Sys Rev Pharm 2020; 11(4): 313 – 318
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

Antibacterial Activity of *Allium sativum* against *Streptococcus mutans* ATCC 25175 in Indonesia

[1]Eriska Riyanti, [2]Ani Melani Maskoen, [1]Roosje Rosita Oewen, [1]Naninda Berliana Pratidina, *[3]Harun Achmad, [4]Yunita Feby Ramadhany

[1]Department Pediatric Dentistry, Faculty of Dentistry, Padjadjaran University, Bandung, Indonesia
[2]Department of Oral Biology, Faculty of Dentistry, Padjadjaran University, Bandung, Indonesia
*[3]Department of Pediatric Dentistry, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia
[4]Clinical Student, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia
Correspondence Author E-mail: harunachmader@gmail.com

Article History: *Submitted: 23.01.2020* *Revised: 20.03.2020* *Accepted: 09.04.2020*

ABSTRACT

Allium sativum is a plant which have a medicinal properties. The use of natural ingredients known to provide significant benefits, especially those from plants grown by the community and cultivated by government institutions. Natural ingredients such as garlic is a plant that is widely grown in the area of Indonesia so easily available at an affordable price. Previous research shows garlic has antibacterial activity against *Streptococcus mutans*. The purpose of this study was determine antibacterial effect of garlic extract against *Streptococcus mutans*. The research method was experimental research to examine the antibacterial activity of the local varieties Ciwidey garlic, local single clove garlic, and garlic imports bought at the market in Indonesia. Third onions extract are made by maceration and would be examined for minimum inhibition concentration and minimum bactericidal concentration by using a microplate reader. The results showed local varieties of local single clove garlic have 9:38% for MIC and 18.75% for MBC, imported garlic 4.69% MIC and 18.75% MBC, while the Ciwidey local garlic have 9:38% for MIC. Conclusion is local single clove garlic and garlic imports have minimum capability and minimum bactericidal concentration inhibition while garlic varieties local Ciwidey only have minimum inhibition concentration ability.

Keywords: *Allium sativum, MIC, MBC, Streptococcus mutans*

Correspondence:
Harun Achmad
Department of Pediatric Dentistry, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia
E-mail: harunachmader@gmail.com

DOI: 10.31838/srp.2020.4.45

INTRODUCTION

Oral health is important because it is part of the overall public health. Teeth is a unity with the other parts of the body. Damage the dental health can affect other parts of the health, so that it can interfere with daily activities, especially in the age of the children. The prevalence of caries in Indonesia is still quite high. This is according to a survey conducted by the Health Research in 2013 , DMF - T index in Indonesia amounted to 4.6.[1,2]

Streptococcus mutans has a major role in the initiation process of the formation of caries , although the bacteria are not the first to colonize the surface of the tooth. The bacteria can produce extracellular polysaccharide.[3,4]

In developing countries, some antibacterial ingredients to prevent caries hard to find and quite expensive. Therefore, it takes the alternative material more effectively and efficiently. Alternative therapy using herbal ingredients can be an effective therapeutic agent. Garlic is one of the herbal ingredients are widely studied and used as a therapeutic agent of various diseases.[5,6]

Garlic (*Allium sativum*) has an antibacterial effect, antifungal, and antiviral. The main content is to be antimicrobial in garlic is allicin, which is produced by enzyme allinase garlic when crushed. According to studies in vitro by Motamayel et al in 2013, extracts of garlic has antibacterial effect against *Streptococcus mutans* and Lactobacillus acidophilus.[7,8] Fieldberg et al showed that allicin has antimicrobial activity by inhibiting the synthesis of RNA , DNA synthesis, and protein synthesis is also partially obstructed.[9,10]

METHODS

Extract Preparation

Three kinds of *Allium sativum* was collected from plantary in Indonesia. Sample were made by simple maceration. Extraction used ethanol 96% as solvent. Result of extraction serve as paste.

The study protocol was reviewed and approved by the Health Research Ethics Committee of Medicine Faculty Universitas Padjadjaran (27/UN6.C1.3.2/KEPK/PN/2015)

Bacteria Rejuvenation

Mueller Hinton on 5% CO_2 37^{0}C during 24 hours in anaerob condition would be used for *Streptococcus mutans* rejuvenation.

Determination of Minimum Inhibition Concentration

After 48 hours of liquid cultures of bacteria determined its optical density at 600 nm and diluted to 0.5 MacFarland standard. At 96 well of plate was added 0.1 ml of media Mueller Hinton, then in column A, B, E, and F plate 96 well was added 0.1 ml of extract of garlic that has been dissolved in phosphate buffer saline pH 7.0 , in column E , F , G and H added 0:01 ml liquid cultures of the bacteria *Streptococcus mutans* 0.5 MacFarland. Minimum inhibition concentration then absorption was measured with a microplate reader (Figure 1).

