ISSN (Print) : 0974-6846
Indian Journal of Science and Technology, *Vol 9(15), DOI: 10.17485/ijst/2016/v9i15/89174, April 2016* ISSN (Online) : 0974-5645

Antimicrobial Activity of Propolis on Different Oral Bacteria

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Abstract

Propolis is a natural substance which is produced from honey bees through collecting and transforming resins and exudates from plants. The propolis can use the medicine field for antibacterial effect. The purpose of our study was to investigate the antibacterial activity for the oral prevention of dental caries. The propolis was from Australia honeybees, diluted 5w% and 10w% in ethanol. The antibacterial activity of the treated propolis against Streptococcus gordonii (S. gordonii), Streptococcus intermedius (S. intermedius) and Streptococcus sanguinis (S. sanguinis) were determined using optical density and colony forming units. All experimental groups were effective against bacteria. Streptococcus intermedius and Streptococcus sanguinis were more effective compared to Streptococcus gordonii. All groups inhibition bacterial growth, but there were no significant differences of different wt% groups. There were significantly low activities treated with propolis. The antibacterial activity indicated propolis against Streptococcus gordonii, Streptococcus intermedius and Streptococcus sanguinis. Propolis possesses several medicinal properties. In this study, the 5 wt% and 10 wt% group of propolis was indicated antibacterial effects, which succeeded in inhibiting the bacteria growth. Our study showed a significant effect related to oral bacteria species. In conclusion, the antibacterial action observed in this study suggests that this propolis substance could be used as an alternative medicine for infectious conditions of the oral disease without causing any major local or systemic adverse effects.

Keywords: Antibacterial Activity, Dental Caries, Microorganisms, Propolis

1. Introduction

Propolis is a natural ingredient collected by honey bees from parts of buds and exudates of plants, which is employed for construction and repair of the honeycomb[1].

Numerous studies have been reported to have antibacterial efficacy, perhaps thousands of years as safe natural antimicrobial substance[2]. The biological activities of propolis have been shown to possess antibacterial, antifungal, antiviral, antiprotozoal, hepatoprotective, antioxidant, antitumor, immunomodulation and anti-inflammatory effects[3].

It is an alternative medicine not used chemical agent and it is possible in supporting conventional process

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of derive form natural product[4]. Antimicrobial agents against oral diseases by microorganisms (bacteria, viruses, or fungi) could play an important part in the prevention of bacterial aspects of dental caries and periodontal diseases. Nowadays, propolis ingredient has been used for the purpose of the oral disease prevention for periodontal diseases and dental caries[5]. As the development of systemic administration of antimicrobials has been reported to cause the development of low resistance, multi-resistant bacteria species, and side effects, the development of new therapies for the treatment and prevention of oral diseases is of a great relevance[6]. By these reasons, propolis can play a major role in the biomedical applications for improving antimicrobial effect in oral cavity. Hence the beneficial properties of propolis are used more efficiently throughout the general public. In our study, the antibacterial effect was evaluated in *Streptococcus* species.

2. Material and Methods

2.1 Material

The propolis was from Australia honeybees. Propolis compounds were dissolved in 5 wt% and 10 wt% ethyl alcohol for .24 h.

2.2 Preparation of Bacteria

The *Streptococcus gordonii (S. gordonii*; KCTC 3286), *Streptococcus intermedius (S. intermedius*; KCTC 5655) and *Streptococcus sanguinis (S. sanguinis*; KCTC 3284) were acquired from the Korea research institute of bioscience and biotechnology.

2.3 Antibaterial Test

All bacteria were cultured on brain heart infusion agar (Brain Heart Infusion agar; Becton, Dickinson and Company, USA) at 37^{0}C. The microorganism-containing solution was diluted to a concentration of 5×10^5 Colony Forming Units per milliliter (CFU/mL). The agar plates were incubated at 37^{0}C for 24 h, respectively. After this incubation period, formations of a Colony Forming Units (CFU) were observed. The tubes were aerobically incubated and then optical density was detected. Plates were read at 600nm in an enzyme-linked immunoassay (ELISA) microplate reader spectrophotometer (Epoch, Biotek Instruments Inc., Winooski, Vermont, U.S.A.) at incubation times for 24h.

