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Isolation and Antibacterial Activity of Sembung (*Blumea balsamifera*) Leaf Essential Oil L., DC

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Abstract

Sembung leaf essential oil is a complex mixture in the form of oily yellow liquid with a unique aroma. It contains various volatile components that have antibacterial, antifungal, antioxidant and cytotoxic activities. This study aims to determine the antibacterial activity of essential oil *Blumea balsamifera* L., DC against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Isolation of essential oil using steam distillation method with 0.21% rendement. Inhibition activity was performed by test tube method with variation concentrations of essential oil were 10%, 20%, 40%, 80%, and 100% for each bacteria. Results of this study showed that the highest antibacterial activity on *Escherichia coli* at 80% concentration with an inhibition zone diameter of 27.53 mm, 100% concentration for *Pseudomonas aeruginosa* with an inhibition zone diameter 25.72 mm and 100% concentration for *Staphylococcus aureus* with inhibition zone diameter 27.31 mm. Essential oil from (*Blumea balsamifera*) L., DC leaves have a strong activity inhibition category against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Keywords: Antibacterial activity; Blumea balsamifera; essential oil; Escherichia coli; Pseudomonas aeruginosa; Staphylococcus aureus.

INTRODUCTION

The use of plants as medicine has recently become increasingly popular in the community to treat the diseases they are experiencing. This is supported by the presence of medicinal ingredients from nature that grow in abundance (Ngajow *et al*., 2013). One of the medicinal plants commonly used by the community, especially in West Pasaman, as traditional medicine, is the Sembung plant *(Blumea balsamifera),* which can be found almost throughout the archipelago (Rahardjo, 2018).

Sembung plants are members of the genus Blumea, and family Asteraceae (Compositae). They have traditionally been used to treat rheumatism, menstrual pain, influenza, bloating, bone pain, diarrhea, canker sores, asthma, and angina pectoris (Dewantari *et al*., 2018). The leaf of the Sembung plant is the most commonly used for treatment. The cleaned leaves can be used as medicine, either boiled and then drunk or applied externally (Tari *et al*., 2016).

The phytochemical identification of the Sembung plant content revealed more than 100 metabolites such as dihydro*flavones, flavonoids, sterols, organic acids, monoterpenes, sesquiterpenes, triterpenes, essential oils, alkaloids, steroids, tannins, and glycosides, The volatile oil content of the Sembung plant is 0.5 percent, and it contains Camphor, cineol, borneol, and landerol (Pang *et al*., 2014; Roger *et al*., 2015).

Sembung leaf essential oil is a complex mixture that takes the form of an oily yellow liquid with a distinct aroma and contains a variety of volatile components with antibacterial, antifungal, antioxidant, and cytotoxic properties (Boligon *et al*., 2013). The majority of the essential oil content in Sembung plants is found in the leaves and stem branches (Jiang *et al*., 2014; Putri *et al*., 2020).

According to research on the antibacterial activity of various variations of Sembung extract, essential oil had the strongest antibacterial effect, with a minimum inhibitory concentration (MIC) of 150 mg/mL for *Bacillus cereus* and 1.2 mg/mL for *Staphylococcus aureus* (He *et al*., 2020). The essential oil extracted from Sembung leaves has the potential to damage cell membranes and degrade cell proteins beyond repair. As a result, bacterial cells lack nutrients and their growth is slowed, causing bacteria to die (Pang *et al*., 2014; Ruhimat, 2015).

The purpose of this study was to isolate and investigate the antibacterial activity of Sembung (Balsamifera) essential oil against the growth of *E. coli*, *P. aeruginosa*, and *S. aureus bacteria.*

MATERIALS AND METHODS

Chemical material

The materials used included sembung leaves obtained from West Pasaman, *Escherichia coli* (Gram negative) ATCC 10531, *Pseudomonas aeruginosa* (Gram negative) ATCC 25619, *Staphylococcus aureus* (Gram positive) ATCC 6538, Nutrient Agar media (Merck®Germany), Physiological NaCl 0.9%, Ethanol 70% (Bratachem, Indonesia), Ethanol 96% (Bratachem, Indonesia), Ethyl acetate (Bratachem, Indonesia), KOH (Merck® Germany), HCl (Merck® Germany), NaOH (Merck® Germany), Phenolphthalein (Merck® Germany), Anhydrous sodium sulfate (Merck® Germany), and distilled water.

