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Antibacterial Activity Mixed Extract Gel Formula Ethanol Leaves of Lime (Citrus Aurantifolia) and Aloe Vera (Aloe Vera) Against Propionibacterium Acnes

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Abstract. Propionibacterium acnes is an anaerobic gram-positive microorganism that is one of the causes of acne. Acne therapies may target inflammation and Propionibacterium acne reduction. Anti-acne drugs may now be manufactured as anti-acne gels and creams. In order to achieve the therapy objective of lowering Propionibacterium acnes, gel formulations must be formulated correctly with additional active components such as antibacterials. Antibacterial compounds may be extracted from plants such as lime and aloe vera. According to (Yusmaini & Bahar, 2018), the chemicals found in lime leaves and aloe vera can inhibit the growth of Propionibacterium acnes as antibacterials. Consequently, this study aimed to assess the antibacterial activity of the ethanol extract gel formula of lime leaves (Citrus aurantifolia), the ethanol extract gel formula of aloe vera (Aloe vera), and the ethanol extract gel formula including lime leaf extract and aloe. The effectiveness of aloe vera against Propionibacterium acnes, as well as the difference in the diameter of the inhibition zone between lime leaf extract gel, aloe vera extract gel, and a combination of lime leaf extract and aloe vera gel in preventing the growth of Propionibacterium acnes. This study is a sophisticated experimental investigation employing a randomized factorial design with two elements: the type of extract and the concentration. The extract type consists of three levels: lime leaf extract, aloe vera extract, and a mixture of lime leaf and aloe vera extract. At the same time, the concentration consisted of six levels, including extract concentrations of 3%, 5%, 7%, and 9%. The negative control utilized aqua dest, and the positive control utilized Clindamycin antibiotics. The ethanol extract gel of lime leaves, aloe vera, and a combination of ethanol extracts of lime leaves and aloe vera exhibited weak and high inhibitory response categories for antibacterial activity. Significant variations were seen in the average width of the inhibitory zones produced by ethanol extracts of lime leaves, aloe vera, and a combination of ethanol extracts of lime leaves and aloe vera against P.acnes.

Keywords : Antibacterial, Aloe vera, Citrus aurantifolia, Gel Formula, *Propionibacterium acnes*

INTRODUCTION

Acne is caused in part by the anaerobic, gram-positive bacteria Propionibacterium acnes. Propionibacterium acnes causes acne by secreting sebum, which can harm the stratum corneum and stratum germination and damage the pore walls (Miratunnisa et al., 2015). Propionibacterium acnes' excessive sebum production can lead to blocked pores or sebum ducts on the skin (Hafsari et al., 2015). Reduce sebum production, skin damage, hair follicle abnormalities, and the quantity of Propionibacterium acnes colonies to treat acne (Ansel, 2008). Acne therapies may target inflammation and Propionibacterium acne reduction. Anti-acne medications are now available in creams and gels.

Gel preparations are topical formulations with a polar basis that do not irritate the skin and have high diffusivity (Sasanti et al., 2012). Gel preparations have a high water content to alleviate irritation caused by sebum buildup in

the pores (Arikaumala et al., 2013). According to Voigt (1994), gel compositions dry quickly and create easily-cleanable films. Gel offers the skin a cooling feeling (Winarti, 2013). In order to achieve the therapy objective of lowering *Propionibacterium acnes*, gel formulations must be formulated correctly with additional active components such as antibacterials.

Antibacterial substances can be obtained from plants such as lime and aloe vera. Lime leaves contain secondary metabolites in alkaloids, phenols, saponins, tannins, steroids, and flavonoids. Flavonoids, tannins, phenols, and saponins in lime leaves have the potential as antibacterials (Anggraito et al., 2018). Meanwhile, aloe vera plants have secondary metabolites such as flavonoids, tannins, phenols, and saponins, which have the potential as antibacterials (Wijaya, 2013). According to (Yusmaini & Bahar, 2018) that the substances contained in lime leaves and aloe vera have the potential as antibacterials that can suppress the development of Propionibacterium acnes. Besides being an antibacterial, Aloe vera is also known to have acted as an anti-inflammatory to speed up acne's healing process. According to research conducted by (Biworo et al., 2013) that aloe vera water extract has the potential as an anti-inflammatory in a rat model of contaminated wounds. Therefore this study aimed to determine the antibacterial activity of the ethanol extract gel formula of lime leaves (Citrus aurantifolia), the ethanol extract gel formula of aloe vera (Aloe vera), and the ethanol extract gel formula mixed with lime leaf extract (Citrus aurantifolia) and aloe. Aloe vera against Propionibacterium acnes, as well as knowing the difference in the diameter of the inhibition zone of the formula of lime leaf extract gel, aloe vera extract gel, and a mixture of lime leaf extract and aloe vera gel in inhibiting the growth of Propionibacterium acnes

