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MIC, MBIC, MBEC Analyses of Garlic Extract (*Allium sativum*) from Indonesian Variety Against Streptococcus *mutans*

Lani Manurung[1], Eriska Riyanti[2], Eka Chemiawan[2], Mieke Hemiawati Satari[2], Meirina Gartika[2]

[1]Graduate School of Pediatric Dentistry, Faculty of Dentistry Universitas Padjadjaran, Indonesia.

[2]Dept of Pediatric Dentistry Faculty of Dentistry Universitas Padjadjaran, Indonesia

Aim: This study was designed to evaluate the inhibition and eradication ability for garlic against *Streptococcus mutans,* the bacteria that cause dental caries. Methods: Garlic extract was obtained from Ciwidey garlic maseration process in etanol 96%. MIC was a test to measure the inhibition ability of garlic extract against *Streptococcus mutans* in planktonic form, and MBIC in biofilms form. MBEC was a test to measure the eradication ability of garlic extract against *Streptococcus mutans* in biofilms form. Statistic analysis using ANOVA followed by post hoc with ρ value <0.05. Results: The garlic extract showed MIC value at 9.39% and MBIC value at 37.5% but was not observed to have eradication activity against *Streptococcus mutans* in biofilms form up to concentration 37.5%. Conclusion: Garlic extract inhibit *Streptococcus mutans* both in planktonic and biofilm form. Garlic extract has no eradication activity against *Streptococcus mutans* in biofilms form up to concentration 37.5%.

Keywords: Garlic extract. *Streptococcus mutans*. Planktonic. Biofilm.

Corresponding author:
Lani Manurung
232, Pinus Raya Street,
Bandung, Indonesia
elemem25@gmail.com

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Introduction

Nature provides many natural ingredients that are nutritious for health, such as garlic, turmeric, ginger and many others[2]. Natural ingredients can be better option because of several advantages such as often having fewer side-effects, better patient tolerance, being relatively less expensive and acceptable due to a long history of use. Garlic (*Allium sativum*) has many uses such as an antioxidant, antiseptic, anti-viral, anti-fungal, anti-cancer, anti-aging, reduce the risk of heart disease, and antibacterial[1]. Garlic contains allicin that well known has antibacterial properties that can inhibit the growth of bacteria which cause disease[3]. However, there is lack of studies regarding the exact value of garlic's inhibition ability against bacteria.

One of the disease that caused by bacteria is dental caries. Dental caries is a multifactorial, chronic bacterial disease, that causes demineralization and destruction of the hard tissues, usually by production of acid by bacterial fermentation of the food debris accumulated on the tooth surface[4]. Bacteria has two forms, which is planktonic form and biofilm form that have different ability, properties and resistance.

Indonesian still have high levels of tooth decay or caries according to Riset Kesehatan Dasar[5]. Garlic has two properties that are useful for reducing caries: antibacterial so it can reduce bacteria's acid production and can stimulate saliva expenditure caused garlic flavor[6,7]. The aim of this study is to find out the inhibition and eradication ability of garlic extract (*Allium sativum)* against *Streptococcus mutans* ATCC 25175 in planktonic and biofilm form.

Material and methods

Garlic extract

The fresh Ciwidey garlic were collected on March 2015. Ciwidey is one of agricultural area in Bandung, Indonesia. The determination of the species was from taxonomy test in Bidang Sumber Daya Sekolah Ilmu dan Teknologi Hayati Institut Teknologi Bandung, Indonesia. 500 gram fresh garlic peeled and cleaned, then soaked in 650 mL ethanol 96%. The next phase is filtered, followed by evaporation of the solvent with a rotary evaporator. Maceration process is performed three times at the Laboratory of Chemistry, University of Padjadjaran, Bandung, Indonesia.

Microbial strain

Microbial strain used was *Streptococcus mutans* ATCC 25175 obtained from UNPAD Laboratory of Chemistry, Bandung, Indonesia. Bacteria were grown in Brain Heart Infusion broth enriched with sucrose 2% for 48 h at 37°C both for planktonic and biofilm assay.

Determination of the Minimum Inhibitory Concentration (MIC)

MICs were determined by modified micro titer broth method in sterile flat bottom 96-well polystyrene plates[8]. Prepared microwell plate formats: media + samples, media, media + sample + bacteria, media + bacteria (made Duplo).

Brain Heart Infusion (BHI) broth 150µL pipette into a microwell plate. Then, pipette 150µL sample (garlic extract) in a microwell plate and performed a serial dilution. Liquid culture of *Streptococcus mutans* 10µL pipetted and put it in a microwell plate then incubated at 37°C for 48 hours. Optical density read by Bio-Rad microplate reader at absorbance 595 nm.

Determination of the Minimum Biofilm Inhibition Concentration (MBIC)

MBICs were determined by the adherence assay in sterile flat bottom 96-well plates. A similar serial dilution was as performed in antibacterial assay. After 48 h incubation, the supernatants were aspired from the wells, rinsed 3 times with *phosphate buffered saline* (*PBS*) and incubated at 70 ^{0}C for 15 min. Then, 200 µL of 0.1% crystal violet was added to the wells and left for 30 min to stain the formed biofilm. The excess stain was rinsed off with PBS 3 times, followed by addition of 200µL of ethanol to the wells. Optical density read by Bio-Rad microplate reader at absorbance 595 nm.

