JNPDD
Journal of Natural Product for Degenerative Diseases
https://journal.univpancasila.ac.id/index.php/Natural

Gel Formulation from a Combination of Sidaguri Leaf Extract (*Sida rhombofolia* L.) and Chinese Petai Leaf (*Leucaena leucocephala* L) as Inhibitors of Acne-causing Bacteria (*Propionibacterium acne* and *Staphylococcus aureus*)

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Received: 30 January 2023 / Accepted: 29 August 2023

ABSTRACT: Acne vulgaris, a chronic inflammatory disease of the pilosebaceous unit, is the most common skin condition. *Propionibacterium acne*s and *Staphylococcus. aureus* are two bacteria that can cause infections. Chinese Petai leaf are known to inhibit acne growth. Sidaguri leaves are also thought to have antibacterial activity. This study aimed to determine the effect of the combination of the two extracts on inhibiting the growth of acne-causing bacteria, also to test the gel formula to meet physical and chemical parameters as well as stability to temperature and storage, as well as to test acute dermal. irritation to rabbits. Each extract with a concentration of 0.19%; 0.39%; 0.78%; 1.56%; 3.125%; 6.25%; 12.5%; 25%; and 50% were incubated and then the inhibition zone was measured, then the minimum inhibition zone concentration of the two extracts was determined to determine the lowest concentration of the extract that still provided antibacterial activity against the tested bacteria using the well-diffusion method. The extract combination is formulated in a gel preparation with Carbopol 940, Propylene glycol, Phenoxyethanol, TEA, and Aquadest as excipients. Test the antibacterial activity of gel preparations using the well method with positive control clindamycin gel. Then evaluation of the formula includes the evaluation of physics, chemistry, and microbiology. The results showed that the combination gel had activity against P. acne and *Staphylococcus. aureus* at a concentration of 3.12% : 1.56%. The combined gel preparation of the two extracts also has a synergistic effect in inhibiting acne bacteria. The formula is resistant to temperature and storage, and it can satisfy chemical and physical parameters.

KEYWORDS: Sidaguri leaf; Chinese Petai Leaf; *Propionibacterium acne*; *Staphylococcus. aureus*; Gel preparations

1. INTRODUCTION

The skin is a "blanket" covering the body's surface and is part of the protection from outside disturbances and stimuli [1]. Smooth facial skin free of skin problems such as acne is synonymous with healthy facial skin. Acne is a skin problem that is common in everyday life even though acne is not a disease that causes death or serious health problems and generally occurs in adolescents until it is possible to appear at all ages [2]. Acne is an inflammation of the skin that can occur due to excessive sebum production which can lead to blockage of the ducts of the oil glands and the formation of blackheads (whiteheads) [3]. If the blockage enlarges, open comedones (blackheads) appear and will interact with acne-causing bacteria which are also normal skin flora such as *Propionibacterium acne*s and *Staphylococcus aureus*. Until now, the causes of acne are not known with certainty, but 4 factors, namely follicular hyperkeratinization, sebum hypersecretion, chronic inflammatory processes in the sebaceous glands, and colonization by the bacteria *Propionibacterium acne*s and *Staphylococcus aureus* have been believed to contribute greatly to the cause of acne [4]. The incidence of acne is around 85% and is mostly at a young age. Acne usually begins in women between the ages of 14 - 17 years

How to cite this article: Andriana, Chaidir, and Deni R. Gel Formulation from a Combination of Sidaguri Leaf Extract (*Sida rhombofolia* L.) and Chinese Petai Leaf (*Leucaena leucocephala* L) as Inhibitors of Acne-causing Bacteria (*Propionibacterium acne* and *Staphylococcus. aureus*). JNPDD. 2023; 1(1): 47-56.

ISSN: 0000-0000
https://doi.org/10.58511/jnpdd.v1i1.4619

and men between the ages of 16 - 19 years. The most common lesions are comedones and papules. Additionally, acne vulgaris (acne) affects more men than women between the ages of 15-44, with 34% of men and 27% of women experiencing the condition [5].

