

Efficacy Test of Lemon Juice (*Citrus Limon*) on Acne to Inhibit the Growth of *Propionibacterium Acnes* 2025

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ABSTRACT

Acne (Acne Vulgaris) is a common skin disease that can cause scars (acne scar). In Indonesia, cases have increased from 60% in 2006 to 90% in 2009. Propionibacterium acnes plays a key role in acne onset and inflammation. Lemon water (Citrus limon) contains limonene and quercetin, which have anti-inflammatory and antibacterial effects against P. acnes. This study aimed to evaluate the effectiveness of lemon water extract added to face toner to inhibit P. acnes growth. Extraction was done via maceration with ethanol and methanol for 120 hours. Extract concentrations tested were 20%, 40%, 60%, 80%, and 100%. Bacterial inhibition was tested using paper disk diffusion on nutrient agar, observed every 12 hours.

A one-way ANOVA showed no significant difference in inhibition zone diameter across concentrations ($p > 0.05$). Methanol extraction produced the highest quercetin content, but face toner with ethanol-extracted lemon water showed better antibacterial activity. Even 1% lemon water extract exhibited bacterial activity; however, P. acnes resisted faster at this lower concentration than at higher ones. The results indicate that lemon water extract, especially when prepared with ethanol, has potential as an ingredient in face toner for controlling P. acnes, although concentration influences bacterial resistance.

Keywords: Lemon Water; Acne; *Propionibacterium acnes* Bacteria

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INTRODUCTION

The fruit of the Rutaceae family (the orange-citrus tribe) is lemon, which is sometimes referred to as citrus lemon (Hazarika, 2025; Miah, 2025). As Indonesia's restaurant, hotel, and culinary industries continue to grow, the country's demand for lemons is also increasing (Hanif et al., 2025; Kesumaputra, 2025). One of the main reasons for the increasing demand for lemons in the country is the healthy lifestyle adopted by many Indonesians (Hanif et al., 2025). In 2020, Indonesia reportedly imported about 40,000 tons of lemons, according to data from the Central Statistics Agency (Priyandita, van der Kley, & Herscovitch, 2022). Companies that use lemons as bread products face challenges because the part of the lemon that is used is the juice (Akanda & Mehjabin, 2025; Aswal, Sati, & Chauhan, 2025). [1] Lemon peel contains a concentration of flavonoids of about 1%–2% [2] and an active component with antibacterial qualities that successfully prevent the growth of *Propionibacterium acnes*. Because *P. acne* produces lipase, which breaks down free fatty acids from skin lipids, it plays an important role in the pathogenesis of acne vulgaris (acne) (AlEdani & Ashour, 2025; Guleria et al., 2025).

The impact of bacteria, hyperkeratosis on the hair infundibulum, and an active sebaceous gland are some of the causes of acne. *P. acnes* is thought to be prevented by using lemon water, a natural ingredient, as a facial toner (AlEdani & Ashour, 2025). To maintain and improve the health of facial skin, especially acne, facial hygiene and facial pH must be considered (Kircik et al., 2025). It is mild, antiseptic, and at the same time can help prevent pores, while facial toner is a skincare product (Panda & Dash, 2024). Use methanol and ethanol to remove lemons from the atmosphere. With a lower degree of turbidity in the tube compared to the germ control tube, the minimum inhibition concentration by lemon water extract with a concentration of

20% is the smallest concentration (Bridget, Ijebor, & Amina, 2025; Taha, Edrees, Ghaly, ElSaid, & Askoura, 2025). The active component of the facial toner application is lemon water extract (Diputra & Nurfatimah, 2025).

To obtain lemon peel extract (*Citrus limon*) with high quercetin content and facial toner with the best concentration of lemon extract as antibacterial and anti-inflammatory to inhibit the growth of *P. acnes* bacteria, this study aims to determine the optimal time and type of solvent.

Previous research has explored the anti-acne potential of *Citrus limon*, but with varying focuses and methodologies (Ramsis, Selim, Elseedy, & Fayed, 2024). Investigated the inhibitory effect of fresh lemon juice against *P. acnes*, reporting inhibition zones and establishing a foundational antibacterial activity (Hin, Mahmood, Amin, Salim, & Yusof, 2025). However, their study did not utilize a standardized extract from the peel, which is known to harbor a higher density of flavonoids (Arshad et al., 2025; Phetkul et al., 2025). Siregar et al. (2021) advanced this by incorporating lemon peel extract into a facial toner, demonstrating its activity against *P. acnes*. Their work confirmed the practical applicability of the extract in a cosmetic formulation but did not systematically optimize the extraction parameters (Gunawardana & Dias, 2025). Fina et al. (2023) specifically focused on lemon peel extract, confirming its efficacy and contributing to the understanding of the peel's superior potential. Meanwhile, while studying bay leaves, highlighted the critical influence of maceration time on extract yield and flavonoid content, a variable often overlooked in anti-acne studies concerning lemon peel (Outten, 2025).

