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Formulation Optimization and Antibacterial Activity of Citronella (*Cymbopogon nardus* (L.) Rendle) Topical Gel Spray against *Staphylococcus aureus* Using a Simplex Lattice Design

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ABSTRACT

Atopic dermatitis is skin inflammation caused by *Staphylococcus aureus* infection. Citronella essential oil has the potential as an active ingredient in spray gel preparations to help overcome atopic dermatitis due to *Staphylococcus aureus* bacterial infection. The aim is to optimize the spray gel formula from citronella essential oil (*Cymbopogon nardus* (L.) Rendle) and its antibacterial activity against *Staphylococcus aureus* bacteria. The methods material optimized are Na-CMC, Propylene glycol, and tween 20 using a *Simplex Lattice Design*. Evaluation of the preparation in the form of organoleptic tests, homogeneity, pH, viscosity, drying time, spraying patterns, yeast mold numbers using the spread plate method, and antibacterial activity against *Staphylococcus aureus* bacteria using the well diffusion method. The results of the concentration of Na-CMC: Propylene glycol: Tween 20 (0.27: 4.72: 10) produced an optimal preparation formula. The spray gel is milky white, has a distinctive lemongrass odor, and is moderate viscosity. The spray gel is homogeneous with a pH of 4.94 ± 0.16 , a viscosity of 76.73 ± 7.01 cPs, and a drying time of 3.60 ± 0.40 minutes. The mold/yeast count test showed that the spray gel was free from mold/yeast contamination and was stable at storage temperatures of 4°C and 40°C. The results of antibacterial activity were able to inhibit *Staphylococcus aureus* bacteria with an inhibition zone diameter of $6.85 \text{ mm} \pm 2.80$ classified as moderate. Conclusion: Na-CMC concentration of 0.27%, Propylene Glycol concentration of 4.72%, and Tween 20 concentration of 10% produced optimal spray gel and inhibited *Staphylococcus aureus*.

Keywords: Antibacterial, Citronella, Simplex Lattice Design, Spray Gel, *Staphylococcus aureus*

INTRODUCTION

Atopic dermatitis is an Inflammation of the skin accompanied by itching and affects certain parts of the body, especially the face in the form of chronic relapsing dermatitis. According to the Pediatric Dermatology Study Group (KSDAI), atopic dermatitis in Indonesia is ranked 1 out of 10 cases of skin diseases that occur in children with a prevalence of 23.67%. The prevalence of dermatitis cases in Indonesia is quite high, namely 67.8%. However, public attention to atopic dermatitis is still lacking (Abdi 2020); (Monalisa et al., 2023).

The causes of atopic dermatitis are caused by intrinsic and extrinsic factors. Intrinsic factors include genetic factors, age, microorganisms, exposure to aeroallergens, and irritants (wool, lotion, disinfectants). One of the main microorganisms that causes atopic dermatitis cases is infection due to *Staphylococcus aureus* bacteria (Indika et al., 2020).

Staphylococcus aureus is a gram positive bacterium commonly detected in patients with atopic dermatitis. According to Iwamoto et al. (2019), approximately 70% of individuals with atopic dermatitis present *S. aureus* colonization on lesioned skin and 39% on nonlesioned skin. Management of atopic dermatitis focuses on reducing or preventing recurrence and minimizing symptom severity during flare ups.

Topical antibiotics are widely used in the community; however, continuous application increases the risk of antimicrobial resistance, and symptoms frequently reappear once treatment is discontinued. Therefore, safer and more effective alternatives are required to inhibit bacterial growth associated with atopic dermatitis, particularly through the utilization of natural ingredients as active compounds in pharmaceutical formulations (Ogonowska et al., 2023; Kim et al., 2019).

Lemongrass (*Cymbopogon nardus* (L.) Rendle) essential oil is a natural ingredient with potential as an alternative active compound in pharmaceutical preparations for managing atopic dermatitis associated with *Staphylococcus aureus*. Due to its content of monoterpenes citronellal, citronellol, and geraniol the oil demonstrates the ability to inhibit bacterial growth by disrupting cytoplasmic membrane functions, leading to impaired metabolic processes, cell wall damage, and ultimately cell death (Olisvelos *et al.*, 2023).

In a previous study, Sari *et al.* (2022) reported that pure lemongrass essential oil exhibits antibacterial activity against *S. aureus*, producing an inhibition zone of 13.20 ± 1.13 mm at a concentration of 10%. However, the direct application of lemongrass essential oil to the skin is less effective for atopic dermatitis treatment because its volatile nature reduces practicality, and its use may cause skin irritation, blistering, or severe allergic reactions.

Therefore, it is necessary to formulate lemongrass essential oil into a pharmaceutical dosage form such as a spray gel (Djajanti & Andi Muhammad Farid, 2023). A spray gel is a gel or hydrogel containing 10–90% aqueous phase, designed to be dispensed as fine liquid droplets through an aerosol applicator or spray pump. Key formulation factors include the selection of appropriate gelling agents and humectants, which function as the primary components determining the characteristics of the gel (Zubaydah *et al.*, 2022).

This study aims to determine the optimal concentration variations of Na-CMC, propylene glycol, and Tween 20 required to produce an effective spray gel formulation containing lemongrass essential oil, to evaluate the physical properties of the resulting preparation, and to assess its antibacterial activity against *Staphylococcus aureus*.

RESEARCH METHODS

The method in this study was a Laboratory Experiment carried out in the Laboratory of the Faculty of Pharmacy, Sultan Agung Islamic

University, the Simplex Lattice Design (SLD) approach and using a post-test only control design research plan on the antibacterial activity test of *Staphylococcus aureus* bacteria.

Equipment

The tools used in this study were analytical scales (Precisa® XB 220A), refrigerator, pH meter (Handylab® pH 11/SET), viscometer (Rion® VT-04), centrifuge tube (onemed®), oven (Froilabo®), centrifugator (Boeco® S-8), stopwatch, spray bottle, Erlenmeyer flask (Pyrex®), petri dish, porcelain dish, loop needle, vernier caliper.

Materials

The materials used are citronella essential oil from Ambarawa District, Semarang Regency, Central Java, Na-CMC, propylene glycol, tween 20, methyl paraben, propyl paraben (Asian Pharmaceutical), distilled water (Brataco), MHA media (Mueller Hinton Agar) (Sigma-Aldrich), *Staphylococcus aureus* ATCC 25923 and pure clindamycin (Phapros).

Plant Determination

This stage aims to verify the identity of the plant to avoid errors in the use of plant types when collecting materials. The determination process was carried out at the Herbal Laboratory of the Faculty of Pharmacy, Great Islamic University of Semarang. The plant used in this study was *Cymbopogon nardus* (L.) Rendle which came from Ambarawa District, Semarang Regency, Central Java.