Until now there is no most appropriate treatment for acne but giving antibiotics has proven to be effective for dealing with acne, but antibiotic retention is increasingly widespread and is an important problem throughout the world [6]. Long-term use of antibiotics can also cause organ damage and immunohypersensitivity, as well as microbial resistance. Natural ingredients that have antibiotic effects that are proven to be effective for treating acne can be the answer to this problem, including the Sidaguri plant (*Sida rhombofolia* L) and Chinese petai (*Leucaena leucocephal* L). The combination of the two plants is thought to be able to accelerate acne healing and improve sebum conditions. Due to its tropical climate, the sidaguri plant is a type of wild plant that thrives in Indonesia. The leaf of a wild plant whose latin name is Sida rhombifolia L it contains useful compounds. Sidaguri leaf contain flavonoids, alkaloids, calcium oxalate, tannins, saponins, phenols, amino acids and essential oils [7]. Sidaguri leaf are empirically known and known for a long time to be able to treat cystic acne quickly. The antibacterial properties of sidaguri leaf can accelerate acne treatment [8]. Previous research by (Andriana) on Sidaguri leaf was that they had anti-anthelmintic activity. Several studies also reported that the ethyl acetate extract of sidaguri leaf (*Sida rhombifolia* L) showed antibacterial activity in all of its fractions, both against gram-positive and gram-negative bacteria [9]. Other studies have found that Sidaguri leaf extract contains antimicrobial compounds that can inhibit growth *Propionibacterium acne*s cause of acne. Minimum concentration of leaf extract sidaguri that can stop the growth of *Propionibacterium acne*s is fifty percent. The maximum concentration of sidaguri leaf extract which further inhibits bacterial growth *Propionibacterium acne*s is 90% [10].

Chinese petai leaf have been proven to be useful in medicine and contain chemical tannins, saponins and alkaloids which have strong antibacterial properties [11]. The 96% ethanol extract of Chinese petai leaf has antibacterial activity with an inhibition zone of 17.33 mm, in the determination of MIC, Chinese petai also has the greatest antibacterial activity with a MIC value of 62.5 µg/mL [12]. Research by Sartinah (2010) proved that 96% ethanol extract of Chinese petai leaf has inhibitory activity against *Staphylococcus aureus* that cause acne. Research by Ristiana (2020) proved that 96% ethanol extract of Chinese petai leaf is effective as an anti-acne against bacteria *Staphylococcos aureus* with the results of the diameter of the inhibition zone of the extract formulation 5%:4.63 mm, 10%:6.1 mm, 15%:7.5 mm, and 1% clindamycin positive control: 23.17 mm. Research on the combination of 96% ethanol extract of sidaguri leaf and Chinese petai Leaf as antibacterial causes of acne has never been done. This research will be carried out with the aim of achieving a synergistic effect to increase antibacterial activity. In addition, several studies have explained that sidaguri leaf apart from being antibacterial can also have the ability to accelerate wound healing and anti-inflammation which is very much needed for the management of acne so that it can be applied as a natural active ingredient for acne treatment. However, the combination of sidaguri and Chinese Petai Leaf extracts in cosmetic dosage forms has not been carried out so that in vitro research data is also not available. Therefore, research was carried out on the two extracts by combining them in a cosmetic preparation in the form of an anti-acne gel.

Dosage form plays a role in the success of treating acne. Gel preparations are suitable for topical therapy for acne, especially for those with oily skin types because they do not exacerbate acne and can reduce it. This is due to the volatile nature of the gel, which dries easily and forms an easy-to-clean layer. possible additional inflammation due to oil buildup in the skin pores [13]. In an effort to formulate natural cosmetic preparations for anti-acne from Sidaguri leaf extract (*Sida rhombofolia* L.) and Chinese petai leaf extract (*Leucaena leucocephala*), it is necessary to make cosmetic preparations that can increase the effectiveness of the use of a combination of extracts as anti-bacterial causes of acne.

