THE EFFECTIVENESS OF EDAMAME (*GLYCINE MAX (L.) MERRILL*) EXTRACT AS ACRYLIC RESIN DENTURE CLEANSER ON THE NUMBER OF CANDIDA ALBICANS

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ABSTRACT

Background: Cleaning in denture base is needed to reduce the Candida albicans colonization and prevent denture stomatitis. Denture cleaning can be mechanically cleaned with a toothbrush, or chemically cleaned by immersion. Another natural ingredient that can be used for immersion is edamame (*Glycine max (L.) Merrill*) extract because it contains saponins, which contain antifungal compounds that can inhibit C. albicans. The purpose of this study was to determine the effectiveness of edamame extract (*Glycine max (L.) Merrill*) as a heat-cured acrylic resin denture cleanser with concentrations of 25%, 50%, 75%, and 100% by soaking for 8 hours against the amount of *C. albicans*.
Method: This research is a laboratory experimental study with 50 samples divided into 5 groups. The research group consisted of immersion acrylic resin samples with *C. albicans* contamination for 8 hours in the control group, namely sterile distilled water and the treatment group, namely edamame extract with concentrations of 25%, 50%, 75%, 100% and then spectrophotometer test to see the absorbance of C. albicans.
Result: The ANOVA test result showed p <0.005 Based on the results of the LSD test, it showed that there was a significant difference (p<0.05) in most groups except the control group against the acrylic group soaked in 25% edamame extract and acrylic soaked in 75% edamame extract against the acrylic group soaked in 50% and 100% of edamame extract
Conclusion: Edamame extract at 100% concentration as acrylic resin denture cleanser was the most effective in reducing the amount of *C. albicans* by soaking for 8 hours

INTRODUCTION

Edentulous is very disturbing a dental and oral health problem and appears in many communities. Edentulous is a condition where one or more teeth are removed from their socket or place.(1) This condition usually occurs due to periodontal disease, trauma and caries. The use of dentures as replacements of edentulous is very important to restore the function of these teeth. Dentures serve to improve the ability to chew, speak, provide support for facial muscles, and improve facial appearance and smile. Dentures are divided into two types, namely fixed dentures and removable dentures.(2)

The use of dentures is closely related to the way the user cleans his dentures. Denture cleaning procedures must be carried out regularly and regularly every day to prevent plaque buildup, clean food debris, calculus, and discoloration of the denture. Unclean dentures can cause bad breath and inflammation of the oral mucosa such as denture stomatitis.(3)

Denture stomatitis can be prevented by cleaning the denture. Currently the price of denture cleaning in the market is relatively expensive. Several studies concluded that the daily use of denture cleaning can cause acrylic resin properties such as discoloration, surface roughness,

hardness, and transverse strength. Therefore, alternative materials are needed as denture cleaning that can be obtained from herbal ingredients.(4)

The herbal ingredient that can be used as a natural denture cleaning is the edamame plant *(Glycine max (L.) Merrill)*. Jember regency is one of the edamame soybean producing areas in East Java Province. Edamame soybeans contain high nutritional value such as protein, carbohydrates, fat, vitamins and other content such as saponins. Saponins have anti-inflammatory and antibacterial activity.(5)

Edamame soybeans contain a fairly high nutritional value, every 100 g of seeds contains 582 kcal, 11.4 g protein, 7.4 g carbohydrates, 6.6 g fat, 100 mg vitamin A or carotene, 0.27 mg B1, B2 0 ,14 mg, B3 1 mg, and vitamin C.(6) Other ingredients of edamame are saponins and flavonoids. Saponins have anti-inflammatory and antibacterial activity. About 2% of saponins (triterpene glycosides) contained in edamame soybeans also have antifungal activity.(7)

Saponins are composed of structural compounds containing steroidal aglycones or triterpenes linked to one or more oligosaccharide moieties. The aglycon or non-saccharide part of the saponin molecule is called genin or sapogenin. Saponins have been proved to have a wide range of antifungal activities through phytochemical tests.(8) According to the study conducted by Ambo et al, the extract of Adas Seed (Foeniculum Vulgare mill.) is an effective denture cleanser to the growth of Candida albicans on acrylic plate because of the fennel seeds which contained medicinal properties such as essential oils, flavonoids and saponins which are useful as antifungals.(9)

Saponins work by disrupting the integrity of *C. albicans* cells. The antifungal properties of saponins come from the formation of polar saponin compounds bonding with lipoproteins and the bonding of non-polar saponin groups with the plasma membrane lipids of fungal cells.(10) Saponins have polar surfactant properties so that they can break down the fat layer on the cell membrane which causes disruption of cell membrane permeability, this results in the diffusion of materials or substances needed by fungi can be disrupted, eventually the cells swell and burst.(11)

