Research Report

Antifungal Activity from Garlic Extract (*Allium sativum*) Against *Candida albicans* Growth

Titien Hary Agustantina, R. Helal Soekartono

Department of Dental Material, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

Background: The accumulation of food waste and plaque causes Candida albicans development, which causes the product of Candida albicans' endotoxin to rise and enter into the mucous membrane, producing inflammation known as denture stomatitis. Many natural ingredients have been transformed into medications, including Allium sativum (garlic), which includes antibacterial allicin components. **Purpose:** The purpose of this study was to investigate inhibitory activity of A. sativum extract toward C. albicans growth. **Methods:** The sample used in this investigation was A. sativum extract at various concentrations of 5%, 25%, 50%, and 75%. The sample was separated into five groups: 5%, 25%, 50%, and 75% A. sativum extract, with plain A. sativum extract serving as the control group. Each group has three specimens. One way analysis of variance (ANOVA) and Tukey honest significant different (HSD) were used to evaluate the data (p<0.05). **Results:** There was significant difference of 5%, 25%, 50%, and 75% A. sativum extract towards C. albicans growth. **Conclusion:** The highest antifungal activity on Candida albicans growth was found in 75% Allium sativum extract.

Keywords: Allium Sativum; Candida albicans; Denture Stomatitis; Dentistry; Medicine

Correspondence: Titien Hary Agustantina, Department of Dental Material, Faculty of Dental Medicine, Universitas Airlangga. Jl. Mayjen Prof. Dr. Moestopo No. 47 Surabaya, 60132 Indonesia. Email: titien-h-a@fkg.unair.ac.id

INTRODUCTION

Dentures play a significant role in rehabilitating the masticatory function and oral health of patients suffering from tooth loss. Fixed dentures and removable dentures are two types of dentures. The benefits of removable dentures are that they are less expensive and simpler to maintain since they are easily removed and placed. Heat cure acrylic resins are one of the materials that may be utilized to produce removable dentures. Heat cure acrylic resins in removable dentures for the oral cavity have the advantages of being easy to produce, having stability in colours, proportions, and aesthetic characteristics. Maintaining the cleanliness of removable dentures is critical.¹ Plaque, stains, and food debris must be removed from dentures. Removable dentures that are cleaned on a regular basis will keep the surfaces of the dentures smooth, giving them more durability against food debris or microorganisms.²

Removable dentures that are not cleaned on a regular basis might be detrimental to one's dental health. Food debris will build and create plaque under these settings. This plaque will promote the growth of microorganisms, one of which being *Candida albicans*.³ *C. albicans* has the capacity to cling to the surfaces of acrylic resin detachable dentures.⁴ *C. albicans*, a pathogen microbe, will emit endotoxin, causing inflammation in the mouth cavity known as dentures stomatitis.⁵ Using a denture cleaner is one of the techniques advised for cleaning removable dentures; however, the material of the denture cleanser must be chosen carefully. Corrosion in dentures containing metal components is a concern since some of them can damage the colors of the denture bases.^{1,2}

Garlic (*A. Sativum*) is a medicinal plant that is frequently utilized for health purposes. Garlic has been shown in studies to be anticancer, antibacterial, antiviral, and antifungal. Garlic has antifungal qualities because it contains allicin sulfur (diallylthiosulfurnate).⁶ This sulfur component enables the use of alternate denture cleaning materials. One study using garlic juice as a denture cleaning found antifungal effectiveness against Candida albicans.⁷ Garlic juice had antifungal efficacy against Candida albicans in her studies. The concentration of garlic juice in their study was 25%, 50%, and 75%, indicating a mean inhibitory zone diameter in a row of 1.61 mm, 3.16 mm, and 13.57 mm.⁸ The quantity of inhibitory zone development of microorganism, indicating the magnitude of antimicroorganism from a compound.⁹

Based on this context, the purpose of this study is to discover alternate methods to garlic juice for obtaining allicin sulfur by employing garlic extract. This method may make it easier to use garlic as an antifungal. As a result, study into the antifungal potential of garlic extract against Candida albicans development is required. This study employed garlic extract with concentrations of 5%, 25%, 50%, and 75%. The purpose of this study was to investigate inhibitory activity of A. sativum extract toward *C. albicans* growth.

