Antibacterial Activity Of Lime Peel (*Citrus Aurantifolia* (Christm.) Swingle) Ethanolic Extract Against Skin Infection Bacteria (*Propionibacterium Acnes* And *Staphylococcus Epidermidis*)

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Abstract.
The skin is the outermost part of the body that serves as a protective body. In addition to protecting the skin of the face, it also acts as a beautifier. The main problem with facial skin is inflammation of the skin, which can cause acne. Bacteria like Propionibacterium acnes, Staphylococcus aureus, Staphylococcus epidermidis, and Pseudomonas aeruginosa can cause acne on the face. Prolonged use of antibiotics as therapy against acne will cause a new problem, namely resistance to antibiotics. Lime peel has many uses, especially as an antibacterial, which makes it a good candidate for use as an antibacterial agent in acne treatment. The goal of this study was to find out if the etTMhanolic extract of lime peel could kill bacteria like Propionibacterium acnes and Staphylococcus epidermidis. The research method used the Kirby-Bauer method and the Streaking methods to analyze the minimum inhibitory concentration and minimum bactericidal concentration. The results showed MIC at a concentration of 1 mg/mL for each bacterium, while MBC at a concentration of 40 mg/mL in Propionibacterium acnes and 50 mg/mL in Staphylococcus epidermidis. The conclusion is that the extract of lime peel in ethanol can kill bacteria.

Keywords: *Antibacterial; Propionibacterium acnes; Staphylococcus epidermidis; and Lime peel.*

I. INTRODUCTION

The skin has a main function as a protector from various kinds of disturbances and external stimuli. This protective function is done by a number of biological processes, such as keratinization and the shedding of dead skin cells, as well as the control of body temperature and the production of sebum and sweat [1], [2]. Acne is a skin disorder due to inflammation accompanied by blockage of the oil glands of the skin and hair (pilosebaceous ducts), which is characterized by the presence of blackheads, papules, and pustules. Acne usually appears on the skin surface of the face, neck, chest, and back when the oil glands on the skin are too active. So that the skin pores will be clogged by excessive fat deposits mixed with sweat, dust, and other dirt, causing blackheads. Comedones that are infected with bacteria cause inflammation called acne, which varies in size from small to large and red in color. Sometimes pus causes pain [3]–[5].Inflammation that occurs in acne can be triggered by pathogenic bacteria that can enter the host's body in several ways, one of which is through open skin areas such as wounds, hair follicles, and sweat gland pockets [6]. The pathogenic bacteria are Propionibacterium acnes, *Staphylococcus epidermidis*, Staphylococcus aureus, and Pityrosporum ovale [7].

Bacteria called Propionibacterium acne can cause infections in acne by releasing hydrolytic enzymes that damage polysebaceous follicles and enzymes that are a key part of the inflammatory process [8]. Staphylococcus aureus can cause skin infections with characteristic signs of inflammation and abscess formation [9].*Citrus aurantifolia* (Christm.) Swingle) is one of the herbal plants used by the community to treat various diseases, one of which is antibacterial. So far, people use lime leaves and fruits as medicine and food preservatives, but they still do not use lime peels. This is because very few people know the uses and contents of the lime peel, so the lime peel is wasted and ends up as waste [10]–[12], In addition, there are studies that prove that both the leaves, fruit, and peel of lime have properties that are useful as antibacterials

because they contain flavonoid compounds [13], [14].Lime peel is one of the wastes that can be used to treat diseases, especially ones that irritate the skin's surface, like acne caused by *S. aureus* and *Propionibacterium acne*, inflammation caused by infection caused by S. epidermidis, and burn infections caused by S. epidermidis. P. aeruginosa [15]–[17]. Therefore, researchers are interested in testing the antibacterial activity of lime peel against acne-causing bacteria, namely *Propionibacterium acnes* and *Staphylococcus epidermidis*.

II. MATERIAL AND METHODS

Apparatus and Materials

An analytical balance, caliper, wire loop, micropipette, paper backer, petri dish, and other glassware were used in this study. The materials used in this study were Muller Hinton Agar (MHA: Himedia), Muller Hinton Broth (MHB: Himedia), Plate Count Agar (PCA), Dimethylsulfoxide, 96% ethanol (pa), aquadest and the test bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis*.

Lime Fruit Peel Sample Preparation

The plant material used was lime peel, which was taken purposively without comparing it with the same plant from other regions. The limes were taken from Sentosa Baru Market, Edan Perjuangan sub-district. Lime peel is separated from other impurities, washed under running water, drained and weighed wet weight. Then it was dried in the dryer to dry and blended until smooth. weighed and stored in a tightly closed container [18].

Plant Identification

Plant identification was carried out at the Medanense Herbarium Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara.