The increased permeability causes a more concentrated intracellular fluid to be pulled out of the cell so that nutrients, metabolic substances, enzymes, proteins in the cell come out and the fungus can die.(12) The purpose of this study was to determine the effectiveness of edamame extract (*Glycine max (L.) Merrill*) as a heat-cured acrylic resin denture cleanser with concentrations of 25%, 50%, 75%, and 100% by soaking for 8 hours against the amount of *C. albicans.*

RESEARCH METHODS

This research is an experimental laboratory research with the post test only control group design. The research group consisted of immersing acrylic resin samples that had been contaminated with *C. albicans* in the control group, namely sterile distilled water and the treatment group, namely edamame extract with concentrations of 25%, 50%, 75%, and 100% for 8 hours. Then all groups were tested by looking at the absorbance value of *C. albicans* which was calculated using a spectrophotometer.

The research was carried out at the Dental and Oral Hospital Technology Laboratory, University of Jember for the manufacture of heat cured acrylic plates, the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, University of Jember and the Biology Laboratory of the Faculty of Pharmacy. The samples used were cylindrical with a diameter of 10 mm and a thickness

of 2 mm as many as 50 samples. The samples were divided into 5 groups with the number of samples in each group of 5 samples

Edamame Extract Making

Edamame extract is a preparation material from edamame seeds from PT Mitratani Dua Tujuh Jember. Edamame seeds are washed and cleaned and then dried by aerating so that the water content is reduced. The dried edamame seeds are blended until they become powder (simplicia). It aims to expand the surface area of edamame seeds so as to increase the number of active substance soluble in the solvent. How to extract the active substance from edamame seeds using ethanol as a solvent.[13]

Edamame seed extract was made using the maceration method by immersion 70% ethanol solvent for 3 days, then filtering to separate the filtrate and residue. The collected filtrate is then placed in the evaporator at 50°C to produce the remaining solvent vapor, thus obtaining a thick extract of edamame seeds.[6]

Heat Cured Acrylic Resin Sample Making

Acrylic resin plate is done by preparing red wax which has been formed cylindrical with a diameter of 10 mm and a thickness of 2 mm. Then prepare the cuvette and the white plaster/plaster of paris dough with a ratio of 75 ml of water: 250 grams of plaster, the dough is stirred for about 30 seconds. After that, put the plaster mixture into the bottom of the cuvette and then vibrate so that no air is trapped. Apply the wax model with Vaseline and place it on the plaster dough with a horizontal position and wait for 15 minutes. After setting the cast on the bottom cuvette, coat the surface of the cast and wax model again with Vaseline and then attach the top cuvette until it is metal to metal. Place the plaster dough on the top of the cuvette then close the cuvette and press it until it hardens. Then open the cuvette and do watering with hot water for about 15 minutes on the night model until the red night decays completely and cleans the mold space. The next step is packing heat cured acrylic resin by mixing heat cured acrylic resin material in a prepared glass, with a polymer ratio: monomer = 3:1, then closing the cup until it reaches the dough stage, then inserting the acrylic resin mixture into the mold space) which had previously been smeared with Could Mould Seal on the surface of the mold, then put cellophane paper on the top of the dough and installed the top cuvette.

Pressing using a hydraulic brench press with a pressure I of 900 psi. Opening the top cuvette, while removing any excess acrylic residue, reattach the cellophane paper before covering with the top cuvette. Then do a second press with a pressure II of 1200 psi and reopen the top cuvette and remove excess acrylic residue and trim, and reinstall the top cuvette without cellophane paper and perform a third press with a pressure of III of 1500 psi. Install the press begel on a cuvette containing acrylic resin that has gone through the packaging stage. Then maintain the pressure of the cuvette with a begel press and immerse it in water for 6-7 hours. Furthermore, for the heat cured acrylic resin curing process, that is by inserting the cuvette in an aluminum pan filled with boiling water (100℃) on a fire of ± 20 minutes according to the factory rules on Acrylic Denture Material brand resin. After that, turn off the heat and leave the cuvette in the pot until the water temperature becomes normal. The last stage is finishing the acrylic resin plate by smoothing the acrylic resin plate and cutting it according to its shape and size.

