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Antimicrobial Activity Test of Ethanol Extract of Senggani Leaves (*Melastoma malabraticum* L) Against *Propionibacterium Acnes* and *Staphylococcus Epidermidis*

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Abstract

Senggani leaves are a plant that can be found in Indonesia, including hilly locations. The people of Marendal, Patumbak subdistrict, believe that senggani leaves may heal minor skin wounds. The goal of this study was to identify the class of secondary metabolite chemical compounds and their antibacterial efficacy against the microorganisms *Propionibacterium acnes* and *Staphylococcus epidermidis* at 5, 10, and 20% concentrations. The ethanol extract of senggani leaves contains secondary metabolite compounds with antibacterial activity. According to the antibacterial test results, a 20% ethanol extract of senggani leaves is effective against *Propionibacterium* with a diameter of 10.7 mm, a 10% in the medium category with a diameter of 9.27 mm, and a 5% in the medium category with a diameter of 8.8 mm. Meanwhile, the diameter of *Staphylococcus epidermidis* at a 20% was 8.77 mm in the medium category, 8.37 mm at 10%, and 8.6 mm at a 5% concentration.

Keywords: Propionibacterium acnes, Senggani leaves, Staphylococcus epidermidis

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1 Introduction

Senggani leaves (*Melastoma malabraticum* L) from the Melastomaceae family. This plant is efficacious as a fever reducer (antipyretic), pain reliever (analgesic), urine laxative (diuretic), treating vaginal discharge (leukorrhea), and can treat various types of cut wounds [1]. One possible solution is to use the active antibacterial compounds found in medicinal plants. The medicinal plant senggani leaves (*Melastoma malabraticum* L) is well-known among Indonesians as a source of therapeutic compounds.

Taking care of your face can mean taking care of your facial skin. In order to take care of facial skin as well as possible, we first need to understand the structure of human skin. The skin is the largest part of the body and the first defense against the surrounding environment. Skin has various functions and uses, including functioning as a thermostat in maintaining body temperature, protecting the body from attacks by microorganisms, and ultraviolet rays, and also playing a role in regulating blood pressure [2]. One of the functions of the skin is to protect it from several things that can damage the skin. One of them is dust and pollution in the environment. Often bacteria from the air will enter and stick to the skin, causing acne. Acne is a persistent infection of the sebaceous follicles that is distinguished by the appearance of comedones, papules, pustules, and cysts in predilection areas [3].

Acne develops as a result of pilosebaceous obstruction and inflammation caused by the bacteria Propionibacterium acnes, Staphylococcus epidermidis, and Staphylococcus aureus [4]. Oily skin will cause clogged pores so anaerobic bacteria will multiply quickly and cause acne. *Propionibacterium acnes* belongs to the Corynebacteria group of bacteria. Propionibacterium acnes, which is found in normal skin flora, plays a role in the pathogenesis of acne by generating lipase, which breaks down free fatty acids from skin lipids. When these fatty acids interact with the immune system, they can cause tissue inflammation and contribute to the onset of acne [5].

Acne is treated by lowering sebum production, skin irritation, follicular irregularities, and the quantity of *Propionibacterium acnes* and *Staphylococcus epidermidis* colonies or their metabolic products. Antibacterial agents such as tetracycline, erythromycin, and clindamycin have been shown to diminish the population of *Propionibacterium acnes* and *Staphylococcus epidermidis* bacteria [6].

Excessive use of antibiotics can cause increased bacterial resistance to certain antibiotics. The high use of antibiotics is the biggest trigger for the emergence of resistance [7]. The development of resistance to drugs is one example of a process carried out by organisms to develop tolerance to new natural environmental conditions. So the treatment of infectious diseases caused by bacteria that are resistant to antibiotics requires new products that have high potential [8]. Traditional medicines, which contain antibacterial qualities, require research to identify novel antibacterial solutions that have the ability to suppress or kill bacteria that are resistant to antibiotics at affordable prices [9].

In several previous studies, senggani leaves have antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* bacteria. The antibacterial activity test findings did not establish an inhibitory zone in the negative control, the positive control was 34.50 mm in the Escherichia coli bacteria and 34.66 mm, 10.83 mm in the ratio 1:1, 15.33 mm in the ratio 1: 2, 13,00 mm in the ratio 2:1 for Escherichia coli bacteria. Meanwhile, in the positive control, the Staphylococcus aureus bacteria created an inhibitory zone of 34.60 mm, 11.16 mm in the 1:1 ratio, 12.00 mm in the 1:2 ratio, and 11.33 mm in the 2:1 ratio. Meanwhile, in Kusumowati's research, the ethanol extract of senggani leaves (*Melastoma* malabraticum L) is effective as an antibacterial and antifungal (*Staphylococcus aureus* bacteria, Escherichia. coli and the fungus *Candida albicans* because the ethanol extract of senggani

leaves contains flavonoid and polyphenol compounds from the results of her research [1].

