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Phytochemical Screening and Antibacterial Test of Bay Leaf Extract (*Syzigium polyanthum* (Wight) Walp.) Against *Streptococcus mutans* Bacteria

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Abstract

Background: Dental caries is a dental health problem that is often found in Indonesian society and is a major concern. Caries is a condition that affects the hard tissue of the teeth, starting with a gradual demineralization process, followed by damage to the organic components of the teeth. Streptococcus mutans is the main agent causing dental caries. Bay leaves are popular among Indonesian people as medicinal plants. Research shows that bay leaves contain secondary metabolites that act as antibacterials. **Objectives:** The purpose of this study was to determine the results of phytochemical screening and antibacterial tests of bay leaf extract against *Streptococcus mutans* bacteria. **Methods:** The study was conducted as a true experimental laboratory with a post-test only control group design. Bay leaves were extracted by maceration using 70% ethanol solvent. The extract obtained was then subjected to a phytochemical screening test. Antibacterial activity tests were carried out using the well diffusion method. This study used 3 concentrations, namely 50%, 75% and 100%. Negative control using sterile distilled water. **Key findings:** The results of the study showed that based on the results of phytochemical screening, bay leaf extract contains secondary metabolites of wagner alkaloids, dragendroff alkaloids, steroids, saponins, tannins and essential oils. Bay leaf extract has no antibacterial effect on Streptococcus mutans. **Conclusions:** The conclusion in this study is that the antibacterial test of bay leaf extract showed negative results with no inhibition zone on the growth of *Streptococcus mutans*.

Keywords: Antibacterial, Bay leaf extract, *Streptococcus mutans*

Introduction

Health problems related to dental and oral health continue to increase in Indonesia. The problem of dental caries is a dental health problem that is often found among Indonesian people and is a major concern. Caries is a condition that affects the hard tissues of the teeth, starting with a gradual demineralization process, followed by damage to the organic components of the teeth, which can actually be prevented. This disease is characterized by chemical dissolution and damage to the hard tissues of the teeth due to acidic substances caused by the process of biofilm metabolism that coats the surface of the teeth [1,2]. Caries is not only caused by one factor, but is caused by a combination of hosts, microorganisms, substrates, and time [3].

The Global Oral Health Status Report (2022) estimates that around 3.5 billion individuals worldwide face dental and oral health problems. Among these numbers, around 2 billion individuals have caries problems in permanent teeth, while 514 million children worldwide have caries in deciduous teeth [4]. Based on the 2023 Indonesian Health Survey (SKI), the prevalence of dental and oral problems in Indonesia, especially those related to decayed, cavities, or painful teeth, reached 43.6%. In Central Java Province, the prevalence of similar dental problems in 2023 is 42.8% [5].

Gram-positive bacteria *Streptococcus mutans* are often found in the human oral cavity and have a significant role in the formation of dental caries. These bacteria produce lactic acid through the process of glycolysis sucrose, which results in the demineralization of enamel. In addition, Streptococcus mutans contributes to the initial colonization process of dental plaque [6]. *Streptococcus mutans* belongs to the group of anaerobic, gram-positive bacteria, with a spherical shape ranging in size from 0.5 to 0.7 microns, and has the ability to form chains during its growth process [7].

Indonesia has about 30,000 species of plants, of which about 2,500 are used as medicinal plants. One of them is bay leaves, which are increasingly popular among the public as a medicinal plant. The use of bay leaves as a medicine has been going on for a long time, and has even become an important ingredient in the field of dentistry. Bay leaf, also known by the scientific name *Syzygium polyanthum* (Wight) Walp or *Eugenia polyantha* Wight, is a medicinal plant that has antimicrobial properties [8].

In Indonesia, bay leaves are widely used because of their affordable price, easy to obtain, and often used as an additional ingredient in cooking. The bay plant also has benefits as an herbal medicine. The chemical content in bay

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leaves includes 0.05% tannins, flavonoids, saponins and essential oils such as eugenol and citral. The active ingredient of *Syzygium polyanthum* (Wight) Walp is thought to have pharmacological effects. Essential oils are known to have analgesic properties, while tannins, flavonoids and saponins show anti-inflammatory and antibacterial activity [8]. Based on this thought, the author is interested in researching phytochemical screening and antibacterial tests of bay leaf extract (*Syzygium polyanthum* (Wight) Walp.) against *Streptococcus mutans* bacteria.

