

Development of Ethanol Extract Combination of Red Shoot Leaves (*Syzygium myrtifolium*) and Bilimbi Leaves (*Averrhoa bilimbi* L.) as an Anti-*Propionibacterium acnes* Agent in Acne Spot Formulation

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Abstract

Propionibacterium acnes is a Gram-positive bacterium and the primary causative agent of acne, which is commonly treated with the antibiotic clindamycin. However, inappropriate use of clindamycin can lead to bacterial resistance. This study explores a natural therapeutic alternative using a combined ethanol extract of red shoot leaves (*Syzygium myrtifolium*) and bilimbi leaves (*Averrhoa bilimbi* L.), formulated into an acne spot gel preparation. The objectives of this research were to identify secondary metabolites and to determine the antibacterial effectiveness of the combined extract against *P. acnes*. Qualitative phytochemical screening, FTIR analysis, and antibacterial activity assays using the disk diffusion method at concentrations of 2.5–10% were conducted to evaluate both the extract and the gel preparation. The results confirmed the presence of flavonoids with O–H (3000–3500 cm⁻¹), C–O, and C=O functional groups, which are associated with antibacterial activity. The antibacterial activity of the extract showed inhibition zones of 6.5–12 mm, proportionally correlated with concentration. Meanwhile, the acne spot gel containing 17.5% of the combined extract exhibited an inhibition zone of 18.5 mm, greater than that of the commercial product (Wardah acne spot, 15.2 mm), indicating strong inhibitory activity (10–20 mm). Quality evaluation of the gel preparation demonstrated that pH, viscosity, and spreadability complied with the Indonesian National Standard (SNI 16-4399-1996) requirements.

Keywords: *Acne Spots*; Antibacterial; Extracts; Acne; *Propionibacterium acnes*.

INTRODUCTION

Cosmetics are materials or preparations intended to be applied to the external parts of the human body, such as the epidermis, hair, nails, lips, and external genital organs, or to the teeth and the mucous membranes of the oral cavity, primarily for cleansing, perfuming, altering appearance, and/or correcting body odor, as well as protecting or maintaining the body in good condition (Yusuf Zainudin & Chabib, 2023). Acne is a common skin disorder that develops due to blockage of the pilosebaceous units, characterized by the presence of comedones, papules, pustules, and scars on the face and other skin areas. One of the main causes of inflammation in acne is the bacterium *Propionibacterium acnes*. In general, acne treatment involves the use of antibiotics as antibacterial agents; however, prolonged antibiotic use may lead to the risk of hypersensitivity reactions in some individuals (Kamra & Diwan, 2017).

Acne spot treatment in the form of a gel is an alternative skincare product formulated to address inflamed acne and to reduce the potential emergence of new lesions in the same skin area. Gel formulations offer

several advantages, including ease of use, simple removal upon application and cleansing, as well as providing a cooling sensation on the skin (Ansong et al., 2023).

Red shoot leaves (*Syzygium myrtifolium*), belonging to the genus *Syzygium* and the family *Myrtaceae*, contain high levels of bioactive compounds with antioxidant and antibacterial properties, which can help reduce skin inflammation and address skin disorders such as psoriasis, eczema, and acne (Suryati et al., 2025). Red shoot leaves contain secondary metabolites such as alkaloids, triterpenoids, flavonoids, and saponins, which may act as antibacterial agents against *Propionibacterium acnes* (Imrawati Imrawati et al., 2026).

Bilimbi leaves (*Averrhoa bilimbi* L.) also possess antibacterial properties. They contain sulfur, tannins, peroxidase, formic acid, calcium oxalate, potassium citrate, flavonoids, and saponins (Mustiqawati et al., 2022). The extract of bilimbi leaves (*Averrhoa bilimbi* L.) exhibits antibacterial activity against *Propionibacterium acnes* (Putri et al., 2024). Previous

studies have shown that the combination of 70% ethanol extract of bilimbi leaves (*Averrhoa bilimbi* L.) with other plants produced an antibacterial inhibition zone of 15.73 mm against *Staphylococcus epidermidis*, which was greater than that of the positive control, Clindamycin HCl (Chaniago et al., 2024). Based on the antibacterial potential of both plants and the absence of studies on the combination of red shoot leaf and bilimbi leaf extracts, this research aims to develop an acne spot formulation derived from the combination of these extracts as a safe and effective alternative treatment for acne.

