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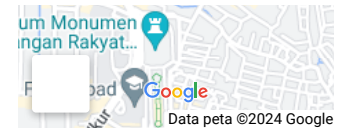
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## ORIGINAL ARTICLE

# Antibacterial effects of tomato ethanol extract (*Solanum lycopersicum* L.) against *S. mutans* and *P. gingivalis*: a laboratory experiment

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## ABSTRACT

**Introduction:** The process of dental plaque formation consists of three stages: first is the pellicle formation; second is the initial colonization of facultative gram-positive one of microbes of *S. mutans*; last is secondary colonization and microbial maturation by *Porphyromonas intermedia*, *P. gingivalis*, *Fusobacterium nucleatum*. Formation of dental biofilm is considered to be the main etiology of chronic periodontitis. Bacteria that are often involved in chronic periodontitis are *P. gingivalis*. Tomatoes (*Solanum lycopersicum* L.) content with lycopene compounds, flavonoids, and saponins have been shown to inhibit or kill bacterial growth. The aim is to analyze the antibacterial effect of servo tomato ethanol extract against *S. mutans* and *P. gingivalis*. **Methods:** This research was a laboratory experimental one with a posttest only control group design where observed variable diameter of the inhibition zone was produced from ethanolic extract of tomato (*Solanum lycopersicum* L.) in various concentrations of 3.125, 6.25, 12.5, 25, 50 and 100% chlorhexidine 0.2% as a positive control, negative control of distilled water against *S. mutans* and *P. gingivalis* on blood agar media. The method used in this study was the well-diffusion test. **Results:** The highest inhibitory zone at 100% concentration with a diameter of 32.10 mm was very strong; lowest inhibition at 3.123% was 3.95 mm, weak classification and against *P. gingivalis* at 3.125%, it was 3.72 mm, weak classification, meanwhile at 100% concentration, it was 9.67 mm, medium classification. The results of the One Way ANOVA statistical test showed a  $p < 0.05$  which had a significant effect in inhibiting the growth of *S. mutans* and *P. gingivalis*. **Conclusions:** The ethanolic extract of tomatoes (*Solanum lycopersicum* L.) as the lycopene compounds, flavonoids, and saponins have been shown to have an antibacterial effect in inhibiting bacterial growth of *S. mutans* and *P. gingivalis*.

## KEYWORDS

tomato ethanol extract (*Solanum lycopersicum* L.), *S. mutans*, *P. gingivalis*, antibacterial

## INTRODUCTION

Oral cavity plays an important role as it is essential for health, since there is a close relationship between oral diseases and other systemic diseases.<sup>1</sup> Oral health is essential to total health and satisfactory quality of life. According to the World Health Organization (2012), oral health has been defined as a state of being free of mouth and facial pain, oral infections and sores, and oral and other diseases that limit an individual's capacity in biting, chewing, smiling, speaking, and psychosocial well-being. Oral conditions like dental caries and periodontal or gum disease continue to plague humanity. Nearly all adults have existing tooth decay, and severe gum disease occurs in 15 to 20% of middle-aged adults.<sup>2</sup> Periodontal disease, caused by oral bacteria has been associated with adverse pregnancy, cardiovascular disease, pulmonary disease, and diabetic outcomes.<sup>3</sup> In addition, based on data from the Basic Health Research (Riskesdas) in 2018, the proportion of the population with dental and oral problems in Indonesia reached 57.6%.<sup>4</sup>

Periodontal disease is an infection of the oral cavity that generally affects the supporting tissues surrounding the teeth. The prevalence of periodontal disease is reported to be 20-50% worldwide. The most common periodontal diseases are gingivitis and periodontitis.<sup>5</sup> Periodontitis is an inflammatory condition of the supporting tissues of the teeth caused by certain microorganisms and causes destruction of the periodontal ligament and alveolar bone accompanied by an increase in probing depth and gingival recession.<sup>6</sup> Based on the 2018 Basic Health Research (Riskesdas), the prevalence of periodontitis in Indonesia is 74.1%, which means that 7 out of 10 people in Indonesia experience periodontitis.<sup>4</sup> One type



of periodontal disease according to the American Academy of Periodontology (AAP) in 1999 is chronic periodontitis.<sup>7</sup>

