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Antibacterial and Antibiofilm Activity of Ethanol Extract of Batak Onion Bulbs (Allium chinense G.Don.) against *Streptococcus mutans* and *Enterococcus faecalis*

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S.mutans **is the main pathogen causing caries, while** ***E.faecalis*** **is the dominant microorganism in dental root canals. Batak onion is one of Indonesia's biological resources frequently used as condiments by Batak tribes and possesses antibacterial compounds such as alkaloids, saponins, and flavonoids. To analyze antibacterial activity test based on Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and antibiofilm from 70% ethanol extract of Batak onion bulbs against** ***S.mutans*** **and** ***E.faecalis.*** **This type of research is a laboratory experiment with a post-test only control group design. Determination of MIC by Kirby Baurer disc diffusion method, determination of MBC by streaking method, and colony tests are calculated using colony counters, determination of antibiofilm by Static Microtiter Plate Assays method, and checking the optical density at around 600nm. Data analysis of MIC, MBC, and antibiofilm using Oneway Anova and Post Hoc LSD tests. MIC on** ***S.mutans*** **and** ***E.faecalis*** **was at a concentration of 0.78% with an average of one of inhibition of 9.00 ± 0.43 mm and 8.06 ± 0.20 mm; no MBC was found because no group was able to reduce bacteria 98-98%, Batak onion bulb extract has antibiofilm ability starting at a concentration of 0.78% with the ability to reduce** ***S.mutans*** **bacteria by 84.55% and 85.73% on** ***E.faecalis.*** **70% ethanol extract of Batak onion bulbs can inhibit bacterial growth and biofilm of** ***S.mutans*** **and** ***E.faecalis.*** **The recommended dose for antibacterial and antibiofilm is a 6.25-50% concentration.**

Keywords: Antibacterial; Antibiofilm; Batak onion bulb; *E.faecalis; S.mutans.*

Indonesia is an agricultural country with more than 30,000 plant species, and about 7,000 of them have been recorded as medicinal plants.[1] Allium genus plants are well known and are often used by the public as a spice and traditional medicine. One of the Allium genus plants that has been widely utilized by the Indonesian people, especially the Batak tribe, is Batak Onion (*Allium chinense* G.Don.).[2]

Batak onions or chives can grow up to 50 cm in height with slender, bright green, thin-walled, 3-5 angled leaves (not round), are not too stiff upright, and have lavender-colored flowers with long stalks and stamens sticking out. Batak onions

grow in clumps and form many bulbs with a solid stalk and a white color. Batak onion bulbs have a diameter of 4-5 cm, are grayish-white to purple, are wrapped in transparent skin, and are white.[3,4]

Allium genus plants have known antimicrobial compounds such as *allicin*, *dialyl disulfide*, *ajoene*, and *3-(allyltrisulfanyl)-2-amino-propanoic acid*. Based on Harborne's phytochemical identification, 70% of Batak onion bulbs' ethanol extract has secondary metabolite compounds of alkaloids, flavonoids, and saponins.[2,5]

Dental caries is one of the most common dental health problems experienced by children and adults.[6] This disease occurs due to the interacting processes of teeth, saliva (the host), microorganisms, substrate, and time. Although the cause of caries is multifactorial, the main trigger is the dominant bacteria *Streptococcus mutans* (*S.mutans*).[7] Caries that are not treated immediately will cause severity, so a root canal or endodontic treatment is needed to handle it. In endodontic treatment, root canal infections usually occur. This infection is usually caused by microorganisms, especially *Enterococcus faecalis* (*E.faecalis*).[8]

Chlorhexidine is an antimicrobial agent effective as a mouthwash in preventing the buildup of caries-causing bacterial plaque and as a root canal irrigation agent in endodontic treatment.[9,10] Although superior, chlorhexidine has the disadvantage of causing discoloration of the teeth when used continuously as a mouthwash and cannot be used as a single root canal irrigation material, so it must be combined with other irrigation solutions. Therefore, other alternative materials are needed to replace chlorhexidine.[9,11]

The study conducted by another researcher demonstrated that the ethanol extract from Allium chinense exhibited significant antioxidant activity. Additionally, the high-content essential oil extract of the plant significantly decreased serum and hepatic levels of total cholesterol.[12] Research shows that Batak onion bulb extract has an antimicrobial response in inhibiting the growth of *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans*.[2] Other reports showed that Batak union bulb extract can inhibit the growth of Methicillin-Resistant *Staphylococcus aureus* (MRSA).[13] Many articles are available for onion studies as antibacterial and antibiolfilm activities. One study that showed Onion (*Allium cepa* L.) had potential antibacterial and antibiofilm activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus cereus*. No research reports on the antibacterial and antibiofilm activity possessed by Batak onion bulb extract against *S.mutans* and *E.faecalis*.[14] Thus, we are interested in conducting antibacterial activity test research based on minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and antibiofilm of 70% ethanol extract of Batak onion bulbs against *S.mutans* and *E.faecalis*.