Based on the aforementioned description, researchers want to investigate the antibacterial activity of an ethanol extract of senggani leaves (*Melastoma malabraticum* L) against Propionibacterium acnes and Staphylococcus epidermidis. The reason is that the widespread use of chemical antibiotics as a therapy to kill bacteria often causes resistance, therefore research is needed on natural ingredients that have antibacterial capabilities that can reduce resistance to antibiotics. To prove whether the leaves (*Melastoma malabraticum* L) which contain the active substances saponins, flavonoids, steroids/triterpenoids, glycosides, and tannins as mentioned above are also able to inhibit the bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis*, one of the bacteria that causes acne, by using ethanol solvent with different concentrations.

2 Methods

2.1 Tools and Materials

The tools used in this research are blender, microscope, glass object, glass deck, rotary evaporator, petri dish, aluminum foil, crucible with lid, incubator, autoclave, electric oven (Memmert), analytical balance, water bath, vortex, test tube, wire loop, hot plate, and azeotrope tool

The materials used are Senggani leaves (Melastoma Malabathricum), *Propionibacterium Acnes* bacterial culture, *Staphylococcus epidermidis* bacterial culture, Mueller Hinton Agar (MHA), Nutrient Agar (NA), 0.5 Mc. Farland I Standard Solution, Chloralhydrate, Toluene, Chloroform, Ethanol 96%, Concentrated Hydrochloric Acid, Mercury (II) Nitrate, Bismuth (II) Nitrate, Glacial Acetic Acid, Potassium Iodide, Iodine, Anhydrous Acetic Acid, 2 N Sulfuric Acid, Iron (II) Chloride 1%, Lead (II) Chloride, Sodium Hydroxide 2 N, Lieberman-Burchard, Dimethyl Sulfoxide (DMSO), Mg Powder, N-Hexane, NaCl 0.9%, $FeCl_3$ 1%, Tetracycline Antibiotics and Aquadest.

2.2 Senggani Plant Identification

Plant identification was carried out at the Medanense Herbarium (MEDA) FMIPA, University of North Sumatra.

2.3 Collection of Senggani Leaf Plant Materials

The plant material was collected on purpose, that is, without comparison to similar plants from other places. The plant material used for this research was senggani leaves (Melastoma malabahtricum L) obtained from Marendal Pasar III.

2.4 Simplicia Preparation

Simplicia is made by making fresh senggani (Melastoma malabatricum L) leaves, then cleaning them of any adhering impurities, then washing them with water until clean and draining them. Next, it is chopped and dried by airing it first, then its wet weight is weighed, then dried in a drying cabinet at a temperature of 40-60°C until the simplicial becomes dry, characterized by being hard and brittle when broken [10]. The dried simplicia is then processed in a blender to make simplicia powder, which is then stored in a tightly sealed plastic container [11].

2.5 Simplicia Characterization

Microscopic and macroscopic examination, determination of water content, determination of water-soluble essence content, determination of soluble essence content in ethanol, determination of total ash content, and determination of acid-insoluble ash content are all part of the Simplicia characterization examination [12].

2.6 Ethanol Extract of Senggani Leaf Preparation

Maceration with 96% ethanol yields Senggani leaf extract. Pour 10 parts simplicia powder (500 g) into a vessel, then 75 parts ethanol filter liquid (5000 ml), cover, and leave for 5 days, covered from sunlight, stirring occasionally. The combination is filtered and the dregs are pressed after 5 days. Wash the dregs with 100 parts ethanol filter fluid to get 100 parts macerate. The liquid was then put into a closed vessel and stored in a cool, dark place for two days before being filtered. The bulk is then

condensed and weighed using a Rotary Evaporator [13].

2.7 Phytochemical Screening

Phytochemical screening was carried out to determine the content of secondary metabolite chemical compounds in fresh Senggani leaves, Simplicia, and ethanol extract of fresh Senggani leaves, including alkaloids, tannins, saponins, steroids/terpenoids, and glycosides [14].