Dental plaque is a soft deposit consisting of bacteria in a glycoprotein matrix of saliva and extracellular polysaccharides. Dental plaque is formed by a biofilm that coats the tooth surface and cannot be removed simply by water rinse.<sup>6</sup> The process of dental plaque formation consists of three stages, namely pellicle formation, First is initial colonization of facultative gram-positive microbes (*S. mutans*, *Actinomyces viscosus* and *Streptococcus sanguis*), then secondary colonization and microbial maturation by *Porphyromonas intermedia*, *P. gingivalis*, *Fusobacterium nucleatum*.<sup>8</sup> Several studies have shown that at the beginning of the formation of dental plaque, gram-positive cocci such as *S. mutans*, *S. sanguis*, *S. mitis*, and *S. salivarius*, are most commonly found.<sup>9</sup> *S. mutans*, is the bacteria that initiates plaque formation and *S. mutans* is the main bacteria that produces extracellular polysaccharide matrix. The presence of *P. gingivalis* in periodontal pockets can predict future disease progression and a significant positive correlation was found between the amount of *P. gingivalis* and pocket depth.

The accumulation of plaque on the tooth surface and gingiva (formation of dental biofilm) at the dentogingival junction (DEJ) is considered to be the main etiology of chronic periodontitis. As the dental biofilm develops, there is an early symptom of an inflammatory reaction at the gingival margin without loss of attachment.<sup>10</sup> The bacteria that are often involved in chronic periodontitis are *P. gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, and *Tannerella forsythia*.<sup>7</sup> The development of chronic periodontitis is influenced by various bacterial species that influence the proinflammatory and immune responses of the host. An increase in the number of periodontal pathogens contributes to the development of a dysbiotic microbial environment which is induced by the inflammation in the periodontal pocket.<sup>6</sup>

Periodontitis treatment can be done by mechanical therapy, namely by surgical and non-surgical plaque debridement followed by patient education to maintain oral hygiene, besides that local irritation factors must also be eliminated.<sup>11</sup> Plaque management consists of the use of mechanical procedures and chemical agents that retard the formation of plaque. Mechanical methods of plaque prevention include toothbrushing, oral hygiene, and professional prophylaxis for interdental washing. The most effective method of plaque control at present appears to be mechanical plaque control. Chemical plaque regulation was used only as an extension and not as a replacement to the mechanical means which further improve the performance of plaque management programs using anti-plaque agents as an adjuvant to mechanical plaque control.<sup>11,12</sup>

One of the ways to prevent plaque chemically is to use active ingredients that have antibacterial power, especially to inhibit the growth of *S. mutans* bacteria.<sup>12</sup> Inappropriate use of antibiotics can also cause bacterial resistance to antibiotics.<sup>4</sup> Various efforts have been made to minimize side effects and reduce antibiotic resistance by utilizing natural ingredients as alternative treatments. Therefore, one alternative for the prevention of dental plaque that can cause periodontitis is by using natural chemical compounds in the form of phytochemical compounds found in tomatoes.<sup>12,13</sup>

