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Antibacterial Activity of Liquid Facial Soap Formulated with Ethanol Extract of Syzygium myrtifolium Leaves against Cutibacterium acnes

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Abstract

Acne is a dermatological condition marked by follicular occlusion and inflammatory pustules, commonly attributed to colonization by *Cutibacterium acnes*. In response to the limitations and side effects of conventional therapies, research has increasingly focused on natural, plant-derived alternatives. Red pucuk leaves (*Syzygium myrtifolium* Walp.) have been reported to contain bioactive phytochemicals—such as flavonoids, saponins, and tannins—with established antibacterial properties. This study investigated the antibacterial efficacy of liquid facial soap formulations containing ethanol extracts of red pucuk leaves against *C. acnes* using a quasi-experimental design. A total of 24 experimental units were prepared, comprising six concentration groups (5%, 10%, 15%, 20%, 25%, and 30%) with four replications each. Additional comparative analysis included a positive control, a base formulation control, and an extract-only control to validate the observed antibacterial effects. Antibacterial activity was assessed via the Kirby-Bauer disc diffusion method, with mean inhibition zone diameters recorded as follows: 28.1 mm (5%), 25.9 mm (10%), 26.8 mm (15%), 27.0 mm (20%), and 30.1 mm for both 25% and 30% concentrations. According to interpretive criteria, all values fell within the category of "very strong" antibacterial activity. These results suggest that the red pucuk extract exerts a concentration-dependent inhibitory effect, peaking at 25–30%. Statistical analysis using simple linear regression revealed a significant positive correlation between extract concentration and inhibition zone diameter ($p = 0.000$, $\alpha < 0.05$), indicating that the increase in extract concentration is scientifically associated with enhanced antibacterial activity against *C. acnes*.

Keywords: Liquid facial soap, Red pucuk leaves, *Syzygium myrtifolium*, *Cutibacterium acnes*, Antibacterial activity

Introduction

Acne vulgaris is a common inflammatory skin disease characterized by a complex pathogenesis involving multiple factors, including sebaceous gland hyperactivity, follicular hyperkeratinization, bacterial colonization, immune system responses, and inflammation[1]. One of the key features of acne is the obstruction of skin pores, which leads to the formation of inflamed pustules[2]. In Indonesia, the prevalence of acne is notably high, affecting approximately 80–85% of adolescents, with peak incidence observed between the ages of 15 and 18 years. Additionally, around 12% of adult women over the age of 25 and 3% of individuals aged 35–44 is also affected[3]

One of the main bacteria involved in the development of acne is *Cutibacterium acnes* (formerly known as *Propionibacterium acnes*). This bacterium normally lives on the skin and can appear in different shapes, such as rods, spheres, or branches. It contributes to acne by producing enzymes and inflammatory substances that damage the outer layers of the skin and the walls of the pores. This leads to a buildup and hardening of oil (sebum) and fatty acids, which can trigger inflammation. Squeezing or picking at acne can worsen the inflammation and cause further skin damage[4].

Effective acne management aims to normalize follicular function, reduce sebum production, minimize bacterial colonization, and alleviate inflammation. Topical anti-acne formulations such as creams, lotions, and facial soaps are widely used[4]. Among these, liquid facial soaps are a popular choice due to their cleansing ability, which helps remove impurities, lower sebum levels on the skin surface, and inhibit microbial growth[5].

To be considered safe and effective, liquid facial soaps must meet specific standards set by regulatory authorities. According to the Indonesian National Standard (SNI, 1996), a quality liquid facial soap should have a pH range between 4.5 and 6.5, be homogenous, and produce foam within an acceptable range (1.3–22 cm). However, many commercial acne treatments rely on synthetic antibiotics (e.g., erythromycin, clindamycin) and active agents (e.g., benzoyl peroxide, salicylic acid), which may cause side effects such as skin irritation, hypersensitivity, and—most notably—antibiotic resistance with prolonged use[6].

