



MOUTHWASH OF *Amaranthus hybridus* L. LEAF EXTRACT WITH ETHYL ACETATE AS A *Streptococcus mutans* ANTIBACTERIAL

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ABSTRACT

Caries and dental plaque are oral and dental problems. Green spinach (*Amaranthus hybridus* L.) can be used as medicine and has antibacterial activity. It also benefits human health and is a nutrition source. The phytochemical results of the leaf extract showed that *A. hybridus* L. contains alkaloids, flavonoids, steroids, saponins, and tannins. This study aimed to determine the inhibitory action of *A. hybridus* L. leaf extract against the growth of *Streptococcus mutans* and to formulate *A. hybridus* L. leaf extract in a mouthwash. The disk diffusion method and brain heart infusion agar medium were used in this study, with five treatment groups: extract concentrations of 25%, 50%, and 100%, positive and negative control were studied. Physical tests, including organoleptic, pH, and viscosity tests, were performed. The pH of *A. hybridus* L. leaf extract in the mouthwash was 4.80, with a viscosity of 1.2456–1.378 cP. The inhibition zones of antibacterial activity against *S. mutans* in the extract concentrations of 25%, 50%, and 100% were 1.13, 1.73, and 2.90 mm, respectively. The ethyl acetate extract of *A. hybridus* L. leaves in mouthwash displayed antibacterial activity against *S. mutans*, with an average inhibition zone of 0.57 mm. The mouthwash formulation produced an inhibition zone with an average F1 of 0.57 mm and F2 of 1.67 mm.
Keywords: *Amaranthus hybridus* L., *Streptococcus mutans*, antibacterial, mouthwash

1. PENDAHULUAN

Dental issues will be addressed to oral health. Oral health tends to be a single factor associated with dental health, such as the number of tooth loss, plaque, etc. The level of oral hygiene is an indicator of oral health. The oral cavity is the "gateway" for germs and bacteria; therefore, it can affect the health of other body organs. According to Riskesdas 2018 data, the percentage of the Indonesian population

with dental and oral problems was 57.6% during the previous 12 months. In fact, only 10.2% received treatment from dentists, whereas 89.8% did not receive dental care. Caries is a type of dental health problem. In Indonesia, the prevalence of caries was 45.3%, according to Riskesdas in 2018 (Ministry of Health of the Republic of Indonesia, 2018).

Caries is caused by the activity of a microorganism on a fermentable

carbohydrate. The signs of caries are the remineralization of tooth tissue and the destruction of organic matter. This condition triggers bacterial invasion and pulp death. The spread of infection to the periapical tissue causes pain. However, remineralization at a very early stage of disease can be stopped (Kidd and Bechal, 2013). Caries and periodontitis are generally caused by dental plaque and are major problems in oral and dental health. Dental plaque is a sticky substance that contains bacteria (Kidd and Bechal, 2013). Bacteria, such as *Streptococcus mutans*, play a major role in the formation of dental plaque by forming extracellular polysaccharides. This bacterium is found in large numbers in the plaque of caries sufferers (Roeslan, 1996).

There are several ways to control dental and oral problems, including brushing, flossing, scaling, and using mouthwash. Mouthwash is a dilute concentration of an antibacterial solution to combat oral microbes and infections, and acts as a cleanser and an antiseptic. Mouthwash plays an important role in the oral hygiene of an individual. It helps to relieve the symptoms of gingivitis and inflamed gums, and to kill pathogenic bacteria (Banu & Gayathri, 2016). Generally, mouthwash contains antibacterial ingredients, including more than 20% alcohol. However, the alcohol content and other chemicals in mouthwash can trigger oral cancer (Mc Cullough and Farah, 2008).

Currently, researchers are studying various medicinal plants as an alternative to reduce chemicals in mouthwash. Green spinach (*Amaranthus hybridus* L.) can be used as medicine and has antibacterial activity. The leaves of *A. hybridus* L. contain

alkaloids, flavonoids, saponins, tannins, and phenols. These bioactive components play an important role as antibacterial agents. This study aimed to determine the inhibitory action of *A. hybridus* L. leaf extract against the growth of *S. mutans*.