Tomatoes (*Solanum lycopersicum* L.) are one type of fruit that is very popular worldwide because of its high consumption, availability, and health benefits.<sup>14</sup> Tomatoes are a source of vitamin A and vitamin C, potassium, fiber, and contain natural chemical compounds such as lycopene,  $\beta$ -carotene, lutein, saponins, tannins, folic acid, and flavonoids.<sup>15</sup> The contents of tomatoes such as lycopene compounds, flavonoids, and saponins have been shown to inhibit or kill bacterial growth.<sup>16</sup> In addition, other ingredients found in tomatoes are saponins and tannins which are in the active compounds group that have antibacterial and antioxidant activities.<sup>17</sup> Flavonoid compounds also have benefits as anti-inflammatory, antioxidant, antitumor, and antibacterial by forming complex compounds with extracellular proteins.<sup>18</sup> The study to determine the effectiveness of the ethanolic extract of tomato (*Solanum lycopersicum* L.) against the growth of *Staphylococcus epidermidis* bacteria in vitro, showed that the diameter of the inhibition zone formed in the positive control was 40.36 mm (potent inhibition), inhibition zones at concentrations of 100, 80, 60, 40, and 20% had solid inhibitory power.<sup>19</sup>

Mouth rinses are the most usual and easy way for antiplaque agents to be delivered. The direct damage to oral health, xerostomia seemed independently associated with mouthwashes, may have an effect on these chemical compounds. Hopefully, the contents of tomatoes such as lycopene compounds, flavonoids, and saponins have been shown to inhibit or kill bacterial growth with natural compounds.<sup>12,13</sup> The purpose of this study was to analyze the antibacterial effect of tomato (*Solanum lycopersicum*) ethanol extract against *S. mutans* and *P. gingivalis*.

## METHODS

This research was a laboratory experimental one with a posttest only control group design where the observed variable was the diameter of the inhibition zone produced from the ethanolic extract of tomato (*Solanum lycopersicum* L.) in various concentrations against *S. mutans* and *P. gingivalis* on blood agar media. In this study, distilled water was used as a negative control, whereas chlorhexidine was used as a positive control. Normality test showed the data was normally distributed then the OneWay ANOVA parametric test was used.

The tomatoes used in this study were obtained from gardens in the Wangunsari area, Lembang, West Bandung, West Java. The tomatoes used for this study were tomatoes which have a maturity level of 90-100% which were picked at the age of 90 days, red in colour. Before performing the research, a plant determination test was carried out on the tomatoes to be used. Plant determination was tested in the

Research Center for Biology-LIPI Bogor, West Java. The extraction method used was the maceration method. Maceration is an extraction method by immersing the material using a solvent that is suitable for the active compound to be taken with low heating. The solvent used in the manufacture of tomato extract was 96% ethanol. Qualitative phytochemical test of the ethanolic extract of tomato (*Solanum lycopersicum* L.) was carried out using the Harborne procedure to determine the content of alkaloids, flavonoids, saponins, steroids, triterpenoids, and tannins.

Alkaloid examination was carried out by taking 2 ml of tomato ethanol extract, then adding 2 ml of chloroform and 5 drops of concentrated ammonia. 3 drops of concentrated  $H_2SO_4$  to chloroform were added. Then 2-3 drops of Dragendorff reagent were added to the mixture. If a red color formed after being dropped, this indicated the presence of alkaloid compounds.<sup>20</sup> Flavonoid examination was carried out by taking 2 ml of tomato ethanol extract, then adding a few drops of concentrated HCl and a little Mg powder to the Wilstater reagent. If it showed a yellow color, it means it is positive for flavonoids. For the  $H_2SO_4$  2N reagent, 2 ml of tomato ethanol extract was given a few drops of  $H_2SO_4$  2N and then heated. If it was red, it was positive for flavonoids.<sup>20</sup> For the 10% NaOH reagent, a few drops of 10% NaOH were added to 2 ml of tomato ethanol extract. If an orange/orange color formed, then it was positive for flavonoids.<sup>20</sup> Saponin examination was carried out by taking 2 ml of tomato ethanol extract, then adding 2 ml of distilled water, and heating at 70°C. After that, it was shaken vigorously for 1 minute. If foam was formed as high as 1-10 cm which was stable for no less than 10 minutes and did not disappear when added with 1 drop of 2N HCl, this indicated the presence of saponin.<sup>20</sup>