2.4 Statistical Analysis

The software (PASW Statistics 18.0; IBM Inc) was used to evaluate significant differences in the antibaterial effect with one-way ANOVA for the antibacterial activity test. In post hoc analysis used the Tukey HSD test.

3. Results

3.1 Colony Forming Units (CFU)

Figure 1 shows the survival test of a bacterial orgasm in the presence of propolis from different bacteria in different wt%. In the control plate, a viable bacteria population of all bacteria showed from approximately 1×10^5 CFU/mL at the beginning to approximately 5×10^5 CFU/mL after 24 h. Meanwhile, all experimental groups were effective against bacteria. *Streptococcus intermedius* and *Streptococcus sanguinis* were more effective compared to *streptococcus gordonii*. All groups inhibited bacterial growth, but there were no significant differences of different wt % groups.

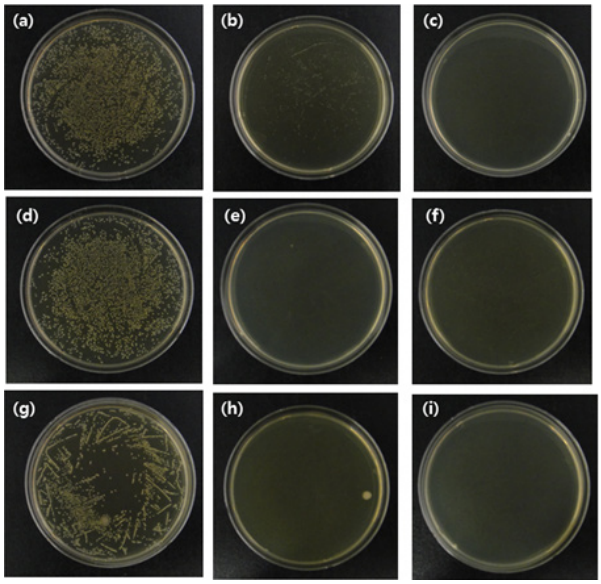

Figure 1. CFU results obtained for test group. (**a**) *Streptococcus gordonii* with 0 wt %. (**b**) *Streptococcus gordonii* with 5 wt%. (**c**) *Streptococcus gordonii* with 10 wt %. (**d**) *Streptococcus intermedius* with 0 wt %. (**e**) *Streptococcus intermedius* with 5 wt %. (**f**) *Streptococcus intermedius* with 10 wt %. (**g**) *Streptococcus sanguinis* with 0 wt %. (**h**) *Streptococcus sanguinis* with 5 wt %. (**i**) *Streptococcus sanguinis* with 10 wt %.

3.2 Antibaterial Activity

We evaluated the antimicrobial of propolis against Streptococcus gordonii, Streptococcus intermedius and Streptococcus sanguinis. Our data indicated that the antibacterial activity of propolis able to inhibit the bacteria growth. There were significantly low activities-treated with propolis (Figure 2, 3, 4). The antibacterial activity of propolis were against Streptococcus gordonii, Streptococcus intermedius and Streptococcus sanguinis ($p<0.05$). There was no significant difference among the propolis-treated groups ($p>0.05$).

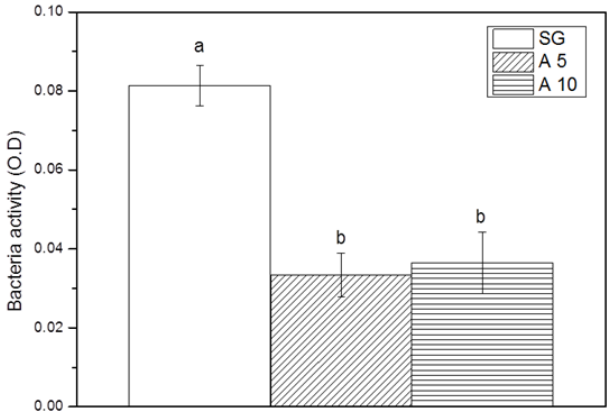

Figure 2. Bacteria activity of *Streptococcus gordonii* test group. SG: 0 wt %, A5:5 wt%, A10:10 wt %. abThere was significant difference within the different alphabet ($p<0.05$).