As a result, there is growing interest in exploring plant-based alternatives with fewer side effects and lower risk of resistance. One such candidate is the red pucuk plant (*Syzygium myrtifolium* Walp.), which contains several secondary metabolites, including

flavonoids, tannins, saponins, steroids, and triterpenoids[7]. These compounds are known for their natural antimicrobial activity and have demonstrated antibacterial effects against a variety of Gram-positive and Gram-negative bacteria, such as *Staphylococcus aureus*, *Escherichia coli*[8], *Streptococcus mutans*[7], and *Salmonella typhi*[9].

Despite the increasing interest in natural alternatives for acne treatment, scientific evidence regarding the antibacterial effectiveness of *Syzygium myrtifolium* (red pucuk leaves) specifically against *Cutibacterium acnes*—a key bacterium in acne pathogenesis—remains limited. Most existing studies have focused on its activity against other bacterial species, leaving a gap in understanding its potential role in acne-specific formulations such as liquid facial soap[10]

The effectiveness of plant extracts largely depends on the extraction method and the type of solvent used. Ethanol is often selected as an extraction solvent due to its polarity, which allows it to efficiently extract phenolic and other bioactive compounds. Compared to methanol and water, ethanol has been shown to yield a broader spectrum of chemical constituents.[11]

Previous research by Syafriana & Wiranti (2022) demonstrated that ethanol extracts of red pucuk leaves exhibited increasing antibacterial activity against *Streptococcus mutans* with higher extract concentrations—producing inhibition zones of 9.04 mm (10%), 10.79 mm (20%), 11.87 mm (40%), and 13.35 mm (80%).

Based on this evidence, the present study aims to evaluate the antibacterial effectiveness of liquid facial soap formulations containing ethanol extracts of red pucuk leaves against *Cutibacterium acnes*. The goal is to determine the potential of this natural ingredient as a safer and more sustainable alternative to synthetic acne treatments.

Materials and methods

This study employed a true experimental design to evaluate the antibacterial effectiveness of liquid facial soap formulations containing varying concentrations of ethanol extract from red pucuk (*Syzygium myrtifolium* Walp.) leaves against *Cutibacterium acnes*, with full control over treatment allocation, randomization, and replication to ensure internal validity.

Sample and Formulation

The test samples consisted of six different facial soap formulations, each containing ethanol extract of red pucuk leaves at concentrations of 5% (Formula I), 10% (Formula II), 15% (Formula III), 20% (Formula IV), 25% (Formula V), and 30% (Formula VI). Each treatment group was replicated four times, resulting in a total of 24 samples. Red pucuk leaves used in this study were freshly harvested, red-tipped, non-wilted, and weighed approximately 4 kg in total.

Preparation of Plant Material (Simplicia)

The red pucuk leaves were cleaned (wet sorting), washed under running water to remove dirt, and then drained. The leaves were subsequently air-dried at room temperature, ground into a fine powder using a blender, and sieved to obtain a uniform particle size [7]

Preparation of Ethanol Extract (Maceration Method)

A total of 500 grams of the powdered leaf material was placed into a glass container and soaked in 96% ethanol, ensuring the material was fully submerged. The maceration process lasted at least three days, during which the solvent was replaced three times or until the filtrate became clear. The resulting solution was filtered through filter paper and collected. The filtrate was then concentrated using a rotary evaporator to obtain a thick ethanol extract of red pucuk leaves.[12]

Antibacterial Activity Testing (Disc Diffusion Method)

The antibacterial activity of each soap formulation was assessed using the Kirby-Bauer disc diffusion method. Sterile filter paper discs impregnated with the soap samples were placed on Mueller-Hinton Agar (MHA) plates previously inoculated with *Cutibacterium acnes*. The plates were incubated under appropriate conditions. The diameter of the inhibition zone around each disc was measured to evaluate the antibacterial efficacy of each formulation. A larger inhibition zone indicated greater antibacterial activity.

Formulation of Liquid Facial Soap

The preparation began by separating the ingredients into two phases: the oil phase and the water phase. Sodium chloride (NaCl) was first dissolved in distilled water and stirred until homogeneous to form Solution 1. Meanwhile, stearic acid and Adeps Lanae were melted until fully liquefied, followed by the addition of glycerin and triethanolamine (TEA) to create Solution 2. Solution 1 was then gradually added to Solution 2 with continuous stirring until a homogeneous mixture was achieved, and additional distilled water was added gradually. The resulting mixture was removed from heat and allowed to cool to room temperature. Once cooled, the ethanol extract of red pucuk leaves and apple fragrance were added with constant stirring. Finally, citric acid was incorporated to adjust the pH to a skin-safe level, and the completed liquid facial soap formulation was transferred into appropriate storage containers (Table 1).