MATERIALS AND METHODS

This laboratory experimental study has a post-test only with a controlled group design. The research samples used in this study were *Streptococcus mutans* ATCC® 25175TM and *Enterococcus faecalis* ATCC® 29212TM, which had been subcultured and isolated at the Universitas Sumatera Utara Hospital Clinical Microbiology Unit, 70% ethanol of Batak onion bulbs with concentrations of 50%; 25%; 12.5%; 6.25%; 3.125%; 1.56%; and 0.78%, and a control group consisting of chlorhexidine 0.2% as a positive control and Dimethyl Sulfoxide (DMSO) as a negative control. All samples were treated three times, so the sample size of this study was 54 samples consisting of 27 samples for *S.mutans* and 27 samples for *E.faecalis*.

Extract preparation

Preparation of the extract begins with making simplisia from 1 kg of Batak onions from Brastagi District. Batak onion bulbs are cleaned in running water, cut, sliced with a slicing knife, and dried in an oven at 25° for 25 hours until completely dry, with characteristic until the water content is less than 10% and easily broken if kneaded.[15] The dried bulbs were crushed with a porcelain pestle and pulverized with a blender (Cosmos CB 282P®), weighted and expected to produce a fine powder of as much as 150 grams, then put in an airtight container.

The extraction of simplisia is done by maceration. 150 grams of simplisia that have been finely added with 1 liter of 70% ethanol

in the vessel then stirred manually with a stick under atmospheric pressure for the first 6 hours and allowed to stand for 18 hours with the vessel closed while occasionally stirring. In the next step, the marinade is filtered to obtain filtrate (Maserat I), and the dregs are collected and accommodated in a white plastic bottle container. Did the same extraction of the dregs in 500 ml of 70% at room temperature for 24 hours while stirring several times, and then filtered and collected them (Maserat II). All filtrates obtained were combined and evaporated with a rotary evaporator RE-2010 at 40°C with 80 rpm until a thick extract was obtained.

Before the antibacterial test, screening was carried out for the phytochemicals in the 70% ethanol extract of Batak onion bulbs. Furthermore, the concentration of the test solution was made by diluting DMSO against the thick extract of Batak onion bulbs to obtain extract concentrations of 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.78%.

MIC Testing

Mueller Hinton Agar media (*Himedia*) is made by dissolving 38 grams of powder with 1 liter of distilled water and then sterilized in an autoclave at 121°C for 15 minutes and then poured into petri dishes. Mueller Hinton Broth media (Himedia) is made by dissolving 21 grams of powder with 1 liter of distilled water, then sterilized in an autoclave at 121°C for 15 minutes, and then poured into Petri dishes.

Making bacterial suspensions is done by taking one dose of *S.mutans* and *E.faecalis* colonies, then each is dissolved in a test tube containing 10 ml of 0.45% NaCl, homogenized with a vortex mixer, and incubated for 1-2 hours so that a McFarland Standard turbidity of 0.5 is obtained. Perform dilutions of each bacterium by mixing 0.1 ml of suspension and 9.9 ml of MHB and homogenizing again with a vortex mixer.

Measurement of antibacterial activity based on the diameter of the inhibition zone was carried out by the Kirby-Baurer disc diffusion method. Paper discs dripped with extracts according to the total sample were placed on Petri dishes containing MHA and bacteria that had been homogenized and then incubated for 24 hours. After incubation, observations were made by measuring the clear zone using calipers. The smallest concentration that has a clear zone is designated as the MIC.

MBC Testing

The Determination of MBC is done by counting the number of bacterial colonies. The clear zone of MIC results from each bacterium was streaked with a sterile cotton swab and then dipped in a test tube containing MHB and allowed to stand for 10 minutes. 1 ml of MHB was taken from the test tube, dripped on sterile petri, and added to PCA. It was then homogenized and incubated in an incubator at 37°C for 24 hours, after which bacterial colonies were counted with a colony counter. The concentration that can reduce bacteria by 98-99% of the initial number of colonies is determined as MBC.