METHOD

This study is an advanced experimental investigation employing a completely random factorial design with two components, extract kind and concentration. The type of extract consisted of three levels, coded F1, F2, and F3: lime leaf ethanol extract, aloe vera ethanol extract, and a blend of lime leaf and aloe vera ethanol extract. While the concentration consisted of six levels, namely extract concentrations of 3%; 5%; 7%; and 9%, the positive control utilized the antibiotic Clindamycin, and the negative control utilized distilled water, which was subsequently coded K1, K2, K3, K4, K5, and K6. Each treatment was administered twice, yielding a total of 34 experimental units. The study was conducted between November 2021 and May 2022. This study employed a sampling method known as purposive sampling, which includes selecting specified criteria. The samples utilized met the inclusion criteria, consisting of lime leaves from fresh lime plants with a dark green hue and aloe vera from aloe vera leaves with a length of 40 cm and a plant age of 1 month after harvesting from Indramayu Regency.

RESULT AND DISCUSSION

Lime leaves were selected from lime plants with the criteria of fresh dark green leaves, while aloe vera plants were selected from aloe vera leaves with a length of ±40 cm and plant age ≥ 1 month after harvest which was taken in Indramayu Regency. Kaffir lime leaves and aloe vera were then extracted with an ethanol solvent to obtain thick extracts of kaffir lime leaves and aloe vera, then tested for phytochemical compounds. The results of calculating the yield of ethanol extract of lime leaves and aloe vera can be seen in table 1.

Table 1. Calculation results of ethanol extract yield
Lime Leaves and Aloe Vera

Type of extract	Wet sample weight (kg)	Thick extract weight (g)	Extract yield (%)
F1	2.00	404.75	40.48 %
F2	1.00	80.96	8.10 %

Information :
F1: ethanol extract from lime leaves
F2: ethanol extract of aloe vera

Based on table 1. The yield of F1 extract was 40.48% from a wet sample weight of 2.00 kg and a thick extract weight of 404.75 g. Meanwhile, the yield of the F2 extract was 8.10% from a wet sample weight of 1.00 kg and a viscous extract weight of 80.96 g. From the calculation of the yield, it can be seen that the results obtained to meet the requirements according to (the Directorate General of POM, 2000) that a good yield is at least 7.2%.

The thick leaves and aloe vera extracts were then subjected to a qualitative test of phytochemical compounds to identify the secondary metabolites in lime leaves and aloe vera. The results of the qualitative test of the phytochemical compounds in the ethanol extract of lime leaves and aloe vera can be seen in table 2.

Table 2. Results of qualitative test of phytochemical compounds on ethanol extract of lime leaves and aloe vera

Extract Type	Secondary Metabolites Compounds	Result	Description
F1	Flavonoid	+	Blackish yellow
	Fenol	+	Black
	Saponin	+	Stable foam is formed
	Tanin	+	Black
F2	Flavonoid	+	Blackish yellow
	Tanin	+	tanned
	Fenol	+	Yellow
	Saponin	+	Foam formed

Information :
F1: ethanol extract from lime leaves
F2: ethanol extract of aloe vera

based on Table 2. It is known that the qualitative test results for the phytochemical compounds in the ethanol extract of lime leaves showed positive results in the tests for flavonoids, phenols, saponins, and tannins. Meanwhile, the ethanol extract of aloe vera showed positive results in the tannin, phenol, and saponin tests. Phytochemical compounds in the form of flavonoids, phenols, saponins, and tannins have antibacterial activity (Anggraito et al., 2018). Apart from being potentially antibacterial, flavonoids also have potential as anti-inflammatories (Kumar and Pandey, 2013). The antibacterial and anti-inflammatory contents of flavonoid compounds can be used as active substances for the acne healing process because acne treatment aims to reduce the number of P. acnes colonies or their metabolic results and inflammation of the skin (Sifatullah and Zulkarnain, 2021).

