KnE Medicine

ICMEDH
2nd International Conference on Medical Health Science
Volume 2023

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Research Article

Optimation of Emulgel Combination of Tea Tree Oil and Lavender Oil: Evaluation and Antibacterial Study

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Abstract.
The terpinene-4-ol contained in tea tree oil has broad-spectrum antimicrobial activity. Lavender oil contains linalool which also has antibacterial properties. So, they can be anti-acne. Both oils are formulated as emulgel hand sanitizers because this form has a better-controlled drug release effect and stability. The emulgel contained tea tree oil at 5% combined with lavender oil at 0.5%, 1%, and 2%. The evaluation consisted of physical and chemical characteristics, irritation, and antibacterial activity on skin bacteria. The results showed that each organoleptic formula had white color and a distinctive aroma of both essential oils. The texture was soft, homogeneous, and oil-in-water emulsion type; the viscosity and pH were within the specification range. All the formulas met the dispersion requirements. For the irritation test result using HET CAM methods, the preparation caused mild irritation, and the value of the irritating score increased when lavender oil levels increased. In the antibacterial test for *Cutibacterium acnes*, the addition of lavender oil intensified the antibacterial activity of tea tree oil. On the other hand, an antibacterial test for *Staphylococcus aureus* and *Staphylococcus epidermis* of lavender oil added to tea tree oil does not influence the antibacterial activity of tea tree oil in emulgel preparation.

Keywords: emulgel of combination tea tree oil and lavender oil, characteristic, irritation effect, antibacterial property

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Published 8 March 2023

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the ICMEDH Conference Committee.

1. Introduction

Acne is a skin disease caused by the overproduction of oil glands which leads to infection and inflammation of the human skin [1]. *Cutibacterium acnes* is an anaerobic pathogen involved in the development of inflammatory acne by its ability to activate complement and its ability to metabolize sebaceous triglycerides to fatty acids, which can chemotactically attract neutrophils [2]. Other bacteria thought to play a role in the incidence of acne are *Staphylococcus aureus* [3] meanwhile *Staphylococcus epidermis*

actually has a beneficial role in limiting *Cutibacterium acnes* over-colonization and inflammation [4].

Tea tree oil extracted from *Melaleuca alternifolia* is one of the most well-known essential oils that is often used to treat skin, respiratory, mouth, and vaginal infections, or used as an antiseptic and disinfectant [5]. The commercial tea tree oil has a minimum content of 30% terpinene-4-ol and a maximum 15% content of 1,8-cineole [6]. Terpinene-4-ol is a compound that has broad-spectrum antimicrobial activity [7]. The lavender essential oil obtained from the flower *Lavandula angustifolia* is widely used as a complementary medicine and as an additive to many over-the-counter cosmetic products [8]. *Linalool* was the most prominent compound found at the highest concentration (37.9%), followed by *linalyl acetate* (33.3%) both of which have antibacterial properties [9].

In this study, both essential oils were formulated into emulgel, hoping the combination of the two essential oils had a synergistic effect. Emulgel shows a better-controlled drug release effect by virtue of the combined effect of gel and emulsion with increased stability [10].

This study was to determine the characteristical, stability, irritating effect, and antibacterial activity of emulgel combined tea tree oil with lavender oil at different levels of lavender oil.

2. Materials and methods

2.1. Materials

The materials used for this emulgel preparation were lavender oil and tea tree oil, obtained from PT.Darjeeling Sembrani Aroma by steam distillation method (*steam distillation*). Carbomer (Ashland), Triethanolamine (Merck), Span 20 (Sigma-Aldrich), Tween 80 (Industria Chimica Panzeri), Propylene glycol (Dow Chemical Pacific), Nipagin (Gujarat-Organic), Nipasol (Gujarat-Organic), Butylated Hydroxytoluene (Sigma-Aldrich), and Water distillate. The irritability test used eggs, NaCl 0.9% solution, and Sodium Lauryl Sulfate (SLS). The antibacterial activity was used by Mueller Hinton Agar, Mueller Hinton Broth, Barium Chloride (BaCl2) 1%, Sulfuric acid 1%, and NaCl 0.9% solution, Clindamycin phosphate gel 1%.

2.2. Tested Microbes.

Cutibacterium acnes, *Staphylococcus aureus,* and *Staphylococcus epidermis* were obtained from the Laboratory of Microbiology, University of Muhammadiyah Malang.

