

DOI: <http://dx.doi.org/10.33846/hn70203>
<http://heanoti.com/index.php/hn>



RESEARCH ARTICLE

URL of this article: <http://heanoti.com/index.php/hn/article/view/hn70203>

Effectiveness of Edamame Extract (*Glycine max L. Merril*) Against *Streptococcus mutans* on Orthodontic Appliances Base

Leliana Sandra Devi^{1(CA)}, Dyah Setyorini², Hanifah Syifa Hasna³, Vanda Ramadhani⁴

^{1(CA)}Department of Orthodontics, Faculty of Dentistry, Universitas Jember, Indonesia;

lelianasandradevi@gmail.com (Corresponding Author)

²Departement of Pediatric Dentistry, Faculty of Dentistry, Universitas Jember, Indonesia;

dyahsetyorini@gmail.com

³Faculty of Dentistry, Universitas Jember, Indonesia; hanifahsyifah@gmail.com

⁴Department of Orthodontics, Faculty of Dentistry, Universitas Jember, Indonesia

ABSTRACT

One of the important components in removable orthodontic appliances is the base. The material that is often used as a base is *self-cured* or autopolymerized acrylic resin (Orthoresin). The base of orthodontic appliances will always be in contact with saliva and become a place of attachment for microorganisms, besides that on the base of removable orthodontic appliances there are two surfaces, namely the unpolished base surface that facing the mucosa and the polished base surface. This unpolished surface causes the surface to become rough so that it can increase the accumulation of dental plaque and become a retention factor for bacteria to stick to one of them is *Streptococcus mutans*. Therefore, it is possible to prevent bacterial colonization with organic or non-organic inhibitors. denture cleaning by immersing the denture in a cleaning solution containing a disinfectant. This research used organic material to be used as an inhibitor with antibacterial content, namely edamame (*Glycine max L. Merril*). As conclusion, edamame extract with concentrations of 50% and 75% effective in inhibiting the growth of *S. mutans* on the basis of removable orthodontic appliances.

Keywords: edamame extract; *S. mutans*; orthodontic appliance base

INTRODUCTION

Orthodontics is a branch of dentistry that studies facial development, variations of the face, jaw and teeth and their corrective treatment to achieve good or normal occlusion⁽¹⁾. Orthodontics comes from the Greek *orthos* which means normal or correct and *dontos* which means tooth. The purpose of orthodontic treatment is to improve the arrangement and position of the teeth to obtain a stable tooth relationship or occlusive function, improve mastication, muscle balance and harmonious facial esthetics. In general, the purpose of orthodontic treatment is to improve aesthetics so that it can overcome psychosocial problems. In addition, a better arrangement of teeth can lead to better standards of oral hygiene⁽²⁾.

There are two types of orthodontic appliances, namely fixed orthodontic appliances and removable orthodontic appliances. Fixed orthodontic appliances are devices that can only be inserted and removed by the treating doctor. Meanwhile, removable orthodontic appliance is a tool that can be installed and removed by the patient himself. For this reason, patient discipline is very important in using removable orthodontic appliances to determine the success of this appliance⁽³⁾. One of the components of removable orthodontic appliances is the base. The material that is widely chosen to make the base is acrylic resin. In removable orthodontics, *self-cured* or autopolymerized acrylic resin (*Orthoresin*) is usually used on the base. This is because *self-cured* has a faster polymerization speed so that it requires a fairly short processing time.

On the base of removable orthodontic appliances there are two surfaces, namely the unpolished base surface that facing the mucosa and the polished base surface. The surface of the base that facing the mucosa is not polished to conform to *anatomical landmarks* and can also be used for retention. This unpolished base surface causes the surface to become rough. Rough surfaces on the base of removable orthodontic appliances can increase the accumulation of dental plaque and susceptible to bacterial retention, one of them *Streptococcus mutans*⁽⁴⁾.

Streptococcus mutans (*S.mutans*) has long been involved in the formation of dental plaque, this bacterium attaches by hydrophobic bonds to the enamel surface⁽⁵⁾. *S. mutans* are gram-positive bacteria, immobile (non-motile), facultatively anaerobic and -hemolytic (total hemolysis shows a clear color on the culture medium for

blood around the colony). *S. mutans* produces extracellular polysaccharides, such as dextran and levan. This substance has a role in the adhesion of plaque and the adhesion of microorganisms. The production of these bacteria will cause demineralization of the enamel and then can lead to caries.

