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Antibacterial Activity of Ethanol Extracts from Sembung Leaves (Blumea Balsamifera) and Purple Leaves (Graptophyllum Pictum) against *Streptococcus Mutans*

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Abstract

Streptococcus mutans is a gram-positive bacterium associated with infectious disorders such as dental caries, cerebral microbleeds, IgA nephropathy, and atherosclerosis. Antibiotics such as amoxicillin and chloramphenicol are employed to address infections induced by this bacterium. The indiscriminate use of antibiotics can lead to bacterial resistance. A potential alternative for this problem is the utilization of herbal medicine sourced from plants like Sambong leaves (Blumea balsamifera) and Caricature leaves (Graptophyllum pictum). This study aims to find out what phytochemicals were in ethanol leaf extract combinations and how well they worked as an antibacterial against S. mutans. This study employs a quasi-experimental design with a testing phase, which involves the formulation of ethanol extracts from Sambong and Caricature leaves in ratios of 3:1, 1:1, and 1:3, as well as phytochemical analyses and antibacterial assessments against S. mutans FNCC 0405. The phytochemical analysis revealed that the two ethanol extracts of the leaf contain flavonoids, saponins, tannins, and phenols. The antibacterial test showed that the inhibitory zones made by sambong and caricature leaf ethanol extracts mixed at 3:1, 1:1, and 1:3 ratios were 4 mm (plus or minus 0 mm), 3.08 mm (plus or minus 0.38 mm), and 1.46 mm (plus or minus 0.71 mm). The positive control, chloramphenicol, had an inhibitory zone diameter of 26.75 ± 0.25 mm. The Kruskal-Wallis test findings indicate a significant difference among the three extract combinations and the positive control ($p<0.05$). The research concludes that the mixed ethanol extract exhibits minimal antibacterial action.

Keywords: *antibacterial, Blumea balsamifera, Graptophyllum pictum, Streptoccocus mutans*

INTRODUCTION

Streptococcus mutans is a bacterial species found in the oral cavity.[1,2] This bacterium is spherical, non-sporulating, immobile, organized in chains, and has a diameter of 0.5-0.7 microns.[3] *Streptococcus mutans* is a gram-positive bacterium with cell walls made up of 40–80% peptidoglycan and as many as 40 layers. In gram-positive bacteria, peptidoglycan is linked to teichoic acid through covalent bond. Teichoic acids possess hydrophilic characteristics and serve as transport mediums for cations across cell membranes.[4]

S. mutans bacteria can cause infectious illnesses, particularly in dental structures, leading to caries formation.[5] This bacterium can induce dental caries by synthesizing substantial quantities of extracellular glucan polymers from sucrose, facilitating bacterial colonization on the hard surfaces of teeth and fostering a conducive bacterial environment. It possesses the capability to transport and metabolize a diverse array of carbohydrates into organic acids (acidogenicity) and can thrive under environmental stress conditions, particularly low pH (acidogenicity). While *S. mutans* is not solely responsible for the onset of dental caries, it can alter the local environment by creating a milieu abundant in extracellular polysaccharides (PSE) and characterized by low pH. This environment will be conducive for the proliferation of other acidogenic and aciduric species. In addition to inducing dental caries, *S. mutans* can also lead to sub-acute bacterial endocarditis, cerebral

microbleeds, IgA nephropathy, and atherosclerosis.[1]

Antibiotics such as amoxicillin and chloramphenicol are generally used to treat infections caused by *S. mutans*.[6,7] Amoxicillin works by inhibiting bacterial cell wall synthesis.[8] Meanwhile, chloramphenicol works by inhibiting bacterial protein synthesis.[9]

Antibiotics can induce several adverse effects on the body, including allergies, nausea, vomiting, and diarrhea.[10–12] The irrational use of antibiotics can lead to bacterial resistance. This resistance causes a medicine ineffective against bacteria, resulting in heightened bacterial resistance to that specific antibiotic.[13] Therefore, we need a herbal remedy composed of natural ingredients that can serve as an alternative to these pharmaceutical treatments.