Equipment

The tools used in this study include analytical balance (Denver-Instrument®), a set of steam distillation apparatus, an incubator (Gallenkamp Plus®), an oven, an autoclave (All American®), an aseptic cabinet, a vortex mixer (Shimadzu® model). vm-100), hot plate (IEC®), refrigerator, calliper (Mitutoyo®), petri dish (Normax®), micropipette (Physiocare concept-Eppendorf research®), loop needle, spritus lamp, pycnometer (Pirex®), and other glassware commonly used in laboratories.

Distillation of Sembung Leaf Essential Oil

Dry samples of Sembung leaves were collected in Kinali, West Pasaman Regency, West Sumatra. By compacting the leaves, the dry sample (20 kg) was placed in a steam distillation apparatus and heated for 6 hours to obtain a distillate mixture of oil and water with three distillations. Anhydrous sodium sulfate was used to bind and separate the water from the essential oil in the resulting oil-water distillate mixture. Allow for 24 hours before filtering and recording the volume of oil obtained (mL) (Widyastuti & Farizal, 2015).

Sembung Leaf Essential Oil Quality Test

Organoleptic Examination

The organoleptic examination includes the shape, colour, and odour of the essential oil produced.

Ethanol Solubility Test

1 mL of essential oil is placed in a tared 10 mL measuring cup, followed by a slow addition of ethanol. Shake the measuring cup after each addition of ethanol. For the first time, a uniform and homogeneous solution is obtained. Keep track of the amount of ethanol you use. Continue to add ethanol until you reach 10 ml.

Specific Weight Calculation

Specific gravity was determined using a pycnometer. Before the essential oil is placed in the instrument, the essential oil must be at the same temperature as the temperature at which the measurement will be made. Measurements were made after the temperature stabilized.

Determination of Acid Numbers

A total of 2 g of essential oil was dissolved in 2.5 mL of neutral ethanol, and then 2-3 drops of phenolphthalein solution were added. Then, it was titrated with 0.1 N KOH until the pink color was steady.

Determination of the Saponification Number

A total of 1 g of essential oil was mixed with 25 mL of 0.5 N KOH solution. Then, it was refluxed in a water bath for 1 hour while shaking frequently. Titrate while hot with 0.5 N HCl and phenolphthalein as an indicator. The end point of the titration is reached when the solution turns cloudy white. Determination of blanks is carried out with the same procedure.

Ester Number Calculation

The ester number is critical in determining the quality of patchouli oil because ester is a component that influences the oil's aroma. The ester number of essential oils is determined by saponifying the esters in an alkaline solution to re-titrate the excess alkali (Saponification Number - Acid Number).

Sembung Leaf Essential Oil Antibacterial Activity Test

The antibacterial activity test of sembung leaf essential oil was carried out by the agar diffusion method using the test bacteria. In a petri dish (Normax®), several cylinders with a diameter of 6 mm were placed. A 1 mL suspension of each bacteria was added to 15 mL of media and placed in a petri dish. After the media solidifies, the cylinder is lifted, thus forming a hole in the media. Sembung leaf essential oil at 10%, 20%, 40%, 80%, and 100% concentrations was placed in the hole and incubated for 24 hours at 37 °C. The results of the observations were indicated by the formation of a clear inhibition area around the hole, and the diameter was measured using a calliper (Mitutoyo®) with three replications (Widyastuti & Farizal, 2015; Septiani *et al*., 2017).

Data analysis

The obtained data were analyzed with one-way ANOVA at a 95% confidence level.

RESULTS AND DISCUSSION

The distillation of 20 kg of Sembung leaves using a water-steam system yielded 42 ml of essential oil with a yield of 0.21%. According to Fakayode & Abobi (2018), the essential oil content of the leaves is not less than 0.19% v/w, so from the calculation of the yield of the Sembung leaf essential oil obtained is concluded that it has good quality.

