Antimicrobial Activities of Propolis against Oral Candidiasis by Candida Albicанс
-Effect of Microbial Inhibition Using Propolis-

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Abstract Propolis is an extremely safe natural antimicrobial substance that has been reported to have powerful antibacterial efficacy. The aim of this study was to evaluate the inhibitory effects of propolis against Candida albicans (C. albicans). Propolis was collected from the honey bee Apis mellifera. The strain of C. albicans was cultivated overnight in liquid media incubated at 37°C. The antimicrobial activity was investigated using phosphate buffered saline (PBS), 3% sodium hypochlorite (NaOCl), 0.1% chorhexidine (CHX), and propolis extracts (5 μl/ml, 10 μl/ml). C. albicans were sensitive to 3% NaOCl, 0.1% CHX, and propolis (5 μl/ml, 10 μl/ml) with zones of inhibition of 15, 14.5, 16, and 17 mm, respectively. The CFU of PBS, 3% NaOCl, 0.1% CHX, 5 μl/ml and 10 μl/ml of propolis led a 1, 7, 7, 5 and 7-log reduction. Among the groups tested, C. albicans was most sensitive to 10 μl/ml of propolis, which showed the largest inhibition zones. Therefore, propolis can be a new antimicrobial therapy for oral mucosa disease in traditional medicine.

Key Words : antimicrobial effect, C. albicans, oral mucosa disease, propolis

1. Introduction

Propolis is known as bee glue and is resinous substance that honey bees extract from flower buds and plant. It is used to reinforce the structural stability in the bee hive[1]. The name comes from the Greek...
'pro', in front, and 'polis' meaning town or city, and bees use propolis to seal their hives against invasion by other insects and the weather[2].

Propolis contains several types of flavonoids, including antibacterial, antifungal, antiprotozoan, antiviral, antitumor, immunomodulation and anti-inflammatory activities as well. Numerous studies also prove its resourceful pharmacological activities: antibacterial, antifungal, antiviral, anti-inflammatory, antitumor, as well as immunomodulatory action, radio-protective, and so forth[3-10]. The chemical composition of propolis is very complex and includes organic compounds such as phenolic compounds and esters, flavonoids in all their forms(flavonols, flavones, flavonones, dihydroflavonols, and chalcones), terpenes, beta-steroids, aromatic aldehydes and alcohols, sesquiterpenes, and stilbene terpenes[11].

Among these functional properties, the propolis contains a large amount of flavonoids in mineral substance and in the various organisms’ substance. These flavonoids have been reported as a powerful antibacterial efficacy[12]. Flavonoids in propolis are well known to be protective of the occurrence of lipid peroxidant not only the hydrolysis but also inhibitors[13]. It is an alternative medicine not chemical agent and it’s possible in supporting conventional process of healing as natural products[14]. Therefore, if ingested, propolis is extremely safe natural antimicrobial substance without any toxic[15]. Despite increasing use of propolis worldwide[16] only a few studies have been carried out to determine the inhibitory effect of propolis against some bacteria and fungi of relevance in dentistry[17].

Recently, Propolis components have been used for the purpose of the oral prevention for dental caries, halitosis, periodontal diseases.

Therefore, natural substance such as green tea components, chitosan, and aloe are used to attempt to add the composition in the mouthwashes products[18,19].

The activities of propolis suggest its possible use in the local treatment of infectious conditions. Maryam et al. reported that propolis is one of disinfective agents for root canal treatment and it is also a new alternative substance as an intracanal medicaments. In their study, it showed that minimum inhibitory concentrations and colony forming units of propolis were significantly less than calcium hydroxide[20].

Oral mucosa disease is one of the most common infectious by a variety of infections in the bacteria, virus, systemic disease, nutritional disorder, and stress[21]. Oral candidiasis is an opportunistic fungus as a form of yeast, which is generally present on the skin and in mucous membranes like a rectum, mouth, and throat. The Candida albicans(\textit{C. albicans}) fungus also can pass through the blood vessels and affect intestines, throat, and heart valves. The most typical species is \textit{C. albicans}, which is a major cause of oral candidiasis or yeast infection in the oral mucosa[22]. Treatment of antibiotics, steroids, and antifungal agents can weaken the immune system and decline in the normal bacteria flora, leading to superinfection[23].