Determination of Minimum Bactericidal Concentration

MIC results from each well that there is no bacterial growth, gained 100 mg/ml and put into petri dishes containing solid media (jelly). Then spread across the surface of the petri dishes), then incubated for 1x24 hours (2x24 hours if not already apparent bacterial colonies growing) at a

temperature of 37^{0}C in anaerobic state. KBM then its absorbtion was measured with a microplate reader (Figure1).

Figure 1: Well in Microplate Reader

	1	2	3	4	5	6	7	8	9	10	11	12
A					Media + sample							
B					Media + sample							
C					Media + sample + bacteria							
D					Media + sample + bacteria							
E												
F					Media + bacteria							
G					Media							
H					Media							

RESULTS

Local garlic of Ciwidey variety has bacteriostatic activity against *Streptococcus mutans* ATCC 25175 with a minimum concentration of 9.38%. Local garlic extract of Ciwidey variety did not have bactericidal activity against ATre 25175 *Streptococcus mutans* bacteria characterized by the growth of *Streptococcus mutans* ATCC 25175 bacteria on agar media. Based on the results of statistical calculations and analysis, local garlic extract of Ciwidey variety gave a MIC value of 9.38% but it was not visually apparent due to the color of the sample so it disturbed the results of the analysis as in Figure 1 and Table 1.

Figure 1: MIC of Garlic Extract Localized Ciwidey Varieties against *Streptococcus mutans*

Table 1: MIC Analysis of Ciwidey Varieties against MIC *Streptococcus mutans*

Well	Concentration (%)											
	37,50	18,75	9,38	4,69	2,34	1,17	0,59	0,29	0,15	0,07	0,04	0,02
Media + Sample	0,158	0,103	0,078	0,066	0,059	0,054	0,049	0,047	0,045	0,045	0,045	0,044
	0,150	0,106	0,078	0,065	0,057	0,050	0,047	0,045	0,045	0,045	0,044	0,045
Media	0,042	0,044	0,044	0,045	0,045	0,044	0,045	0,044	0,042	0,042	0,044	0,045
	0,043	0,043	0,044	0,044	0,044	0,044	0,044	0,043	0,042	0,043	0,044	0,044
Media + Sample + Bacteria	0,183	0,153	0,118	0,120	0,140	0,162	0,128	0,089	0,079	0,075	0,071	0,065
	0,182	0,165	0,112	0,128	0,161	0,173	0,141	0,094	0,076	0,073	0,070	0,066
Media + Bacteria	0,063	0,070	0,066	0,066	0,069	0,065	0,069	0,068	0,068	0,069	0,067	0,064
	0,065	0,065	0,067	0,068	0,069	0,068	0,069	0,066	0,067	0,064	0,067	0,067

Then the single local locally extracted garlic and imported garlic have bacteriostatic and bactericidal activity against *Streptococcus mutans* ATCC 25175. The local garlic extract of single cloves has a bacteriostatic effect on *Streptococcus mutans* ATCC 25175 at a minimum concentration of 9.38% and a bactericidal effect on *Streptococcus bacteria* ATCC mutans 25175 at a minimum concentration of 18.75%. Based on the results of the calculation and statistical analysis, the local single clove garlic extract gave a MIC value of 9.38% but it was not visually apparent due to the colored and turbid samples which disturbed the analysis results such as in Figure 2 and Table 2.

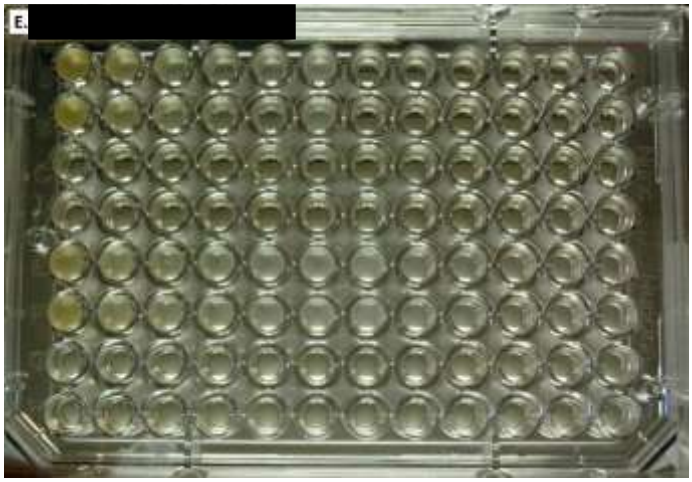

Figure 2: MIC Garlic Extract Localized Single Siung against *Streptococcus mutans*