2. MATERIALS AND METHODS

2.1 Materials

The materials of this study were green spinach leaves (*A. hybridus* L.), ethyl acetate, distilled water, physiological NaCl 0.9%, cotton swab, aluminum foil, ammonia, chloroform, 2N hydrochloric acid, Mayer's reagent, Wagner's reagent, magnesium powder, amyl alcohol, reagent iron (III) chloride, Liebermann–Buchard reagent, *S. mutans* (obtained from the Central Laboratory of Padjajaran University Bandung ATCC 25175), brain heart infusion broth (BHI-B) medium, brain heart infusion agar (BHI-A) medium, Bacteriological Agar, paper disks, chlorhexidine gluconate 0.2%, dimethyl sulfoxide (DMSO), Minosep®gargle, glycerin, sorbitol, peppermint oil, and distilled water.

2.2 Research Procedure

2.2.1 Sample Preparation

Green spinach leaves (*A. hybridus* L.) were obtained from the Bumi Agung Permai 1 Housing Estate, Serang–Banten. The wet sample was covered with a black fabric and dried completely under the sun. Then, sample was powdered and sieved to obtain simplicia, a natural material that presented in the dry form.

2.2.2 Sample Extraction

The sample was extracted by the maceration method using ethyl acetate (Maiyo, 2008). The dried green spinach

leaves were ground to a powder weighing 2.050 g. Then, the sample was placed in a glass container, soaked with ethyl acetate at a ratio of 1:6, and incubated for 24 hours, followed by stirring. The extraction process was repeated at least twice using the same type and volume of solvent. All the macerate was collected and evaporated to obtain a thick extract. Then, the percentage of the extract yield was calculated.

2.2.3 Antibacterial Activity Test of Extract Sterilization

The antibacterial test was performed under sterile conditions to kill microorganisms on tools and materials. Sterilization was conducted by using an autoclave at 121°C for 15 minutes.

Making brain heart infusion broth (BHI-B) medium

The BHI-B medium was made by adding 1.85 g of BHI-B powder and 50 ml of distilled water into an Erlenmeyer flask. The BHI-B mixture was boiled and stirred until homogeneous, then sterilized in an autoclave at 121°C for 15 minutes (Suryani *et al.*, 2019).

Making brain heart infusion agar (BHI-A) medium

The BHI-A medium was made by dissolving 3.7 g of BHI-B powder and 2 g of Bacteriological Agar in 100 ml of distilled water. This mixture was boiled and stirred until a homogeneous solution was obtained. Sterilization was conducted in an autoclave at 121°C for 15 minutes. Then, it was poured into Petri dishes at a thickness of 2 mm (Suryani *et al.*, 2019).

Making agar medium oblique

A volume of 5 ml of BHI-A medium was poured into each sterile test tube and covered with aluminum foil. The media samples were sterilized in an autoclave at 121°C for 15 minutes, and then left at room temperature for \pm 30 minutes until they solidified at an inclination of 30°. Oblique agar media was used for the inoculation of bacteria (Handayani, *et al.*, 2017).

Bacterial rejuvenation

S. mutans bacteria from the pure culture were taken one ose needle and inoculated by scratching on the medium. Bacterial cultures in each agar tube were incubated at 37°C for 18–24 hours.

Preparation of turbidity standards for McFarland solution

A volume of 9.5 ml of 0.36 N H₂SO₄ solution was mixed with 0.5 ml of 1.175% BaCl₂ 2.H₂O solution in an Erlenmeyer flask, then shaken until a turbid solution was obtained. This turbidity was used as a standard to measure the turbidity of the test bacterial suspension (Handayani, *et al.*, 2017).

Bacterial suspension

The rejuvenated *S. mutans* bacteria were suspended using 0.9% sterile NaCl. The turbidity level was measured according to McFarland's standard solution.

