Majalah Kedokteran Gigi Indonesia
Vol 9 No 1 – April 2023
ISSN 2460-0164 (print), ISSN 2442-2576 (online)
Available online at https://jurnal.ugm.ac.id/mkgi
DOI: http://doi.org/10.22146/majkedgiind.77132

RESEARCH ARTICLE

An Inhibition effect of immersion in effervescent garlic ethanol extract (*Allium sativum* L.) against *Staphylococcus aureus* growth on heat cured acrylic

Dian Praba Ramadhanti*, Eka Prasasti Nur Rachmani✉, Aris Aji Kurniawan***

*Department of Dentistry, Jenderal Soedirman University, Purwokerto, Central Java, Indonesia
**Department of Pharmacy, Jenderal Soedirman University, Purwokerto, Central Java, Indonesia
*Jl Dr Soeparno, Purwokerto, Central Java, Indonesia; ✉ correspondence: eka.rachmani.unsoed@gmail.com

Submitted: 17th August 2022; Revised: 9th December 2022; Accepted: 23rd February 2023

ABSTRACT

Denture stomatitis is an infection of the mucosa caused by bacteria such as *Staphylococcus aureus* (*S. aureus*) accumulating on the denture. Garlic (*Allium sativum*) contains antibacterial compounds that can be used as an alternative denture cleanser. The purpose of this study was to determine the inhibition effect of immersion in effervescent garlic ethanol extract (*Allium sativum*) against *Staphylococcus aureus* growth on heat cured acrylic dental plate. This research was a laboratory experiment *in vitro* using 40%, 50%, and 60% effervescent garlic ethanol extract. The samples were 12 pieces (n = 12) of heat cured acrylic plates which were divided into 3 treatment groups then incubated in *S. aureus* suspension for 24 hours and then immersed in an effervescent garlic ethanol extract for 6 hours. Bacterial colonies were counted using a colony counter and the data were analyzed using the One-way ANOVA and LSD Post hoc tests. The statistical analysis showed that the number of *S. aureus* colonies decreased along with an increased concentration of garlic ethanol extract. The results of the analysis showed that the 60% effervescent garlic ethanol extract had a significantly lower mean number of colonies compared to the 40% and 50% effervescent garlic ethanol extracts ($p \leq 0.05$). This research concluded that effervescent garlic ethanol extract prevented the growth of *S. aureus* on the heat cured acrylic dental plate.

Keywords: antibacterial; denture stomatitis; effervescent; garlic extract; *Staphylococcus aureus*

INTRODUCTION

Individuals with tooth loss may experience a variety of consequences including changes in aesthetic features, jaw issues, and nerve disorders. Removable dentures have a component in the form of a base that supports the denture elements. Denture materials that are often used to date are heat cured acrylic.[1] However, the microporosity in heat cured acrylic, continuous use of dentures, and neglected denture hygiene can cause bacterial accumulation and, if left for a long time, will increase the number of bacterial and fungal colonies that will cause inflammation called denture stomatitis.[2,3]

Denture stomatitis is an inflammatory disease in the palatal mucosa of the oral cavity that is in contact with the denture base. It is characterized by a reddish hue and a slippery texture.[4] In fact, it is very easy for microorganisms to attach to the surface of the denture, allowing them to form a biofilm and plaque. The most common microbial cause of denture stomatitis is *Candida albicans*, but some bacteria also cause denture stomatitis such as *Prevotella sp, Veillonella sp, Streptococcus mutans*, and *Staphylococcus aureus*. Soft tissues continuously exposed to dental plaque will cause inflammation in the denture bearings.[5] Based on the research, among the bacterial groups, *S. aureus* was the most common bacteria found in denture wearing patients with denture stomatitis.[6]

Denture stomatitis can be prevented by performing adequate denture cleaning and denture care.[7] One of the easy ways is to use denture cleaners in the form of effervescent cleaners. Some of the advantages of using effervescent denture cleaners include being easy to use, being able to clean areas that cannot be reached by a toothbrush, being able to clean food residue and stains, and not scratching the denture.[1] The common materials