Therefore, this study aims to fill these gaps with the specific aim of determining the optimal maceration time (24, 48, 72, and 120 hours) to obtain the highest quercetin content of lemons, determine the best solvent (96% ethanol vs methanol) to extract bioactive compounds from lemon peel with optimal antibacterial activity, and test the antibacterial efficacy of the optimized lemon peel extract. which has been incorporated into the formulation of facial toner, against the growth inhibition of *Propionibacterium acnes*. The benefits of this research are twofold. In practical terms, this research provides a scientific basis for developing a more effective natural anti-acne toner through the optimization of the extraction process. Academically, this study contributes new data on the relationship between maceration parameters, phytochemical content, and biological activity in *Citrus lemon* skins, while offering a more detailed methodology for future research of natural ingredients.

METHOD

This research used *Propionibacterium acnes* to measure the efficacy of lemon juice extract and qualitatively evaluate secondary metabolites. The filtrate was extracted from lemon juice using maceration with 96% ethanol as the solvent. The lemons were washed, cut in half, and manually squeezed to collect about 2 liters of juice.

The extraction process involved several steps. First, lemon juice was collected by squeezing and divided into two containers. It was then dried for five hours. Next, the juice was macerated in 96% ethanol and subsequently filtered using a sterile filter. The filtrate was concentrated using a rotary evaporator. Finally, a water flow technique was applied for four hours.

The maceration process lasted for five days (120 hours) during the study. After maceration, the lemon extract was used for testing against *Propionibacterium acnes*.

RESULTS AND DISCUSSION

The antibacterial effect on *Propionibacterium Acnes* bacteria can be ascertained from the results of the lemon water extraction procedure (citrus limon). 20%, 40%, 60%, 80%, and 100% extraction results, as well as (+) control with chloramphenicol and control (-) with DMSO, are disc paper used in petri dish. The next graph shows the clear zone that surrounds the paper disc in the petri dish in case of an antimicrobial effect.

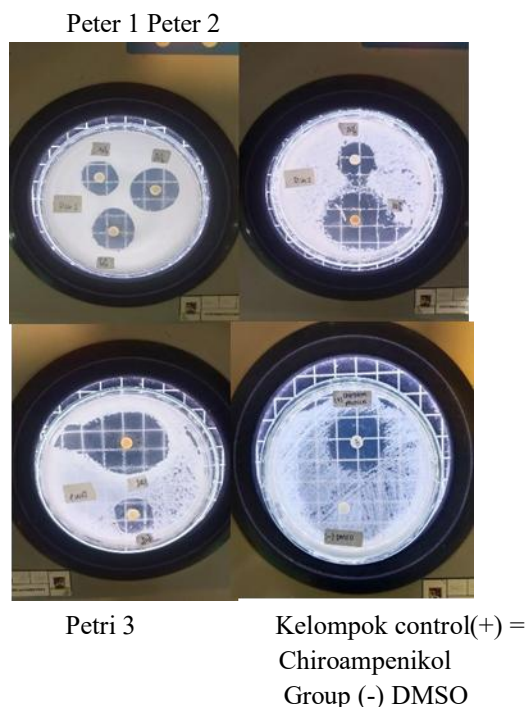


Figure 1. Diameter of Lemon Water Extract (Citrus Limon) Inhibition Zone Against Propionibacterium Acnes Bacteria

Source: Documentation of primary research results, 2025

Lemon water (Citrus limon) extracts of 20%, 40%, 60%, 80%, and 100% were found to be effective against *Propionibacterium Acnes* (Petri dish image 1&2). *Propionibacterium Acnes* bacteria are more likely to grow in the clear zone seen in 100% lemon water extract (Petri dish image 3). In the positive control of chloramphenicol against the bacteria *Propionibacterium Acnes*, there is a clear zone (Figure 4). Regarding the bacteria *Propionibacterium Acnes*, the clear zone is absent on the negative control of DMSO (Figure 4).