Antibacterial activity test with diffusion method

The inhibition test of *A. hybridus* L. leaf extract on the growth of *S. mutans* was conducted using the agar disk diffusion method. *S. mutans* bacteria were smeared on BHI-A media in a smooth layer. A paper disk was saturated with ethyl acetate extract of *A. hybridus* L. leaves, at

concentrations of 25%, 50%, and 100%; the negative control was DMSO and the positive control was 0.2% Chlorhexidine. The test samples were incubated for 24 hours, after which the clear zone was measured with a caliper (Handayani, *et al.*, 2017).

Table 1. Mouthwash Formulation of Ethyl Acetate Extract with *A. hybridus* L. Leaves (Handayani, *et al.*, 2017).

Material	F0	F1	F2	Function
Ethyl acetate extract of <i>A. hybridus</i> L.	0	30	40	Active substance
Glycerin	5	5	5	Humectant
Sorbitol	8	8	8	Sweetener
Peppermint oil	0.15	0.15	0.15	Taste
Distilled water	100 ml	100 ml	100 ml	Solvent

F0 : Mouthwash without concentration of *A. hybridus* L. leaf extract

F1 : Mouthwash with 30% concentration of *A. hybridus* L. leaf extract

F2 : Mouthwash with 40% concentration of *A. hybridus* L. leaf extract

The mouthwash was made by adding the ethyl acetate extract of *A. hybridus* L. leaves into a mortar. Then, glycerin was added and mixed until it dissolved. Sorbitol, peppermint oil, and 100 ml of distilled water were added and mixed until a homogeneous mixture was obtained. The mouthwash formulation of the ethyl acetate extract of *A. hybridus* L. leaves was evaluated to determine the stability of the preparation (Table 1). This evaluation was conducted by observing the test preparation over 4 weeks of storage time at room temperature, including organoleptic, pH, and viscosity observations.

2.3 Organoleptic Observations

The formulation samples were observed over 4 weeks of storage, including color, aroma, and taste, at room temperature.

2.4 pH Tests

The pH was measured using a pH meter. The pH that appeared on the screen

Mouthwash making

The *A. hybridus* L. leaf extract concentrations of the mouthwash were 30% and 40%.

as a stable value was recorded. pH tests were conducted over 4 weeks. According to Hidayanto *et al.* (2017), the quality standard of an herbal mouthwash is a pH range between 5 and 7.

2.5 Viscosity Tests

The viscosity was evaluated by using an Ostwald viscometer. First, the Ostwald viscometer was cleaned with water. Then, it was rinsed with alcohol and distilled water was added (as a comparison liquid). The water was pipetted into the upper limit. A stopwatch was used to measure the time the sample took to pass from the upper limit to the lower limit. A similar method was followed for the mouthwash sample. This test was carried out for 28 days, with data collected on days 0, 7, 14, 21, and 28. Viscosity was calculated by the following formula:

$$\frac{\eta_2}{\eta_1} = \frac{\rho_2 \cdot t_2}{\rho_1 \cdot t_1}$$

η_1 = viscosity of water (cP)

- η_2 = viscosity of the liquid (cP)
- ρ_1 = density of water (g/ml)
- ρ_2 = density of the liquid (g/ml)
- t_1 = flow time of water (s)
- t_2 = flow time of liquid (s)

Water viscosity: 0.89cP (Rowe et al., 2009).

2.6 Antibacterial Activity of Mouthwash with *A. hybridus* L. Leaf Extract

The inhibition test of *A. hybridus* L. leaf extract with ethyl acetate in mouthwash against the growth of *S. mutans* was conducted using the agar diffusion method. *S. mutans* was smeared on BHI-A media until smooth and added with the mouthwash formulation of *A. hybridus* L. leaves at concentrations of 30% and 40%, and negative and positive controls (Minosep®gargle). Next, the samples were incubated for 24 hours and the clear zone formed was measured with a caliper.

2.7 Statistical Analysis

The data from the inhibition zone measured after 24 hours of incubation were collected and analyzed using the analysis of variance (ANOVA) test, with a significance level of 0.05. All data were analyzed by using SPSS 23 software, New York.