Majalah Kedokteran Gigi Indonesia. April 2023; 9(1): 67-72
ISSN 2460-0164 (print)
ISSN 2442-2576 (online)

used as commercial denture cleaners are chemicals which, in the long term, can change the physical properties of acrylic resin, making it easy for plaque and bacteria to accumulate, thus eventually causing inflammation. Therefore, the use of medical plants as denture cleaners has started to be developed.[8]

Garlic (*Allium sativum*) is one type of medicinal plant that has an antibacterial activity from its allicin content because of its enzymatic alliinase activity and has the potential to produce antimicrobial effects.[9] Garlic is a type of medicinal plant that has broad spectrum antibacterial properties so it is effective on both Gram positive and Gram negative bacteria.[10] The antibacterial effect in garlic is due to the presence of allicin and its derivatives in the form of diallyl sulfide and diallyl trisulfide which are able to overcome and regulate oxidative stress conditions by binding to or deactivating oxidizing agents, reducing cell viability in bacterial infections, and inhibiting the formation of enterotoxins in *Staphylococcus* bacteria.[11,12] In addition to allicin, garlic also contains flavonoids, tannins, and saponins which are bacteriostatic to inhibit bacterial growth. Based on this background, the researchers were interested in investigating the effect of immersion with effervescent garlic ethanol extract (*Allium sativum*) against *Staphylococcus aureus* growth inhibition on heat cured acrylic dental plate.

MATERIALS AND METHODS

This was an *in vitro* laboratory experiment with post-test only without control group design. The research was conducted at the Dental Engineering Laboratory, Faculty of Dentistry, Universitas Gadjah Mada; Pharmacy Biology Laboratory and Pharmacy Laboratory, Faculty of Health Sciences; and Microbiology Laboratory, Faculty of Medicine, Jenderal Soedirman University in April-June 2022. The study began with an ethical clearance application to the Health Research Ethics Commission (KEPK), Faculty of Medicine, Jenderal Soedirman University and ethical permit was obtained with number 015/KEPK/PE/III/2022.

The samples of this study were 12 pieces (n=12) of heat cured acrylic resin plates with a cylindrical shape with a diameter of 10 mm and thickness of 2 mm which were divided into 3 groups, namely groups immersed in 40%, 50%, and 60% effervescent garlic ethanol extracts, each of which consisted of 4 pieces. The heat cured acrylic resin plates were manufactured using a stainless-steel mold to form a master model using red wax. The bottom of the cuvette was smeared with vaseline and filled with plaster dough until it was full. The wax model was smeared with vaseline and placed on top of the plaster, then left until the plaster hardened. The top surface of the plaster and wax model was smeared with vaseline, then the top cuvette was installed until metal-to-metal contact was achieved. The plaster dough was poured into the cuvette and sealed with a cap, then left until the plaster hardened. The wax removal was carried out by placing the cuvette in boiling water for 5 minutes. The cuvette that had been lifted was opened and the mold space was cleaned by pouring hot water. The entire surface of the plaster was smeared with cold mold seal (CMS) and left to dry. The heat cured acrylic resin dough was made using a polymer:monomer ratio of 3:1 and stirred in a mixing jar and then sealed until it entered the dough stage. The acrylic resin dough was placed in the mold space until it was completely filled and given cellophane plastic. The cuvette was pressed with a cuvette press; any excess acrylic resin was removed by opening the cuvette. The cuvette was pressed again until there was no residual acrylic resin and sealed until metal-to-metal contact was achieved. Curing was done by immersing the cuvette in 15 liters of boiling water at 100 °C for 30 minutes, then allowing it cool at a room temperature.[7] The sample was removed from the cuvette, followed by finishing and polishing until the surface was smooth and shiny.

The garlic ethanol extract was prepared using the maceration method in which the simplicia powder was immersed in a 70% ethanol solution. A total of 1000-gram garlic simplicia was used with ratio of 1:5 simplicia to solvent. The garlic powder was soaked in 70% ethanol solution then stirred occasionally for 30 minutes and left for 24 hours in a closed place. The solution was then filtered and remaceration was carried out for 3x24

hours. The collected filtrate liquid was evaporated using a rotary evaporator at a temperature of 50 °C, then the substrate was put into a water bath at a temperature of 70 °C so the ethanol could evaporate and a thick extract was obtained.[4,13]

The effervescent granules of the garlic ethanol extract were made by forming acid granules, garlic ethanol extract granules, and base granules. The garlic ethanol extract was mixed with dextrin and stirred until homogeneous to form granules. The acid granules were made by mixing acidic substances in the form of tartaric acid, citric acid, starch, partial PVP, and the garlic ethanol extract granules. The mixed ingredients were stirred until homogeneous to form acid granules. The base granules were made by mixing the remaining base substances in the form of PVP with sodium bicarbonate and starch. These ingredients were mixed until homogeneous and not sticky, then sieved using a 14-mesh sieve to obtain the same granule size. The effervescent granules were dried in an oven at 60 °C for 2 hours.[14] The effervescent granules were stored in a dry and tightly closed place.

