Journal of
Fundamental and Applied Pharmaceutical Science
https://journal.umy.ac.id/index.php/jfap

Antibacterial Activity of *Abrus precatorius* L. Leaves Against *Streptococcus mutans* ATCC 25175 Bacteria

Nadia Aisy Andika*, Kusumaningtyas Siwi Artini, Tatiana Siska Wardani
Faculty oh Healthy Sciences, Universitas Duta Bangsa Surakarta, Jl. Pinang No. 47, Jati, Cemani, Kec. Grogol, Kabupaten Sukoharjo, Jawa Tengah

Abstract

One of the herbal plants that have medicinal properties is Abrus precatorius L or commonly known as Saga in Indonesia. Empirically, the boiled water of saga leaves is widely used as an ingredient in cough medicine, cancer sores and swollen tonsils. The chemical constituents of antibacterial activity in the saga leaves are glycosides (abrusoside AD and abrusgenin), flavonoids and saponins (glycerin). This study aims to determine the antibacterial activity of extracts and fractions from saga leaves and the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the most active fraction of saga leaves on the growth of Streptococcus mutans ATCC 25175. Saga leaf powder was macerated using 96% ethanol, then fractionated using n-hexane, ethyl acetate, water as solvent, 96% ethanol extract, and n-hexane fraction. The antibacterial activity test using the diffusion method showed that the extract, n-hexane fraction, ethyl acetate fraction, and water fraction of saga leaves had antibacterial activity against Streptococcus mutans. The most active fraction was the ethyl acetate fraction, with a concentration of 50% with an average inhibition zone diameter of 12.2 mm. The ethyl acetate fraction from saga leaves had the most active antibacterial activity compared to ethanol extract, n-hexane fraction, ethyl acetate fraction, and water fraction, as seen from the average diameter of the inhibition zone obtained. The test results of the dilution method of the ethyl acetate fraction of saga leaves showed a Minimum Inhibitory Concentration of 12.5% and a Minimum Killing Concentration of 25%.

Keywords: *Abrus precatorius*. L; antibacterial; extract and fraction; Streptococcus mutansATCC25175

Data of article
Received : 17 Sept 2022
Reviewed : 22 Dec 2022
Accepted : 27 Mar 2023

DOI
10.18196/jfaps.v3i2.16206

Type of article:
Research

INTRODUCTION

One of the herbal plants that have medicinal properties is *Abrus precatorius* L. The saga plant belongs to the Leguminosae plant family and is a type of herbaceous plant with small stems that propagates to the host in a twisted manner. This plant grows wild in forests and fields or is deliberately kept in the yard.[1]

Saga plants contain flavonoid and steroid compounds in the leaves. The chemical constituents of antibacterial activity in saga leaves are glycosides (abrusoside AD

* *Corresponding author, e-mail: nadiayaya216@gmail.com*

and abrusgenin), flavonoids and saponins (glycerin). Empirically, saga leaves boiled in hot are widely used as a cough medicine for cancer sores and swollen tonsils.[2]

Among the 300 species of bacteria in the oral cavity, *Streptococcus mutants* are the bacteria that mostly cause dental caries.[3,4] *Streptococcus mutant* is a gram-positive bacterium that forms dental plaque in the form of a sticky substance containing bacteria and products that form on the tooth surface.[5]

In previous studies, it was known that saga leaf extract *((Abrus precatorius* L.) was able to inhibit the growth of gram-positive bacteria, namely *Staphylococcus aureus*, *Streptococcus beta-hemolytic, Streptococcus pneumonia,* so that it could be seen that saga leaves had the potential as antibacterial.[6,7]

Based on the description above, a study was conducted on the antibacterial extract and the active fraction of *Abrus precatorius* L. leaf against the growth of *Streptococcus mutans* ATCC 25175 to determine which of the solvents had the greatest inhibitory power and to determine the value of the Minimum Inhibitory Concentration (MIC) and its Minimum Bactericidal Concentration (MBC).