3. RESULTS

3.1 *A. hybridus* L. Leaf Extract with Ethyl Acetate

A total of 2.05 g of green spinach leaves were used to yield an extract using 12,000 ml of ethyl acetate, based on the maceration method. The yield was 5.682% and the extraction yield was 116.5 g.

3.2 Phytochemical Screening

Table 2 show the results of phytochemical screening.

Table 2. Phytochemical screening

Group	Extract
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Steroids	+
Terpenoids	-

3.3 *A. hybridus* L. Leaf Extract with Ethyl Acetate against *S. mutans*

The inhibitory test was conducted using the agar diffusion method. The inhibition diameters of *A. hybridus* L. leaf extract on the growth of *S. mutans* after 24 hours of incubation are shown in Figure 1.

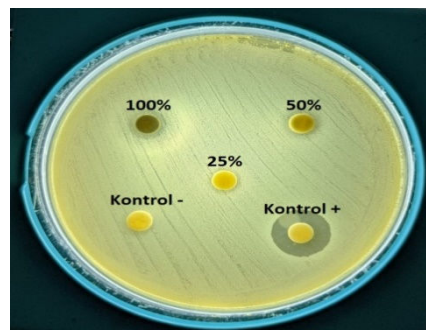


Figure 1. Inhibitory tests of *A. hybridus* L. leaf extract against *S. Mutans*

Table 3 shows the inhibition diameter from each concentration of the extract.

The largest average inhibition zone was found at 100% concentration, with an inhibition zone of 2.9 mm. The inhibition zones at 50% and 25% concentrations were 1.73 and 1.13 mm, respectively. No

inhibition zone was observed in the negative control group, whereas the positive control group showed an inhibition zone of 10.13 mm.

Table 3. Inhibition Diameters of *A. hybridus* L. Leaf Extract on the Growth of *S. mutans*

Repetition	Inhibition Zone Diameter (mm)				
	Extract concentration (25%)	Extract concentration (50%)	Extract concentration (100%)	+ Control (Chlorhexidine) 0.2%	-Control (DMSO)
I	1.50	2.00	3.20	10.30	0
II	1.00	1.80	3.10	10.05	0
III	0.90	1.40	2.40	10.05	0
Mean	1.13	1.73	2.9	10.13	0

3.4 Evaluation of Mouthwash with *A. hybridus* L. Leaf Extract

3.4.1 Organoleptic Observations

Organoleptic evaluations of the mouthwash included the color, aroma, and taste of *A. hybridus* L. leaves as active ingredients (Table 4). The aroma in formulas 1.2 and 3 were mint from the additional ingredients (peppermint oil).

There was a difference in taste between F0, F1, and F2. Formulation 0 (without the extract) and formulation 1 had a sweet mint taste, whereas formulation 2 had a sweet and sour taste because of the high concentration of the extract. The results of observations over 4 weeks did not change in the three formulations, including color, aroma, and taste.

Table 4. Observations of Organoleptic Tests of Mouthwash with *A. hybridus* L. Leaf Extract Over 4 Weeks of Storage

Week	Mouthwash	Color	Aroma	Taste
0	F0 (Control)	Clear	Mint	Sweet mint
	F1 (30% extract)	Blackish green	Mint	Sweet mint
	F2 (40% extract)	Blackish green	Mint	Sweet-sour
1	F0 (Control)	Clear	Mint	Sweet mint
	F1 (30% extract)	Blackish green	Mint	Sweet mint
	F2 (40% extract)	Blackish green	Mint	Sweet-sour
2	F0 (Basic)	Clear	Mint	Sweet mint
	F1 (30% extract)	Blackish green	Mint	Sweet mint
	F2 (40% extract)	Blackish green	Mint	Sweet-sour

Mouthwash of *Amaranthus hybridus* L. Leaf Extract...

