



The Effectiveness of Mugwort Leaf Extract and Gotu Kola Leaf Extract against Acne Bacterial Activity

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ABSTRACT

Acne is a problem among teenagers generally caused by bacterial infections such as *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. Mugwort (*Artemisia vulgaris* L.) and Gotu Kola (*Centella asiatica* L.) extracts have antibacterial activity formulated in anti-acne gel preparations. The purpose of this study was to test and obtain a gel formula of mugwort leaf extract and gotu kola leaf extract which effectively kills and controls the growth of acne-causing bacteria. The methods used in this study include simplicity making, leaf extraction, making anti-acne gel preparations, testing pH, homogeneity, inhibition, and data analysis. The extract obtained by maceration was made in three concentrations, namely 2.50; 5.00; and 10.00%. The results showed that the pH value of the gel preparations obtained was in the range of 8.08-9.96. Mugwort leaf extract, gotu kola leaf, and mugwort-gotu kola have a distinctive smells with a thick texture at a concentration of 2.50% and a liquid texture at a concentration of 10.00%. Inhibition tests showed that the mugwort extract sample had a value of 16-18 mm, while the gotu kola extract and mugwort-gotu kola had a value of >14 mm. Mugwort leaf extract is able to inhibit the growth of acne-causing bacteria. This success was obtained because the extracts of mugwort and gotu kola leaves contain flavonoids that can inhibit the growth of bacteria that cause acne. Mugwort leaf extract gel with a concentration of 2.50 and 5.00% had the largest zone of inhibition in inhibiting *P. acnes* bacteria compared to other concentrations.

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1. INTRODUCTION

Acne is an inflammation characterized by the presence of blackheads, small or large lumps, nodules, and cysts on the skin of the face. Acne is usually caused by bacterial infections such as *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. Records of the Indonesian cosmetic dermatology study show that there were 60% of acne sufferers in 2006 and 80% in 2007 (Purwanindyah & Nelva, 2013). In both developed and developing countries, acne sufferers are higher in women than men, with a peak incidence at the age of 15 years (Lyn et al., 2016).

Acne medications that are usually used have antibacterial active compounds from both chemical and natural ingredients. Antibiotics used as an effective way of treating acne include clindamycin, tetracycline, and erythromycin (Guay, 2007). Inappropriate use of antibiotics can cause resistance (Sholih et al., 2010). Anti-acne preparations are better formulated in gel preparations because they have good diffusion capabilities on the skin so that the topical effect is obtained immediately after the active ingredients penetrate the skin's semipermeable membrane (Anggraini et al., 2013). A gel is a topical preparation that uses a polar base formulation so that it is easily accepted by the skin. The diffusion power generated by the gel is better than cream because of its ability to pass through the skin membrane more effectively than cream preparations (Anggraini et al., 2013). Mugwort (*Artemisia vulgaris L.*) is a plant rich in essential oils and sesquiterpenoid lactones and is a good source of flavonoids, coumarins, and phenolic acids (Ekiert et al., 2020). This plant is widely used by the community as traditional medicine and also medicine to treat facial skin. Phytochemical screening in the leaf extract of *Artemisia vulgaris L.* showed the presence of saponins, glycosides, flavonoids, proteins, and triterpenoids. It has antibacterial properties (Thangjam et al., 2020).

