can be seen in **Table IV**.

Table IV. Viscosity Test

Cycle to-	Dosage	Spindel	Rm	Viskositas (Cps)		
				Replication 1	Replication 2	Replication 3
0	Base	06	5	36400	36200	35600
	Formula 1	06	10	17100	16300	15500
	Formula 2	06	5	27400	29600	30400
	Formula 3	06	10	12700	13300	14800
6	Base	06	4	36250	37750	38750
	Formula 1	06	10	11900	12100	12700
	Formula 2	06	10	14900	15100	16000
	Formula 3	06	10	13700	13800	13500

Based on the results of the observation of the flow property curve obtained in cream preparations, namely the type of non-Newtonian flow properties in the form of ticsotropic flow, where the downflow property curve is on the left side of the ascending curve. The nature of the tixotropic flow is known by a decrease in viscosity in the application of force and the turning point value becomes small. The typotropic flow type is considered to be an ideal system for topical preparations (Wardiyah, 2015). Tixotropic flow properties has a high consistency in the container, but little force can be removed from the container easily and spreads easily when used on the skin (Pricillya et al., 2019). So that when applied to the skin, the gel spreads easily (Chandra & Fitria, 2019).The results of flow property can be seen in **Figure 2**.

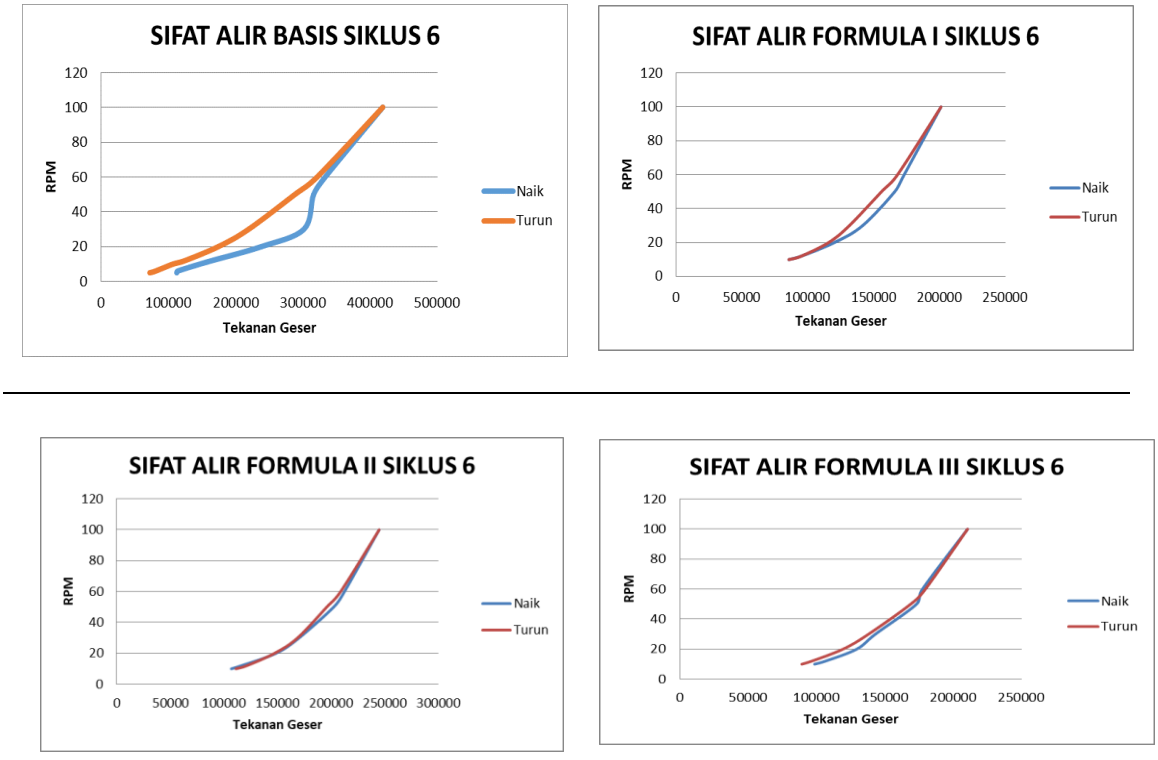


Figure 2. The results of flow property creams

CONCLUSION

The combination of tea tree and lemon oil in the ratio of 1: 1, 1: 2, and 2: 1 has antibacterial activity against *Propionibacterium acnes* with antibacterial inhibitory zones of 1.23 cm, 1.12 cm, and 1.12 cm. The formulas of tea tree and lemon oil combination cream preparations can be formulated into cream preparations.

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