The preparation of anti-acne gel from ethanol extract of lime leaves and a mixture of ethanol extract of lime leaves and aloe vera used the formula according to Andika et al. (2021), while the manufacture of anti-acne gel from the ethanol extract of aloe vera used the formula according to Roroningtyas et al. (2012).The anti-acne gel that will be made must go through an evaluation process first in order to find the best formula from the specified base. The evaluation results of the anti-acne gel preparation of ethanol extract of lime leaves, aloe vera, and a mixture of lime and aloe vera extracts can be seen in Tables 3, 4, and 5.

Table 3. Results of Evaluation of Lime Leaf Ethanol Extract Gel Preparations

No.	Ingredients	K1	K2	K3	K4
1.	Form	Semi-solid	Semi-solid	Semi-solid	Semi-solid
2.	Color	Dark green	Dark green	Dark green	Dark green
3.	Smell	Lime special	Lime special	Lime special	Lime special
4.	Homogeneity	No grain	No grain	No grain	No grain
5.	pH	4.9	4.9	5.3	5.3
6.	Spread Power	5.00 cm	5.00 cm	5.30cm	5.30cm
7.	Stickiness	09.20 seconds	09.20 seconds	63.00 seconds	93.00 seconds
8.	Protection	No smudges	No smudges	No smudges	No smudges

		on every second	on every second	on every second	on every second
9.	Viscosity	11980 cP	13070 cP	11570 cP	9067 cP

Table 4. Hasil Evaluasi Sediaan Gel Ekstrak Etanol Lidah Buaya

No.	Ingredients	K1	K2	K3	K4
1.	Form	Semi-solid	Semi-solid	Semi-solid	Semi-solid
2.	Color	Yellow	Yellow	Yellow	Yellow
3.	Smell	No smell	No smell	No smell	No smell
4.	Homogeneity	No grain	No grain	No grain	No grain
5.	pH	4.7	5.0	5.1	5.2
6.	Spread Power	5.00 cm	5.10cm	5.50cm	5.60cm
8.	Stickiness	50.57 seconds	76.00 seconds	77.00 seconds	80.00 seconds
9.	Protection	No smudges on every second	No smudges on every second	No smudges on every second	No smudges on every second
10.	Viscosity	32000 cP	39530 cP	41000 cP	44470 cP

Table 5. Results of Evaluation of Mixed Gel Preparations Ethanol Extract of Lime and Aloe Vera Leaves

No.	Ingredients	K1	K2	K3	K4
1.	Form	Semi-solid	Semi-solid	Semi-solid	Semi-solid
2.	Color	Dark green	Dark green	Dark green	Dark green
3.	Smell	The dominant smell of lime	The dominant smell of lime	The dominant smell of lime	The dominant smell of lime
4.	Homogeneity	No grain	No grain	No grain	No grain
5.	pH	4.9	5.0	5.3	5.4
6.	Spread Power	5.10cm	5.20cm	5.20cm	5.30cm
8.	Stickiness	30.11 seconds	30.26 seconds	58.59 seconds	73.00 seconds
9.	Protection	No smudges on every second	No smudges on every second	No smudges on every second	No smudges on every second
10.	Viscosity	37330 cP	43000 cP	39330 cP	35600 cP

Information :
K1 : extract concentrations of 3%;
K2 : extract concentrations of 5%
K3 : extract concentrations of 7%
K4 **:** extract concentrations of 9%

The results of the gel evaluation in Tables 3, 4, and 5 show that the evaluation results for gel preparations of ethanol extract of lime leaves, aloe vera, and a mixture of ethanol extracts of lime leaves and aloe vera, namely gel preparations at K1, K2, K3, and K4 has a semi-solid form, has a distinct lime odor, is dark green and yellow, has no coarse grains, and does not stain every second in the protection test. According to (Depkes RI 2020) that the requirement for homogeneity in gel preparations is that they do not contain coarse grains that can be felt. The form of semi-solid gel follows the definition of gel according to (Allen & Ansel, 2013), which is a semi-solid preparation consisting of small or large molecules dispersed in an aqueous liquid made like jelly with a gel-forming agent. As for the pH of the ethanol extract gel preparations of lime leaves, aloe vera, and a mixture of ethanol extracts of lime