Staphylococcus aureus bacteria were obtained from the Microbiology Laboratory, Faculty of Medicine, Jenderal Soedirman University. These bacteria were taken using 1 sterile tube and then inoculated in 10 ml BHI-B media until the turbidity was reached according to the McFarland Standards 0.5 (1.5 x 10^8 CFU/ml). The *Staphylococcus aureus* bacterial suspension was then incubated for 24 hours at 37 °C.[15,16]

The acrylic resin plate was immersed in distilled water for 24 hours to reduce the residual monomer. The acrylic resin plate was sterilized using an autoclave at 121 °C and then immersed in BHI-B for 1 hour. The acrylic resin plate was inserted into 10 ml of *Staphylococcus aureus* suspension using sterile tweezers, then incubated for 24 hours at 37 °C and the bacteria were cultured to determine *S. aureus* attached to the acrylic resin plate. The acrylic resin plate was treated by immersing it for 6 hours in 200 ml effervescent garlic ethanol extract solution at a concentration of 40%, 50%, and 60%.[17] The acrylic resin plate was rinsed for 15 seconds using phosphate buffer saline, then put in 10 ml of 0.9% NaCl with a dilution to 10^{-3} and vibrated using a vortex for 30 seconds so *S. aureus* could be released from the acrylic resin plate. A total of 0.1ml *S. aureus* suspension was taken using a micropipette, then dripped on Mannitol Salt Agar (MSA) and distributed using drigalski-spatel. The MSA medium was incubated for 24 hours at 37 °C. Colony calculations were carried out using a colony counter.[18]

The data analysis was done using the Statistical Package for The Social Sciences (SPSS) software. The normality of the data was tested using the Shapiro Wilk test and the homogeneity was tested using the Levene's test. The results were then analyzed using One-Way ANOVA and followed by the Least Significant Difference (LSD) post hoc test.

RESULTS

The results showed different average number of *Staphylococcus aureus* colonies in each group. The average number of *S. aureus* colonies decreased with an increase in the garlic ethanol extract concentration as shown in Figure 1. The results of the mean number of *S. aureus* colonies in each group can be seen in Table 1.

The results showed that the highest average number of *Staphylococcus aureus* colonies was in the P1 group, namely 1.3 x 10^5 CFU/ml. The lowest average number of *S. aureus* colonies was in the P3 group, namely 7.0 x 10^4 CFU/ml.

The results of the hypothesis testing with the One Way ANOVA showed sig. of 0.000 ($p \leq 0.05$), so the mean number of *S. aureus* colonies had a significant difference in which at least two groups had a significant difference. The next test was the LSD post hoc test and the results are in Table 2.

The results of the LSD post hoc test showed that groups P1, P2, and P3 had a significant difference in the mean number of *S. aureus* colonies because $p \leq 0.05$. The difference in the mean number of *S. aureus* colonies could mean that the P1, P2, and P3 groups had different inhibition effects against the growth of *S. aureus* in which the percentages of inhibition were 99.91%,

Majalah Kedokteran Gigi Indonesia. April 2023; 9(1): 67-72
ISSN 2460-0164 (print)
ISSN 2442-2576 (online)

Table 1. *One way ANOVA* results and average colonies *S. aureus*

No	Groups	N	Mean ± SD (CFU/ml)	Sig.
1	P1	4	$1.3 \times 10^5 \pm 1.3 \times 10^4$	
2	P2	4	$1.0 \times 10^5 \pm 1.5 \times 10^4$	0.000*
3	P3	4	$7.0 \times 10^4 \pm 1.9 \times 10^4$	