Distillation

Citronella essential oil is produced by water distillation, in which the plant material is heated in water for several hours to release the volatile components. The resulting condensate is collected, the oil is separated from the water using a separating funnel, and residual moisture is removed with anhydrous sodium sulfate (Sugiyanto *et al.*, 2022).

Spray Gel Formulation

The concentration of the citronella essential oil spray gel formulation based on the Handbook of Pharmaceutical Excipients Fifth Edition can be seen in Table 1.

Table 1. Lemongrass Essential Oil Spray Gel Preparation Formula

No.	Material Name	Amount	Function
1	Citronella essential oil (%)	10	Active ingredients
2	Na-CMC (%)	0.25 – 1	Basis
3	Propylene glycol (%)	1 – 15	Humectant
4	Tween 20 (%)	1 – 10	Surfactant
5	Methylparaben (%)	0.2	Preservative
6	Propylparaben (%)	0.025	Preservative
7	Aquadest	q.s	Solvent

Making Spray Gel

The making of spray gel preparations begins with preparing the tools and materials, and then weighing the required ingredients according to the specified concentration. Na-CMC as a gelling agent is dispersed into some hot water and homogenized to form a transparent gel mass in a glass beaker. In a separate beaker, propylene glycol is added to methyl paraben and propyl paraben and then stirred until homogeneous. Then the two beakers are mixed and tween 20 is added and mixed until homogeneous. When the mixture is homogeneous, add citronella essential oil and homogenize, then stir until completely mixed and homogeneous and add distilled water to the limit of 100 ml (Zubaydah *et al.*, 2022).

Evaluation of Spray Gel Preparations

The Evaluation Stage of Spray Gel Preparations Includes Organoleptic Tests, Homogeneity Tests, Spray Pattern Tests, Drying Time Tests (Zubaydah *et al.*, 2022), pH Tests, Viscosity Tests, and Stability Tests (Tari and Indriani 2023).

Yeast and Mold Number Test (YAC)

This test was carried out using Potato Dextrose Agar (PDA) media during a 6-day incubation period at a temperature of 25°C. The analysis of mold and yeast was calculated in units of colonies/ml with the principle of the mold number test, namely the growth of mold after the sample was inoculated on the media made and incubated at a temperature of 25°C and observed. The media used was Potato Dextrose Agar (PDA). After incubation, the colonies that grew were calculated and expressed in colonies/ml (Sundari and Fadhliani 2019).

Antibacterial Activity Test

The antibacterial activity test stage on spray gel preparations includes Sterilization of equipment (Hermawati *et al.*, 2023), Bacterial Rejuvenation (Ulfa *et al.*, 2023), and Preparation of Mc.Farland Standard Solution (Rosmania and Yanti 2020), and Preparation of Test Bacterial Suspension (Ulfa *et al.*, 2023), Preparation of MHA Media (Ulfa *et al.*, 2023), Negative Control (Hermawati *et al.*, 2023), Positive Control (Hermawati *et al.*, 2023), Media Control (Hermawati *et al.*, 2023) and Antibacterial Activity Test of Spray Gel Preparations From Lemongrass Essential Oil Against *Staphylococcus aureus* Bacteria (Wulandari and Ariyani 2020).

Data Analysis

The results of the physical evaluation of the viscosity and drying time tests on modified formulas 1 to 17 were analyzed using the Design-Expert version 13 application with the Simplex Lattice Design approach to obtain the optimum range by looking at the desirability degree point (1.0).

RESULTS AND DISCUSSION

Distillation

Extraction is carried out to separate a secondary metabolite compound contained in a plant from its mixture using an appropriate solvent. The selection of the water distillation method in this study is a very easy process, a very simple method, namely by boiling the time required is short, and the material to be distilled is put into a kettle filled with water and then heated (Porawati *et al.*, 2019). The distillation results can be seen in Table 2.

Table 2. Results of Distillation of Lemongrass

No.	Information	Results	SNI 06-3593-1995
1	Total sample	15.02 kg	-
2	Essential oil weight	23.8702 grams	-
3	Essential oil volume	27 ml	-
4	%yield	0.1589%	0.97 – 1.2%.
5	Specific gravity	0.8804 grams	0.880-0.902 grams
6	Color	Clear Yellow	Yellow - Brownish
7	Odor	Specifically fragrant lemongrass	Typical of fragrant lemongrass
8	Shape	Liquid	clear and non-thick liquid

This distillation process uses 15.02 kg of fresh lemongrass, and the volume of essential oil produced is 27 ml with an essential oil weight of 23.8702 grams. By knowing the weight of the essential oil, the value of the essential oil yield can be calculated. The yield requirement for lemongrass essential oil according to

SNI 06-3593-1995 (BSN, 1995) is 0.97-1.2%. The yield obtained in this study was 0.1589%, which did not meet the requirements for a good yield because it was lower than the requirement. In a study conducted by Juliarti *et al.* (2020), a higher yield of 0.99% was obtained.

It is reported that the differences in citronella essential oil yields are caused by various factors such as the extraction method used, soil elevation, soil fertility, plant age, distillation method, and location. According to Murni & Rustin (2020), the best time to harvest lemongrass is in the morning because this allows the essential oils in the lemongrass to be preserved. In other words, harvesting is carried out when the plants have not yet begun photosynthesis.

This study obtained the results of citronella essential oil with a clear yellow color and a distinctive smell of citronella in liquid form. These results are by SNI 06-3593-1995 (BSN, 1995), stating that the organoleptic requirements for citronella essential oil are clear and non-thick liquid, yellow-brown to light yellowish brown, with a distinctive smell of citronella. Essential oils that are newly obtained after the distillation process are usually colorless or yellowish. If the essential oil is left in the air for too long and exposed to sunlight, the color will change to dark, the shape will be thick and finally form resin. Color changes in essential oils are influenced by evaporation, oxidation, compound components contained in the oil, and also impurities that are mixed in (Suarantika *et al.* 2023).

The measurement of the specific gravity of essential oils obtained a result of 0.8804 g/ml. This result is by SNI 06-3593-1995 (BSN, 1995), namely the requirements for the specific gravity of citronella essential oil, which is 0.880-0.902 grams. The specific gravity of citronella essential oil is defined as the ratio between the weight of citronella essential oil and the weight of water in the same volume of water with the same volume of oil. Specific gravity is associated with the weight fraction of the components contained in patchouli oil. The higher the mass fraction contained in the oil, the greater the specific gravity value of citronella essential oil (Suarantika, *et al.* 2023).

Optimization of Spray Gel Formula

The simplex lattice design method is one of the approaches available in Design Expert 13.0 software which is used to optimize formulations with various combinations of different materials. The biodiesel synthesis process is carried out based on the experimental design created by the program. Some functions of the simplex lattice design method include determining the formula, optimizing

variables in the formulation, determining the number of trials (runs), and keeping the total concentration constant. ANOVA (Analysis of Variance) analysis is used to evaluate the significance of the relationship between response variables and to identify the most appropriate model. The desirability value describes how well the program can meet the criteria set in the final product.