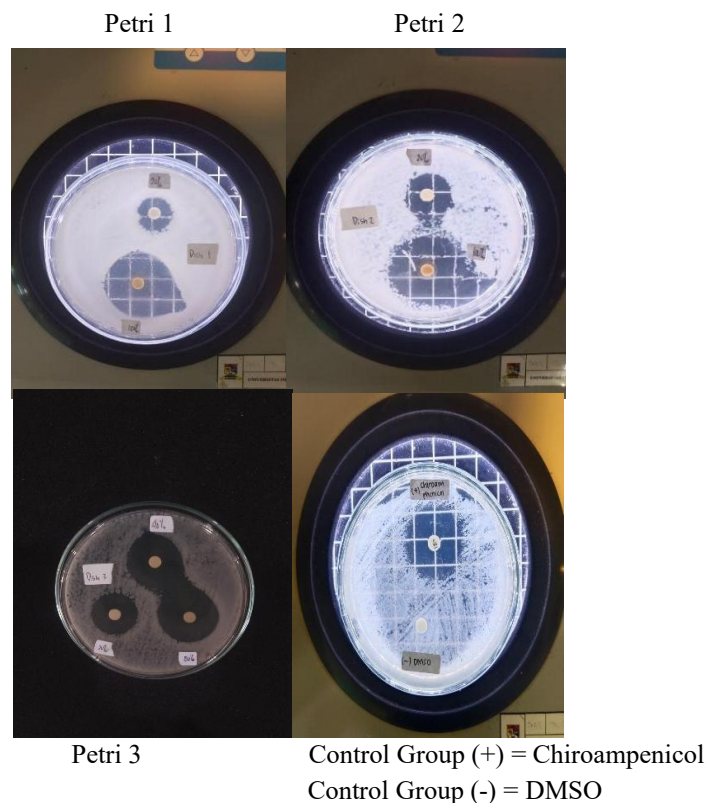


Figure 2. Diameter of Lemon Water Extract Inhibition Zone (Citrus Lemon) Against Propionibacterium Acnes Bacteria.

Source: Documentation of primary research results, 2025

Compared to Propionibacterium Acnes bacteria, Zona found that the inhibition zones on lemon water extract were 20%, 40%, 60%, 80%, and 100% (Figures 1, 2, and 3). Compared to the bacteria Propionibacterium Acnes (petri images 1&2), the clear zone, which is made entirely of lemon water extract, is more challenging. Clear zone shown in the successful eradication of Propionibacterium Acnes bacteria by Chloramphenicol (Petri Figure 4). Regarding the bacteria Propionibacterium Acnes, the clear zone is not visible on the negative control of DMSO (Petri Figure 4).

Results of Measurement of Inhibition Zone of Propionibacterium Acnes Bacteria

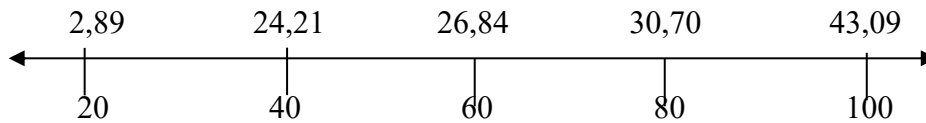
The measurement uses a caliper on the diameter of the straight clear zone that surrounds the filter paper in one milliliter to obtain the average shown in the table below.

Table 1. Diameter of the inhibition zone of *Propionibacterium Acnes bacteria*.
Diameter of Inhibition Zone of *Propionibacterium Acnes Bacteria*

Concentration	Jamming Zone Diameter (mm)			Average
	Dish I	Dish II	Dish III	
20%	17,94	21,09	23,65	2,89
40%	22,35	26,97	23,30	24,21
60%	27,05	29,08	24,39	26,84
80%	29,31	31,80	30,98	30,70
100%	39,44	41,73	48,1	43,09
Chloramphenicol	28,61	0	0	0
DMSO(-0	0	0	0	0

Source: Data processed from primary research results, 2025

The clear zone diameter of *Propionibacterium Acnes* bacteria with the lowest lemon water extract is 20%, or an average of 2.89 mm, according to the table above, while the clear zone diameter of *Propionibacterium Acnes* bacteria with the highest lemon water extract is 100%, or an average of 43.09 mm. Findings of the average zone of inhibition of *Propionibacterium Acnes* bacteria diagram:



Furthermore, Shapiro-Wilk is used in data distribution research.

Table 2. Test Distribution Data of Clear Zone Diameter of *Propionibacterium Acnes*

			Concentration	Testing Normality
Diameter Zona Bening: <i>Propionibacterium Acnes</i>			20%	0,760
			40%	0,374
			60%	0,852
			80%	0,586
			100%	0,498
			Kontrol (+)	1,000
			Kontrol (-)	1,000

Source: Data processed from primary research results, 2025

Propionibacterium Acnes bacteria are considered normal if their diameters are 20%, 40%, 60%, 80%, and 100% $p > 0.05$, according to the results of the normality test in the table above (Shapiro-Wilk test). For additional analysis, the Kruskal-Wallis and Maan Whitney tests were used.