2. MATERIALS AND METHODS

2.1 Materials

Chinese Petai Leaf and sidaguri leaf extracts, 96% ethanol, aluminum foil, Whatman filter paper no. 1, phytochemical screening reagent, Mc. Farland Standard no 0.5, propylene glycol, carbopol 940, distilled water, pure culture *Staphylococcus aureus* fwhich is a collection of the Laboratory Unit, DMSO, *Propionibacterium acne*s , Nutrient Agar (NA). glassware used to test the effectiveness of bacteria, such as glasses, Petri dishes, and test tubes, washed with running water, then drained and heated in an oven at 150°C for 2 hours.

2.2 Preparation of extract

The extraction process used is maceration where simplicia is extracted using 96% ethanol with a ratio of 1:10, then filtered using Whatman filter paper no. 1. The extraction process is continued until the filtrate is clear, colorless. The collected filtrate was evaporated and concentrated using a vacuum rotary evaporator to form a thick extract. The thick extract obtained was packaged in a tightly closed container and stored in the refrigerator.

2.3.Regeneration

Bacteria Rejuvenation of bacteria is carried out in a way, bacterial colonies are taken from pure strain colonies using a sterile loop needle and then streaked on TSA media to *S. aureus* bacteria then incubated at 37°C for 24 hours and BHIA for *P. acne.* The bacteria were then incubated at 37°C for 48 hours.

2.4 Preparation of Bacterial Suspension

Sterile 0.9% NaCl was put into a test tube, then the bacterial culture was taken using a loop needle then put into 0.9% NaCl solution then shaken until turbidity was obtained which was adjusted to Mc. Farland turbidity standard 0.5 (10^8 CFU/mL) . If it is not dense enough, add colonies and add 0.9% NaCl.

2.5 Antibacterial Activity Test

Chinese Petai Leaf and Sidaguri leaf extracts were each weighed according to the concentration to be tested, namely 0.19%; 0.39%; 0.78%; 1.56%; 3.125%; 6.25%; 12.5%; 25%; 50% then dissolved in 1 mL of sterile aquadest. The extract was then put into the agar well as much as 50µL (each variation in concentration) and given a positive control and negative control then incubated for 1x24 hours at 37°C (for *S. aureus*) and for *P. acne* incubated for 2x24 hours at 37°C put in an anaerobic jar. After incubation, the diameter of the inhibition zone was measured by looking at the area of the clear zone using a caliper. The positive control used clindamycin and the negative control used sterile aquabides.

2.6 Single Extract MIC Determination Test

The minimum inhibitory concentration of Chinese Petai Leaf and sidaguri leaf extracts was determined to determine the lowest levels of extracts that still provide antibacterial activity against the tested bacteria by the well-diffusion method.

2.8 Preparation of Gel Formulas

Condensed extracts of sidaguri leaf and Chinese Petai Leaf leaf were dissolved in distilled water until homogeneous using a homogenizer for 10 minutes. Carbopol 940 was grown for 24 hours in distilled water containing a combination of extracts covered with aluminum foil. Phenoxyethanol was dissolved in propylene glycol then added to the carbopol 940 base, stirred until homogeneous using a homogenizer. Neutralization was carried out using triethanolamine (TEA) until a thick and clear gel base was obtained with a pH of 4.5 – 6.5 then stirred again until homogeneous. Add the volume of the gel using distilled water up to 100 g then stir again until homogeneous. The preparations that have been made are then put into a tightly closed container for evaluation of the gel.

2.9 Diameter Test of Inhibitory Power of Test Gel Formula

Antibacterial activity of gel preparations was carried out using the well method. Each medium which was still in the form of liquid was put into the bacterial inoculum as much as 1 mL (BHIA media for *P. acne* and TSB media for *S. aureus*). After that, it was poured into a 20 mL sterile petri dish and allowed to solidify. After the agar has solidified, holes are made in the media by inserting a sterile mount. After that each hole was added with each formula as much as 0.5 grams. Mediklin® gel was used for the positive control, while F1 (base) was used for the negative control.. Each petri dish was then incubated at 37°C for 2x24 hours under anaerobic conditions to *P. acne* in an anaerobic jar, while for *S. aureus* bacteria incubated for 1x24 hours at 37°C.