Currently, there are many plants that can be used as antibacterial agents. One of the plants that can be used as an antibacterial is the edamame plant. Edamame (*Glycine max L. Merrill*) is one of the leading commodities in Jember Regency and has penetrated the international market. Edamame contains nine essential amino acids that the body needs, rich in fiber, vitamin B and C, calcium, iron, and folic acid. Edamame also contains phytochemical components that can be used as antibacterial, namely isoflavones, steroids, and saponins⁽⁶⁾.

Edamame has a higher isoflavone content than ordinary soybeans so it has a higher antibacterial effect⁽⁷⁾. Anticaterial effect on edamame works in 3 ways through inhibition of nucleic acid synthesis, inhibition of cytoplasmic membranes, and inhibit energy metabolism⁽⁸⁾.

Edamame extract had antibacterial effect on *Staphylococcus aureus* at a concentration of 50 mg/ml⁽⁹⁾. In previous studies on *stainless steel* with different concentrations of *S. mutans*, it was proved that the greater the concentration of edamame extract it will get bigger percentage decrease number of *S. mutans colonies*. The average value of the percentage decrease in the number of *S. mutans* colonies from the largest was the edamame extract group with concentrations of 75 and 100 mg/ml at 100% and for the edamame extract group with a concentration of 50 mg/ml at 75.96%⁽¹⁰⁾.

Based on the description above, it is necessary to conduct research on the effectiveness of edamame extract on the number of *S. mutans* on the basis of orthodontic appliances. So by knowing the effectiveness of edamame extract on the number of *S. mutans* on the basis of orthodontic appliances, it can be considered by dentists or orthodontic appliance users in maintaining healthy teeth and oral cavity.

METHODS

This type of research was an in vitro experimental laboratory with a post test only control group design. The sampling technique used was total population sampling. The manufacture of orthodontic appliance base samples begins with the manufacture of molds. The molds were made using red wax with a size of 65x10x2 mm as many as 12 samples and then placed on a tray. Then make a blue plaster dough and then pour it around the red wax to form a mold. After the cast hardens, the red wax is slowly removed. Then smear the surface of the mold using a cold mold seal (CMS) separator. Then stir the orthoresin with the ratio of powder: liquid is 3: 1 according to the manufacturer's instructions. Then stirred using a spatula until it reaches the dough stage. Then it is poured into a mold that has been smeared with CMS and then let it be. After the resin reaches the final setting stage at room temperature, The resin is slowly removed from the mold and then smoothed out all the sharp edges with a Fraser bur. The plate has two surfaces / sides, on one side a smoothing process is carried out, starting with the use of sandpaper (grid 5000) then the plate is polished using pumice and kryet materials to produce a smooth and shiny orthoresin base surface. While on the other side there is no smoothing and polishing.

Making edamame extract by washing edamame seeds with running water until clean then drained so that the water is reduced and then dried by aerating at room temperature. After that it is blended until it becomes a powder. After it was deemed smooth enough, the edamame was removed from the blender and then sieved to obtain a really fine powder so that it could be an active ingredient that could be extracted⁽¹¹⁾. Then the maceration process was carried out with 70% ethanol solvent in a ratio (1:5) gr/ML. The maceration process was carried out for 3 days and stirred every 24 hours until homogeneous. After 3 days the solution was removed then filtered and the filtrate was taken. The filtrate obtained was then concentrated with a rotary evaporator at a temperature of 50°C for 3-4 hours until it turned into a thick edamame seed extract⁽¹²⁾.

All samples were sterilized by autoclave 121°C for 15 minutes, then immersed in artificial saliva for 1 hour. All samples were put in a test tube containing *S. mutans*, then incubated for 24 hours in an anaerobic jar at 37°C. The sample was then taken with tweezers and soaked. The sample was divided into 3 groups, the group was soaked in distilled water, the group was soaked in 50% and 75% edamame extract. Each was immersed for 2 minutes in a glass beaker. Next, it was removed and rinsed with phosphate buffered saline (PBS) 2 times. Then, the sample was immersed in Mueller-Hinton Broth (MHB) media and vibrated with a vortex for 30 seconds⁽¹³⁾. Then 0.5 ml was taken and diluted into 4.5 ml of sterile distilled water to multiples of 10⁻³⁽¹⁴⁾ then take 0.1 ml of bacterial suspension with a yellow tip attached to a micropipette of *S. mutans* suspension then put it into Mueller-Hinton Agar (MHA) media on each petridish then spread, then incubate for 24 hours in an anaerobic jar at 37°C⁽¹⁵⁾. Next, the number of *S. mutans* was calculated using a colony counter with units of CFU/plate⁽¹⁶⁾.