leaves and aloe vera K1, K2, K3, and K4, respectively, were 4.9; 5.3; 4.7; 5.1; and 5.2. Then in the spreadability test, the results obtained at K1, K2, K3, and K4 were 5.00 cm; 5.20cm; 5.30cm; 5.10cm; 5.50cm; and 5.60 cm. According to (Kaur et al., 2010), the results of a good spreadability test are 5-7 cm. In the adhesive test, the results were obtained for K1, K2, K3, and K4 for 09.20 seconds, 15.37 seconds, 63.00 seconds, 93.00 seconds, 50.57 seconds, 76.00 seconds, 77.00 seconds, 80.00 seconds, 30.11 seconds, 30.26 seconds, respectively. , 58.59 seconds, and 73.00 seconds. Good adhesion to gel preparations, which is more than 1 second. The longer the preparation is attached to the skin, the longer the active substance will be in contact with the skin and is expected to provide optimal therapeutic effects (Voight, 1984). The viscosity of the ethanol extract gel preparations of lime leaves, aloe vera, and the mixture on K1, K2, K3, and K4 were 11980 cP, 13070 cP, 11570 cP, 9067 cP, 32000 cP, 39530 cP, 41000 cP, respectively. 44470 cP, 37330 cP, 39330 cP, and 35600 cP. A good gel preparation has a viscosity of 6000-50000 cP or 6-50 Pa.S (Ardana et al., 2015).

The antibacterial test of the ethanol extract gel preparation of lime leaves, aloe vera, and a mixture of lime and aloe vera was carried out on pure isolate Propionibacterium acnes ATCC 11827. The results of the antibacterial test can be seen in table 6.

Table 6. Antibacterial Test Results of Ethanol Extract of Lime Leaves, Aloe Vera, and a Mixture of Lime Leaves and Aloe Vera against P. Acnes ATCC 11827

Treatment	Average Inhibition Zone Diameter (mm)	Inhibition Response Category (Greenwood, 1995)
F1K1	3.45	Not Inhibiting
F1K2	8.90	Not Inhibiting
F1K3	9.72	Not Inhibiting
F1K4	7.00	Not Inhibiting
F1K5	10.15	Weak
F1K6	0.00	Not Inhibiting
F2K1	12.40	Weak
F2K2	5.85	Not Inhibiting
F2K3	4.80	Not Inhibiting
F2K4	5.00	Not Inhibiting
F2K5	10.35	Weak
F2K6	0.00	Not Inhibiting
F3K1	35.20	Strong
F3K2	32.25	Strong
F3K3	29.90	Strong
F3K4	33.70	Strong
F3K5	23.95	Strong
F3K6	0.00	Not Inhibiting

Information :
K1 : extract concentrations of 3%;
K2 : extract concentrations of 5%
K3 : extract concentrations of 7%
K4 **:** extract concentrations of 9%
K5 : the positive control utilized the antibiotic Clindamycin
K6 : the negative control utilized distilled water.

Based on table 6, it was found that the average diameter of the inhibition zone in the F1K6, F2K6, and F3K6 treatments for P.acnes was all negative control, which was 0.00 mm in the non-inhibitory response category. The F1K1, F1K2, F1K3, F1K4, F2K2, F2K3, and F2K4 treatments also showed an average diameter of the inhibition zone <10 mm, namely 3.45 mm, 8.90 mm, 9.72 mm, 7.00 mm, 5.85 mm, 4.80 mm, respectively. And 5.00mm. The F1K5, F2K1, and F2K5 treatments had an average inhibition zone diameter of 10.15 mm, 12.40 mm, and 10.35 mm, respectively, with a weak inhibition zone response category. Then in the treatment F3K1, F3K2, F3K3, F3K4, and F3K5, the diameter of the inhibition zone was > 20 mm, namely 35.20 mm, 32.25 mm, 29.90 mm, 33.70 mm, 23.95 mm, respectively, in the category of solid resistance response. The average diameter of the inhibition zone with a

number < 10 mm, according to (Greenwood, 2003), can be categorized as having a non-inhibiting barrier response category, while those with an inhibition zone diameter of 10-15 are categorized as weak, then those with an inhibition zone diameter of 16-20 and > 20 can be categorized weak and strong resistance response.