Methods

This type of research is true experimental laboratory with a research design using a post-test only control group design. The population in this study is Streptococcus mutans which is bred and available in the laboratory, while the sample in this study is *Streptococcus mutans* which is placed in several petri dishes which are treated with bay leaf ethanol extract with a concentration of 50%, 75%, 100%, and control using sterile aqueducts. This study uses a sampling technique in the form of a simple random sampling technique.

Tools and Materials

The tools used in this study are petri dishes, autoclaves, erlenmeyer tubes, test tubes, calipers, incubators, bunsenes, vortexes, measuring cups, ose, cork borers, spreaders, vacuum rotary evaporators, micropipettes, hot plate magnetic stirrer and laminar air flow. The materials used in this study were Streptococcus mutans ATCC 25175, bay leaf ethanol extract, 70% ethanol, dragendroff, NaCl 0.9%, sterile aqueducts, MHAA media, McFarland 0.5 suspension, spirtus, masks, handscoons, markers, and filter paper.

The Course of Research

Sterilization of the appliance

The sterilization process of research equipment and materials is carried out by wrapping the equipment first. Sterilization uses an autoclave with a pressure parameter of 15 atm, heated to a temperature of 121ºC for a time interval of 15 minutes [9].

Bay Leaf Determination Test

Bay leaf determination to know and confirm that the bay leaf used is a species of *Syzygium polyanthum*.

Main Ingredient Preparation

A total of 2 kg of young bay leaves are washed. Furthermore, the leaves are dried without direct exposure to sunlight for 7 days. After the drying process is complete, the bay leaves are blended until smooth so that bay leaf simplicia is obtained [10].

Manufacture of Bay Leaf Ethanol Extract

Simplicia powder is extracted by the maceration method using 2.5 liters of solvent for 3x24 hours in a ratio of 1:10. On the first day, the researchers soaked the simplicia in 1.2 liters of 70% ethanol. Then on the second day with 700 ml of solvent. On the third day with 600 ml of solvent. Then, separate the mafiber from the solvent using a vacuum rotary evaporator at a temperature of 60°C [11].

Bay Leaf Phytochemical Screening Test

Flavonoid identification

A sample of 70% ethanol extract of bay leaves as much as 2 ml was dissolved in 2 ml of methanol, then added Mg powder and 5 drops of concentrated HCl. The formation of red or orange indicates the presence of flavonoid compounds.

Tannin identification

A total of 2 ml of bay leaf extract is dissolved in 10 ml of aqueduct, then heated for 5 minutes and filtered. The filtrate obtained is plus 4-5 drops of FeCl3 2.5 %. The presence of tannins occurs a change in color to dark blue or greenish-black [12].

Identification of essential oils

A total of 1 mL of the test solution is evaporated on a porcelain cup until only residue remains. The presence of essential oils is indicated by a special odor.

Saponin identification

Mix 2 mL of extract and 10 mL of aquadest, then whisk for 30 seconds. Stable foam formation (does not disappear in 30 seconds) indicates the presence of saponins.

Identification of terpenoids and steroids

Mix 2 mL of extract (from each solvent) with 10 drops of acetic acid and 2 drops of sulfuric acid. The formation of a red or purple color indicates the presence of terpenoids, while the green color indicates a steroid [13].

Identification of alkaloids

In the first tube, 2 to 3 drops of Wegner reagent are added, while in the second tube, 5 drops of Dragendorff reagent are given. Positive results of the alkaloid test were indicated by reddish-brown deposits in the Wagner test and red or orange deposits in the Dragendorff test [14].

Dilution of Extracts

Bay leaf ethanol extract at 100% concentration will be diluted using sterile aqueducts to obtain three concentrations, namely 50%, 75%, and 100% with calculation %= x 100b/(v)

Description:
% : Variation in the concentration of bay leaf ethanol extract in percent
b : Mass of bay leaf extract (100%)v : Total volume of dilution

Concentration 50%
Weighing as much as 5 grams of extract then dissolved with sterile aqueducts up to 10 ml.

Concentration 75%
Weighing as much as 7.5 grams of extract then dissolved with sterile aqueducts up to 10 ml.

100% Concentration

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Weighing as much as 10 grams of extract is then dissolved with sterile aqueducts up to 10 ml.

Mueller Hinton Agar Media Manufacturing Techniques (MHA)

Weigh the MHA media, then put it in the erlenmeyer pump. Sterile Aquades as much as 315 ml is then added to the pumpkin. The solution is then heated on a hotplate while stirring with a magnetic stirrer until it reaches a boiling point and is evenly mixed. After that, put the media in an autoclave, cover it until the temperature inside can gradually increase until it reaches a temperature of 121°C and leave it at that temperature for 15 minutes. Then, remove the MHA media from the autoclave and pour as much as 15 ml into each sterile petri cup and let it harden.