Gotu kola (*Centella asiatica L.*) is a stemless plant and has green leaves shaped like a fan. Gotu kola is one of the medicinal plants that is widely known to the public. Gotu kola contains asiaticoside, acetic acid, and madeksaic acid which can promote wound healing, so it is used as a source of active ingredients in the treatment of skin that is dull, wrinkled, or showing signs of aging (Primastuti, 2013). Other properties of asiaticoside in gotu kola can also accelerate and trigger the growth of collagen in the skin so that it can improve skin regeneration when skin damage occurs due to acne (Sikareepaisan et al., 2008). According to research, gotu kola extract gel with a concentration of 5.00% is a more stable formula and complies with the requirements of the homogeneity and dispersion test (Budi & Rahmawati, 2019). However, the research of Budi and Rahmawati (2019) was limited to gotu kola leaf gel and had not been tested on bacteria. In the research by Anggraini et al. (2019), gotu kola and paspagan leaf extracts were bacteriostatic against *Escherichia coli* and bactericidal against *Micrococcus luteus* with an inhibition zone diameter of 25 mm. Ethanol and methanol extracts of *Artemisia iwayomogi* and *Artemisia princeps* have antibacterial activity on *S. aureus* and *P. acnes* (Park & Oh, 2019). In the research of Sun et al. (2011), mugwort dye dipped in wool can suppress the growth of *E. coli* and *S. aureus* bacteria. Essential oil from *Artemisia* has a strong antibacterial effect against *E. coli* bacteria (Soetjipto et al., 2019). However, some of these studies only tested the leaf extract and had not been made into a gel. In addition, the bacteria used are not acne-causing bacteria. Because there has been no further development of the mugwort leaf gel formula, and the bacteria studied by the gotu kola leaf extract were only *E. coli* and *M. luteus*, we intend to develop an alternative antibiotic drug in the form of a gel formula of mugwort leaf extract and gotu kola and then its effectiveness will be tested against acne-causing bacteria such as *P. acnes*, *S. aureus*, and *S. epidermidis*. In addition, the results of the comparison of antibacterial activity in gotu kola and mugwort leaf extracts can later be

used as antibacterial drugs that can be used by the community. In contrast to other studies, the novelties of this study are (i) the combined gel formulation between mugwort leaf and gotu kola leaf; (ii) testing of mugwort leaf gel and gotu kola leaf on *P. acnes*, *S. aureus*, and *S. epidermidis* bacteria.

2. METHODS

This research was conducted in Bandung, West Java, for three months. It started with sample making, data collection, and data analysis. The sample preparation consisted of two stages, namely the manufacture of mugwort leaf extract and gotu kola leaf; manufacture of control gel preparations, mugwort leaf extract, gotu kola leaf extract, and a mixture of mugwort and gotu kola extracts. The data collection was done through pH test, homogeneity test, organoleptic test, and inhibition zone test.

2.1. Tools and materials

The tools and materials used in this study were petri dishes, measuring cups, beakers, scaled glass, mortar and pestle, inoculation needles, labels, paper disks, bunsen, test tubes, slides, glass covers, loop needles, microscopes, marker, dropper, digital scale, pH meter, incubator, gel container, mugwort leaf, gotu kola leaf, 70% of ethanol, *P. acnes*, *S. aureus*, and *S. epidermidis* bacteria, nutrient medium agar, spiritus, viscolam, glycerin, propylene glycol, dimethylol dimethyl hydantoin (DMDM hydantoin), triethylamine (TEA), clindamycin, and distilled water.

2.2 Research Stages

The stages of research carried out included:

- (i) **Research Sample.** The research sample used were fresh leaves of mugwort and gotu kola plants taken from several places in Indonesia: Cianjur, Pangandaran, and Bandung areas.
- (ii) **Simplicity Making.** Wet sorting was done on mugwort and gotu kola leaves, then drained. The leaves were then dried using a desiccator at 45°C to constant weight. The process is continued by grinding the dried simplicia into powder.
- (iii) **Extract Making.** Mugwort leaf extract and gotu kola leaf extract were made using maceration and evaporation methods. In the maceration process, the dried sample was mixed with 70% of alcohol and allowed to stand for 16 hours. The ratio of dried sample and 70% of alcohol was 1:10. Then the samples were evaporated using the steam method at a temperature of 45°C until it thicked and was weighed. After that, the extracts were used as an anti-bacterial material for the sample to be tested.
- (iv) **Gel Making.** The anti-acne gel was made using mugwort leaf extract and gotu kola leaf extract with a concentration of 2.50; 5.00; and 10.00% for each extract. The gel formula dosage is shown in **Table 1**. The preparation of the anti-acne gel began by dissolving the extract using some glycerin, mixing viscolam and water, adding some glycerin and propylene glycol, and adding DMDM hydantoin, mix until homogeneous, then added TEA. We did the same way for gotu kola leaf extract. The gel was stored in a sealed gel container at room temperature.
- (v) **pH Test.** The pH test or the acidity level of the gel preparation is carried out using a pH meter to ensure that the gel does not irritate the skin.
- (vi) **Homogeneity Test.** The gel homogeneity test was carried out to see the even distribution of the content contained in each component in the gel preparation. The test was carried out by leveling the gel preparation on the surface of the glass object for 3 times of

repetitions. This shows that all components are well mixed in each formula so that the gel looks homogeneous, the texture is smooth and not rough.