The closer to 1.0, the desirability value indicates that the program is increasingly able to produce products that are in accordance with the wishes. (Ramadhani *et al.* 2017). The data obtained in Design Expert 13 in the study obtained 17 formulas that met the optimal formula requirements. The most optimal formula concentration is the result of measuring the viscosity and drying time tests of 17 formulas. The images and results of the optimization of the Design Exper 13 spray gel preparation formula can be seen in Figure 1 and Table 3.

The results of the viscosity test of 17 spray gel preparation formulas in formula optimization obtained the smallest viscosity value of 76.6 ± 2.70 cPs and the largest was 600.7 ± 0.91 cPs. The viscosity test on the spray gel preparations made showed different viscosity values. So it can be concluded that differences in the concentration of Na-CMC, Propylene Glycol, and Tween 20 affect the viscosity of the spray gel preparation. Viscosity can be influenced by several factors such as the concentration of the gelling agent, the manufacturing temperature, and the particle size. A good spray gel viscosity is less than 150 cP so that the preparation can be sprayed easily using a spray bottle and spread well (Angelia *et al.* 2022). The results of the drying time test of 17 spray gel preparation formulas in formula optimization obtained the smallest drying time of 2.95 ± 0.47 and the largest of 10.14 ± 0.51 . The drying time test showed different values. So it can be concluded that the difference in concentration of Na-CMC, Propylene Glycol, and Tween 20 affects the drying time of the spray gel preparation. A good drying time for a spray gel preparation is less than 5 minutes so that the preparation does not stick to the skin and provides comfort when used (Devi *et al.*, 2020). 3D Surface viscosity and drying time tests can be seen in Figure 2.



Figure 1. 17 Optimization Formulas

Table 3. Optimization of Spray Gel Preparation Formula Design Exper 13

No.	Formula	Material		
		Na-Cmc (%)	Propylene Glycol (%)	Tween 20 (%)
1	F1	0.25	10.6828	4.06721
2	F2	0.25	9.54777	5.20223
3	F3	0.871316	13.1287	1
4	F4	0.337797	8.22892	6.43328
5	F5	0.629311	7.02889	7.3418
6	F6	0.629311	7.02889	7.3418
7	F7	0.25	12.4828	2.26721
8	F8	0.25	5.99764	8.75236
9	F9	1	4	10
10	F10	0.25	4.75	10
11	F11	1	8.8586	5.1414
12	F12	0.629311	7.02889	7.3418
13	F13	0.367764	13.6322	1
14	F14	1	8.8586	5.1414
15	F15	0.871316	13.1287	1
16	F16	1	11.1415	2,85853
17	F17	0.629311	7.02889	7.3418

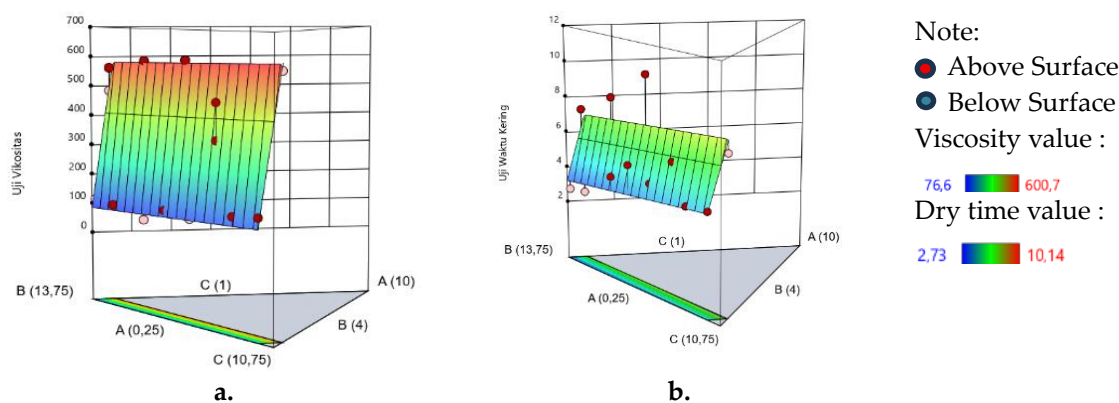


Figure 2. 3D Surface Viscosity Test (a) and Drying Time (b)

Optimum Formula Verification

Based on the results of viscosity and drying time tests, the most optimal formula is the formula with a maximum desirability value of 1.0. If a formula has a desirability value closer to 1, it indicates the program's ability to produce the desired product more perfectly (Ramadhani *et al.*, 2017). The results of the optimal formula after physical evaluation of the spray gel preparation from citronella essential oil were obtained in Table 4 and Figure 3.

So from the recommendation of design expert 13, the optimal formula obtained is formula 1 with a desirability value of 0.858 with a concentration of Na-CMC concentration of 0.27%; Propylene Glycol of 4.72%; and Tween 20 of 10%. Optimization is carried out by finding the limits (goals) of the desired response criteria with a range that is possible to obtain. In this study, the criteria used are viscosity value and drying time. SLD is processed using Design Expert as software to perform optimization (Ramadhani *et al.*, 2017).

Table 4. Optimal Formulation of Spray Gel Preparation

No	Formula	Na-CMC	Propylene Glycol	Tween 20	Desirability
		Konsentrasi (%)			
1	F1	0.27	4.73	10	0.858
2	F2	0.30	13.7	1	0.697

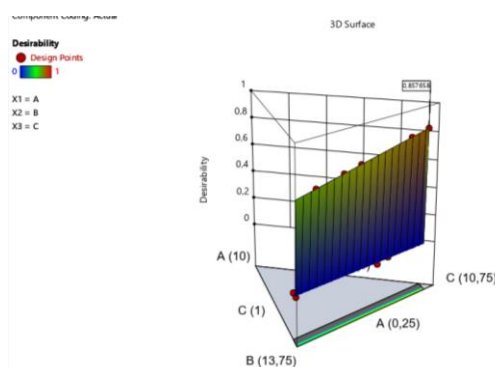


Figure 3. 3D Surface Optimal Desirability Formula Value

Evaluation of Lemongrass Essential Oil Spray Gel Preparation

The gelling agent used in this study was Na-CMC, the humectant used was propylene glycol, and the surfactant used was Tween 20. These ingredients affect viscosity, homogeneity, produce a clear base, and are capable of forming a strong film layer, increasing penetration into the skin so that the drug diffusion coefficient to the stratum corneum also

increases and the rate of drug release is fast (Zubaydah *et al.*, 2022).

After obtaining the optimal formulation of the spray gel preparation, an evaluation of the preparation was carried out in the form of organoleptic tests, homogeneity, pH, viscosity, drying time, spraying pattern, yeast mold numbers, and antibacterial activity against *Staphylococcus aureus* bacteria. The results of the evaluation of the spray gel preparation can be seen in Table 5.

