

Antibacterial Activity Testing *Propionibacterium acnes* Combination of 70% Ethanol Extract of Starfruit Leaves (*Averrhoa bilimbi* L.) and Jarak Pagar Leaves (*Jatropha curcas* L.)

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Abstract

Acne is an inflammation accompanied by blockage of oil gland ducts that gather and cause blackheads. The onset of acne can be caused by the bacterium *Propionibacterium acnes*. Flavonoid compounds and tannins contained in star fruit leaves and jatropha leaves can be used as antibacterials. Both extracts from the plant can be combined to achieve antibacterial effects that can be used as an alternative to acne treatment. The purpose of this study was to determine the antibacterial activity of star fruit leaf extract and jatropha leaf and determine the optimal concentration resulting from the antibacterial activity of the extract combination. The method used is experimental and antibacterial tests in vitro. The results of antibacterial tests on the combination of jatropha leaf extract and star fruit leaf extract against *P. acnes* had the most optimal average inhibitory zone of 9,642 mm with a ratio of 1:2 at a concentration of 10%.

Keywords: *Averrhoa bilimbi* L.; combination extract; *Jatropha curcas* L.; *Propionibacterium acnes*.

INTRODUCTION

Acne is inflammation accompanied by blockage of the pilosebaceous duct which causes sebum not to come out and collect which then swells and blackheads appear. The onset of acne is caused by stressful conditions, smoking, the influence of food intake with high fat and calorie content, frequent consumption of fast food, lack of attention to skin hygiene, increased physical activity, psychological influences, and *Propionibacterium acnes* bacterial infections (Febriani et al., 2022).

P. acnes includes rod-shaped gram-positive bacteria with normal skin flora that plays a role in acne formation (Rahmi et al., 2015). The mechanism of acne formation by *P. acnes* is that bacteria can damage the stratum corneum and stratum germinativum by secreting chemicals that can break down the pore wall. In addition, fatty acids and skin oils can be blocked and then hardened, causing inflammation that if touched will expand (Afifi et al., 2018). Giving antibiotics such as clindamycin can be done to reduce the growth of *P. acnes* (Asdika et al., 2020). Clindamycin as an antibiotic works by preventing peptide binding so that it can inhibit bacterial protein synthesis. Clindamycin is a broad-spectrum antibiotic that has bacteriostatic or bactericidal effects depending on the concentration of the drug, the degree of infection and the type of organism

(Novaryatiin, 2016; Jayanti, 2021). However, excessive use of antibiotics can lead to increased bacterial resistance to antibiotics (Rahmi et al., 2015).

The development of natural ingredients as an alternative to acne treatment can be done to prevent side effects of synthetic drugs, especially preventing the possibility of bacterial resistance. Examples of natural ingredients such as star fruit leaves and castor leaves have antibacterial effects that are beneficial for acne expansion (Afifi et al., 2018; Juliansyah and Paotonan, 2017).

Based on research by Afifi et al. (2018), star fruit leaf extract can be used as an antibacterial against *P. acnes* at a concentration of 150 ppm; 175 ppm; and 200 ppm with an inhibitory zone diameter of 9.88 mm; 10.8 mm; and 11.76 mm expanded (Afifi et al., 2018). In addition, based on Juliansyah and Paotonan, (2017), it was stated that jatropha leaf extract also had antibacterial activity against *P. acnes* at a concentration of 15% with an inhibitory zone of 10.24 mm (Juliansyah and Paotonan, 2017)

Inhibition of bacterial growth in both plants is produced by compounds contained in each plant. Both plants contain flavonoid compounds and tannins that can have antibacterial potential. Flavonoids as antibacterial work by forming complex compounds with extracellular and dissolved proteins so that they can damage bacterial

cell membranes followed by the release of intracellular compounds. While tannins work by constricting the cell wall so that it interferes with cell permeability and causes cell wall damage (Amalia et.al., 2017).

MATERIALS AND METHODS

Tools and Materials

The equipment used is a petri dish (OneMed), test tube, cotton, falcon, micropipette (Socorex), micropipette tips, ose needle, spider, tweezers, disc, bunsen, vortex (Gemmy VM-300 Vortex Mixer), LAF (Panasonic), autoclave (Hirayama Hve-50), incubator and caliper (Taffware).