2.10 Evaluation of Gel Preparations

Formula evaluation includes evaluation of physics, chemistry, and microbiology. The physical evaluation included organoleptic examination, spreadability, homogeneity, viscosity, and irritation test on the preparation. Chemical evaluation includes determination of pH. Microbiological evaluation included

determining the antibacterial effectiveness of the combination gel preparation of Chinese Petai Leaf leaf and sidaguri extract against *P. acnes* and *S. aureus*.

2.11 Acute Skin Irritation Test

Acute skin irritation was performed on three rabbits, before starting the test, the test animals were acclimatized in the experimental room for approximately 5 days and the animals were placed in individual cages. At least 24 hours before the test, animal hair is shaved on the back 10 x 15 cm against exposure to the test preparation. Shaving starts from the scapula area to the base of the spine and down the middle of the body on each side. The test area on the rabbit's back was divided into 4 sections, each measuring ± 2x3 cm. The locations were divided into 2 blank locations and 2 gel preparation application locations. All test animals were observed for the presence or absence of erythema and edema, response assessments were carried out at 1, 24, 48, and 72 hours after the patch was opened. The test site was examined and observed for changes in skin reaction to the test substance and assessed by giving 0 to 4 the severity of the observed skin reaction.

2.12 Data Analysis

The data obtained will then be analyzed. Data stability test using ANOVA.

3. RESULTS AND DISCUSSION

3.1 Standardization simplicia

The results of quality standardization simplicity is obtaine dash content 2.17% sidaguri leaf, 2.82% chinese petai leaf. water content 7.40% sidaguri leaf, 4.29% chinese petai leaf. ash is insoluble in acid 0.21% sidaguri leaf, 0.90% chinese petai leaf. ALT 8.0 x 10^4 CFU/g sidaguri leaf, 1.0X10^4 Chinese petai leaf. ash dissolves in acid 98.71%, 99.01% Chinese petai leaf.

3.2 Phytochemical screening

The phytochemical screening from Chinese petai leaf extract and leaf extract sidaguri contains alkaloids, flavonoids, saponin, tanin,terpenoids, and steroids.

3.3 Results antibacterial activity of leaf extracts chinese and leaf sidaguri

Antibacterial activity testing was carried out using the agar well diffusion method, where each well was given 40 µL of the test extract. The positive control used clindamycin powder and the negative control used 10% DMSO solution. The results of measuring the average diameter of the inhibition zone of Chinese petai Leaf and sidaguri leaf extracts can be seen in Tables 1 and 2.

Table 1.Activity antibacterial Chinese Petai Leaf extract

Extract	Concent (%)	*P. acnes*			*S. aureus*			Average ± Sd *P. acne*	Average ± Sd *S. aureus*
Chinese Petai Leaf Leaf	50	21.35	21.1	21.05	20.15	20.15	20.55	21.17±0.16	20.28±0.23
	25	18.3	18.45	18.1	19.2	19.35	19.65	18.28±0.18	19.40±0.23
	12.5	17.3	17.8	17.2	17.3	17.05	17.25	17.43±0.32	17.20±0.13
	6.25	14.3	14.45	14.2	13.25	14.05	13.85	14.32±0.13	13.72±0.42
	3.125	11.4	11.45	11.5	12.4	12.05	12.35	11.45±0.05	12.27±0.19
	1.56	-	-	-	-	-	-	-	-
	0.78	-	-	-	-	-	-	-	-
	0.39	-	-	-	-	-	-	-	-
	0.19	-	-	-	-	-	-	-	-
	K+	38.15	38.05	38.25	36.05	36.1	36.1	38.15±0.1	36.08±0.02
	K-	-	-	-	-	-	-	-	-