Table 5. Results of Evaluation of Spray Gel Preparation

No.	Evaluation Results of Spray Gel Preparation	
1	Evaluation of supplies	Results
2	Organoleptic	Milky white, typical of citronella essential oil, and slightly thick
3	Homogeneity	Homogeneous
4	pH	4.94±0.16
5	Viscosity (cPs)	76.73±7.01
6	Dry time (minutes)	3.60±0.40
7	Spray Pattern	
	Distribution pattern	Spread
	Diameter	2.5–6 cm
	Spray weight	0.13 gram

Based on the results of organoleptic test observations of the spray gel preparation in this study, the results were milky white, had a distinctive smell of citronella essential oil, and were slightly thick. The results obtained were by research conducted by Angelia *et al.* (2022), which stated that a good spray gel preparation is a preparation that meets the requirements, namely in liquid form, with no phase separation, colored according to the active ingredients used, and does not have a pungent odor. Similar results were also obtained by Martihandini & Sutiswa (2023), namely that the spray gel preparation from citronella essential oil was white, had a distinctive smell of citronella, and was in the form of a thick liquid.

The results of observations of the homogeneity test of the spray gel preparation in this study obtained homogeneous results, no lumps were

seen on the glass object, and there were no cashier grains in the preparation. A good topical preparation is a preparation that is homogeneous because it does not cause skin irritation (Angelia *et al.*, 2022). The results of the homogeneity test have met the requirements for a good spray gel preparation. The results of this study are on the research conducted by Martihandini & Sutiswa (2023), which obtained homogeneous spray gel preparation results.

The results of the pH measurement of the spray gel preparation in this study obtained a pH of 4.94 ± 0.16, which met the range of 4.5-6.5. According to Angelia *et al.* (2022), the pH of topical preparations must be in accordance with human skin, which is around 4.5-6.5. so that the resulting spray gel preparation has good stability and does not irritate the skin. The results of the pH test in this study have met the requirements for a good spray gel

preparation. The results obtained in this study are smaller than the study conducted by Martihandini & Sutiswa (2023) which obtained pH results in the range of 6.71 - 6.93. This is because the study used Carbopol 940 as a gelling agent. Where this Carbopol can be dispersed in aqueous media in the form of free acid. So that it can increase the pH of the spray gel preparation.

The results of the measurement of the viscosity of the spray gel preparation in this study showed good results, namely 76.73 ± 7.01 because the viscosity was less than 150 cP. According to Angelia *et al* (2022), the viscosity of a good spray preparation must be less than 150 cP so that the preparation can be sprayed easily using a spray bottle and produce good distribution. The results of the viscosity test have met the requirements for a good spray gel preparation. The results of the study were smaller than the study conducted by Martihandini & Sutiswa (2023) where the results of the viscosity test were in the range of 109.5 ± 0.8 - 113.5 ± 0.5 cPs. The difference in viscosity value results is due to the study using Carbopol 940 where the high pH will affect the viscosity of the preparation and the presence of additional ingredients in the form of triethanolamine which will neutralize the Carbopol dispersion so that it will produce a gel with high viscosity (Martihandini & Sutiswa, 2023).

The results of the drying time measurement on the spray gel preparation in this study showed good results, namely 3.60 ± 0.40 because a good spray gel must have a drying time of less than 5 minutes so that the preparation does not stick to the skin and provides comfort when used by consumers (Angelia *et al.*, 2022) The results of the drying time test have met the requirements for a good spray gel preparation. The results obtained in this study were greater than the study conducted by Angelia *et al.*, (2022) which obtained results of 2.77 ± 0.28 minutes. The difference in the results of the drying time test was due to the study using HPMC and HEC as gelling agents.

The results of the spray pattern test in the study varied. The variation in the spray pattern obtained from the spray gel preparation is influenced by the spray distance and viscosity of the preparation. The spraying distance will always be directly proportional to the size of the spray pattern produced. The smaller the spray distance, the smaller the spray pattern formed. At a distance of 15 cm and 20 cm, it produces a spray pattern that is elongated and spread when sprayed and a large diameter of 5 cm and 6 cm. At a distance of 3 cm, it produces a small

diameter of 2.5 cm. The spray diameter with variations in spray distance aims to determine the optimal spray distance and can provide good distribution, namely 5-7 cm. The results obtained show that a good spray pattern is at a distance of 15 cm and 20 cm (Zubaydah *et al.*, 2022). The results of this study are by the research conducted by Martihandini & Sutiswa, (2023), which obtained the results of a circular pattern evenly with small particles.

The purpose of the spray weight examination is to ensure the weight is released from the preparation. In this study, the spray weight value was 0.13 grams. The spray weight of the spray gel preparation met the good requirements, namely 0.11-0.35 grams (Zubaydah *et al.*, 2022). The results of the spray pattern test have met the requirements for a good spray gel preparation. The results of this study are consistent with the research conducted by Zubaydah *et al.* (2022), which obtained the spray weight results of 0.12 grams.

Stability Test of Citronella Essential Oil Spray Gel Preparation

In this study, a stability test was carried out using the Freeze-thaw cycling test method. This test was carried out at two different temperatures. Cycles 1, 3, and 5 were stored at a temperature of 4°C for 12 hours. Cycles 2, 4, and 6 were stored at a temperature of 40 °C for 12 hours. Then, observations were made on the evaluation of the citronella essential oil spray gel preparation. The results of the spray pattern test in the Stability Test of Spray Gel Preparations can be seen in [Table 6](#).

The results of observations on the evaluation of the citronella essential oil spray gel preparation in the organoleptic test from cycles 1 to 6 obtained consistent results, namely, the essential oil spray gel preparation was milky white, slightly liquid, and had a distinctive citronella odor. Thus, the spray gel preparation did not experience any changes in color, odor, or shape. From these results, it can be concluded that the spray gel is organoleptically stable and there is no reaction of the ingredients so no changes occur (Tari and Indriani 2023).

The results of observations on the evaluation of the citronella essential oil spray gel preparation in the homogeneity test from cycles 1 to 6 showed that the spray gel preparation was homogeneous, no lumps were visible, and there were no coarse grains in the preparation. Good topical preparation is homogeneous and does not irritate the skin (Martihandini and Sutiswa 2023).