2.8 Antibacterial Test of Ethanol Extract of Senggani Leaf

The agar diffusion method, specifically paper discs, was employed in the inhibitory power test. Prepare a petri dish that has been oven-stripped. Pour in 20 mL of homogenized MHA medium and wait for the agar to solidify. The petri dish that contains the media and has solidified on the surface is streaked with the bacterial suspension using a sterile hose, using a zig-zag method. Then take the paper disc using tweezers which was previously heated over a Bunsen fire. Pipetted 250 µL of each extract paper disc with a predetermined concentration, namely 5%, 10%, 20%, DMSO as a negative control, and Tetracycline as a positive control. After that, place it on the surface of the agar media carefully using tweezers and mark each concentration location. Then the media was incubated in an incubator at 37 °C for 24 hours. After that, the zone of inhibition formed around the disc was measured using a digital caliper. Characterized by a clear zone around the disc [15].

3 Results and Discussions

The sample used in this research was Senggani (Melastoma malabatricum L) leaves. The wet weight of senggani leaves obtained was 5000 g, then the sample weight after drying was 2500 g and the simplicia powder obtained was 500 g. The extraction method used was maceration using 96% ethanol solvent, resulting in a thick extract of 96 g blackish-green in color with a distinctive odor.

3.1 Simplicia Characterization Examination

The results of the examination of the characterization of Senggani leaf powder can be seen in Table 1.

Table 1 Characterization Examination of Senggani Leaf Simplicia Powder

No	Parameter	Check up result%	MMI % Requirements
1	Water content	8	≤10
2	Water marine essence content	40	≥7
3	Ethanol soluble essence content	7.6	≥3
4	Total ash content	7	≤15
5	Acid insoluble ash content	0.6	≤1

An assessment of the water content of simplicia powder was performed based on the table above to determine the water content present in simplicia powder. The water content requirement for simplicia is generally no more than 10% because if the water content exceeds 10% it will easily grow mold and bacteria [16]. The results of the characterization examination showed that the water content of simplicia was 8%. Check the water and ethanol soluble essence levels in simplicia powder to see the amount that can be extracted with water and ethanol solvents from simplicia [17].

The water-soluble essence content of senggani leaf simplicia powder was 40%, while the ethanol-soluble essence content was 7.6%, according to the results of the characterisation. To measure the level of inorganic compounds in simplicia, the total ash content of senggani leaf simplicia powder was examined, yielding a total ash content of 7%. The findings of the acid insoluble ash content characterisation were used to determine which compounds in the sample were acid resistant, and an acid insoluble ash content of 0.5% was achieved [18].

3.2 Phytochemical Screening

Phytochemical screening was carried out to determine secondary metabolites of phytochemical compounds contained in senggani leaf plants. The results of the phytochemical screening of senggani leaf powder and extract by looking at the presence of alkaloid, saponin, tannin, flavonoid, steroid/triterpenoid, and glycoside compounds which show the presence of all these compounds are attached in Table 2.

Table 2 Phytochemical Screening of Senggani Leaf Powder and Extract

No	Inspection	Powder Yield	Extract Results
1	Alkoloid	+	+
2	Saponin	+	+
3	Tannin	+	+
4	Flavonoids	+	+
5	Steroids/Triterpenoids	+	+
6	Glycosides	+	+

Information :
+ : contains the substance being examined
- : does not contain the substance examined

Secondary metabolite chemical components found in senggani leaf powder and extract include alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids, and glycosides, as shown in Table 2. When Bouchardat's reagent is added, a blackish-brown precipitate forms, and when Dragendorf's reagent is introduced, a reddish-brown precipitate forms, indicating the presence of alkaloid compounds. The orange color in the separated amyl alcohol layer indicates the presence of flavonoid components, proving that senggani leaf powder and extract contain flavonoid chemical compounds. The existence of saponin components is demonstrated by the height of the foam obtained from senggani leaf powder and extract, which is 2 cm, demonstrating that it exceeded the minimal limit for saponin foam, which is 1 cm. With the use of $FeCl_3$ reagent, the presence of tannin compounds is revealed by the presence of a blackish-green color, indicating that senggani leaf powder and extract positively contain tannin compounds. Furthermore, the production of a green hue suggests the presence of steroid/triterpenoid chemicals, indicating that senggani leaf powder and extract positively contain steroid components. The development of a purple ring with the addition of molish reagent indicates the presence of sugar compounds in senggani leaf powder and extract.

