The effectiveness of edamame extract (*glycine max l. merrill*) against the inhibition of *streptococcus mutans* in orthodontic material

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***Abstract*: Orthodontic appliances consist of brackets and *Elastomeric Chain* Generation II (ECG II). Fixed orthodontic appliances can increase the amount of oral biofilm and bacterial colonization on the tooth surface. The bracket enhances bacterial adhesion due to its high surface free energy. The *Elastomeric Chain* is susceptible to stretching during use which shows the presence of microporosity shaped like a honeycomb pattern due to the release of filaments when stretched.** Objective**: The objective of this study was to determine the power of edamame extract (*Glycine max L. merril*) as an antibacterial in inhibiting *Streptococcus mutans* (*S. mutans*) on metal brackets and EGC II and to determine the concentration of edamame extract which was effective in inhibiting the growth of *S. mutans* on metal brackets and EGC II.** Research Methods**: This type of research is experimental laboratory with post test only control group design. This research is divided into 3 groups, namely control group (sterile distilled water), 50% edamame extract group, and 75% edamame extract group. Observations were made by counting the number of colonies on MHA solid media using the Total Plate Count (TPC) method.** Results**: The results showed that the control group did not have any inhibition on the growth of *S. mutans* bacteria. Between the 50% and 75% groups, the one with the greatest inhibition was the 75% group.** Conclusion**: The conclusion of this study is that the antibacterial power of 75% concentration of edamame (Glycine Max L. merril) extract was effective in inhibiting the number of *S. mutans* colonies on metal brackets and EGC II**

Index Terms*: Metal Bracket, *Elastomeric Chain* Generation II, Edamame Extract, *Streptococcus mutans

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I. INTRODUCTION

Fixed orthodontic treatment is a treatment carried out in the field of dentistry that aims to achieve an aesthetically pleasing dentofacial appearance by eliminating crowding of teeth, correcting rotational and apical deviations of the teeth, correcting the incisal relationship and creating a good occlusive relationship. Fixed orthodontic appliances have components including bands, brackets, orthodontic adhesives, bow wires and *elastomeric chains* [1,2].

The bracket serves to deliver the required force to the teeth [3]. The brackets used must be manufactured with accuracy, both in terms of shape, level of strength and level of corrosion resistance and biocompatibility. The brackets that are most often used are metal-based brackets, because they are not easily fractured, resistant to deformation and are more economical [4]. However, metal brackets have the disadvantage of having a high surface free energy compared to ceramic brackets, thereby increasing bacterial adhesion [5].

Elastomeric Chain Generation II (ECG II) is a renewal of *Elastomeric Chain* Generation I (ECG I) which has the characteristics of an ideal elastomeric material, because it takes longer to maintain style, but has fewer color variants and chain types [6]. *Elastomeric Chain* Generation II (ECG II) can experiencing susceptibility to stretching during daily use which shows the presence of microporosity with a honeycomb-like formation due to filament shedding when ECG II stretches. The honeycomb pattern on the surface of ECG II can be a site for plaque retention and bacterial colonization. Bacterial colonization can also be caused by malocclusion, poor oral hygiene, and a cariogenic diet exacerbated by poor fixed orthodontic components, thereby leading to susceptibility that creates more surface area for bacterial retention [7].

One of the bacteria that colonizes fixed orthodontic appliances the most is *Streptococcus mutans* (*S. mutans*), which has been proven to be one of the main pathogens causing caries. Metal orthodontic brackets induce changes in the oral cavity such as increased plaque accumulation and increased *S. mutans* colonization. Colonization of *S. mutans*, which is a foreign substance on the surface of the *elastomeric chain*, can enter into the microporosity formed due to stretching of the *elastomeric chain*. *S. mutans* that enter the microporosity are proven to be able to damage the *elastomeric chain* structure so that force decay can occur. Colonization of *S. mutans* also produces acid which can cause discoloration of the *elastomeric chain* [8]. Bacterial colonization can be prevented by cleaning using organic or inorganic inhibitors. However, non-organic inhibitors can cause side effects such as tooth discoloration and resistance [9]. The use of natural ingredients is considered to have fewer side effects compared to chemicals, besides the price is more affordable [10]. This reason became the basis for searching and developing various antibacterial studies from organic materials, one of which was antibacterial in edamame ingredients.