Table 6. Results of Spraying Pattern Test

No.	Test	Cycle to -	Spraying Distance				
			3	5	10	15	20
1	Distribution pattern	1	Spreading	Spreading	Spreading	Spreading	Spreading
		2	Spreading	Spreading	Spreading	Spreading	Spreading
		3	Spreading	Spreading	Spreading	Spreading	Spreading
		4	Spreading	Spreading	Spreading	Spreading	Spreading
		5	Spreading	Spreading	Spreading	Spreading	Spreading
		6	Spreading	Spreading	Spreading	Spreading	Spreading
2	Diameter (cm)	1	2	2,5	4,5	5	5
		2	2,5	3,5	5	5,2	5,5
		3	2	3	3,5	4,5	6,5
		4	3	3	4,5	5,5	5,5
		5	2,5	3	4,5	6	7
		6	2,5	4	5,5	7	8,5
3	Weight of spray (grams)	1			0,11		
		2			0,12		
		3			0,12		
		4			0,13		
		5			0,15		
		6			0,12		

Observation results on the evaluation of citronella essential oil spray gel preparations in the pH test Based on the results of the pH test on the spray gel preparation, different results were obtained from cycle 1 to cycle 6 in replications 1, 2, and 3. However, the pH measurement results obtained have

met the pH requirements for the spray gel preparation, namely 4.5-6.5. So that the spray gel preparation produced is stable and safe to use and does not irritate the skin because it is to the pH of human skin (Angelia *et al.* 2022). The pH test results can be seen in Table 7 and Figure 4.

Table 7. Results of The pH test

pH test table		
Cycle to-	Results	Standard
1	6,12 ± 0,03	4,5-6,5 (Angelia <i>et al.</i> , 2022)
2	6,01 ± 0,02	
3	6 ± 0,06	
4	5,20 ± 0,04	
5	5,76 ± 0,04	
6	5,62 ± 0,07	

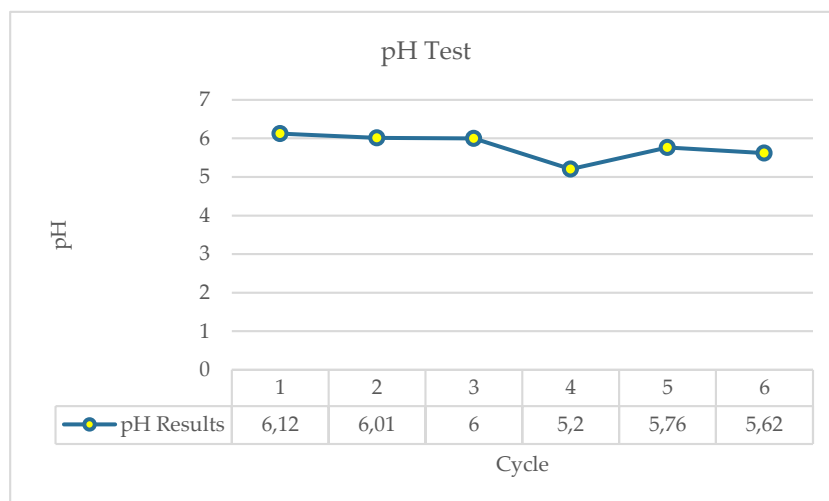


Figure 4. Results of pH Test on Stability Test of Spray Gel Preparation

Based on the results of the viscosity test on the spray gel preparation, the results met the requirements for the spray gel viscosity value, which is less than 150 cPs. So that the spray gel preparation is stable can be sprayed easily using a spray bottle

and can spread well on the skin (Martihandini and Sutiswa 2023). The results of the viscosity test on the Stability Test of the Spray Gel Preparation can be seen in Table 8 and Figure 5.

Table 8. Results of Spraying Pattern Test

Viscosity test table (cPs)		
Cycle to -	Results	Standard
1	75,6 ± 3,01	< 150 cPs (Angelia <i>et al.</i> , 2022).
2	59,2 ± 4,93	
3	67,2 ± 4,99	
4	55,5 ± 3,61	
5	67 ± 2,89	
6	51,2 ± 5,73	

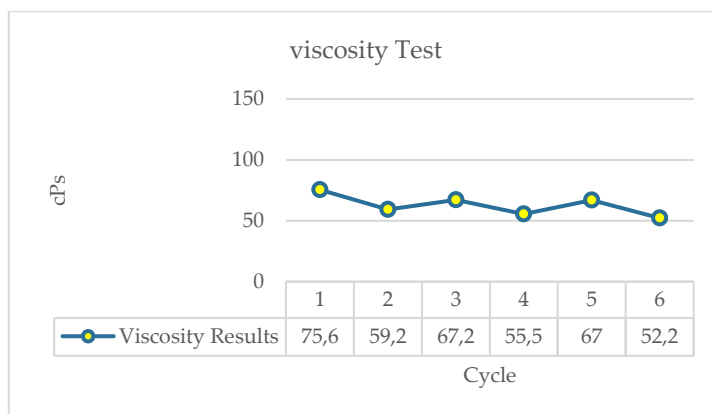


Figure 5. Viscosity Test Results in the Stability Test of Spray Gel Preparations

Based on the results of the drying time measurement on the spray gel preparation, it has met the drying time requirements, which are less than 5 minutes and are stable from cycles 1 to 6. Angelia *et al.*, (2022) stated that a good spray gel has a drying

time of less than 5 minutes so that it does not stick to the skin and is more comfortable to use. The results of the drying time test in the Stability Test of Spray Gel Preparations can be seen in Table 9 and Figure 6.

Table 9. Results of Spraying Pattern Test

Dry Time Test Table (Minutes)		
Cycle to-	Results	Standard
1	2.54 ± 00	< 5 menit (Angelia <i>et al.</i> , 2022).
2	2.37 ± 00	
3	3.01 ± 00	
4	2.30 ± 00	
5	2.59 ± 00	
6	2.21 ± 00	

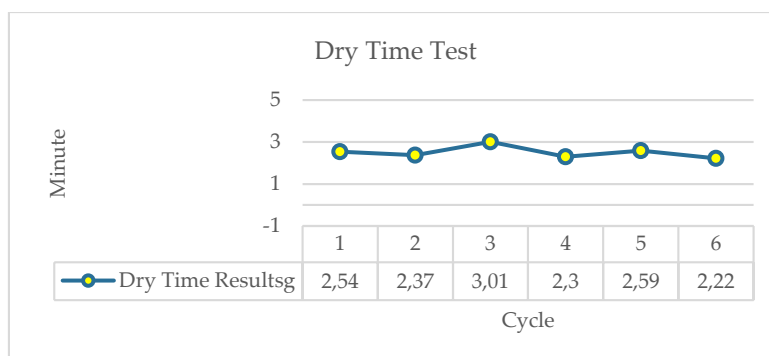


Figure 6. Results of Drying Time Test in Stability Test of Spray Gel Preparation

Based on the results of the spray pattern test on the spray gel preparation with the Freeze-thaw stability test for 6 cycles, the results of the spread pattern were obtained, namely spread and stable diameter from cycle 1 to cycle 6. The factors that affect the spray pattern are viscosity and spray distance. The spray weight results have also met the spray weight requirements for the spray gel preparation, namely 0.11-0.38 grams. Based on the results of the stability test of the citronella essential oil spray gel preparation, it can be concluded that the spray gel preparation from citronella essential oil has good stability in storage (Zubaydah *et al.*, 2022).