Antibiofilm Testing

Antibiofilm testing used the Static Microtiter Plate Assay method by culturing each bacterial suspension in Trypticase Soy Broth (TSB) + 1% Glucose. 100µL of a 70% ethanol extract solution of various concentrations of Batak onion bulbs and 100µL of each bacterial suspension was put into a 96-well microplate and incubated at 37°C for 48 hours. After incubation, the contents of the microplate were removed, rinsed with Phosphate Buffered Saline (PBS) and fixed with a bunsen. Crystal violet solution (1% w/v) was given to each microplate container of 200 µL, incubated for 15 minutes at room temperature, and then rinsed with PBS. After drying, enter 200 µL of 96% ethanol in each wheel and incubate again. The microplate is then inserted into the microplate reader with a wavelength of 600nm. Repeat three times, and then the percentage is calculated according to Costa et al. (2016):[12]

$$\% \text{ Antibiofilm} = ((\text{Negative control OD} - \text{Sample OD}) / (\text{Negative control OD}) \times 100\%$$

OD=Optical Density

Test Analysis

The collected data were statistically examined With the aid of Statistical Product Service Solutions (SPSS). The Shapiro-Wilk method was used to evaluate the normality of the data, and one-way ANOVA and LSD post hoc tests with a significance threshold of $p<0.05$ were used to continue the data analysis.

RESULTS

According to the results of phytochemical screening, the secondary metabolites contained in the 70% ethanol extract of Batak onion bulbs are flavonoids, alkaloids, saponins and glycosides.

Antibacterial testing based on the diameter of the inhibition zone obtained MIC at a concentration of 0.78% with an average inhibition zone of *S.mutans* 9.00±0.43 mm and *E. faecalis* 8.06±0.20 mm (Table 1).

From the measurement of bacterial colonies, no MBC was found because all test concentrations could not reduce bacteria by 98-99%. The largest percentage of bacterial reduction produced was in the 50% extract with a percentage reduction of 94.67% in *S.mutans* and 84.19% in *E.faecalis* (Table 2).

Antibiofilm measurements obtained the results that 70% ethanol extract of Batak onion bulbs at a concentration of 0.78% was able to form antibiofilm against *S.mutans* and *E.faecalis* with a percentage of 84.55% and 85.73% (Table 3). This antibiofilm increased as the concentration increased to 12.5% and decreased antibiofilm reduction at 25% and 50% concentrations.

In Table 4, the results from the Post Hoc Test (LSD) show a comparison of concentrations that have significant differences from 70% ethanol extract of Batak onion bulbs against *S.mutans*

Table 1. Antibacterial activity based on the inhibition zone diameter of 70% ethanol extract of Batak onion bulbs against *Streptococcus mutans* and *Enterococcus faecalis*

Treatment Group	*Streptococcus mutans*		*Enterococcus faecalis*	
	Mean diameter of inhibition zone (mm)	p	Mean diameter of inhibition zone (mm)	p
50%	24.66±0.75	0.000*	13.86±0.55	0.000*
25%	20.83±1.36		12.56±0.41	
12.5%	13.53±0.50		11.16±0.87	
6.25%	12.40±0.36		10.03±0.60	
3.125%	10.13±0.30		9.53±0.35	
1.56%	9.76±0.47		8.96±0.32	
0.78%	9.00±0.43		8.06±0.20	
Chlorhexidine 0.2%	12.8±0.36		17.90±0.30	
DMSO	0.00±0.00		0.00±0.00	

*Statistically significant ($p<0.05$)

Table 2. Antibacterial activity based on bactericidal activity of 70% ethanol extract of Batak onion bulbs against *Streptococcus mutans* and *Enterococcus faecalis*

Treatment Group	*Streptococcus mutans*			*Enterococcus facealis*		
	Number of colonies (X±SD)(CFU/ml)	% Reduction	p	Number of colonies (X±SD)(CFU/ml)	% Reduction	p
50%	67±12.85	94.67%	0.000*	182±3.78	84.19%	0.000*
25%	117±4.72	90.67%		290±6.55	74.80%	
12.5%	213±13.11	83.05%		371±4.50	67.77%	
6.25%	309±4.16	75.42%		484±8.02	57.95%	
3.125%	406±8.14	67.70%		571±8.18	50.39%	
1.56%	522±5.56	58.47%		662±22.00	42.48%	
0.78%	641±12.50	49.00%		755±7.63	34.40%	
Chlorhexidine 0.2%	0±0.00	100.00%		0±0.00	100%	
DMSO	1257±156.61	0%		1151±46.51	0%	

*Statistically significant ($p<0.05$)

($p<0.05$). Post hoc inhibition of *S.mutans* group (I) all have significant differences except at concentrations of 12.5% against control (+), 6.25% against control (+), 3.125% against 1.56%, and 1.56% against 0.78%. Almost all post-hoc test results of MBC (II) had significant differences, except at concentrations of 50% against 25% and 50% against the control (+). The antibiofilm group (III) almost all had significant differences, except at concentrations of 50% against 25%, 50% against