Sembung leaf essential oil distillation using steam-water distillation yields a yellow liquid with a distinct aroma (typical of sembung). These findings are consistent with previous research (Jiang *et al*., 2014; Putri *et al*., 2020). One of the most important criteria in determining the quality and purity of essential oils is specific gravity. In this study, the average density of Sembung leaf essential oil was 0.9213. (Table 1). Most of the essential oils have specific gravity ranging from 0.696–1.88 (Rompas *et al*., 2016). The specific gravity of the essential oil used in this study falls within this range, indicating that the essential oil is quite good in terms of specific gravity.

Table 1. Results of physical and chemical testing of Sembung leaf essential oil.

No	Type of Test	Outcome
1	Description	liquid
	Color	yellow
	Odor	typical of sembung
2	Specific gravity	0.9213
3	The solubility in Ethanol 96%	Brown solution 1:1
4	The solubility in Ethanol 70%	Brown solution 1:3
5	Acid value	6.171
6	Saponification value	75.735
7	Ester value	69.564

The solubility of sembung essential oil in 96 percent ethanol was 1:1, while the solubility of sembung essential oil in 70 percent ethanol was 1:3. (Table 1). The type of chemical components contained in the essential oil determines its solubility in alcohol. Essential oils, in general, contain a variety of volatile alcohols, aldehydes, ketones, and ethers (Widyastuti & Farizal, 2015).

The acid number is also an important factor in determining the oil's quality (Syamsudin *et al*., 2018). In the distillation of the Sembung essential oil test, the acid number was 6.171. (Table 1). The function of determining the acid number is to determine an oil's acidity index. The acid number of sembung leaf essential oil indicates that the acid content in the oil is still low, implying that the oil does not contain many free acids or fatty acids.

The essential oil of sembung leaf has 75.735 saponifications (Table 1). Because the saponification number is inversely proportional to the average molecular weight of the triglyceride constituent acids, it can be used to detect the presence of triglyceride esters whose fatty acids contain less than 16 carbon atoms or more than 18 carbon atoms (Widyastuti & Farizal, 2015). The ester number can be calculated using the saponification number. The ester number indicates how much alkali is required for ester saponification. The presence of an ester number in the oil indicates that the oil has a pleasant aroma, with Sembung leaf essential oil having an ester value of 69.564. (Table 1). The high number of esters in sembung oil is due to the large number of triglyceride esters that form saponification when an oil is dissolved in ethanol and a base is added. most fatty acids are found in the simplest oils because their building blocks are triglycerides, which are also known as fats, neutral fats, or triglycerides. Triglycerides are glycerol esters with three fatty acid molecules. Triglycerides are insoluble in water but soluble in nonpolar solvents such as chloroform, benzene, and ether. Saponification is the hydrolysis of triacylglycerol by KOH and NaOH, which results in a mixture of K+, Na+, and glycerol. When triacylglycerol is exposed to air, it produces a product that causes taste and odour to deposit in oils, which frequently become rancid (Widyastuti & Farizal, 2015).

The method used to test the antibacterial activity of Sembung essential oil is the diffusion method with a 6 mm diameter well technique against Gram positive bacteria *S. aereus* and Gram negative bacteria *E. coli* and *P. aeruginosa*. The antibacterial activity of sembung leaf essential oil was tested at concentrations of 10%, 20%, 40%, 80%, and 100%. The negative control used was ethyl acetate (Bratachem). The selection of ethyl acetate was because ethyl acetate did not have an antibacterial effect, so it did not affect antibacterial test results. It is also used as a solvent for essential oils.

Hanh *et al*., (2021) identified 50 essential oil constituents in sembung leaves, with the highest content being borneol, caryophylle, ledol, tetracyclo [6,3,2,0,(2.5).0(1.8)tridecan-9-ol, 4, 4-dimethyl]. Borneol is thought to be the active compound of Sembung leaf essential oil which has antibacterial activity. Borneol belongs to the bicyclic monoterpene group which has a phenolic -OH group. Compounds with phenolic -OH groups can damage cell membranes by forming protein complexes through hydrogen bonds (Rompas *et al*., 2016).

