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Physical Quality Stability Test and Antibacterial Activity Test of *Face Toner Roll On* Formulation of *Centella Asiatica* (L.) Urb. Leaf Extract Againts *Propionibacterus acnes*

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ABSTRAK

Acne affects all ages, from teenagers to adults, and is generally caused by bacteria. One of the main causes is Propionibacterium acnes, a gram-positive bacterium that lives in the skin and sebaceous follicles. Therefore, a natural solution for treating acne is gotu kola leaves, which are blended into a liquid roll-on face toner. Its flavonoids, tannins, saponins, and alkaloids act as antibacterial agents against Propionibacterium acnes. This study used a laboratory experiment. The results showed that the physical stability test of gotu kola leaf extract roll-on face toner (Centella asiatica (L.) urb) in formulation 1 (7.5%), formulation 2 (10%), and formulation 3 (12.5%) successfully withstood the test, maintained stable pH and viscosity, and exhibited no significant changes in color and odor upon storage. The gotu kola leaf extract roll-on face toner demonstrated very strong antibacterial activity against Propionibacterium acnes. Formula F3 (12.5%) had the largest inhibition zone diameter of 46.27 mm. However, based on ANOVA and post hoc tests, there was no significant difference between F2 (10%) and F3 (12.5%) compared to the positive control (sig > 0.05), so both formulas had comparable antibacterial effectiveness to the positive control.), so that both formulas had antibacterial effectiveness comparable to the positive control.

KEYWORDS : *Activity, Antibacterial, Face Toner Roll On, Propionibacterium Acnes, Physical Quality Stability.*

1. INTRODUCTION

The skin is the outer layer of the human body that is exposed to external factors. It is essential to take care of it, not only because it serves as the body's outermost barrier but also because it contributes to beauty. Healthy, clear, and radiant skin is the desire of many men and women [1]. One of the most common skin conditions among teenagers is acne. Acne is

a skin problem characterized by infection and inflammation of the sebaceous glands. Some antibiotics commonly used to treat acne include clindamycin, tetracycline, and others. However, excessive use of antibiotics can lead to resistance. Therefore, alternative treatments for acne are needed, namely those derived from natural ingredients in the form of medicinal plants [1].

One medicinal plant in Indonesia with antibacterial properties that can fight acne-causing bacteria is pegagan (*Centella asiatica* (L.) Urb.). The pegagan plant (*Centella asiatica* (L.) Urb.) contains many active ingredients such as saponins, triterpenoids, and chemical compounds divided into several groups, such as flavonoids, essential oils, amino acids, and triterpenoids. Some antibiotics commonly used to treat acne include clindamycin, tetracycline, and others. However, excessive use of antibiotics can lead to resistance. Therefore, alternative treatments for acne are needed, namely those derived from natural ingredients in the form of medicinal plants.

One medicinal plant in Indonesia with antibacterial properties that can fight acne-causing bacteria is gotu kola (*Centella asiatica* (L.) Urb.). The gotu kola plant (*Centella asiatica* (L.) Urb.) contains many active ingredients such as saponins, triterpenoids, and chemical compounds divided into several groups, such as flavonoids, essential oils, amino acids, and triterpenoids. The results of the study showed that the average diameter of the highest inhibition zone at a concentration of 80% was 19.5 mm with a strong category. *Centella asiatica* (L.) Urb.) leaf extract was formulated in the form of a gel preparation that has an antibacterial activity effect that causes acne, namely *Staphylococcus aureus* bacteria. Another content of the centelloid triterpenoid plant has antibacterial activity and provides defense against pathogenic infections. Other benefits of the centella asiatica plant (*Centella asiatica* (L.) Urb.) are as a pain reliever, sedative, antimicrobial and antidepressant. The pennywort plant (*Centella asiatica* (L.) Urb.) contains pentacyclic triterpenoid aglycones, a group of saponin glycoside compounds, collectively known as centelloids. The pennywort plant (*Centella asiatica* (L.) Urb.) has properties such as lightening cellulite, reducing wrinkles, eliminating dark spots on the face, and reducing wrinkles on the skin caused by aging.

The ease of application of gotu kola herbal extract in acne treatment can be made in one dosage form, namely a face toner roll-on. Face toner roll-on is one pharmaceutical preparation that can be chosen to treat acne. It is applied by rubbing a ball coated with the face toner roll-on formula onto the skin affected by acne. Face toner roll-on is basically a liquid cosmetic formulation designed as a substitute for facial cleansing or after facial cleansing. It also acts as a moisturizer to control sebum production and can help percutaneous absorption that acts as a barrier to increase skin hydration, some even function as medicine. In addition to perfecting facial cleansers, face toner roll-on can also be used with the addition of important active ingredients such as anti-acne. Face toner roll-on formulas are usually water-based, while other additional ingredients include active substances, humectants, emollients, surfactants, fragrances, and preservatives. This study actually focuses on face toner roll-on that functions as an anti-acne.

Various previous studies have described gotu kola leaf extract, such as Ointu, that gotu kola leaf extract (*Centella asiatica* (L.) Urb.) with concentrations of 2%, 4%, 8% and 10% can inhibit the growth of Propionibacterium acnes. The results of antibacterial activity testing showed that gotu kola leaf extract was able to inhibit the growth of Propionibacterium acnes bacteria, a concentration of 2% had an inhibitory power of 6 mm, a concentration of 4% had an inhibitory power of 8 mm, a concentration of 8% had an inhibitory power of 10 mm and at a concentration of 10% with an inhibition zone diameter of 12 mm, it was included in the strong category.