Sembung leaf (*Blumea balsamifera*) and purple leaf (*Graptophyllum pictum*) are therapeutic herbs with possible antibacterial properties against S. mutans bacterium. This is due to the presence of secondary metabolites in sembung and purple leaves, including flavonoids, saponins, tannins, and phenols. The content of secondary metabolites is recognized for its antibacterial effect against *S. mutans*. [14,15]

Jayadewata (2022) conducted research on the effectiveness of combining sembung and purple ethanol extracts as an antibacterial agent against *S. aureus*. When the ethanol extract ratios of sembung and purple leaves were 1:3, 1:1, and 3:1, the study found that the two extracts together had average inhibitory powers of 14 mm, 16 mm, and 12 mm. According to this study, the ethanol extract of sembung leaves had an average inhibition zone of 9.8 mm. On the other hand, the purple leaf extract did not kill *S. aureus* bacteria.[16]

A study by Polakitan (2017) using sembung leaf ethanol extract as an antibacterial agent against S. mutans revealed an average inhibition zone diameter of 15 mm. The average diameters of the inhibition zone of purple leaf ethanol extract against S. mutans for concentrations of 2%, 3%, and 4% were 12.60 mm, 13.73 mm, and 14.82 mm, respectively.[17] The average diameter is into the robust group.[18]

The data indicates that sembung leaves and purple leaves have antibacterial activity against S. mutans. Nonetheless, investigations into the antibacterial efficacy of the combined extracts from the two varieties of leaves have not been conducted. A combination of two distinct extracts could result in a more extensive zone of inhibition than one particular extract.[19] Thus, it is necessary to investigate the potential antibacterial activity of the combination of sembung and purple leaf extracts against *S. mutans*.

METHOD

This study used a quasi-experimental design methodology. The study was conducted in the Research Laboratory of the Faculty of Medicine and Health Sciences and the Research Laboratory of the Faculty of Agriculture, both at Warmadewa University, Denpasar, Bali. For this study, we used sembung leaves, purple leaves, 96% ethanol, a blender, mesh 20, a rotary evaporator, Whatman No. 42 filter paper, a glass beaker, a tray, an oven, a jar, aluminum foil, a tube, a Bunsen burner, a cotton swab, an inoculum tube, a vortex mixer, a petri dish, a blank disc, micropipette tips, a micropipette, an Erlenmeyer flask, tweezers, a tube rack, a caliper, a chloramphenicol disc, an amoxicillin disc, a pure culture of Streptococcus mutans FNCC 0405 bacteria, and LB media (which contain yeast extract, sodium chloride, casein enzyme hydrolysate, and agar).

Specimens of sembung leaves and purple leaves were collected from the Peguyangan region, North Denpasar, Denpasar City, Bali, at an elevation of 500 meters above sea level. Sampling is conducted by taking into consideration the growth and geographical

locations of the plant. The collected samples meet the following criteria: undamaged young leaves, free from insect infestation, and not wilted.

Preparation of Extracts

The extraction process starts with the collection of fresh sembung and purple leaves. The leaves are rinsed with running water until free of dirt and other organic matter. The leaf desiccation process was conducted for 24 hours in an oven at 50 °C to eliminate the moisture content from the two leaves. The dried sembung and purple leaves were pulverized in a blender and filtered through a 20-mesh screen to yield a fine powder.[16]

The fine powder obtained was then extracted using the maceration method with 96% ethanol as the solvent. The ratio between fine powder and ethanol is 1:8. After mixing the fine leaf powder and ethanol in a jar, close the container tightly and let it sit at a stable temperature for 3x24 hours. Then, using a stir stick, stir every 24 hours. After 3x24 hours, filtering was carried out using Whatman No. filter paper. 42. The filtrate obtained was then evaporated using a vacuum rotary evaporator at a speed of 100 rpm and a temperature of 50 °C. The thick extract resulting from the evaporation process will be weighed using an analytical balance to determine the amount of extract produced.[14,16]

Phytochemical Screening

Phytochemical screening of extracts was carried out using a qualitative method using the following color reaction:

a. Flavonoids
   The analysis of flavonoid components in sembung and violet leaf extracts involved taking 2 ml of each dissolved sample and heating it for roughly 5 minutes in a test tube. Subsequently, 0.1 gram of magnesium metal and 5 drops of strong hydrochloric acid were introduced after heating each component. If the solution exhibits a yellow-orange to red color, it indicates a positive presence of flavonoids.[20,21]
b. Saponins
   The analysis of saponins was conducted by dissolving the extract in 10-20 mL of distilled water and stirring for 15 minutes. The formation of a 1 cm layer of foam signifies the presence of saponin. [20,21]
c. Tanin
   The analysis of tannin compounds involved collecting 2 cc of the dissolved sample and heating it in a test tube for roughly 5 minutes. Subsequently, add three to four drops of 1% FeCl3. If each solution exhibits a greenish-brown or blackish-blue hue, it indicates a positive presence of tannin. [20,21]
d. Fenol
   The analysis of phenolic components was conducted by combining 2 ml of extract with 3-4 drops of 1% ferric chloride (FeCl3) solution in a test tube. The blue-black hue indicates the presence of phenol. [20,21]

