



Formulation and Activity Test of Anti-Acne Toner Containing Lemongrass Essential Oil (*Cymbopogon nardus*) against *Propionibacterium acnes* Bacteria

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ABSTRACT

Acne (*Acne vulgaris*) is caused by colonization of *Propionibacterium acnes* bacteria on the skin. The use of natural ingredients with antimicrobial activity for acne medication is an attractive alternative because it is considered safer than chemical ingredients. Lemongrass shows antibacterial activity. Lemongrass acne gel preparations can inhibit the growth of *Propionibacterium acnes* bacteria. This study aims to formulate the preparation and antibacterial activity of lemongrass essential oil toner. The method used is an experimental method. Variations in the concentration of lemongrass essential oil (*Cymbopogon nardus*) were tested at 10%, 20%, and 30%. The essential oil was produced using steam distillation. The physical quality of the toner formulation was tested using organoleptic testing, homogeneity testing, pH testing, and viscosity testing. This was followed by irritation testing, hedonic testing, and antibacterial activity testing of the toner formulation. Data analysis was performed using One-way ANOVA and Post Hoc. The acne-fighting toner based on lemongrass essential oil exhibited good physical characteristics, including a slightly opaque white color, a distinctive lemongrass aroma, a liquid texture that absorbs easily into the skin, and visual homogeneity. pH and viscosity tests showed significant differences between formulations, but all remained within safe ranges. Irritation tests showed that all formulations are safe for use as they did not cause negative reactions on the volunteers' skin. Based on hedonic testing, the formulation with a 20% concentration was most preferred by panelists in terms of aroma and color. Antibacterial activity testing showed that all concentrations (10%, 20%, and 30%) were able to inhibit the growth of *Propionibacterium acnes*, with effectiveness increasing as the concentration increased. The 10% formulation already demonstrated moderate antibacterial activity, while the 20% and 30% formulations fall into the strong category. Therefore, the lemongrass essential oil toner is effective and safe for use as an acne treatment, with an optimal concentration of 20%.

Keywords: Antibacterial, *Cymbopogon nardus*, *Propionibacterium acnes*, Toner Formulation.

ABSTRAK

Jerawat (*Acne vulgaris*) disebabkan oleh kolonisasi bakteri *Propionibacterium acnes* di kulit. Penggunaan bahan alami dengan aktivitas antimikroba untuk obat antijerawat menjadi alternatif yang menarik karena dirasa lebih aman dibanding bahan kimia. Serai wangi menunjukkan aktivitas antibakteri. Sediaan gel antijerawat serai wangi dapat menghambat pertumbuhan bakteri *Propionibacterium acnes*. Penelitian ini bertujuan merumuskan sediaan dan aktivitas antibakteri dari toner minyak atsiri serai wangi. Metode yang digunakan adalah metode eksperimental. Dilakukan variasi konsentrasi minyak atsiri serai wangi (*Cymbopogon nardus*) sebesar 10%, 20%, 30%. Pembuatan minyak atsiri dilakukan menggunakan metode destilasi uap. Pengujian mutu fisik sediaan toner dengan uji organoleptis, uji homogenitas, uji pH, uji viskositas. Kemudian, dilanjutkan dengan uji iritasi, uji hedonik, dan aktivitas antibakteri sediaan toner. Analisis data menggunakan One way ANOVA dan Post Hoc. Toner antijerawat berbahan dasar minyak atsiri serai wangi menunjukkan karakteristik fisik yang baik, yakni berwarna putih agak pekat, beraroma khas serai wangi, memiliki tekstur cair yang mudah menyerap di kulit, serta homogen secara visual. Hasil uji pH dan viskositas menunjukkan adanya perbedaan signifikan antar formula, namun tetap dalam rentang yang aman. Uji iritasi menunjukkan bahwa seluruh formulasi aman digunakan karena tidak menimbulkan reaksi negatif pada kulit sukarelawan. Berdasarkan uji hedonik, formulasi dengan konsentrasi 20% paling disukai panelis dari segi aroma dan warna. Uji aktivitas antibakteri menunjukkan bahwa semua konsentrasi (10%, 20%, dan 30%) mampu menghambat pertumbuhan *Propionibacterium acnes*, dengan efektivitas yang meningkat seiring naiknya konsentrasi. Formulasi 10% sudah menunjukkan aktivitas antibakteri kategori sedang, sedangkan 20% dan 30% masuk kategori kuat. Oleh karena itu, toner minyak atsiri serai wangi efektif dan aman digunakan sebagai sediaan antijerawat, dengan konsentrasi optimal pada 20%.