***C. albicans* test**

The first test of *C. albicans* carried out immersion of acrylic resin samples in sterile distilled water for 48 hours to reduce the remaining

monomer, then sterilized by autoclaving at 121^{0}C for 15 minutes then immersed in artificial saliva for 1 hour and rinsed with Phosphate Buffered Saline twice. After rinsing, the samples were put into a suspension of C. albicans and then incubated for 24 hours at 30C. The acrylic resin samples were removed and rinsed with PBS twice, then followed by immersion for 8 hours in the treatment group, namely edamame extract with concentrations of 25%, 50%, 75%, 100% and the control group, sterile distilled water. The sample was removed and rinsed with PBS twice and then put in a test tube containing saubouraud broth and vibrated for 30 seconds then the absorbance value was calculated on the spectrophotometer.

The data that has been entered first is tested for normality using the *Shapiro-Wilk* test. This test is used to determine normally distributed data, and then the Levene test is used to continue the homogeneity test to determine the data that has been entered into each set of homogeneous samples. If the data analysis results show that the data are normally distributed and homogeneous, use *One-way Annova* to perform statistical tests on the treatment group and the control group. Then, it proceeds to the *Least Significant Difference* (LSD) test to determine the most effective dose to reduce the number of *C. albicans*.

RESULT

The results have been obtained by calculating the absorbance value of *C. albicans* on acrylic resin plates immersed in 25%, 50%, 75% and 100% concentration of edamame extract, and the absorbance value of *C. albicans* in Sabouraud medium has been obtained sabouraud dextrose broth (SDB), see Table 1.

Table 1. Absorbance values of *C. albicans* in SDB media in the control group and the treatment group by immersion in edamame extract concentrations of 25%, 50%, 75%, and 100%

Sample Group	Control	Immersion with Edamame Extract			
		25%	50%	75%	100%
1	.152	.158	.143	.149	.148
2	.151	.157	.151	.148	.140
3	.155	.152	.148	.141	.137
4	.154	.153	.149	.142	.141
5	.161	.149	.150	.146	.142
Median	.155	.154	.148	.145	.142
Standard Deviation	.0037	.0037	.0031	.0035	.0041

Based on Table 1, the highest average absorbance value was found in the immersion group with distilled water (control) which was 0.155 and the lowest average absorbance value was found in the immersion group with 100% concentration of edamame extract, which was 0.142. All absorbance valuesobtained in Table 1 are then converted to obtain the number of *C. albicans* cells on the acrylic resin plate which is presented in Table 2.

Table 2. The number of *C. albicans* cells on acrylic resin after immersion (×108 CFU/ml) in the control group and the treatment group with 25%, 50%, 75%, and 100% concentration of edamame extract immersion

Sample Group	Control	Immersion with Edamame Extract			
		25%	50%	75%	100%
1	.062	.101	.006	.043	.039
2	.058	.091	.056	.039	-.012
3	.080	.062	.039	-.004	-.029
4	.078	.068	.045	.002	-.004
5	.115	.041	.049	.027	.002
Median	.078	.072	.039	.021	-.001
Deviation Standard	.0228	.0237	.0194	.0215	.0251

Based on Table 2, the highest average number of *C. albicans* cells was found in the water immersion group, which was 0.078x108 CFU/ml, followed by the 25% edamame extract immersion group, 0.072x108 CFU/ml, then the edamame extract immersion group with a concentration of 0.072x108 CFU/ml. 50% was 0.039x108 CFU/ml and the 75% edamame extract immersion group was 0.021x108 CFU/ml. The immersion group with 100% concentration of edamame extract showed the lowest average number of *C. albicans* cells, which was -0.001x108 CFU/ml.

The ANOVA test result showed p <0.005 Based on the results of the LSD test, it showed that there was a significant difference (p<0.05) in most groups except the control group against the acrylic group soaked in 25% edamame extract and acrylic soaked in 75% edamame extract against the acrylic group soaked in 50% and 100% of edamame extract

DISCUSSION

The completed research is a laboratory experimental study designed to determine the effectiveness of 25%, 50%, 75% and 100% Edamame extract as a heat cured acrylic resin denture cleaning on the number of C colonies. albicans. Each sample in the previous research was contaminated with *C. albicans* and then immersed in edamame extract for 8 hours. The difference in the concentration of edamame is to determine the concentration of edamame extract which is more effective as a heat-cured acrylic resin denture cleaning.

The results of the research that have been obtained, Table 1 shows the average value of the absorbance of *C. albicans* and its media which was measured using a UV-Vis spectrophotometer. The absorbance value is a value that indicates the number of light absorbed by the media solution in each tube to see the density of microbial cells which will be seen as the turbidity of the medium. The absorbance valuesobtained in Table 1 show that the highest average absorbance value was in the negative control group, namely distilled water and the lowest average absorbance was in the 100% edamame extract immersion group. This indicates that the media turbidity in the control group is the highest, and also indicates that the number of *C. albicans* in the control group is the highest.