Examination of Dried Powder Characteristics

Examination of dried powder characteristics includes determination of water content; determination of water-soluble extract content; determination of ethanol soluble extract content; determination of total ash content; and determination of acid insoluble ash content [19].

Preparation of Lime Peel Ethanolic Extract

500 g dry lime peel powder was weighed and placed in a glass container, then 75 parts solvent with 96% ethanol (pa) was added and allowed to stand for 5 days. Then it was macerated again with 25 parts of the remaining solvent for 2 days. A rotary evaporator was used to filter and concentrate the extract until a thick extract was made [20].

Phytochemical Screening

Phytochemical screening of simplicia and ethanolic extract of lime peel includes examination of flavonoid compounds [21], alkaloids [18], saponins [18], tannins [21], glycosides [18], and steroids/triterpenoids [22] (Harbone, 1996).

Preparation of Various Concentration of ethanol extract of lime peel

The extract was weighed up to 1.5 g, then 10 mL of dimethyl sulfoside was added and stirred until dissolved, yielding a concentration of 150 mg/mL. Dilutions of various concentrations were carried out [23].

Sterilization of Apparatus and Materials

The tools used in testing the antibacterial activity were sterilized before use. Glass utensils were sterilized using an oven at 170°C for 1 hour. At the same time, materials like test media were sterilized in an autoclave for 15 minutes at 121°C [24].

Test of Minimum Inhibitory Concentration (MIC)

Determination of the minimum inhibitory concentration as bacteriostatic antibacterial activity was tested by inserting 0.1 ml of bacterial inoculum into a petri dish then adding 15 ml of media and homogenizing it. The media is allowed to solidify and the paper discs that have been given a concentration are placed on the surface of the media. Then incubated for 24 hours at a temperature of 36-37°C. After incubation, the diameter of the inhibition zone was measured using a caliper (mm) [20], [23].

Test of Minimum Bactericidal Concentration (MBC)

Furthermore, the determination of the minimum kill concentration was carried out using the streaking method from the inhibition zone formed and then suspended with 1 mL NB and planted on Plate Count Agar media. At a temperature of 36-37°C for 24 hours, the number of colonies growing at each concentration was counted and compared to the number of colonies growing in the negative control. The difference that shows a percentage decrease of 98% – 99.99% is determined as the value of the MBC [25], [26].

Data Analysis

All data is presented in the form of an average with a standard deviation value. The analysis of variance test using SPSS IBM v.26 saw a comparison of values between treatments and negative controls.

III. RESULTS AND DISCUSSION

Plant identification

The results of plant identification were carried out at the Herbarium Medanencse laboratory, with the identification results stating that the plant used in this study was lime peel (Citrux hystrix) with identification letter no.

Examination of Simplicia Characteristics of Lime Peel

Macroscopic examination of lime peel (*Citrus aurantifolia* (Christm.) Swingle) reveals a yellowish green lime peel that has a slippery texture on the surface. Examination of simplicia characteristics can be seen in table 1.

Table 1. Data on the characterization of lime peel simplicia

No.	Parameter	Result* (%)	Indonesian Herbal Pharmacopoeia Requirements Edition I (Depkes RI, 2013)
1.	Water content	8,64	≤ 10%
2.	Water Soluble Extract Level	26,67	≥ 4,4%
3.	Content of Soluble Essence in Ethanol	18,14	≥ 15,4%
4.	Total Ash Content	5,62	≤ 16,6%
5.	Acid Insoluble Ash Content	0,35	≤ 0,7%

Examining the amount of water in simplicia is done to find out how much water it has and to make sure it is of good quality, since the amount of water in simplicia will affect the growth of fungi if it has a high-water content. The result of determining the water content in lime peel simplicia is 8.64%, which means the results obtained still meet the requirements according to the Indonesian herbal pharmacopoeia in 2013, which is less than 10%. Water content that exceeds 10% will trigger microbial growth in simplicia, especially fungal growth, thereby accelerating damage to simplicia [27].The determination of juice content in simplicia using two types of solvents, namely water and ethanol, is completed.

The water soluble extract content determination aims to determine how many polar chemical compounds are contained in simplicia, whereas the ethanol solvent determination aims to determine chemical compounds dissolved in ethanol, both polar and non-polar [28]. The result of the determination of the water-soluble extract content in lime peel simplicia was 26.67%, while the ethanol soluble extract content was 18.14%. This shows that lime peel simplicia contains more polar compounds than non-polar compounds [29].The determination of ash content aims to determine the physiological ash content (internal minerals) derived from plant tissue contained in simplicia. acid insoluble ash content to indicate the amount of silicate, especially sand, present in simplicia by dissolving total ash in hydrochloric acid [30]. A determination of ash content in simplicia showed a total ash content of 5.62% and an acid-insoluble ash content of 0.35%.