Kata Kunci: Antibakteri, *Cymbopogon nardus*, *Propionibacterium acnes*, Formulasi Toner.

INTRODUCTION

Acne (*Acne vulgaris*) is a common skin disorder, especially during puberty, which is characterized by hormonal changes in the body. According to data from the World Health Organization (WHO) (Okoro et al., 2016), approximately 80% of individuals aged 11 to 30 years experience acne. This condition is caused by the colonization of *Propionibacterium acnes* bacteria on the skin, which triggers blockages in hair follicles, thereby hindering sebum secretion, causing inflammation, enlarged pores, and ultimately dryness. Factors that trigger the onset of acne include genetics, hormonal activity during the menstrual cycle, stress, hyperactive sebaceous gland activity, hygiene, diet, and the use of cosmetics (Try et al., 2021).

One approach to addressing acne problems is the use of safe and effective skincare products. Skincare involves the use of products specifically formulated for different skin types to maintain healthy facial skin (Maarif et al., 2019). The use of natural ingredients with antimicrobial activity is an attractive alternative as they are perceived to be safer than chemical ingredients. Essential oils from natural sources can be found in various fields such as pharmaceuticals, food, fragrances, and beauty products. These oils are a preferred alternative to chemical substances, as they exhibit fewer harmful effects on humans or the environment (Naeem et al., 2018).

Essential oil from lemongrass has long been used in traditional medicine and skin care product formulations. The active compounds such as geraniol, citral, citronellal, and citronellol (Chanthai et al., 2012; Dewi & Hanifa, 2021) contribute to the biological activity of this plant. Based on research by Innsan et al. (2011), lemongrass exhibits antiviral, antibacterial, and antifungal activity. This antibacterial capability is also demonstrated in a study by Winato et al. (2019), which reported an inhibitory effect of 11.7 mm on bacterial growth at a concentration of 10%. This forms the basis for the development of a facial toner formulation based on lemongrass essential oil as an acne-fighting agent. In the study by Nurlina et al. (2022), the development and formulation of lemongrass leaf ethanol extract into a pharmaceutical preparation in the form of an acne-fighting gel were conducted. The results, according to the antibacterial activity test against *Propionibacterium acnes*, showed that the lemongrass leaf acne gel formulation could inhibit the growth of *Propionibacterium acnes* bacteria with an average inhibitory zone of 20.5 mm, indicating strong efficacy. This study aims to develop lemongrass essential oil in the form of a toner.

Toner is a type of cosmetic product used to cleanse the face of dirt and dead skin cells, as well as provide a refreshing sensation to the skin (Puspawati, Suirta, & Bahri, 2016; Siregar, 2020; Yuliasuti, Indriapuspa, & Pratiwi, 2023). In addition, toner plays an important role as the first step in a facial skincare routine (Purdiyanti et al., 2023). Liquid-based toners have a lighter texture compared to gels. This makes them absorb faster, leave no heavy residue, and are ideal for oily or combination skin prone to acne. Toner formulations containing lemongrass essential oil not only have antimicrobial benefits but also help balance skin pH, provide a soothing effect, and control excessive sebum production. This study aims to formulate a facial toner containing lemongrass essential oil as an active ingredient and evaluate its antibacterial activity against *Propionibacterium acnes*, the main bacteria that causes acne.

RESEARCH METHODS

This study used an experimental method. In this study, variations in the concentration of lemongrass essential oil (*Cymbopogon nardus*) were tested at 10%, 20%, and 30%. The materials used in this study were distilled water, glycerin, propylene glycol, DMDM hydantoin, Triethanolamine (TEA), 1% DMSO, Tween 80, citric acid, clindamycin discs, blank discs, *Propionibacterium acnes* bacteria, Mueller Hinton Agar medium, McFarland 0.5, NaCl, 90% alcohol, and nutrient agar medium.

Essential oil production is carried out using the steam distillation method, using lemongrass that has been cleaned, dried, and cut into pieces. Next, the material is placed in a boiler that has been set to a temperature of 70°C to 100°C and left for 3-4 hours. The steam emitted from the boiler is connected to a condenser, causing the liquid to separate using a separator based on the oil's density (Wijayati et al., 2023).

