Antimicrobial Effect of Propolis Drops On Oral Pathogens: An In Vitro Study

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ABSTRACT

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Background: The aim of this in vitro study was to evaluate the antimicrobial effectiveness of various propolis drops found easily in Turkey against two oral pathogens; Streptococcus mutans and Enterococcus faecalis.

Methods: Seven different propolis drops (Group 1 to 7: Umay Herbal Organic Propolis, Bee'o Up [15%], Propoli EVSP, Brazilian Green Liquid Propolis, Eğriçayır Propolis, Bee'o Up [30%], Biostore Propolis) were included in the study. Antimicrobial effect of drops was investigated by the agar plate diffusion method. Petri dishes were prepared with Brain-Heart Infusion broth. 100 µl of microorganisms and 10 µl of each propolis were planted to these petri dishes from a broth culture. Inhibition zones formed on the 24th hour were measured in mm's and each experimental group was photographed. Data were compared by Mann Whitney-U test.

Results: Only water-based propolis of the present study (Group 1) formed no inhibition zone against both oral pathogens and alcohol-based groups 4 and 5 formed no zone only against the latter. Group 5 showed significantly the highest antimicrobial effect against S. mutans, however that group showed no influence on E. faecalis. Group 6 was found significantly more effective on E. faecalis than other propolis drops (p<0.05).

Conclusion: All of the groups showed antimicrobial effect on either one of the oral pathogens except for the water-based propolis drop. Compared to E. faecalis, tested propolis drops had affected S. mutans more. Thus, propolis extracts could be used as a more natural and harmless prevention method to dental caries specifically.

KEYWORDS

Antimicrobial effectiveness, Inhibition zone, Oral pathogens, Propolis

The oral cavity contains various kinds of species including bacteria and fungi.¹ All of these compromises the oral microbiota on equilibrium and the excessive amount of any species could create oral diseases. Gathering of species to form oral biofilm is an important virulence factor in the oral cavity, where many microbial species share the same ecological field, compete for resources and thus, influence each other. To explain the importance, it is clear that oral biofilm is mainly responsible for many oral diseases.² Regarding dental caries, the main etiological agent is Streptococcus mutans (S. mutans) among other Streptococcus groups which have been described and divided by gene sequence comparisons as pyogenic, mitis, anginosus, mutants, salivarious, and bovis.² S. mutans plays a central role in the development of cariogenic biofilms, mainly due to its acid-tolerant and acidogenic characteristics. This microorganism uses dietary sucrose for the carbohydrate fermentation process that creates a low-pH oral environment resulting in enamel and dentin demineralization.³ Apart from Streptococcus species, Enterococcus faecalis

ÖZ

Farklı Propolis Damalarının Oral Patojenlere Karşı Antimikrobiyel Etkinliğinin Değerlendirilmesi: Bir İn Vitro Çalışma

Amaç: Bu in vitro çalışmanın amacı, Türkiye’de kolaylıkla bulunan çeşitli propolis damalarının iki oral patojene (Streptococcus mutans ve Enterococcus faecalis) karşı antimikrobiyel etkilerinin değerlendirilmesidir.

Gereç ve Yöntemler: Çalışmaya yedi farklı propolis daması (1'ten 7'ye gruplar: Umay Herbal Organic Propolis, Bee'o Up [%15], Propoli EVSP, Brazilian Green Liquid Propolis, Eğriçayır Propolis, Bee'o Up [%30], Biostore Propolis) dahil edildi. Bakteriler 100 µl, her bir propolis önerdiği ise 10 µl olarak çekilde yaşa ekim teknigi ile Brain-Heart Infusion agar basıverilerek ekildi. Petri kutuları 24 saatlik inkübesinde bırakıldı ve sonuçta oluşan zon çapları ölçüldü. Her deney grubu fotoğraftandı. İstatistiksel analiz Mann Whitney-U testi kullanılarak yapıldı.

Bulgular: Su bazlı propolis (Grup 1) iki oral patojene karşı da inhibisyon bölgesi oluşturulamadı. Alkol bazlı propolislerden ise, yalnızca 4. ve 5. gruplar E. faecalis’ e karşı etkinlik gösterememştir. S. mutanslara karşı en yüksek antimikrobiyel etki 5. grup’ta görülürken, aynı grup E. faecalis’ü üzerinde herhangi bir etki gösterememiştir. 6. grup, E. faecalis’üne diğer propolis damalarından anılamı olarak daha etkil bir davranış göstermiştir (p<0.05).


ANAHTAR KELİMELER

Antimikrobiyel etkinlik, İnhibisyon alanı, Oral patojenler, Propolis

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... (E. faecalis), which is the predominant human enterococcus, is also important for oral health, since it is mainly an indicator of failures of endodontic treatments. E. faecalis localizes in root canals of devitalized teeth and is responsible for apical inflammation. Moreover, E. faecalis has also related to oral diseases, such as caries, periodontitis, and peri-implantitis in certain studies as well. Recently, Anderson et al. have reported that oral isolates had the highest percentages of virulence genes as well as extracellular enzymes and a capacity to form biofilms. Due to E. faecalis' resistance to common endodontic irrigants, chlorhexidine (CHX) is generally used for root canal therapies instead of calcium hydroxide and it showed significant microbial reduction.