3	F0 (Basic)	Clear	Mint	Sweet mint
	F1 (30% extract)	Blackish green	Mint	Sweet mint
	F2 (40% extract)	Blackish green	Mint	Sweet-sour
4	F0 (Basic)	Clear	Mint	Sweet mint
	F1 (30% extract)	Blackish green	Mint	Sweet mint
	F2 (40% extract)	Blackish green	Mint	Sweet-sour

3.5 pH Tests

As shown in **Table 5**, the pH value of each mouthwash was different. The pH

measurements from formula 0, 1, and 3 were 4.39–5.18, 4.50–4.80, and 3.7–4.06, respectively.

Table 5. pH Values Over 4 Weeks of Storage

Mouthwash	pH value					SNI standard (1995)
	Week 0	Week 1	Week 2	Week 3	Week 4	
F0	4.39	4.77	4.82	4.87	5.18	4.5–10.5
F1	4.50	4.68	4.70	4.78	4.80	
F2	3.77	4.02	4.03	4.05	4.06	

3.6 Viscosity Tests

Viscosity tests were conducted using the Oswald viscometer. The viscosity of the mouthwash was tested as it affected the gargling in the mouth. The closer the viscosity level of the mouthwash

formulation to the viscosity level of water, the more comfortable the mouthwash is for gargling. The viscosity of water used as a standard in the calculation of viscosity was ±1 cP (**Table 6**).

Table 6. Viscosity Values Over 4 Weeks of Storage

Mouthwash	Viscosity (cP)					Viscosity Standard (Handayani et al., 2017)
	Week 0	Week 1	Week 2	Week 3	Week 4	
F0	1.2456	1.2275	1.2456	1.2433	1.2569	± 1 cP
F1	1.2568	1.2456	1.2612	1.2612	1.2547	
F2	1.3945	1.3877	1.3915	1.4041	1.3978	

3.7 Bacterial Test Results of Mouthwash with the Addition of *A. hybridus* L. Leaf Extract against *S. Mutans*

Table 7 shows the average diameter of the paper disks dipped in the mouthwash with *A. hybridus* L. extract

during the 24-hour incubation period (Figure 2). The average inhibition zones of F1 and F2 were 0.57 and 1.67 mm, respectively. In addition, the negative control did not inhibit the growth of *S. mutans* bacteria, whereas the positive control displayed an average inhibition

diameter of 10.15 mm. The purpose of using the positive control was to compare its inhibition diameter with that of our mouthwash.

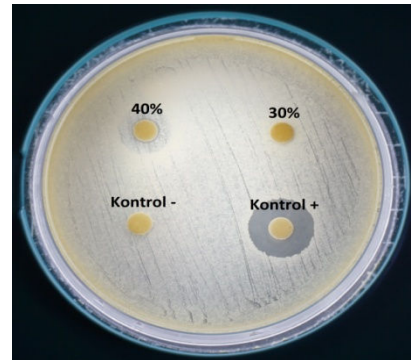


Figure 2. Test results of antibacterial activity of mouthwash formulation with *A. hybridus* L. leaf extract against *S. mutans* after 24 hours of incubation at 37°C

Table 7. The Inhibition Zone of Mouthwash Formulation with the Addition of *A. hybridus* L. Leaf Extract on the Growth of *S. mutans*

Repetition	Inhibition zone (mm)			
	F1 (30%)	F2 (40%)	Positive Control (C+) (Minosep®gargle)	Negative Control (C-)
I	0.40	1.00	10.10	0
II	0.60	2.00	10.35	0
III	0.70	2.00	10.00	0
Mean	0.57	1.67	10.15	0

F1 : Mouthwash with 30% concentration of *A. hybridus* L. leaf extract

F2 : Mouthwash with 40% concentration of *A. hybridus* L. leaf extract

C+ : Positive control (Minosep®gargle)

C- : Negative control

4. DISCUSSIONS

4.1 Extraction Results

Simplicia powder of *A. hybridus* L. was macerated using ethyl acetate as solvent. Maceration is the most widely used and simplest method of extracting compounds. Maceration was chosen because it has several advantages, including the absence of a heating process. Therefore, labile compounds are not damaged by heat (Wardhani and Sulistiyani, 2012). We used ethyl acetate as a solvent as it is a solvent with low toxicity and semi-polar to attract alkaloids, flavonoids, saponins, tannins, polyphenol, and triterpenoids (Maiyo, 2008). The result of maceration was 116.5 g, with an extract yield of 5.682%. According to Sani *et al.*

(2014), the extract yield was calculated based on the ratio of the final weight to the initial weight, multiplied by 100%.