Tannin examination was carried out by taking 2 ml of tomato ethanol extract then mixing it with 2 ml of distilled water and heating at 100°C. Next, the solution was cooled and filtered. The filtrate obtained was added with 2-3 drops of 1%  $FeCl_3$  solution. If the solution formed a blackish green or dark blue color, this indicated the presence of tannin compounds.<sup>20</sup> Phenolic glycoside examination was carried out by taking tomato ethanol extract which was added to 5%  $FeCl_3$  until a color change occurred, then the color was compared with the pure extract. If a darker color formed, it meant it was positive for phenolic content.<sup>20</sup> Triterpenoid and steroid examination was carried out by taking 2 ml of tomato ethanol extract, then dissolving it in 0.5 ml of chloroform and adding 0.5 ml of anhydrous acetic acid. Next, 1-2 ml of concentrated  $H_2SO_4$  was added to this mixture through the walls of the tube. If a brownish or violet ring formed at the border of the two solvents, this indicated the presence of triterpenoids. If a bluish green color formed, it indicated the presence of steroids.<sup>20</sup>

The method used in this study was the well-diffusion test. The test bacteria were obtained from the Microbiology Laboratory, Universitas Padjadjaran, Bandung. The procedure for making a suspension of *S. mutans* bacteria was as follows: (1) One ose of *S. mutans* colony was taken for culture; (2) Then the *S. mutans* bacteria were taken using a sterile tube and put into 10 ml of bulyon solution aseptically; (3) The bacterial suspension was homogenized using a homogenizer until the turbidity of the test bacterial suspension obtained was in accordance with the 0.5 McFarland standard (around  $1.5 \times 10^8$  CFU/mL). The turbidity indicated the growth of bacteria.<sup>21</sup>

The procedure for making a suspension of *P. gingivalis* was as follows: (1) Bacteria on Blood Agar plate (BAP) medium were incubated at 37°C for 24 hours; (2) *P. gingivalis* bacteria that had been cultured on medium using a sterile ose needle were taken and inoculated into the bulyon solution; (3) The suspension was homogenized using a vortex until it reached a turbidity level equivalent to Mc Farland 0.5 which was  $1.5 \times 10^8$  colony forming unit (CFU)/ml.<sup>8</sup> The petri dish was closed and put into the incubator to be incubated at 37°C for 24 hours. The diameter of the inhibition zone was measured using a caliper in millimeters from one side to the other that was opposite over the center of the wellbore. The same procedure as the sample above was repeated four times.

## RESULTS

Tomato fruit used in this study was extracted and then carried out a qualitative phytochemical test with the following results (Table 1).

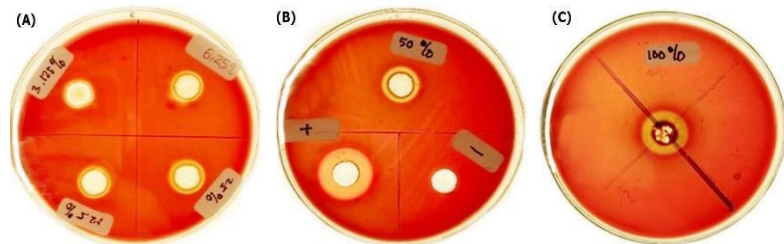
**Table 1.** Results of qualitative phytochemical testing of tomato ethanol extract

| Active biological component of tomato ethanol extract | Results of phytochemical test |
|---|-------------------------------|
| Alkaloid  | +                             |
| Phenolic  | +                             |
| Flavonoid   | +                             |
| Saponin   | -                             |
| Steroid   | -                             |
| Tannin  | +                             |
| Triterpenoid  | +                             |

Note: (+) positive, significantly visible color change; (-) negative, no visible color change