METHOD

Materials

This research utilized aluminum foil, bacterial culture of *Streptococcus mutans* ATCC 25175, *Abrus precatorius* L leaf powder, antibiotic disc ciprofloxacin 5µg/ml, TLC plate, cotton, sterile gauze, sterile disc paper, filter paper, *Mueller Hinton Agar* (MHA) media, Nutrien Broth media (NB), 0.9% NaCl, 10% DMSO, 96% ethanol solvent, ethyl acetate solvent, n-hexane solvent, aqua dest, tissue, and cotton.

Plant Determination

The *Abrus precatorius* L. leaves were taken from the Tawangmangu area, Karanganyar Regency, Central Java. The Abrus precatorius L. was determined at the Biology Laboratory of Setia Budi University, Surakarta.

Simplicia Preparation

The *Abrus precatorius* L. leaves were washed using running water and separated from the attached dirt. Drying was done directly under the hot sun covered with black cloth. Pollination was done using a blender. They were sifted using mesh number 40.[6]

Extraction

The simplicial powder was weighed as much as 550 grams, then put into a maceration bottle and added with 96% ethanol as a solvent in a ratio (1:10). The bottle was stored in a place protected from sunlight for 5 days. The macerate was concentrated using a rotary evaporator at a temperature of approximately 40°C to obtain a thick ethanol extract.[6]

The concentrated extract from the maceration was then fractionated using a solvent of different polarity. Fractionation was carried out using the LLF (Liquid-Liquid Fractionation) method with *n*-hexane (no*n*-polar solvent), ethyl acetate (semi-polar solvent), and water (polar solvent) using a separating funnel. The thick extract of saga leaves was weighed as much as 10 g, then dissolved with 75 ml of water solvent (replicated 3 times), and fractionated with 75 ml of *n*-hexane solvent (replicated 3 times), the residue obtained from the *n*-hexane fraction followed by fractionation with 75 ml of ethyl acetate solvent each (replicated 3

times). The result was the ethyl acetate fraction, and the residue obtained from the ethyl acetate fraction is the water fraction. The results of each fraction were evaporated with a water bath.[8]

Phytochemical Screening Test

Phytochemical tests of the ethanolic extract of saga leaves were carried out, including tests for alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids.

Antibacterial Activity Test

Bacterial Rejuvenation

Culture *Streptococcus mutans* ATCC 25175 was rejuvenated by using the method to tilt it by scratching it on the surface of the media *Mueller Hinton* so that *it* tilted in a zig-zag manner and then incubated at 37°C for 24 hours using an incubator.

Preparation of Streptococcus mutans suspension ATCC 25175

Culture *Streptococcus mutans* ATCC 25175 was suspended in a tube containing sterile 0.9% NaCl solution and incubated at 37°C for 15 minutes, then compared the turbidity of the suspension with standard Mc. Farland.[9]

Making Variable Concentration of Saga Leaf Extract

A 50% concentration of mother liquor was made from the thick extract of saga leaves, and each fraction with 10% DMSO as solvent. The dilution was continued until the test solution concentration series was 25% w/v, 12.5% w/v, and 6.25% w/v. Furthermore, for the MIC and MBC testing, the sample with the greatest antibacterial activity was selected, then the concentration was made at 25% w/v, 12.5% w/v, 6.25% w/v, 3.12% w/v, 1, 56% w/v.

Determination of Bacterial Inhibitory Zones Disc Diffusion Method

Mueller Hinton Agar (MHA) media was poured into 10 ml Petri dishes each and allowed to solidify as a base layer. Furthermore, Streptococcus mutans bacteria was suspended in a petri dish with a micropipette under aseptic conditions. Furthermore, the disc paper dipped in the test material was placed on the plate, positive control disk ciprofloxacin and negative control DMSO 10%. The Petri dish was incubated for 24 hours at 30–37°C. The inhibition zone formed around the paper disc was measured with a caliper.