Table 2: MIC Analysis of Garlic Extract Localized Single Siung against *Streptococcus mutans*

Well	Concentration (%)											
	37,50	18,75	9,38	4,69	2,34	1,17	0,59	0,29	0,15	0,07	0,04	0,02
Media + Sample	0,280	0,209	0,172	0,121	0,120	0,221	0,063	0,058	0,054	0,052	0,050	0,050
	0,318	0,208	0,168	0,109	0,081	0,162	0,057	0,053	0,050	0,049	0,048	0,050
Media	0,046	0,049	0,048	0,050	0,051	0,053	0,049	0,050	0,049	0,049	0,049	0,049
	0,046	0,047	0,047	0,048	0,048	0,049	0,048	0,048	0,049	0,049	0,049	0,049
Media + Sample + Bacteria	0,256	0,194	0,172	0,138	0,151	0,150	0,190	0,123	0,099	0,080	0,071	0,063
	0,280	0,189	0,147	0,140	0,142	0,144	0,188	0,131	0,101	0,082	0,070	0,065
Media + Bacteria	0,058	0,062	0,064	0,066	0,066	0,067	0,063	0,062	0,066	0,064	0,061	0,059
	0,056	0,061	0,064	0,065	0,064	0,065	0,065	0,062	0,063	0,061	0,061	0,058

Furthermore, imported garlic extract has a bacteriostatic effect on the Streptococcus mutans ATCC 25175 with a minimum concentration of 4.69% and a bactericidal effect on the *Streptococcus mutans* ATCC 25175 with a minimum concentration of 18.75%. Based on the results of the calculation and statistical analysis, imported garlic extract gave a MIC value of 4.69% but it was not visually apparent due to the colored and turbid samples so that it disturbed the analysis results such as in Figure 3 and Table 3.

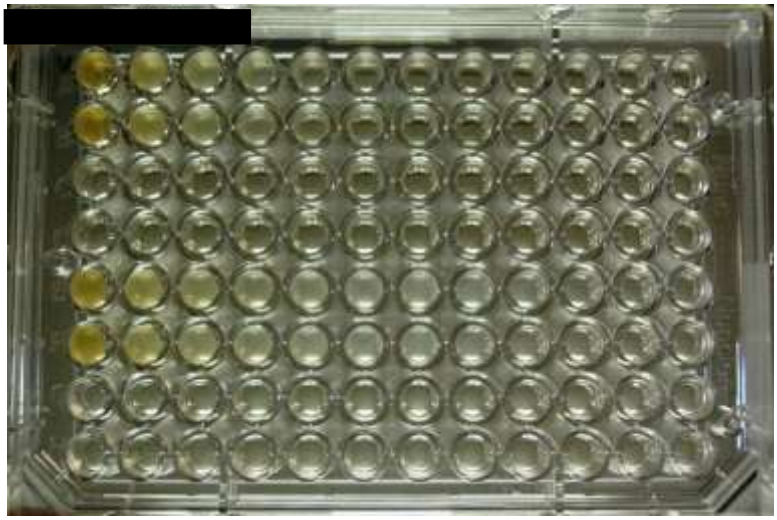

Figure 3: MIC Garlic Extract Imported against *Streptococcus mutans*

Table 3: Results of MIC Analysis of Imported Garlic Extract against *Streptococcus mutans*

Well	Concentration (%)											
	37,50	18,75	9,38	4,69	2,34	1,17	0,59	0,29	0,15	0,07	0,04	0,02
Media + Sample	0,269 0,245	0,270 0,286	0,204 0,217	0,158 0,164	0,112 0,105	0,083 0,078	0,065 0,065	0,059 0,057	0,055 0,053	0,053 0,052	0,051 0,049	0,049 0,050
Media	0,048 0,047	0,048 0,047	0,042 0,047	0,051 0,048	0,051 0,048	0,053 0,050	0,051 0,049	0,050 0,049	0,050 0,049	0,049 0,049	0,049 0,049	0,049 0,049
Media + Sample + Bacteria	0,227 0,248	0,256 0,256	0,203 0,200	0,152 0,163	0,173 0,195	0,163 0,161	0,192 0,174	0,163 0,133	0,110 0,096	0,087 0,079	0,068 0,067	0,061 0,061
Media + Bacteria	0,060 0,058	0,063 0,060	0,063 0,059	0,064 0,063	0,065 0,064	0,063 0,062	0,065 0,062	0,064 0,062	0,066 0,063	0,066 0,062	0,062 0,061	0,058 0,057

Phytochemical research on the three varieties of garlic obtained data of secondary metabolite content as in Table 4.