The absorbance value of each sample was then converted to obtain the number of *C. albican*s

cells on the acrylic resin plate which is presented in Table 2. Table 2 shows the highest average number of *C. albicans* cells in the negative control group, which is .078x10^8 CFU/ml. This indicates that the negative control immersed in aquadest has the lowest antifungal activity, because aquadest does not have antifungal ability. This is supported by the research of Kurniawati et. al., (2016) on the inhibition of the growth of *C. albicans*, which stated that sterile distilled water used as a negative control did not provide an inhibition zone.(9) Then the immersion group with 25% edamame extract was 0.072x10^8 CFU/ml, the immersion group with 50% edamame extract was 0.039x10^8 CFU/ml, the immersion group with 75% edamame extract was 0.021x10^8 CFU/ml. The decrease in the average number of *C. albicans* cells at a concentration of 75% proves that at this concentration it has a greater antifungal effect due to an increase in the concentration of edamame extract. Then, the lowest average number of *C. albicans* cells was seen in the 100% concentration of edamame extract immersion group, which was -0.001x10^8 CFU/ml. This indicates that immersion of 100% concentration of edamame extract was the most effective in reducing the number of *C. albicans* compared to the lower concentration of 75%. The negative value obtained in the calculation was due to the absorbance value of *C. albicans* in SDB medium which was smaller than the absorbance value of SDB media without fungi, so it could be concluded that after treatment no more *C. albicans* cells were found in the media.

One Way ANOVA test results show a significance value of less than 0.05 (Sig. = 0.000). The test results showed that between each group there was a significant difference in the number of *C. albicans* cells so that it could be continued withLSD test. The results of the analysis of the LSD test data showed that there was a significant difference ($p<0.05$) in most groups except the control group against the 25% edamame extract immersion group and the 75% edamame extract immersion group against the edamame extract immersion group 50% and 100%. There were not significant differences in several groups due to the decrease in the number of *C. albicans* cells between the groups that were not much different. Based on the results of the research, it can be seen that increasing the concentration of edamame extract was proven to affect the number of *C. albicans* cells. The higher the concentration of edamame extract, the higher the antifungal effect. These results are in accordance with the opinion of Igboabuchi and Ilodibia (2018) and Abdel-Hady et. al., (2019) which stated that it was known that edamame content was able to inhibit the growth of *C. albicans*. (13)(14) Substances contained in edmame that act as antifungals are saponins.

The mechanism of saponins as antifungals is by lowering the surface tension of the sterol membrane of the fungal cell wall. The decrease in surface tension causes this sterol membrane to increase cell permeability, thereby disrupting the absorption of substances needed by fungi for growth so that cells will swell and burst.(15) Saponins increase the production of H_2O_2 and cause membrane lipid peroxidation, thereby increasing cell membrane permeability, and causes lysis of cell membranes, thereby inhibiting fungal growth. (16) Antifungal in saponins is from the ability of complex molecules with sterols in fungal membranes, causing the formation of pores in the lipid bilayer which can eliminate membrane integrity and increase cellular permeability.(17) The increased permeability causes a more concentrated intracellular fluid to be pulled out of the cell so that nutrients, metabolic substances, enzymes, proteins inside the cell come out and the fungus can die. (12)

The results showed that immersion edamame extract in concentrations of 25%, 50%, 75%, and 100% could be recommended as a denture cleaning because it has antifungal effects and is acceptable in the oral cavity.

CONCLUSION

Edamame extract at 100% concentration as acrylic resin denture cleanser was the most effective in reducing the amount of *C. albicans* by soaking for 8 hours.

