

Carbonated Hydroxyapatite Containing Propolis as an Antibacterial Agent Candidate against *Aggregatibacter actinomycetemcomitans*

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ABSTRACT

One of the periodontal pathogenic bacteria that can cause periodontitis and alveolar bone destruction is *Aggregatibacter actinomycetemcomitans*. An alveolar bone defect can be treated using a bone graft. Carbonated hydroxyapatite (CHA) is an alloplastic graft material. Alloplastic materials do not have vascularization, which will increase the risk of bacterial adhesion. Therefore, adding an antibacterial agent is needed to prevent bacterial adhesion, which will improve periodontal healing. Propolis is a natural ingredient that has broad-spectrum antibacterial activity and does not cause bacterial resistance. This study aimed to assess the antibacterial activity of carbonated hydroxyapatite after being incorporated with propolis against *Aggregatibacter actinomycetemcomitans*. Carbonated hydroxyapatite was embedded into four different concentrations of propolis solution (2.5%, 5%, 7.5%, and 10%). An antimicrobial assay against *Aggregatibacter actinomycetemcomitans* was done using the disc diffusion test method. The inhibition zone was measured to determine the antibacterial ability of the specimens. The inhibition zone was found on the carbonated hydroxyapatite incorporated with propolis at all concentrations. Carbonated hydroxyapatite incorporated with 10% propolis showed the largest inhibition zone. Data analysis using the Kruskal-Wallis test showed a significant difference between the groups tested ($P < .05$). In conclusion, carbonated hydroxyapatite incorporated with propolis has antibacterial activity against *Aggregatibacter actinomycetemcomitans*

Keywords: *Aggregatibacter actinomycetemcomitans*; Carbonated hydroxyapatite; Propolis

INTRODUCTION

One of the periodontal pathogenic bacteria that can cause periodontitis and alveolar bone destruction is *Aggregatibacter actinomycetemcomitans*. An alveolar bone defect can be treated using a bone graft. Carbonated hydroxyapatite (CHA) is an alloplastic graft material. Alloplastic materials do not have vascularization, which will increase the risk of bacterial adhesion. Therefore, adding an antibacterial agent is needed to prevent bacterial adhesion, which will improve periodontal healing. Propolis is a natural ingredient that has broad-spectrum antibacterial activity and does not cause bacterial resistance. This study aimed to assess the antibacterial activity of carbonated hydroxyapatite after being incorporated with propolis against *Aggregatibacter actinomycetemcomitans*. Carbonated hydroxyapatite was embedded into four different concentrations of propolis solution (2.5%, 5%, 7.5%, and 10%). An antimicrobial assay against *Aggregatibacter actinomycetemcomitans* was done using the disc diffusion test method.

The inhibition zone was measured to determine the antibacterial ability of the specimens. The inhibition zone was found on the carbonated hydroxyapatite incorporated with propolis at all concentrations. Carbonated hydroxyapatite incorporated with 10% propolis showed the largest inhibition zone. Data analysis using the Kruskal-Wallis test showed a significant difference between the groups tested ($P < .05$). In conclusion, carbonated hydroxyapatite incorporated with propolis has antibacterial activity against *Aggregatibacter actinomycetemcomitans*.

METHODOLOGY

Materials

Preparation of carbonated hydroxyapatite incorporated with propolis

The carbonated hydroxyapatite used in this study was 10 mg Gama-CHA (Yogyakarta, Indonesia). The propolis solution was made using pure propolis (Propolis Brazilian® - Minas Gerais, Brazil) and sterile aquabidest, using the gradual dilution technique. The dilution was carried out until the propolis concentrations of 2.5%, 5%, 7.5%, and 10% were obtained. The carbonated hydroxyapatite was then embedded into each

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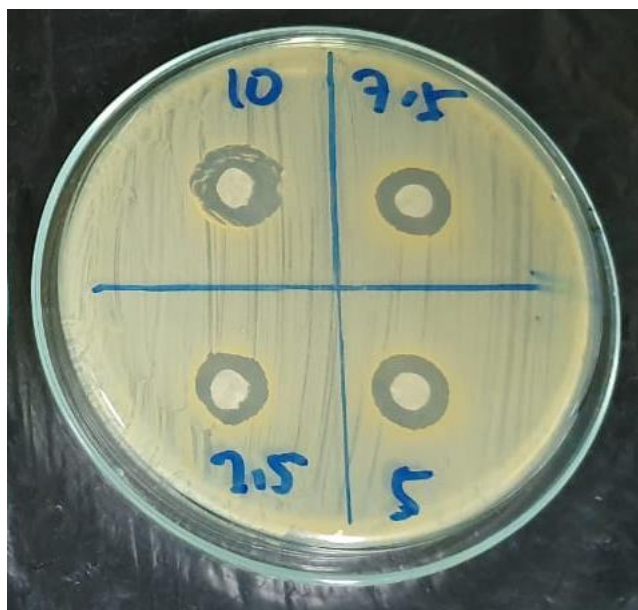


Figure 1. Antimicrobial assay of carbonated hydroxyapatite incorporated with propolis at different concentrations against *A. actinomycetemcomitans*.

concentration of propolis solution for 24 h at room temperature. After 24 h, the specimens were then put into an incubator for another 24 h. All specimens were then sterilized using ethylene oxide.