**Table 2.** Results of Measurement of Inhibitory Zone Diameter of *S. mutans*

| %                | 1 <sup>st</sup><br>repetition | 2 <sup>nd</sup><br>repetition | 3 <sup>rd</sup><br>repetition | 4 <sup>th</sup><br>repetition | Mean ± SD<br>(mm) |
|------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------|
| Negative control | 0.0                           | 0.0                           | 0.0                           | 0.0                           | 0.0±0.5           |
| 3.125            | 3.125                         | 4.0                           | 3.9                           | 4.1                           | 3.95±0.5          |
| 6.25             | 6.25                          | 4.2                           | 4.4                           | 4.6                           | 4.48±0.5          |
| 12.5             | 12.5                          | 4.7                           | 4.7                           | 5.2                           | 4.80±0.5          |
| 25               | 25                            | 5.0                           | 5.2                           | 5.4                           | 5.150±0.5         |
| 50               | 50                            | 5.2                           | 6.3                           | 6.7                           | 6.00±0.5          |
| 100              | 100                           | 29.3                          | 32.6                          | 30.6                          | 32.10±0.5         |
| Positive control | Positive control              | 8.4                           | 7.7                           | 8.2                           | 7.85±0.5          |

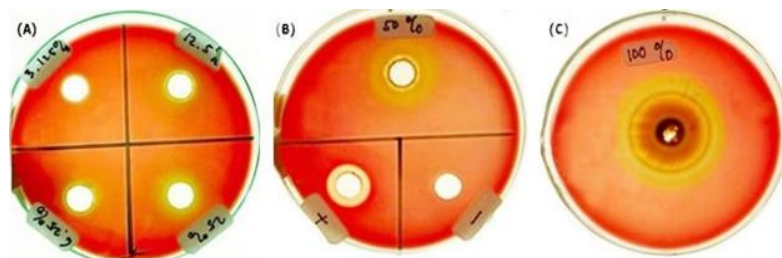


**Figure 1.** The observation results of the diameter of the inhibition zone of tomato ethanol extract against *S. mutans* at a concentration of (A) 3.125%, 6.25%, 12.5% and 25% concentration of tomato ethanol extract, (B) 50% concentration of tomato ethanol extract, positive control and negative control, (C) 100% concentration of tomato ethanol extract.

Based on Table 2 and Figure 1, it is shown that the diameter of the largest inhibition zone for *S. mutans* bacteria was found in the 100% concentration of tomato ethanol extract with a diameter of 32.10 mm. The results of the measurement of the second largest inhibition zone were chlorhexidine 0.2% as a positive control with a diameter of 7.85 mm. While the diameter of the smallest inhibition zone was found in the negative control of distilled water with a diameter of 0.00 mm.

**Table 3.** Results of Measurement of Inhibition zone diameter of *P. gingivalis*.

| %                | 1 <sup>st</sup><br>repetition | 2 <sup>nd</sup><br>repetition | 3 <sup>rd</sup><br>repetition | 4 <sup>th</sup><br>repetition | Mean±SD<br>(mm) |
|------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-----------------|
| Negative Control | 0.0                           | 0.0                           | 0.0                           | 0.0                           | 0±0.5           |
| 1.325            | 3.9                           | 3.5                           | 3.7                           | 3.8                           | 3.72±0.5        |
| 6.25             | 4.6                           | 4.9                           | 4.7                           | 4.6                           | 4.70±0.5        |
| 12.5             | 5.0                           | 5.9                           | 4.8                           | 4.8                           | 5.12±0.5        |
| 25               | 5.9                           | 5.6                           | 5.3                           | 5.1                           | 5.47±0.5        |
| 50               | 5.9                           | 6.3                           | 5.3                           | 6.1                           | 5.90±0.5        |
| 100              | 8.9                           | 10.6                          | 10.1                          | 9.1                           | 9.67±0.5        |
| Positive Control | 12.2                          | 10.6                          | 10.1                          | 10.8                          | 10.92±0.5       |



**Figure 2 .** The results of the observation of the diameter of the inhibition zone of tomato ethanol extract against *P. gingivalis* at a concentration of (A) 3.125, 6.25, 12.5 and 25% of tomato ethanol extract, (B) 50% concentration of tomato ethanol extract, positive control and negative control, (C) 100% concentration of tomato ethanol extract.