2. METHOD

This type of research is a laboratory experimental study. This study aims to determine whether the face toner roll-on extract of *Centella asiatica* (L.) Urb. has antibacterial activity against Propionibacterium acnes. Experimental laboratory research, authentic experiments, or study center research is conducted in a laboratory.[2] *Centella asiatica* (L.) Urb. leaf extract was tested under controlled laboratory conditions to determine its ability to inhibit Propionibacterium acnes. The location and research were conducted at the Pharmaceutical Preparation Technology Laboratory, Pharmacognosy Laboratory and Microbiology Laboratory of Strada Indonesia University and the research time was January – March 2025.

The population of this research consisted of *Centella asiatica* leaves cultivated in Pagerwojo, Tulungagung Regency, East Java Province. The study employed a purposive sampling method to select samples intentionally based on specific criteria. However, the probability sampling approach was also utilized to ensure representative data collection.

Variables refer to the characteristics or attributes possessed by individuals, objects, or situations that vary among subjects or groups. This section outlines both the independent and dependent variables in detail. Identifying variables accurately is essential for all research stages, particularly in defining operational concepts and conducting data analysis. The independent variable in this study was the roll-on face toner formulation containing Gotu Kola (*Centella asiatica* (L.) Urb.) leaf extract, tested at three concentration levels: F1 (7.5%), F2 (10%), and F3 (12.5%).

Organoleptic evaluation was carried out through direct observation of the toner's physical characteristics, including color, odor, and texture. Each formulation was evenly applied onto a glass slide to ensure the mixture was homogeneous and free from particle clumps. The pH measurement was performed using a pH meter, which was immersed in the sample and allowed to stabilize before reading. Ideally, the pH of face toner products should range between 4.5 and 6.5.

Viscosity testing was conducted using a viscometer equipped with spindle number 1 at a speed of 60 rpm. The toner sample was placed in a beaker, and the spindle was immersed in the preparation. Each formulation was tested three times to obtain an accurate viscosity value. The data obtained will then be analyzed statistically using SPSS with the following stages:

Normally Test

A normality test was performed to verify whether the data were normally distributed, allowing for the application of parametric statistical analysis. The Shapiro–Wilk test was used for this purpose.

Hypothesis :
H_0 : The data are normally distributed; H_1: The data are not normally distributed.
Decision criteria:
If $p > 0.05 \rightarrow H_0$ is accepted, H_1 is rejected. If $p \leq 0.05 \rightarrow H_1$ is accepted, H_0 is rejected.

Homogenity Test

The homogenity test was conducted to determine whether the sample data had uniform variance, assuming that the samples were drawn from the same population. Levene's test was employed to assess homogenity.

Hypothesis :
H_0 : The data have equal variances (homogeneous); H_1 : The data have unequal variances (not homogeneous).
Decision criteria:
If $p > 0.05 \rightarrow H_0$ is accepted, H_1 is rejected. If $p \leq 0.05 \rightarrow H_1$ is accepted, H_0 is rejected.

One-Way ANOVA Test

A one-way ANOVA was applied to analyze differences in inhibition zones among the various concentrations of *Centella asiatica* leaf extract formulations, with a positive control used as a comparison for antibacterial activity [3] To identify specific differences between treatments, a Tukey post hoc test was subsequently conducted. Furthermore, a paired sample *t*-test was used to evaluate the stability of the formulations by comparing pH values and antibacterial activitefore and after the cycling test.

3. RESULT AND DISCUSSION

The samples used in this study came from Tulungagung Regency, Pagerwojo District, Kradinan Village, East Java Province. Furthermore, at the Batu Materia Medika Herbal Laboratory, gotu kola leaves were identified. The identification results of the gotu kola leaf plant used were the species *Centella asiatica* (L.) Urb and the family Umbelliferae (Apiaceae). Appendix 1 shows the certificate of identification results from the determination test. The results of the determination state that.

Kingdom : Plantae
Division : Magnoliophyta

Class : Dicotyledonae

Order : Umbellales

Family : Umbelliferae (Apiaceae)

Genus : *Centella*

Species : *Centella asiatica* (L.) Urb

Researchers cultivated *Centella asiatica* (L.) Urb in a field in Pagerwojo District. Once the plants were mature enough, they harvested the plants, selected the leaves, and trimmed any branches that were slightly mixed with the leaves. The next step was to wash the leaves under running water to clean them. This resulted in fresh *Centella asiatica* (L.) Urb ready for drying. Five kg of fresh *Centella asiatica* (L.) Urb were dried and then pollinated using a blender. Afterward, they were sieved using a number 60 sieve to obtain 2 kg of powder. The next step was to test the water content using a moisture balance, resulting in a result of 6.83%, which meets the water content requirement of <10%.

The results obtained from testing the *Centella asiatica* (L.) Urb extract were dark green in color, thick in consistency, and had a distinctive *Centella asiatica* (L.) Urb aroma. The steps involved extracting gotu kola (*Centella asiatica* (L.) Urb.) leaves using a maceration method. Then, the gotu kola leaf powder was dissolved in 70% ethanol at a ratio of 1:10 for 3 x 24 hours, stirring occasionally, in a dark bottle protected from light. The resulting macerate was filtered and concentrated with a rotary evaporator to obtain a thick gotu kola (*Centella asiatica* (L.) Urb.) leaf extract. The extract was then heated in a water bath to produce a thick extract.