MATERIALS AND METHODS

Materials

The equipment used included an analytical balance, oven, rotary evaporator, Petri dishes, paper discs, autoclave, laminar air flow (LAF) cabinet, pH meter, viscometer, and Fourier Transform Infrared (FTIR) spectrometer. The materials used comprised young red shoot leaves obtained from PT Multielok Cosmetic Cikande, bilimbi (*Averrhoa bilimbi*) leaves from Cilegon Tegal Wangi, absolute ethanol, concentrated HCl, magnesium powder, distilled water, a pure culture of *Propionibacterium acnes*, nutrient agar, 0.9% physiological NaCl solution, the antibiotic clindamycin, Wardah acne spot product (as a comparator), carbomer, butylene glycol, phenoxyethanol, and AMP-95.

Preparation Sample

The red shoot leaves and bilimbi leaves were cleaned, washed, and dried in an open room without direct sunlight for seven days, then ground into powder (Iwansyah et al., 2021). Extraction was carried out using the maceration method with absolute ethanol at a ratio of 1:10. The powdered leaves (50 g of each) were combined and macerated with 1000 mL of absolute ethanol for 24 hours with stirring every 6 hours. The remaceration process was performed twice until a clear filtrate was obtained. The filtrate was concentrated using a vacuum rotary evaporator and further evaporated on a water bath at a temperature of 40–50 °C.

Phytochemical Screening and Characterization

The flavonoid test was carried out by adding 2 mg of magnesium powder and 3 drops of concentrated HCl to the extract; a positive result was indicated by the formation of an orange-red color (Safrina et al., 2022). Extract characterization was performed using FTIR with the ATR method.

Antibacterial Activity Test of the Extract

The antibacterial test was conducted using the disk diffusion method with 6 mm paper disks on nutrient agar media inoculated with *P. acnes*. Sterile distilled water was used as the negative control, clindamycin as the

positive control, the Wardah acne spot product as a comparator, and the test extract at concentrations of 2.5%, 5%, 7.5%, and 10% prepared in 10% DMSO. Incubation was carried out at 37 °C for 24 hours, after which the inhibition zones were measured using a vernier caliper.

Acne Spot Formulation

The gel formulation was prepared by mixing carbomer (1 g), distilled water, butylene glycol (15 g), AMP-95 (3 g), phenoxyethanol (0.2 g), and the combined extract at the optimum concentrations determined from the antibacterial test, namely 10% (F1), 12.5% (F2), 15% (F3), and 17.5% (F4). The mixture was stirred until homogeneous.

Evaluation of the Acne Spot Preparation

The evaluation included an organoleptic test to observe aroma, color, and texture; a homogeneity test by observing the presence or absence of coarse particles; a spreadability test using a 200 g load (standard 5–7 cm); a pH test within the skin-acceptable range of 6–8; and a viscosity test using a viscometer at 20 rpm with a standard range of 2,000–50,000 cP.

Antibacterial Activity Test of the Acne Spot Preparation

The test was conducted in the same manner as the antibacterial assay of the extract, using gel formulations at concentrations of 10%, 12.5%, 15%, and 17.5% against *P. acnes* with the disk diffusion method and incubation at 37 °C for 24 hours.

RESULTS AND DISCUSSION

In this study, the samples used were red shoot leaves (*Syzygium myrtifolium*) and bilimbi leaves (*Averrhoa bilimbi* L.) that were sorted and washed with running water, then dried in an open area without direct exposure to sunlight for 7 days to prevent damage to the chemical components of the leaves due to high temperatures. The dried red shoot and bilimbi leaves (1:1) were extracted using the maceration method with 96% ethanol as the solvent at a ratio of 1:10 for 24 hours, and the maceration was repeated twice using fresh solvent. The solvent used was 96% ethanol because ethanol has the ability to extract compounds over a wide polarity range, from polar to nonpolar compounds, is less toxic than other organic solvents, is not easily contaminated by microorganisms, and is relatively inexpensive. After the simplicia were macerated, the extract was concentrated or evaporated using a rotary evaporator at a temperature of 50 °C until a viscous extract was obtained. The resulting thick extract amounted to 80 g, with an extraction yield of 80%. The extraction results showed that the high percentage yield of the combined ethanol extract of red

shoot leaves and bilimbi leaves met the requirements for a good plant extract yield which is greater than 10% (Kementrian