Based on Table 3 and Figure 2, it was found that the diameter of the largest inhibition zone for *P. gingivalis* was 10.925 mm in the treatment with positive control. The result of the measurement of the diameter of the next largest inhibition zone was in the treatment of 100% tomato ethanol extract of 9.675 mm. While the smallest inhibition zone produced by the negative control is 0 mm. Based on the classification of inhibition according to Davis and Stout, the results of the measurement of the diameter of the inhibition zone of tomato ethanol extract against *S. mutans* were in the weak category for concentrations of 3.125, 6.25, 12.5%, and negative control, and the medium category for tomato ethanol extract with concentration of 25, 50%, and positive control of chlorhexidine 0.2%, very strong category for 100% concentration of tomato ethanol extract (Table 4). Meanwhile, the results of the measurement of the diameter of the

inhibition zone of tomato ethanol extract against *P. gingivalis* were in the weak category for tomato ethanol extract with concentrations of 3.125 and 6.25%, and the medium category for tomato ethanol extract with concentrations of 12.5, 25, 50 and 100% (Table 4).

**Table 4.** Classification of inhibition zones of tomato ethanol extract against *S. mutans* and *P. gingivalis*

| Tomato ethanol extract (%) | Inhibition Zone to <i>S. mutans</i> Diameter Mean (mm) | Classification of Inhibition Zone to <i>S. mutans</i> | Inhibition Zone to <i>P. gingivalis</i> Diameter Mean (mm) | Classification of Inhibition Zone to <i>P. gingivalis</i> |
|----------------------------|--|---|--|---|
| 3.125                      | 3.95   | Weak  | 3.72   | Weak  |
| 6.25                       | 4.48   | Weak  | 4.70   | Weak  |
| 12.5                       | 4.80   | Weak  | 5.12   | Medium  |
| 25                         | 5.15   | Medium  | 5.47   | Medium  |
| 50                         | 6.00   | Medium  | 5.90   | Medium  |
| 100                        | 32.10  | Very Strong   | 9.67   | Medium  |

Note: Classification of inhibition according to Davis and Stout, the results of the measurement of the diameter of the inhibition zone in millimeter.

The results of the normality test showed that the data were normally distributed. Results of the one way analysis of variance test, the p-value was <0.05 which indicated that there were significant differences in the inhibition zones of the seven treatments as seen from the average inhibition zone. Next, a follow-up One Way ANOVA test was performed in the form of a post hoc test to find out which treatment group was the most significant. The treatment of 100% tomato ethanol extract was found to be the most significant with an average value of inhibition on *S. mutans* of 32.10 mm and on *P. gingivalis* of 9.675 mm.

## DISCUSSION

Based on Table 1, the servo tomato ethanol extract contained alkaloid, phenolic, flavonoid, tanin, triterpenoid, while it did not contain saponins and steroids. This is in accordance with previous study about tomatoes containing natural chemical compounds such as lycopene,  $\beta$ -carotene, lutein, saponins, tannins, folic acid, and flavonoids.<sup>15</sup> The presence of saponin content was indicated by the height of the foam. The height of this foam is affected by the time and extraction temperature. Extraction temperature that is too high will cause damage to the ethanolic extract of tomatoes. Extraction time that is too long can cause the extract to be oxidized, while if the extraction time is too fast it can result in not all bioactive compounds being extracted from tomatoes. Steroid content which was also not detected can be because the solvent used was less able to extract steroids in tomatoes. The results of the literature review reported that the highest concentration of steroids was found in the ethyl acetate extract. In addition, the results of the steroid extraction process could also be influenced by the conditions of natural ingredients, extraction methods, sample particle size, and storage time.