Creation of Mc. Farland Turbidity Standards

Using the standard concentration of Mc. Farland turbidity 0.5 by preparing 0.05 ml of BaCl2, then mixing it with 9.95 ml of 1% H 2SO4 solution. Next, the mixture is shaken until homogeneous and produces a noticeable turbidity [6].

Bacterial Suspension Manufacturing Techniques

Using sterile ose, take the incubated test bacterial colony from agar media, then insert it into a 0.9% NaCl solution contained in a sterile test tube. Then homogenization was carried out using a vortex for 15 seconds. Next, observe the turbidity by comparing it to a standard turbidity of 0.5 Mc Farland (equivalent to 3x108 CFU/mL) [6].

Bacterial Inhibition Test

Prepare 24 petri dishes containing MHA media. Make one well hole using a cork borer with a thread diameter of 6 mm. Streptococcus mutans suspension is applied to the surface of the media in a zig-zag manner with a sterile cotton swab. Drop 20 µl of bay leaf extract with concentrations of 50%, 75%, and 100%, and sterile aqueducts as negative control, then insert each one into the sink hole. Then incubation is carried out for 24 hours at a temperature of 37°C. After the incubation process is complete, the clear zone formed around the wells is measured using calipers [15].

Measurement of Bacterial Inhibition Zone

The determination of the inhibition force is carried out by measuring the diameter of the inhibition zone, which is the area clear or clear areas that do not show the growth of microorganisms around the subsum. Measurements were made using calipers through the three-line withdrawal method, namely two lines that are perpendicular to each other and pass through the center of the sump hole, and one additional line that forms a 45° angle between them. Each measurement is repeated three times at different points. The first, second, and third measurement data were then averaged, so that the barrier zone data for the first petri dish was obtained. The same method is applied to each petri dish whose resistance zone will be measured.

Data Analysis

After laboratory research was carried out on the test material in the form of bay leaf extract using the well diffusion method to test the antibacterial activity against *Streptococcus mutantans* bacteria, observations were made to determine whether or not there is an inhibition zone formed around the well as an indication of antibacterial activity. The data is presented in the form of a table.

Results and Discussion

Determination Results

The plant determination process in this study shows that the bay leaf plant used is the *Syzygium polyanthum* (Wight) Walpers type.

Extraction Results

The process of making this extraction uses 250 grams of simplicia powder. The extraction results were obtained as much as 255.5 grams.

Phytochemical Screening Test Results

The results of phytochemical tests of bay leaf extract (*Syzygium polyanthum* (Wight) Walpers) in this study are qualitatively presented in Table 1.

Antibacterial Activity Test Results

Testing of the antibacterial activity of bay leaf extract against Streptococcus mutans bacteria was carried out using the sewage diffusion method at concentrations of 50%, 75%, and 100%, with sterile aqueducts as negative control. The results of the measurement of antibacterial activity are presented in Table 2. In the results of the above test, the results were obtained that bay leaf extract at concentrations of 50%, 75%, and 100% and negative control of sterile aqueducts did not have an antibacterial effect against Streptococcus mutans. This is evidenced by the absence of the diameter of the barrier zone around the wells that contain bay leaf extract in various concentrations and sterile aqueducts.

Description of Extraction Results

This study used bay leaves as samples. A total of 2.5 liters of ethanol 70% is used to extract 250 grams of dried simplicia powder through the maceration method. After the filtration process, the resulting fiber is evaporated using a vacuum rotary evaporator. The extraction of bay leaf simplicia is carried out using the maceration method with

70% ethanol solvent. The selection of 70% ethanol as a solvent is based on the grounds that its polarity properties allow this solvent to attract a wide variety of compounds, both polar and non-polar. The higher the ethanol content, the less polarity it will be. A solvent with a level of polarity similar to the extracted substance will be more effective in attracting and dissolving the substance. Simplicia smoothing aims to enlarge the surface area of the particles, so that the contact between simplicia and solvents increases. This makes it easier for the solvent to penetrate the structure of the simplicia and allows the extraction of

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larger amounts of active compounds. This study uses the maceration method for extraction, because this technique does not involve heating, so temperature-sensitive chemical compounds (thermobilis) are maintained and do not suffer damage [16,17].