Methods

Antimicrobial assay

Aggregatibacter actinomycetemcomitans (ATCC 43718) were obtained from the laboratory of microbiology Dentistry Faculty Universitas Airlangga, Surabaya. The bacteria were cultured in brain heart infusion broth media and then incubated for 24 h. The bacterial suspension turbidity was compared with the 0.5 McFarland standard, which equals 1.5×10^8 colony forming units (CFU mL⁻¹). A petri dish was prepared using nutrient agar. Then the surface was inoculated with bacteria using a sterile swab.

The antimicrobial assay was done using the disc diffusion test and replicated five times. Specimens were put on the agar surface, and the inhibition zone was measured using a caliper after 24 h. The carbonated hydroxyapatite that was not embedded into the propolis solution was used as a control.

Statistical analysis

The data obtained from the antimicrobial assay were analyzed with the Kruskal–Wallis test

and continued with the least significant difference (LSD) posthoc test. The significance value was set at $P < .05$.

RESULTS AND DISCUSSION

There are several methods to test the antimicrobial ability of a material, such as the broth dilution test, antimicrobial gradient method, disc diffusion test, and automated instrument systems (Jorgensen & Ferraro, 2009). The antimicrobial assay used in this study was the disc diffusion test. An antimicrobial assay for carbonated hydroxyapatite incorporated with propolis against *A. actinomycetemcomitans* was done *in vitro*.

Figure 1 shows the inhibition zone on carbonated hydroxyapatite incorporated with four different concentrations of propolis. The mean diameter of the inhibition zone at a concentration of 10%, 7.5%, 5%, and 2.5% are 12.7 mm, 10.58 mm, 8.9 mm, and 8 mm, respectively.

The carbonated hydroxyapatite specimen that was not embedded in propolis had no inhibition zone, as seen in Figure 2. The mean value and standard deviation of each inhibition zone are shown in Table I. The diagram for the mean value of the inhibition zone in all tested groups is shown in Figure 3.

The statistical analysis using the Kruskal–Wallis test showed a significant difference ($P < .05$)

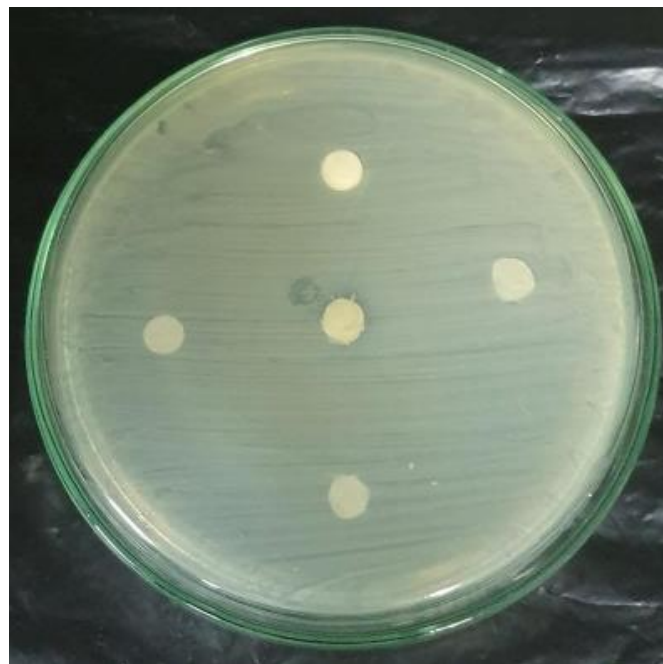


Figure II. Antimicrobial assay of carbonated hydroxyapatite against *A. actinomycetemcomitans*.