Based on Table 2 and Figure 1, it is shown that the diameter of the largest inhibition zone for *S. mutans* bacteria was found in the 100% concentration of tomato ethanol extract with a diameter of 32.10 mm. *S. mutans* were in the weak category for concentrations of 3.125, 6.25, 12.5, and negative control, and the medium category for tomato ethanol extract with concentration of 25, 50, and positive control of chlorhexidine 0.2%, very strong category for 100% concentration of tomato ethanol extract (Table 4) Previous study showed methanol tomato fruit (*Solanum lycopersicum* L) extract concentration of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12,5 mg/ml against *S. mutans* had 0 mm zones of growth inhibition.<sup>22</sup> This study used cold maceration extraction using methanol, containing alkaloids, flavonoids, glycosides, saponins, tannins, steroids, phlabotannins, terpenoids and tannins were present in tomato fruit extract, but, anthraquinones and phlabotannins were absent in methanolic extract.<sup>22</sup>

Among the secondary metabolites studied, alkaloids and polyphenols have shown strong antimicrobial activity. Polyphenols are one of the most numerous and diverse groups of secondary metabolites; their antioxidant properties provide the basis for antimicrobial effects. Alkaloids provided the underlying structure for the development of several antibiotics with a diverse range of action.<sup>23</sup> *S. mutans* can alter the local environment by forming an extracellular polymeric substances (EPS)-rich and low pH milieu, thereby creating a favorable niche for other acidogenic and aciduric species to thrive. *S. mutans* strains are more frequently isolated from dental plaque of individuals with bacteremia.<sup>24</sup>

Periodontitis is an infection-driven inflammatory disease in which the composition of biofilms plays a significant role. Dental plaque accumulation at the gingival margin initiates an inflammatory response that, in turn, causes microbial alterations and may lead to drastic consequences in the periodontium of susceptible individuals.<sup>25</sup>

Based on Table 3 and Figure 2, it was found that the diameter of the largest inhibition zone for *P. gingivalis* was 10.92 mm in the treatment with positive control. The result of the measurement of the diameter of the next largest inhibition zone was in the treatment of 100% tomato ethanol extract of 9.67 mm. Meanwhile, the results of the measurement of the diameter of the inhibition zone of tomato ethanol extract against *P. gingivalis* were in the weak category for tomato ethanol extract with concentrations of 3.125 and 6.25, and the medium category for tomato ethanol extract with concentrations of 12.5, 25, 50 and 100 (Table 4). *P. gingivalis* bacteria have a double defense system, namely the outer membrane and the inner membrane which is cytoplasmic. The outer membrane of this bacterium is composed of the outer membrane protein A (OmpA-like) and porins. Triterpenoid compounds in tomatoes damage *P. gingivalis* cells by reacting with porins in OmpA of *P. gingivalis*. The reaction between OmpA, porin and terpenoids will form a strong polymeric bond that damages the porin and OmpA of *P. gingivalis*.<sup>26-28</sup>

Based on the results of the study, the higher the concentration of tomato ethanol extract, the larger the diameter of the inhibition zone produced by *S. mutans*. The presence of an antibacterial effect was indicated by the formation of an inhibition zone around the well which was filled with tomato ethanol extract. The increase in the diameter of the inhibition zone proportional to the increase in concentration could be due to the number of bioactive compounds having an antibacterial effect which increased with the increase in extract concentration. According to expert opinion, the magnitude of the inhibitory activity depends on the rate of diffusion of the antibacterial compound content and the diameter of the inhibition zone tends to increase as the concentration of the extract increases<sup>16</sup>.