Based on the basic toner formulation from the research by Muhsinin et al. (2023), the formulation of the lemongrass essential oil acne-fighting toner in this study is as shown in Table

1 below. The addition of essential oil is based on the percentage of each formulation relative to distilled water (w/v).

Table 1. Formula for lemongrass oil toner

Materials	Formula 1	Formula 2	Formula 3	Function
Glycerin	2 ml	2 ml	2 ml	Humectant
Propylene Glycol	3 ml	3 ml	3 ml	Humectant
DMDM Hydantoin	0.6 ml	0.6 ml	0.6 ml	Preservative
TEA	0.1 ml	0.1 ml	0.1 ml	pH Regulator
Essential Oil	10% (3 ml)	20% (6 ml)	30% (9 ml)	Active Ingredient
Distilled Water	30 ml	30 ml	30 ml	Solvent

Physical quality testing of toner preparations using organoleptic testing, homogeneity testing, pH testing, and viscosity testing. This is followed by irritation testing, hedonic testing, and antibacterial activity testing of toner preparations. Organoleptic testing is carried out by physical observation, including color, shape, and aroma. pH measurement is performed using a pH meter, and the acceptable pH range for toner formulations is 4.5–6.5 (Olisvelos et al., 2023). Homogeneity testing was performed by observing the solubility of the ingredients. Viscosity testing of the toner formulation was conducted using a viscometer with spindle number 1 at a speed of 60 rpm. The procedure involved first placing the toner formulation into a glass beaker with a specific volume. The spindle is then lowered until it is submerged in the toner formulation, and the test can be repeated three times for each formulation (Karami et al., 2023).

Furthermore, irritation testing was conducted directly on humans by applying the preparation to the inner arm. After 24 hours, symptoms such as swelling, redness, and itching on the skin were observed. If no unwanted allergic reactions occurred after 24 hours, the cosmetic product was deemed safe for use (Hilmarni et al., 2022). Meanwhile, hedonic testing or preference testing for the toner formulation was conducted by presenting the scent, color, and texture by spraying it. The formulation was tested by spraying it on the face. The test was conducted on 30 panelists who regularly use toner (Siregar, 2020). The panelists were women aged 20–25 years who regularly use toner. The anti-irritation test was conducted with the issuance of an ethical clearance letter from Moewardi General Hospital, which was deemed ethically acceptable with letter number 2.784/XII/2024.

Antibacterial activity testing of the toner preparation began with sterilizing the medium using an autoclave at 121°C for 15 minutes, while glassware was sterilized in an oven at 170–180°C for 2 hours. Sterile cotton swabs were dipped into the bacterial suspension and then inoculated onto Mueller Hinton Agar medium using the spread plate method. The petri dish was marked into 5 sections as boundaries between samples and left for 10 minutes to allow the bacteria to spread. The paper discs were soaked for 15 minutes in various concentrations of the sample, negative control (F0), and positive control (antibacterial toner with salicylic acid). After that, the discs are placed in Petri dishes containing media and cultures, then incubated for 24 hours at 37°C. The inhibition zones are observed and measured around the paper discs (Kotimah, 2023).

Data analysis was performed using one-way ANOVA to compare the significant inhibitory values between formulations. Next, post hoc analysis was performed using Tukey to determine which formulations had significant differences.

RESULTS

A total of 40 kg of lemongrass (*Cymbopogon nardus*) was selected and cleaned, then chopped to facilitate the distillation process by opening the oil glands. Chopping was carried out before drying to reduce the water content in the material. The longer the drying time, the more water is reduced (A'yun et al., 2020). After drying, the process continued with distillation. Lemongrass distillation was carried out using the steam distillation method. The dried samples were placed in a pot to be vaporized and produce oil. This study was conducted at the Trolodringo Aromatic Garden, Tawangmangu, Central Java, with a distillation time of 3–4 hours. The distillation product, a mixture of oil and water, is separated using a separator funnel. To facilitate separation, NaCl is added so that the oil still mixed with water can separate completely (Bahri et

al., 2016). From the distillation of 40 kg of fresh weight of fragrant lemongrass plants, 115 ml of essential oil was obtained. The percentage of essential oil obtained was 0.28%, calculated using the v/w percentage of essential oil. This percentage does not meet the Indonesian National Standard of 0.6%–1.2% (A'yun et al., 2020).