MATERIALS AND METHODS

Garlic extract (UPT Materia Medica, Batu), *C. albicans*, Sabouraud Broth, carboxy methyl cellulose natrium (CMC-Na), Nutrient Agar, and Sterilized Aquadest, petri dish, spreader, ose, anaerob jar, mortar and pestle, beaker glass, reaction test tube, incubator, and Vernier caliper were utilized in this study. The method of producing basic gelatin CMC-Na begin by generating a CMC-Na 3 percent suspension in 100ml using the following method: sterilized aquadest 60ml (at room temperature 27°C) put in beaker glass, then poured in mortar and gradually add CMC-Na 3g powder on the water surfaces. CMC-Na powder was left for 10-15 minutes until dispersed and a soft gelatin clear mass was obtained. CMC-Na gel was mixed with a pestle until homogenous, then water was progressively added until 40ml was reached.

Garlic gel is created by combining garlic extract with a base of CMC-Na 3 percent gel in a ratio determined by the organization. Group 1 (control): no garlic gel, Group 2: garlic gel with 5% concentrate (garlic extract 0.5ml with CMC-Na 3% gel 9.5 ml), Group 3: garlic gel 25% (garlic extract 0.5ml with CMC-Na 3% gel 7.5 ml), Group 4: garlic gel 50% (garlic extract 5 ml with CMC-Na 3% gel 5 ml), Group 5: garlic gel 75% (garlic extract 7.5 ml with CMC-Na 3 percent gel 2.5 ml).

The technique of generating *C. albicans* suspension involves taking a colony of *C. albicans* from the laboratory with sterilized ose and placing it in a response test tube containing Sabouraud Broth 0.5ml medium. Then, place this response test tube in an incubator at 37°C for 48 hours, or until *C. albicans* suspension is obtained. This suspension was diluted with sterilized aquadest until it reached the Mc Farland standard of 10^8 CFU/ml. *C. albicans* inhibition test utilizing dilution technique. The *C. albicans* suspension was swabbed and dispersed in the petri dish on nutritional agar. Make a well out of nutritional agar, then place garlic gel in each group. Each group's petri dish was placed in an incubator at 37°C for 48 hours. The potensial of inhibitory growth was assessed by measuring the inhibition zone using a Vernier caliper while gazing at the transparent surface of nutrient agar. The statistical analysis of study result was done by mean of one way analysis of variance (anova); if there is a significant difference (p<0.05), continued with post-hoc tukey Honestly Significant Difference (HSD).

RESULTS

The diameter inhibitory zone of C. albicans growth showed in Table 1. The formation of an inhibition zone demonstrates that garlic extract has inhibitory potential as an antifungal against C. albicans. Except for group 5 and the control group, each treatment group had a C. albicans growth inhibition zone (Figure 1). The greater the quantity of garlic extract, the larger the width of the C. albicans inhibitory growth zone. The Kolgomorov-Smirnov test results demonstrate that this research data is normally distributed, and the Levene test results show that this research data is homogeneous (p>0.05). The p value (p<0.05) result of the one-way anova test indicates that there are significant variations in the inhibitory zone of garlic extract against C. albicans. The investigation of the significance of the difference between each treatment group, the posthoc Tukey HSD test was applied, as shown in Table 2.

 Table 1.
 Means (mm) and standard deviation of garlic extract against *Candida albicans* growth.

Groups	n	Means	Standard Deviation
1	3	0.00000	0.000000
2	3	7.06433	0.005508
3	3	9.03667	0.004041
4	3	12.03367	0.005033
5	3	17.29967	0.001528



Figure 1. The potential inhibition garlic extract result with difference concentration against *Candida albicans* growth. A. 5%, 25% and 50%. B. 70% and control group.

Indonesian Journal of Dental Medicine Volume 4 Issue 2 2021; 60-62

	Group 1	Group 2	Group 3	Group 4	Group 5
Group 1	-	0.0001	0.0001	0.0001	0.0001
Group 2	0.0001	-	0.0001	0.0001	0.0001
Group 3	0.0001	0.0001	-	0.0001	0.0001
Group 4	0.0001	0.0001	0.0001	-	0.0001
Group 5	0.0001	0.0001	0.0001	0.0001	-

 Table 2.
 Result of data analyze using Post-hoc Tukey HSD.

The Post-Hoc Tukey HSD result demonstrates that there was a significant difference between all of the treatment groups (p<0.05). This result indicates that there is a substantial variation in the inhibition zone of garlic extract against *C. albicans* growth between each treatment group.