Table 3. Antibiofilm activity of 70% ethanol extract of Batak onion bulbs against *Streptococcus mutans* and *Enterococcus faecalis*

Treatment Group	*Streptococcus mutans*			*Enterococcus faecalis*		
	Average *Optical Density* (OD)	% Reduction	p	Average *Optical Density* (OD)	% Reduction	p
50%	0.142±0.009	83.99%	0.001*	0.098±0.006	84.55%	0.000*
25%	0.136±0.017	84.66%		0.091±0.003	86.02%	
12.5%	0.116±0.004	86.92%		0.086±0.001	86.60%	
6.25%	0.118±0.001	86.69%		0.088±0.002	87.19%	
3.125%	0.121±0.003	86.35%		0.092±0.002	87.48%	
1.56%	0.124±0.001	86.02%		0.097±0.003	86.75%	
0.78%	0.137±0.007	84.55%		0.105±0.001	85.73%	
Chlorhexidine 0.2%	0.000±0.000	100,00%		0,0000±0,000	100.00%	
DMSO	0.887±0.007	0%		0,6544±0,063	0%	

* Statistically significant ($p<0.05$)

Table 4. LSD post hoc test results of differences in inhibition zone diameter, bactericidal, and antibiofilm activity test of 70% ethanol extract of Batak onion bulbs against *Streptococcus mutans*

Concentration		25%	12.5%	6.25%	3.125%	1.56%	0.78%	C(+)	C(-)
50%	I	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
	II	0.265	0.003*	0.000*	0.000*	0.000*	0.000*	0.134	0.000*
	III	0.305	0.001	0.001*	0.004	0.010*	0.389	0.000*	0.000*
25%	I	-	0.000	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
	II	-	0.040	0.000*	0.000*	0.000*	0.000*	0.014*	0.000*
	III	-	0.007	0.015	0.042	0.083	0.866	0.000*	0.000*
12.5%	I	-	-	0.038	0.000*	0.000*	0.000*	0.164	0.000*
	II	-	-	0.038	0.000*	0.000*	0.000*	0.000*	0.000*
	III	-	-	0.743	0.419	0.251	0.005*	0.000*	0.000*
6.25%	I	-	-	-	0.000*	0.000*	0.000*	0.440	0.000*
	II	-	-	-	0.037	0.000*	0.000*	0.000*	0.000*
	III	-	-	-	0.628	0.405	0.010*	0.000*	0.000*
3.125%	I	-	-	-	-	0.478	0.038	0.000*	0.000*
	II	-	-	-	-	0.016*	0.000*	0.000*	0.000*
	III	-	-	-	-	0.724	0.029	0.000*	0.000*
1.56%	I	-	-	-	-	-	0.147	0.000*	0.000*
	II	-	-	-	-	-	0.013	0.000*	0.000*
	III	-	-	-	-	-	0.060	0.000*	0.000*
0.78%	I	-	-	-	-	-	-	0.000*	0.000*
	II	-	-	-	-	-	-	0.000*	0.000*
	III	-	-	-	-	-	-	0.000*	0.000*

* Statistically significant ($p<0.05$)
Description I : Inhibition zone diameter
II : bactericidal activity
III : Antibiofilm activity

Table 5. LSD post hoc test results of differences in inhibition zone diameter, bactericidal, and antibiofilm activity test of 70% ethanol extract of Batak onion bulbs against *Enterococcus faecalis*

Concentration		25%	12.5%	6.25%	3.125%	1.56%	0.78%	C(+)	C(-)
50%	I	0.003*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
	II	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
	III	0.610	0.403	0.501	0.691	0.925	0.627	0.000*	0.000*
25%	I	-	0.002*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
	II	-	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
	III	-	0.741	0.869	0.909	0.676	0.324	0.000*	0.000*
12.5%	I	-	-	0.008*	0.000*	0.000*	0.000*	0.000*	0.000*
	II	-	-	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
	III	-	-	0.867	0.657	0.457	0.194	0.000*	0.000*
6.25%	I	-	-	-	0.206	0.012*	0.000*	0.000*	0.000*
	II	-	-	-	0.000*	0.000*	0.000*	0.000*	0.000*
	III	-	-	-	0.780	0.562	0.253	0.000*	0.000*
3.125%	I	-	-	-	-	0.155	0.001*	0.000*	0.000*
	II	-	-	-	-	0.000*	0.000*	0.000*	0.000*
	III	-	-	-	-	0.761	0.381	0.000*	0.000*
1.56%	I	-	-	-	-	-	0.030	0.000	0.000
	II	-	-	-	-	-	0.000	0.000	0.000
	III	-	-	-	-	-	0.563	0.000	0.000
0.78%	I	-	-	-	-	-	-	0.000	0.000
	II	-	-	-	-	-	-	0.000	0.000
	III	-	-	-	-	-	-	0.000	0.000