Determination of Minimum Inhibitory Level (MIC) and Minimum Bactericidal Concentration (MBC) in the Most Active Fraction by Dilution Method

Prepared 8 test tubes. The stock solution concentration was 50%, then diluted with 10% DMSO solvent. Aseptically from the stock solution, a series of concentrations were made below that of 25% (tube 1); 12.5% (tube 2); 6.25% (tube 3); 3.12% (tube 4); 1.56% (tube 5); positive control (tube 6) and negative control (tube 7),

The NB media used was put in 2 ml in each tube. Furthermore, aseptically, 4 ml of the stock solution to be tested was put into the stock tube, then 2 ml of the tube was pipetted and put into tube 1. 2 ml of tube 1 was pipetted and put into tube 2, and so on until the test tube 5. On test tube 5, 2 ml was discarded. Test tube 6 was added with 1 ml of ciprofloxacin as a positive control, and test tube 7 was filled with 1 ml of 10% DMSO as a negative control. The entire test tube was then added to the bacterial suspension *Streptococcus mutans* ATCC 25175 and incubated for 24 hours at 37°C in an incubator. The smallest concentration of test material in the tube that showed the absence of turbidity in the tube was

called the Minimum Inhibitory Concentration (MIC). The Minimum Bactericidal Concentration (MBC) was calculated by inoculating the sample in the tube on *Mueller Hinton Agar* (MHA) media in a petri dish, then incubated at 37°C for 24 hours.

Data Processing and Analysis Method

The data obtained from the test results are presented in tabular form and compared with the table classification of bacterial growth inhibition response.[10]

Table 1. Classification of Bacterial Growth Inhibitory Responses

Bright zone diameter	Growth inhibition response
> 17 mm	Strong
12–16 mm	Medium
7–11 mm	Weak
0 mm	No inhibition

The data was then processed using SPSS (Statistical Product for Service Solutions) version 23 with the One-Way ANOVA (Analysis of Variance) test.

RESULTS AND DISCUSSION

Determination Results

Plant determination aims to determine the correctness of the plant and avoid errors in the material collection. The determination was carried out at the Biology Laboratory, Setia Budi University, Surakarata. Based on the determination results, the sample was *Abrus precatorius* L or commonly known as Saga in Indonesia

Drying Loss

The test used an oven at a temperature of 105°C for 30 minutes with a drying shrinkage of 3.33%. The test results met the drying shrinkage parameter of not more than 10%.[11]

Extraction Results

The results of the extraction of saga leaf powder were 550 grams, which was found to weigh 69.43 grams with a yield of 12%. The yield obtained met the requirements of the Indonesian herbal pharmacopeia, which was not less than 7.2%.[11] The results of the calculation of the average percentage yield of saga leaf extract, namely the *n*-hexane fraction of saga leaves of 22.121%, ethyl acetate fraction of saga leaves of 24.401% and water fraction of saga leaves of 25.861%.

Water Content Test Results

The moisture content was tested with a moisture balance at a temperature of 105°C for 5 minutes. The result showed the water content of the Saga leaf extract was 9.02%. These results met the requirements of the Indonesian Ministry of Health (2000), which stated that the percentage of water content should be not more than 10%.

Ethanol Free Test Results

Ethanol-free testing on saga leaf extract has been carried out utilizing the esterification test. The results were positive for ethanol-free saga leaf extract as it did not smell the distinctive odor of ethanol esters when tested.[12]

Phytochemical Screening Test Results

Based on the phytochemical screening test results, the ethanolic extract of saga leaves was positive for flavonoid compounds, saponins, steroids, phenols and tannins. The results of the phytochemical screening test can be seen in Table 2.

Table 2. Phytochemical Screening Test Result, Saga Result, Saga Leaf Extract (*(Abrus precatorius* linn.)