Antibacterial Activity Test

The antibacterial activity test of the ethanol extract from sembung and purple leaves was conducted aseptically using the Kirby-Bauer method in a laminar airflow. This procedure commences with the preparation of Luria Bertani (LB) media. Ten grams of Luria Bertani agar were dissolved in 250 mL of sterile distilled water within an Erlenmeyer flask and subsequently sterilized for 15 minutes at 121 °C. Subsequently, transfer the fluid into a petri dish and allow it to cool.[22]

The next phase entails preparing a bacterial suspension. Firstly, a colony of S. mutans FNCC-0405 bacteria with a loop is put into a test tube containing physiological NaCl. The tube is then left to sit at 37°C for 24 hours. The incubation results are subsequently diluted to 1.5 x 10^8 CFU/ml (CFU: Colony Forming Unit) or to the

standard concentration of 0.5 McFarland. Two hundred microliters of Streptococcus mutans were suspended in pre-prepared LB media and disseminated using a sterile cotton swab.[22]

As a positive control, chloramphenicol and amoxicillin were used, along with ethanol extracts from a mixture of sembung leaves and purple leaves at three different concentration ratios (3:1, 1:1, and 1:3), as well as extracts of sembung leaves and purple leaves separately. Twenty microliters of negative samples were applied to each sterile paper disc with a diameter of 6 mm, resulting in seven test groups.[6,9,16] The paper discs were subsequently positioned over the pre-prepared LB media and incubated at 37°C for 24 hours. Each test was conducted in triplicate.[22] The clean zone surrounding the disc was subsequently measured with a caliper to assess the inhibitory efficacy of the bacteria present. The interpretation of antibacterial inhibition zone test findings is categorized into four groups according to the dimensions of the inhibition zone. The inhibitory zone is classified as weak if its diameter measures 0–5 mm. The inhibitory zone is classified as moderate if its diameter ranges from 5 to 10 mm. An inhibitory zone is considered strong if it measures between 10 and 20 mm. If the inhibitory zone is ≥20 mm, it is considered very strong.[18]

RESULT

During the extraction phase of sembung leaves utilizing 96% ethanol solvent via the maceration technique, 4.16 grams of concentrated extract were obtained from 80.66 grams of finely powdered sembung leaves, resulting in a yield of 5.2%. The extraction of purple leaves yielded 2.57 grams of concentrated extract from 84.277 grams of purple leaf powder, resulting in a yield of 3%, as indicated in Table 1 below.

Table 1. Extraction results of sembung leaves and purple leaves

Sample Type	Berat Sampel	Extract Weight	Rendement
Sembung leaves	80,66 g	4,16 g	5,2%
Purple leaves	84,27 g	2,57 g	3%

The secondary metabolite groups or active compounds tested in this study were tannins, flavonoids, saponins, and phenols. The results of phytochemical screening of sembung and purple leaf extracts showed that they contained tannins, flavonoids, phenols, and saponins (Tables 2 and 3). This can be proven by the presence of color changes and the formation of precipitates in the tested solution (Figures 1 and 2).

Table 2. Results of phytochemical screening of sembung leaves

Parameter	Reagent	Colour	Sembung Leaf Ethanol Extract		
			Test 1	Test 2	Test 3
Flavonoids	Mg and HCL	Yellow Orange	+	+	+
Saponins	Aquadest	Foamy	+	+	+
Tannins	$FeCl_3$ 1%	Greenish brown	+	+	+
Phenols	$FeCl_3$ 1%	Bluish Black	+	+	+

Table 3. Results of phytochemical screening of purple leaves

Parameter	Reagent	Colour	Purple Leaf Ethanol Extract		
			Test 1	Test 2	Test 3
Flavonoids	Mg and HCL	Yellow Orange	+	+	+
Saponins	Aquadest	Foamy	+	+	+
Tannins	$FeCl_3$ 1%	Greenish brown	+	+	+
Phenols	$FeCl_3$ 1%	Bluish Black	+	+	+

Information
(+): There are active compounds

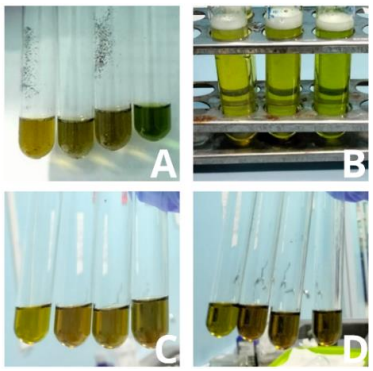


Figure 1. Phytochemical test results of ethanol extract of sembung leaves A: flavonoids, B: saponins, C: tannins, and D: phenols