* Statistically significant ($p<0.05$) ;
Description I : Inhibition zone diameter
II : bactericidal activity
III : Antibiofilm activity

0.78%, 25% against 1.56%; 25% against 0.78%; 12.5% against 6.25%; 12.5% against 3.125%; 12.5% against 1.56%; 6.25% against 3.125%; 6.25% against 1.56%; 3.125%against 1.56%: and 1.56% against 0.78%.

In Table 5, the results from the post hoc test (LSD) show a concentration comparison that has a significant difference from 70% ethanol extract of Batak onion bulbs against *E.faecalis* ($p<0.05$). Post hoc group inhibition zone (I) against *E.faecalis* mostly has significant differences, except at concentrations of 6.25% against 3.125% and 3.125% against 1.56%. In the post hoc test of MBC (II), all of them have significantly different concentration comparisons ($p<0.05$). Almost all concentration comparisons in the post hoc antibiofilm group (III) have significant differences except in the concentration group against the control group.

DISCUSSION

70% ethanol as a solvent was determined because ethanol is a universal solvent that can dissolve all secondary metabolite compounds contained in simplicia and has lower toxicity than another organic solvent. 70% ethanol can dissolve phytochemical compounds more optimally because 70% ethanol still contains quite a lot of water (30%), which helps the extraction process so that some of these compounds can be attracted to ethanol, and some are attracted to water.[16,17]

The results of the antibacterial activity test based on the diameter of the inhibition zone of 70% ethanol extract of Batak onion bulbs show that Batak onion bulbs have antibacterial activity ($p<0.05$) against *S.mutans* and *E.faecalis* bacteria. The observation of the inhibition zone and the formation of a clear zone around the disc

evidence this. There are research results stated that the higher the extract concentration, the greater the inhibition zone formed due to the greater content of antibacterial active compounds.[18] The research is in line with the results of this study with the increasing inhibition zone value from the smallest to the largest concentration against *S.mutans* and *E.faecalis* (Table 1).

The results of antibacterial research based on MBC in this study did not find MBC concentrations in *S.mutans* and *E.faecalis* except in the positive control group (chlorhexidine 0.2%). According to research by Nasri et al., a good MBC value comes from the lowest extract concentration, which can reduce bacteria by 98%-99%.[19] The highest extract concentration in this study was at a concentration of 50%, reducing the number of bacterial colonies 94.67% in *S.mutans* and 84.19% in *E.faecalis*. This shows that the 50% Batak onion bulb extract concentration can still not fulfill the MBC value requirements. Still, it does not rule out the possibility that at concentrations above 50% Batak onion bulb extract can kill *S.mutans* and *E.faecalis*.

This study is in line with research regarding antibacterial extracts of Batak onion bulbs with 70% ethanol solvent at concentrations of 12.5%, 25%, 50%, and 70% against *Escherichia coli*, which reported that the highest inhibition zone diameter value was found in the 70% concentration with a value of 8.70 mm. In comparison, the smallest inhibition zone diameter was found in the 12.5% concentration treatment with a value of 6.53 mm.[20] This study is also consistent with earlier research on antibacterial extracts of Batak onion with 96% ethanol solvent at concentrations of 100%, 50%, 25%, 12,5%, and 6,25% against Methicilin-Resistant *Staphylococcus aureus* (MRSA) with inhibition of bacterial growth occurring at concentration levels of 50% and 100% with inhibition zone diameters of 8,695 mm and 10,545 mm, respectively. In contrast, extracts with 25%, 12,5%, and 6,25% concentration levels showed no inhibition.[13] In this study, an extract of Batak onion bulbs using 70% ethanol solvent with different bacteria, namely *S.mutans,* at a concentration of 0.78%, providing a better inhibition zone with an inhibition zone diameter value of 9.00 mm. In comparison, the 0.78% extract concentration provided an inhibition zone of 8.06 mm against *E.faecalis*.