N = total sample
*p ≤ 0.05 = significant different

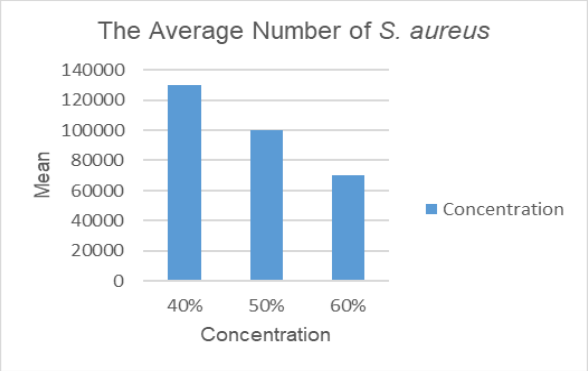

Figure 1. The average number of *S. aureus*

Table 2. Post hoc LSD Results

Groups	P1	P2	P3
P1		0.025*	0.000*
P2			0.012*
P3			

*p≤0.05= significant different

99.93%, and 99.95%, respectively of the initial bacterial count of 1.5×10^8 CFU/ml.

DISCUSSION

The results showed that groups P1, P2, and P3 had a decreasing average number of *S. aureus* colonies with an increasing concentration of the effervescent garlic ethanol extract. After treated with the effervescent garlic ethanol extract, the lowest number of colonies was in group P3 (immersion in the 60% effervescent garlic ethanol extract), while the highest number of colonies was in group P1 (immersion in the 40% effervescent garlic ethanol extract). These results indicated that the effervescent garlic ethanol extract had an inhibition effect against the growth of *S. aureus* on the heat cured acrylic resin denture plate as the concentration of the garlic ethanol extract increased. The average number of *S. aureus* colonies decreased significantly in the P3 group compared to the P1 and P2 groups. This is because the P3 group was immersed in the 60% extract so the antibacterial content in the extract was higher than that of the 40% and 50% effervescent garlic ethanol extracts. The antibacterial activity of an extract will increase as the concentration of the extract increases. Garlic extract contains antibacterial compounds, so the ethanol extract of garlic can reduce the growth of *S. aureus* bacteria.

The results of this study are line with the results of previous studies by Salim and Soleha which showed that the diameter of the inhibition zone of *Staphylococcus aureus* bacteria by garlic ethanol extract at a concentration of 20% was in the weak category (11.05 mm), that at 40% was in the moderate category (19.17 mm), which increased at 60% (32.45 mm), and the highest at 80% (36.12 mm) in the strong category.[19] Fahmi et al stated that the diameter of the inhibition zone of *Staphylococcus aureus* bacteria with garlic ethanol extract will increase at a concentration of 250 g/ml, i.e., 6.5 mm; 500 g/ml, i.e., 13.3 mm; 750 g/ml, i.e., 15 mm; and 1000 g/ml, i.e., 17.8 mm; all of them fell in the moderate category.[20]

Garlic contains allicin, flavonoid, tannin, and saponin compounds which have antibacterial activity as bacteriostatic. The ethanol extract of garlic contains organosulfur compounds that are antimicrobial, i.e., allicin and ajoene which are able to inhibit DNA and RNA synthesis, denature proteins, and damage bacterial cell membranes by dissolving fat in cell walls. Damage in the cell membrane can inhibit the activity and synthesis of enzymes in the metabolic process, so the growth and development of bacteria do not occur.[21] The flavonoid and tannin compounds in garlic extract function in inhibiting bacterial growth by changing the protein structure and nucleic acid synthesis, damaging the cytoplasmic membrane in bacterial cells, and changing the permeability of the cell membrane. Denatured protein will cause the cell

wall to become unstable, thus disrupting its function and causes bacterial cell lysis.[4]

According to Baron et al in Widiastiti, an antibacterial can be declared bactericidal if the minimum mortality of the test bacteria is 99.99% and can be declared bacteriostatic if it is less than 99.99%.[22] Based on the results of this study, the effervescent garlic ethanol extracts at concentrations of 40%, 50%, and 60% could be declared bacteriostatic because they were able to reduce the growth of *S. aureus* by 99.91%; 99.93%; and 99.95% after immersion for 6 hours.