Results of Phytochemical Screening

Phytochemical screening in this study aimed to identify the secondary metabolite compounds present in the combined ethanol extract of red shoot leaves (*Syzygium myrtifolium*) and bilimbi leaves (*Averrhoa bilimbi* L.). The results of the phytochemical screening can be seen in Table 1.

Table 1. The results of the phytochemical screening of the combined ethanol extract of red shoot leaves (*Syzygium myrtifolium*) and bilimbi leaves (*Averrhoa bilimbi* L.).

Sample	Test	Reagents	Results	Note
The combined ethanol extract of red shoot leaves and bilimbi leaves.	Flavonoid	Mg powder and concentrated HCl.	+	A color change to orange-brown.

The phytochemical screening test showed that the combined ethanol extract of red shoot leaves (*Syzygium myrtifolium*) and bilimbi leaves (*Averrhoa bilimbi* L.) was positive for flavonoids. A positive reaction was indicated by a color change to orange-red or orange-brown after the addition of magnesium powder and HCl, which forms flavilium salts through interaction with the carbonyl groups of flavonoids. These results are consistent with a study which used 96% ethanol as the solvent (Supriatno et al., 2024).

FTIR Test Results

FTIR analysis was performed to identify the functional groups of compounds present in the combined ethanol extract of red shoot leaves (*Syzygium myrtifolium*) and bilimbi leaves (*Averrhoa bilimbi* L.) based on their respective spectra.

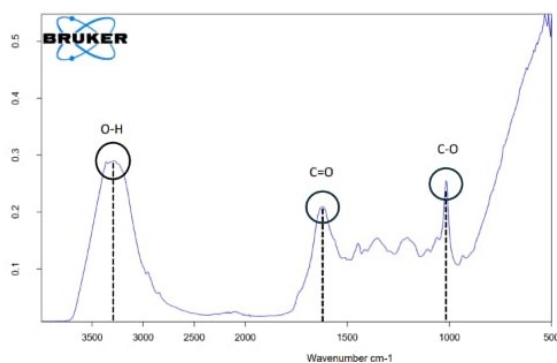


Figure 1. FTIR Spectrum of the Combined Extract of Red Shoot Leaves and Bilimbi Leaves.

The FTIR spectrum analysis of the ethanol extract combination of red shoot leaves (*Syzygium myrtifolium*) and bilimbi leaves (*Averrhoa bilimbi* L.) (Figure 1) showed a broad absorption band at 3000–3500 cm^{-1} , indicating the presence of O–H groups characteristic of flavonoids, supported by the presence of C–O and C=O functional groups (Suteja et al., 2025). The identification of these flavonoid compounds suggests the antibacterial potential of the combined leaf extract.

Antibacterial Activity Test Results

The antibacterial activity test was conducted using the disc diffusion method with various extract concentrations, a positive control (the antibiotic clindamycin), and a negative control (sterile distilled water). Clindamycin (a macrolide antibiotic) inhibits bacterial protein synthesis at the ribosomal level, particularly against *Propionibacterium acnes*. Sterile distilled water was selected as the negative control because it has no antibacterial activity, thereby ensuring that any inhibition zone observed originated from the sample rather than from technical factors.

The results of the antibacterial activity test of the ethanol extract combination of red shoot leaves and bilimbi leaves against *P. acnes* are presented in Figure 2.