The difference in the diameter of the inhibition zone resulting from each concentration and extract is influenced by various factors, including the content of phytochemical compounds that have the potential as antibacterials contained in plant extracts. Flavonoid compounds in lime leaf and aloe vera plant extracts have the potential to damage the cytoplasmic membrane and precipitate proteins in bacterial cells so that they can affect the bacterial enzyme metabolism system (Afifi et al., 2018). Phenol compounds act as enzyme inhibitors causing toxicity to bacterial cells, and tannin compounds can inhibit bacterial growth by inactivating bacterial adhesion. Saponin compounds can interfere with the permeability of bacterial cell membranes, which will damage the cell membrane so that the essential components in the cell will come out. Namely, proteins and nucleic acids cause bacteria to lyse and cause cell death (Cahyanta et al., 2020). The concentration of antibacterial substances affects the growth of bacteria, so the higher the concentration contained in the extract, it will affect the growth of bacteria, and the value of the inhibition zone obtained will be even greater (Afifi et al., 2018).

Positive control in the K5 treatment using Clindamycin antibiotics. The average diameter of the inhibition zone in the F1K5 and F2K5 treatments was 10.15 mm and 10.35 mm, with a weak inhibition response category. Meanwhile, F3K5 has an average inhibition zone diameter of 23.95 mm with a strong resistance response. This shows that there are differences in the categories of inhibition even though using the same antibiotic. The turbidity of the bacterial suspension can cause this compared to the McFarland standard. If the suspension is too turbid, the resulting diameter will be smaller because it is influenced by the density of the bacterial cells. The thickness of the media, which results in a lack of diffusion power into the media, can also affect the diameter of the resulting inhibition zone (Zeniusa et al., 2019). The rate of diffusion of antibiotics, the concentration of antibiotics, the sensitivity of bacteria to antibiotics, and the interaction of antibiotics with the media will affect the zone of inhibition of bacterial growth (Sarlina et al., 2017).

The average value of the diameter of the inhibition zone on all types of extracts with various concentrations of K1, K2, K3, and K4 were analyzed using the Friedman test. This is because the homogeneity test results show that the data is not homogeneous, with a p-value of 0.010 <0.050. The Friedman test results obtained a p-value of 0.000 <0.050. This shows a significant difference in the average diameter of the inhibition zone between lime leaf extract, aloe vera, and a mixture of lime leaf and aloe vera extracts. Therefore, it is necessary to carry out further Mann-Whitney tests on each type of extract. The results of the Mann-Whitney test can be seen in table 7.

Table 7. Mann Whitney Test Results Mean Inhibition Zone Diameter of Ethanol Extracts of Lime Leaves, Aloe Vera, and Mixture of Lime Leaves and Aloe Vera Against P.acnes

Treatment Extract	*p-value*	Description
The mean diameter of the inhibition zone of the ethanol extract of lime and aloe vera leaves against P.acnes 0.839 > 0.050	0.839 > 0.050	There was no significant difference
The mean diameter of the inhibition zone of the ethanol extract of aloe vera and the mixture of ethanol extract of lime leaves and aloe vera against P.acnes 0.004 < 0.050	0.004 < 0.050	There is a significant difference
Mean Diameter of Inhibition Zone of Lime Leaf Ethanol Extract and Mixture of Lime Leaf and Aloe Vera Ethanol Extract to P.acnes 0.004 < 0.050	0.004 < 0.050	There is a significant difference

Table 7 shows that the p-value of lime leaf extract with a mixture of lime leaf extract and aloe vera is 0.004 <0.050. This shows a significant difference in the average diameter of the inhibition zone in lime leaf extract and a mixture of lime leaf extract and aloe vera. Then the aloe vera extract with a mixture of lime leaf extract and aloe vera also has a p-value of 0.004 <0.050. Lime leaf extract with aloe vera extract has a p-value of 0.839 > 0.050. This shows no difference in the average diameter of the inhibition zone in lime leaf extract and aloe vera extract.

CONCLUSION

The antibacterial activity of the ethanol extract gel of lime leaves, aloe vera, and a mixture of ethanol extracts of lime leaves and aloe vera had weak and strong inhibition response categories. The average diameter of the inhibition zone between the ethanol extracts of lime leaves, aloe vera, and a mixture of ethanol extracts of lime leaves and aloe vera showed significant differences to Propionibacterium acne.

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