2.3. Instruments

Basic20+(Crison) pH meter, digital analytical balance (Metter Toledo), scatter power testing equipment, viscosity testing equipment (Brookfield Engineering Lab. INC), incubator, refrigerator, microscope, laminar airflow cabinet.

2.4. Formula Design

In this formulation, three formulas of emulgel tea tree oil combined with lavender oil with different variations in the levels of lavender essential oil, namely used F1was 0.5%; F2 was 1%, and F3 was 2%, for tea tree oil using a fixed concentration in each formula of 5%, design of the three formulas is as follows:

Table 1: Formula of emulgel combination tea tree oil and lavender oil.

Ingredients	Function	F1 (%) (w/w)	F2 (%) (w/w)	F3 (%) (w/w)
Lavender Oil	Active ingredients	0.5	1	2
Tea tree Oil	Active ingredients	5	5	5
Carbomer	Gelling agent	1	1	1
Triethanolamine	Alkalizing agent	q.s	q.s	q.s
Span 20	Emulgator	2.34	2.34	2.34
Tween 80	Emulgator	2.66	2.66	2.66
Propylene glycol	Humectant	10	10	10
Nipagin	Preservative	0.1	0.1	0.1
Nipasol	Preservative	0.1	0.1	0.1
BHT	Antioxidant	0.03	0.03	0.03
Distilled water	Solvent	until 100	until 100	until 100

The methods of making emulgel

First, dispersed the carbomer into distilled water and used triethanolamine to adjust the pH by 7. Prepare the oil phase by mixed butylhydroxytoluene, propyl paraben, Span 20, lavender essential oil, tea tree essential oil, and the aqueous phase by mixing tween 80, propylene glycol, and nipagin. Pour the oil phase into the water phase under stirring at 200 rpm for 10 minutes or until an emulsion. The emulsion mass was put into the gel mass under mechanical stirring (200 rpm) for 10 minutes. This is replicated 3 times.

2.5. Evaluation

2.5.1. Characteristic Test

*Organoleptic test.*Organoleptic testing of emulgel included inspection for consistency, color and odor [11].

*Homogeneity test.*The emulgel was measured at 0.1 gram and then spread evenly on transparent glass [12].

pH determination. Measurement of pH is carried out with a pH meter at room temperature (Phad *et al.*, 2018). The pH of the preparation must be in the pH range regulated by SNI number 16-4399-1996, that is 4.5-8.0 for topical preparations [13].

Viscosity test. Viscosity measurements were carried out using a Brookfield viscometer. Good viscosity requirements are between 2000-50000 cPs [14].

Spreadability test. A total of 1 gram emulgel was placed in the center of transparent glass and given a load (50g, 100g, and 150g). Good spreadability of topical preparations ranges from 5-7 cm [15].

*Emulsion type test.*The emulgel dripped on an object-glass, then dripped with methylene blue solution, then covered with transparent glass, and observed under a microscope [16].

2.5.2. Irritation test

The fertilized leghorn eggs were placed in an incubator at 37oC and ensuring that the air cavity of the egg was at the top, the eggs rotated for teen days. After that, eggs that did not contain live embryos were discarded, the air cavity egg was the mark, then the outer shell using sterile scissors was cut. The outer shell is softened by 0.9% NaCl solution to facilitate the cutting process, then the outer membrane of the egg is moistened with warm 0.9% NaCl solution. Put it back into the incubator for 5-20 minutes. A total of 300 mg of the sample was placed on the CAM and allowed to stand for 20 seconds. After 20 seconds, using a sterile solution of NaCl 0.9%, CAM was cleaned, then observations for 300 seconds. The data obtained in the HET-CAM test is calculated using the formula (1), if the score is 0 -0.9 is not irritating, 1-4.9 is mild irritation, 5-8.9 or 5-9.9 is moderate irritation, and 9-21 or 10-21 is severe irritation [17].

$$T = \frac{301-H}{300} X\ 5 + \frac{301-L}{300} X\ 7 + \frac{301-C}{300} X\ 9 \quad \text{.....(i)}$$

which T = irritation score

H = time required to cause hemorrhage (sec)

L = time required to cause lysis (sec)

C = time required to cause coagulation (sec)

2.5.3. Antibacterial activity

Antibacterial activity of emulgel contained tea tree oil 5% (F0), tea tree oil combined lavender oil at 5% and 0,5% (F1); 5% and 1% (F2); 5% and 2% (F3) on bacterial strains tested by agar diffusion technique, inhibition zone was measured in millimeters using a metric ruler from the edge of the well to the end of the inhibition zone. Clindamycin phosphate gel 1% was a positive control, and emulgel base was the negative control.