Table 1: Liquid Facial Soap Formulation from Ethanol Extract of Red pucuk Leaves (Syzygium myrtifolium Walp.)

Material	Function	F0	FI	FII	FIII	FIV	FV	FVI
Red Pucuk leaves	extract Active ingredient	-	1 gr	2 gr	3 gr	4 gr	5 gr	6 gr
Stearic acid	Foam stabilizer agent	0.15 gr	0.15 gr	0.15 gr	0.15 gr	0.15 gr	0.15 gr	0.15 gr
Adeps lanae	Alkalizer and saponification	0.1 gr	0.1 gr	0.1 gr	0.1 gr	0.1 gr	0.1 gr	0.1 gr
TEA	Base	0.03 ml	0.03 ml	0.03 ml	0.03 ml	0.03 ml	0.03 ml	0.03 ml
Glycerin	Humectant	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
NaCl	Foam former	0.334 gr	0.334 gr	0.334 gr	0.334 gr	0.334 gr	0.334 gr	0.334 gr
Fragrance	Fragrance	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Citric acid	Acidity regulator	0.02 gr	0.02 gr	0.02 gr	0.02 gr	0.02 gr	0.02 gr	0.02 gr
Distilled warer	Solvent	Add 20 ml	Add 20 ml	Add 20 ml	Add 20 ml	Add 20 ml	Add 20 ml	Add 20 ml

Preparation of McFarland Turbidity Standard

The McFarland turbidity standard was prepared following the method described by Kuswiyanto (2015). A total of 0.05 mL of 1.175% barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$) was mixed with 9.95 mL of 1% sulfuric acid (H_2SO_4) and homogenized thoroughly. The resulting suspension mimics the turbidity of a bacterial suspension containing approximately 10^8 CFU/mL. This standard was stored in a sealed test tube to prevent evaporation and kept in a dark environment at room temperature. The standard can be used for up to six months, and should be shaken well before use.

Preparation of *Cutibacterium acnes* Suspension

The bacterial suspension was prepared as described by Dewi et al. (2020). A volume of 10 mL of sterile 0.9% sodium chloride (NaCl) solution was placed into a sterile test tube. A bacterial colony was obtained from a pure *C. acnes* culture using a sterile inoculating loop and transferred into the NaCl solution. The suspension was mixed thoroughly using the same sterile loop. The turbidity of the bacterial suspension was then adjusted to match the McFarland 0.5 turbidity standard.

Inoculation of *C. acnes* on Mueller Hinton Agar (MHA)

Using a sterile cotton swab dipped into the adjusted bacterial suspension, the swab was allowed to absorb the liquid, then gently pressed against the inner wall of the test tube while rotating to remove excess fluid (Kuswiyanto, 2015). The MHA plate was inoculated by streaking the swab over the entire surface using three strokes while rotating the plate 90° between the first and second streak, and 45° between the second and third streak. Plates were then left to rest for 5–15 minutes to allow absorption of the suspension into the agar before applying the test discs.

Preparation of Antibacterial Discs

According to the method of Novaryatiin et al. (2019), sterile paper discs were immersed in 20 mL of each facial soap formulation containing 5%, 10%, 15%, 20%, 25%, and 30% ethanol extract of red pucuk leaves. The discs were then placed on the surface of MHA plates previously inoculated with *C. acnes*. Plates were incubated at 37°C for 24 hours.

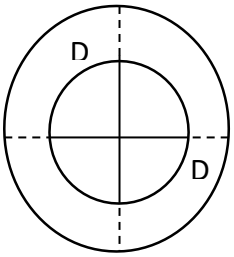
For the preparation of control discs, paper discs were respectively soaked in a commercially available antibacterial facial soap (positive control), the soap base without ethanol extract of red pucuk leaves (base control), and pure ethanol extract of red pucuk leaves without the soap formulation (extract control), then each disc was placed on Mueller-Hinton Agar (MHA) plates inoculated with the test bacteria and incubated at 37°C for 24 hours.