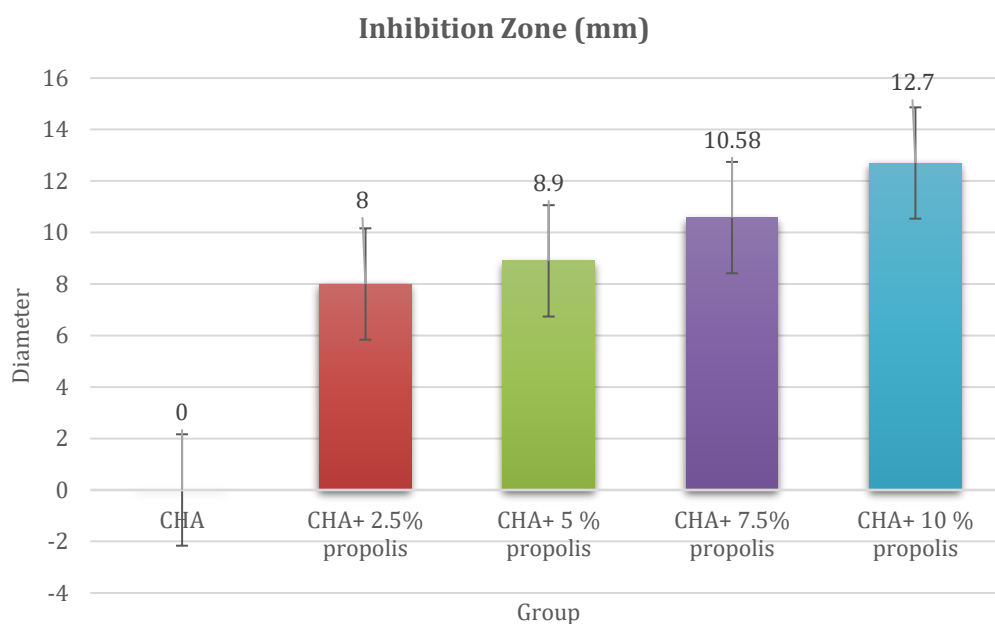


Figure 3. The mean value of the inhibition zone in all groups.

between all tested groups. A LSD posthoc test showed that there was a significant mean difference from each of the tested groups.

The data showed that carbonated hydroxyapatite incorporated with 10% propolis had the highest antibacterial ability. The diameter of the inhibition zone showed that there was a positive correlation between the concentration of

propolis used in the incorporation specimen and its antibacterial activity.

As a natural ingredient, propolis is proven to have antimicrobial ability without causing bacterial resistance or eliminating oral microflora (Ghasemi *et al.*, 2017). The antimicrobial property of propolis is mainly contributed by its flavonoid compound (Przybyłek and Karpinski, 2019).

Table I. The mean value and standard deviation of the inhibition zone

Group	n	Mean ± SD
CHA	5	0
CHA + 2.5% propolis	5	8 ± 0.15
CHA + 5% propolis	5	8.9 ± 0.15
CHA + 7.5% propolis	5	10.58 ± 0.13
CHA + 10% propolis	5	12.7 ± 0.21

Besides flavonoids, phenolic acids and esters also play an essential role in propolis's antimicrobial activity. Due to its sophisticated, active ingredients, the antimicrobial property of propolis may result from a synergistic effect between the compounds (Grenho *et al.*, 2015). A previous study demonstrated that propolis could suppress the virulence factors of bacteria. The virulence factors that were suppressed in that study were lipase and coagulase enzymes. Besides suppressing these virulence factors, the same study proved that propolis was also capable of inhibiting the formation of biofilm by bacteria (Bueno-Silva *et al.*, 2013).

Virulence factors are metabolites of bacteria that are crucial for its survival while inside the host environment (How *et al.*, 2016). Lipase is one of the virulence factors that are capable of converting host tissue into nutrients needed for its growth (Akca *et al.*, 2016). In contrast, coagulase is an enzyme secreted by bacteria as its defense mechanism against the immune system of the host (McAdow *et al.*, 2012). Biofilm formation is important for bacterial growth because once it is formed, bacteria are protected from the process of phagocytosis (Grenho *et al.*, 2015).

Several active ingredients of propolis, such as caffeic acid, benzoic acid, and cinnamic acid, act on the cell membrane or cell wall and result in the structural and functional damage of bacteria (Przybyłek and Karpinski, 2019). Other mechanisms that might contribute to the antibacterial property of propolis are the disintegration of the bacterial cytoplasm, cytoplasmic membrane and cell wall, partial bacteriolysis, and inhibition of bacterial protein synthesis (Akca *et al.*, 2016).

In this study, carbonated hydroxyapatite that was embedded in the propolis solution showed a significant antibacterial effect against *A. actinomycetemcomitans*. The antibacterial effect is represented by the inhibition zone found during the experiment. The inhibition zone was shown to occur in a dose-dependent manner.

This phenomenon suggests that embedding carbonated hydroxyapatite into propolis allowed the absorption of its active ingredients and showed an antibacterial effect.

The results of this study are similar to the previous study that investigated the antibacterial effect of nano-hydroxyapatite that was embedded in propolis extract. The study proved that embedded nano-hydroxyapatite had an antibacterial effect against *S. aureus*, both in the planktonic and sessile state, through the formation of biofilm (Grenho *et al.*, 2015). Unembedded nano-hydroxyapatite did not have this ability, similar to the findings in this current study.