The first way to use the colony counter is that the petridish is placed on the colony counter in an inverted position right in the middle of the section where there is a checkered line. The tool is divided into 4 quadrants, from 64 boxes 30 boxes are taken randomly so that each quadrant is taken 7-8 boxes. Then a valid count of the number of bacterial colonies was carried out with a limit of 30-300 colonies per petridish. The results of the colony calculation from 30 boxes were entered into the formula to obtain the total number of colonies in the petridish with units of CFU/plate:

$$N = M/30 \times r^2$$

Note:

N: Total number of colonies in petridish (CFU/plate)

M: Total number of colonies from 30 randomly selected boxes

r : Petridish radius (cm)

The number of colonies obtained was then calculated by calculating the percentage decrease in the number of *S. mutans* colonies on the orthodontic appliance base sample between the untreated group (control group) and the treatment group to determine the effectiveness of edamame extract using the formula:

$$Mr = (M1-M2)/M1 \times 100\%$$

Note:

Mr: Decrease in the number of colonies

M1 : Number of colonies in the untreated group (control)

M2 : Total number of colonies in the treated group

The data obtained were then analyzed using the Shapiro-Wilk normality test and the Levene-test homogeneity test. Furthermore, the non-parametric Kruskal Wallis Test was carried out to see the differences in all groups and continued with the Mann Whitney U test to see significant differences in each of the two study groups⁽¹⁷⁾.

RESULTS

The results of the study on the effectiveness of edamame extract (*Glycine max L. Merrill*) in inhibiting the growth of *S. mutans* on basis of orthodontic appliances are shown in Figure 1.

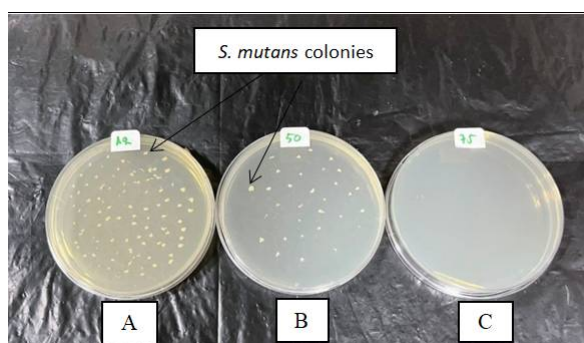


Figure 1. Research results on the basis of orthodontic appliances ; (a) immersion in distilled water (control group); (b) Soaking in 50% edamame extract; (c) Soaking in 75% edamame extract

The average value of the number of *S. mutans* colonies on the basis of orthodontic appliances growing on MHA solid media. The lowest number of *S. mutans* colonies was found on the base of orthodontic appliances soaked in edamame extract with a concentration of 75%. The average value of the number of *S. mutans* colonies in a row from the most effective was the soaking group with 75% edamame extract because there were no *S. mutans* colonies growing on MHA solid media, the immersion group with 50% edamame extract was 33, 75 cfu/ml, and the control group was 126.25 cfu/ml (Table 1).

Table 1. The average value of the number of *S. mutans* colonies on the basis of orthodontic appliances immersed in distilled water and extract of edamame (*Glycine max L. Merrill*)

Research group	N	X±SD
Control	4	126.25±5.25
Concentration 50%	4	33.75±2.22
Concentration 75%	4	0±0

Note:

N : Number of samples

X : The average number of colonies of *S. mutans*

SD : Standard deviation

The percentage decrease in the number of *S. mutans* colonies on the basis of orthodontic appliances between the control group and the treatment group by soaking the base in edamame extract (*Glycine max L. Merrill*). The largest percentage value of the decrease in the number of *S. mutans* colonies was the immersion

group with 75% edamame extract concentration of 100%, then the immersion group with 50% edamame extract concentration of 73.2% (Table 2)

Table 2. The percentage decrease in the number of *S. mutans* colonies on the basis of orthodontic appliances between the control group and the treatment group

Control group	Treatment group	% \pm SD
Control	Concentration 50%	73,2% \pm 3.6
Control	Concentration 75%	100% \pm 3.5

The results of the Shapiro-Wilk normality test for each group showed a significance value of more than 0.05 so that it could be interpreted that the data were normally distributed. The results of the Levene homogeneity test showed a significance value of less than 0.05 ($p < 0.05$), which means the data is not homogeneous. From the results of the normality and homogeneity test, it shows that the data is normally distributed and not homogeneous, so the non-parametric statistical test uses the Kruskal Wallis Test. The results of the Kruskal Wallis test obtained a significance value of 0.006 which means less than 0.05 ($p < 0.05$) so it can be concluded that there are differences in results between all research groups in inhibiting the growth of *S. mutans* on the basis of orthodontic appliances. Then the statistical test was continued with the Mann Whitney U-Test to see a significant difference between the two research groups.