Research on Batak onion bulb extracts against bacteria and different solvents has also previously been carried out by Rubiatik et al., who reported that methanol extracts of Batak onion bulbs with concentrations of 0%, 1%, 5%, 10%, 15%, 20%, and 25% began to show inhibitory capability against St*aphylococcus aureus* at a concentration of 1% with an inhibition zone diameter of 5 mm.[21] A research stated that a 96% ethanol extract from Batak onion bulbs with a concentration of 2.5% inhibited the growth of *Escherichia coli*.[2] Other research stated MRSA stopped growing when 96% ethanol extract from Batak onion bulbs was added at a concentration of 50%.[13] T. Rhetso also stated that extract of Batak onion bulbs with Gas Chromatography-Mass Spectrometry (GC-MS) analysis at a concentration of 1 ìg/ml could inhibit the growth of *Staphylococcus aureus*, Pseudomonas aeruginosa, and *Aspergillus niger*.[22] All the research results still show that the extract of Batak onion bulbs (*Allium chinense* G.Don.) has antibacterial activity that can inhibit bacterial growth, even though the test bacteria and solvents differ.

The results of the phytochemical screening test in this study showed that a 70% ethanol extract of Batak onion bulbs contained secondary metabolite compounds in the form of flavonoids, alkaloids, glycosides and saponins. Antibacterial and antibiofilm activity occurs due to the content of active compounds in the 70% ethanol extract of Batak onion bulbs that function as antibacterials. Flavonoids have a mechanism of action in inhibiting bacterial cell membrane function by forming complex compounds against extracellular proteins. Then, bacterial cell proteins will be denatured, causing the cell membrane to be damaged and intracellular compounds to come out. Alkaloids can interfere with the components of the preparation of the cell wall and bacterial DNA, causing the bacterial cell wall layer to not form intact and experience the cell's death. Saponins can block the formation or transportation of each component to the cell wall and release cell contents, ultimately inhibiting or killing bacterial cell growth.[23-25]

Based on the Indonesian Pharmacopoeia VI edition, the diameter of the inhibition zone

formed from an extract against bacteria is considered satisfactory if the diameter produced is greater than 14 mm.[26] In this study, the diameter of the inhibition zone formed was greater than 14 mm against *S.mutans* at concentrations of 25% (20.83 ± 1.36 mm) and 50% (24.66±0.75 mm), while *E.faecalis* bacteria were only found in the positive control (chlorhexidine 0.2%). However, if the results of the diameter of the inhibition zone are classified according to David and Stout, namely:[23] a). the category of no inhibition zone; b). weak inhibition is the zone of less than 5 mm; c). moderate is the inhibition zone of 5-10 mm; d). strong is the inhibition zone of 10-20 mm; e). very strong, namely the inhibition zone of 20-30 mm, then the antibacterial extract of Batak onion bulbs against *S.mutans* concentrations of 0.78%, 1.56%, and 3.125% are in the category of moderate inhibition, concentrations of 6.25% and 12.5% are in the category of strong inhibition. Extracts with concentrations of 25% and 50% are in the category of very strong. On *E.faecalis* obtained, extract concentrations of 0.78%, 1.56%, and 3.125% were included in the moderate inhibition category, and concentrations of 6.25%, 12.5%, 25%, and 50% were included in the strong inhibition category.

In this study, the extract of Batak onion bulbs with a concentration of 6.25% is the optimal extract concentration because the concentration of 6.25% is the first extract concentration with a strong inhibition category and is the smallest concentration significantly different in inhibition with a concentration of 1.56% in the LSD post hoc test against *E.faecalis* ($p < 0.05$). In *S.mutans*, it was also found that the concentration of 6.25% had a strong inhibition category and was the smallest concentration significantly different in inhibition against *S.mutans* bacteria against the concentration of 3.125% (has moderate inhibition) based on the LSD post hoc test ($p<0.05$) (Table 5 and Table 6).

The accumulation of biofilm occurs in two stages. The first stage is cell growth and forming Extracellular Polymeric Substances (EPS), so biofilm cells can accumulate. The second stage is detachment or reattachment, using extracellular organs and proteins to sense and attach to the surface. Therefore, inhibiting or killing cells from increasing and preventing the formation of EPS is a way to prevent biofilm growth through antibacterial substances contained in an extract.[27] The presence of antibiofilm activity in this study is due to the 70% ethanol extract of Batak onion bulbs, which contains flavonoid, alkaloid, and saponin phytochemical compounds that work synergistically to damage biofilms and then kill planktonic bacteria that protect biofilms so that they can effectively inhibit biofilm formation.[28]