DISCUSSION

The purpose of this study is to investigate whether garlic extract has the potentiality to inhibit C. albicans growth. The highest size inhibition zone of garlic extract against C. albicans growth was in group 5, i.e. 17.29967 mm, whereas the smallest size inhibition zone of garlic extract against C. albicans growth was in group 2, i.e. 7.06433 mm. The outcome of data analysis with Post-Hoc Tukey HSD Shows that group 5 with a concentration of garlic extract of 75% had the largest potential inhibition compared to the other groups with concentrations of 50%, 25%, and 5%. This finding was consistent with previous study that stated the higher the garlic content, the stronger the prevention of C. albicans growth. Because of its potential as an antifungal found in garlic, allicin (diallylthiosulfnate) inhibits C. albicans growth. Allicin is synthesized from the amino acid nonprotein alliin (S-allylcysteine sulfoxide), which is catalyzed by the alliinase enzyme. Allicin is a bioactive molecule and one of the primary components of garlic that acts as an antifungal.6

Allicin acts as an antifungal by causing physical damage to the fungal cell wall, particularly *C. albicans.*¹⁰ Allicin damages *C. albicans*' cell walls by inhibiting lipid production. Lipids are a component of the cell wall of *C. albicans*. The permeability of the fungal cell wall membrane increases under situations of restricted lipid synthesis.⁸ When the wall of *C. albicans* is broken, allicin enters the membrane cell and mitochondria, inhibiting cell development and possibly leading to cell death. Allicin has the ability to alter the structure of *C. albicans* proteins, resulting in cell function loss.¹¹

According to the findings of this study, garlic extract with a concentration of 75% has the highest average inhibitory zone of 17.29967 mm, whereas garlic extract with a concentration of 50% produced an average inhibitory zone of 12.03367 mm. The average size of these two garlic extract concentrations is classified as having a high inhibitory potential. The findings of this study also revealed that the higher the concentration of garlic extract, the bigger the inhibition zone of *C. albicans* growth.

ACKNOWLEDGEMENT

The authors would like to thank Faculty of Dental Medicine, Universitas Airlangga, Surabaya for the supporting this study.

REFERENCES

- Shen C, Rawls HR, Esquivel-Upshaw J. Phillips' Science of Dental Materials. 13th ed. St. Louis: Missouri: Elsevier; 2013. 593, 633, 877p.
- Eakle WS, Hatrick CD. Dental Materials: Clinical Applications for Dental Assistants and Dental Hygienists. 3rd ed. St. Louis: Missouri: Elsevier; 2016. 634–5p.
- Wirayuni KA, Nugrahini S. Jumlah Koloni Candida Albicans Pada Plat Resin Akrilik Heat Cured Setelah Dilakukan Perendaman Ekstrak Daun Kemangi (Ocimum Bassilicum Linn) 50%. Interdental J Kedokt Gigi. 2020 Dec 10;16(2):61–3.
- Nannochloropsis DE, Terhadap O, Kepadatan P, Pada K, Mandibula O, Azmi F, et al. Efektifitas Perbandingan Kombinasi Clindamycin. Dent J Kedokt Gigi [Internet]. 2015 Feb 1 [cited 2020 Jul 20];9(1):64–73. Available from: http://journal-denta.hangtuah.ac.id/index.php/denta/article/ view/17
- Ammar Y, Swailes D, Bridgens B, Chen J. Influence of surface roughness on the initial formation of biofilm. Surf Coatings Technol. 2015 Dec;284:410–6.
- Leontiev R, Hohaus N, Jacob C, Gruhlke MCH, Slusarenko AJ. A Comparison of the Antibacterial and Antifungal Activities of Thiosulfinate Analogues of Allicin. Sci Rep. 2018;8(1):6763.
- 7. Nirwana I, Agustantina TH, Asymal A. Antifungal Activity of Freshly Squeezed Garlic as Denture Cleanser on Candida Albicans Growth. J Int Dent Med Res. 2018;11(2):639–42.
- 8. Mahmudah RB, Pribadi P, Agusta HF. Uji Aktivitas Antifungi Perasan Bawang Putih (Allium sativum L.) Terhadap Candida albicans. J Farm Sain dan Prakt. 2017;3(1).
- Oroh SB, Kandou FEF, Pelealu J, Pandiangan D. Uji Daya Hambat Ekstrak Metanol Selaginella delicatula dan Diplazium dilatatum Terhadap Bakteri Staphylococcus aureus dan Escherichia coli. J Ilm SAINS. 2015 Feb 10;15(1):52–8.
- Burian JP, Sacramento LVS, Carlos IZ. Fungal infection control by garlic extracts (Allium sativum L.) and modulation of peritoneal macrophages activity in murine model of sporotrichosis. Braz J Biol. 2017 Nov;77(4):848–55.
- Natasya C, Miftahullaila M, Sinamo S, 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 Oct 5;7(3):25–30.