REFERENCES

1. Siagian K V. *Kehilangan sebagian gigi pada rongga mulut*. e-CliniC. 2016;4(1).
2. Pongibidan. Inlay, *Crowns and bridges a clinical hand book*. 4th Ed. London: Wright Bristol; 2013 pp. 59.
3. Lahama L, Wowor VNS, Waworuntu OA. *Denture Stomatitis Pada Pengguna Gigi Tiruan Di Kelurahan Batu Kota Manado*. Pharmacon. 2015;4(4):71–81.
4. Sari KI, Dewi W, Jasrin TA, Sumarsongko T. *Kebersihan Gigi Tiruan pada Lansia, Suatu Tinjauan Metode dan Bahan*. J Mater Kedokt Gigi. 2018;7(1):1.
5. Hasanah AN, Sutejo IR, Suswati E. *The Effectiveness of Edamame Seed (Glycine max L. Merril) Ethanolic Extract to Fibroblast Count on Second Degree Burn Wound Healing*. J Agromedicine Med Sci. 2019;5(3):154.
6. Siddiq HBHF, Rosida, Prabawati EF. *Uji Aktivitas Antioksidan Ekstrak Etanol Biji Edamame (Glycin max (L) Merril) dengan Metode DPPH*. J Ilm Farm AKFAR. 2016;1(1).
7. Salgado JM & Donado-Pestana CM. *Soy as a functional food. Soybean and nutrition*, El-Shemy, H.(ed.) InTech. 2011:21-44
8. Ningsih, Gati, Nugrahani*. Pengaruh Lamanya Waktu Ekstraksi Remaserasi Kulit Buah Durian Terhadap Rendemen Saponin Dan Aplikasinya Sebagai Zat Aktif Anti Jamur. Konversi* , vol. 4, no. April, 2015, pp. 1–9.
9. Ambo, Andhika, Rahman. *Pengaruh Berbagai Konsentrasi Ekstrak Biji Adas (Foeniculum Vulgare Mill.) Sebagai Denture Cleanser Terhadap Pertumbuhan Candida Albicans Pada Plat Akrilik*. Odonto : Dental Journal, vol. 2, no. 2, 2015, p. 62, doi:10.30659/odj.2.2.62-67.
10. Kurniawati A, Mashartini A, Fauzia IS. *Perbedaan Khasiat Antijamur antara Ekstrak Etanol Daun Kersen (Muntingia calabura L.) dengan Nistatin terhadap Pertumbuhan Candida albicans*. J PDGI [Internet]. 2016;65(3):74–7. Available from: http://jurnal.pdgi.or.id/index.php/jpdgi/article/view/147
11. Arundhina E, Soegihardjo CJ, Sidharta BBR. *Aktivitas Ekstrak Etanol Daun Alamanda (Allamanda cathartica L.) Sebagai Antijamur terhadap Candida albicans dan Pityrosporum ovale Secara In Vitro*. J Ilmu Farm. 2012;(2006):1–15.
12. Septiadi T, Pringgenies D, Radjasa OK. *Uji Fitokimia dan Aktivitas Antijamur Ekstrak Teripang Keling (Holoturia atra) Dari Pantai Bandengan Jepara Terhadap Jamur Candida albicans*. Diponegoro J Mar Res. 2013;2(2):76–84.
13. Puspitasari AD dan Prayogo LS. *Perbandingan metode ekstraksi maserasi dan sokletasi terhadap kadar flavonoid total ekstrak etanol daun kersen (Muntingiacalabura).* Jurnal Ilmu Farmasi &Farmasi Klinik. 2016;13(2):16-23.
14. Rambet LG, Waworuntu O, Gunawan PN. *Uji Konsentrasi Hambat Minimum (Khm) Perasan Murni Bawang Putih (Allium Sativum) Terhadap Pertumbuhan Candida Albicans*. Pharmacon. 2017;6(1):16–23.
15. Igboabuchi NA, Ilodibia C V, Tamuli P. *A Study on the Antioxidant and Antimicrobial Activities of Seed and Leaf Extracts of Glycine max (L) Merr*. Asian J Res Bot [Internet]. 2018;1(1):1–8. Available from: http://www.journalrepository.org/media/journals/AJRIB_113/2018/Mar/Ilodibia112018AJRIB40123..pdf
16. Abdel-Hady H, El-Wakil EA, Nasr SM. *Characterization and evaluation of antimicrobial and cytotoxic activities of glycine max methanol extract*. Int J Pharm Res. 2019;11(2):418–26.
17. Sadowska B, Budzyńska A, Wieckowska-Szakiel M, Paszkiewicz M, Stochmal A, Moniuszko-Szajwaj B, et al. New pharmacological properties of Medicago sativa and Saponaria officinalis saponin-rich fractions addressed to Candida albicans. J Med Microbiol. 2014;63(PART 8):1076–86.
18. Jiang X, Feng K, Yang X. *In vitro antifungal activity and mechanism of action of tea polyphenols and tea saponin against rhizopus stolonifer*. J Mol Microbiol Biotechnol. 2015;25(4):269–76.
19. Khafidhoh Z, Dewi SS, Iswara A. *Efektvitas infusa kulit jeruk purut (Citrus hystrix DC.) terhadap pertumbuhan Candida albicans penyebab sariawan secara in vitro*. 2nd Univ Res Coloquium 2015. 2015;2:31–7.