Note: Positive control (+) = Clindamycin; Negative control (-) = DMSO 10%; SD = Standard Deviation

Table 2. Activity antibacterial leaf extract sidaguri

Extract	Concent (%)	*P. acnes*			*S. aureus*			Average ± Sd *P. acne*	Average ± Sd *S. aureus*
Sidaguri Leaf	50	25.2	25.15	25.15	19.1	19.05	19.25	25.17±0.03	19.13±0.10
	25	23.15	23.2	23.15	18.25	18.1	18.1	23.17±0.03	18.15±0.09
	12.5	22.05	22.1	22.15	16.35	16.2	16.2	22.10±0.05	16.25±0.09
	6.25	21.45	21.35	21.4	14.15	14.15	14.15	21.40±0.05	14.15±0.01
	3.125	17.3	17.45	17.5	11.15	11.5	11.2	17.42±0.10	11.23±0.10
	1.56	11.3	11.25	11.25	9.2	9.05	9.15	11.27±0.03	9.13±0.08
	0.78	-	-	-	-	-	-	-	-
	0.39	-	-	-	-	-	-	-	-
	0.19	-	-	-	-	-	-	-	-
	K+	38.2	2.2	28.15	36	36.1	36.05	31.52±0.1	36.05±0.05
	K-	-	-	-	-	-	-	-	-

Note: Positive control (+) = Clindamycin; Negative control (-) = DMSO 10%; SD = Standard Deviation

3.4 Gel Formulation Results

Carbopol 940 was chosen as a gelling agent because carbopol has good stability and does not experience changes in viscosity over a long time or is caused by temperature and is not easily contaminated by bacteria. The use of 2% carbopol is based on optimization results to determine the best formula and 2% is the best result obtained. Propylene glycol functions as a humectant to maintain gel stability by absorbing moisture from the environment and reducing water evaporation from preparations so that it can maintain skin moisture and prevent dry skin. Phenoxyethanol acts as a preservative that needed in gel formulas considering the high water content in gel preparations can cause microbial contamination. Triethanolamine is used to neutralize the pH (alkalizing agent). because the sidaguri and Chinese Petai Leaf extracts are acidic so triethanolamine is needed to stabilize the pH. besides that TEA also functions to develop carbopol 940 and gel to stabilize it.

Table 3. Antibacterial Activity of Gel Preparations

Formula	Week-	Temperature	Inhibition zone diameter (Mean ± SD) mm	
			P. acne	*S.aureus*
F1 (Sidaguri Leaf Extract 1.56% : Chinese Petai Leaf Leaf 3.12%)	0		13.60 ± 0.08	16.86 ± 0.05
	12	4^{0}C	13.30 ± 0.22	16.38 ± 0.31
		27^{0}C	13.22 ± 0.12	16.23 ± 0.18
		40^{0}C	12.92 ± 0.42	16.18 ± 0.06
F0 (Base)	0		0.00 ± 0.00	0.00 ± 0.00
	12	4^{0}C	0.00 ± 0.00	0.00 ± 0.00
		27^{0}C	0.00 ± 0.00	0.00 ± 0.00

	40⁰C	0.00 ± 0.00	0.00 ± 0.00
Negative Control		0.00 ± 0.00	0.00 ± 0.00
Positive control		16.30 ± 0.28	41.02 ± 0.03