Table 3. Results of Mann Whitney U-test between research groups

Research group	K	KP1	KP2
K	-	0.021*	0.014*
KP1		-	0.014*
KP2			-

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 treatment group 1, the control group and the treatment group 2, and the treatment group 1 and the treatment group 2. This indicates a significant difference between the study groups in inhibiting the growth of *S. mutans* on the basis of orthodontic appliances.

DISCUSSION

The results of this study showed that all concentrations of edamame extract could inhibit the growth of *S. mutans* on the basis of removable orthodontic appliances. The average value of the number of *S. mutans* colonies from the most effective was the soaking group with an extract with a concentration of 75% edamame extract because there were no *S. mutans* colonies growing on solid media, the immersion group with 50% edamame extract was 33.75 cfu/ml, and the water immersion group (control group) was 126.25 cfu/ml. The difference in the number of *S. mutans* colonies in the edamame extract was influenced by the content of the active compound. The content of active compounds contained in the edamame extract with a concentration of 75% more than the concentration of 50%. The higher the concentration, the more the active compound content.

Edamame contains a lot of nutrients such as protein, fat, carbohydrates, fiber, calcium, potassium, iron, ascorbic acid, vitamin E and also contains secondary metabolites that can be used as antibacterial, including: isoflavones, steroids, and saponins. Edamame is generally safe for consumption. However, if someone allergic to beans in general and to other soy-based products including soy sauce, tofu etc. avoid consuming edamame. Like in the case of all food items, exercise moderation. Excessive intake of edamame can cause constipation, stomach pain and diarrhea. Edamame contains genistein, daidzein, and glycitein. Isoflavones in edamame work as antibacterials including *S. mutans* in 3 ways, namely, inhibition of nucleic acid synthesis, inhibition of cytoplasmic membranes, and inhibit energy metabolism⁽⁹⁾.

Nucleic acids are macromolecules composed of nucleotide polymers. The process of nucleic acid synthesis includes replication, transcription, recombination and transmission of gene information related to changes in DNA structure. Isoflavones work by inhibiting the synthesis of nucleic acids by inhibiting the DNA topoisomerase enzyme which plays a role in the DNA replication process. DNA topoisomerase enzyme is a target for bioactive materials that have antibacterial activity. With the inhibition of the DNA topoisomerase enzyme, the processes in the cell will stop, resulting in cell death⁽¹⁸⁾.

The mechanism of action of isoflavones by inhibiting cell cytoplasmic membranes is to form complex compounds with extracellular and dissolved proteins so that they can damage bacterial cell membranes and are followed by the release of intracellular compounds. Another study stated that the mechanism of isoflavones inhibits cell membrane function by disrupting cell membrane permeability and inhibiting binding enzymes such as ATPase and phospholipase. The mechanism of inhibition of the cell cytoplasmic membrane will cause cell membrane lysis⁽¹⁹⁾.

Isoflavones can inhibit energy metabolism by inhibiting the use of oxygen by bacteria. Isoflavones inhibit cytochrome C reductase so that the formation of metabolism is inhibited. This causes the energy needed by

bacteria for macromolecular biosynthesis to decrease and will eventually lead to bacterial cell death. Edamame also contains steroids, the role of steroids as antibacterial can cause lysosomal leakage and interact with phospholipids so that it can cause bacterial membrane integrity to decrease and bacterial cells to become fragile⁽²⁰⁾. Saponins contained in edamame also act as antibacterial. Saponins at low concentrations can inhibit bacterial growth by increasing cell membrane permeability. Saponins at high concentrations can cause cell death due to bacterial cell lysis⁽²¹⁾.

Based on the explanation above, it can be seen that the antibacterial power of edamame extract (*Glycine max L. Merrill*) is effective in inhibiting the growth of *S. mutans* on the basis of removable orthodontic appliances because it contains active compounds that act as antibacterial, namely isoflavones, steroids, and saponins. Reducing the number of *S. mutans* on the base of orthodontic appliances is expected to reduce plaque buildup so as to minimize the occurrence of caries.

CONCLUSION

The conclusions in this study are edamame extract (*Glycine max L. Merrill*) with concentrations of 50% and 75% effective in inhibiting the growth of *S. mutans* on the basis of removable orthodontic appliances.