Table 2. Results of organoleptic testing of lemongrass oil toner preparations

Aspects evaluated	Replication		
	I	II	III
Color	White	White	White
Odour	Typical Lemongrass	Typical Lemongrass	Typical Lemongrass

Table 2 shows that the results of organoleptic testing, which indicate that the color and smell of toner made with lemongrass oil after three replications are uniform, namely white in color and with a distinctive lemongrass smell.

Table 3. Results of pH testing of lemongrass oil toner preparations

Formulation (Concentration)	Replication			Means	Standard Deviation
	I	II	III		
1 (10%)	5.10	5.50	5.55	5.38	0.247
2 (20%)	6.12	6.15	6.13	6.13	0.015
3 (30%)	6.33	6.30	6.32	6.31	0.015

Table 3 shows that pH testing or acidity testing is carried out using a calibrated pH meter to check or ensure the safety of preparations used on the skin. Facial toners have a good pH ranging from 4.5 to 6.5. After conducting pH testing at each concentration, the results showed that the formulation at a 10% concentration had an average pH of 5.38; the 20% formulation had a pH of 6.13; and the 30% formulation had a pH of 6.31.

Table 4. Results of viscosity testing of lemongrass oil toner preparations

Formulation (Concentration)	Replication (cP)			Means (cP)	Standard Deviation (cP)
	I	II	III		
1 (10%)	2.70	2.72	2.73	2.71	0.015
2 (20%)	2.97	2.95	2.96	2.96	0.014
3 (30%)	3.97	3.98	3.95	3.96	0.015

Based on Table 4 shows that the results obtained from three tests, the average viscosity for toner preparations with a concentration of 10% was 2.71 cP, a concentration of 20% was 2.96 cP, and a concentration of 30% was 3.96 cP. The standard viscosity for a good facial toner is less than 5 cP, so the viscosity test in this study meets the criteria for a good toner.

Additionally, the anti-irritation test was conducted by applying the formulation to the inner arm of the participants and leaving it for 24 hours, with 30 volunteer participants (Siregar, 2020). Irritation can occur, characterized by itching, redness, and swelling. The results showed that the essential oil-based toner formulations 1, 2, and 3 did not cause itching, swelling, or redness in the panelists.

Table 5. Percentage of hedonic test results of citronella oil toner preparation

Formulation (Concentration)	Aspects evaluated							
	Odour (%)				Color (%)			
	1	2	3	4	1	2	3	4
1 (10%)	3	10	50	37	-	3	60	37
2 (20%)	-	17	83	-	-	-	70	30
3 (30%)	-	23	43	33	-	-	63	37

Table 5 shows that the hedonic or preference testing was conducted on 30 volunteer panelists who were given the treatment, after which the panelists assessed the product based on aroma and color criteria, giving scores of 1-4, where 1 meant very dislike, 2 meant slightly like, 3 meant like, and 4 meant very like. Based on the results obtained, it can be concluded that in terms of color, the highest percentage was on scale 3 (like) at 83% for formulation 2 with 20% essential oil content, while for color intensity, the highest result was on scale 3 for formulation 2 at 70%.