Determination Results	**Determination Results**	**Determination Results**
Plant determination aims to determine the correctness of the plant and avoid errors in the material collection. The determination was carried out at the Biology Laboratory, Setia Budi University, Surakarata. Based on the determination results, the sample was *Abrus precatorius* L or commonly known as Saga in Indonesia	Plant determination aims to determine the correctness of the plant and avoid errors in the material collection. The determination was carried out at the Biology Laboratory, Setia Budi University, Surakarata. Based on the determination results, the sample was *Abrus precatorius* L or commonly known as Saga in Indonesia	Plant determination aims to determine the correctness of the plant and avoid errors in the material collection. The determination was carried out at the Biology Laboratory, Setia Budi University, Surakarata. Based on the determination results, the sample was *Abrus precatorius* L or commonly known as Saga in Indonesia
Drying Loss	**Drying Loss**	**Drying Loss**
The test used an oven at a temperature of 105°C for 30 minutes with a drying shrinkage of 3.33%. The test results met the drying shrinkage parameter of not more than 10%.[11]	The test used an oven at a temperature of 105°C for 30 minutes with a drying shrinkage of 3.33%. The test results met the drying shrinkage parameter of not more than 10%.[11]	The test used an oven at a temperature of 105°C for 30 minutes with a drying shrinkage of 3.33%. The test results met the drying shrinkage parameter of not more than 10%.[11]
Extraction Results	**Extraction Results**	**Extraction Results**
The results of the extraction of saga leaf powder were 550 grams, which was found to weigh 69.43 grams with a yield of 12%. The yield obtained met the requirements of the Indonesian herbal pharmacopeia, which was not less than 7.2%.[11] The results of the calculation of the average percentage yield of saga leaf extract, namely the *n*-hexane fraction of saga leaves of 22.121%, ethyl acetate fraction of saga leaves of 24.401% and water	The results of the extraction of saga leaf powder were 550 grams, which was found to weigh 69.43 grams with a yield of 12%. The yield obtained met the requirements of the Indonesian herbal pharmacopeia, which was not less than 7.2%.[11] The results of the calculation of the average percentage yield of saga leaf extract, namely the *n*-hexane fraction of saga leaves of 22.121%, ethyl acetate fraction of saga leaves of 24.401% and water	The results of the extraction of saga leaf powder were 550 grams, which was found to weigh 69.43 grams with a yield of 12%. The yield obtained met the requirements of the Indonesian herbal pharmacopeia, which was not less than 7.2%.[11] The results of the calculation of the average percentage yield of saga leaf extract, namely the *n*-hexane fraction of saga leaves of 22.121%, ethyl acetate fraction of saga leaves of 24.401% and water

Determination Results	Determination Results	Determination Results
fraction of saga leaves of 25.861%.	fraction of saga leaves of 25.861%.	fraction of saga leaves of 25.861%.

Chromatography (TLC) Test Results

Thin Layer Chromatography (TLC) test was carried out to confirm the presence of secondary metabolites in the extract and the fraction of saga leaves.

Table 3. Thin Layer Chromatography Test Result of Saga Leaf Extract and Fraction (*Abrus precatorius* Linn.)

Chemical Content	Test Material	Mobile phase	Stain Color	UV 366	Rf	Reference
Alkaloids (Dragondorf stain viewer)	Extract	Toluene: Ethyl acetate: Diethylamine (7:2:1)	Brownish-yellow	Brownish-yellow	0.45	13
	F. Water	Toluene: Ethyl acetate: Diethylamine (7:2:1)	Brownish-yellow	Brownish-yellow	0.59	13
	F. Ethyl Acetate	Toluene: Ethyl acetate: Diethylamine (7:2:1)	Brownish-yellow	Brownish-yellow	0.61	13
	F. *n*-hexane	Toluene: Ethyl acetate: Diethylamine (7:2:1)	-	-	-	13
Flavonoids (Ammoniac vapor stain viewer)	Extract	Butanol : Acetic acid : Water (4:5:1)	Yellowish Green	Greenish blue	0.80 0.61	14
	F. Water	Butanol : Acetic acid : Water (4:5:1)	Yellowish Green	Greenish blue	0.76	14
	F. Ethyl Acetate	Butanol : Acetic acid : Water (4:5:1)	Yellowish Green	Greenish blue	0.78 0.58	14
	F. *n*-hexane	Butanol : Acetic acid : Water (4:5:1)	Yellowish Green	Greenish blue	0.62	14
Tannins ($FeCl_3$ stain viewer)	Extract	Toluene: Ethyl acetate (3:1)	Black	Dark blue	0.61	14
	F. Water	Toluene: Ethyl acetate (3:1)	Black	Dark blue	0.64	14

Chemical Content	Test Material	Mobile phase	Stain Color	UV 366	Rf	Reference
	F. Ethyl Acetate	Toluene: Ethyl acetate (3:1)	Black	Dark blue	0.72	14
	F. *n*-hexane	Toluene: Ethyl acetate (3:1)	Black	Dark blue	0.60	14

Antibacterial Activity Test of Saga Leaf Extract and Fraction (*(Abrus precatorius* L.) Disc Diffusion Method

Antibacterial activity tests were carried out on each extract and fraction of saga leaves from concentrations of 50%, 25%, 12.5%, and 6.25%. The measurement results are listed in Table 4.