Edamame (*Glycine max L. Merrill*) is one of the leading commodities in Jember Regency which is well known in the international market. Edamame which was originally only cultivated in Japan, Taiwan, and China. Edamame cultivation is now developing in Indonesia, especially in Jember which has become the largest edamame provider in Indonesia and is one of the main export commodities from Jember [11]. Edamame has higher levels of isoflavones (flavonoid derivatives) than ordinary soybeans,

so it has more potential as antibacterial [12].The high production of edamame, the flavonoid content in edamame which is proven to have antibacterial power, and edamame which is a natural ingredient made the authors interested in using edamame because of its few side effects [13].

Based on the description above, it is necessary to conduct a study on the power of edamame extract to decrease *S. mutans* colonies on metal brackets and *Elastomeric Chain* Generation II (ECG II). So, by knowing the power of edamame extract on the number of bacterial colonies in *S. mutans* on metal brackets and ECG II, it can be taken into consideration by dentists or for orthodontic appliance users in maintaining oral and dental health. Thus, the hypothesis from this study was that the antibacterial power of edamame (*Glycine Max L. merril*) extract was effective in inhibiting the number of colonies on metal brackets and *Elastomeric Chains* Generation II.

II. MATERIAL AND METHODS

The type of this research is an in vitro experimental laboratory with a post test only control group design. Edamame extract was made using the maceration method. The dried edamame seeds were blended into powder and then macerated with 70% ethanol solution (1:5) for 3 days. Stirring was carried out 2 times a day in the morning and evening. The maceration results were filtered using filter paper and then concentrated using a rotary evaporator at a temperature of 50°C to obtain a thick extract with a concentration of 100%. The viscous extract obtained was then diluted using ethanol as a solvent to obtain concentrations of 50% and 75%.

The suspension of *S. mutans* was made by taking 1 ose of *S. mutans* in a test tube containing 1 ml of MHB. The test tube was covered with cotton and then put into an Anaerobic jar and then incubated at 37ºC for 24 hours. The growth of *S. mutans* was characterized by the presence of turbidity which was adjusted to the standard of 0.5 Mc Farland.

The preparation of the *elastomeric chain* on the sample was carried out using 5 holes, then stretched on both sides of the acrylic plate with 3 mm bolts installed and when stretched it had a distance of about 25 mm due to the average value of the hook bracket distance from the canines to the molars in most patients. The reason for choosing the five holes of the *elastomeric chain* (Table 6) is because the retraction of the canines has been obtained from a simulation that would require a force of 250N after the extraction of the premolars. The study was conducted using 3 sample groups, with each group consisting of 4 samples, so that the samples used in this study amounted to 12 units. Furthermore, stretching is carried out on each bolt from the treatment group. The samples of metal brackets are 12 pieces and made of stainless steel on the premolars with 0.022 inch slots and roth precision.

Metal brackets and *Elastomeric Chains* Generation II (ECG II) were sterilized in an autoclave at 121 C for 15 minutes, then immersed in artificial saliva for 1 hour to form a pellicle on the metal bracket and ECG II. Metal brackets were exposed to *Streptococcus mutans* (*S. mutans*) by being put into a test tube containing a suspension of *S. mutans* then put into an anaerobic jar and incubated at 37oC for 24 hours. Ceramic brackets were immersed in 50% and 75% edamame extract for 2 minutes, while the control group was immersed in distilled water. Rinsed in PBS solution 2 times by immersing slowly. Metal bracket and ECG II were included in 5 ml MHB. Vibrate with a vortex for 30 seconds to dissolve the bacteria in the MHB. The results of the vibration were taken 0.5 ml and then diluted into 4.5 ml of sterile distilled water to multiples of 10-3. Take 0.1 ml of *S. mutans* suspension then put it into MHA media on each petridish according to the concentration of edamame extract and then flatten using a glass spreader. The petridish containing MHA media was then put into an anaerobic jar and incubated at 37°C for 24 hours. Perform the calculation of *S. mutans* using a colony counter with units of CFU/ml. The data were analyzed using the Shapiro-Wilk normality test and the Levene homogeneity test, followed by the Kruskall Wallis and Mann Whitney non-parametric test.

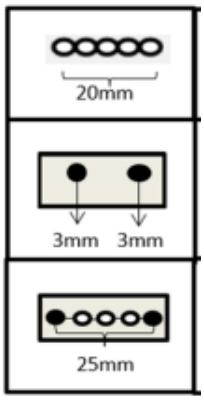

Figure 1. Sample Preparation of *Elastomeric Chain* on Number of Samples

III. RESULTS

Antibacterial effectivity of edamame extract against the number of *S. mutans* on metal bracket

Based on the results of observations and calculations of the number of bacterial colonies using the Total Plate Count (TPC) method using a colony counter, the average number of colonies for each research group can be seen in Table 1.