- (vii) **Organoleptic Test.** The organoleptic test in this study was carried out by 10 panelists to observe the physical form, namely the texture, smell, and color of the gel preparation.
- (viii) **Bacterial Culture.** The slant agar of bacteria *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Propionibacterium acnes* was activated at 37°C for 1 hour and grown on nutrient agar (NA) medium at 37°C for 48 hours.
- (ix) **Inhibitory Test.** The inhibition test was carried out using the diffusion method, by soaking the paper disc in various concentrations of the gel extract of the Gotu kola leaf extract and the mixture (2.50; 5.00; and 10.00%). Bacteria were streaked on NA medium using a sterile cotton swab, then all the soaked discs were implanted on NA medium. Clindamycin antibiotic gel was used as a positive control, and a gel without leaf extract or clindamycin was used as a negative control. Incubation was carried out at 37°C for 48 hours. The zone of inhibition was measured using a ruler and repeated three times for each treatment and control.
- (x) **Data analysis.** The data analysis was carried out by calculating the inhibition zone of the gel extract of mugwort leaf, gotu kola leaf, and mixture against *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Propionibacterium acnes* bacteria. The inhibition data results were calculated using the standard deviation and presented in a tabular form.

Table 1. Gel formula dosage.

No.	Materials	Formula Dosage % (b/v)				
		K-	P1	P2	P3	K+
1	Extract	-	2.50	5.00	10.00	-
2	Clindamycin	-	-	-	-	1
3	Viscolam	8	8	8	8	8
4	Propylene Glycol	10	10	10	10	10
5	Glyserin	5	5	5	5	5
6	TEA (gtt)	25	25	25	25	25
7	DMDM Hydantoin	0.60	0.60	0.60	0.60	0.60
8	Aquadest	100	100	100	100	100

3. RESULTS AND DISCUSSION

3.1. Extract Yield Calculation

Extract yield calculation can use the formula below

$$\frac{\text{Thick extract weight}}{\text{Simplicity weight}} \times 100\%$$

$$\text{Gotu Kola Extract Yield} : \frac{10}{88.2} \times 100\% = 11.37\%$$

$$\text{Mugwort Extract Yield} : \frac{11}{132} \times 100\% = 11.36\%$$

3.2. pH, Homogeneity and Organoleptic Test

Mugwort, gotu kola, and mugwort-gotu kola leaf extract gel were tested using pH test, homogeneity test and organoleptic test to find out and analyze how well the gel was made. Based on the results of the pH test, the pH range of mugwort leaf extract gel, gotu kola leaf,

a mixture of mugwort and gotu kola leaves, positive and negative controls obtained a pH range of 8.08-9.96, where the results were higher than the recommended pH of the skin which ranges from 4.5-6.5 (Mappa *et al.*, 2013). The homogeneity test was carried out to determine the homogeneity of the gel preparation indicated by the presence or absence of a coarse texture such as coarse granules in the gel (Budi & Rahmawati, 2009). Based on the results of the homogeneity test on the gel in **Figure 1**, it was found that all the gels had good homogeneity because of their smooth texture and the absence of coarse grains so all of the active substances were evenly mixed. An organoleptic test was carried out to determine the physical form of the gel by looking at the texture, aroma, and color of the gel preparation.

Based on the observations in **Figure 2** and **Table 2**, the mugwort leaf extract gel has a brownish color with a thick texture for concentrations of 2.50% and 5.00%, liquid texture for a concentration of 10.00%, and has a distinctive smell of mugwort leaves which is quite pungent. The gel of gotu kola leaf extract has a green color with a thick texture for a concentration of 2.50%, liquid texture for a concentration of 5.00 and 10.00%, and has a distinctive odor of gotu kola leaves. The mixed gel between mugwort leaf and gotu kola leaf has a brownish-green color with a thick texture for a concentration of 2.50 and 5.00%, liquid texture for a concentration of 10.00%, and has a distinctive smell of a mixture of mugwort and gotu kola leaves.