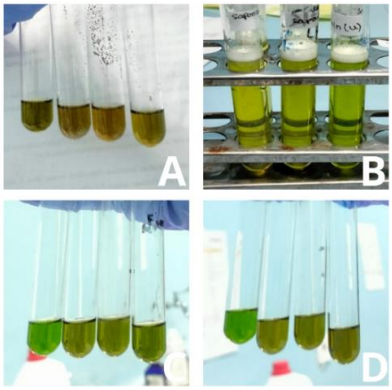


Figure 2. Phytochemical test results of ethanol extract of purple leaves A: flavonoids, B: saponins, C: tannins, and D: phenols

Researchers evaluated the efficacy of the sembung leaf ethanol extract and the combination ethanol extract against *S. mutans* FNCC 0405 to determine their bactericidal properties. Both exhibited a region where bacterial growth was inhibited (Figure 3 and Table 4). The ethanol extract of sembung leaves has an average bacterial inhibition zone diameter of 5.83 ± 0.07 mm, indicating that it is moderately antibacterial. In mixtures of 3:1, 1:1, and 1:3, the ethanol extract of sembung and purple leaves showed average inhibitory zone diameters of 4 ± 0 mm, 3.08 ± 0.38 mm, and 1.46 ± 0.71 mm. The three combinations of ethanol extracts are classified as exhibiting poor antibacterial activity. The results indicate that the singular extract of sembung leaves exhibits superior antibacterial activity compared to the combined ethanol extracts of sembung and purple leaves. Nonetheless, the mean diameter of the inhibition zone remains significantly lower than that of the positive control, chloramphenicol (26.75 ± 0.25 mm), which is classified as exhibiting very strong antibacterial activity. At the same time, neither the purple leaf ethanol extract nor the amoxicillin-positive control could stop the *S. mutans* FNCC 0405 bacteria.

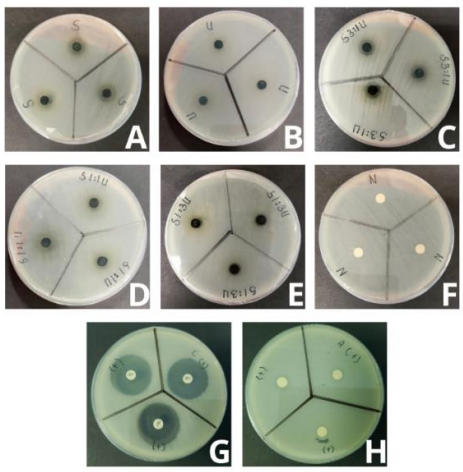


Figure 3. Antibacterial activity test results: A: ethanol extract of sembung leaves, B: purple leaf extract, C: combination of extracts in a ratio of 3:1, D: combination of extracts in a ratio of 1:1, E: combination of extracts in a ratio of 1:3, F: control negative (ethanol), G: positive control (chloramphenicol), and F: positive control (amoxicillin) against *S. mutans* FNCC 0405.

Table 4. Antibacterial activity test results

Test Bacteria	Sample	Mean Inhibition Zone Diameter (mm) ± SD	Interpretation
***Streptococcus mutans* FNCC 0405**	Sembung leaf ethanol extract	5,83 ± 0,07	Moderate
	Purple leaf ethanol extract	0	-
	Combination of ethanol extract of sembung leaves and purple leaves 3:1	4 ± 0	Weak
	Combination of ethanol extract of sembung leaves and purple leaves 1:1	3,08 ± 0,38	Weak
	Combination of ethanol extract of sembung leaves and purple leaves 1:3	1,46 ± 0,71	Weak
	Negative control	0	-
	Positive control (chloramphenicol)	26,75 ± 0,25	Very Strong
	Positive control (amoxicillin)	0	-

DISCUSSION

Extract yield is the percentage comparison between the produced extract and the starting simplicia. A higher yield value indicates an increased production of extract.[23] The yields of sembung leaves and purple leaves in the ethanol extract are 5.2% and 3%, respectively. The yield percentage is comparatively low in relation to the Indonesian Herbal Pharmacopoeia's stipulation that a quality extract must produce no less than 10%.[24]

The low yield percentage can be affected by various factors, including temperature, extraction duration, and the solvent employed. The elevated temperature and prolonged extraction duration will correlate directly with an increased yield percentage. An increase in temperature and extraction duration will result in a greater yield percentage.[25] Nonetheless, prolonged extraction may yield suboptimal results because the solvent used to extract secondary metabolite chemicals reaches a saturation point. Once the solvent attains its saturation point, it is unable to optimally extract secondary metabolite chemicals.[26]

This research employs a maceration approach that avoids high temperatures, resulting in an extended duration for the extraction of secondary metabolite chemicals. The maceration duration in this investigation may have been insufficient, resulting in a low yield percentage. Also, the extract yield may be low if the maceration solvent has reached its saturation point, which stops the best extraction of secondary metabolite chemicals.