CONCLUSION

This study proved that carbonated hydroxyapatite has antibacterial activity after the incorporation of propolis. The antibacterial property possessed by the incorporated specimen was directly proportional to the propolis concentration used in the embedding process. However, additional studies are still needed to investigate its antibacterial activity against other periodontal pathogenic bacteria and its effect on the formation of biofilm.

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REFERENCES

- Akca, A.E., Akca, G., Topçu, F.T., Macit, E., Pıkdöken, L. & Özgen, I.Ş, 2016, 'The comparative evaluation of the antimicrobial effect of propolis with chlorhexidine against oral pathogens: An in vitro study', *BioMed Research International*, 2016, 1–8.
- Bashutski, J.D. & Wang, H.L., 2009, 'Periodontal and

- endodontic regeneration'. *Journal of Endodontics*, 35(3), 321–328.
- Filho, H.N., Pinto, T.F., De Freitas, C.P., Ribeiro-Junior, P.D., Dos Santos, P.L. & Matsumoto, M.A., 2014, 'Autogenous bone grafts contamination after exposure to the oral cavity', *Journal of Craniofacial Surgery*, 25(2), 412–414.
- Fine, D.H., Kaplan, J.B., Kachlany, S.C. & Schreiner, H.C., 2006, 'How we got attached to *Actinobacillus actinomycetemcomitans*: A model for infectious diseases'. *Periodontology 2000*, 42(1), 114–157.
- Ghasemi, F.S., Eshraghi, S.S., Andalibi, F., Hooshyar, H., Kalantar-Neyestanaki, D., Samadi, A. & Fatahi-Bafghi, M, 2017, 'Anti-Bacterial effect of propolis extract in oil against different bacteria', *Zahedan Journal of Research in Medical Sciences*, 19(3), e7225.
- Grenho, L., Barros, J., Ferreira, C., Santos, V.R., Monteiro, F.J., Ferraz, M.P. & Cortes, M.E., 2015, 'In vitro antimicrobial activity and biocompatibility of propolis containing nanohydroxyapatite', *Biomedical Materials (Bristol)*, 10(2), 025004.
- Hara, K., et al., 2016, 'Fabrication and physical evaluation of gelatin-coated carbonate apatite foam', *Materials*, 9(9), 1–9. <https://doi.org/10.3390/ma9090711>
- Henderson, B., Ward, J.M. & Ready, D., 2010, 'Aggregatibacter (*Actinobacillus*) actinomycetemcomitans: A triple A* periodontopathogen?', *Periodontology 2000*, 54(1), 78–105.
- How, K.Y., Song, K.P. & Chan, K.G., 2016, 'Porphyromonas gingivalis: An overview of periodontopathic pathogen below the gum line', *Frontiers in Microbiology*, 7(FEB), 1–14.
- Jebahi, S., et al., 2012, 'Biologic response to carbonated hydroxyapatite associated with orthopedic device: Experimental study in a rabbit model', *Korean Journal of Pathology*, 46(1), 48–54.
- Jorgensen, J.H. & Ferraro, M.J., 2009, 'Antimicrobial susceptibility testing: a review of general principles and contemporary practices', *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 49(11), 1749–1755.
- Bueno-Silva, B., Koo, H., Falsetta, M.L., Alencar, S.M., Ikegaki, M. & Rosalen, P.L., 2013, 'Effect of neovestitol-vestitol containing Brazilian red propolis on biofilm accumulation in vitro and dental caries development in vivo', *Biofouling*, 29(10), 1–20.
- McAdow, M., Missiakas, D.M. & Schneewind, O., 2012. *Staphylococcus aureus* secretes coagulase and von willebrand factor binding protein to modify the coagulation cascade and establish host infections. *Journal of Innate Immunity*, 4(2), 141–148.
- Meimandi-Parizi, A., Oryan, A., Sayahi, E. & Bigham-Sadegh, A., 2018, 'Propolis extract a new reinforcement material in improving bone healing: An in vivo study', *International Journal of Surgery*, 56(April), 94–101.
- Przybyłek, I. & Karpinski, T.M., 2019, 'Antibacterial properties of propolis', *Molecules*, 24(2047), 1–17. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6600457/>
- Kaboosaya, B., Wulansari, L.K., Aoki, K. & Kasugai, S., 2017, 'Ligation period required to induce periodontitis in mice: Analysis with micro-computed tomography', *Journal of Oral Tissue Engineering*, 15(1), 25–34. https://www.jstage.jst.go.jp/article/jarde/15/1/15_15_25/_article/-char/ja/
- Winkler, H. & Haiden, P., 2016, 'Allograft bone as antibiotic carrier'. *Journal of Bone and Joint Infection*, 2(1), 52–62.