The smallest concentration of the sample that is able to inhibit the growth of the test bacteria with the formation of a clear zone is the value of the Minimum Inhibitory Concentration (MIC) of the sample. The MIC levels of essential oil samples against *E. coli*, *P. aeruginosa*, and *S. aureus* bacteria were at a concentration of 10%, with the diameter of each inhibition zone being 21.3150 mm; 22.4550 mm; and 20.8433 mm (Tables 2, 3, and 4). The diameter of the clear zone produced by the three test bacteria differed according to the amount of concentration given.

Table 2. The Average diameter of the inhibition zone of sembung essential oil against *E. coli.*

Concentration %	Inhibition Zone Diameter (mm) ($\bar{x} \pm SD$)
10	20.8433±.73777
20	22.8650±.45632
40	25.0933±2.17097
80	27.5217±.54259
100	24.5250±4.28266

Table 3. Average diameter of the inhibition zone of Sembung essential oil against *P. aeruginosa.*

Concentration %	Inhibition Zone Diameter (mm) ($\bar{x} \pm SD$)
10	22.4550±1.41733
20	23.0800±1.25654
40	22.4283±2.02095
80	24.5167±1.48918
100	25.7183±2.06037

Tabel 4. Average diameter of the inhibition zone of Sembung essential oil against *S. aureus.*

Concentration %	Inhibition Zone Diameter (mm) ($\bar{x} \pm SD$)
10	21.3150±.66006
20	24.0950±1.84031
40	18.6817±1.51319
80	25.6767±2.21854
100	27.3050±1.08316

The highest clear zone (inhibition) in *E. coli* bacteria was at an 80 percent concentration with a diameter of 27.5217 mm, while the lowest clear zone was at a 10% concentration with a diameter of 20.8433 mm (Table 2). The highest clear zone in *P. aeruginosa* was at 100% concentration and had a diameter of 25.7183 mm, while the lowest clear zone was at 40% concentration and had a diameter of 22.4283 mm (Table 3), while the highest clear zone in *S. aureus* bacteria was at 100% concentration. At 40% concentration, the lowest clear zone was 18.6817 mm (Table 4).

The inhibition zone with a small diameter had low antibacterial activity, whereas the inhibition zone with a large diameter had high antibacterial activity. The components of the substances contained in medicinal plants that could weaken, strengthen, improve, or change the inhibition zone caused the large increase and decrease. Furthermore, the quality and quantity of substances found in medicinal plants (Syamsudin *et al*., 2018).

According to research (Syamsudin *et al*., 2018), the antimicrobial activity of medicinal plants against *S. aureus* bacteria had an inhibitory zone value that increased and decreased at different concentrations. Seed components, inoculum size, and bacterial metabolic activity are all factors that influence antibacterial activity (Bujung *et al*., 2017).

This is possible because the test solution has non-polar properties. Because of its low solubility in polar media, increasing the concentration of the test solution used makes it more difficult for the test solution to dissolve or diffuse into polar media. It can also occur as a result of several factors that influence the diameter of the inhibition zone, including the organism's sensitivity, the culture medium, the incubation conditions, and the rate of diffusion (Jamaludin *et al*., 2017).

The following is how these criteria are determined based on the category of inhibition according to (Khan *et al*., 2019): A diameter of less than 10 mm is said not to inhibit the growth of the test bacteria (T), 11-15 mm is considered weak (L), and 16-20 mm is considered moderate. (S) and a diameter greater than 20 mm are classified as strong (K). It can be concluded that the inhibitory power of essential oil from the leaves of the sembung plant against *E. coli*, *P. aeruginosa*, and *S. aureus* is strong.

The diameter of the inhibition zone produced by the essential oil of sembung leaves was found on E. coli and P. aeruginosa with a significance value of P> 0.05, indicating that H0 was rejected and H1 was accepted. This means that, based on the inhibition zone, the essential oil of sembung leaves did not have a significant difference in antibacterial potency. Based on the diameter of the resulting inhibition zone, the significance value of S. aureus P0.05 indicates that H1 is rejected and H0 is accepted. This means that the antibacterial potential of Sembung leaf essential oil varies significantly depending on the inhibition zone.

CONCLUSIONS

The study's findings indicate that the essential oil of sembung *(Blumea balsamifera)* L., DC leaves has antibacterial potential against *E. coli*, *P. aeruginosa*, and *S. aureus* with strong inhibition criteria.

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

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