The content of tannins, saponins, flavonoids, and phenols in the individual extracts of sembung and purple leaves were assessed through phytochemical screening in this study. Phytochemical screening will reveal the concentration of secondary metabolites present in an extract.[27] The screening is conducted by combining the extract with various reagents in a reagent tube.[20] It was found that both the ethanol extract from sembung leaves and the ethanol extract from purple leaves contain tannins, flavonoids, saponins, and phenols following phytochemical screening. These four compounds exhibit antibacterial action against *S. mutans*.[14,15,21]

The flavonoid content can stop the production of nucleic acids, mess up the way bacterial cell membranes work, and stop S. mutans bacteria from using energy *S. mutans*.[28] Flavonoids limit nucleic acid synthesis by disrupting the production of bacterial DNA and RNA via the buildup of nucleic acid bases. Flavonoids also damage bacterial cell membranes by making

membranes less functional by combining complex molecules with proteins that are outside of cells and those that dissolve in water. Flavonoids can also obstruct bacteria's utilization of oxygen, impeding their energy metabolism and leading to mortality.[29]

The saponin content in sembung and purple leaf extracts induces hemolysis in bacterial cells by enhancing membrane permeability. Elevated membrane permeability causes cell membrane instability, leading to cellular lysis.[4]

Tannins include free phenolic hydroxyl groups that form stable interactions with proteins.[15] These chemicals can obliterate bacterial cell membranes by interfering with peptidoglycan production. This situation will result in bacterial cell lysis and death.[30]

The phenolic compound exhibits antibacterial properties. Phenol will compromise cell membranes and denature membrane proteins.[14] The formation of hydrogen bonds will compromise the protein's structure. This disrupts the permeability of the cell wall and cytoplasmic membrane, both made of protein, leading to bacterial cell lysis.[28]

The antibacterial activity test findings of the combined ethanol extracts of sembung and purple leaves revealed variations in the diameter of the inhibition zones across each comparison. The results showed that the median diameter of the inhibitory zone was 4 ± 0 mm when sembung and purple ethanol extracts were mixed in a 3:1 ratio. At a 1:1 ratio, the amalgamation of ethanol extracts from sembung and purple leaves produced an average bacterial inhibitory zone diameter of 3.08 ± 0.38 mm. At a ratio of 1:3, the combination of ethanol extracts from sembung leaves and purple leaves exhibited the smallest average inhibition zone diameter, measuring 1.46 ± 0.71 mm. The inhibitory zone diameter for these three extract combinations is ≤ 5 mm, categorizing them as possessing poor antibacterial activity.[18]

This study also tested the antibacterial activity of each individual extract. Sembung leaf ethanol extract has an average inhibitory zone diameter of 5.83 ± 0.07 mm, which is categorized as moderate antibacterial activity. In each comparison, the average diameter of the inhibition zone is greater than the diameter of the bacterial inhibition zone formed in the combination of ethanol, sembung, and violet extracts. This is possibly due to the higher levels of secondary metabolites contained in sembung leaves compared to the combination of extracts. However, this must first be proven through quantitative phytochemical screening, which can determine the levels of secondary metabolites contained in an extract.

The purple leaf ethanol extract test results did not show any bacterial inhibitory effect. This is not in accordance with research conducted by Rahman (2017), which stated that purple leaves were able to inhibit the growth of S. mutans bacteria. In this study, the ethanol extract of sembung leaves had an average diameter of the inhibition zone of the ethanol extract of purple leaves against S. mutans for concentrations of 2%, 3%, and 4%, respectively: 12.60 mm, 13.73 mm, and 14.82 mm. This difference is likely due to several things, such as differences in the length of time maceration is carried out, differences in the solvent used, and differences in where the leaves grow. Ethanol 70% is the solvent used in research conducted by Rahman with a maceration time of 5 days using a solvent-to-sample ratio of 1:5. This research used leaves originating from Enreakang Regency.[17] In addition, the absence of bacterial inhibition in the test using purple leaf ethanol extract could be due to the fact that purple leaf ethanol extract has low levels of secondary metabolites, so it cannot inhibit bacterial activity. Secondary metabolite levels influence the extract's effectiveness. The higher the level of secondary metabolites contained in an extract, the higher its effectiveness.[31] Nonetheless, this must be confirmed by assessing the concentrations of secondary metabolites produced via quantitative phytochemical analyses. It's possible that the test using an ethanol extract of purple leaves didn't show any bacterial inhibition because the media

conditions weren't ideal, which stopped the formation of a bacterial inhibition zone in the medium. This study exhibited a limitation as one test group was evaluated solely on a single medium utilizing the triplicate approach.