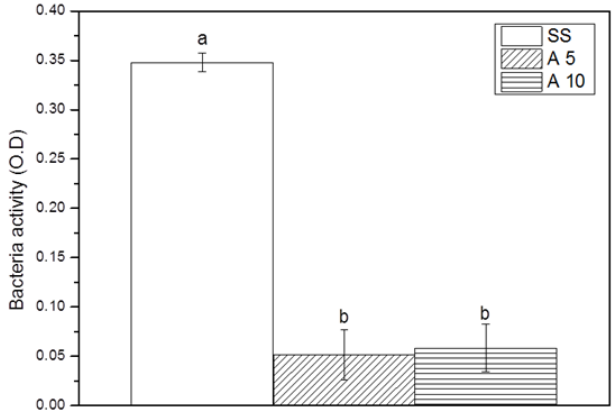

Figure 3. Bacteria activity of *Streptococcus sanguinis* test group. SS: 0 wt %, A5:5 wt%, A10:10 wt %. abThere was significant difference within the different alphabet ($p<0.05$).

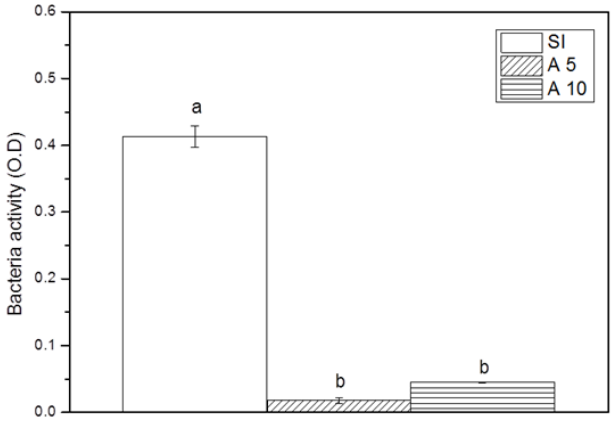

Figure 4. Bacteria activity of *Streptococcus intermedius* test group. Si: 0 wt %, A5:5 wt%, A10:10 wt %. abThere was significant difference within the different alphabet ($p<0.05$).

4. Conclusions

Oral diseases such as periodontal disease and dental caries are the most chronic common oral health problem in humans[7]. In particular, dental caries can progress into a series of complicated oral diseases, which can negatively effect on the individual's health-related quality of life[8]. It is characterized by the accumulation and growth of oral bacteria on tooth surfaces, resulting in the dental plaque formation and demineralization of tooth enamel[9]. Mutans streptococci (*S. mutans*) is the main etiologic agent in the formation of dental caries in humans. The bacterial infection lead to disease in healthy humans.

Natural substances are efficient because of them being less toxic alternative and constitute a promising source for new medicines. In[10] mention that the use of propolis against *S. mutans* is inhibited activity and growth of *S. mutans* from various regions in Brazil. Similarly, *in vivo*, In[11] stated that the Brazilian propolis possessed significant antimicrobial activity against cariogenic bacteria such as *S. mutans* in the oral cavity. The propolis inhibits bacterial growth by preventing enzyme activity and cell division, thus resulting in antimicrobial properties[12]. These inhibitory action of propolis can explain partially the synergism of propolis with drugs that act by inhibiting protein synthesis of RNA-polymerase[13]. Several studies have reported the propolis substance of natural agents can

inhibit bacterial metabolism, resulting in antibacterial, anti nuclear and fungicidal properties[14,15].

In this study, the 5 wt% and 10 wt% group of propolis was indicated antimicrobial efficacy, which proved of inhibited the growth in the bacteria organisms. Propolis of 10 wt% concentration showed an growth inhibition of microorganisms tested. Especially, in this study resulted from propolis that significant antibaterial effect related to oral bactera species. Hence, the antibaterial effect observed in this study suggests that this propolis could be used as an alternative therapy for infectious conditions of the oral cavity without causing any side effect. In conclusion, we were showed that propolis exhibited satisfying antimicrobial activities, biological effects, and may be suitable for use in pharmacological therapy. Propolis is recommendation for antibacterial effect from nature product and it is possible in the development product for the prevention of the oral disease.

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