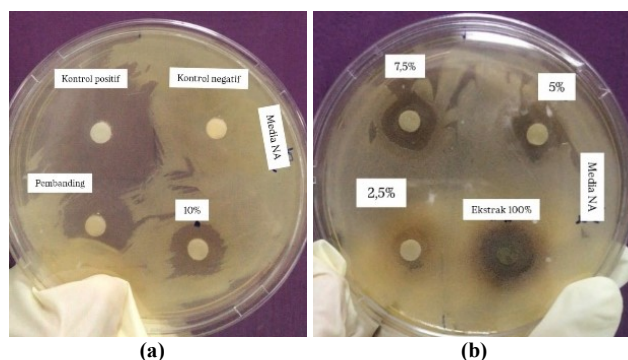


Figure 2. Results of the antibacterial activity test of the ethanol extract of red shoot leaves and bilimbi leaves against *Propionibacterium acnes*; Testing of the positive control, negative control, product comparator, and 10% concentration extract (a); Testing of extract samples at concentrations of 2.5%, 5%, 7.5%, and 100% (b)

Figure 2 shows that the ethanol extract combination of red shoot leaves and bilimbi leaves has potential as an antibacterial agent against *Propionibacterium acnes*, as indicated by the formation of a clear inhibition zone. The smallest inhibition zone was 6.5 mm at a concentration of 2.5%, and the largest was 12 mm at a concentration of 10%, demonstrating a proportional relationship between concentration and antibacterial activity. This is because higher concentrations contain greater amounts of active compounds, which more effectively inhibit bacterial growth.

This antibacterial activity is influenced by secondary metabolites, particularly flavonoids, which act by damaging the bacterial cell membrane through the formation of extracellular protein complexes and by inhibiting energy metabolism and oxygen utilization in

bacteria, thereby preventing bacterial proliferation (Zhang et al., 2025).

Table 2. Results of the antibacterial activity test of the ethanol extract of red shoot leaves and bilimbi leaves against *Propionibacterium acnes*.

	concentration (%)	Inhibition Zone (mm)
The combined ethanol extract of red shoot leaves and bilimbi leaves.	2.5	6.5
	5	8
	7,5	10
	10	12
	100	20
Positive control	<i>Clindamycin</i>	32
Negative control	<i>Aquadest</i>	-
comparator	Wardah <i>acne spot</i>	16.5

The antibacterial inhibition zones were classified into four categories: weak (<5 mm), moderate (5–10 mm), strong (10–20 mm), and very strong (>20 mm) (Sanam et al., 2022). The combined ethanol extract of red shoot leaves and bilimbi leaves against *Propionibacterium acnes* showed moderate activity at a concentration of 7.5% and strong activity at a concentration of 10% (Zakiah et al., 2025).

The 10% concentration, which exhibited the greatest antibacterial activity (Table 2), was determined as the optimum concentration for the acne spot gel formulation. The results of the antibacterial activity test of the acne

spot gel formulated with the combined extract against *P. acnes* are presented in Figure 3.

Figure 3 shows that the acne spot gel formulated with the combined ethanol extract of red shoot leaves and bilimbi leaves has potential antibacterial activity against *Propionibacterium acnes*, with an optimal clear inhibition zone observed at an extract concentration of 17.5%.

The use of an initial 10% concentration in the gel formulation was intended to anticipate the possible degradation of secondary metabolite compounds present in the extract when mixed with the chemical ingredients used in the acne spot formulation (Wahdi et al., 2026)

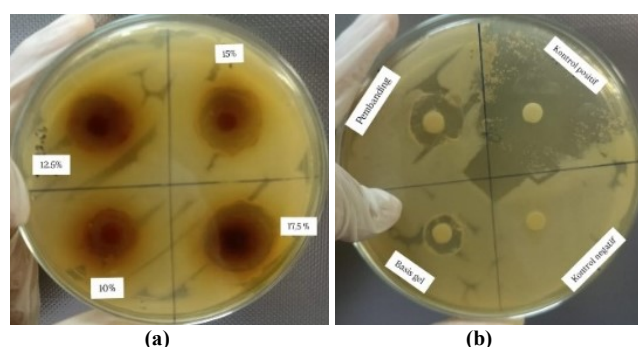


Figure 3. Results of the antibacterial activity test against *Propionibacterium acnes*: Testing on formulations F1, F2, F3, and F4 (a); Testing on the acne spot gel base, positive control, negative control, and product comparator (b)

Table 3. Results of the antibacterial activity test of the acne spot gel base + ethanol extract of red shoot leaves and bilimbi leaves against *Propionibacterium acnes*.