Measurement of Inhibition Zones

Following the 24-hour incubation period, zones of inhibition were observed as clear areas around the discs, indicating antibacterial activity. The diameters of these zones were measured both horizontally and vertically using a milimeter ruler, and the mean values were recorded. The results were interpreted according to the guidelines from the Kementerian Kesehatan 2014[13].

$$\frac{Dv + Dh}{2}$$

information:
Dv: Vertical diameter
Dh: Horizontal diameter



Statistical Analysis

Univariate Analysis

Univariate analysis was conducted to describe the characteristics of each research variable. This analysis involved processing the data to determine descriptive statistics for each treatment concentration, including the minimum and maximum values, mean, and standard deviation.

Bivariate Analysis

Bivariate analysis was used to assess the relationship between two variables, specifically the concentration of the ethanol extract and the diameter of the bacterial inhibition zone. Prior to analysis, tests for normality and homogeneity were conducted to ensure the assumptions of linear regression were met. This analysis was performed using a simple linear regression test with SPSS software[14].

Results

Table 2: Phytochemical Screening Results

Phytochemical Test	Result	Description
Flavonoids	Purple	Positive (+)
Saponins	Stable Foam	Positive (+)
Tannins	Greenish Black	Positive (+)

Table 2 shows that*Syzygium myrtifolium*leaf extract is positive for flavonoids, saponins, and tannins. The presence of these phytochemical compounds is important as they have antibacterial properties that may contribute to the effectiveness of the liquid facial soap in fighting *Cutibacterium acnes*. Flavonoids are known to disrupt bacterial metabolism, saponins increase the permeability of bacterial cell membranes, and tannins have cytotoxic effects on bacterial membranes and disrupt the activity of important enzymes.

Table 3: Organoleptic Test of Liquid Facial Soap Ethanol Extract of Red Pucuk Leaves (Syzygium myrtifolium Walp.)

Concentration	Color	Odor	Consistency
FI	Reddish brown	Pleasant fragrance	Liquid
FII	Reddish brown	Pleasant fragrance	Liquid
FIII	Reddish brown	Pleasant fragrance	Liquid
FIV	Reddish brown	Pleasant fragrance	Liquid
FV	Reddish brown	Pleasant fragrance	Liquid
FVI	Reddish brown	Pleasant fragrance	Liquid
Positive Control (PC)	Clear	Pleasant fragrance	Liquid
Base Control (BC)	White	Odorless	Liquid
Extract Control (EC)	Reddish brown	Characteristic of extract	Semi-solid

Table 3 shows the organoleptic test was conducted to evaluate the physical characteristics of the

liquid facial soap formulations. The results show that all formulas (FI to FVI) have a brownish-red colour, pleasant aroma, and liquid consistency. This brownish-red colour is due to the presence of anthocyanins in the extract, which also have antioxidant activity and can protect the skin from damage due to ultraviolet radiation. The positive control (PC) has a clear colour with a pleasant aroma and liquid consistency, while the base control (BC) is white and odourless with a liquid consistency. The extract control (EC) was brownish-red in colour with extract characteristics and semi-solid in form..

Table 4: pH Test of Liquid Facial Soap Ethanol Extract of Red Pucuk Leaves (Syzygium myrtifolium Walp.)

	FI	FII	FIII	FIV	FV	FVI	PC	BC	EC
R1	5	5	5	5	5	5	5	6	5
R2	5	5	5	5	5	5	-	-	-
R3	5	5	5	5	5	5	-	-	-
R4	5	5	5	5	5	5	-	-	-
Average	5	5	5	5	5	5	5	6	5

Table 4 shows the pH measurement is very important to ensure the liquid facial soap is safe and effective for the skin. The results show that the average pH for all formulas (FI to FVI) is 5. This pH value corresponds to the pH standard for healthy facial skin (4.5-6.5), which helps maintain the skin's natural barrier function and prevents irritation or bacterial overgrowth. The positive control had a pH of 5 and the base control had a pH of 6.