The ingredients used in this study were star fruit leaf extract, jatropa leaf extract, sterile aquadest, *Nutrient Agar* (NA), sterile NaCl, clindamycin phosphate, *Mc. Farland* standard solution, and *Propionibacterium acnes* bacteria (ATCC 11827).

Procedures

Simplisia Extraction

The extraction simplisia of star fruit leaf and jatropa leaves was carried out using the maceration method and the resulting filtrate was then concentrated. The maceration process is carried out by soaking simplistic using 70% ethanol solvent in a ratio of 1:10. Soaking is carried out for 3 days and stirred every 1×24 hours for 5 minutes. After that, filtration is carried out to separate the pulp and filtrate. The resulting filtrate is then concentrated using a rotary evaporator at a temperature of 50° C at a speed of 60 rpm (Hasim et al., 2019). Calculate the resulting yield.

$$\% \text{ Yield} = \frac{\text{Dry Extract}}{\text{Simplisia Weight}} \times 100\%$$

Antibacterial Activity Test of Combination of Star Fruit Leaf Extract and Jatropa Leaf

Testing the antibacterial activity of the extract combination was carried out 5x repeats using several concentration comparisons, namely, 1:1; 1:2, and 2:1. Before the testing stage, it is necessary to carry out bacterial rejuvenation. Rejuvenated bacteria are made of a bacterial suspension which is then implanted over the surface of the NA media by the streak method. Then the disc is immersed and placed on the surface of the substrate already planted with bacteria. After that, it is incubated at 36-37°C for 24 hours (Puspita, 2021). The resulting inhibitory zone is then calculated using the following formula (Hasanah & Novian, 2020):

$$\text{Obstacles Zone} = \frac{(V_D - H_D) + (H_D - D_D)}{2}$$

VD: Vertical Diameter

HD: Horizontal Diameter

DD: Disc Diameter

RESULTS AND DISCUSSION

Simplisia Extraction

Simplisia extraction was carried out using the maceration method according to the provisions for making extracts in the Indonesian Herbal Pharmacopoeia Edition II using 70% ethanol solvent with a ratio of 1:10 (Kemenkes RI, 2017).

Table 1. Extract Yield.

Parameter	Starfruit Leaf Extract	Jatropa Leaf Extract
Sample Weight	1.180 grams	1.380 grams
Extract Weight	176,53 grams	244,66 grams
% Rendemen	14,96%	17,729%

Extraction of star fruit leaves and jatropa leaves yielded yield values of 14.96% and 17.729%. Extraction was carried out for 3 days using 70% ethanol solvent. 70% ethanol solvent is used because it can attract more active compounds than other solvents. In addition, ethanol is polar which can dissolve polar compounds such as flavonoids and tannins (Hasanah & Novian, 2020). The more polar the solvent used, the more easily dissolved polar compounds such as flavonoids and tannins. So it can be concluded that the extraction results using 70% ethanol will produce a higher yield value compared to 96% ethanol (Nahor et al., 2022).

The yield produced from star fruit leaf extract is different from previous studies conducted by Aryantini et al. (2017) with a yield value of 16.83%. The difference is due to the difference in extraction time. Longer extraction time will result in higher extract yield because the chance of contact between solvent and material becomes greater (Andriani, et al., 2019). While the yield value produced in jatropa leaf extract is also different from previous studies by Saraswati and Karina, (2016) with a yield value of 11.28% using 96% ethanol solvent. The difference in the level of polarity of the solvent used can cause the yield value produced to be different. The more polar the solvent used, the more easily dissolved polar compounds such as flavonoids and tannins (Nahor et al., 2022).