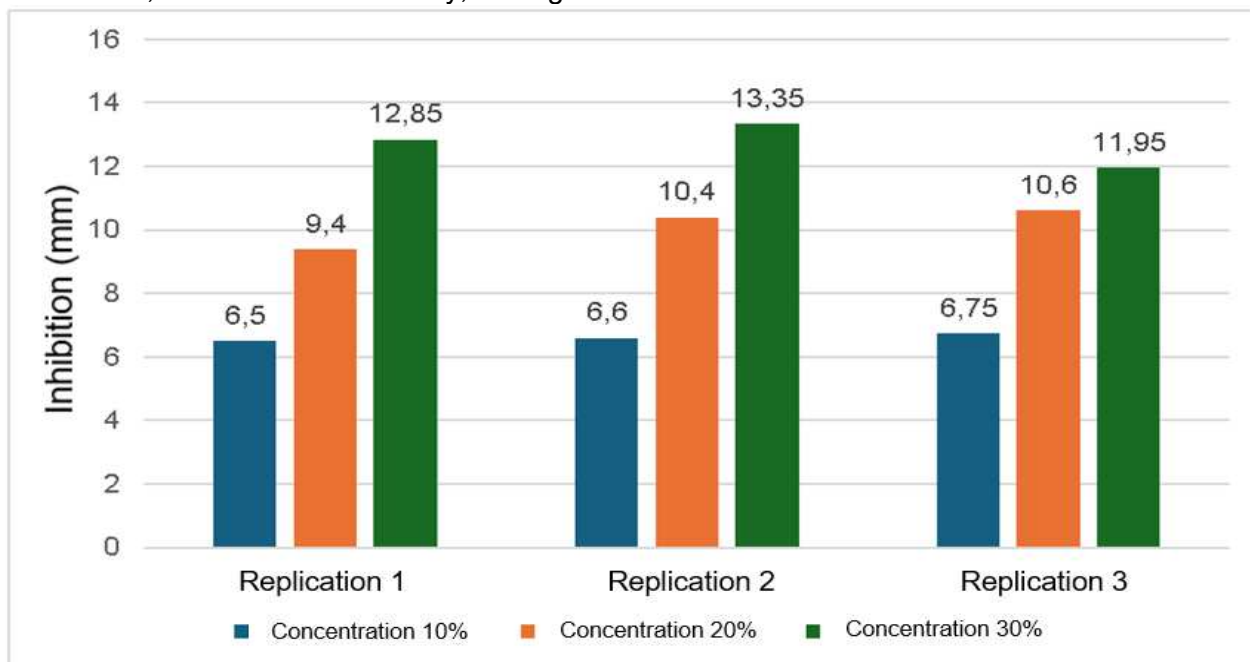


Figure 1. Results of antibacterial activity testing of lemongrass oil toner preparations

Antibacterial testing was conducted by performing three replications for each concentration. As shown in Figure 1, an increase in concentration affects the inhibitory power. The higher the concentration given, the greater the inhibitory power value. Furthermore, more detailed information regarding the results of the antibacterial activity test of lemongrass oil toner preparations.

Table 6. Results of antibacterial activity testing of lemongrass oil toner preparations

Test substance	Formulation (Concentration)	Inhibitor Power (mm)			Means (mm)	Category
		I	II	III		
Toner Supplies	1 (10%)	6.5	6.6	6.75	6.61	Moderate
	2 (20%)	9.4	10.4	10.6	10.13	Strong
	3 (30%)	12.85	13.35	11.95	12.71	Strong
F0	0	0	0	0	0	
Antibacterial Toner	2µg	4.95	8.35	6.35	6.55	Moderate

Testing the activity of anti-acne essential oil toner preparations against the growth of *Propionibacterium acnes* bacteria using the disk diffusion method to determine the Minimum Inhibitory Concentration (MIC) using Mueller Hinton Agar (MHA) medium. The test was conducted by pouring the bacterial suspension onto the solidified medium and allowing it to stand for 10 minutes so that the bacteria diffused into the medium. Disk diffusion discs were soaked in samples at concentrations of 10%, 20%, 30%, and a positive control of the acne-fighting toner formulation containing salicylic acid, with a negative control using 1% DMSO. The disk paper was placed on the marked medium, then incubated for 24 hours, and the test results were obtained and calculated.

The results of this test indicate that the absence of bacterial growth around the disk indicates antibacterial activity or an inhibitory effect against *Propionibacterium acnes* bacteria.

Subsequently, the diameter of the clear zone was measured in the antibacterial facial toner sample containing salicylic acid, which has anti-acne properties, as a positive control. The results of the antibacterial activity testing of the lemongrass essential oil toner formulation. The analysis using one-way ANOVA on the toner formulation test showed a significant value of 0.000, indicating that the lemongrass essential oil toner formulation made in three formulas affects the growth of *Propionibacterium acnes* bacteria.

Table 7. Results of the post hoc test of the toner formulation

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
F1	F2	-3.51667*	.71671	.004	-5.8754	-1.1579
	F3	-6.10000*	.71671	.000	-8.4587	-3.7413
	KP	.06667	.71671	1.000	-2.2921	2.4254
	KN	6.61667*	.71671	.000	4.2579	8.9754
F2	F1	3.51667*	.71671	.004	1.1579	5.8754
	F3	-2.58333*	.71671	.031	-4.9421	-.2246
	KP	3.58333*	.71671	.004	1.2246	5.9421
	KN	10.13333*	.71671	.000	7.7746	12.4921
F3	F1	6.10000*	.71671	.000	3.7413	8.4587
	F2	2.58333*	.71671	.031	.2246	4.9421
	KP	6.16667*	.71671	.000	3.8079	8.5254
	KN	12.71667*	.71671	.000	10.3579	15.0754
KP	F1	-.06667	.71671	1.000	-2.4254	2.2921
	F2	-3.58333*	.71671	.004	-5.9421	-1.2246
	F3	-6.16667*	.71671	.000	-8.5254	-3.8079
	KN	6.55000*	.71671	.000	4.1913	8.9087
KN	F1	-6.61667*	.71671	.000	-8.9754	-4.2579
	F2	-10.13333*	.71671	.000	-12.4921	-7.7746
	F3	-12.71667*	.71671	.000	-15.0754	-10.3579
	KP	-6.55000*	.71671	.000	-8.9087	-4.1913

Description: * The mean difference is significant at the 0.05 level.