The duration of immersing acrylic resin plates in denture cleaners can also affect the inhibition of microorganism colony growth. Immersion time is categorized into long term (6-8 hours at night) and short term (15-45 minutes after eating). This study used a minimum long-term immersion time of 6 hours.[23] Research by Miftahullaila et al found that after the immersion of acrylic resin plates for 6 and 8 hours, there was a significant difference in the number of microorganism colonies, meaning that the longer the immersion time, the better the results.[17]

This study has a limitation, namely the temperature and humidity during the storage of the effervescent garlic ethanol extract granules could not be controlled, so it might affect the quality of the effervescent granules. The quality of the effervescent granules could be maintained by adjusting the temperature and humidity at low conditions so there is no damage to the effervescent granules during storage. In fact, the good effect of drug preparations might decline significantly due to improper storage.[24] Based on the results of the research, it is important to conduct further research on the inhibition effect of effervescent garlic ethanol extract against *Staphylococcus aureus* growth with a longer variation of immersion time.

CONCLUSION

Based on the results of the study, it can be concluded that effervescent garlic ethanol extract (*Allium sativum*) can inhibit the growth of *Staphylococcus aureus* on heat cured acrylic dental plate. The effectiveness of the effervescent garlic ethanol extract at a concentration of 60% in *S. aureus* growth inhibition on the acrylic resin plate was better than the effervescent garlic ethanol extract at 40% and 50% concentrations.

CONFLICT OF INTEREST

The authors declare no conflict of interest with the data contained in the manuscript.

REFERENCES

1. Alveno A, Ashrin MN, Damaiyanti DW. Pengaruh effervescent ekstrak kulit nanas dalam menghambat pertumbuhan Candida albicans pada resin akrilik heat cured. Dent J Kedokt Gigi. 2016; 10(2): 135–141.
2. Kaomongkolkit R, Wongviriya A, Daroonpan P, Chansamat R, Tantanapornkul W, Palasuk J. Denture stomatitis and its predisposing factors in denture wearers. JIDMR. 2017; 10(1): 89-94.
3. Machmud E. Effectiveness of roselle effervescent tablets as traditional medicinal plants in preventing growth of candida albicans colonies and Streptococcus mutans. J Contemp Dent Pract. 2018; 19(8): 925–928. doi: 10.5005/jp-journals-10024-2358.
4. Gosal L, Hutomo S, Sooai CM. Kemampuan ekstrak etanol bawang putih (Allium sativum L.) dalam menghambat perlekatan bakteri Pseudomonas aeruginosa. J Med Health. 2021; 3(1): 1–8. doi: 10.28932/jmh.v3i1.3143
5. Hannah VE, O'Donnell L, Robertson D, Ramage G. Denture stomatitis: causes, cures and prevention. Prim Dent J. 2017; 6(4): 46–51. doi: 10.1308/205016817822230175
6. Rodriguez-Archilla A, Garcia-Galan C. Etiological factors related to denture stomatitis: A meta-analysis. Dent Med Res. 2020; 8(2): 37. doi: 10.4103/dmr.dmr_26_20.
7. Izzah R, Kf IWA, Sukmana BI. Pengaruh perendaman ekstrak daun kemangi 12, 5% dan batang pisang mauli 25% terhadap kekerasan permukaan resin akrilik. Dentin J Kedokt Gigi. 2019; 3(3): 68–74.
8. Rahayu I, Fadriyanti O, Edrizal. Efektivitas pembersih gigi tiruan dengan rebusan daun sirih