Table 1. The average value of the number of *S. mutans* colonies on metal brackets

Research Group	N	X±SD
Control	4	107.75±5.31
Consentration 50%	4	33±2.94
75%	4	0±0

Based on Table 1, The number of *S. mutans* colonies on metal bracket in succession from the smallest was the immersion group with 75% edamame extract where no colonies were found *S. mutans* grown on MHA solid media, the immersion group with 50% edamame extract was 33 cfu/ml and the control group of 107.75 cfu/ml.

Table 2. Percentage decrease in the number of *S. mutans* colonies on metal bracket

Control Group	Treatment Group	%±SD
Control	Consentration 50%	65,09%±3.6
Control	Consentration 75%	100%±3.5

Table 2 shows the percentage decrease in the number of *S. mutans* colonies on metal brackets between the control group and the treatment group by immersing the metal bracket in edamame (*Glycine Max L. Merril*) extract. The percentage value of the decrease in the number of *S. mutans* colonies from the largest was the soaking group with 75% edamame extract concentration of 100%, then the immersion group with 50% edamame extract concentration of 65.09%.

Antibacterial effectivity of edamame extract against the number of *S. mutans* on *Elastomeric Chain* Generation II

Table 3. The average value of the number of *S. mutans* colonies on *Elastomeric Chain* Generation II

Research Group	N	X±SD
Control	4	105.75±8.01
Consentration 50%	4	31.25±1.70
75%	4	0±0

Based on Table 3, the lowest number of *S. mutans* colonies was found in ECG II which was immersed in edamame extract at a concentration of 50% and 75%, respectively. The average value of the number of *S. mutans* colonies in succession from the smallest was the edamame extract group with a concentration of 75% because no *S. mutans* colonies were found, the edamame extract group with a concentration of 50% was 31.25 cfu/ml and the control group was 105.75. cfu/ml

Table 4. Percentage of the decrease in the number of *S. mutans* colonies on the *Elastomeric Chain* Generation II

Control Group	Treatment Group	%±SD
Control	Concentration 50%	70.44%±3.7
Control	Concentration 75%	100%±3.5

Table 4 shows that there was a percentage decrease in the number of *S. mutans* colonies in the *Elastomeric Chain* Generation II between the control group and the treatment group by soaking the *elastomeric chain* in edamame *(Glycine Max L. merrill*) extract. The percentage value of the decrease in the number of *S. mutans* colonies from the largest was the soaking group with 75% edamame extract concentration of 100%, the immersion group with 50% edamame extract concentration of 70.44%.

The Statistical Test

The results of the Saphiro Wilk normality test showed a significance value of more than 0.05 ($p>0.05$), which means that the data is normally distributed. The results of the Levene homogeneity test showed a value of less than 0.05 ($p <0.05$), which means the data are not homogeneous. Based on the results of the analysis of normality and homogeneity tests, it was found that the data were normally distributed but not homogeneous, so Kruskal Wallis nonparametric statistical test was performed. The results of the Kruskal Wallis test showed that there was a significant difference between the sample groups ($p<0.05$). Mann Whitney U-Test statistical test was conducted to see significant differences between the two study groups. The results of the Mann Whitney test showed that the significant value was <0.05 ($p<0.05$). The results of the analysis can be interpreted that the control group could not inhibit the growth of *S. mutans* bacteria. Meanwhile, 50% concentration and 75% concentration were able to inhibit the growth of *S. mutans*, but 75% concentration had a greater ability to inhibit the growth of *S. mutans* compared to 50% concentration.

Table 5. Result of Mann Whitney U-Test between research group

Research Group	K	KP1	KP2
Control	-	0.021*	0.014*
Concentration 50%		-	0.014*
Concentration 75%			-

The results of statistical tests between research groups using the Mann Whitney U-Test showed a significant difference between the two research groups with a significance value of less than 0.05 ($p < 0.05$). There was a significant difference between the control group and the 50% concentration group, the control group and the 75% concentration group , and the 50% concentration group and the 75% concentration group. This indicates a significant difference between the study groups in inhibiting the growth of *S. mutans* on the orthodontic materials.