Table 7 shows that in F1 vs F2, F1 vs F3, F1 vs K-, F2 vs F1, F2 vs F3, F2 vs K+, F2 vs K-, F3 vs F1, F3 vs F2, F3 vs K+, F3 vs K-, K+ vs F2, K+ vs F3, K+ vs K-, K- vs F1, K- vs F2, K- vs F3, K- vs K+, there are significant differences, as evidenced by significance values less than 0.05. However, in F1 vs K+ and K+ vs F1, there are no significant differences, as evidenced by significance values greater than 0.05.

DISCUSSION

Based on the results of organoleptic testing, the toner has a white color and a distinctive lemongrass scent. According to Karami et al. (2023), if the toner has a thick consistency, it may cause a sticky sensation during use, making the user uncomfortable when applying the toner. In contrast, this lemongrass oil toner has a slightly opaque white color with a liquid texture, making it easy to absorb into the skin. Furthermore, based on the physical properties of the toner, the prepared toner formulation results in a homogeneous or perfectly mixed toner. The findings of Karami et al. (2023) indicate that a homogeneous toner formulation is one where all components are fully dissolved and all particles are evenly distributed, resulting in a clear toner appearance. Furthermore, based on physical testing of pH and viscosity, this lemongrass oil toner exhibits good properties. After analysis using ANOVA, both pH and viscosity showed a significant value of 0.000, indicating a significant difference between each formulation.

Then, irritation and hedonic tests were conducted with 30 volunteers. Regarding the irritation test results, none of the 30 panelists who applied the three toner formulations showed signs of itching, swelling, or redness, so the formulation can be assumed to be safe for use.

Meanwhile, regarding the results of the hedonic test, which was based on aroma and color aspects, it was concluded that the most preferred formulation was formulation 2 or the 20% concentration of lemongrass essential oil.

Based on the results obtained after three repetitions, it was found that a toner preparation with a concentration of 10% was able to inhibit the growth of *Propionibacterium acnes* bacteria. According to Simanjuntak & Tarigan (2014), antibacterial activity in inhibition is influenced by concentration and bacterial type, so that the higher the concentration used, the larger the inhibition zone produced, as the ability to inhibit bacterial growth increases and the inhibition zone formed becomes broader. The inhibitory effect on *Propionibacterium acnes* bacteria is influenced by the presence of compounds with antibacterial properties in lemongrass essential oil (*Cymbopogon nardus*). The results obtained are consistent with the research conducted by Dewi & Hanifa (2021), which stated that at a concentration of 10%, it can already inhibit bacteria, as evidenced by an inhibition zone diameter of 11.7 ± 0.57 mm, thus falling into the strong category. Inhibition zones less than 5 mm fall into the weak category, 5–10 mm into the moderate category, 10–20 mm into the strong category, and over 20 mm into the very strong category (Dewi & Hanifa, 2021).

CONCLUSION

The acne-fighting toner made from lemongrass essential oil exhibits good physical characteristics, namely a slightly thick white color, a distinctive lemongrass aroma, a liquid texture that is easily absorbed by the skin, and visual homogeneity. pH and viscosity test results show significant differences between formulas, but remain within a safe range. Irritation tests indicate that all formulations are safe for use as they do not cause negative reactions on volunteer skin. Based on hedonic testing, the formulation with a 20% concentration was most preferred by panelists in terms of aroma and color. Antibacterial activity testing showed that all concentrations (10%, 20%, and 30%) were able to inhibit the growth of *Propionibacterium acnes*, with effectiveness increasing as the concentration increased. The 10% formulation already demonstrated moderate antibacterial activity, while the 20% and 30% formulations fall into the strong category. Therefore, the lemongrass essential oil toner is effective and safe for use as an acne treatment, with an optimal concentration of 20%.

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