Table 4: Data of Secondary Metabolite Content in Garlic

No	Secondary metabolites	Test Method	Garlic Test Results		
			Single	Import	Ciwidey
1	Phenolic	Reactor $FeCl_3$ 5%	+	+	+
2	Flavonoid	a. Reactor Concentrated HCl +Mg	-	-	-
		b. Reactor H_2SO_4 2N	-	-	-
		c. Reactor NaOH 10%	-	-	-
3	Steroid	Reactor Lieberman-Bourchard	-	-	-
4	Triterpenoid		+	-	+
5	Saponin	Reactor HCl + H_2O	+	+	-
6	Tanin	Reactor $FeCl_3$ 1%	-	-	-

Qualitative data from research on secondary metabolite content shows that all three varieties of garlic contain phenolic compounds. Imported garlic does not contain triterpenoids while in local cloves single cloves and local garlic varieties Ciwidey has triterpenoids. In local single garlic cloves and imported garlic contain saponins that are not owned by the local garlic variety Ciwidey.

DISCUSSION

Garlic is a layer of coriander plants or collated cloves, has a pseudo stem formed from the midrib and belongs to the genus Allium. According to Ross et al, in recent centuries garlic has been known to have an effect as an alternative medicine. Historically, garlic has been used for centuries to cure infectious diseases.[11,12] Garlic is available in capsule and powder form, as an additional food or supplement. Louis Pasteur was the first to explain the antibacterial effect of garlic. Allium group, especially garlic (*Allium sativum*) shows the effect of broad-spectrum antibiotics against Gram-positive and Gram-negative bacteria.[13,14,15]

Garlic is used as a remedy for digestive diseases, respiratory infections, skin diseases, wounds, and so on. From several scientific articles in the world, garlic has benefits for health. Biological responses from garlic include antibacterial, antifungal, antiviral, antioxidant, anti-cancer, antiaging effects, reduction of risk factors for cardiovascular disease.[16,17] Based on the results that the three varieties of garlic has a difference in the effectiveness of the power to inhibit and kill bacteria *Streptococcus mutans.* At the local garlic varieties Ciwidey just as effective inhibition without killing against *Streptococcus mutans*, while the single local garlic cloves and garlic imports has the power effectiveness of inhibiting and killing the bacteria *Streptococcus mutans.*[18,19]

The order of the three varieties of garlic studied bacteriostatic effect against the bacteria *Streptococcus mutans* which is first imported garlic with a minimum concentration of 4.69%, which is able to provide a bacteriostatic effect against the bacteria *Streptococcus mutans*, followed by local garlic cloves and garlic single local Ciwidey varieties with a minimum concentration of 9.38%. While the order of the garlic varieties are on the bactericidal effect against the bacteria *Streptococcus mutans* are two varieties of garlic is garlic imported and local garlic cloves single has the same minimum concentration of 18.75%, on a local garlic varieties Ciwidey not found effectiveness for bactericidal.[20]

The active component of garlic is allicin extract which is a chemical compound containing sulfur. Sulfur compounds in garlic is believed to be related to several factors such way of cultivation of garlic. The different ways of cultivation and harvest garlic after the process greatly affect the primary content of sulfur in garlic.[21]

Some of the factors mentioned above cultivation to note is grown in the highlands or low, fertilization, ambient temperature, soil texture, soil pH, rainfall and humidity.

CONCLUSION

Local single clove garlic and garlic imports have minimum capability and minimum bactericidal concentration inhibition while garlic varieties local Ciwidey only have minimum inhibition concentration ability.

ACKNOWLEDGEMENT

The authors would like to thank Ministry of Research Technology and Higher Education Republic of Indonesia for funding this study by means of Superior Research University Grant, Padjadjaran University (Grant no. 393/UN6.R/PL/2015).

CONFLICTS OF INTEREST

The authors have no potential conflicts of interest to declare.

