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ANTIBACTERIAL ACTIVITY TEST OF FENNEL LEAVES AND STEMS (*Foeniculum vulgare* Mill.) AGAINST BACTERIA *Cutibacterium acnes* AND *Staphylococcus epidermidis*

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ABSTRACT

Background: Acne vulgaris is a common skin problem that often affects adolescents and young adults, caused by the bacteria *Cutibacterium acnes* and *Staphylococcus epidermidis*. The use of synthetic antibiotics for acne treatment can lead to resistance; therefore, it is necessary to develop alternative natural ingredients that can act as a companion or complementary therapy, such as fennel plants (*Foeniculum vulgare* Mill.). **Objective:** To identify and compare the antibacterial activity of ethanol extracts of fennel leaves and stems against *C.acnes* and *S.epidermidis*. **Methods:** Extraction is carried out by maceration using 96% ethanol. Antibacterial tests were carried out in vitro using disc diffusion methods with concentrations of 50%, 75%, and 100%. Tetracycline 1% is used as a positive control, and DMSO 10% as a negative control. **Results:** Extracts of fennel leaves and stems exhibited antibacterial activity. The diameter of the inhibition zone increased with increasing extract concentration, with the leaves extract showing greater antibacterial activity than the stems extract. **Conclusion:** The results of the antibacterial activity test showed that ethanol extracts of leaves and fennel stems were able to inhibit the growth of *C. acnes* and *S. epidermidis*, with an average inhibition zone of leaf extract that was more optimal than that of stems.

Keywords: Acne vulgaris; *Cutibacterium acnes*; *Foeniculum vulgare* Mill; Inhibition Zone; *Staphylococcus epidermidis*

INTRODUCTION

Acne vulgaris is one of the most common dermatological conditions, particularly affecting adolescents, and may significantly impair self-esteem. The highest prevalence of acne vulgaris has been reported among female adolescents aged 14-17 years, reaching 83-85%[1]. Acne development is influenced by multiple factors, including genetic predisposition, stress, dietary habits, sebaceous gland activity, exposure to chemical agents, and bacterial infection[2]. *C. acnes* and *S. epidermidis* are pus-forming microorganisms that play a crucial role in the pathogenesis of various forms of acne vulgaris.

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The use of topical and oral antibiotics has been the standard therapy for moderate acne vulgaris for more than five decades. Antibiotics such as clindamycin, tetracycline, and erythromycin are among the most effective agents for acne management[2]. However, increasing rates of antibiotic resistance have been reported worldwide, with more than 50% of *C. acnes* strains showing resistance to topical macrolides[3]. This condition has encouraged the exploration of natural antibacterial agents derived from medicinal plants native to Indonesia. One such plant is fennel (*Foeniculum vulgare* Mill.), which is widely cultivated in Indonesia and has been traditionally utilized for its medicinal properties across various plant parts, including the roots (*radix*), leaves (*folium*), stems (*caulis*), and seeds (*semen*)[4]. This study specifically investigates the leaves and stems of fennel.

Fennel leaves contain relatively high levels of essential oils that demonstrate antibacterial potential against *S. aureus* [5]. The essential oil derived from fennel leaves has also shown antibacterial activity against *S. epidermidis*, with an inhibition zone diameter of 15.5 ± 0.5 mm[6]. Furthermore, Elkiran and Telhuner reported that fennel stems contain fenchyl acetate (35.3%), limonene (26.8%), trans-limonene oxide (8.5%), and endo-fenchyl acetate (4.6%), compounds that are potentially responsible for antibacterial activity[7]. Although previous studies have indicated that fennel leaves possess relatively higher levels of secondary metabolites compared to other plant parts, scientific reports that specifically and comparatively evaluate the antibacterial activities of fennel leaves and stems against *S. epidermidis* and *C. acnes* remain limited. This lack of information highlights a research gap, warranting further investigation to assess and compare the antibacterial potential of fennel leaf and stem extracts against both bacteria.