The antibacterial activity of the formula was shown by the formation of an inhibition zone against *Propionibacterium acne* and *S. aureus* when the combined gel extract was tested for activity. The formation of this inhibition zone is due to the active compounds in the gel base diffusing through the media to prevent bacterial growth. The combined extract gel preparation experienced a decrease in the area of the inhibition zone. This is because the extract used is diluted with a gel base so that its activity decreases.The best antibacterial activity test results were the combination of sidaguri and Chinese petai leaf extracts at a ratio of 1:1 against *Propionibacterium acnes* and *Staphylococcus aureus* bacteria with respective inhibition zones and formulated in gel dosage forms. Analysis of the paired t-test was performed to see the difference in the area of the antibacterial inhibition zone produced by the combination gel extract and base without extract at various storage temperatures for 12 weeks. Interpretation of the analysis results is a significant value < 0.05. so there is a significant difference in the area of the inhibition zone produced by the combined extract gel against storage temperature. The significance value < 0.05 indicated that there was no significant difference in the area of the inhibition zone produced by the combined extract gel with respect to storage temperature. The results of the analysis obtained had a significance value of < 0.05 which means that there was a significant effect on the diameter of the inhibition of the gel combination of extracts on *P.acne* and *S.aureus* bacteria on storage temperature.

3.5 Results Gel Evaluation

Preparations Organoleptic tests were carried out to determine physical changes in the dosage formula including color, odor, and shape as a Stability Parameter A Preparation. The results of the evaluation of the organoleptic test showed that the gel did not change. both in terms of homogeneity. odor and color for twelve weeks at all temperatures. This indicates that the resulting gel remains stable during storage. To ensure that the gel remains stable and is not affected by the surrounding environment. the gel is stored in a tightly closed container at constant room temperature.The evaluation results of the pH test were carried out to determine the pH of the preparation and monitor the pH value during storage at 4⁰C, 27⁰C, and 40⁰C. The pH resulting from the gel for 12 weeks at all temperatures ranged from 5.45 to 6.10. The results of measuring the pH of the gel at room temperature. The heat and cycling test for 12 weeks showed varied pH values. However. the resulting pH still meets the skin pH requirements of 4.5 – 6.5.

The results of the evaluation of the viscosity test showed that the viscosity value decreased. although not significantly, at room temperature. heat and *cycling test*. The decrease in viscosity is due to the influence of the polymer on temperature changes. When the gel is stored at hot temperatures. the polymer chain forms a spherical shape (*disentangle*) resulted in decreased gel viscosity (dilute). Whereas if a gel is stored at cold temperatures. the polymer chains will shorten and join each other and over time the gel will shrink (*entangle*) resulting in a change in viscosity [51]. The results of the evaluation of the spreadability test on the gel after storage showed an increase in the spreadability compared to the initial formula before storage. This increase is influenced by the environmental conditions of gel storage. The consistency of the gel will be affected if the storage environment is unstable. The spreadability of the gel can also be affected by variations in base concentration in the formula. Due to the increase in viscosity and strengthening of the inter bonding polymer. the spreadability of the gel decreases with increasing base concentration. The results of the evaluation of spreadability obtained at temperature 4⁰C, 27⁰C, and 40⁰C. which ranges from 5.90 – 6.3 cm. This shows that the spreadability of all the formulas obtained meets the requirements so that the gel will spread properly when applied.

3.6 Gel Preparation Stability Test

Testing the antibacterial activity of gel preparations (F1) was carried out at week 0 and week 12 at various temperatures against bacteria *P.acne dan S.aureus*. The method used was agar-well diffusion method with positive control clindamycin and gel base (F0) as negative control. Clindamycin can treat serious infections caused by anaerobic and non-anaerobic bacteria. Clindamycin has a mechanism of action by inhibiting protein synthesis from bacteria by inhibiting ribosome translocation. Clindamycin will bind to 50S from bacteria which results in inhibition of peptide bonds [55]. The results of measuring the average diameter of the inhibition zone gel of the combination of sidaguri and Chinese Petai Leaf leaf extracts

temperature at 4^0C, 27^0C, and 40^0C.

The paired t-test analysis was carried out to see the difference in the area of antibacterial inhibition zones produced by a combination gel of extract and base without extract at various storage temperatures for 12 weeks. Interpretation of the analysis results is a significant value < 0.05. There is a significant difference in the area of the inhibition zone produced by the combined extract gel against storage temperature. The significance value < 0.05 indicated that there was no significant difference in the area of the inhibition zone produced by the combined extract gel with respect to storage temperature. The results of the analysis obtained have a significance value of < 0.05 which means that there is a significant effect on the diameter of the inhibitory power of the extract combination gel on bacteria *P.acne dan S.aureus* to storage temperature.