REFERENCES

1. Durairaj S, Srinivasan S, Lakshmana PP. (2009). In vitroantibacterial activity and stability of garlic extract at different pH and temperature.. Electronic J of Bio5(1), 5-10.
2. Dhinahar S, Lakshmi T. (2011) Role of botanicals as antimocrobial agents in management of dental infections – A review. Int Jof Pharma and Bio Sciences Oct-Dec 2(4), 690-704.
3. Eja ME, Asikong BE, Abriba C, Arikpo GE, Anwan EE, Enyi-Idoh KH. (2007). A comparative assessment of the antimicrobial effects of garlic (*Allium sativum*) and antibiotics on diarrheagenic organism. Southeast Asian J Trop Med Public Health. Maret 38(2),343-348.
4. Fani M, Kohanteb J, Dayaghi M. (2007). Inhibiory activity of garlic (*Allium sativum*) extract on multidrug-resistant *Streptococcus mutans.* J of Indian Society of Pedodontics and Prev Dent Vol 12 Issue 4, 164-168.
5. Featherstone JDB. (2003). The caries balance: contributing faktors and early detection. J of California Dent Assoc February,1-8.
6. Hamada S, HD Slade. (1980). Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiological review June, 331-84.
7. Harini k, Babu S, Ajila V, Hegde S. (2013). Garlic: It's role in oral and systemic health. NUJHS Des 3, 17-22.
8. Jeon JG, Rosaln PL, Falsetta ML, Koo H. (2011). Natural products in caries research: current (limited) knowledge challanges and future Perspective. Caries Res 45, 243-263.
9. Kidd EAM, Joyston-Bechal S. (1992). Fundamental of caries: Disease and prevention [Sumawinata dan Faruk, trans]. EGC, 111-112.
10. Kudva S, Prabhakar S, Pai V, Tegginamani A. (2012). Effect of garlic extract on salivary pH: a clinical study. Arch Orofac Sci 7, 1-8.
11. Lester K. (2010). Zoocin A and lauricidin in combination selectively inhibit Streptococcus mutans in a bifilm model. Disertasion. Otago University, Dunedin, New Zealand. November, 25-30.

12. Lee HJ, Park HS, Kwon TY, Hong SH. (2011). Effect of garlic on bacterial biofilm formation on orthodontic wire. Angle Orthodontist 81, 895-900.
13. Lynch DJ. (2010). An Analysis of the role of glucan binding proteins in *Streptococcus mutans* biofilm architecture and caries development. Disertasion. Iowa University, 1-29.
14. Achmad H, Ramadhany S, Suryajaya FE. (2019). Streptococcus Colonial Growth of Dental Plaque Inhibition Using Flavonoid Extract of Ants Nest (Myrmecodia pendans): An in Vitro Study. Pesquisa Brasileira em Odontopediatria e Clinica Integrada. ISSN 1519-0501, 19(1):e4250.
15. Mei ML, Chu CH, Low KH, Che CM, Lo ECM. (2013). Caries arresting effect of Silver Diamine Fluoride on dentine carious lesion with *Streptococcus mutans* and *Lactobacillus acidophilus* dual-species cariogenic biofilm. Med Oral Patol Oral Cir Bucal Nov 18, 824-831.
16. Achmad H, Singgih MF, Andries S, Handayani H, Sumintarti. (2019). Analysis of ascorbic acid in gingival handling of childrens mouth cavity. Indian Journal of Public Health Research and Development. 10(5): 610-615.
17. Motamayel FA, Hassanpour S, Alikhani MY, Poororajal J, Salehi J. (2013). Antibacterial effects of eucalyptus (*globulus Labill*I and garlic (*Allium sativum*) extracts on oral cariogenic bacteria. J of Microbiology Research May 1, 12-17.
18. Pinkham JR, Casamassimo PS, Fiels HW, McTigue DJ, Nowak AJ. (2005). Pediatric Dentistry: Infancy Through Adolescence 4th ed. St. Louis. Elsevier Saunders, 199-204.
19. Achmad H, Pratiwi R, Sugiharto S, Handayani H, Singgih MF, Mudjari S. (2019). Analysis of Risk Factors of Biopsychosocial with Early Childhood Caries (ECC) in Indonesian Pre-School Children. Pesquisa Brasileira em Odontopeditria e Clinica Integrada. 19 (1): 4432.
20. Stewart RE, Hale KJ. (2003). The Paradigm shift in etiology, prevention, and management of dental caries : its effect on the practice of clinical dentistry. J Cal Dent Assoc 31, 247-51.
21. Mei, L., Du, W., Guo, L.-J., Mi, L., Deng, Y.-T., Zhou, S.-Y., Gao, W. Association of plasma nucleotide levels with severity of coronary arterial lesion and left ventricular systolic function in acute myocardial infarction (2015) Journal of Cardiovascular Disease Research, 6 (2), pp. 60-66. DOI: 10.5530/jcdr.2015.2.3
22. Achmad H, Pratiwi R, Sumintarti, Mudjari, Rahma M. (2019). Identification of early childhood caries in children's preschool based on demographic risk factor and ph saliva. Indian Journal of Public Health Research and Development. 10 (5): 598-603.