After calculating the drying loss, the drying process resulted in a loss of 7.75% of water content and compounds. The average calculation results can be seen in Appendix 4.c. The drying loss of the *Centella asiatica* (L.) Urb extract exceeded the parameters specified in the Indonesian Herbal Pharmacopoeia, Edition II. The drying loss did not exceed 10%. This can occur depending on the temperature and humidity in the workspace/laboratory, the temperature and air pressure in the oven, the size and shape of the sample, and the size of the container/bottle.

The next test was an ethanol-free test. The finished gotu kola leaf extract was added with concentrated H_2SO_4 and 1% CH_3COOH. A positive test for gotu kola leaf extract was considered ethanol-free if there was no characteristic ethanol ester odor. The results of this

ethanol-free test for gotu kola leaf extract indicated that it was ethanol-free, as evidenced by the absence of the characteristic ethanol odor. The results of the phytochemical screening test of *Centella asiatica* (L.) Urb extract in the tube test image in the appendix show that the *Centella asiatica* (L.) Urb extract tested positive for flavonoids due to the formation of a brownish-yellow color after testing with the reagent. A stable foaming test for the positive sample indicates the presence of saponins. The formation of a blackish-green color indicates the presence of tannins. No yellow precipitate formed, indicating the negative *Centella asiatica* (L.) Urb sample, indicating the presence of alkaloids. Meanwhile, TLC testing was performed on the positive compounds from the phytochemical screening test using the reagent test. Identification by TLC used a 1x7 cm2 silica 60 F254 plate. The ethanol extract of *Centella asiatica* (L.) Urb. leaves was spotted at a distance of approximately 1 cm from the bottom edge of the plate using a capillary tube. The TLC plate was then inserted into a chamber containing a saturated mobile phase. The eluent was allowed to rise to the edge of the TLC plate. The TLC plate was then removed and air-dried. The spots formed on the silica gel were observed under UV light at a wavelength of 366 nm. Each spot was then observed, including the number of spots and the calculation of the Rf value. The 366 nm UV light produced spots with a dark background, allowing the spots to fluoresce and be visually observed.

The production of the Gotu Kola leaf extract roll-on face toner consists of three formulations with different extract concentration. Formulation 1 contains 7.5% Gotu Kola leaf extract and is dark green to black in color. Formulation 2 contains 10% Gotu Kola leaf extract and is dark green to black in color. Formulation 3 contains 12.5% Gotu Kola leaf extract and is dark green to black in color. The pH stability test data of the face toner roll on gotu kola leaf extract was tested for normality using Shapiro-Wilk and resulted in the finding that the data was not normally distributed because the sig value was 0.000 <0.05:

Table 1. Normality Test of pH Stability Data

FORMULASI	STATIST	DF	SIG.	STATIST	DF	SIG.
FORMULASI 1	.428	12	.000	.584	12	**.000**
FORMULASI 2	.474	12	.000	.453	12	**.000**
FORMULASI 3	.447	12	.000	.552	12	**.000**

a. Lilliefors Significance Correction
b. Skor is constant when Formulasi = K+. It has been omitted.
c. Skor is constant when Formulasi = K-. It has been omitted.

The data was then tested for homogeneity, and the significance value was 0.018<0.05, thus concluding that the data variance was not homogeneous.

Table 2. Homogenity

LEVENE STATISTIC	DF_1	DF_2	**SIG.**
3.255	4	55	.018

Then, a Kruskal-Wallis test was performed on the pH stability data. This was because the data was neither normally distributed nor homogeneous. The Kruskal Wallis test results in the table above indicate a p-value of 0.000. When the p-value is <0.05, a significant difference can be seen between the pH of formulations 1, 2, and 3, as well as the K- and K+ concentrations. This is different if the p-value is >0.05. If the p-value is >0.05, there is no significant difference between the pH of formulations 1, 2, and 3, as well as the K- and K+ concentrations.

The average viscosity data obtained was used to determine the difference in viscosity of the gotu kola leaf extract roll-on face toner in each formulation with different concentrations of gotu kola leaf extract. Based on the Shapiro-Wilk test, the data obtained are normally distributed, as the sig value is >0.05. The data was then tested for homogeneity, and the significance value was 0.000<0.05, thus concluding that the data variance was not homogeneous. The table below shows the results:

Table 3. Viscosity Stability Data Homogeneity Test

LEVENE STATISTIC	DF_1	DF_2	SIG.
9.479	4	55	.000

Then, the analysis was carried out using the Kruskal Wallis method. The data analysis yielded a p-value of 0.000, with a significance level of 0.05, where 0.000 <0.05. Therefore, this test indicates a difference in viscosity between the two formulations.

The Kruskal-Wallis test results were followed by a post-hoc test on each formulation, with the following results. Based on the LSD post-hoc test, it can be concluded that the formulation with no significant difference was formulation 2 with K+, and vice versa. This is because the sig>0.05. There were significant differences between formulation 1 and formulation 2, formulation 3, K+, and K-. The asterisk in the mean difference column indicates the average difference between the two formulations being compared. The data obtained for the average inhibition zone diameter was used to determine the difference in inhibition zone diameter of the gotu kola leaf extract face toner roll-on in each formulation with different concentrations of gotu kola leaf extract. First, the data were tested for

normality using the Shapiro-Wilk test using SPSS 26. The Shapiro-Wilk test confirmed the data were normal, as the p-value was >0.05. The following are the SPSS results:

Table 4. Normality Test of Inhibition Zone Data

FORMULASI	STATIST	DF	SIG.	STATIST	DF	SIG.
F1	.298	3	.	.915	3	**.435**
F2	.195	3	.	.996	3	**.881**
F3	.302	3	.	.910	3	**.417**
K+	.204	3	.	.993	3	**.846**

a. Lilliefors Significance Correction
b. Skor is constant when Formulasi = K+. It has been omitted.
c. Skor is constant when Formulasi = K-. It has been omitted.