Table 1. Phytochemical Screening Test Results

Parameter	Content	Remarks
Alkaloid Wagner	+	Positive when Orange-Red Deposits Form
Alkaloid Dragendroff	+	Positive when Orange-Red Deposits Form
Flavonoid	-	Positive if there is a change in color Pink - red
Steroids	+	Positive if there is a change in color to Green
Terpenoid	-	Positive if there is a change in color red - purple
Saponin	+	Positive when Foam Forms
Tanin	+	Positive if there is a change in blue/purplish blue color
Essential oils	+	Positive when there is a special smell

Table 2. Growth inhibition zone diameter measurement results *Streptococcus mutans* from bay leaf extract

Extract Concentration	Inhibition zone (mm)						Average zone Inhibition (mm)
	R1	R2	R3	R4	R5	R6	
Control -	0	0	0	0	0	0	0
50%	0	0	0	0	0	0	0
75%	0	0	0	0	0	0	0
100%	0	0	0	0	0	0	0

Description : R1: Replication 1, R2: Replication 2, R3: Replication 3, R4: Replication 4, R5: Replication 5, R6: Replication 6, Control (-): sterile aqueducts

Phytochemical Screening Results

The results of phytochemical screening in this study revealed that bay leaf extract contains the alkaloid compounds wagner, dragendroff, steroids, saponins, tannins and essential oils. Meanwhile, compounds from the flavonoid and terpenoid groups showed negative results. The results of this study show a difference compared to research that has been conducted by Siagian et al. [18], showing that bay leaf extract contains flavonoids, glycosides, saponins, tannins, and triterpenes/steroids.

Alkaloid tests with wagner reagents gave positive results indicated by the formation of reddish-brown deposits, which indicated the presence of potassium-alkaloids. There are free electron pairs in nitrogen that make up alkaloid compounds and in Wagner reagents contain iodine and potassium iodide. The nitrogen atoms in alkaloid compounds have free electrons that can bond coordinate covalent with the metal ions K^+ of potassium tetraiodomercurate (II), the main component of the Wagner reagent of the reddish-brown complex precipitate between potassium and alkaloids [14].

Steroid tests on bay leaf extract with the addition of 10 drops of CH3COOH and 2 drops of H2SO4 showed positive signs of steroid presence indicated by a change of color to green. Steroid tests give positive results due to the presence of a condensation process followed by binding with carbocation. This process begins with the release of the hydrogen group and its electrons, causing a shift in the double bond and resonance, producing electrophiles and carbocations that then attack through electrophilic additions which are then accompanied by the release of hydrogen atoms, so that the hydrogen group and its electrons are released, prolonging the conjugation in the compound, thus forming a red ring [19].

The saponin test is done by mixing the extract into 10 mL of aquadest, then whipping for up to 30 seconds and leaving for a while. The sample showed a positive result containing saponins due to the formation of foam that did not disappear within 30 seconds. The presence of polar and nonpolar groups in a compound causes the active properties of the surface. In saponins, this property causes the formation of micelles when shaken with water, where the polar parts will interact with the water, while the nonpolar parts will interact with each other on the inside, resulting in a foam-like appearance [13].

A positive tannin test is indicated by the formation of a dark blue color after the addition of FeCl3 solution, which indicates the presence of hydrolyzed tannin content. This

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color change results from the chemical interaction between FeCl3 and the hydroxyl group in tannin compounds. The interaction between tannins and Fe^{3+} ions results in the formation of complex compounds that cause such discoloration [13].

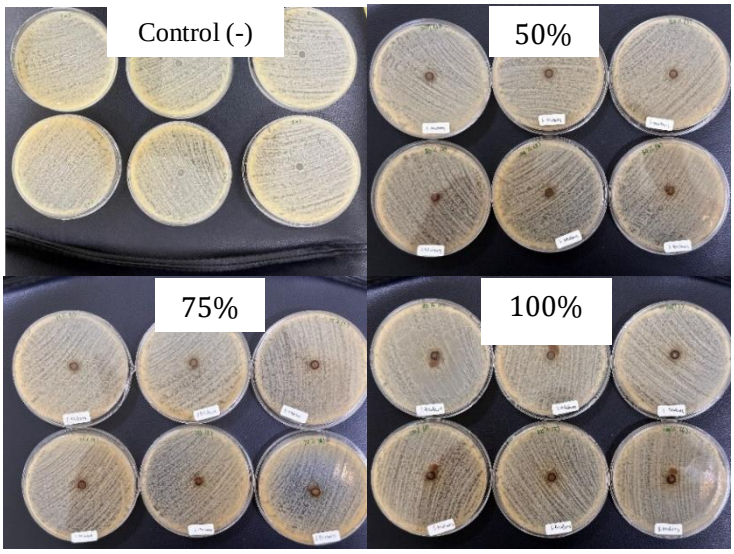

Figure 1. The test results of bay leaf extract against *Streptococcus mutans bacteria* concentration of 50%, 75%, 100% and negative control

The essential oil test gave a positive result indicated by the distinctive smell produced by the residue of the extract which had been compared with the essential oil of pure bay leaf.