The results of the antibiofilm test showed that 70% ethanol extract of Batak onion bulbs was able to inhibit biofilm formation at a concentration of 0.78% by 84.55% against *S.mutans* and 84.71% against *E.faecalis* and continued to increase until a concentration of 12.5%. The antibiofilm properties decreased at 25% and 50%, which is not in line with the greater the concentration, the higher the antibacterial effect. This can occur because the more chemical content in an extract (high concentration at a certain limit), the higher the antimicrobial effect, but if the content of compounds dissolved in the extract is too high (too high concentration), it can cause saturation, thus affecting the antimicrobial power of the extract. These results follow the hyperbolic curve theory, namely regarding the relationship between concentration and drug effects, which states that the higher the concentration of the drug, the higher the drug effect, but if it reaches the maximum effect at a certain concentration (optimal concentration), then the drug effect will decrease at a concentration higher than the optimal concentration.[29]

CONCLUSION

The MIC of 70% ethanol extract of Batak onion (*Allium chinense* G.Don.) against the growth of S.Mutans and E.faecealis is at a concentration of 0,78% with an average inhibition zone diameter of 9.00±0.43 mm and 8.06±0.20 mm. The MBC and the Antibiofilm activity with 98-99% reduction in this study has not been found among the concentrations of the tested extracts. The highest concentration of 70% ethanol extract of Batak onion in this study is 50%, which killed and reduced the biofilm of S.Mutans at 94.67% and 83.99% and killed and reduced the biofilm of *F.Faecalis* at 84.19% and 84.55%. Further research is required to prove that the concentration of 6.25%-50% of 70% ethanol extract of Batak onion is antibacterial against *S.mutans* and 50% against *E.faecalis*.

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Conflict of Interest

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REFERENCES

1. Asra R, Mahya I, Adriani F, Silalahi M, IA Fijridiyanto, AT Maryani. Diversity of medical plants sold in the traditional markets in Jambi, Indonesia. Biodiversitas 2023, 24(3):1544-50
2. FG Naibaho, M Bintang, FH Pasaribu. Antimicrobial activity of *Allium chinense* G.Don. Current biochemistry 2015, 2(3):129-138
3. Amadou A Bah, F Wang, Z huang, IH Shamsi, Q Zhang, G Jilani, S Hussain, N Hussain, E Ali. Phyto-characteristic, cultivation and medical prospects of Chinese jiaotou (*Allium chinense*). International jounal of agriculture & biology 2012, 14(4); 650-657
4. Y Pin Lin, L Yun Lin, H Yu Yeh, C Hung Chuang. Antihyperlipidemic activity of *Allium chinense* bulbs. Journal of food and drug analysis 2016. 24(3); 516-26
5. Supomo, H Warnida, BM Sahid. Perbandingan metode ekstraksi ekstrak umbi bawang rambut (*Allium chinense* G.Don.) menggunakan pelarut etanol 70% terhadap rendemen dan skrining fitokimia. Jurnal Riset Kefarmasian Indonesia 2019, 1(1): 30-40
6. FS Susilo, D Aripin, AA Suwargiani. Practices of oral health maintenance, caries protective factors and caroes experience in adult. Padjajaran Journal of Dentistry 2021. 33(2): 170-180
7. Utamaningyas A, HT Pramesti, FF Balafif. The *Streptococcus mutans* ability to survive in biofilm dan during dental caries formation: scoping riview. Journal of Syiah Dentistry society 2022. 7(2): 150-58
8. AA Nurbianti, Alhawaris, S Yani. Daya hambat ekstrak etanol batang akar kuning (*Arcangelisia flava* (L) Merr) terhadap pertumbuhan bakteri *Streptococcus mutans, Porphyromonas gingivalis* dan *Enterococcus faecalis*. Mulawarman dental journal 2021, 1(2): 76-89
9. S Deviyanti. Potensi larutan chitosan 0,2% sebagai alternatif bahan irigasi dalam perawatan saluran akar gigi (kajian pustaka). JITEKGI 2018. 14(1): 6-10
10. R Novamaura, F Meliawaty, Jeffrey. Comparison of the effects of hexetidine and chlorhexidine mouthwash on the plaque index. Odonto: Dental journal 2022, 9(2): 323-33
11. N Aini, HY Mandalas, K Edinata. Perbandingan Efektivitas Berkumur Dengan *Chlorhexidine* dan Obat Kumur yang Mengandung Daun Sirih (*Piper betle*) Terhadap Penurunan Indeks Plak Pasien Pengguna Alat Ortodontik Cekat. SONDE (Sound of dentistry) 2021, 6(2): 45-57
12. 12. Ung-Pin Lin, Li-Yun Lin, Hsiang-Yu Yeh, Cheng-Hung Chuang, Sheng-Wen Tseng, Yue-Horng Yen, Antihyperlipidemic activity of *Allium chinense* bulbs. Journal of food and drug analysis 2016; 24(3): 516-26
13. Aretha, T & Jusuf , NK. In vitro antimicrobial activity of bawang batak bulbs (*Allium Chinense* G. Don) against Methicillin-Resistant *Staphylococcus Aureus*. Sumatera medical journal 2019; 2(2): 67-71
14. Kavita Sharma, Neelima Mahato, Yong Rok Lee. Systematic study on active compounds as antibacterial and antibiofilm agent in aging onions. Journal of food and drug analysis 2018; 26(2): 518-28
15. Widarta, I.W.R., & Wiadnyani, A.A.I.S. Pengaruh metode pengeringan terhadap aktivitas antioksidan daun alpukat. Jurnal aplikasi teknologi pangan 2019; 8(3): 80-85.
16. Ibrahim, S.T.P., Simbala, H., & Lebang, J. Acute toxicity test of combination extracts of dayak onion (*Eleutherine americana* Merr.) and Yaki pinang (*Areca vestiaria* Giseke) against heart organs of wistar male white rat (*Rattus norvegicus*). PHARMACON 2023; 12(1): 48-56.
17. Sani, R.N., Nisa, F.C., Andriani, R.D., & Maligan, J.M. Yield analysis and phytochemical screening ethanol extract of marine *Microalgae tetraselmis* chuii. Jurnal Pangan dan Agroindustri 2014; 2(2): 121-6.
18. Lingga AR, Pato U, Rossi E. Uji antibakteri ekstrak batang kecombrang (*Nicolaia speciosa* Horan) terhadap *Staphylococcus aureus* dan *Escherichia coli*. JOM Faperta 2016; 3(1): 1-15
19. Nasri, Harahap U, Silalahi J, Satria D. Antibacterial activity of lactic acid bacteria isolated from dengke naniura of carp (*Cyprinus carpio*) against diarrhea-causing pathogenic bacteria. Biodiversitas 2021; 22(8): 3098-104
20. Siregar DJS, Tasfin M, Warisman. Uji antimikrob ekstrak bawang Batak (*Allium chinense* G. Don.) terhadap diameter zona hambat bakteri *Escherichia coli* dan *Salmonella typhi*. EKSAKTA: Jurnal Penelitian dan Pembelajaran MIPA 2020; 5(1): 69-80.
21. Rubiatik S, Sartini, Lubis R. Skrining fitokimia dan uji antimikroba ekstrak kasar bawang Batak (*Allium Cinense*) terhadap bakteri *Staphylococcus aureus* dan *Escherichia coli*.