Table 4. Activity Test Result of Saga Leaves (*(Abrus precatorius L.)* Against *Streptococcus mutans* Bacteria Using The Diffusion Method

Test Material	Concentration (%)	Barrier Zone Diameter (mm)			Average	Category
		P1	P2	P3		
Extract	50%	10.0	10.5	10.5	10.3	Weak
	25%	9.5	9.5	10	9.6	Weak
	12.5%	8.0	8.5	8.5	8.3	Weak
	6.25%	8.0	8.0	8.5	8.2	Weak
Water Faction	50%	10.0	10.0	10.5	10.2	Weak
	25%	9.0	9.5	9.5	9.3	Weak
	12.5%	8.0	8.0	8.5	8.2	Weak
	6.25%	7.0	7.0	7.5	7.2	Weak
Ethyl Acetate Fraction	50%	12.0	12.0	12.5	12.2	Medium
	25%	9.5	10.0	10.0	9.8	Weak
	12.5%	8.0	8.0	8.5	8.2	Weak
	6.25%	7.0	7.0	7.5	7.2	Weak
***n*- hexane fraction**	50%	8.0	8.5	8.5	8.3	Weak
	25%	7.5	8.0	8.0	7.8	Weak
	12.5%	7.0	7.0	7.5	7.1	Weak
	6.25%	5.0	5.0	6.5	5.5	Weak
+ control (ciprofloxacin)	0.0005%	21	21	22	21.3	Strong
Control –	10%	0	0	0	0	No inhibited

Information:
P1: Repetition 1
P2: Repetition 2
P3: Repetition 3
Positive Control: Ciprofloxacin disk 5 g/ml (0.0005%)
Negative Control: DMSO 10%

According to the response classification table of bacterial growth inhibition zones, the inhibitory power of positive control disk ciprofloxacin of 21.3 mm belongs to the strong category.[10] The ethyl acetate fraction of saga leaves at a concentration of 50% of 12.2 mm belonged to the medium category. In comparison, the ethanol extract of saga leaves at a concentration of 50% of 10.3 was in the weak category, the water fraction of saga leaves at a concentration of 50% of 10.2 mm was in the weak category, and the n-hexane fraction of saga leaves at a concentration of 50% of 8.3 mm was categorized as weak. The negative control DMSO 10% of 0 mm had no inhibition. These results revealed that the most active fraction was the ethyl acetate fraction. Ethyl acetate solvent attracted antibacterial compounds in Saga leaves, namely flavonoids, phenols, and glycosides. The higher the concentration series of saga leaf extract and fraction is, the stronger the inhibitory response to the growth of *Streptococcus mutants* bacteria will be. It aligns with the statement of Frazier and Westhof,[15] denoting that concentration can affect the effectiveness of an antimicrobial substance. The increase in the concentration of the extract causes a greater number of antimicrobial compounds to diffuse into the agar media, so an increase in the inhibition zone is expected

Chemical compounds in the extract and fraction of saga leaves that are considered to have antibacterial activity against *Streptococcus mutants* bacteria are alkaloids, flavonoids, and saponins. The ability of flavonoid compounds as antibacterial is influenced by the difference in polarity between the lipids that make up bacterial DNA and the alcohol groups on the flavonoid compounds, which cause damage to the bacterial DNA structure so that bacterial cells undergo lysis and die.[16] In alkaloid compounds, there are nitrogen groups that, when in contact with bacteria, will change the genetic balance in bacterial DNA. Thus, the bacterial cell nucleus will be damaged and lysed, leading them to die.[17]