REFERENCES

1. Alawiyah. Komplikasi dan Resiko yang Berhubungan dengan Perawatan Orthodonti. Jurnal Imiah WIDYA. 2017;4(1):256-261.
2. Sari C, Kiki. Persepsi Kepuasan Pasien Perawatan Ortodontik Berdasarkan Keahlian Operator Menurut Jenjang Profesionalisme. 2013.
3. Iswari HS. Relaps dan Pencegahannya dalam Ortodonti. 2012;29(319):53-9.
4. Manson JD, Eley BM. Buku Ajar Periodonti. Jakarta: Hipokrates; 2012.
5. Mounika S, Jagannathan N, Murali. Association of *Streptococcus mutans* and *Streptococcus sanguis* in act of Dental Caries. Journal of Pharmaceutical Sciences and Research. 2015;7(9):764-766.
6. Arifah NH, Sutejo IR, Suswati E. The Effectiveness of Edamame Seed (*Glycine max L. Merrill*) Ethanol Extract to Fibroblast Count on Second Degree Burn Wound Healing. Journal of Agromedicine and Medical Sciences. 2019;5(3):152-159.
7. Rosiana NM, Amareta DI. Karakteristik Yogurt Edamame Hasil Fermentasi Kultur Campuran Bakteri Asam Laktat Komersial sebagai Pangan Fungsional Berbasis Biji-Bijian. Jurnal Ilmiah Inovasi. 2016;16(2):33-37.
8. Albert DRP, Neethirajan S, Xue J, Shi J. Characterization Of Antimicrobial Efficacy Of Soy Isoflavones Against Pathogenic Biofilms. LWT- Food Science and Technology. 2015;63(1):859-865.
9. Adamczak A, Ożarowski M, Karpiński TM. Antibacterial Activity of Some Flavonoids and Organic Acids Widely Distributed in Plants. Journal of Clinical Medicine. 2020;9(1):109.
10. Igboabuchi NA, Ilodibia CV. A Study on the Antioxidant and Antimicrobial Activities of Seed and Leaf Extracts of *Glycine max (L) Merr.* Asian Journal of Research in Botany. 2018;1(1):1-8.
11. Ningrum LS. Efektivitas Ekstrak Edamame (*Glycine max L. Merrill*) Dalam Menghambat Pertumbuhan *Streptococcus Mutans* Pada Kawat Ortodonti Berbahan Stainless Steel. Skripsi. Jember: Fakultas Kedokteran Gigi Universitas Jember. 2020.
12. Puspitasari AD, Prayogo LS. Perbandingan Metode Ekstraksi Maserasi dan Sokletasi terhadap Kadar Flavonoid Total Ekstrak Etanol Daun Kersen (*Muntingiacalabura*). Jurnal Ilmu Farmasi dan Farmasi Klinik. 2016;13(2):16-23.
13. Siddiq HBHF, Prabawati EF. Uji Aktivitas Antioksidan Ekstrak Etanol Biji Edamame (*Glycine max L. Merrill*) Dengan Metode DPPH. Jurnal Ilmiah Kesehatan. 2016;1(1):27-31.
14. Kim EH, Kim SL, Kim SH, Chung IM. Comparison of isoflavones and anthocyanins in soybean (*Glycine max (L.) Merrill*) seeds of different planting dates. J Agric Food Chem. 2012;60:10196-202.
15. Zhou X, Li Y. Atlas of Oral Microbiology from Health Microflora to Disease. San Diego: Elsevier Inc.; 2015.
16. Al-Jumaily EFA, Al-Seubehawy HMZ, Al-Toraihy FA. Isolation And Identification Of *Streptococcus mutans* Produced Glucosyltransferase And Cell Associated Glucosyltransferase Isolated From Dental Caries. International Journal of Current Microbiology and Applied Sciences. 2014;3(6):850-864.
17. Syahdrajat T. Panduan Penelitian untuk Skripsi Kedokteran dan Kesehatan. Yogyakarta: Pedhe Offset; 2018.
18. Xie Y, Yang W, Tang F, Chen X, Ren L. Antibacterial activities of flavonoids: Structure-activity relationship and mechanism. Curr. Med. Chem. 2015;22:132-149.
19. Górnaiak I, Bartoszewski R, Króliczewski J. Comprehensive Review Of Antimicrobial Activities Of Plant Flavonoids. Phytochem. Rev. 2019;18:241-272.
20. Swantara IMD. Sterol Ganggang Laut. Denpasar: Diktat Kuliah Program Studi Magister Kimia Terapan Program Pascasarjana; 2015.
21. Prihatiningtyas W, Mariani Y, Oramahi AH, Yusro F, Sisillia L. Uji Aktivitas Antibakteri Ekstrak Etanol Kulit Batang Mangga Kweni (*Mangifera Odorata* Griff.) Terhadap *Escherichia coli* ATCC 25922 dan *Staphylococcus aureus* ATCC 25923. Jurnal Tengkuwang. 2018;8(2):59-74.