IV. DISCUSSION

The results showed that the number of bacterial colonies in the group soaked with 50% and 75% edamame extract both decreased on metal brackets and *Elastomeric Chain* Generation II (ECG II) (Fig.2, Fig.3). This is because edamame contains many nutrients such as iron, isoflavones (flavonoid derivatives), vitamin E, ascorbic acid, calcium, potassium, protein, fat, carbohydrates, fiber. Phytochemical components in edamame consist of isoflavones (0.1-3%), sterols (0.23-0.46%), and saponins (0.17-6.16%) [15]. The difference in the number of colonies of S. mutans bacteria in the edamame extract was influenced by the content of the active compound. The content of the active compounds contained in the 75% edamame extract was higher than the 50% concentration. The results are in accordance with Lestari et al [14] who concluded that the higher the concentration, the more active compounds contained.

Isoflavones are active ingredients derived from flavonoids which has antibacterial ability. Isoflavones in edamame consist of genistein, daidzein, and glycitein. Isoflavones work as antibacterial, especially *S. mutans* by inhibiting energy metabolism, inhibition of nucleic acid synthesis and inhibition of cytoplasmic membranes [15]. Isoflavones can also inhibit nucleic acid synthesis by inhibiting enzymes in DNA topoisomerase, so that processes in cells will stop and cause bacterial cell death [16].

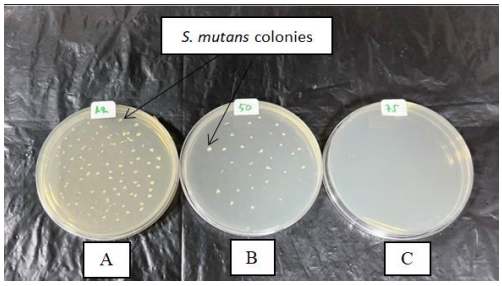

Figure 2. Research results on metal brackets (a) Immersion in distilled water (control group); (b) Soaking in 50% edamame extract; (c) Soaking in 75% edamame extract

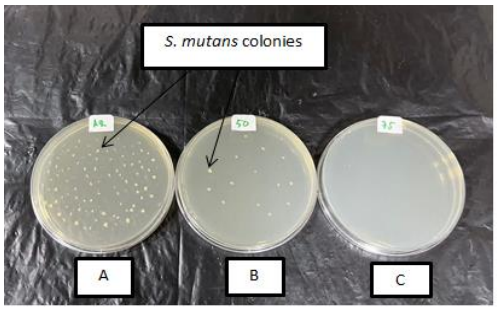

Figure 3. Research results on *Elastomeric Chain* Generation II a) Immersion in distilled water (control group); (b) Soaking in 50% edamame extract; (c) Soaking in 75% edamame extract

As an antibacterial agent, isoflavones work in inhibiting cell cytoplasmic membranes by forming complex compounds with extracellular proteins that can dissolve compounds in bacterial cell membranes, causing bacterial cell membranes to be damaged and intracellular compounds to exit. The mechanism of inhibition of the cell cytoplasmic membrane will cause cell membrane lysis [17]. Isoflavones (Daidsin, Daidzein, Genistein, Genistin) can also inhibit oxygen consumption by bacteria and inhibit the formation of metabolism in cytochrome reductase C, thereby reducing the energy needed by bacteria for macromolecular biosynthesis and causing cell death [18]. Isoflavones in edamame can interfere with the permeability of bacterial cell membranes by reducing hydrophobicity on the cell surface, resulting in leakage from the membrane and causing bacterial death [19].

In addition to containing isoflavones, edamame also contains saponins that can act as antibacterial compounds. The content of saponins in edamame can cause the leakage of enzymes and proteins from within the bacterial cells. Saponins can reduce the surface tension of bacterial cell walls and damage cell membrane permeability. Another ingredient that has antibacterial properties is steroids. The mechanism of steroids as antibacterials is related to lipid membranes and sensitivity to steroid components that cause leakage in

liposomes. Steroids can interact with cell phospholipid membranes which are permeable to lipophilic compounds, causing decreased membrane integrity and cell membrane morphology to change which causes cell brittleness and lysis [20].

V. CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that the antibacterial power of the 75% concentration of edamame (*Glycine Max L. merril*) extract was effective in inhibiting the number of *S. mutans* colonies on metal brackets and *Elastomeric Chains* Generation II.

VI. ACKNOWLEDGMENT

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