METHODS

1. Types of Equipment and Materials

The tools used in this study include beaker glass (Iwaki), analytical scales (Ohaus), autoclaves (Hirayama), Laminar Air Flow (BioSafety Cabinet), waterbaths, rotary evaporators, moisture analyzers (Ohaus), UV-Vis spectrophotometers, incubators, and ose needles.

The materials used in this study included fennel (*Foeniculum vulgare* Mill.) leaf and stem simplicia collected from Trenggana Street, Denpasar, Bali, Indonesia. Other materials used were 96% ethanol, dimethyl sulfoxide (DMSO), tetracycline, distilled water (aquadest), *C. acnes* (ATCC 12228), *S. epidermidis* (FNCC 0048), nutrient agar (Himedia), and nutrient broth.

2. Fennel Leaf and Stem Extraction

Leaf and stem simplicia, weighing 250 g and 350 g, respectively, were macerated separately in 96% ethanol at a 1:10 (w/v) ratio for 72 h at room temperature. The resulting macerates were filtered and concentrated using a rotary evaporator to obtain viscous extracts[8].

The extract yield was calculated as the ratio of the weight of the dried extract obtained to the initial weight of the simplicia used. The yield was expressed as a percentage (%) using the following equation. Extract yield (%) = (weight of dried extract / initial weight of simplicia) × 100 [9].

3. Loss On Drying Test

A total of 1-2 g of simplicia was weighed and placed in a previously dried and tared porcelain crucible. The sample was evenly distributed to form a thin layer and dried in an oven at 105°C until a constant weight was achieved. After each drying cycle, the crucible was cooled to room temperature in a

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desiccator before reweighing. The drying and weighing steps were repeated until no further change in weight was observed[10].

4. Moisture Content Result

A 1 g sample was weighed using a moisture analyzer cup. The moisture analyzer was set to 105°C, and the sample was analyzed until the moisture content result was obtained and recorded[11].

5. Screening Phytochemistry

a. Flavonoids

10 g of simplicia powder were mixed with 10 mL of hot water, boiled for 5 minutes, and filtered while hot. An aliquot of 5 mL of the filtrate was then treated with 0.1 g of magnesium powder, 1 mL of concentrated hydrochloric acid, and 2 mL of amyl alcohol. The mixture was shaken and allowed to separate. A positive result for flavonoids was indicated by the appearance of a red, yellow, or orange color in the amyl alcohol layer [12].

b. Steroid or Triterpenoid Test

A total of 100 mg of fennel leaf extract was dissolved in chloroform and filtered. Five milliliters of the filtrate were evaporated to obtain a residue, which was then treated with two drops of acetic anhydride and one drop of concentrated sulfuric acid. The appearance of a blue or green color indicated the presence of steroids, whereas a purple or orange color indicated the presence of triterpenoids[13].

c. Tannin

A total of 0.5 g of extract was placed into a test tube and dissolved in a small amount of distilled water. The solution was heated in a water bath and then added to a mixture of 1% gelatin solution and 10% sodium chloride solution (1:1, v/v). The formation of a white precipitate indicated a positive result for tannins [14].

d. Phenols

1 mL of the solution was treated with two drops of 5% $FeCl_3$ solution. The formation of a green, blue, or black color indicated a positive result for phenolic compounds[15].

e. Saponins

A total of 0.2 g of viscous extract was dissolved in 4 mL of an appropriate solvent and shaken vertically for 10 s. The formation of stable foam with a height of 1–10 cm that persisted for 10 min and did not disappear after the addition of 2 N HCl indicated a positive result for saponins[16].

f. Alkaloids

A total of 0.5 g of simplicia powder was mixed with 1 mL of 2 N HCl and 9 mL of distilled water, heated in a water bath for 2 min, cooled, and filtered. Aliquots of 0.5 mL of the filtrate were placed into three separate test tubes and treated with two drops of Mayer, Wagner, and Dragendorff reagents, respectively. A positive result for alkaloids was indicated by the formation of a white precipitate (Mayer), brown to black precipitate (Wagner), or orange precipitate (Dragendorff)[12].