Table 5: Average Inhibition Zones and Interpretation of Antibacterial Activity

Concentration	Average Inhibition Zone (mm)	SD	Interpretation
FI	23.1 mm	1.24	Very Strong
FII	25.9 mm	1.11	Very Strong
FIII	26.8 mm	0.91	Very Strong
FIV	27.0 mm	0.70	Very Strong
FV	30.1 mm	1.68	Very Strong
FVI	30.1 mm	0.50	Very Strong

Table 5 shows the antibacterial activity test was conducted using the Kirby-Bauer disc diffusion method to measure the ability of the liquid facial soap formulations in inhibiting the growth of*Cutibacterium acnes*. The results showed that all concentrations (FI to FVI) exhibited "Very Strong" antibacterial activity with zones of inhibition ranging from 23.1 mm to 30.1 mm. Formula FV and FVI (25% and 30%) showed the highest zone of inhibition of 30.1 mm. This indicates that the antibacterial effectiveness increases as the concentration of red shoot leaf extract increases.

Table 6: ANOVA Test of the Effectiveness of Liquid Facial Soap Formulation with Ethanol Extract of Red Pucuk Leaves (Syzygium myrtifolium Walp.) Against Cutibacterium acne Bacteria

Model		*Sum of square*	*Df*	*Mean Square*	*F*	*Sig*
1	*Regression*	129.880	1	129.880	86.668	.000[b]
	Residual	32.969	22	1.499		
	Total	162.850	23			

ANOVA analysis was used to determine if there was a significant difference in effectiveness between the various liquid facial soap formulations in inhibiting the growth of*Cutibacterium acnes*bacteria. The significance value (Sig.) was 0.000, which is less than 0.05. This indicates that there is a significant difference in effectiveness between the various liquid facial soap formulations in inhibiting the growth of*Cutibacterium acnes* bacteria (Table 6).

Table 7: Coefficient of Effectiveness Test of Liquid Facial Soap Formulation of Ethanol Extract of Red Pucuk Leaves (Syzygium myrtifolium Walp.) Against Cutibacterium acne Bacteria

		Unstandardized Coeffiecients		*Standardized Coefficients*		
Model		*B*	*Std. Error*	*Beta*	*t*	*Sig.*
1	(*Constant*)	22.437	.570		39.375	.000
	Konsentrasi	1.362	.146	.893	9.310	.000

The coefficient of effectiveness test was performed to measure the relationship between extract concentration and zone of inhibition. The significance value (Sig.) for concentration was 0.000, which is less than 0.05. The regression coefficient (B) for concentration was 1.362, indicating a positive relationship between extract concentration and zone of inhibition. This means that the higher the concentration of red shoot leaf extract, the greater the zone of inhibition produced against*Cutibacterium acnes* bacteria (Table 7).

Discussion

This study investigated the antibacterial effectiveness of liquid facial soap formulations containing various concentrations of ethanol extract from red pucuk leaves (*Syzygium myrtifolium*) against *Cutibacterium acnes*, a bacterium commonly associated with acne pathogenesis. Six formulations were evaluated, each incorporating increasing concentrations of the extract: 5%, 10%, 15%, 20%, 25%, and 30%.

The ethanol extract of *Syzygium myrtifolium* exhibited a moisture content of 7.79%, meeting the Indonesian Ministry of Health's standard of less than 10%. This low moisture content is advantageous as it reduces the risk of microbial contamination and enhances the shelf-life of the extract by minimizing spoilage. Organoleptic evaluation of the soap formulations revealed a reddish-brown color, liquid consistency, and a characteristic herbal aroma, all of which are consistent with the natural properties of the red pucuk leaf extract. The reddish-brown coloration is attributed to the presence of anthocyanins, which possess antioxidant activity that may help mitigate oxidative stress, promote skin cell regeneration, and protect the skin from damage caused by ultraviolet radiation[15].