3. Result

3.1. Characteristic.

Organoleptic test. The emulgel F1, F2, and F3 have a soft texture, homogenous, white color, and an aromatic combination of tea tree and lavender oil and no phase separation (Figure 1).

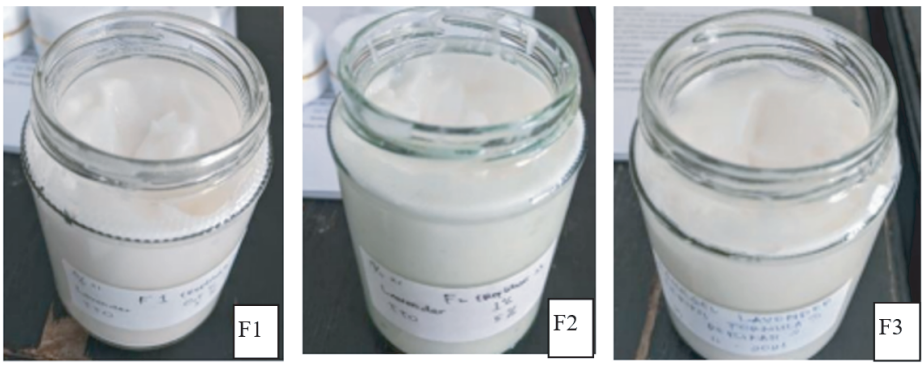

Figure 1: Emulgel contained tea trea oil 5% and lavender oil 0.5% (F1), emulgel contained tea tree oil 5%+lavender oil 1% (F2), and emulgel contained tea tree oil 5%+lavender oil 2% (F3).

Homogeneity test. Each emulgel has an even white appearance and no coarse grains when the emulgel is applied to the surface of the slide so that all emulgel formulations are homogenous.

pH test. The pH of F1 is 6.14±0.30, F2 is 6.90±0.43, and F3 is 7.11±0.46. There is no difference in pH on all emulgel formulas (Anova One Way, a=0.05), so increasing levels of lavender essential oil do not influence the pH value of the preparation.

Viscosity test. By an instrument of Brookfield viscometer with spindle number 64 at a speed of 12 rpm, the viscosity of emulgels was performed. The F1 has a viscosity of

Table 2: The characteristic of emulgel combined tee tree oil and lavender oil (average ± SD, n = 3).

Parameters	F1	F2	F3
Organoleptic	White color; aromatic; soft texture	White color; aromatic; soft texture	White color; aromatic; soft texture
Homogeneity	Homogenous	Homogenous	Homogenous
pH	6.14 ± 0.30	6.90 ± 0.43	7.11 ± 0.46
Viscosity (cPs)	36,167 ± 1,155	43,750 ± 500	45,667 ± 1,607
Rheology	Pseudoplastic	Pseudoplastic	Pseudoplastic
Spreadability (cm)	5.07 ± 0.25	4.9 ± 0.36	4.9 ± 0.27
Emulsion type	Oil-in-water (o/w)	Oil-in-water (o/w)	Oil-in-water (o/w)

36,167±1,155 cPs, F2 has a viscosity of 43,750±500cPs, and F3 has a 45,667±1,607cPs. The viscosity of F2 and F3 is the same, and it is higher than F1(Anova Oneway, a=0.05).

Rheology inspection. The result of measuring viscosity at speed variation is 3 rpm, 6 rpm, and 12 rpm can be seen in Figure 2. Based on Figure 2, the flow properties of emulgel are pseudoplastic because the higher the stirring speed, the more liquid the emulgel becomes.

Spreadability test. The spreadability of F1 is 5.07±0.25cm, F2 is 4.9±0.36cm, and F3 is 4.9±0.27. There is no difference in spreadability on all formulas (Anova One Way, α=0.05).

Emulsion type test. When the methylene blue reagent dripped onto the emulgel produced a homogeneous color. So the emulsion type of the emulgel preparation of a combination of lavender oil and tea tree oil is oil in water (O/W).