3.3. Resistance Test

The diameter of the inhibition zone was measured after the agar medium was incubated for 30-48 hours using a ruler and can be seen in **Figure 3**. The measured zone is the diameter of the clear zone around the paper disc, measurements are made from various sides of the circle which are then averaged. The results of the inhibition test of mugwort and gotu kola leaf extracts can be seen in **Table 3**. In this study, clindamycin was used as a positive control which served as a comparison in determining the ability of the extract to inhibit bacteria. Clindamycin is an antimicrobial that is both bacteriostatic and bactericidal and has a mechanism to kill bacteria by preventing protein synthesis from bacteria (Putra *et al.*, 2017). The inhibition zones in the gel mixture and 1% of clindamycin in *P. acnes*, *S. epidermidis*, and *S. aureus* were 19.0 ± 0.17 , 20.0 ± 0.40 , and 25.0 ± 0.73 , respectively, which is shown in **Table 3**.



Figure 1. Homogeneity test on each gel preparation.

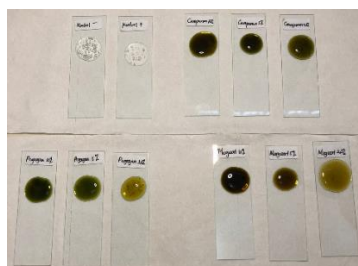
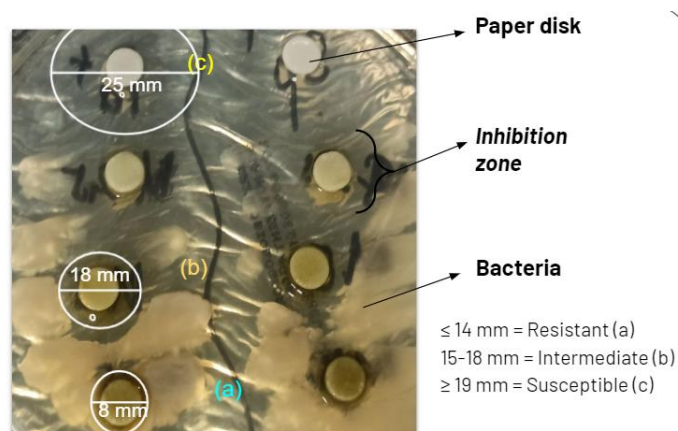


Figure 2. Organoleptic test on each gel preparation.

Table 2. Gel preparation test results.

No.	Variable		Organoleptic Test			Homogeneity Test	pH Test
	Extract	Concentration	Color	Smell	Texture		
1	Mugwort	2.50%	Yellowish Brown	Distinctive Smell	A Bit Thick	Homogeneous	9.93
		5.00%	Brown	Distinctive Smell	Thick	Homogeneous	9.96
		10.00%	Dark Brown	Pungent Smell	Quite Liquid	Homogeneous	9.94
2	Gotu Kola	2.50%	Yellowish Green	Berbau tidak menyengat	Really Thick	Homogeneous	8.72
		5.00%	Green	Smells	Quite Liquid	Homogeneous	8.08
		10.00%	Dark Green	Pungent Smell	Liquid	Homogeneous	8.96
3	Mugwort-Gotu Kola mixture	2.50%	Brownish Green	Distinctive Smell	Thick	Homogeneous	9.18
		5.00%	Dark Green	Distinctive Smell	A Bit Thick	Homogeneous	9.06
		10.00%	Greenish Brown	Pungent Smell	Liquid	Homogeneous	9.27
4	Clindamycin Control (+)		Clear	Smells	Thick	Homogeneous	8.79
5	Control (-)		Clear	Smells	Thick	Homogeneous	9.08

**Figure 3.** Inhibition zone of positive control and mugwort leaf extract gel 2.50; 5.00; and 10.00% in *P. acnes* (Pa) and *S. epidermidis* (Sp).

