Antimicrobial Activity of Korean Propolis Extracts on Oral Pathogenic Microorganisms

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Propolis has been used as a natural remedy in folk medicine worldwide. The antibacterial, antiviral, antifungal, and antiprotozoal aspects of its antimicrobial properties have been widely investigated. However, few studies focused on its applications in dentistry. Many dental diseases are related to various microorganisms in the oral cavity. In this study, we assessed the antimicrobial activity of Korean propolis extract, collected from 6 different regions, on oral pathogenic microorganisms. The propolis samples, collected from 6 different regions (P1: Uijeongbu, P2: Ansan, P3: Hongcheon, P4: Iksan, P5: Gwangju, and P6: Sangju), were dissolved in ethanol at two different concentrations (10 and 50 mg/ml). Three oral bacteria (Streptococcus mutans, Staphylococcus aureus, and Enterococcus faecalis) and one fungus (Candida albicans) were activated in general broth for 24 hours. Microorganisms were diluted and spread onto agar plates, onto which sterilized 6 mm filter papers with or without each propolis sample were placed. After 24 hours of incubation, clear zones of inhibition were observed. All tests were performed in triplicate. The propolis samples showed significant antibacterial and antifungal activity on oral pathogenic microorganisms; in addition, low-concentration groups showed outstanding antimicrobial efficacy on the 4 different microorganisms. Among the samples, P6 had significantly higher antibacterial activity than that of the others against three different bacteria. In particular, a high concentration of P6 showed a significant antifungal effect. In conclusion, we confirmed that Korean propolis has an inhibitory effect on oral pathogenic bacteria and fungi. Therefore, we suggest the possibility of developing oral medicine and oral care products based on Korean propolis.

Key Words: Antimicrobial activity, Microorganisms, Oral diseases, Propolis

Introduction

Propolis has been known for its antibacterial properties in folk medicine, and recently it found application in medicine and cosmetics 1). It is generally composed of resin and balsam (around 50%), wax (30%), essential and aromatic oils (10%), pollen (5%), and other substances 2). There is subtle variability in composition, based on several factors such as botanical sources and geographical zones 3).

The oral cavity is a suitable habitat for many microorganisms because of the wet environment and abundance of nutrients. Furthermore, in the oral cavity, soft and hard tissues coexist and form a specific environment that provides both aerobic and anaerobic conditions, in which various microorganisms interact. If the overall oral bacteria decrease because of excessive intake of antibiotics, this may lead to opportunistic infections by fungi. Thus, various factors such as diseases, medicines, or irradiation can cause different oral diseases due to changes in oral microbial flora.

Among oral bacteria, Streptococcus mutans, a major dental caries-inducing bacterium, occupies the largest portion 4). Enterococcus faecalis, on the other hand, plays a pivotal role in establishing a microbial community by connecting various bacteria in the oral cavity 5-7). Staphylococcus aureus, a Gram-positive staphylococcus typically found in the respiratory system, on the skin, and in the oral cavity, is also found in the periodontium where it may
cause periodontal disease\textsuperscript{8,9}. \textit{Candida albicans}, the most common fungus in the oral cavity and the gastrointestinal tract, causes opportunistic infections by quickly expanding due to host environmental changes induced by various factors\textsuperscript{10,11}.

Regarding health, products based on natural components have recently gained focus\textsuperscript{12-14}. The biological activity and chemical constituents of propolis have been widely studied, and propolis has been applied using various household items. However, few studies have been conducted on Korean propolis, and insufficient studies assessed its antimicrobial effects on microorganisms that inhabit the oral cavity\textsuperscript{15-17}.

In this study, we investigated the antimicrobial potential of Korean propolis extracts on standard microorganism strains that cause major oral diseases.

\section*{Materials and Methods}

\subsection*{1. Microbial samples}
\textit{S. mutans} (KCTC 5365), \textit{S. aureus} (KCTC 1916), \textit{E. faecalis} (KCTC 5290), and \textit{C. albicans} (KCTC 17712) were purchased from the Biological Resource Center in Korea Research Institute of Bioscience and Biotechnology (KRIBB). Four types of strains were cultured in Brain Heart Infusion (BHI) broth (Difco; BD, Franklin Lakes, NJ, USA) and Bacto Agar (BD) at 37°C.