The ethanol extracts of sembung leaves and purple leaves in a 3:1 ratio exhibit the most substantial bacterial inhibition zone, whereas the 1:3 ratio of these extracts results in the least significant bacterial inhibition zone. This finding may be attributed to the single purple leaf extract's lack of bacterial inhibitory properties against S. mutans. The larger the ratio of sembung leaf extract to purple leaf extract, the more substantial the bacterial inhibition zone formed.

The combination of sembung and purple leaf extracts has antibacterial properties due to the presence of secondary metabolites in both leaves, including tannins, flavonoids, saponins, and phenols. The flavonoid chemicals in the ethanol extract mixture can stop the production of nucleic acids, mess up the way bacterial cell membranes work, and stop *S. mutans* from using energy.[28] Saponin chemicals can induce lysis in bacterial cells by compromising their cell membrane permeability.[32] Tannin chemicals disrupt the bacterial cell membrane to combat bacteria.[4] Phenols eradicate *S. mutans* bacteria by compromising the cell membrane and denaturing membrane proteins.[14]

This study also used negative and positive controls as comparisons. Ethanol (96%), as a negative control, did not show bacterial inhibition. Meanwhile, the positive controls used, namely amoxicillin and chloramphenicol, showed different results. Amoxicillin did not inhibit the *S. mutans* bacteria, whereas chloramphenicol had an inhibition zone diameter of 26.75 ± 0.25 mm.

The lack of an inhibitory zone for amoxicillin may be attributed to S. mutans bacteria, which exhibit resistance to the antibiotic amoxicillin.[33] Amoxicillin belongs to the beta-lactam class of antibiotics. S. mutans can neutralize this category of antibiotics by acting on the beta-lactamase enzyme. The beta-lactamase enzyme functions by hydrolyzing the beta-lactam ring in antibiotics, rendering them ineffective against bacteria.[34]

The difference between the positive control and the combination of sembung and violet leaf ethanol extracts may be attributed to the latter's insufficient quantity of secondary metabolites that function as antibiotics. Nonetheless, this study did not evaluate the concentrations of secondary metabolites present in the ethanol extract of the leaves. This represents a significant limitation of the research. Quantitative phytochemical screening is necessary to ascertain the concentration of secondary metabolite chemicals present in an extract.

The diameter of the bacterial inhibitory zone was analyzed using SPSS. The Kruskal-Wallis test indicated a significant difference between the group receiving ethanol extracts from both sembung leaf and purple leaf and the positive control (p value <0.05). Variations in the extract combination ratios will influence the inhibitory zone's diameter for S. mutans bacteria. Furthermore, this indicates that when combined in various ratios, the ethanol extracts of sembung leaves and purple leaves have antibacterial properties, albeit less effective than chloramphenicol, which served as the positive control.

Dunn's post-hoc follow-up test results indicated a significant difference between the 3:1 and 1:3 extract combination groups. This is probably due to variations in antibacterial efficacy between sembung leaves and purple leaves. The single extract antibacterial assay indicated that the sembung leaf extract exhibited moderate antibacterial activity, while the purple leaf extract demonstrated no antibacterial activity. The combination of extracts with a higher ratio of sembung leaves (3:1) has markedly distinct antibacterial activity compared to the combination with a higher ratio of purple leaves (1:3). Additionally, it was determined that the 1:3 extract combination group with the positive control (chloramphenicol) and the 1:1 extract

combination group with the positive control (chloramphenicol) exhibited significant differences. This might be because there are a lot fewer antibacterial agents in the extract mixture than in chloramphenicol alone. The test results indicated no significant difference between the 3:1 extract combination group and the positive control (chloramphenicol). The results suggest that the 3:1 combination possesses promise for further advancement as an antibacterial agent.

This research has various weaknesses, including the absence of quantitative phytochemical screening and gas chromatography-mass spectrometry (GC-MS) analysis. Furthermore, antimicrobial testing was not conducted on each group across various media in this investigation. Quantitative phytochemical screening can quantify the secondary metabolites present in an extract. This enables researchers to examine the influence of secondary metabolite levels on the extent of the zone inhibiting bacterial growth. The GC-MS test can accurately identify secondary metabolite molecules, allowing for the detection of all secondary metabolites with antibacterial properties. Evaluating test groups across various media helps mitigate bias resulting from compromised media.