Phytochemical screening of simplicial and ethanol extract of lime peel

As shown in table 2, the phytochemical screening looked at the amount of alkaloids, flavonoids, glycosides, saponins, tannins, and steroids and triterpenoids.

Table 2. Phytochemical screening of simplicia and ethanol extract of lime peel

No.	Secondary Metabolites	Reagents	Result	
			Simplicia	Extract
1.	Alkaloids	Dragendroff	+	+

		Bouchardat	+	+
		Meyer	+	+
2.	Flavonoids	Mg Powder + Amil Alcohol + HCl (p)	+	+
3.	Glycoside	Molish + H_2SO_4	+	+
4.	Saponins	Hot water / shaking	+	+
5.	Tannins	$FeCl_3$	+	+
6.	Triterpenoid/Steroids	Lieberman-Bourchat	+	+

Information : + = positive contains a group of compounds.

Based on the results of the phytochemical screening examination, it showed that both simplicia and ethanol extracts contained groups of alkaloids, flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids. Flavonoid compounds and tannins have antibacterial properties. Secondary metabolites of flavonoids and tannins work as antibacterials by messing with the parts of the bacterial cell wall (peptidoglycan) in bacterial cells. This keeps the cell wall layer from forming fully, which kills the bacterial cell [31].

Testing the Minimum Inhibitory Concentration (MIC) of lime peel ethanol extract against *Propionibacterium acnes* and *Staphylococcus epidermidis*

Testing the antibacterial activity of the ethanolic extract of lime peel showed that there was an effective inhibition of the growth of *Propionibacterium acnes* and *Staphylococcus epidermidis* bacteria at a concentration of 50 mg/mL (P. acnes) with an inhibition zone diameter of 10.23 ± 0.15 mm, while in S. epidermidis bacteria it was effective at a concentration of 70 mg/mL with an inhibition zone diameter of 10.17 ± 0.15 mm. The results of the measurement of the diameter of the inhibition zone for each concentration can be seen in table 3.

Table 3. Results of Measurement of Inhibition Zone Diameter of Lime Peel Ethanol Extract Against *Propionibacterium acnes* and *Staphylococcus epidermidis*.

Concentration (mg/mL)	Diameter Inhibition Zones (mm)							
	Propionibacterium acnes				*Staphylococcus epidermidis*			
	P1	P2	P3	X ± SD	P1	P2	P3	X ± SD
Control -	6,0	6,0	6,0	6,00 ± 0,00	6,0	6,0	6,0	6,00 ± 0,00
0,5	6,0	6,0	6,0	6,00 ± 0,00	6,0	6,0	6,0	6,00 ± 0,00
1	7,2	7,4	7,1	7,23 ± 0,15	6,8	6,7	6,7	6,73 ± 0,06
5	8,2	8,0	7,9	8,03 ± 0,15	7,2	7,3	7,2	7,23 ± 0,06
10	8,3	8,2	8,6	8,37 ± 0,21	7,7	7,8	7,7	7,73 ± 0,06
20	8,9	8,8	8,7	8,80 ± 0,10	8,4	8,5	8,0	8,30 ± 0,26
30	9,2	9,0	8,9	9,03 ± 0,15	8,9	8,7	8,7	8,77 ± 0,12
40	9,9	9,7	9,6	9,73 ± 0,15	9,5	9,4	9,0	9,30 ± 0,26
50	10,4	10,1	10,2	10,23 ± 0,15	9,7	9,8	9,4	9,63 ± 0,21
60	10,9	10,6	10,5	10,67 ± 0,21	10,0	9,9	9,8	9,90 ± 0,10
70	11,2	11,0	10,8	11,00 ± 0,20	10,3	10,2	10,0	10,17 ± 0,15
80	11,3	11,2	11,0	11,17 ± 0,15	10,6	10,7	10,4	10,57 ± 0,15
90	11,6	11,4	11,3	11,43 ± 0,15	11,0	10,9	10,8	10,90 ± 0,10
100	11,7	11,8	11,7	11,73 ± 0,06	11,2	11,4	11,0	11,20 ± 0,20
150	12,3	12,2	12,1	12,20 ± 0,10	11,4	12,0	11,4	11,60 ± 0,35
Control +	31,4	30,7	31,3	31,13 ± 0,38	28,6	27,5	28,4	28,17 ± 0,59

In Figure 1, it can be seen that the antibacterial activity of the ethanolic extract of lime peel increased with increasing concentration, where the diameter of the inhibition zone formed was larger at higher concentrations. The greater the concentration of the extract, the larger the diameter of the inhibition zone that is formed. The smallest concentration that was still able to inhibit bacterial growth was the minimum inhibitory concentration (MIC) at a concentration of 1 mg/mL with an inhibition zone diameter of 7.23 ± 0.15 mm in *Propionibacterium acnes* and 6.73 ± 0.06 mm in *Staphylococcus epidermidis*. The inhibition zone thus formed is classified in the weak category. Where if the inhibition zone is formed, 0-5 mm is included in the weak category, 5-10 mm is included in the strong category, and above 20 mm is included in the very strong category [32], [33].