The data obtained were tested for data analysis. Data analysis used the One-Way ANOVA (Analysis of Variances) test and continued with the Post Hoc Test using the Tukey method. The Kolmogorov-Smirnov One Sample test results obtained a significant result of 0.201 > 0.05, indicating the hypothesis is accepted. It was concluded that the data were normally distributed, so the ANOVA analysis of variance could continue. The Homogeneity of Variances test results were 0.056 > 0.05, indicating that Ho is accepted. It means that the four samples had the same variance or were homogeneous. The results of the significance of the ANOVA test data were 0.000 <0.05, indicating that the four samples had differences in the diameter of the inhibition zone, followed by the Post Hoc Test.

The Post Hoc Tukey test revealed no significant difference in comparing 50% saga leaf extract with 50% water fraction (P>0.05). In comparison, the ethyl acetate fraction and 50% *n*-hexane fraction had a significant difference (P<0.05). Post Hoc Tukey test of extract, *n*-hexane fraction, ethyl acetate fraction and water fraction of saga leaf with positive ciprofloxacin control showed significant differences (P<0.05). The factor that influenced the formation of a greater inhibitory power of ciprofloxacin was because ciprofloxacin had an antibacterial effect (broad spectrum). It was categorized in the bactericidal quinolone group (kills

bacteria) and was quite effective for gram-positive bacteria.[18]

Results of MIC and MBC Test of the Most Active Fraction of Saga Leaves (*(Abrus precatorius* L.) Dilution Method

The MIC and MBC tests used the ethyl acetate fraction from saga leaves. The MIC test was carried out to identify the smallest amount of active antibacterial substances that could inhibit the growth of the tested organisms. The results of the MIC observations can be seen in Table

Table 5. Results of Observation of MIC Ethyl Acetate Fraction of Saga Leaves ((Abrus precatorius linn.) against Streptococcus mutans by Liquid Dilution Method

No.	Tube	Information
1	25%	Clear
2	12.5%	Clear-MIC
3	6.25%	Turbid
4	3.125%	Turbid
5	1.5625%	Turbid
6	C+ (Ciprofloxacin 0.0005%)	Clear
7	C- (DMSO 10%)	Turbid

These results concluded that the Minimum Inhibitory Concentration (MIC) of the ethyl acetate fraction of saga leaves was 12.5%, as it showed clear tube results. A clear tube was obtained in the positive control of ciprofloxacin, indicating that the antibiotic ciprofloxacin could inhibit the growth of Streptococcus mutans ATCC 25175. Meanwhile, the negative control of DMSO 10% produced a tube that looked cloudy, proving that the solvent could not inhibit the growth of Streptococcus mutans ATCC 25175 bacteria.

After obtaining the MIC value, the Minimum Bactericidal Concentration (MBC) test continued. The inoculation results to determine the MBC are summarized in Table 6 and Figure 1.

Table 6. Inoculation Results of Antibacterial Activity of Ethyl Acetate Fraction by Dilution against Bacteria Streptococcus mutans ATCC 25175

The concentration of Ethyl Acetate Fraction	Replication		
	I	II	III
25%	-	-	-
12.5%	+	+	+
6.25%	+	+	+
3.12%	+	+	+
1.56%	+	+	+
Control (-)	+	+	+
Control (+)	-	-	-

Description (+) : There is bacterial growth
(-) : No bacterial growth
Positive control : Ciprofloxacin
Negative control : DMSO 10%