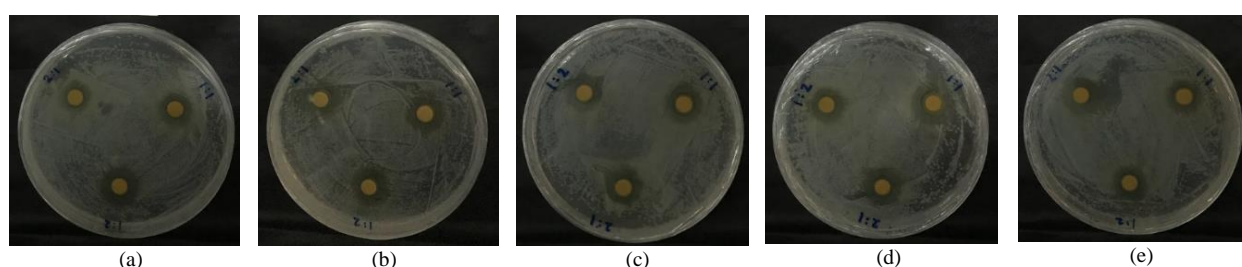
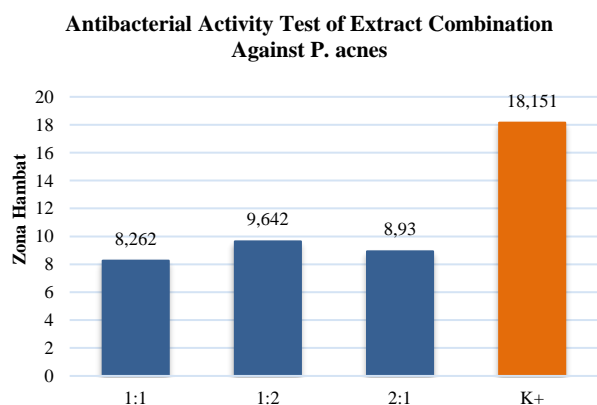
Antibacterial Activity Test of Combination of Star Fruit Leaf Extract and Jatropa Leaf

Based on antibacterial activity testing, the combination of extracts in Table 2 shows that the combination of star fruit leaf extract and jatropa leaf from each concentration comparison has antibacterial activity against *P. acnes*. The inhibitory zone of the three comparisons showed the results of the medium inhibitory zone category. This is based on the diameter category of the inhibitory zone ≤5 mm is included in the weak category, 5-10 mm diameter is medium, 11-20 mm is strong, and ≥21 mm diameter is solid (Winastri et al., 2020).

Table 2. Results of Measurement of the Inhibition Zone of the Combination of Star Fruit Leaf Extract Wuluh and Jatropa Leaf Against *P. acnes*.

Replication	Obstacles Zone (mm)				
	K-	K+	Concentration Comparison		
			1 : 1	1 : 2	2 : 1
1	0	18,625	9,56	7,975	8,75
2	0	20,425	7,77	9,505	8,93
3	0	17,525	9,895	9,915	10,075
4	0	17,25	7,345	9,255	9,01
5	0	16,93	6,74	10,66	7,91
Average	0	18,151	8,262	9,462	8,93

Note: K+ : Positive Control, K- : Negative Control

**Figure 1.** Antibacterial Activity Test of Combination of Star Fruit Leaf Extract and Jatropa Leaf against *P. acnes*.**Figure 2.** Inhibitory Zone of Combination Extracts.

Based on testing the antibacterial activity, it can be seen in Table 2 that the ratio of 1:2 produces the largest inhibitory zone against *P. acnes*, which is 9.642 mm when compared to the ratio of 1:1 (8.262 mm) and 2:1 (8.93 mm). In the negative control, DMSO 67% did not produce an inhibitory zone which showed the solvent used did not affect the growth inhibition of *P. acnes*. A positive control treatment of 1% clindamycin phosphate resulted in an average inhibitory zone of 18.151 mm (Figure 2).

The inhibitory zone produced in the combination extract test is influenced by the presence of flavonoid compounds and tannins that work as antibacterials. Flavonoid compounds have antibacterial abilities by damaging the bacterial cytoplasmic membrane which causes bacterial cells to lose shape and lysis occurs. In addition, tannin compounds can inhibit bacterial growth

through their ability to react with bacterial cell membrane proteins and form complex bonds that can turn off bacterial adhesin (Winastri et al., 2020).

CONCLUSIONS

The combination of star fruit leaf extract and castor leaf can be combined and has antibacterial activity against *Propionibacterium acnes*. The combination of extracts at a ratio of 1:2 produced the highest inhibitory zone compared to other concentration comparisons.

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Competing Interests: There are no competing interests

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