Based on Riasari et al. (2020), the clindamycin inhibition zone tested at the three concentrations was categorized as susceptible. The measurement results of the inhibition zone can be seen in **Figure 3** and **Table 3**. From the results, we know that 2.50% of mugwort extract gel had an inhibition zone of *P. acnes*, *S. epidermidis*, and *S. aureus*, respectively, which was 18.0 ± 0.05 , 18.0 ± 0.30 , and 16.0 ± 0.15 , so based on Riasari et al. (2020) it was categorized as intermediate. Similar to 2.50% of mugwort leaf extract gel, 5.00% of mugwort leaf extract gel was included in the intermediate category because it had an inhibitory zone in the range of 15-18 mm. Meanwhile, the gotu kola extract gel, mixture, and negative control were categorized into resistance because the area of the inhibition zone was less than 14 mm.

Table 3. Results of measurement of inhibitory power of gel preparations.

No.	Variable	Bacterial Inhibition Zone \pm SD (mm)			Description
		<i>P. acnes</i>	<i>S. epidermidis</i>	<i>S. aureus</i>	
1	Negative control	7.60 \pm 0.20	8.00 \pm 0.20	11.00 \pm 0.15	Resistant
2	Positive control	19.00 \pm 0.17	20.00 \pm 0.40	25.00 \pm 0.73	Susceptible
3	2.50% of mugwort extract	11.00 \pm 1.00	18.30 \pm 0.20	6.00 \pm 0.00	Resistance
	5.00% of mugwort extract	17.60 \pm 0.06*	14.00 \pm 0.00	6.60 \pm 0.11	*Intermediate
	10.00% of mugwort extract	6.00 \pm 0.00	9.60 \pm 0.40	6.00 \pm 0.00	Resistant
4	2.50% of gotu kola extract	8.60 \pm 0.57	17.00 \pm 0.42*	6.00 \pm 0.00	*Intermediate
	5.00% of gotu kola extract	19.30 \pm 0.15*	25.00 \pm 1.00*	6.00 \pm 0.00	*Susceptible
	10.00% of gotu kola extract	14.60 \pm 0.41	15.00 \pm 1.00*	6.00 \pm 0.00	*Intermediate
5	2.50% of mugwort-gotu kola extract	12.50 \pm 0.07	12.50 \pm 0.63	6.00 \pm 0.00	Resistant
	5.00% of mugwort-gotu kola extract	8.50 \pm 0.07	14.50 \pm 0.07	6.00 \pm 0.00	Resistant
	10.00% of mugwort-gotu kola extract	10.00 \pm 0.00	19.50 \pm 0.07*	6.00 \pm 0.00	*Susceptible
6	2.50% of mugwort gel	18.00 \pm 0.05	18.00 \pm 0.30	16.00 \pm 0.15	Intermediate
	5.00% of mugwort gel	18.00 \pm 0.30	17.00 \pm 0.11	17.00 \pm 0.32	Intermediate
	10.00% of mugwort gel	11.00 \pm 0.40	9.00 \pm 0.17	13.00 \pm 0.20	Resistant
7	2.50% of gotu kola gel	10.00 \pm 0.28	9.00 \pm 0.20	11.60 \pm 0.11	Resistant
	5.00% of gotu kola gel	11.60 \pm 0.11	10.00 \pm 0.00	13.00 \pm 0.43	Resistant
	10.00% of gotu kola gel	10.60 \pm 0.11	9.30 \pm 0.15	10.60 \pm 0.30	Resistant
8	2.50% of mugwort-gotu kola gel	9.60 \pm 0.11	9.30 \pm 0.25	14.30 \pm 0.05	Resistant
	5.00% of mugwort-gotu kola gel	9.60 \pm 0.05	9.60 \pm 0.05	12.30 \pm 0.15	Resistant
	10.00% of mugwort-gotu kola gel	8.60 \pm 0.11	8.30 \pm 0.05	11.00 \pm 0.20	Resistant

4. CONCLUSION

Mugwort leaf extract gel with a concentration of 2.50 and 5.00% was proven to be able to inhibit the growth of acne-causing bacteria because it has the largest inhibition zone in inhibiting *P. acnes* bacteria compared to other concentrations.

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6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. The authors confirmed that the paper was free of plagiarism.

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