The results of the inhibition zone normality test revealed a p-value >0.05. This indicates that the hypothesis stating that the inhibition zone data is normal is accepted. Conversely, if the p-value <0.05 indicates that the inhibition zone data is not normal. However, if the p- value >0.05 is obtained, it is concluded that the inhibition zone data is normal. A homogeneity test was then performed, and the significance value was 0.065 >0.05, thus concluding that the data variance is homogeneous. The results of the SPSS analysis are as shown in the table below:

Table 5. Inhibition Zone Data Homogeneity Test

LEVENE STATISTIC	DF_1	DF_2	SIG.
3.141	4	10	.065

Based on the homogeneity test, the inhibition zone data were homogeneous, as evidenced by a p-value >0.05. The result of this homogeneity test was 0.065 >0.05. Therefore, the conclusion is that the inhibition zone data variance is homogeneous. The data were analyzed using SPSS 26 using the one-way ANOVA method. The data analysis yielded a p-value of 0.000, with a significance level of 0.05, where 0.000 <0.05. Therefore, this test indicates a difference in the inhibition zones of each formulation: F1, F2, F3, K+, and K-. The results of the ANOVA data analysis are as shown in the table below:

Table 6. Results of the ANOVA Test for Inhibition Zones

	SUM OF SQUARES	DF	MEAN SQUARE	F	SIG.
BETWEEN GROUPS	1528.735	4	382.184	31.577	**.000**
WITHIN GROUPS	121.032	10	12.103		
TOTAL	**1649.767**	**14**			

Based on the results of the ANOVA test above, it can be concluded that the p-value is 0.000. This value is certainly <0.05. Therefore, it can be concluded that there is a difference in the inhibition zones between each formulation, F1, F2, F3, K+, and K-. In this physical quality test, the researchers confirmed that the *Centella asiatica* (L.) Urb used in this study originated from Tulungagung Regency, Pagerwojo District, Kradinan Village,

East Java Province, where the plant is cultivated. The researchers then submitted Centella asiatica (L.) Urb to the Batu Materia Medika Herbal Laboratory for identification. The plant identification results indicated that the type of gotu kola used is the *Centella asiatica* (L.) Urb species and belongs to the Umbelliferae (Apiaceae) family. Appendix 1 shows the certificate of identification results from the determination test. The next step was to use 5 kg of wet *Centella asiatica* (L.) Urb, which was then dried and powdered using a blender. Afterward, it was sieved using a number 60 sieve, resulting in 2 kg of powder. The next step was to test the water content using a moisture balance, resulting in a result of 6.83%, which meets the water content requirement of <10%.

The results of the *Centella asiatica* (L.) Urb extract test were dark green in color, thick in consistency, and with a distinctive *Centella asiatica* (L.) Urb aroma. The *Centella* asiatica (L.) Urb. leaves were extracted using a maceration method, resulting in a yield of 19.59%. The *Centella asiatica* (L.) Urb. powder was then dissolved in 70% ethanol at a ratio of 1:10 for 3 x 24 hours, occasionally shaking, in a dark bottle protected from light. The filtered macerate was concentrated using a rotary evaporator to obtain a thick extract of *Centella asiatica* (L.) Urb. leaves, then heated in a water bath until a thick extract was obtained.

The drying loss was determined three times. The drying loss of the resulting *Centella asiatica* (L.) Urb. extract was calculated using the formula below. After calculating the drying loss, it was found to be 7.75%. The drying process resulted in a loss of water content and compounds of 7.75%. The average calculation results can be seen in Appendix 4.c. The drying loss of the resulting *Centella asiatica* (L.) Urb. extract did not exceed the parameters stipulated in the Indonesian Herbal Pharmacopoeia, Edition II. The drying loss level did not exceed 10%. This is due to strict control in the oven, resulting in a drying process that meets the specified requirements.

Maceration is a simple extraction method that does not involve heating. The process involves soaking powdered medicinal plants in a solvent. The solvent then penetrates through the cell walls into the cell cavities containing the active ingredients. The active ingredients dissolve due to changes in concentration between the solution outside and inside the cells. The active ingredients dissolve, releasing a more concentrated solution.[4] This study used 70% ethanol as the solvent due to its universality. The purpose of 70% ethanol is to moisten the powdered sample, opening the pores, and allowing the compounds to be easily released. Then, the gotu kola leaf powder was dissolved in 70% ethanol at a ratio of 1:10 for 3 x 24 hours, stirring occasionally, in a dark bottle protected from light. The filtered macerate

was concentrated using a rotary evaporator to obtain a thick extract of *Centella asiatica* (L.) Urb. leaves. This was then heated in a water bath to produce a thick extract.

The next step was the ethanol-free test. The finished *Centella asiatica* (L.) Urb. leaf extract was added with concentrated H_2SO_4 and 1% CH_3COOH. A positive test for ethanol-free extract was achieved if the characteristic ester odor of ethanol was absent. This is consistent with the journal's statement that an extract is considered ethanol-free if the characteristic ester odor of ethanol is absent[2]. Afterward, a test tube test was performed as an initial phytochemical screening. The phytochemical screening results for *Centella asiatica* (L.) Urb. extract tube test were positive for flavonoids, as a brownish-yellow color was formed after testing with the reagent. Stable foaming of the sample indicated the presence of saponins. The formation of a blackish-green color indicated the presence of tannins. No yellow precipitate formed, indicating the presence of alkaloids. The test tube results indicated that *Centella asiatica* (L.) Urb extract contained flavonoids, saponins, and tannins. The alkaloid test was not visible because the reaction involves ligand replacement, where the nitrogen with the lone pair of electrons in the alkaloid forms a coordinate covalent bond with the corresponding ion. A slight deficiency in the test tube analysis, possibly due to the absence of alkaloids, was observed.