In addition, based on research conducted by Suhartati and Nuryanti, it stated that the higher the solvent content in the extract, the lower the active compound content.<sup>29</sup> If more solvent content is used than the extract, the less bioactive compounds contained in tomatoes will have so that the antibacterial effect is weaker than large concentrations. Tomato ethanol extract with concentrations of 50, 25, 12.5, 6.25 and 3.125% indicated that the smaller inhibition zone formed could be due to the decreasing activity of lycopene and other bioactive compounds. Based on the results of phytochemical testing, the biologically active compounds contained in tomato include alkaloids, flavonoids, phenolics, tannins and triterpenoids. The main bioactive compounds in tomato extract that have antibacterial effects are carotenoids and phenolic compounds. The most carotenoid compound in tomatoes is lycopene. Meanwhile, the phenolic compounds found in tomatoes are phenolic acids (caffeic, chlorogenic, sinapic, p-coumaric dan ferulic acids) and flavonoids (quercetin, rutin, kaempferol dan naringenin).<sup>30</sup>

The work mechanism of flavonoids as *S. mutans* antibacterial compounds is by inhibiting cell membrane function, inhibiting nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, inhibition of the attachment and biofilm formation, inhibition of the porin on the cell membrane, alteration of the membrane permeability, attenuation of the pathogenicity, and inhibition bacterial energy metabolism. When inhibiting cell membrane function, flavonoids form complex compounds from extracellular proteins that can damage the cell membrane of *S. mutans* bacteria and are followed by the release of these bacterial intracellular compounds. In inhibiting nucleic acid synthesis, rings A and B of flavonoid compounds have an important role in the process of inter classification or hydrogen bonding, namely by accumulating nucleic acid bases so that they can inhibit the formation of RNA and DNA. Inhibition of energy metabolism is done by inhibiting the use of oxygen by bacteria. Energy itself is needed by bacteria for the biosynthesis of macromolecules so that when their metabolism is inhibited, the molecules of *S. mutans* bacteria cannot develop into complex molecules.<sup>31</sup>

Alkaloid, flavonoid and phenol compounds can be antibacterial because they can damage the cell membrane of *P. gingivalis* bacteria by denaturing and coagulating proteins. These compounds enter the bacterial cell through the cell wall and bacterial cytoplasm. In it, these compounds cause clumping or coagulation of protoplasmic constituent proteins. The n, the metabolism becomes inactive and bacterial growth is inhibited. The presence of phenolic compounds in low amounts causes the formation of phenol protein complexes with weak bonds and will decompose. Furthermore, phenol penetration occurs into cells and causes protein denaturation. Phenol compounds in high amounts can cause protein coagulation and cell membrane lysis occurs.<sup>23,31</sup>

Lycopene is known as a carotenoid red pigment that has the effect of inhibiting bacterial growth. Recent studies have reported that lycopene acts as an antibacterial agent that works by influencing reactive oxygen species (ROS) to mediate bacterial DNA damage. Lycopene can increase ROS levels, especially hydroxyl radicals that can damage bacterial DNA, and cause cell filamentation due to limitation of cell division due to activation of the DNA repair system (ROS response) which is characterized by the increase of RecA expression. Furthermore, lycopene induces OH- compounds in DNA damage that cannot be repaired by the DNA repair system (ROS response) so that it can have an antibacterial effect.<sup>32</sup> In this study, the diffusion method used was the well diffusion method. The well diffusion method was used because it could produce a larger diameter and stronger inhibition zone to inhibit the growth of *S. mutans* and *P. gingivalis* bacteria. This is because in the well method there will be an osmolarity process from a higher extract concentration. The holes formed in the blood agar medium filled with the exact concentration experienced a more thorough and more homogeneous osmolarity process than using disc diffusion.<sup>33</sup>

The limitation of this research was that it was only found for minimum inhibitory concentration (MIC). It is recommended that future research examines the minimum bactericidal concentration accompanied by a standard curve.

## CONCLUSION

The ethanolic extract of tomatoes (*Solanum lycopersium* L.) as the lycopene compounds, flavonoids, and saponins have been shown to have an antibacterial effect in inhibiting bacterial growth of *S. mutans* and *P. gingivalis*.

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