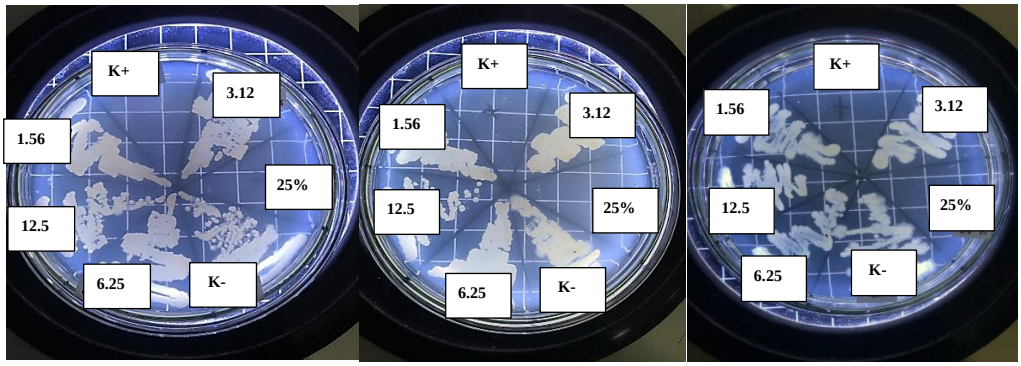

Figure 1. Results Streaking on Media *Mueller Hinton Agar* to Determine MBC (Personal documentation, 2022)

Based on the table and figure above, it is known that the three replications revealed the same results, namely, the ethyl acetate fraction with a concentration of 25% and the positive control of ciprofloxacin produced a clear area. Therefore, it can be concluded that the Minimum Kill Concentration (KBM) of the ethyl acetate fraction of saga leaves against *Streptococcus mutans* bacteria was 25%.

CONCLUSION

Based on the research results, it can be concluded that the ethanol extract, *n*-hexane fraction, ethyl acetate fraction and water fraction of saga leaf *(Abrus precatorius* L.) had antibacterial power against the growth of *Streptococcus*

mutans ATCC 25175. The ethyl acetate fraction of saga leaf was the most active fraction in inhibiting the growth of *Streptococcus mutans* bacteria ATCC 25175 with an inhibitory value of 11.87 mm, categorized in the strong category at a concentration of 50%. The Minimal Inhibitory Concentration (MIC) of the ethyl acetate fraction of saga leaves was 12.5% , while the Minimum Kill Concentration (MBC) of the ethyl acetate fraction of saga leaves was 25% against *Streptococcus mutans* ATCC 25175.

ACKNOWLEDGMENT

With sincerity and humility, the author would like to thank Mrs. Tatiana Siska Wardani, S.Farm., M.Farm, as Head of the Undergraduate Study Program Pharmacy at Universitas Duta Bangsa Surakarta as well as a mentor who has given lots of constructive advice and Mrs. apt. Kusumaningtyas Siwi Artini, S.Farm., M.Sc as the supervisor who has provided much direction and motivation for the author.

REFERENCES

1. Pramiastuti O, Sri Rejeki D. Uji Antibakteri Kombinasi Ekstrak Daun Belimbing Wuluh (Averrhoa bilimbi L) Dan Daun Kersen (Muntingia calabura L) Terhadap Staphylococcus aureus. J Ilm Farm. 2020;9(2):2020–53. https://doi.org/10.30591/pjif.v9i2.2026
2. Osmeli D, Yuhernita Y. KANDUNGAN SENYAWA KIMIA, UJI TOKSISITAS (Brine Shrimp Lethality Test) DAN ANTIOKSIDAN (1,1-diphenyl-2-pikrilhydrazyl) DARI EKSTRAK DAUN SAGA (Abrus precatorius L.). Makara J Sci [Internet]. 2009;13(1). https://doi.org/10.7454/mss.v13i1.378
3. Alhassan A, Ahmed Q. Averrhoa bilimbi Linn.: A review of its ethnomedicinal uses, phytochemistry, and pharmacology. J Pharm Bioallied