Next, a TLC test was performed. UV 366 nm observation produced spots with a dark background, allowing the spots to fluoresce and be visually observed.[5] This TLC test confirmed that gotu kola leaves contain flavonoids, saponins, tannins, and alkaloids. TLC analysis for flavonoid compounds, as proposed by Dewi et al.,[6] that TLC analysis with aquadest methanol (6:4) produced 1 sample spot with a spot distance of 6.2 cm and an eluent distance of 8 cm with an Rf value of 0.775 and a quercetin spot with a spot distance of 7.6 cm and an eluent distance of 8 cm with an Rf value of 0.95. According to research by Muksin Maulana the results of determining the best eluent for alkaloids were ethyl acetate: methanol: water (6:4:2) ethanol extract eluent, for flavonoids it was n-butanol: acetic acid: water (4:1:5) ethanol extract. According to [4], the results of the TLC test were almost the same as Muhsin's, namely flavonoids: Water:Butanol: Glacial Acetic Acid (5:4:1), alkaloids: Ethyl Acetate:Methanol:Water (4:6:2), saponins: Water:N-Butanol (1:1), tannins: Water:Butanol:Glacial Acetic Acid (5:4:1).

The next step was to make a face toner roll-on with gotu kola leaf extract. There were four formulations with different extract concentrations. The positive formulation was the one that did not contain gotu kola leaf extract and had a transparent white color. Formulation 1 was

the preparation containing 7.5% gotu kola leaf extract and a dark green-black color. Formulation 2 was the preparation containing 10% gotu kola leaf extract and a dark green-black color. Formulation 3 was the preparation containing 12.5% gotu kola leaf extract and a deep dark green-black color. This concentration becomes more apparent as the percentage of gotu kola leaf extract increases.[7]

The next step is to evaluate the preparation using a cycling test and stability tests at room temperature and 40°C. The cycling test involves storing the Face Toner Roll-On preparation for six 24-hour cycles at 4°C and 40°C. Two storage temperatures constitute one cycle. Physical and chemical stability are then observed, including organoleptic tests for color, odor, and shape, as well as homogeneity, viscosity, and pH tests on the preparation from before to after testing.

Viscosity testing found that thicker extract formulations will produce higher viscosity values than lighter extract formulations. The viscosity value of a toner should be less than 5 cPs (centipoise). This standard indicates that the toner has a low viscosity, making it easy to apply and quickly absorbed into the skin without leaving a sticky feeling. Too high a viscosity will make the toner feel thick and difficult to spread, while too low a viscosity can make the toner too runny, making it difficult to control during application. Viscosity testing is typically performed with a Brookfield viscometer at a specific speed (60 rpm) and using an appropriate spindle. In addition to meeting the viscosity limit of <5 cPs, the toner must also be physically stable, meaning its viscosity does not change significantly after stability tests (e.g., temperature cycling tests).[8] A toner with a suitable viscosity ensures comfortable application, stability of the preparation, and effective delivery of active ingredients to the skin. The pH quality test of the formulations on day 0, after undergoing the Kruskal Wallis test, showed no difference in pH between the formulations, with a p value > 0.05. The data analysis yielded a p-value of 0.151, with a significance level of 0.05, where 0.151 > 0.05. However, a post-hoc analysis revealed that this difference only occurred when compared to K-. The post-hoc test concluded that the F1:F2:F3:K+ ratio yielded a p value > 0.05. F1, F2, and F3 had no significant pH differences when compared to K+. This is the correct position, as the formulations have no significant pH differences compared to K+ or the positive control.

In the viscosity test, the Kruskal Wallis test revealed a p-value of 0.000, with a significance level of 0.05, where 0.000 < 0.05. The post hoc test results revealed that formulations with no significant differences were Formulation 2 and Formulation 3,

Formulation 2 and K+, and vice versa. This was because the sig>0.05. There were significant differences between Formulation 1 and Formulation 2, Formulation 3, and K+ and K-, due to the sig<0.05. Regarding viscosity, based on the ANOVA and post hoc test results, Formulation 2 showed no significant difference from the positive control, while Formulations 1 and 3 showed significant differences from the positive control. Physical stability factors such as temperature and storage duration can also affect the viscosity of a preparation, resulting in viscosity differences between the first formulation and the positive control during the physical quality test.

Bacterial identification was then performed through biochemical tests. The bacterial isolates were inoculated onto Triple Sugar Iron Agar (TSIA) media by inoculating perpendicularly on the butt side and by streaking continuously on the slant side. Then the culture was incubated at 37°C for 24 hours and the color change of the media was observed.[9] The results of the biochemical test showed that the slant part of the media was red and the butt part was yellow, so it can be concluded that the bacteria are only able to ferment glucose, but are unable to ferment lactose and sucrose. The yellow color on the butt (base) indicates the occurrence of glucose fermentation which produces acid, so the pH becomes acidic. The red color on the slant (slant) indicates that on the surface, the bacteria do not ferment lactose or sucrose, so the pH remains or becomes alkaline due to aerobic peptone breakdown.[10]