3.7 Microbial Contamination Test

The testing for microbial contamination was carried out at week 0 and 12 on preparations stored at 4^0C, 27^0C, and 40^0C. The results of the microbial contamination test using the ALT method on gel preparations showed negative results up to 10^{-3} with negative control gel base without extract. According to regulation of the Head of the Indonesian Food and Drug Agency (BPOM) Number 17 (2014) that the microbial contamination requirements for cosmetics contain no more than 10^3 colonies/g [54].

3.8 Statistical Analysis

Paired sampel t-test This research was carried out to see the difference in the area of antibacterial inhibition zones produced by a combination gel of extract and base without extract at various storage temperatures for 12 weeks. Interpretation of the analysis results is a significant value < 0.05. so there is a significant difference in the area of the inhibition zone produced by the combined extract gel against storage temperature. The significance value < 0.05 indicated that there was no significant difference in the area of the inhibition zone produced by the combined extract gel with respect to storage temperature. The results of the analysis obtained have a significance value of < 0.05 which means that there is a significant effect on the diameter of the inhibitory power of the extract combination gel on *P.acne dan S.aureus* to storage temperature.

3.9 Irritation Test Animal

Table 4. Irritation test results

Time	Rabbit 1						Rabbit 2						Rabbit 3					
	4^0C		27^0C		40^0C		4^0C		27^0C		40^0C		4^0C		27^0C		40^0C	
	E	U	E	U	E	U	E	U	E	U	E	U	E	U	E	U	E	U
24 h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48 h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72 h	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Amount	**0**	**0**	**0**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Description: E = Erythema U = Udema

The results in table 4 show that there was a slight erythema reaction but it was not obvious. but did not cause edema after 3 days of treatment. with clear boundaries but did not cause edema at 48 and 72 hours of treatment. This result is not classified as dangerous because basically the skin sensitivity of experimental animals is slightly different from human skin. On the first and second days, the three rabbits did not experience erythema and none experienced edema. On the third day, rabbit number 1 experienced erythema with clear borders and edema. This result is not classified as dangerous because basically the skin sensitivity of experimental animals is slightly different from human skin. Irritation test is done to avoid side effects on the skin. The irritation test in this study was carried out in vivo in 3 experimental rabbits at 24, 48, and 72 hours after receiving the test preparation. observation of the irritation test was carried out by observing skin reactions with two observation parameters. namely the degree of erythema, redness, and edema that occurred. The

possibility of delayed irritation reactions was checked at 24, 48, and 72 hours after the bandage was removed. Then the results of these observations were given a score of 0 to 4 according to the level of severity. The degree of irritation is calculated based on the calculation of the observed value.

4. CONCLUSION

The 96% ethanol extract of sidaguri leaf can inhibit growth *Propionibacterium acne*s at a concentration of 1.56%, against *S. aureus* at a concentration of 1.56% and 96% ethanol extract of Chinese Petai Leaf leaf can inhibit the growth of *Propionibacterium acne*s at a concentration of 3.125%. against *S. aureus* at a concentration of 3.125%. The combination of sidaguri and Chinese Petai Leaf leaf extracts has better activity against bacteria *Propionibacterium acne*s and *S. aureus* in comparison (1.56%: 3.125%) compared to a single extract. Gel preparation combination of sidaguri and Chinese Petai Leaf leaf extracts with a comparative concentration (1.56%: 3.125%) has antibacterial activity against bacteria *Propionibacterium acne*s and *S. aureus*. The gel formula of a combination of sidaguri and Chinese Petai Leaf leaf extracts can fulfill physical and chemical parameters and is stable to temperature 4^0C, 27^0C, 40^0C and storage time for 12 weeks. The gel combination of 96% ethanol extract of sidaguri leaf and Chinese Petai Leaf leaf had very mild responses.

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