Table 3. Kruskal-Wallis Test Clear Zone Diameter *Propionibacterium Acnes*

		Uji Kruskal-Wallis
Diameter Zona Bening: <i>Propionibacterium Acnes</i>		0,0128

Source: Data processed from primary research results, 2025

The table above shows the data that may be obtained from the results of the Kruskal-Wallis Test test, which is 0.0128. In other words, the difference between the growth of *Propionibacterium Acnes* and the treatment group was shown by $p = 0.0128$ ($p < 0.05$).

Table 4. Mann-Whitney Test Whole Concentration of Lemon Water Extract Against Propionibacterium Acnes Bacteria

Concentration	By Maan-Whitney
20%	0,500
40%	0,500
60%	1,000
80%	0,500
100%	0,500
Kontrol (+)	-
Kontrol (-)	-

Source: Data processed from primary research results, 2025

All concentrations of 20%, 40%, 60%, 80%, and 100% lemon water extract had significance levels greater than 0.05, according to the results of the Mann-Whitney test shown in the table above. This shows that there was no significant difference between each group of lemon water extract concentrations and Propionibacterium Acnes bacteria when compared to the corresponding group.

Discussion

The study is being conducted as an in vitro laboratory experiment. The 96% ethanol maceration technique was used to assess the antibacterial activity of citrus lemon, also known as lemon water extract, against the bacteria Propionibacterium Acnes.

The extraction was carried out using the maceration method which involves splitting two liters of lemon into two tubes (containers) and using 96% ethanol for three hours. This process is repeated several times. After that, sterile gauze and a filter are used with the extracts obtained. Evaporation is then carried out at 70°C for two hours at 48 rpm using a rotary evaporator. Once the combined results have been made into a single tube (container), the maceration procedure is completed in two steps or in a similar way. The thick extract is made by raising the temperature to 80°C in a water bath for four hours.

The antibacterial activity against Propionibacterium Acnes bacteria was then evaluated using Mueller-Hinton Agar (MHA) bacterial cultures and extracted data. Filter the catenswaab is used for 1x24 hours in a petri dish that has previously been evenly exposed to Propionibacterium Acnes bacteria. After adding the antimicrobial agents DMSO and chloramphenicol, the filter paper is put into an incubator set at 37°C for use within 1x24 hours. Calipers are a tool that can be used to reduce the buffer zones around filter paper in MHA media, according to antibacterial activity data.

The study's findings revealed Propionibacterium Acnes bacteria in the inhibit brought on by lemon (Citrus lemon water extract. According to the findings of certain studies, citric acid, flavonoid compounds, alkaloids, tannins, and essential oils, including limonene found in lemons, have antimicrobial activity against the bacteria Proponibacterium Acnes (Hartini S, 2020). The diameter of the zone can reach about 10 mm, with groups of 5 mm, for each concentration of 20%, 40%, 60%, 80%, and 100% mg/L. Lemon (Citrus limon) scented face wash is a mild and gentle-based facial cleanser that prevents dull skin, according to research (Eliana Kesuma dewi et al., 2020). Kursia et al. (2021). Glycerin is one of the ingredients that

has antibacterial properties. (Riha 2021) The lifestyle of young women tends to appear with charm and perfection, so they should have cosmetics. The findings of the study, according to Riyanisusan (2020), are 25% (993 mm), 50% (12.46 mm), 75% (14.83), and 100% (16.4 mm) of orange lemons. The highest resistance is found at 100% concentration.

In this study, DMSO functioned as a negative control and chloramphenicol, a positive control that functioned as an antibacterial agent against *Propionibacterium Acnes*. The findings showed that gram-positive bacteria were found at the average inhibition zone derived from the chloramphenicol positive control, which was about 28.61 mm, after one repetition. However, the findings of the study show that lemon extract (*Citrus limon*) has an antibacterial action against the gram-negative bacteria *Propionibacterium Acnes*, which is about 0 mm.

CONCLUSION

Research has shown that *Citrus limon* (lemon water extract) possesses antibacterial properties against *Propionibacterium acnes*, with higher extract concentrations (up to 100%) producing greater inhibition zones compared to lower concentrations (20%). This suggests that lemon extract can effectively inhibit *P. acnes* growth, making it a promising antibacterial agent for acne treatment. Future research should focus on evaluating the stability and safety of lemon-based topical preparations to ensure their efficacy and suitability for clinical use.

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