Meanwhile, the antibacterial activity test of *Centella asiatica* (L) urb pennywort leaf extract face toner roll on was conducted using the disc method against *Propionibacterium acnes* bacterial isolates with COA (Certificate of Analysis) the name of the bacteria can be seen in Appendix 2 and the area of the bacterial inhibition zone. Antibacterial testing was conducted by: Preparing tools and materials for the antibacterial activity test by soaking disc paper in 7.5% formulation, 10% formulation, 12.5% formulation, negative control (aquadest) and positive control (point clear acne rohto) for 15 minutes. The negative control (aquadest) was chosen because aquadest is neutral and does not contain active substances that can affect bacterial growth or other biological activities. Thus, aquadest serves as a basic comparison to ensure that the effects observed in the test are truly caused by the pennywort leaf extract toner preparation, not by solvents or other factors. This negative control typically does not produce an inhibition zone in the antibacterial test, so its results serve as a reference for assessing the activity of the tested preparation. Meanwhile, the positive control (Point Clear Acne Rohto) was chosen because this product has been proven to have antibacterial activity

or therapeutic effects on skin conditions such as acne. The positive control serves as a standard comparison for measuring the effectiveness of the toner preparation containing gotu kola leaf extract.[11] With a positive control, researchers can compare the antibacterial activity or benefits of the tested toner relative to well-known and widely used products. This helps assess whether the new preparation has comparable or superior potency compared to commercial products.

Of the five formulations, the negative control formulation, distilled water, fell within the 0 mm range, indicating no antibacterial activity. The positive control was used to compare the effectiveness of the gotu kola leaf extract roll-on face toner, *Centella asiatica* (L.) urb, against *Propionibacterium acnes*. Point Clear Acne Rohto was chosen as the positive control because this product is typically used for acne therapy by modifying or inhibiting the growth and protein synthesis of acne-causing bacteria.

The 7.5% formulation, 10% formulation, and 12.5% formulation indicate that the *Centella asiatica* (L) urb pennywort leaf extract face toner roll-on preparation with various concentrations of pennywort leaf extract enters the 30-50 mm range [12], [13] based on the bacterial inhibition zone activity criteria, has a very strong category in antibacterial activity against *Propionibacterium acnes*.[6] The positive control here takes the product because the product has the strongest zone among the designed formulations.

The results of the ANOVA and post hoc tests indicated that formulations with no significant differences were Formulation 1 and Formulation 2, Formulation 2 and Formulation 3, Formulation 2 and K+, Formulation 3 and K+, and vice versa. This was because the sig>0.05. Other significant differences were found because the sig<0.05. The results of the ANOVA and post hoc tests revealed that Formulation 1 had a significant difference in inhibition zones compared to the positive control, while Formulations 2 and 3 did not have significant differences in inhibition zones compared to the positive control. This is because Formulation 1 may contain extracts or active ingredients at lower concentrations or different types of compounds than the positive control.

Bacterial growth inhibition responses are classified into several categories: weak (≤5 mm), moderate (6-10 mm), strong (11-20 mm), and very strong (>20 mm) (Permadani, 2015). Based on the observational data obtained, the growth inhibition response of S. aureus bacteria in the extract treatment at concentrations of 10%, 20%, 30%, 40%, and 50% showed an average inhibition zone diameter of 10.10 mm; 12.56 mm; 13.08 mm; 14.57 mm, and

15.97 mm, respectively, categorized as strong. According to [7] inhibition of bacteria with a diameter of ≤ 5 mm is considered weak, inhibition of bacteria with a diameter of 6-10 mm is considered moderate, inhibition of bacteria with a diameter of 11-20 mm is considered strong, and inhibition of bacteria with a diameter of ≥ 21 mm is considered very strong.[12]

Meanwhile, the antibacterial strength criteria for *Propionibacterium acnes*[14] include a very strong inhibition zone with a diameter of >20 mm, a strong inhibition zone with a diameter of 10-20 mm, a moderate inhibition zone with a diameter of 5-10 mm, and a diameter of 0-5 mm, which is categorized as a weak inhibition zone. Therefore, for formulations 2 and 3, whose inhibition zones based on ANOVA and post hoc tests show no difference compared to the positive control, they can be categorized as having a very strong inhibition zone. Meanwhile, formulation 1, whose inhibition zone according to ANOVA and post hoc tests shows a difference compared to the positive control, is also categorized as very strong. The difference that occurs may only be a few points but is not below 20 mm because it is influenced by the concentration of the active ingredient.

This is in accordance with research presented by Ointu, where gotu kola leaf extract with formulations of 2%, 4%, 8%, and 10% was able to inhibit the growth of *Propionibacterium acnes* bacteria.[15] Gotu kola leaf extract with formulations of 2%, 4%, 8%, and 10% was proven to be able to inhibit the growth of *Propionibacterium acnes* bacteria. The results showed that gotu kola leaf extract contains chemical compounds, namely flavonoids, saponins, tannins, and triterpenoids. Tannin and flavonoid compounds have antimicrobial benefits by coagulating bacterial protoplasm so that stable bonds form with bacterial proteins. These compounds function to inhibit *Propionibacterium acnes* bacteria.[16] This activity was also confirmed through inhibition zone testing, where the higher the extract concentration, the greater the resulting inhibition. Therefore, the 7.5%, 10%, and 12.5% formulations indicate that the gotu kola leaf extract roll-on face toner can be used to inhibit the growth of *Propionibacterium acnes* bacteria.