4.2 Mouthwash Formulation with *A. hybridus* L. Leaf Extract

Mouthwash products need glycerin, sorbitol, peppermint oil, and aquadest. Glycerin was used to increase the solubility of extracts in water; 5–20% of glycerin is used in mouthwash for a certain comfortable sensation in the mouth. Humectants in mouthwash keep the ingredients from evaporating into the air (Handayani *et al.*, 2017). Sorbitol was used as a sweetener to give a sweet taste to the mouthwash. The addition of peppermint oil was chosen for its fresh aroma; it also increased the sensation in the mouth.

4.3 pH Tests

The pH test was conducted to determine whether our mouthwash met the pH standards of commercial mouthwash. The pH value greatly affects the type of bacteria. Most bacteria have an optimum pH value of 6.5–7.5 (Lukas, 2012). Yosephine *et al.* (2013) stated that the pH of the mouthwash should range from 5 to 6. If the pH is < 5, it is too acidic and causes more bacterial growth. On other hand, if the pH is > 6, it is too alkaline and can lead to fungal growth, causing canker sores. The pH of our mouthwash was tested using a pH meter. The mouthwash formulation in this study had the smallest pH of 3.77 in F2, whereas the highest pH of 5.18 was found in F0. According to SNI No. 12-3524-1995, the pH requirement for mouthwash is 4.5–10.5. The pH ranges of F0 and F1 fulfilled the pH requirements for mouthwash according to SNI, whereas F2 did not meet the pH requirements. In our mouthwash formulation, the ethyl acetate extract of *A. hybridus* L. leaves tends to be acidic. Mouthwash of *A. hybridus* L. leaves is influenced by the pH extract. According to Rowe *et al.* (2009), the pH value in mouthwash is also influenced by the use of sorbitol, with a pH value of 4.5. The sorbitol formulation has the largest concentration of 8% sorbitol. Therefore, it affects the pH value of the preparation.

4.4 Viscosity Tests

The viscosity value is influenced by the specific gravity of a liquid. According to Rowe *et al.* (2009), additives also affect the viscosity of a mouthwash. Glycerin had a viscosity of 1.143 cP at the 5% concentration of *A. hybridus* L. Sorbitol had a viscosity of 1.2 cP at a concentration of

10%. The viscosity analysis in the first week showed that the viscosity value of the mouthwash of *A. hybridus* L. leaf extract ranged from 1.2456 to 1.3945 cP. The highest and smallest viscosity values were found in F2 and F0, respectively. The viscosity value of the mouthwash with *A. hybridus* L. leaves was higher than that of water (Table 6). It can be seen that the higher concentration of the extract resulted in the higher viscosity value. In addition, the addition of glycerin and sorbitol affected the viscosity value. The viscosity value was relatively unstable over 4 weeks of storage. This was also because a solution has a shorter shelf life than solid dosage forms. The mouthwash solution was easily decomposed by temperature and light. Moreover, it was affected by the solution concentration, dissolved molecular weight, and pressure.