3.3 Antibacterial Test Results

The average results of the anti-bacterial test inhibition zone on the ethanol extract samples of senggani leaves can be seen in Table 3.

Table 3 Test Results of Senggani Leaf Extract Against Bacteria

Bacteria	Sample		U1	U2	U3	Average	Inhibition zone category
Propionibacterium acne	Extract	5%	9.8	9.2	7.4	8.8	Currently
		10%	11.0	9.3	7.5	9.27	Currently
		20%	12.3	11.3	8.5	10.7	Strong
	Control (Tetracycline)	(+)			22.4		
		(-)			0		
Staphlylococcus epidermidis	Extract	5%	8.8	9.9	7.6	8.77	Currently
		10%	8.6	9.2	7.3	8.37	Currently
		20%	8.5	9.1	8.2	8.6	Currently
	Control (Tetracycline)	(+)			21.9		
		(-)			0		

The ethanol extract of senggani leaves possesses antibacterial action against acne-causing bacteria, specifically *Propionibacterium acnes* and *Staphylococcus epidermidis*, according to the test results on 3. The antibacterial efficacy of paper discs in preventing the growth of *Propionibacterium acnes* and *Staphylococcus epidermidis* bacteria was investigated in this study utilizing the agar diffusion method. The reason for selecting this method is because it is quick, easy, and simple to use. Because DMSO has no antibacterial effect against bacteria, it was utilized as a negative control. Dimethyl sulfoxide (DMSO) is an organosulfur molecule that is soluble in a variety of organic solvents and water and can dissolve both polar and nonpolar substances. Aside from that, because DMSO is non-toxic, it will not interfere with observations. Tetracycline was employed as a positive control. Tetracycline has bacteriostatic properties. The method of action of tetracycline is by binding to the 30S ribosomal subunit. Then, by limiting aminoacyl-tRNA access to the acceptor site (A) in the mRNA-ribosome complex, bacterial production is inhibited.

An inhibitory zone against *Propionibacterium acnes* bacteria was found in ethanol extracts of senggani leaves at doses of 5,

10, and 20%. At each concentration, the average diameter of the clear zone created around the paper disc is 5% (8.8 mm), 10% (9.27 mm), and 20% (10.7). Each concentration, notably 5 and 10%, produces a moderate inhibitory action. Meanwhile, the inhibitory reaction is classified as strong at a dose of 20%. The extract created the biggest inhibition zone at a concentration of 20%, then the inhibitory power fell again at a concentration of 5%. At a concentration of 5%, the biggest zone of inhibition is created in *Staphylococcus epidermidis* bacteria. The average diameter of the clear zone generated around the disc paper is 5% (8.77 mm), 10% (8.37 mm), and 20% (8.6 mm), with a mild resistance response. Of the two bacteria, the zone of inhibition was no greater than the positive control, in the *Propionibacterium acnes* bacteria, the clear zone formed was 22.4 mm, and in the *Staphylococcus epidermidis* bacteria, the clear zone formed around the disc was 21.9.

Several active antibacterial compounds contained in this plant which are thought to have antibacterial inhibitory properties include flavonoids, tannins, and triterpenoids. Flavanoids are polar chemicals that dissolve quickly in polar solvents like ethanol, methanol, butanol, and acetone. The most common type of phenolic substance is flavonoids. Phenolic compounds are efficient at suppressing the growth of viruses, bacteria, and fungi [19].

4 Conclusions

According to the findings of this study, the secondary metabolite substances found in senggani leaves are alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids, and glycosides. The results of the antibacterial test research showed that the ethanol extract of senggani leaves at a concentration of 20% showed a strong category with a diameter of 10.7 mm, at a concentration of 10% showed a medium category with a diameter of 9.27 mm, and at a concentration of 5% showed a medium category with a diameter of 8.8 mm. *Propionibacterium acnes* bacteria and for a 20% concentration of *Staphylococcus epidermidis* bacteria it shows a medium category with a diameter of 8.77 mm, at a concentration of 10% it shows a medium category with a diameter of 8.37 mm and at a concentration of 5%, it shows a medium category with a diameter of 8.6 mm.

5 Declarations

5.1 Acknowledgements

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5.2 Author contribution

M. Rasyid Ridho as researcher, collection of library data, Zulmai Rani, and Ziza Putri Aisyia Fauzi preparation of manuscript data and revision. Haris Munandar Nasution as director, supervisor, and manuscript coordinator.

5.3 Conflict of Interest

The authors declare no conflict of interest.

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