25% dan 50% terhadap pertumbuhan Candida albicans pada lempeng resin akrilik polimerisasi panas. J B-Dent. 2014; 1(2): 142–149.
9. Ali DQ, Saputera D, Budiarti LY. Perbandingan daya hambat ekstrak bawang putih dengan sodium hipoklorit terhadap Streptococcus mutans pada plat akrilik. Dentino J Kedokt Gigi. 2017; 1(1): 16–21.
10. Fufa BK. Anti-bacterial and anti-fungal properties of garlic extract (Allium sativum): a review. Microbiol Res J Int. 2019; 28(3): 1–5. doi: 10.9734/MRJI/2019/v28i330133.
11. Mnayer D, Fabiano-Tixier AS, Petitcolas E, Hamieh T, Nehme N, Ferrant C, Fernandez X, Chemat F. Chemical composition, antibacterial and antioxidant activities of six essentials oils from the Alliaceae family. Molecules. 2014; 19(12): 20034–20053. doi: 10.3390/molecules191220034.
12. Shang A, Cao SY, Xu XY, Gan RY, Tang GY, Corke H, Mavumengwana V, Li HB. Bioactive compounds and biological functions of garlic (Allium sativum L.). Foods. 2019; 8(7): 1–31. doi: 10.3390/foods8070246.
13. Gull I, Saeed M, Shaukat H, Aslam SM, Samra ZQ, Athar AM. Inhibitory effect of Allium sativum and Zingiber officinale extracts on clinically important drug resistant pathogenic bacteria. Ann Clin Microbiol Antimicrob. 2012; 11(8): 1–6.
14. Asiani TW, Sulaeman TNS, Kurniawan DW. Formulasi tablet efervesen dari ekstrak etanol kelopak bunga rosella (Hibiscus sabdariffa L .). Pembangunan. 2012; 1–9.
15. Arniati, Haris A, Werorilangi S. Uji antibakteri patogen ekstrak sponge menggunakan metode high throughput screening (HTS) dengan indikator MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). Pros Simp Nas Kelaut dan Perikan II Univ Hasanuddin. 2015; 144–149. Available from: https://www.researchgate.net/publication/269107473_What_is_governance/link/548173090cf22525dcb61443/download%0Ahttp://www.econ.upf.edu/~reynal/Civil wars_12December2010.pdf%0Ahttps://think-asia.org/handle/11540/8282%0Ahttps://www.jstor.org/stable/41857625. ISBN: 978-602-71759-1-4.
16. Wikananda IDARN, Hendrayana MA, Pinatih KJP. Efek antibakteri ekstrak ethanol kulit batang tanaman cempaka kuning (M. champaca L.) terhadap pertumbuhan Staphylococcus aureus. E-Jurnal Med. 2019; 8(5).
17. Miftahullaila M, Sinamo S, Natasya C, Nurul N, Griselda J. Pengaruh waktu perendaman plat resin akrilik dalam perasan murni bawang putih terhadap jumlah koloni Candida albicans. J Kedokt dan Kesehat Publ Ilm Fak Kedokt Univ Sriwij. 2020; 7(3): 25–30. doi: 10.32539/JKK.V713.11483.
18. Ayu ZP, Pintadi S. Daya antibakteri ekstrak jintan hitam dan daun sirih terhadap Staphylococcus aureus pada plat gigi tiruan. Insisiva Dent J. 2020; 9(1): 19–25. doi: 10.18196/di.9113.
19. Salim, Hari Hardana Utama dan Soleha TU. Pengaruh Aktivitas Antimikroba Ekstrak Bawang Putih (Allium sativum) Terhadap Bakteri Gram Positif (Staphylococcus aureus) dan Gram Negatif (Escherichia coli) Secara In Vitro. Fak Kedokt Univ Lampung. 2017; 7: 66–70.
20. Fahmi YI, Andriana A, Hidayati DS. Uji daya hambat ekstrak bawang putih (Allium sativum) terhadap bakteri (Staphylococcus aureus). J Kedokt. 2019; 4(2): 82.
21. Pujoharjo P, Herdiyati Y. Efektivitas antibakteri tanaman herbal terhadap Streptococcus mutans pada karies anak. J Indones Dent Assoc. 2018;1(1):51–6.
22. Widiastiti M, Putra IWWP, Duniaji AS, Darmayanti LP. Analisis potensi beberapa larutan pengencer pada uji antibakteri teh temu putih (Curcuma zedoaria (Berg) Roscoe) terhadap Escherichia coli. Media Ilm Teknol Pangan. 2019; 6(2): 117–25.
23. Winardhi A, Saputra D, Dewipuspitasari. Perbandingan nilai kekasaran permukaan resin termoplastik poliamida yang direndam larutan sodium hipoklorit dan alkalin peroksida. Dentin J Kedokt Gigi. 2017; 1(1): 45–49.
24. Kurniawan RA, Jiwintarum Y, Dwihartati L. Pengaruh perbedaan suhu terhadap potensi tablet siproflaksasin (Eksperimen dengan bakteri Escherichia coli). 2015; 9(2): 1525–1533.