Based on the results of the stability test from organoleptic observations, homogeneity, viscosity, pH, drying time, and spraying patterns, a citronella essential oil spray gel preparation was obtained which had good stability because it met the requirements for a good spray gel preparation so that the preparation could be safely stored at a storage temperature of 4°C and 40°C (Zubaydah *et al.*, 2022).

Mold and Yeast Number Test

Based on the results of the mold and yeast number test on the citronella essential oil spray gel preparation, the results are shown by counting the number of colonies that grow on the petri dish from the dilution results. According to PPOMN (2006), Petri dishes that show the number of colonies between 10-150 from the selected dilution and the number of colonies on the two petri dishes or more will be counted and multiplied by the dilution factor.

In this study, five levels of dilution were carried out, namely concentrations of 10⁻¹ to 10⁻⁵. According to Rahayu *et al.* (2022), the use of NaCl in dilution is because. NaCl is a buffer solution with a normal pH, so it can maintain the physiological balance of microbes. The purpose of dilution is that the embedded mold/yeast can be reduced in density. This test also used Potato Dextrose Agar (PDA) media, where according to Imansyah & Alam (2021), PDA is the main choice of media for growing mushrooms. The results of the mold and yeast number test can be seen in Table 10 and Figure 7.

Table 10. Results of the Yeast Mold Number Test for Spray Gel Preparations

No.	Yeast Mold Number Test Results		
	Dilution	Number (Colony)	Amount
1	10 ⁻¹	4	No More Than 2 x 10 Colonies/MI (BPOM, 2023)
2	10 ⁻²	1	
3	10 ⁻³	2	
4	10 ⁻⁴	0	
5	10 ⁻⁵	0	

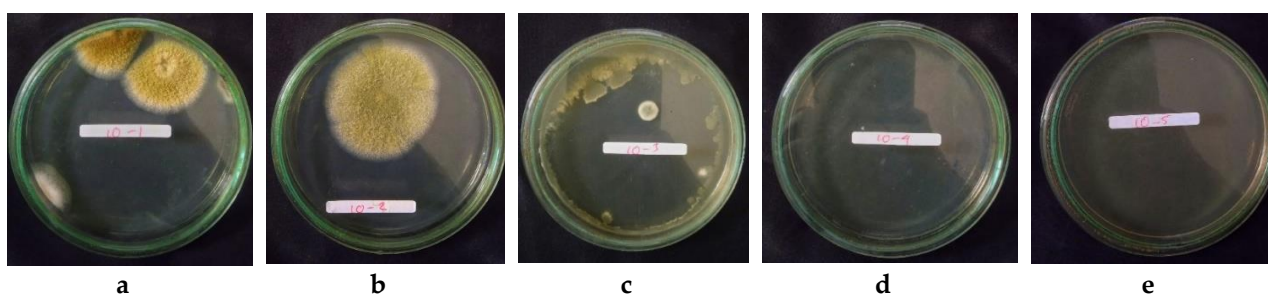


Figure 7. Results of Yeast Mold Number Testing at Dilution 10⁻¹ (a), Dilution 10⁻² (b), Dilution 10⁻³ (c), Dilution 10⁻⁴ (d), Dilution 10⁻⁵ (e)

In this study, five levels of dilution were carried out, namely concentrations of 10⁻¹ to 10⁻⁵. According to Rahayu *et al.* (2022), the use of NaCl in dilution is because. NaCl is a buffer solution with a normal pH, so it can maintain the physiological balance of microbes. The purpose of dilution is that the embedded mold/yeast can be reduced in density. This test also used Potato Dextrose Agar (PDA) media, where According to Imansyah & Alam, (2021), PDA is the main choice of media for growing fungi. Based on the results of the study, it was found that none of the plates showed several 10-150 colonies,

because all plates had several columns <10 colonies. So if the colonies obtained on the plate are less than 10 colonies, the mold and yeast number values are obtained as less than one multiplied by the lowest dilution factor or <1 x 10¹ (Rahayu *et al.* 2022). So that the results of the yeast mold test for the citronella essential oil spray gel preparation are <1 x 10 colonies/ml, these results have met the requirements with Indonesian Food and Drug Authority No. 29 of 2023, the yeast mold number (AKK) is not more than 2 x 10 colonies/ml. So it can be concluded that the spray gel preparation guarantees that it does not

contain mold/yeast or is free from mold/yeast contamination that can interfere with the stability and safety of the citronella essential oil spray gel preparation.

Antibacterial Activity Test

This study used the good diffusion method because it is effective in testing the inhibitory ability of antibacterial substances compared to other

methods. After all, the samples inserted into the wells that have been made produce an osmosis process that can diffuse to the bottom of the media surface (Sefriyanti *et al.*, 2020) The results of the antibacterial test of the spray gel preparation from citronella essential oil against *Staphylococcus aureus* bacteria can be seen in Table 11 and Figure 8.

Table 11. Antibacterial Activity Test Results

No.	Antibacterial Activity Test Results	
	Concentration	Average inhibition zone (mm)
1	Spray gel preparation	6.85±2.80
2	Control (+)	26.72±1.35
3	Control (-)	-

Note: Control (+) = clindamycin, Control (-) = spray gel preparation without active ingredients

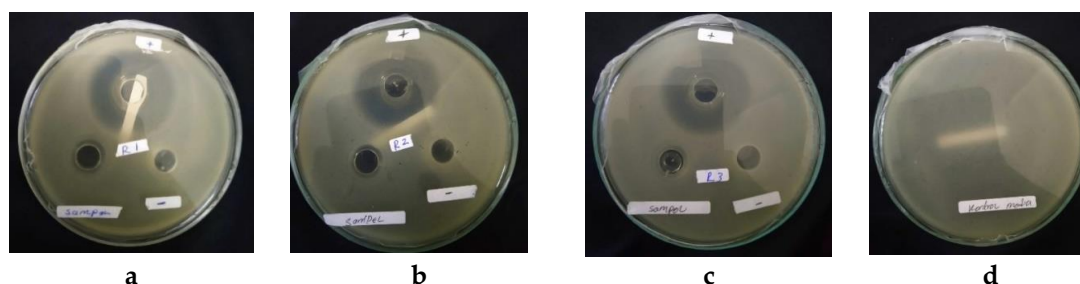


Figure 8. Results of Antibacterial Activity Testing of Replication 1 (a), Replication 2 (b), Replication 3 (c), and Media Control (d)

The differences in inhibition zone diameter obtained in this study not only show numerical variations but also reflect differences in the effectiveness of formulations in delivering lemongrass essential oil as an antibacterial agent. The inhibition zone of 6.85 ± 2.80 mm produced by the spray gel formulation falls into the moderate inhibition category, but its effectiveness is greatly influenced by the structure and characteristics of the spray gel formulation. In the spray gel preparation, the essential oil is trapped in a gel matrix that tends to release the active substance gradually. This slower release pattern can limit the diffusion rate of the essential oil into the agar medium, resulting in a relatively smaller inhibition zone. Factors such as gel viscosity, material homogeneity, and the strength of the essential oil bond to the gel base also affect the diffusion effectiveness.