CONCLUSION

The research findings indicate that the ethanol extract of sembung leaves and purple leaves contains tannins, flavonoids, saponins, and phenols. There was a weak antibacterial effect against S. mutans in the ethanol extract of sembung leaves (B. balsamifera) and purple leaves (G. pictum) when the ratios were 3:1, 1:1, and 1:3. The inhibition zones were 4 ± 0 mm, 3.08 ± 0.38 mm, and 1.46 ± 0.71 mm, respectively. A future study might focus on formulating sembung leaf ethanol extract as an antibacterial agent against S. mutans. Using quantitative phytochemical screening, we must determine the quantity of secondary metabolites present in the ethanol extract of purple and sembung leaves. This will assist us in determining how secondary metabolite concentrations influence S. mutans' bactericidal activity. The gas chromatography-mass spectrometry (GC-MS) technique must be employed to identify the secondary metabolite chemicals present in the ethanol extract of purple and sembung leaves.

REFERENCE

1. Lemos JA, Palmer SR, Zeng L, Wen ZT, Kajfasz JK, Freires IA, dkk. The Biology of Streptococcus mutans . Microbiol Spectr. 2019;7(1):1–26.
2. Prasasti CA, G BT, Hasibuan SY, Hutagalung MH., Molek M. Perbandingan Ekstrak Daun Mangga Bacang dengan Ekstrak Daun Pepaya dalam Menghambat Pertumbuhan Streptococcus Mutans. Jurnal Ilmiah Kesehatan Sandi Husada. 2021;10(1):235–40.
3. Putri DK, Nizar M. Uji Aktivitas Antibakteri Beberapa Jamu untuk Pengobatan Karies Gigi yang Disebabkan oleh Streptococcus mutans. Jurnal Kesehatan Pharmasi. 2019;1(1):11–6.
4. Rahman FA, Haniastuti T, Utami TW. Skrining Fitokimia dan Aktivitas Antibakteri Ekstrak Etanol Daun Sirsak (Annona muricata L .) pada Streptococcus mutans ATCC 35668. 2017;3(1):1–7.
5. Warganegara E, Restina D. Getah Jarak (Jatropha curcas L.) sebagai Penghambat Pertumbuhan Bakteri Streptococcus mutans pada Karies Gigi. Majority. 2016;5(3):62–7.
6. Supari I, Leman M, Zuliari K. Efektivitas antibakteri ekstrak biji bengkuang (Pachyrrhizus erosus) terhadap pertumbuhan Streptococcus mutans secara in vitro. Pharmacon. 2016;5(3):33–9.
7. Wardaniati I, Gusmawarni V. Uji

Aktivitas Antibakteri Ekstrak Etanol Propolis terhadap Streptococcus mutans. Jurnal Farmasi Higea. 2021;13(2):115–23.
8. Ayuningtyas JEP, Astuti P, Fatmawati DWA. Aktivitas Antibakteri Kombinasi Vitamin C dan Amoksisilin sebagai Bahan Alternatif Intrakanal Medikamen terhadap Enterococcus faecalis secara In Vitro. Pustaka Kesehatan. 2021;9(1):60.
9. Nabila AIN, Yusran, Oktarlina RZ. Perbandingan Penggunaan Kloramfenikol dan Levofloksasin pada Pengobatan Konjungtivitis Bakterial. Medula. 2021;11(4):353–6.
10. Eveliani BV, Gunawan S. Profil Ketepatan Penggunaan Antibiotik pada Karyawan Universitas Tarumanagara. Jurnal Muara Medika dan Psikologi Klinik. 2021;1(1):30–9.
11. Octavia R, Laila, Kumil W, Saptarina N, Estikomah SA. Evaluasi Terapi Antibiotik Pada Pasien Diare Akut Balita Di Instalasi Rawat Inap Rumah Sakit Tentara Dr . Soedjono Magelang Tahun 2018. Pharmaceutical Journal of Islamic Pharmacy. 2021;5(2):63–71.
12. Parisa N, Parulian T, Adelia RAA. Rasionalitas Penggunaan Azitromisin pada Pasien ISPA di Rumah Sakit Moh. Hoesin (RSMH) Palembang. Jurnal Mandala Pharmacon Indonesia. 2022;8(1):34–48.
13. Sukertiasih NK, Megawati F, Meriyani H, Sanjaya DA. Studi Retrospektif Gambaran Resistensi Bakteri terhadap Antibiotik. Jurnal Ilmiah Medicamento. 2021;7(2):108–11.
14. Polakitan IR, Fatimawali, Leman MA. Uji Daya Hambat Ekstrak Daun Sembung Rambat (Mikania micrantha) terhadap Pertumbuhan Streptococcus mutans. Jurnal Ilmiah Farmasi. 2017;6(1):1–8.
15. Kurniawati A. Pengaruh Kumur Ekstrak Daun Ungu terhadap Jumlah Bakteri dalam Saliva. Stomatognatic (Jurnal Kedokteran Gigi Universitas Jember). 2018;15(2):43–6.
16. Jayadewata A, Natania E, Puspita N, Paramitha I, Wiraputra P, Gunarta M. Laporan akhir penelitian reguler. 2022;(April).
17. Rahman. Uji Efek Antibakteri Ekstrak Daun Wungu (Graptophylum pictum Griff) Asal Kabupaten Enrekang terhadap Pertumbuhan Streptococcus mutans. Media Analis Kesehatan. 2017;8(2):111–7.
18. Davis WW, Stout TR. Disc Plate Method of Microbiological Antibiotic Assay. 1971;22(4):659–65.
19. Usman I, Stefany J, Ismail ER. Aktivitas Antibakteri Kombinasi Ekstrak Daun Kemangi dan Daun Binahong terhadap Streptoccocus mutans. Media Farmasi. 2019;15(2):107–11.
20. Sangi M, Runtuwene MRJ, Simbala HEI, Makang VMA. Analisis Fitokimia Tumbuhan Obat Di Kabupaten Minahasa Utara. Chem Prog. 2008;1(1):47–53.
21. Amalia A, Sari I, Nursanty R. Aktivitas Antibakteri Ekstrak Etil Asetat Daun Sembung (Blumea balsamifera (L.) DC.) terhadap Pertumbuhan Bakteri Methicillin Resistant Staphylococcus aureus (MRSA). Jurnal UIN Ar-Raniry. 2017;5(1):387–91.