Sample	Formulation	Inhibition Zone (mm)
Acne spot gel base + combined ethanol extract of red shoot leaves and bilimbi leaves.	F1	10
	F2	14
	F3	15
	F4	18.5
Acne spot gel base	-	6
Positive Control	<i>Clindamycin</i>	29
Negative Control	<i>Aquadest</i>	-
Comparator	Wardah <i>acne spot</i>	15.2

Table 3 shows that the acne spot gel based on the combined extract remains effective, with inhibition zones ranging from 10 mm (10% concentration) to 18.5 mm (17.5% concentration) against *Propionibacterium acnes*. This activity is higher than that of the commercial product Wardah acne spot (15.2 mm), indicating that the secondary metabolite compounds in the extract remain stable even when formulated with other chemical ingredients.

Acne Spot Formulation

The acne spot gel formulation (Table 4) uses the ethanol extract combination of red shoot leaves and bilimbi leaves as the active ingredient at concentrations of 10–17.5%. The excipients include carbomer (thickening agent), butylene glycol (humectant), AMP-95 (emulsifier), phenoxyethanol (preservative), and distilled water (solvent). The results of the gel preparation evaluation are presented in Table 4.

Table 4. Formulation of an acne spot gel based on the ethanol extract combination of red shoot leaves and bilimbi leaves

Ingredients	Weight (g)			
	F1	F2	F3	F4
Ethanol extract combination of red shoot leaves and bilimbi leaves	10	12,5	15	17,5
Carbomer	1	1	1	1
Butylene glycol	15	15	15	15
AMP-95	3	3	3	3
Phenoxyethanol	0,2	0,2	0,2	0,2
Aquadest	70,8	68,3	65,8	63,3

The physical evaluation of anti-acne gel preparations is conducted to ensure quality, stability, and user comfort on the skin. This evaluation includes organoleptic properties, homogeneity, pH, viscosity, spreadability, and adhesion, which influence the effectiveness and safety of the formulation. Through these tests, it can be determined whether the formulated gel meets the

required standards for topical preparations (Ningrat, 2024).

Table 5 shows that the acne spot gel based on the combined extract meets the SNI 16-4399-1996, with a pH of 4.5–8.0 and a viscosity of 2000–50,000 cP. The spreadability test resulted in values of 5–5.3 cm, which comply with the standard range of 5–7 cm, ensuring optimal gel distribution when applied to the skin.

Table 5. Evaluation test of the acne spot gel formulation based on the ethanol extract combination of red shoot leaves and bilimbi leaves.

Sample	Organoleptic		Homogeneity	Spreadability(cm)	pH	Viscosity (cP)
	Colour	Smell				
Basis	transparent	Not pungent	No lumps	3,2	6,0	38920
F1	Brownish yellow	pungent	No lumps	5	6,2	44700
F2	Light brown	pungent	No lumps	5,1	6,3	46100
F3	Dark brown	Very pungent	No lumps	5,2	6,5	47200
F4	Blackish brown	Very pungent	No lumps	5,3	6,7	47400

CONCLUSIONS

The ethanol extract combination of red shoot leaves (*Syzygium myrtifolium*) and bilimbi leaves (*Averrhoa bilimbi* L.) contains secondary metabolites in the form of flavonoids that can be used as antibacterial agents. The ethanol extract combination of red shoot leaves (*Syzygium myrtifolium*) and bilimbi leaves (*Averrhoa bilimbi* L.) can be developed as an active antibacterial ingredient in acne spot formulations as an alternative to replace harmful chemical substances. Based on the research results, the formulation showed inhibition zones against *Propionibacterium acnes* at concentrations of 10% (10 mm), 12.5% (14 mm), 15% (15 mm), and 17.5% (18.5 mm).

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Authors' Contributions: Micha Mahardika & Isna Lailatusholihah designed the study. Micha Mahardika, Miftahul Jannah, and Isna Lailatusholihah carried out the laboratory work. Miftahul Jannah analyzed the data.

Micha Mahardika & Isna Lailatusholihah wrote the manuscript. All authors read and approved the final version of the manuscript

Competing Interests: The authors declare that there are no competing interests.

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