By these reasons, propolis can use the bio-medical applications for antimicrobial effect. Therefore, the aim of this study was to evaluate the antimicrobial effect of propolis against \textit{C. albicans}.

2. Materials and methods

2.1 Propolis preparation

Propolis was collected from the honey bee \textit{Apis mellifera} in Geochang County, Gyeongsan gnam-do, Southeast Korea.

2.2 Bacterial strains and culture conditions

The strain of \textit{C. albicans}(KCTC 7965/ATCC 10231) was used as the test organism. The strain of \textit{C. albicans} was grown in yeast mold broth(Difco, USA) and cultivated over night in liquid media incubated at 37°C. The cell was diluted in phosphate buffered saline(PBS) to a final concentration approximately
5×10⁶ colony-forming units (CFU) per milliliter.

2.3 Antimicrobial effects

Antimicrobial activity was investigated using an agar diffusion method. Sterilized filter paper discs (Advantec Toyo Kaisha, Ltd) of 8 mm diameter were impregnated with 100 μl of PBS, 3% Sodium hypochlorite (NaOCl), 0.1% Chlorhexidine (CHX), and propolis extracts (5 μl/ml, 10 μl/ml). There were control tubes with the liquid medium (without propolis) as negative controls and PBS, 3% NaOCl, and 0.1% CHX as positive controls. The inhibitory zone was considered the distance (mm) from the outside margin of the samples to the initial point of microbial growth. Each liquid medium was cultured into tubes and these cultures were incubated at 37°C for 24 h, and a single test tube was used for evaluating CFU. The inhibition assays were performed in sterile 96-well plates, and the optical density (OD) was detected using micro plate reader (BioTek Instruments, Winooski, VT, USA) at the wavelength of 550 nm. All tests were performed in triplicate.

2.4 Statistical analysis

Statistical analysis was performed using SPSS (IBM Co., Armonk, NY, USA). The clean zone and logarithms of each C. albicans plate count were taken and analysis using one-way analysis of variance (ANOVA) to check the difference in bacterial inhibition among the groups. P value of <0.05 was considered statistically significant.

3. Results and Discussion

Oral C. albicans have been found as normal flora in 30-45% of healthy adults [24]. An opportunistic infection is any infection caused by a pathogen that doesn’t normally lead to disease in healthy humans. When immune system is reduced, the number of C. albicans rapidly increased to induce the oral candidiasis [25]. Particularly, it usually occurs in children, the elderly and people with impaired immunity [26] and the people who are poor oral hygiene, have systemic disease, and cancer patients undergoing chemotherapy [27].

3.1 Antibacterial activity of C. albicans

The susceptibility of C. albicans to YM broth, PBS, 3% NaOCl, 0.1% CHX, and propolis (5 μl/ml, 10 μl/ml) was evaluated in solid and liquid culture. In a disc diffusion assay, C. albicans were sensitive to 3% NaOCl, 0.1% CHX, and propolis (5 μl/ml, 10 μl/ml) with zones of inhibition of 15, 14, 5, 16 and 17 mm, respectively. Table 1 provides the mean values of inhibition zones diameter obtained for each tested groups by diffusion method.

![Table 1](attachment:table1.png)

The antimicrobial activity was represented as followed. -; no inhibitory effect, +; 8.1-10.0 mm, ++; 10.1-13.0 mm, +++; 13.1-160 mm, ++++; over 160 mm

As the concentration of the propolis loaded on the disc increased, the clean zone around the paper disc also increased. Among the tested groups, the most sensitive to C. albicans was 10 μl/ml of propolis, which showed the highest inhibition zones [Fig. 1].
The number of counted colonies after 24h incubation is demonstrated in Fig. 2(A). The CFU of PBS, 3% NaOCl, 0.1% CHX, 5 μl/ml and 10 μl/ml of propolis led 1, 7, 7, 5 and 7-log reduction of C. albicans, respectively [Fig. 2(B)].