The next step was to conduct a six-cycle stability test. Formulation 1, containing 7.5% gotu kola leaf extract, was dark green to black in color. Formulation 2, containing 10% gotu kola leaf extract, was dark green to black in color. Formulation 3, containing 12.5% gotu kola leaf extract, was dark green to black in color. The results of this stability test indicated that the gotu kola leaf extract roll-on face toner was quite stable. Stability is quite important because it significantly affects a product and its quality.

The Kruskal Wallis test results on the pH stability data showed a p-value of 0.000. When the p value <0.05, it can be stated that there is a significant difference between the pH of formulation 1, formulation 2, formulation 3, K- and K+. Then the post hoc LSD results show that F1: F2: F3 results are all sig> 0.05. This can be stated that there is no significant difference between formulation 1 with formulation 2 and formulation 3. However, the comparison with K+ sig<0.05. This can be stated that there is a significant difference between formulation 1, formulation 2 and formulation 3 with K+ in terms of pH.

In terms of pH testing, the pH data shows that the face toner roll-on with gotu kola leaf extract ranges from 5>pH>5.5. This is actually almost the same as the skin's pH, which is 4>pH>5.5. The pH of the face toner roll-on with gotu kola leaf extract ranges from 5 to 5.5. While the skin's pH ranges from 4 to 5.5. Ideally, the pH level of topical preparations should be the same as the skin's pH level. If the toner's pH is too high compared to the skin's pH (more alkaline than the normal skin pH of 4–5.5), then several effects that can occur on the skin are: 1) The skin becomes dry and feels tight, a pH that is too high can damage the skin's natural protective layer (acid mantle), so that the skin loses its natural moisture and easily becomes dry [17]. 2) Increases the risk of irritation and itching. Dry skin conditions due to a toner pH that is too high also make the skin more easily irritated, itchy, and can even trigger redness and a burning sensation. 3) The skin becomes more sensitive. Damage to the acid mantle due to an overly alkaline pH makes the skin more susceptible to allergens, pollution, and irritants from other skincare products. 4) Increases the risk of infection and acne. High skin pH disrupts the balance of good bacteria on the skin's surface, allowing acne-causing bacteria like *Propionibacterium acnes* to thrive and cause infections or acne. 5) Decreases the quality and protective function of the skin. If the skin's protective layer is damaged, the skin is more susceptible to inflammation, dehydration, and even premature aging.[17]

If the toner's pH is too low compared to the skin's pH (more acidic than normal skin pH, which is below 4), then some of the effects that can occur on the skin are: 1) The skin becomes too oily. A pH that is too low (too acidic) can trigger excess oil production on the skin, making the face look oilier and feel sticky; 2) The appearance of acne. Skin conditions that are too acidic can create an ideal environment for acne-causing bacteria to thrive, increasing the risk of acne; 3) Irritation and inflammation. A pH that is too low can cause the skin to become inflamed, feel sore to the touch, and be more easily irritated or burnt; 4) The skin feels sore and sensitive. The skin's protective layer (acid mantle) can be disrupted if the pH is too acidic, making the skin more sensitive to irritants, pollution, and microorganisms;

and 5) The risk of skin infections. An imbalance in pH, whether too low or too high, can reduce the skin's ability to fight harmful bacteria and microorganisms, increasing the risk of skin infections such as impetigo, cellulitis, or rashes [1]. Regarding viscosity, the Kruskal-Wallis test, followed by post-hoc LSD, revealed that formulation 2 and K+ did not show significant differences, and vice versa. This was due to a sig value >0.05. However, there were significant differences between formulations 1 and 2, formulation 3, and K+ and K-. This was due to a sig value <0.05.

Toner viscosity should generally be in the low range, for example < 5 cPs (centipoise) so that the toner remains easy to apply and absorbs into the skin without feeling heavy or sticky. Temperature significantly affects toner viscosity. At high temperatures, viscosity tends to decrease because the substance molecules become more mobile, while at low temperatures viscosity increases because the molecules become stiffer and denser. Viscosity testing during the stability period is usually analyzed using statistical tests such as ANOVA and Kruskal Wallis to determine whether there are significant changes during storage. Stable toner shows viscosity values that do not differ significantly across various test cycles. Therefore, the conclusion that can be drawn is that a good formulation is formulation 2, which shows no significant difference with K+.

4. CONCLUSION

Physical quality stability test *of Centella asiatica* (L) urb leaf extract roll on face toner in formulation 1 with a concentration of 7.5%, formulation 2 with a concentration of 10%, and formulation 3 with a concentration of 12.5% were able to withstand the stability test, had a stable pH and viscosity, color and odor did not show significant changes in storage, based on the Kruskal Wallis and post hoc tests it was stated that only formulation 2 with a concentration of 10% had no difference with the positive control, because sig>0.05, it can be said that formulation 2 with a concentration of 10% meets the requirements so that it can be used by consumers well. Face toner roll on gotu kola leaf extract showed antibacterial activity against Propionibacterium acnes with a very strong category. Formula F3 (12.5%) had the largest inhibition zone diameter of 46.27 mm. However, based on ANOVA and post hoc tests, there was no significant difference between F2 (10%) and F3 (12.5%) compared to the positive control (sig > 0.05), so both formulas had antibacterial effectiveness comparable to the positive control.

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6. CONFLICT OF INTEREST

All authors declare that there is no potential conflict of interest with regard to the research, authorship, and/or publication of this article.

7. REFERENCE

1) M. R. A. N. Karami, S. Malahayati, N. Hidayah, and S. Budi, "Formulasi Dan Uji Stabilitas Sediaan Toner Anti Jerawat Ekstrak Bunga Melati (Jasminum sambac L.)," JFS, vol. 1, no. 2, pp. 68–76, Aug. 2023, doi: http://10.63004/jfs.v1i2.235.