3.2. Irritation effect

The result of the irritation test of emulgel using HET CAM methods is in Table 3. Score irritation of Fo, emulgel tea tree oil 5% is 3.10±0.94 ; F1, emulgel tea tree oil 5% + lavender oil 0.5% is 3.18±0.21; F2, emulgel tea tree oil 5% + lavender oil 1% is 4.12±0.89; F3, emulgel tea tree oil 5% + lavender oil 2% is 4.74±0.23. There was a mild irritation in all emulgel formulations. While the score of positive control, SLS 5% solution is 11.89±0.17, its score category as severe irritation, while distilled water is a negative control has 0±0, its category not irritated.

3.3. Antibacterial activity

The antibacterial activity of emulgel on *Cutibacterium acnes, Staphylococcus aureus*, and *Staphulococcus epidermis* are in Figure 3. The inhibition zone of emulgels for the

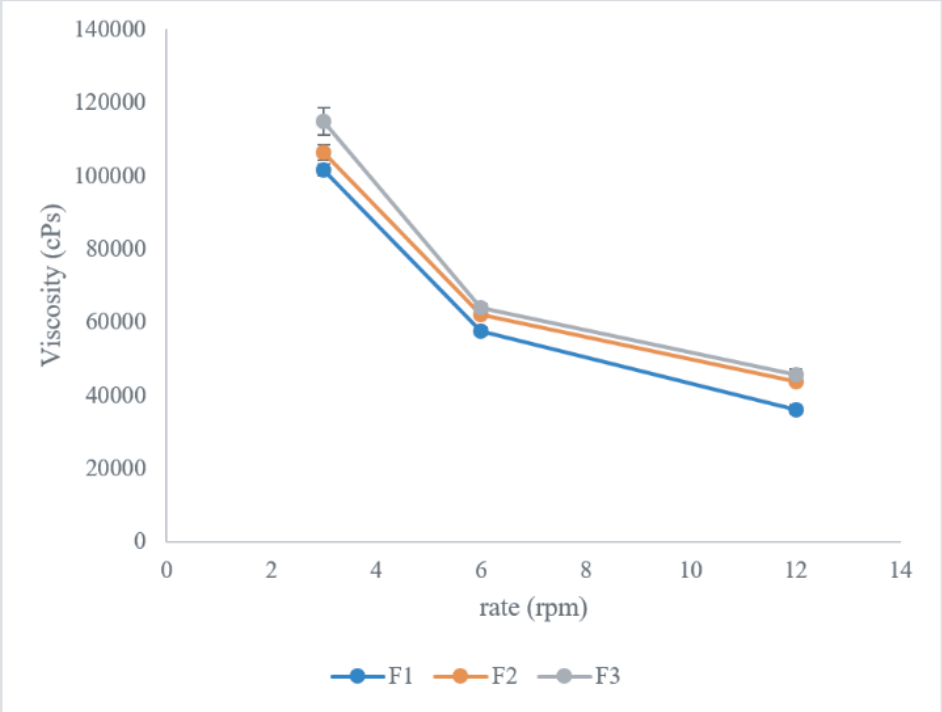

Figure 2: The rheological of the emulgel of F1, F2, and F3 shows a pseudoplastic flow.

TABLE 3: Emulgel irritation test results.

Sample	Score (average±SD)	Irritation level
Fo	3.10±0.94	mild irritation
F1	3.18±0.21	mild irritation
F2	4.12±0.89	mild irritation
F3	4.74±0.23	mild irritation
Positive control	11.89±0.17	severe Irritation
Negative control	0±0	no irritation

growth of bacteria is in Table 4. Clindamycin phosphate 1,2% is a positive control and distilled water is a negative control.

The inhibition zone on the growth of *Cutibacterium acnes* of F0 is 2.12±0.44mm, F1 is 3.93±0.41mm, F2 is 3.19±0.37mm, and F3 is 3.19±0.37mm. The positive control, gel of Clindamycin phosphate at 1.2% is 10.07±mm. On *Staphylococcus aureus*, the inhibition zone of F0 is 2.62± 0.33mm, F1 is 2.23±0.45mm, F2 is 2.15±0.44mm, F3 is 1.86±0.64mm, and the positive control is 18.26±1.04mm. On *Staphylococcus epidermis*, the inhibition zone of F0 is 4.14± 1.88mm, F1 is 4.39±2.11mm, F2 is 2.98±0.91mm, F3 is 3.44±1.21mm, and the positive control is 14.2±1.38mm.