Fig. 3 shows the OD measurement at each tested groups. In a broth culture, the cell number of was significantly reduced in 3% NaOCl, 0.1% CHX and 10 μl/ml of propolis. The 10 μl/ml concentration of propolis was showed anti-microbial effects, which succeeded in inhibiting the growth of the microorganisms.

The disinfectants such as NaOCl and CHX had good antibacterial activity. Both disinfectants significantly reduced the cultivable number of bacteria. NaOCl is an effective antimicrobial agent[28] and an excellent organic tissue solvent[29], but has a highly toxic effect on the periapical tissues[30]. CHX is a broad-spectrum antimicrobial agent[31] that has substantive antibacterial activity and relatively low toxic effects[32]. However, it does not dissolve organic tissue[33]. In addition, the systemic administration of antimicrobials has been reported to cause the development of multiresistant microorganisms, interbacterial transfer of resistance determinants, and side effects[34].

The exploration for improved therapeutic efficacy will open newer agents in drug delivery[35]. Due to resistance to antibiotics by pathogens, recent research has been directed towards the use of traditional medicine/natural products for treatment and control of infections. The application of natural agents can inhibit bacterial colonization, growth, and metabolism[36]. Propolis is one of such products that is being tested on pathogens. There are a number of studies documenting the biocidal functions of propolis, its extracts and constituents. The propolis antibacterial activity is very
significant, since many of the assayed bacteria present resistance against antibiotics in clinical use[37]. Ghisalberti[38] found that propolis possesses several medicinal properties, i.e. antibacterial, anti-ulcer and fungicidal properties. Park et al.[39] showed the presence of the inhibitory effects of propolis on cariogenic bacteria. Several studies have demonstrated an in vitro inhibition of Streptococcus mutans group growth by propolis from different geographical origins[40,41].

We demonstrated the antibacterial effectiveness of propolis in the oral mucosa disease including oral candidiasis. In this study, the control and PBS groups did not show any difference in the number of bacteria at observation periods. However, the antibacterial activity of 3% NaOCl and 0.1% CHX was similar as the same time. A significant reduction in the cultivable numbers of bacteria was achieved with the use of 3% NaOCl, 0.1% CHX and 10μl/ml propolis. The remarkable antibacterial activities had greater than the inhibition zones diameter of 3% NaOCl and 0.1% CHX.

The propolis used showed good capacity of diffusion in agar. It succeeded in inhibiting the growth of the microorganisms. It seems that the antimicrobial activity of propolis occurs in a dose-dependent manner. According to the findings of the study presented here, it is concluded that the 10μl/ml propolis was superior to CHX in terms of bacterial elimination against C. albicans strain.

The development of new agents for the treatment of the oral diseases is of great relevance. Furthermore, the microorganisms studied in this work are of great relevance in dentistry and are involved with oral mucosa lesions. The propolis is expected to bring its therapeutic activity to the patient with greater comfort in order to benefit oral health.

Propolis agent for the present study showed a significant role related to oral diseases, further study is also required to identify the effect of propolis with the different Streptococcus mutans. Dodwad et al showed that the study was to investigate the effectiveness of a propolis-containing mouthrinse in inhibition of plaque formation and improvement of gingival health. This present study suggested that propolis might be used as a natural mouthwash. Therefore, our findings could recommend to be used as a propolis gargle products as an alternative to chemical mouthwashes[42]. Therefore, the antimicrobial action observed in this new formulation suggests its use as an alternative adjuvant therapy for infectious conditions of the oral cavity, without causing major local or systemic adverse effects. It will be able to be efficient against oral infections by C. albicans. A step further should be given to evaluate the cytotoxicity of this propolis.

4. Conclusion

Propolis can be useful as an antimicrobial agent in traditional medicine worldwide and in the development of oral hygiene products for the prevention of the oral mucosa disease including oral candidiasis.

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