A negative flavonoid test is indicated by not experiencing a discoloration of the sample extract to pink or red. The terpenoid test is negative because the extract does not undergo a color change to red or purple. In this study, the sample did not show positive results because variations in topography or the location where the plant grows can affect the amount of its secondary metabolite content. One of the environmental factors in question is the change or difference in the height of the growing location. The higher a place, the greater the environmental pressure experienced by plants. Under such stressful conditions, plants tend to increase the production of secondary metabolites [20].

Antibacterial Activity Test Results

The results of the antibacterial activity test of bay leaf extract at concentration variations of 50%, 75%, 100%, as well as negative control repeated six times, showed that this extract did not show antibacterial activity against the growth of *Streptococcus mutans.* The inability of the extract to inhibit bacterial growth is characterized by the absence of an inhibition zone around the pores. These results prove that bay leaf extract is not effective in inhibiting the growth of *Streptococcus mutans*. This is not in line with the research conducted by Haerunnisa et al. [15], which showed that bay leaf extract with concentrations of 4%, 6%, 7.33%, 8%, and 10% is able to inhibit the growth of *Streptococcus mutans* bacteria with weak to moderate activity and produces a significant inhibition zone for *Streptococcus mutans* bacteria at concentrations of 10%, 30%, 60%, and 90% [21].

Bay leaf extract is supposed to have antibacterial activity. However, in this study, bay leaf extract did not show the ability to inhibit the growth of *Streptococcus mutans* bacteria. The absence of antibacterial activity from bay leaf extract is suspected to be influenced by several factors, namely:

The virulence factor of the test bacteria is suspected to affect the results of testing the antibacterial activity of bay leaf extract. The cell wall structure of gram-positive bacteria, such as *Streptococcus mutans*, consists of thick, rigid peptidoglycans with tight peptide bonds between glycan chains, which makes it difficult for compounds to penetrate from the outside. Gram-negative bacteria have a thinner layer of polysaccharides and peptidoglycans, and the outer layer is made up of lipoproteins, phospholipids, and lipopolysaccharides. This is in accordance with studies conducted by Maarisit et al. [22] and Hendy et al. [23], which showed that garlic palmitic acid (*Allium sativum*) at concentrations of 0.5%, 2%, 4%, 8%, and 12% had no antibacterial power at the concentrations tested against *Streptococcus mutans* ATCC 25175.

Uncontrolled growth factors in bay leaves, such as the type of fertilizer, watering frequency, light intensity, and other factors, can affect the content of antibacterial substances in the sample. The low levels of these antibacterial substances are not strong enough to damage cell membranes or interfere with the physiological function of bacteria. Therefore, the extract is not able to form an inhibition zone on the growth of *Streptococcus mutans* [24].

In addition to the above factors, the possibility of resistance also needs to be considered. This study used *Streptococcus mutans* ATCC 25175 as the test bacterial strain. Such strains of bacteria are based on having the ability to form biofilms. The ability to form biofilms increases the persistence of caries, resulting in high antimicrobial resistance and a lacking immune response. Biofilm is a complex mixture of extracellular polysaccharides (EPS), and the microbial communities that

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develop on the surface of the tooth play a significant role in the occurrence of dental caries. Research indicates that Streptococcus mutans shows resistance to the antibiotics amoxicillin and cefritriason [25-27]. The study reported that Streptococcus mutans bacteria showed a resistance rate of 50% to the antibiotics cefotaxime, tetracycline, levofloxicocine, while resistance to chloramphenicol antibiotic resistance has reached 100% [28]. These antibiotics work through mechanisms of inhibition of cell wall synthesis and protein synthesis in bacteria, which have similar mechanisms of action to most of the secondary metabolites found in bay leaf extract.

Conclusion

Based on the results of the study, it can be concluded that the phytochemical screening test of 70% ethanol extract of bay leaf (*Syzygium polyanthum* (Wight) Walpers) has secondary metabolites of wagner alkaloids, dragendroff alkaloids, steroids, saponins, tannins and essential oils. Antibacterial tests of bay leaf extract showed negative results in the absence of an inhibition zone on the growth of *Streptococcus mutans*.

Supplementary Material

None

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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