22. Peethambar P, Sowjanya, Konde S, Agarwal M, Prasad S. Anti microbial effect of prebiotic containing tooth paste against streptococcus mutans and lactobacillus: An invitro evaluation. Indian Journal of Microbiology Research. 2022;9(1):41–9.
23. Wijaya H, Novitasari, Jubaidah S. Perbandingan Metode Ekstraksi terhadap Rendemen Ekstrak Daun Rambui Laut (Sonneratia caseolaris L. Engl). Jurnal Ilmiah Manuntung. 2018;4(1):79–83.
24. Kemenkes. Farmakope Herbal Indonesia. Kementrian Kesehatan RI. 2017. 1–539 p.
25. Syamsul ES, Amanda NA, Lestari D. Perbandingan Ekstrak Lamur Aquilaria malaccensis dengan Metode Maserasi dan Refluks. Jurnal Riset Kefarmasian Indonesia. 2020;2(2):97–104.
26. Yuliantari N, Widarta I, Permana I. Pengaruh Suhu dan Waktu Ekstraksi Terhadap Kandungan Flavonoid dan Aktivitas Antioksidan Daun Sirsak (Annona muricata L.) Menggunakan Ultrasonik. Media Ilmiah Teknologi Pangan. 2017;4(1):35–42.
27. Yanti S, Vera Y. Skrining Fitokimia Ekstrak Daun Belimbing Wuluh (Averrhoa Bilimbi). Jurnal Kesehatan Ilmiah Indonesia. 2019;4(2):41–6.
28. Bontjura S, Waworuntu OA, Siagian KV. Uji Efek Antibakteri Ekstrak Daun Leilem (Clerodendrum minahassae l .) Terhadap Bakteri Streptococcus mutans. Jurnal Ilmiah Farmasi. 2015;4(4).
29. Nomer N, Duniaji A, Nocianitri K. Kandungan Senyawa Flavonoid dan Antosianin Ekstrak Kayu Secang (Caesalpinia sappan L.) serta Aktivitas Antibakteri terhadap Vibrio cholerae. Jurnal Ilmu dan Teknologi Pangan. 2019;8(2):216–25.
30. Handayani F, Sundu R, Sari RM. Formulasi dan Uji Aktivitas Antibakteri Streptococcus mutans dari Sediaan Mouthwash Ekstrak Daun Jambu Biji (Psidium guajava L.). Jurnal Sains dan Kesehatan. 2017;1(8):422–33.
31. Hastuti N, Wardani M. Identifikasi Senyawa Kimia Potensial Berkhasiat Obat dari Kulit Batang Shorea ovalis (korth.) Blume Menggunakan Analisis Gas Kromatografi. Majalah Farmasi dan Farmakologi. 2020;24(3):72–6.
32. Sadiah HH, Cahyadi AI, Windria S. Kajian Daun Sirih Hijau (Piper betle L) sebagai Antibakteri. Jurnal Sain Veteriner. 2022;40(2):128–38.
33. Al-Shami IZ, Al-Hamzi MA, Al-Shamahy HA, Majeed A. Efficacy of some Antibiotics against Streptococcus Mutans Associated with Tooth Decay in Children and their Mothers. Online Journal of Dentistry & Oral Health. 2019;2(1).
34. Donaliazarti. Mekanisme Resistensi terhadap Anti Mikroba. Collaborative Medical Journal. 2022;5(3):37–45.