Jurnal BioLink 2015; 2(1): 1-9.

22. Rhetso, Thejanuo & Shubharani, R. & Roopa,M. & Venkataramegowda, Sivaram. Chemical constituents, antioxidant, and antimicrobial activity of *Allium chinense* G. Don. Future Journal of Pharmaceutical Sciences 2020; 6(1); 1-9.
23. Supomo, Soemarie YB, Saihatin U. Uji aktivitas antibakteri ekstrak etanol umbi bawang rambut (*Allium chinense* G. Don.) terhadap bakteri *Escherichia coli* ATCC 8939. Media Sains 2017; 10(2): 189-95.
24. Nomer NMGR, Duniaji AS, Nocianitri KA. 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.
25. Kapondo GL, Fatimawali, Jayanti M. Isolasi, identifikasi senyawa alkaloid dan uji efektivitas penghambatan dari ekstrak daun sirih (*Piper betle* L.) terhadap bakteri *Staphylococcus epidermidis*. *e*-Biomedik 2020; 8(2): 180-6.
26. Departemen Kesehatan Republik Indonesia. Farmakope Indonesia. Ed 6. Jakarta: DEPKES RI, 2020: 48.
27. E Kining, S Falah, N Nurhidayat. The In Vitro Antibiofilm Activity of Water Leaf Extract of Papaya (*Carica papaya* L.) against *Pseudomonas aeruginosa*. Current Biochemistry 2016. 2(3): 150-63.
28. SN Aini, R Effendy, I Widjiastuti. Konsentrasi Efektif Ekstrak Daun Salam (*Syzygium polyanthum Wight*) terhadap Hambatan Biofilm *Enterococcus faecalis*. Conservative dentistry journal 2016. 6(2): 87.
29. Rosyada AG, Prihastuti CC, Sari DNI, Setiawati, Ichsyani M, Laksitasari A, et al. Aktivitas antibiofilm ekstrak etanol kulit bawang merah (*Allium cepa* L.) dalam menghambat pembentukan biofilm *Staphylococcus aureus* ATCC 25923: penelitian eksperimental laboratoris. Jurnal Kedokteran gigi UNPAD 2023, 35(1); 33-40.