In contrast, research by Flora Yulen Pia Rumlus *et al.* (2022) using liquid soap preparations produced an inhibition zone of 8.7 mm. Liquid soap has more fluid physical properties and does not form a matrix like gel, allowing essential oils to disperse and diffuse more quickly into the agar medium. This makes it easier for the active substance to reach *Staphylococcus aureus* bacteria, resulting in a larger inhibition zone diameter. Thus, the difference in the inhibition zone between the two studies is better

understood as a result of differences in the delivery systems of the active ingredients. The spray gel provides a more controlled but slower release, while the liquid soap allows for faster and more widespread diffusion of the active ingredient, resulting in a larger inhibition zone.

The positive control used in this study was clindamycin with a concentration of 1%. In the positive control study, it produced an inhibitory diameter of $26.72 \text{ mm} \pm 1.35$. The selection of clindamycin a positive control is because clindamycin is one of the therapies that can be used to treat atopic dermatitis infections. Clindamycin has a mechanism by which it prevents bacterial protein synthesis and is included in both bacteriostatic and bactericidal. (Herdiansyah *et al.*, 2023).

The negative control used in this study was the spray gel base, namely Na-CMC. The results showed that the antibacterial test with Na-CMC did not form an inhibition zone in the media. This is in accordance with the research of Mangkey *et al* (2023) that Na-CMC does not have antibacterial activity against *Staphylococcus aureus* bacteria.

Media control in this study was conducted to determine whether this test was free from external contamination. Media control aims to prove that the test bacteria used in this study can grow and survive

after treatment, thus proving that the method is correct (Astutiningsih *et al.*, 2014).

CONCLUSION

The concentration of Na-CMC is 0.27%; propylene glycol 4.72%; and Tween 20 10% can produce an optimal citronella essential oil spray gel formula. Manufacture of citronella essential oil spray gel (*Cymbopogon nardus* (L.) Rendle). The result is a milky white color, a distinctive citronella aroma, and a slightly thick consistency. This formulation is homogeneous, with pH, viscosity, and drying time that meet standards. This spray gel formulation is stable at 4°C and 40°C storage temperatures and is free from fungal/yeast contamination. The citronella essential oil spray gel formulation (*Cymbopogon nardus* (L.) Rendle) exhibits antibacterial activity against *Staphylococcus aureus* ATCC 25923 with an inhibition zone diameter of 6.85 mm ±2.80 and is classified as moderate. In the future, this preparation has the potential to be developed through long-term stability testing or in vivo efficacy testing to ensure more comprehensive performance and safety.

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REFERENCES

- Abdi, Dian Amelia. 2020. "Dermatitis Atopik." *Wal'afiat Hospital Journal* 1(2):40.
- Angelia, Angelia, Gracia Rinika Putri, Amalia Shabrina, And Nuraini Ekawati. 2022. "Formulasi Sediaan Spray Gel Ekstrak Kulit Jeruk Manis (*Citrus Sinensis* L.) Sebagai Anti-Aging." *Generics: Journal Of Research In Pharmacy* 2(1):44–53.
- Astutiningsih, Christina, Wahyuning Setyani, And Himawan Hindratna. 2014. "Uji Daya Antibakteri Dan Identifikasi Isolat Senyawa Katekin Dari Daun Sirih." *Jurnal Farmasi Sains Dan Komunitas* 11(2):50–57.
- Devi, Anggi Meliana, Aulia Fikri Hidayat, And Sani Ega Priani. N.D. 2020 "Formulasi Sediaan Spray Gel Mengandung Nanoemulsi Minyak Cengkeh (*Syzygium Aromaticum* L.) Untuk Kandidiasis Oral." 567–74.
- Djajanti, Agust Dwi Djajanti, And Andi Muhammad Farid. 2023. "Formulasi Dan Uji Aktivitas Sediaan Gel Handsanitizer Minyak Atsiri Sereh (*Cymbopogon Citratus*) Terhadap Bakteri *Pseudomonas Aeruginosa*." *Jurnal Kesehatan Yamasi Makassar* 7(1):17–27.
- Herdiansyah, Al Fadil, La Ode Bariun, And Citra Dewi. 2023. "Uji Aktivitas Antibakteri Ekstrak Etanol Daun Suruhan (*Peperomia Pellucida* L.Kunth) Terhadap Bakteri *Staphylococcus aureus* Dan *Staphylococcus Epidermidis*." *Jurnal Pharmacia Mandala Waluya* 2(2):106–16.
- Hermawati, Ema, Ardy Tanfil T, Pra Panca, And Bayu Chandra. 2023. "Aktivitas Antibakteri Minyak Atsiri Sereh Wangi (*Cymbopogon Citratus*) Terhadap Bakteri *Escherichia Propionibacterium Acne*, *Staphylococcus aureus* Antibacterial Activity Of Citrus Essential Oil (*Cymbopogon Citratus*) Against *Escherichia Coli*, *Propionib.*" 12(2):11–16.
- Imansyah, Maulana Z., And Gusti Alam. 2021. "Jurnal Kesehatan Yamasi Makassar." *Jurnal Kesehatan Yamasi Makasar* 5(2):121–27.
- Indika, Raihan, Linda Adriani, And Wulandari. 2020. "Faktor Yang Mempengaruhi Kejadian Dermatitis Pada Bayi." *Darussalam Indonesian Journal Of Nursing* 1(1):42–53.
- Iwamoto, Kazumasa, Masaya Moriwaki, Ryu Miyake, And Michihiro Hide. 2019. "Staphylococcus aureus In Atopic Dermatitis: Strain-Specific Cell Wall Proteins And Skin Immunity." *Allergology International* 68(3):309–15.
- Juliarti, Anna, Nurheni Wijayanto, Irdika Mansur, And Trikoesoemaningtyas. 2020. "Analisis Rendemen Minyak Serai Wangi (*Cymbopogon nardus* L.) Yang Ditanam Dengan Pola Agroforestri Dan Monokultur Pada Lahan Revegetasi Pasca Tambang Batubara." *Jurnal Sylva Lestari* 8(2):181–88.
- Kim, J., Kim, B. E., & Leung, D. Y. M. (2019). Pathophysiology of atopic dermatitis: Clinical implications. *Allergy and Asthma Proceedings*, 40(2), 84–92.
- Mangkey, Tasya E. L., Paulina V. Y. Yamlean, And Jainer Pasca Siampa. 2023. "Formulation And Test Of Antibacterial Activity Of Ethanol Extract Of Avocado Peel (*Persea Americana* Mill.) Using Na-Cmc And Carbopol Base Against *Staphylococcus Aureus*." *Pharmacon* 12(1):127–32.
- Martihandini, Nooryza, And Shandra Isasi Sutiswa. 2023. "Formulation Of Spray Gel Hand Sanitizer From Citronella Oil Using Gelling Agent Carbopol 940 And Humectant Propylene Glycol." *Indonesian Journal Of Pharmaceutical Education* 3(3):447–55.
- Murni, & Rustin, L. (2020). Karakteristik Kandungan Minyak Atsiri Tanaman Serai Wangi