Sci [Internet]. 2016;8(4):265. https://doi.org/10.4103/0975-7406.199342

4. Silva BR Da, Freitas VAA De, Nascimento-Neto LG, Carneiro VA, Arruda FVS, Aguiar ASW De, et al. Antimicrobial peptide control of pathogenic microorganisms of the oral cavity: A review of the literature. Peptides. 2012 Aug 1;36(2):315–21. https://doi.org/10.1016/j.peptides.2012.05.015
5. Lemos JA, Quivey RG, Koo H, Abranches J. Streptococcus mutans: a new Gram-positive paradigm? Microbiology [Internet]. 2013;159(Pt 3):436. https://doi.org/10.1099/mic.0.066134-0
6. Nisak SK, Pambudi DB, Waznah U, Slamet S. Uji Antibakteri Ekstrak Etanol Daun Saga (Abrus Precatorius L.) Terhadap Bakteri Streptococcus mutans ATCC 31987 DAN Staphylococcus aureus ATCC 25923PK/5. Pros Semin Nas Kesehat [Internet]. 2021;1:2031–7.
7. Pertiwi RD, Kristanto J, Praptiwi GA. UJI AKTIVITAS ANTIBAKTERI FORMULASI GEL UNTUK SARIAWAN DARI EKSTRAK DAUN SAGA (Abrus precatorius Linn.) TERHADAP BAKTERI Staphylococcus aureus. J Ilm Manuntung [Internet]. 2016;2(2):239–47. https://doi.org/10.51352/jim.v2i2.72
8. Sulistyarini II, Sari DA, Rahardian MRR. ANTI-BACTERIAL ACTIVITY TEST OF ETHANOL EXTRACT, N-HEXANE FRACTION, ETHYL ACETATE FRACTION AND WATER FRACTION FROM DRAGON FRUIT STEM (Hylocereus polyrhizus) AGAINST METHICILLIN-RESISTANT Staphylococcus aureus (MRSA). J Ilmu Kesehat [Internet]. 2021;9(2):162–71. https://doi.org/10.30650/jik.v9i2.2284
9. Wardani TS, Nisa TC, Artini KS. Antibacterial Activity Test of N-Hexan, Ethyl Acetate and Water from Ethanol Extract of Kitolod Leaf (Isotoma Longiflora (L.) C. Presl.) AGAINST Staphylococcus Aureus ATCC 25923. Proc Int Conf Nurs Heal Sci [Internet]. 2022;3(1):9–16.
10. Lesmana H, Saleh M, Thioritz E, Miko H, Sopianah Y. The Resistance of Bandotan (Ageratum Conyzoides) Leaf Extract and Siwak Stem Extract on the Growth of Butterial Streptococcus Mutans. J Phys Conf Ser [Internet]. 2020;1477(6):062027. https://doi.org/10.1088/1742-6596/1477/6/062027
11. Depkes RI. Parameter Standar Umum Ekstrak Tumbuhan Obat (Edisi 1). Jakarta: Direktorat Jenderal Pengawasan Obat dan Makanan. Direktorat Pengawasan Obat Tradisional,; 2000.
12. Raymon M, Taebe B, Ali A, Khairuddin K. Antibacterial Activity Test of Manila Sawo Fruit Extract (Achras zapota L.) with Various Liquid Filters Against Salmonella typhimurium. J Pharm Med Sci. 2016;1(1):6–11.
13. Harborne J. Metode Fitokimia: Penentuan Cara Modern Menganalisa Tumbuhan (Edisi II). Bandung: Institut Teknologi Bandung; 2003.
14. Zaini M, Shofia V, Studi P, Farmasi D-I, Kalimantan PU, Analis D-I, et al. SKRINING FITOKIMIA EKSTRAK CARICA PAPAYA RADIX, PIPER ORNATUM FOLIUM DAN NEPHELIUM LAPPACEUM SEMEN ASAL KALIMANTAN SELATAN. J Kaji Ilm Kesehat dan Teknol [Internet]. 2020;2(1):15–27. https://doi.org/10.52674/jkikt.v2i1.30
15. Frazier W, Westhoff D. Food microbiology. Hill M, editor. New York; 1998.
16. Dzoyem JP, Hamamoto H, Ngameni B, Ngadjui BT, Sekimizu K. Antimicrobial action mechanism of flavonoids from

Dorstenia species. Drug Discov Ther. 2013 Apr 30;7(2):66–72.
17. Fauzia LA. Testing the Effect of Water from Avocado Leaves (Persea gratissima) against Streptococcus mutans from Saliva by Thin Layer Chromatography (TLC) and Minimum Inhibitory Concentration (MIC). Nusant Med Mag. 2008;41(3):173–8.
18. Andersson MI, MacGowan AP. Development of the quinolones. J Antimicrob Chemother [Internet]. 2003 May 1;51(suppl_1):1–11. https://doi.org/10.1093/jac/dkg212