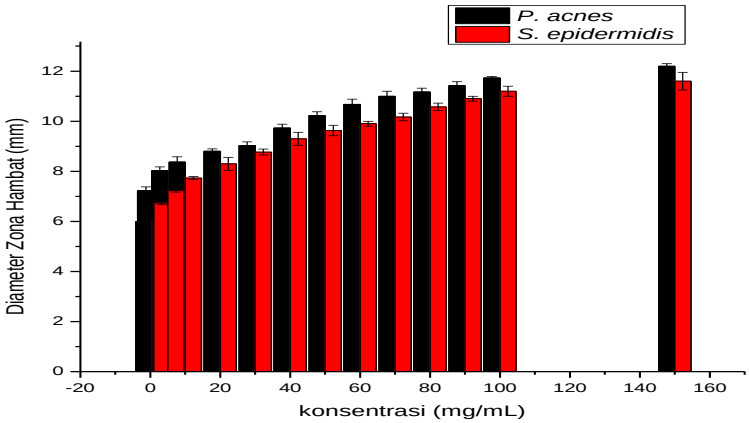

Fig 1. Effect of concentration of ethanolic extract of lime peel on the inhibition of *Propionibacterium acnes* and *Staphylococcus epidermidis*

Testing the Minimum Bactericidal Concentration (MBC) of lime peel ethanol extract against *Propionibacterium acnes* and *Staphylococcus epidermidis*

Testing the minimum bactericidal concentration by the streaking method from the inhibition zone formed and then planted on Plate Count Agar media to see the number of colonies growing at each concentration was calculated using a colony counter. The percentage reduction value of the difference between the treatment and the negative control is compared again with the number of colonies in the negative control. If it shows a value of 98-99.99%, then it is declared as the minimum killing concentration (MBC) [23], [34].In table 4, it can be seen that the percentage reduction value is 98.24% in *Propionibacterium acnes* with a concentration of 40 mg/mL, while in *Staphylococcus epidermidis* the percentage reduction value is 98.80% at a concentration of 50 mg/mL. This happens because of secondary metabolites from the ethanolic extract of lime peel, which can damage the bacterial cell wall so that they can enter the bacterial cell and interfere with the metabolism of the bacterial cell so that lysis and death occur [35], [36].

Table 4. Calculation of Minimum Bactericidal Concentration Value of Lime Peel Ethanol Extract Against *Propionibacterium acnes* and *Staphylococcus epidermidis*

Concentration (mg/mL)	*Propionibacterium acnes*				*Staphylococcus epidermidis*			
	Count	different	% Reduksi	Log Reduksi	Count	different	% Reduksi	Log Reduksi
Control -	2391	0	0,00	0,00	2495	0	0,00	0,00
0,5	2233	158	6,61	0,82	2244	251	10,06	1,00
1	1952	439	18,36	1,26	2135	360	14,43	1,16
5	1639	752	31,45	1,50	1673	822	32,95	1,52
10	784	1607	67,21	1,83	904	1591	63,77	1,80
20	565	1826	76,37	1,88	686	1809	72,51	1,86
30	432	1959	81,93	1,91	443	2052	82,24	1,92
40	42	2349	98,24	1,99	66	2429	97,35	1,99
50	21	2370	99,12	2,00	30	2465	98,80	1,99
60	17	2374	99,29	2,00	20	2475	99,20	2,00
70	12	2370	99,12	2,00	19	2476	99,24	2,00
80	10	2381	99,58	2,00	13	2482	99,48	2,00
90	7	2384	99,71	2,00	12	2483	99,52	2,00
100	6	2385	99,75	2,00	9	2486	99,64	2,00
150	1	2390	99,96	2,00	5	2490	99,80	2,00
Control +	0	2391	100,00	2,00	0	2495	100,00	2,00

The occurrence of this minimum killing concentration is due to the mechanism that occurs against the test bacteria from secondary metabolites contained in the ethanol extract of lime peel [37]. The mechanism of secondary metabolites of alkaloids is to damage cells and enter cells so that they interfere with metabolism that occurs in cells [38]. Another mechanism of sap-onin processing is to form foam and reduce the surface tension of bacterial cells. So, other things also cause the cells of bacteria to break, which kills the bacteria [39]–[41].

IV. CONCLUSION

Ethanol extract of lime peel showed antibacterial activity by inhibiting and killing the bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis*. Recommendation for future research may to make formulation of acnes preparation like gel or nanogel to treat acnes skin infection.

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none

Conflict of Interest

All author declared that there was no conflict of interest.

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