6. Ethanol-Free Test

One milliliter of viscous extract was placed into a test tube, followed by the addition of two drops of concentrated sulfuric acid (H_2SO_4) and two drops of acetic acid, and then heated. The extract was considered ethanol-free if no characteristic ester odor of ethanol was detected[17].

7. Antibacterial Activity Test

a. Sample Preparation

Stock solutions of the leaf and stem extracts were prepared by dissolving the viscous extract in 10% DMSO (w/v). Extract concentrations of 50%, 75%, and 100% were obtained by serial dilution of the stock solution with 10% DMSO. All

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concentrations were prepared in triplicate and used for antibacterial testing by the disc diffusion method[4].

b. Sterilization of equipment and media

In this study, glassware such as petri dishes and inoculating needles, as well as media materials, were sterilized using the steam method in an autoclave for 15 minutes[4].

c. Bacterial Subculture

This study used two pathogenic strains, *Cutibacterium acnes* (ATCC 6919) and *Staphylococcus epidermidis* (FNCC 0048). A single colony was aseptically subcultured onto agar plates using a sterile inoculating loop. The plates were incubated at 37°C for 24 h. *C. acnes* was grown under anaerobic conditions using an anaerobic jar with gas-generating sachets, whereas *S. epidermidis* was cultured aerobically on nutrient agar[18].

d. The turbidity of the bacterial suspension was adjusted to 0.5 McFarland.

The antimicrobial activity was evaluated using the disc diffusion method following the guidelines of the Clinical and Laboratory Standards Institute Briefly, bacterial suspensions (≈ $5x10^8$ CFU/mL; OD_{625}= 0.5) were spread on agar plates, and paper disc impregnated with plant extracts, a positive control (tetracycline), or a negative control (10% DMSO) were subsequently placed on the surface[18].

e. Antibacterial Assay

The bacterial suspension was adjusted to the 0.5 McFarland standard and evenly spread onto the surface of agar medium using a sterile cotton swab. Sterile paper discs were impregnated with extract solutions at concentrations of 50%, 75%, and 100% for 20 min. Tetracycline (1%) was used as the positive control, while 10% DMSO served as the negative control. All discs were aseptically placed on the inoculated agar using sterile forceps. The plates were incubated at 37°C for 24 h in an inverted position. Antibacterial activity was determined by measuring the diameter of the inhibition zones around each disc. All tests were performed in triplicate[19].

8. Data Analysis Results

Data were expressed as mean ± standard deviation (SD). Data were analyzed using SPSS version 27. Normality was assessed using the Shapiro-Wilk test due to the small sample size (≤50). Homogeneity of variance was evaluated using Levene's test, which indicated that the data were not homogeneous ($p < 0.05$).

RESULTS

1. Fennel Leaf and Stem Extraction

Following extraction, the yield was calculated to determine the ratio between the weight of the obtained simplicia or extract and that of the raw material. The results (Table 1) indicated that the fennel leaf extract produced a higher yield than the fennel stem extract.

Table 1. Extract Yield

Plant Parts for Extract	Weight of Simplisia Powder (g)	Weight of Extract (g)	Extract Yield (%)
Leaf	350	30.9	**8.82%**
Stems	250	19.73	**7.89%**

2. Loss on Drying Results

In this study, the average loss on drying of leaf simplicia was 6.50%, while that of fennel stem simplicia was slightly lower at 6.37% (Table 2). Both values were below the acceptable limit of 10%, indicating that the simplicia met quality standards for moisture content after drying.