The pH of the liquid soap formulations was measured at 5.0, which falls within the optimal pH range for facial skin (4.5–6.5). This slightly acidic pH is crucial in maintaining the skin's natural barrier function. Formulations with a pH above 8 may disrupt the acid mantle, leading to dryness and increased susceptibility to bacterial growth, while excessively acidic products may also cause irritation[16]. Therefore, the tested formulation is considered safe and suitable for daily use, particularly for individuals with acne-prone skin.

Foaming ability, evaluated through foam height measurement, yielded a value of 1.5 cm, which complies with the Indonesian National Standard (SNI) acceptable range of 1.3 to 22 cm. This level of foam is generally considered sufficient for effective cleansing and is preferred by consumers.

The antibacterial activity of the formulations was assessed using the Kirby-Bauer disc diffusion method, with each formulation tested in quadruplicate to ensure reproducibility and accuracy. All formulations demonstrated inhibitory effects against *C. acnes*, with increasing concentrations of the red pucuk leaf extract resulting in larger inhibition zones. Notably, the 25% and 30% extract formulations (formulas V and VI) produced the most pronounced antibacterial effects, with inhibition zones categorized as "very strong." These findings indicate that antibacterial efficacy increases in a dose-dependent manner, reaching optimal performance at a concentration of 25%.

Interestingly, the pure ethanol extract (100%) exhibited a smaller inhibition zone (30 mm) than formulas V and VI. This phenomenon is likely due to the higher viscosity of the undiluted extract, which may hinder its diffusion through the agar medium and subsequently reduce its bioavailability on the testing disc[17]. A commercially available liquid facial soap used as the positive control exhibited a larger inhibition zone of 40 mm, likely attributable to the presence of synthetic antimicrobial agents. Nevertheless, the plant-based extract offers a safer alternative, with reduced risk of skin irritation or antibiotic resistance. The negative control, consisting of the soap base without any extract, showed no inhibition zone, confirming that the antibacterial activity observed was solely due to the presence of the red pucuk leaf extract.

Phytochemical screening of *Syzygium myrtifolium* extract revealed the presence of several bioactive constituents, including flavonoids, tannins, saponins, and essential oils. Each of these components contributes to the extract's antibacterial properties through distinct mechanisms. Flavonoids interfere with bacterial metabolism by inhibiting nucleic acid synthesis, compromising membrane integrity, and impairing energy metabolism, which collectively suppress bacterial growth[18]. Saponins increase bacterial membrane permeability, leading to leakage of intracellular contents and eventual cell lysis[19]. Tannins act through cytotoxic effects on the bacterial membrane and chelation of metal ions, thereby disrupting critical enzymatic activities[20]. In addition, essential oils present in the extract contribute to antibacterial activity by penetrating the phospholipid bilayer of bacterial membranes and disrupting their function, leading to inhibited bacterial growth[21].

The synergistic action of flavonoids, tannins, and saponins likely enhances the antibacterial efficacy of *Syzygium myrtifolium* beyond that of individual compounds. A positive correlation between extract concentration and antibacterial activity was observed, with 25% identified as the optimal formulation for effective diffusion and maximal inhibition. These results support the potential use of *S. myrtifolium* extract as a natural antibacterial agent in facial soap targeting *Cutibacterium acnes*. Upon topical application, its bioactive compounds may penetrate the skin and provide local antimicrobial and anti-inflammatory effects; however, further pharmacokinetic studies are needed to clarify absorption, stability, and tissue accumulation

This study has several limitations, including the absence of toxicity testing, lack of stability evaluation of the liquid soap formulation, and no in vivo assessment of the red pucuk leaf (*Syzygium myrtifolium*) extract. Future research is needed to address these aspects to ensure product safety, long-term effectiveness, and clinical applicability.

Conclusion

Based on the effectiveness test results, the liquid facial soap containing ethanol extract of red pucuk leaves (*Syzygium myrtifolium*) demonstrated antibacterial activity categorized as very strong. Furthermore, statistical analysis using a simple linear regression test confirmed that the ethanol extract in the formulation had a statistically significant effect in inhibiting the growth of *Cutibacterium acnes*. A minimum concentration of 25% is recommended as the optimal level to achieve maximal antibacterial effectiveness

Conflict of interest

Authors state no conflict of interest.

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