Table 4: The antibacterial activity of emulgel measured by the diameter of the inhibition zone.

Inhibition zone (mm) (average ± SD, n=3)			
Sample	*Cutibacterium acnes*	*Staphylococcus aureus*	*Staphylococcus epidermis*
F0	2.12±0.44	2.62 ± 0.33	4.14±1.88
F1	3.93±0.41	2.23±0.45	4.39±2.11
F2	3.19±0.37	2.15±0.44	2.98±0.91
F3	3.56±0.16	1.86±0.64	3.44±1.21
Positive control	10.07 ± 1.12	18.26 ± 1.04	14.2±1.38
Negative control	0 ± 0	0 ± 0	0 ± 0

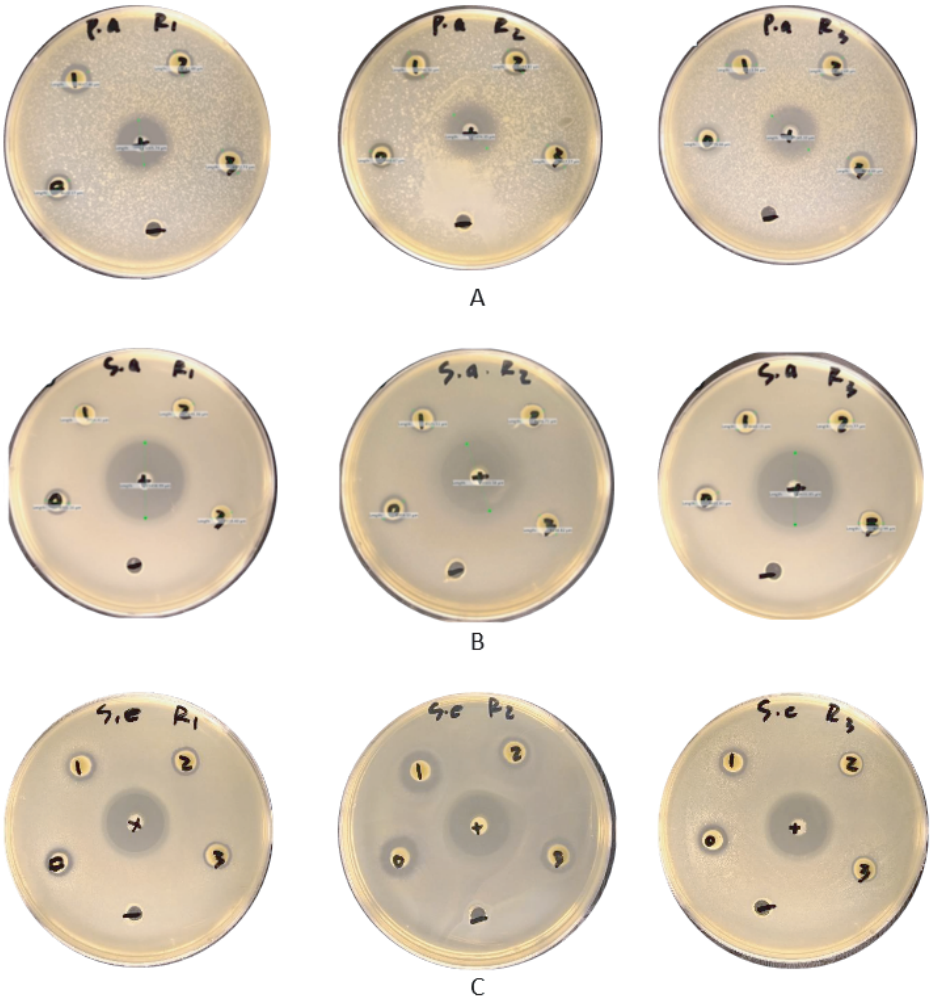

Figure 3: The antibacterial activity of emulgel on the growth of *Cutibacterium acnes* (A), *Staphylococcus aureus* (B), and *Staphylococcus epidermis* (C) measured by the diameter of the inhibition zone (mm).