\subsection*{2. Propolis extracts}
Korean propolis samples, which were collected from various regions of Korea (Fig. 1), were provided by Dong-A University. In brief, crude propolis was extracted with ethanol for 24 hours. The suspensions were separated, concentrated in a rotary evaporator, and redissolved in ethanol. We used two different concentrations of each propolis sample (10 and 50 mg/ml), and dimethyl sulfoxide (DMSO) was used as the negative control.

\subsection*{3. Measurement of microbial growth curve}
Each microorganism was serially diluted 10-fold using BHI broth, and 100 μl of all dilutions were seeded into a 96-well microplate. Microbial growth was determined by following changes in optical density (OD\textsubscript{600}) at 1, 3, 5, 7, and 18 hours, using a spectrophotometer (Epoch Take3; BioTek, Winooski, VT, USA). Before analysis, the plates were tapped carefully. BHI broth without bacteria was used as blank.

\subsection*{4. Agar diffusion test}
About 15 ml of BHI agar was poured onto sterile 90 mm Petri dishes (SPL, Pocheon, Korea) and allowed to solidify at room temperature. A 0.1 ml aliquot of each microbial suspension was spread on the agar plates separately. Six-millimeter filter papers were immersed in 0.1 ml of various propolis samples, and were placed onto the surface of the agar plates. The plates were incubated for 24 hours at 37°C. The inhibition zones around each group were measured with Vernier calipers (Mitutoyo, Kawasaki, Japan).

\subsection*{5. Statistical analysis}
All experiments were repeated at least 3 times independently. Data are expressed as the mean value±standard deviation. One-way analysis of variance (ANOVA) was performed to detect the significant effects of variables, followed by Tukey’s test (IBM SPSS Statistics 23.0; IBM Co., Armonk, NY, USA). The differences were considered significant at p < 0.05.

\section*{Results}

\subsection*{1. Growth curves of oral microorganisms}
Growth curves of four different oral microorganisms are
shown in Fig. 2. All microorganisms were incubated for 20 to 22 hours at 37°C. According to the curve graph, the last exponential phase of each microorganism was used for this study.

2. Antimicrobial activity by agar diffusion test

All propolis samples showed significant antibacterial and antifungal activity in oral pathogenic microorganisms, and there was no zone of inhibition in the negative control. Low concentration groups (10 mg/ml) showed antimicrobial effects in all tested microorganisms (Table 1, Fig. 3). Propolis from P1 and P6 significantly suppressed S. mutans at both concentrations (p < 0.05). The sample P6 had significantly higher antibacterial activity than that of the others against three different bacteria strains. The high concentration of the P6 sample has also shown an antifungal effect (p < 0.05).

**Discussion**

Many microorganisms that inhabit the oral cavity, including bacteria and fungi, may cause oral health problems. Due to the diversity of the oral microbiome, few specific treatments exist. As the focus on health increases, an increasing amount of research is performed on finding alternative treatments. The multifunctional properties of propolis have been applied in oral health products such as mouth rinses and toothpastes\textsuperscript{16,18,19}. Although antibacterial and antifungal effects were found, the evidence was weak and, in some studies, mouth rinse that contained propolis showed no clinical effect\textsuperscript{20}. Thus, in the context of propolis application, further research on the connection between its antimicrobial properties and chemical composition is required.

Flavonoids, aromatic acids, diterpenic acids, and phenolic compounds are known as the major components responsible for the biological activities of propolis and ethanol extracts of propolis\textsuperscript{21}. The flavonoid and phenol content of propolis has been previously studied\textsuperscript{3,22}, and a correlation between them and the effect of propolis has been found; however, the antibacterial effects of propolis could not be described based on its composition.