Determination of the Minimum Biofilm Eradication Concentration (MBEC)

MBECs were determined by the adherence assay in flat bottom 96-well plates as performed on biofilm formation inhibition assay[9]. After 48 h incubation of bacteria suspension in media used (without samples), the supernatants were aspired from the wells. A serial dilution of samples in media were then added to the wells and incubated for another 24 h in 37° C. Further treatments were as performed on the biofilm formation inhibition assay.

Results

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Biofilm Inhibitory Concentration (MBIC)

Results summary of the Minimum Inhibitory Concentration (MIC) is as described in table 1.

Table 1. Results of the Minimum Inhibitory Concentration (MIC).
M = media
S = samples (garlic extract)
P = solvent
B = bacteria (Streptococcus mutans)

	Garlic extract concentration (% v/v)											
	37.5%	18.76%	9.38%	4.69%	2.34%	1.17%	0.59%	0.29%	0.15%	0.07%	0.04%	
M+S		0.266	0.305	0.273	0.245	0.240	0.2405	0.250	0.349	0.265	0.240	0.226
M+P						0.285						
M+S+B	0.214	0.283	0.274	0.383	0.732	1.416	0.862	0.401	0.358	0.359	0.376	
M+P+B						0.301						

Results summary of the Minimum Biofilm Inhibitory Concentration (MBIC) is as described in table 2.

Table 2. Results of the Minimum Biofilm Inhibitory Concentration (MBIC).

	Garlic extract concentration (% v/v)											
	37.5%	18.76%	9.38%	4.69%	2.34%	1.17%	0,59%	0,29%	0,15%	0,07%	0,04%	0,02%
M+S	0.255	0.167	0.125	0.165	0.113	0.124	0.113	0.283	0.105	0.105	0.100	0.098
M+P	0.087											
M+S+B	0.2545	0.419	0.266	0.311	0.292	0.271	0.243	0.2675	0.266	0.1795	0.093	0.091
M+P+B	0.2162											

The result showed MIC value at 9.39%. and MBIC at 37.5%, so that garlic extract has inhibitory ability against *Streptococcus mutans* ATCC 25175 in planktonic and biofilm form.

Determination of the Minimum Biofilm Eradication Concentration (MBEC)

Results summary of the Minimum Biofilm Eradication Concentration (MBEC) is as described in Table 3.

Table 3. Results of the Minimum Biofilm Eradication Concentration (MBEC).

	Garlic extract concentration (% v/v)									
	37.5%	18.76%	9.38%	4.69%	2.34%	1.17%	0.59%	0.29%	0.15%	0.07%
M+S	0.1915	0.146	0.105	0.1055	0.1035	0.124	0.0975	0.106	0.107	0.172
M+P	0.087									
M+S+B	0.2075	0.1505	0.1275	0.2335	0.159	1.6655	1.9935	0.113	0.115	0.103
M+P+B	0.1466									

The result showed that garlic extract not observed to have eradication activity against *Streptococcus mutans* ATCC 25175 in biofilms form up to concentration 37.5% which is the highest concentration on this study.

Discussion

There are five stages in the process of biofilm formation (Figure 1): At the first stage, planktonic cells create reversible attachment to a biomaterial surface and/or host cell surface. The cells are still susceptible to antimicrobial agents. At the second stage, the adhesion of subsequent microorganism to surface becomes irreversible and the cells begin to secrete EPS. The EPS reducing the amount of antimicrobial available to interact with biofilm, act as an adsorbent or reactant, as well as their structure physically reduces the penetration of antimicrobial agents by walling off access to regions of the biofilm[10,11].

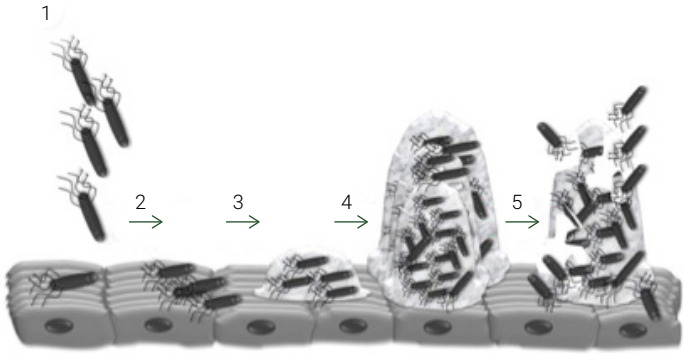

Figure 1. Stages in Biofilm Formation[10].

Third stage is the maturation stage where the amount of ECM increases around the microcolonies, due a continued secretion of EPS. It is possible to observe a mature biofilm containing cavities that serve as transport channels of water and planktonic cells throughout the biofilm community, at the fouth stage, and also provides a unique environment for optimum nutrient absorption and waste disposal.

Biofilms detachment can occurs in two ways (stage 5): continual dispersal of single cell or small portion of the biofilms (erosion); and, where large pieces of the biofilms are significantly lost (sloughing)[12]. This disruption can expand the infection once the microbial cells are liberated and can colonize another location/surface. All this five stages explain why bacteria in biofilm formation more difficult to inhibit or eradicate than bacteria in planktonic formation.

In conclusion, as according to Karygianni journal[2], Yu-Ying journal[6], and El-Samarrai and Rashad journal[7], the result of this study revealed that garlic extract have inhibition ability against *Streptococcus mutans* ATCC 25175 in planktonic and biofilm form, but was not observed to have eradication activity against *Streptococcus mutans* ATCC 25 175 in biofilms form up to concentration 37.5%.

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