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Table 2. Lost On Drying Test Results

Replication	Result (%)		Information
	Simplicial of fennel stem	Simplicia of fennel leaves	
1	7.51%	7.15%	Condition <10% (Eligible)
2	6.01%	6.06%	
3	5.61%	5.57%	
Average	**6.37%**	**6.50%**	

3. Moisture Content Yield

The determination of moisture content in the extract was conducted to establish an acceptable range of residual water in the material. As shown in Table 3, the measured moisture content values indicate that the extracts contained low levels of residual moisture, which is important for minimizing the risk of fungal or mold growth that could compromise the biological activity of the extract during storage.

Table 3. Moisture Content Test Results

Replication	Moisture Content Yield %		Information
	Leaf Extract	Stem Extract	
1	3.0 %	3.0 %	Condition < 15% (Eligible)
2	2.0 %	2.9 %	
3	3.0 %	3.0 %	
Average	**2.6 %**	**2.9 %**	

4. Ethanol-Free Test Results

This test is conducted to ensure that the condensed extract produced is completely pure and free of ethanol. The results of the ethanol-free test demonstrated that the condensed extracts of fennel leaves and stems did not exhibit any ester-like odor. Esters are characterized by distinctive aromas, commonly described as floral, fresh, or fruity.

5. Screening Phytochemistry Result

Phytochemical screening was conducted to qualitatively identify the secondary metabolite compounds present in the simplicia and in the extracts of fennel leaves and stems.

Table 4. Phytochemical Screening Test

Metabolite compounds	Reaction Results			
	LS	SS	LE	SE
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Tannin	+	+	+	+
Steroids/ Triterpenoids	+	+	+	+
Alkaloids	-	-	-	-
Phenols	+	+	+	+

Information : (+) there are metabolite compounds; (-) no metabolite compounds; (LS) Leaf simplicia; (SS) Stem simplicia; (LE) Leaf extract; (SE) Stem extract.

6. Antibacterial Activity Test Results

The antibacterial activity of fennel leaf and stem extracts against *C. acnes* and *S. epidermidis* was evaluated using the disk diffusion method. The inhibition zone diameters obtained for each extract concentration are summarized in Tables 5-8. Tetracycline was used as the positive control, while DMSO served as the negative control.

The results showed that both leaf and stem extracts exhibited antibacterial activity against *C. acnes* and *S. epidermidis*. At concentrations of 75% and 100%, the leaf and stem extracts produced a significant increase in inhibition zone diameters against *C. acnes* and *S. epidermidis* compared to lower concentrations. However, only the 100% concentration of both leaf and stem extracts showed a significantly greater inhibition zone diameter compared to all other concentrations.

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Table 5. Results of *C. acnes* Leaf Inhibition Zone

Treatment	Inhibition Zone Diameter (mm)			Average ± SD (mm)	Category
	R1	R2	R3		
Leaf 50%	1.6	1	0	0.87 ± 0,80[a]	Weak
Leaf 75%	6.5	5.5	7.3	6.43 ± 0,90[b]	Medium
Leaf 100%	8.8	6.5	8.4	7.90 ± 1,23	Medium
C-Positive	26.6	26.1	27	26.56± 0.45	Very Strong
C-Negative	0	0	0	0 ± 0	None

Category: Weak < 5 mm; Medium 5-10 mm; Strong 10-20 mm; Very Strong >20 mm. [ab]Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$).

Table 6. Results of *C. acnes* Stem Inhibition Zone

Treatment	Inhibition Zone Diameter (mm)			Average ± SD (mm)	Category
	R1	R2	R3		
Stem 50%	0	0	0	0 ± 0[a]	None
Stem 75%	3.5	4	8.5	5.33 ± 2.75[b]	Medium
Stem 100%	5.1	5.5	8.5	6.37 ± 1.86[b]	Medium
C-Positive	28.5	23.5	25.5	25.83 ± 2.51	Very Strong
C-Negative	0	0	0	0 ± 0	None

Category: Weak < 5 mm; Medium 5-10 mm; Strong 10-20 mm; Very Strong >20 mm. [ab]Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$).