2) A. H. Rosyida, A. P. Pramono, M. Bahar, and O. S. Puspita, "Analisis perbandingan daya hambat ekstrak pirolisis dan ekstrak maserasi tembakau (Nicotiana tabacum L.) var virginia terhadap Candida albicans secara in vitro," Jurnal Kedokteran Syiah Kuala, vol. 22, 2022.

3) Sugiyono, Metode Penelitian Kuantitatif Kualitatif Dan R&D. Bandung: Alfabeta, 2017.

4) C. E. E. Putri, D. M. Wulandari, U. H. Hasyim, I. Hasyim, and M. S. Ramadhan, "Optimasi Waktu Maserasi Pada Ekstraksi Daun Pegagan (Centella Asiatica) Terhadap Uji Aktivitas Antioksidan," in Seminar Nasional Sains dan Teknologi 2024, Jakarta, 2024.

5) S. A. Pratiwi, N. Februyani, and A. Basith, "Skrining dan Uji Penggolongan Fitokimia dengan Metode KLT pada Ekstrak Etanol Kemangi (Ocium basilicum L) dan Sereh Dapur (Cymbopogon ciratus)," Pharmacy Medical Journal, vol. 6, no. 2, p. 2023.

6) K. E. K. Dewi, N. Habibah, and N. Mastra, "Uji Daya Hambat Berbagai Konsentrasi Perasan Jeruk Lemon Terhadap Bakteri Propionibacterium acnes," j. sains. teknologi., vol. 9, no. 1, pp. 86–93, Jun. 2020, doi: http:// 10.23887/jstundiksha.v9i1.19216.

7) E. Mora and A. Fernando, "Optimasi Ekstraksi Triterpenoid Total Pegagan (Centella asiatica (Linn.) Urban) yang Tumbuh di Riau," Jurnal Penelitian Farmasi Indonesia, vol. 1, no. 1, 2012.

8) M. Z. Imansyah and S. Hamdayani, "Uji Aktivitas Ekstrak Etanol Daun Sirih Cina (Peperomia pellucida L.) Terhadap Bakteri Propionibacterium acnes," Jurnal Kesehatan Yamasi Makassar, vol. 6, no. 1, 2022.

9) P. Kusumaningsih and G. Mustika, "Evaluasi Tiga Metode Identifikasi Bakteri Staphylococcus Sciuri Dari Pindang Tongkol (Euthynnus Affinis).," in Sintesa Prosiding, 2020.

10) S. Kursia, I. Imrawati, A. Halim, S. Sasmita, and F. Hanifah, "Identifikasi Biokimia Dan Aktivitas Antibakteri Isolat Bakteri Asam Laktat Dari Limbah Sayur Sawi (Brassica juncea L.)," kesehatan, vol. 0, no. 0, pp. 51–60, Apr. 2020, doi: http://10.24252/kesehatan.v0i0.11626.

11) D. Yuliastuti, C. Indriapuspa, and P. Yuli Pratiwi, "Uji Aktivitas Antibakteri Toner Minyak Atsiri Kulit Jeruk Purut (Cytrus hystrix Dc) terhadap Propionibacterium acnes," MFI, vol. 18, no. 2, pp. 101–106, Sep. 2023, doi: http://10.53359/mfi.v18i2.223.

12) L. Ayu, H. Kuspradini, and R. Ruga, "Potensi Aktivitas Antibakteri Ekstrak Diklorometana Dan Metanol Bunga Terompet Emas (Allamanda cathartica L .) Terhadap Bakteri Staphylococcus aureus ATCC 25923 Potential Antibacterial Activity Of Dichloromethane And Methanol Extracts Of The Golden Trump,"Jurnal Atomik, vol. 9, no. 2, pp. 78–83, 2024.

13) N. P. Dewi, A. T. Vani, D. Abdullah, M. Oktora, and F. Marisa, “Uji Kuersetin Pada Ekstrak Etanol Lidah Buaya (Aloe Vera Barbadensis) Dengan Metode Kromatografi Lapis Tipis,” Nusantara Hasana Journal, vol. 1, no. 11, pp. 59–69, 2022.

14) P. D. M. Jayanti, “Uji Aktivitas Antibakteri Fraksi Etanol dan N-heksan kulit Bawang Merah (Allium cepa L.) terhadap bakteri Propionibacterium acnes penyebab jerawat,” Stikes Bhakti Husada Mulia Madiun, vol. 18, 2021.

15) R. Ointu, “Uji Aktivitas Ekstrak Daun Pegagan Terhadap Bakteri Propionibacterium acnes,” Skripsi, Universitas Negeri Gorontalo, Gorontalo, 2018.

16) V. Starlista, E. F. Apriani, B. Untari, and A. Setyawan, “Uji Aktivitas Antibakteri secara In-Vitro Gel Submikro Partikel Pembawa Ekstrak Etanol Daun Pegagan (Centella Asiatica (L.) Urban) terhadap Propionibacterium Acnes,” JPS, vol. 25, no. 2, p. 136, Aug. 2023, doi: http://10.56064/jps.v25i2.798.

17) M. Noor, S. Malahayati, and K. Nastiti, “Formulasi Dan Uji Stabilitas Sediaan Toner Wajah Ekstrak Buah Pare (Momordica charantia L) Sebagai Anti Jerawat Dengan Variasi Surfaktan,” JRKI, vol. 5, no. 1, pp. 133–145, Feb. 2023, doi: http://10.33759/jrki.v5i1.330.