**Table 1. Zones of Inhibition (mm)**

<table>
<thead>
<tr>
<th></th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
<th>p</th>
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<tbody>
<tr>
<td><strong>PE 10 mg/ml</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>S. mutans</td>
<td>9.10±0.17\textsuperscript{a}</td>
<td>8.55±0.13\textsuperscript{b}</td>
<td>8.97±0.45\textsuperscript{a}</td>
<td>8.08±0.28\textsuperscript{c}</td>
<td>8.07±0.21\textsuperscript{b}</td>
<td>8.97±0.26\textsuperscript{a}</td>
<td>0.001</td>
</tr>
<tr>
<td>S. aureus</td>
<td>7.50±0.13\textsuperscript{b}</td>
<td>7.48±0.19\textsuperscript{b}</td>
<td>7.62±0.15\textsuperscript{b}</td>
<td>8.05±0.09\textsuperscript{b}</td>
<td>8.32±0.18\textsuperscript{b}</td>
<td>8.30±0.30\textsuperscript{a}</td>
<td>0.000</td>
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<tr>
<td>E. faecalis</td>
<td>7.47±0.29\textsuperscript{b,c}</td>
<td>7.25±0.22\textsuperscript{c}</td>
<td>7.68±0.20\textsuperscript{b,c}</td>
<td>7.72±0.28\textsuperscript{b,c}</td>
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<td>8.28±0.28\textsuperscript{b}</td>
<td>0.005</td>
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<tr>
<td>C. albicans</td>
<td>7.93±0.51\textsuperscript{a}</td>
<td>7.97±0.42\textsuperscript{a}</td>
<td>7.65±0.22\textsuperscript{b}</td>
<td>7.98±0.13\textsuperscript{a}</td>
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<td>8.32±0.16\textsuperscript{b}</td>
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<td><strong>PE 50 mg/ml</strong></td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>S. mutans</td>
<td>9.97±0.65\textsuperscript{a}</td>
<td>9.33±0.38\textsuperscript{b,c}</td>
<td>10.43±0.81\textsuperscript{b}</td>
<td>10.32±0.30\textsuperscript{b}</td>
<td>9.83±0.57\textsuperscript{b}</td>
<td>10.88±0.39\textsuperscript{b}</td>
<td>0.060</td>
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<td>8.17±0.23\textsuperscript{b}</td>
<td>7.94±0.13\textsuperscript{b}</td>
<td>8.17±0.12\textsuperscript{b}</td>
<td>8.72±0.14\textsuperscript{b}</td>
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<td>9.38±0.18\textsuperscript{b}</td>
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<tr>
<td>E. faecalis</td>
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<td>8.03±0.15\textsuperscript{b}</td>
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<td>8.42±0.25\textsuperscript{b}</td>
<td>8.50±0.30\textsuperscript{b}</td>
<td>0.029</td>
</tr>
<tr>
<td>C. albicans</td>
<td>7.95±0.18\textsuperscript{b}</td>
<td>8.35±0.49\textsuperscript{b}</td>
<td>7.78±0.51\textsuperscript{b}</td>
<td>8.12±0.13\textsuperscript{b}</td>
<td>8.25±0.30\textsuperscript{b}</td>
<td>9.33±0.13\textsuperscript{b}</td>
<td>0.001</td>
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Values are presented as mean±standard deviation. All experiments were repeated at least 3 times independently.


*±Values in the same row with different superscript small letters are significantly different at p < 0.05.
Furthermore, the solution used for propolis extraction also influences the contents of propolis, in turn affecting the effects of propolis. Various basic solvents such as ethanolic base, aqueous base, and glycolic extracts are used for propolis extraction. In one study, the antibacterial effect of aqueous propolis on Streptococcus mitis has been demonstrated. It was also found that the glycol extract of propolis, associated with calcium hydroxide, was able to completely eliminate Enterococcus faecalis and Candida albicans.

Ethanolic extracts of propolis samples showed high inhibitory effects against Gram-positive cocci (S. mutans, S. aureus, and E. faecalis), but had a weak effect against yeast (C. albicans). In addition, low activity against Gram-negative cocci has been previously reported. The cariogenic process of S. mutans was disturbed by propolis, and its plaque index and polysaccharide formation were reduced in in vivo studies. Duarte et al. suggested that the cariostatic properties of propolis are associated with its effect on acid production and the acid tolerance of streptococci.

In the present study, all samples showed the highest antibacterial effect on S. mutans at both concentrations, and, among the samples, P4 showed the highest effect. P4 and P6 had an antibacterial effect against S. aureus, while P4, P5, and P6 had an antibacterial effect against E. faecalis. Interestingly, all test samples showed a similar antifungal effect against C. albicans at both concentrations; however, P6 showed a significantly higher, concentration-dependent effect. In particular, the propolis sample collected in Sangju (P6) showed a stronger antibacterial activity than the samples collected in the other areas. Finally, all samples showed significant results compared to the control results.

Unlike for many natural-based remedies, data on the biological effects and toxicity of propolis with regard to allergic reactions do exist. Regarding allergic reactions, propolis is relatively non-toxic. Although the composition of Korean propolis has been shown to be similar to that from other countries, the differences in its chemical composition based to its geographical distribution pattern make it difficult to commercialize it clinically. Therefore, further studies are needed to determine the quality and...
safety control criteria for propolis for its routine clinical use.

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References


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