Table 7. Results of *S. epidermidis* Leaf Inhibition Zone

Treatment	Inhibition Zone Diameter (mm)			Average ± SD (mm)	Category
	R1	R2	R3		
Leaf 50%	5.25	2.5	6.5	4.75 ±2.04[a]	Weak
Leaf 75%	11.3	7.5	5	7.93 ± 3.17[a]	Strong
Leaf 100%	12	7.5	11.5	10.33 ± 2.46[b]	Strong
C-Positive	21.5	20.5	21	21 ±0.5	Very Strong
C-Negative	0	0	0	0 ± 0	None

Category: Weak < 5 mm; Medium 5-10 mm; Strong 10-20 mm; Very Strong >20 mm. [ab]Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$).

Table 8. Results of *S. epidermidis* Stem Inhibition Zone

Treatment	Inhibition Zone Diameter (mm)			Average ± SD (mm)	Category
	R1	R2	R3		
Stem 50%	3.5	5	5.5	4.66 ±1.04[a]	Weak
Stem 75%	5	6.3	5.75	5.68 ±0.65[a]	Medium
Stem 100%	11	9.5	10	10.16 ±0.76[b]	Strong
C-Positive	20.5	23	22.5	22 ± 1.32	Very Strong
C-Negative	0	0	0	0 ± 0	None

Category: Weak < 5 mm; Medium 5-10 mm; Strong 10-20 mm; Very Strong >20 mm. [ab]Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$).

DISSCUSION

Based on the antibacterial activity assays against *C. acnes* and *S. epidermidis* (Tables 5-8), fennel leaf extract exhibited higher antibacterial activity than the stem extract at all tested concentrations. For both bacterial strains, increasing extract concentrations were directly proportional to increases in inhibition zone diameters, indicating a concentration-dependent antibacterial effect. Against *C. acnes*, the leaf extract began to show antibacterial activity at a concentration of 50%, categorized as weak to moderate, and increased to a moderate category at concentrations of 75% and 100%. In contrast, the stem extract demonstrated lower activity,

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producing no inhibition zone at 50% and reaching only a moderate category at higher concentrations. This pattern suggests that antibacterial active compounds in the leaf extract are more effective in inhibiting the growth of *C. acnes* than those present in the stem extract[20].

Similar results were observed in assays against *S. epidermidis*. Leaf extracts at concentrations of 75% and 100% produced inhibition zones classified as strong, whereas stem extracts at the same concentrations exhibited moderate to strong activity with smaller inhibition zone diameters. These findings further confirm that the leaf extract possesses superior antibacterial potential compared to the stem extract.

The stronger antibacterial activity of the leaf extract is likely associated with its higher content of secondary metabolites, including flavonoids, phenolics, tannins, saponins, and terpenoids, which were detected more dominantly in the leaves based on phytochemical screening. These compounds are known to disrupt bacterial cell membranes, alter membrane permeability, and interfere with bacterial enzymatic systems, ultimately inhibiting bacterial growth. Although the antibacterial activity of the stem extract was lower than that of the leaf extract, the present study demonstrates that fennel stems still exhibit inhibitory effects against *C. acnes* and *S. epidermidis* at certain concentrations. These findings address gaps in earlier studies and contribute additional scientific evidence regarding the antibacterial potential of fennel stems[21].

CONCLUSION

The results of this study demonstrate that ethanol extracts of fennel leaves and stems exhibit antibacterial activity against *C. acnes* and *S. epidermidis*. Among the two plant parts, the leaf extract consistently produced larger inhibition zones than the stem extract, indicating superior antibacterial effectiveness. These findings suggest that fennel leaves possess greater potential as a natural antibacterial agent compared to fennel stems, likely due to their higher content of bioactive secondary metabolites.

CONFLICT OF INTEREST

There are no conflicts of interest in this research. This research article was written independently. All authors have no financial or personal relationships with other individuals or organizations that could inappropriately influence this research.

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