- (*Cymbopogon nardus* L.). *Prosiding Seminar Nasional Biologi Di Era Pandemi COVID-19, September*, 227–231. <http://journal.uin-alauddin.ac.id/index.php/psb/>
- Monalisa, Devi, Rizky Kurniawan, And Yuni Rahmayanti. 2023. "Karakteristik Penyakit Dermatitis Atopik Di Rumah Sakit Pertamedika Ummi Rosnati Periode Tahun 2017-2022." *Jurnal Sains Riset* 13(2):566–76.
- Ogonowska, Patrycja, Klaudia Szymczak, Joanna Empel, Małgorzata Urbaś, Agata Woźniak-Pawlikowska, Wioletta Barańska-Rybak, Dariusz Świetlik, And Joanna Nakonieczna. 2023. "Staphylococcus aureus From Atopic Dermatitis Patients: Its Genetic Structure And Susceptibility To Phototreatment." *Microbiology Spectrum* 11(3).
- Olisvelos, K. A., Dwi Adityarini, And A. Prasetyaningsih. 2023. "Potensi Minyak Sereh Wangi (*Cymbopogon Nardus*) Sebagai Antibakteri Terhadap Propionibacterium Acnes Dan *Staphylococcus aureus* Pada Sediaan Gel Antijerawat." *Jurnal Pro-Life* 10(1):682–95.
- Porawati, Hilda, And Ari Kurniawan. 2019. "Rancang Bangun Alat Penyuling Minyak Atsiri Tumbuhan Nilam Metode Distilasi Air Dan Uap." *Jurnal Inovator* 2(1):20–23.
- Rahayu, Titi Pudji, Laely Fitriyati, And Putri Amalia. 2022. "Uji Cemaran Mikroba Angka Lempeng Total (Alt) Pada Sediaan Jamu Gendong Di Pasar Karanganyar Kabupaten Kebumen." *Prosiding 16th Urecol: Seri Mipa Dan Kesehatan* 1642–54.
- Ramadhani, Reshita Amalia, Dody Herdian Saputra Riyadi, Bayu Triwibowo, And Ratna Dewi Kusumaningtyas. 2017. "Review Pemanfaatan Design Expert Untuk Optimasi Komposisi Campuran Minyak Nabati Sebagai Bahan Baku Sintesis Biodiesel." *Jurnal Teknik Kimia Dan Lingkungan* 1(1):11–16.
- Rosmania, Rosmania, And Fitri Yanti. 2020. "Perhitungan Jumlah Bakteri Di Laboratorium Mikrobiologi Menggunakan Pengembangan Metode Spektrofotometri." *Jurnal Penelitian Sains* 22(2):76.
- Sari, Irma, Misrahanum Misrahanum, Meutia Faradilla, Cut Mutia Ayuningsih, And Hilda Maysarah. 2022. "Antibacterial Activity Of Citronella Essential Oil From *Cymbopogon Nardus* (L.) Rendle) Against Methicillin Resistant *Staphylococcus Aureus*." *Indonesian Journal Of Pharmaceutical And Clinical Research* 5(1):16–22.
- Sefriyanti, Afghani Jayuska, And Andi Hairil Alimuddin. 2020. "Uji Aktivitas Antibakteri Minyak Atsiri Sereh Wangi (*Cymbopogon Bernadus* L.) Terhadap Bakteri *Escherichia Coli* Dan *Staphylococcus Aureus*." *Jurnal Kimia Khatulistiwa* 8(4):1–4.
- Suarantika, Farendina, Vinda Maharani Patricia, And Hanifa Rahma. 2023. "Karakterisasi Dan Identifikasi Senyawa Minyak Atsiri Pada Sereh Wangi (*Cymbopogon nardus* (L.) Rendle) Dengan Kromatografi Gas-Spektrometri Massa." *Jurnal Mandala Pharmacoon Indonesia* 9(2):514–23.
- Sugiyanto, Didik, Yefri Chan, Fahmi Aldi, Hernando Christian, Teknik Mesin, Fakultas Teknik, Universitas Darma Persada Jl Taman Malaka Selatan, Pondok Kelapa, Duren Sawit, And Kota Jakarta Timur. 2022. "3 Sugiyanto, Didik., Dkk; Rancang Bangun Alat Penyuling Daun Serai Menggunakan Metode Steam Hydrodistillation Rancang Bangun Alat Penyuling Daun Serai Menggunakan Metode Steam Hydrodistillation." *Jurnal Teknik Mesin* 8(1):13–18.
- Sundari, Sri, And Fadhlani. 2019. "Uji Angka Lempeng Total (Alt) Pada Sediaan Kosmetik Lotion X Di Bbpom Medan." *Jurnal Biologica Samudra* 1(1):25–28.
- Tari, Mayang, And Ony Indriani. 2023. "Formulasi Dan Uji Stabilitas Fisik Sediaan Krim Ekstrak Sembung Rambat (*Mikania Micrantha Kunth*)." *Babul Ilmi_Jurnal Ilmiah Multi Science Kesehatan* 15(1):192–211.
- Ulfa, Maria, Sukriani Kursia, And Mardayani Novita Sari. 2023. "Uji Aktivitas Antibakteri Dari Ekstrak Etanol Daun Adam Hawa (*Rhoeo Discolor* L.Her) Terhadap Propionibacterium Acnes, *Staphylococcus aureus* Dan *Staphylococcus Epidermidis*." *Medfarm: Jurnal Farmasi Dan Kesehatan* 12(1):67–76.
- Wos Zubaydah Wos Zubaydah, Rini Novianti, And Astrid Indalifiany. 2022. "Pengembangan Dan Pengujian Sifat Fisik Sediaan Spray Gel Dari Ekstrak Etanol Batang *Etingera Rubroloba* Menggunakan Basis Gel Na-Cmc." *Journal Borneo* 2(2):38–49.
- Wulandari, And Lilies Wahyu Ariyani. 2020. "Nanogel Minyak Biji Bunga Matahari (*Helianthus Annuus*) Sebagai Antibakteri Terhadap Bakteri *Staphylococcus Aureus*." *Jurnal Ilmiah Cendekia Eksakta* 63–66.