Antibacterial activity to *Cutinacterium acnes* of F1, F2, and F3 is more than F0. Meanwhile, the antibacterial activity of F0, F1, F2, and F3 in *Staphylococcus aureus* and *Staphylococcus epidermis* is not different (Anova Oneway, a=0.05). Thus, the addition of

lavender oil increases the antibacterial activity of tea tree oil to *Cutinabacterium acnes* but does not influence the antibacterial activity of tea tree oil to the *Staphylococcus aureus* and *Staphylococcus epidermis*.

4. Discussion

4.1. Characteristic

It has been producing an antibacterial emulgel formulation containing the active ingredients of tea tree oil and lavender oil in level variation. It is a homogeneous semisolid, aromatic, and has a soft texture. The pH of the emulgel formulas F1, F2, and F3 is 6 - 7. So, they meet the requirements for pH topical preparations at pH 4.5 - 8.

Viscosity is one of the characteristic parameters of emulgel preparations. Based on the viscosity requirements of other semisolid preparations, namely cream, according to the SNI 16-43-1996, the viscosity value is at 2000 -50000 cPs, and the results of the measurement viscosity of all emulgel formulations are at 36000-46000 cPs, so they meet the requirements. The flow properties of all emulgel formulations are pseudoplastic. The greater the force or shearing stress applied, the lower the viscosity of the emulgel[18]. By having this non-Newtonian pseudoplastic flow property, emulgel is easy to use on the skin because pressing when spreading the preparation on the skin will reduce the viscosity of the emulgel so that it can facilitate the spread. The spreadability of all emulgel formulations around 5 cm has met the requirements for good spreadability of semisolid preparations in 5-7 cm [15]. Furthermore, the result examination of the emulsion type of all emulgel formulations is oil in water type. So all emulgel formulations are non-sticky and non-greasy.

4.2. Irritation effect

Using HET CAM test methods to examine the irritation effect of formulae resulted in a mild irritation reaction in the emulgel of F0, F1, F2, and F3. The irritation reaction emulgel preparations could be due to essential oils. An increase in the concentration of lavender oil increased the irritation score. The lavender essential oil contains linalool compounds, which have the potential to cause irritation reactions. Linalool undergoes autoxidation and produces hydroperoxides (7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol and 6-hydroperoxy-3,7-dimethylocta-1,7-diene-3-ol) which responsible for the immunotoxic properties. Linalool is a lipophilic monoterpene that penetrates human skin, and its

hydroperoxides form a strong affinity for proteins. Likewise, limonene in tree tea oil is oxidized to limonene hydroperoxide, which causes skin irritation [19]. In general, tea tree oil in preparations is less than 10%, but to ensure safety, a concentration below 5% [5].

The skin irritant reaction is lower than eyes due to the skin having a physical barrier such as a layer of stratum corneum that helps fight the penetration of irritants and allergens, whereas the eyes have a thin and transparent vascular mucous membrane called the conjunctiva as a defense against irritants [20], [21]. So, even though preparations of emulgel combination of tea tree oil and lavender oil have mild irritation categories, which score in the range of 1-4.9, it may be safe to use on the skin, but for sensitive skin is not recommended [22].

4.3. Antimicrobial activity

Linalool and linalyl acetate contained in lavender oil and terpene-4-ol contained in tree oil have antibacterial activity [23]. In mixed essential oils, interactions between the compounds can produce a synergistic, additive, indifferent, or antagonistic effect [24]. Emulgel-contained tea tree oil combined with lavender oil had better antibacterial activity than emulgel-containing tea tree oil alone in the growth of *Curinebacterium acne*. However, increasing levels of lavender oil did not affect the antibacterial activity of emulgel. The interaction between tea tree oil and lavender oil may be additive or synergistic was not determined because in this study a test for lavender oil was not done. Different results on *Staphylococcus aureus* and *Staphylococcus epidermis*, the addition of the lavender oil to tea tree oil did not affect its antibacterial activity results this is similar to those Stockley et. al. [25] that the antibacterial effect on Staphylococcus aureus by tea tree oil and a combination of tree tea oil was not different.

5. Conclusions

Emulgels containing tea tree oil and lavender oil had good characteristics but give a mild irritating effect on the CAM. Only in *Corinebacterium acne* was the addition of lavender oil to the emulgel increased antibacterial activity, while in *Staphylococcus aureus* and *Staphylococcus epidermis,* they did not have any effect.

Acknowledgements

Thank you to the Faculty of Health Sciences, University of Muhammadiyah Malang, which has assisted in funding this research through the Block Grant in 2021.

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