4.5 Activity Test for Mouthwash with *A. hybridus* L. Leaf Extract

Mouthwash with *A. hybridus* L. leaf extract can be used as an alternative to commercial mouthwash, as it is made with natural ingredients and without alcohol. *S. mutans* was chosen as the test bacterium because it is the major oral species that causes dental caries (Handayani *et al.*, 2017). The higher concentration of *A. hybridus* L. leaf extract resulted in a larger diameter of the inhibition zone (Figure 2). Pelczar and Chan (2008) stated that the activity of an antimicrobial agent is influenced by the concentration of antimicrobial substances, the number of microorganisms, temperature, species of microorganisms, the presence of organic matter, and pH. After an incubation period of 24 hours, the inhibition area remained

clear at a concentration of 40% of extract, but the concentration of 30% extract did not look clear. This result is supported by Akhyar's (2010) research, which stated that a bioactive compound is effective in inhibiting microbial growth, but is not lethal. The anti-bacterial activity is evaluated by an inhibition zone after an incubation period of 48 hours. On the other hand, a bioactive compound is said to be bactericidal if the bioactive compound is effective enough to kill microbes and inhibit microbial growth. Bactericidal activity is characterized by a clear zone of inhibition after 48 hours of incubation.

The secondary metabolites of *A. hybridus* L. are alkaloids, flavonoids, tannins, saponins, and steroids. The mechanism of action of secondary metabolites is by interfering with peptidoglycan synthesis. Therefore, cell wall formation is reduced, causing lysis of bacterial cells owing to osmotic and physical pressure. According to research by Rabbani *et al.* (2014), diameters of clear zones of 5, 5–10, 10–20, and >20 mm indicate weak, moderate, strong, and very strong antibacterial activity. Based on these standards, the inhibitory activity of *A. hybridus* L. leaf extract against *S. mutans* is in the weak category.

According to research by Rabbani *et al.* (2014), Gram-positive bacteria have a simple cell envelope structure, including a cytoplasmic membrane and a thick layer of peptidoglycan. In contrast, Gram-negative bacteria have cell envelopes with multilayered and complex structures. The cell structure causes Gram-positive bacteria to be more susceptible to

antimicrobial agents. The differences between the inhibition zone of *A. hybridus* L. ethyl extract and commercial mouthwash were analyzed using ANOVA. The ANOVA test showed that *A. hybridus* L. extract had a significant difference between groups. The presence of secondary metabolites in the extract is an important factor in inhibiting bacterial growth.

The antibacterial mechanism of alkaloids is by interfering with the peptidoglycan components in bacterial cells. Therefore, the bacterial cell wall is not fully formed (Azwana *et al.*, 2019). The antibacterial mechanism of flavonoids involves complex compounds and dissolved proteins. Flavonoids act on phospholipids so that they are unable to maintain the shape of the bacterial cell membrane. Therefore, the bacterial cell membrane leaks and the bacteria die. The mechanism of action of tannin is to interfere with peptidoglycan synthesis. It affects the formation of cell walls and destroys bacterial cells. Saponins as antibacterial agents reduce the surface tension of the bacterial cell wall, resulting in increased permeability or cell leakage (Handayani *et al.*, 2017). The mechanism of action of steroids as antibacterial agents is related to lipid membranes and sensitivity to steroid components that cause leakage in liposomes. Steroids can interact with phospholipid membranes that are permeable to lipophilic compounds. They decrease membrane integrity and cell membrane morphology, causing cell fragility and lysis.

In addition, *Amaranthus* species have been reported to have antibacterial

properties and it has the potential to block dental staining due to coffee (Abbasi et al, 2013; Iskandar et al, 2013; Peter and Gandhi, 2017). Hence, those findings supported this present study in investigating the bacterial activity of *A. hybridus* on *S. aureus* and its potential to be a mouthwash.

5. CONCLUSION

The extract of *A. hybridus* L. leaves with ethyl acetate extract inhibited the growth of *S. mutans* bacteria, with inhibition zones at 25% (1.13 mm), 50% (1.73 mm), and 100% concentration (2.9 mm). The formulation of *A. hybridus* L. extract in mouthwash was effective in inhibiting the growth of *S. mutans* causing dental caries, with inhibition zones in formula 1 (0.57 mm) and formula 2 (1.67 mm). Further studies are warranted to evaluate the physical and chemical tests by using the other dosage of *A. hybridus* L. leaf extract for their activity against *S. mutans*.

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