Since oral diseases, such as dental caries, are among the most common non-communicable diseases, great importance on any prevention method should be taken into consideration by health care providers. As there are more than 700 species of bacteria in the oral environment that could cause demineralization reactions and persistent inflammations, it is advisable to use natural products that have been contributing significantly to drug development as well. Among these, different kinds of propolis are such products that are valuable for their biological properties, including antimicrobial, antifungal and antitumor effects with direct application in dentistry. Propolis is a resinous hive product collected by Apis mellifera bees. Additively, it contains bioactive molecules and generally used for oral ulcers. However, regarding its effects on inhibiting glucosyltransferase enzymes and diminishing acid production of S. mutans, it could be used as an anticaries agent as well. For instance, Duarte et al. showed less acid production and tolerance of cariogenic Streptococci after propolis extracts in smooth surface caries of rats. De Luca et al. had also found in an in vivo study that propolis varnish prevented smooth-surface enamel caries and showed low cell toxicity. Hence, propolis could affect cariogenic bacteria directly and help to prevent not only oral ulcers but also other oral diseases as well.

Considering that there is a lack of studies including commonly consumed propolis products in Turkey, the aim of the present in vitro study is to compare the antimicrobial effect of these products against leading oral pathogens, S. mutans and E. faecalis. CHX and chloramphenicol were used as positive controls. The null hypothesis was that there is no significant difference among the propolis groups.

MATERIALS AND METHODS

Material selection

Seven different propolis drops that were commonly consumed in Turkey were chosen to be used in the present study. Numbers of the groups and contents of each propolis drop are given in Table 1.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Propolis Type</th>
<th>Content Description</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Umay Herbal Organic Propolis</td>
<td>Water, organic propolis extract, crude propolis (44.4%)</td>
<td>Drop</td>
</tr>
<tr>
<td>2</td>
<td>Bee’o Up</td>
<td>Water, glycol, propolis (15%)</td>
<td>Drop</td>
</tr>
<tr>
<td>3</td>
<td>Propoli EVSP</td>
<td>Water, glycerol, L-ascorbic acid, propolis extract (%20), green tea and grapes aroma</td>
<td>Drop</td>
</tr>
<tr>
<td>4</td>
<td>Brazilian Green Liquid Propolis</td>
<td>Water, propolis (10%)</td>
<td>Drop</td>
</tr>
<tr>
<td>5</td>
<td>Ejnçayır Propolis</td>
<td>Water, alcohol (67%), crude propolis (30%)</td>
<td>Drop</td>
</tr>
<tr>
<td>6</td>
<td>Bee’o Up</td>
<td>Water, grain alcohol, propolis (30%)</td>
<td>Drop</td>
</tr>
<tr>
<td>7</td>
<td>Biostore Propolis</td>
<td>Water, Glycerol, propolis (10%)</td>
<td>Drop</td>
</tr>
</tbody>
</table>

Microorganisms

Microorganisms used in the present study were Streptococcus Mutans ATCC 25175 and Enterococcus Faecalis ATCC 29212. Bacterial strains were supplied by Scientific Research Projects Coordination Unit of Istanbul University (Project No: 30073).

In vitro experiment

Properly lyophilized bacterial strains were diluted according to Mac-Farland 0.5 and incubated at 37°C for 24 hours. 18 standard petri dishes were prepared with Brain-Heart Infusion broth. 100 µl of each microorganism were planted to these petri dishes from a broth culture. Then, 10 µl of each propolis was planted to 6 mm diameters of discs. As a negative control, 10 µl of saline solution, and as the positive controls, 10 µl of chloramphenicol and 2% CHX were impregnated in each disc. The total amount of antimicrobials was calculated to be lower than 30 µg. Both propolis and antimicrobial discs were placed on petri dishes with a sterilized forceps. For each bacterial and propolis sample, the experiment was performed in duplicate. Then, the dishes were incubated in the oven at 37°C for 24 hours. Eventually, inhibition zones were measured in mm's. and each experimental group was photographed.

Statistical Analysis

Data were analyzed through SPSS 24.0 software (SPSS Inc., Chicago, IL, USA). The normality of data was examined by Kolmogorov-Smirnov and Shapiro-Wilk tests. The data from zones of inhibition of each bacterium related to groups were compared by a
non-parametric Mann Whitney-U test. The level of significance was set to 0.05.

RESULTS

Mean zones of microbial growth inhibition by propolis drops are shown in Table 2.

Table 2.

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>S. Mutans</th>
<th>E. Faecalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Umay Organic Propolis</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>Bee'o Up 19%</td>
<td>20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Propoli EVSP</td>
<td>14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>Brazilian Green Liquid</td>
<td>15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>Eğriçayır</td>
<td>30&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>Bee'o Up 30%</td>
<td>24&lt;sup&gt;e&lt;/sup&gt;</td>
<td>18&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>Biostore</td>
<td>12&lt;sup&gt;f&lt;/sup&gt;</td>
<td>8&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pos. Control</td>
<td>CHX</td>
<td>34&lt;sup&gt;g&lt;/sup&gt;</td>
<td>34&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pos. Control</td>
<td>Chloramphenicol</td>
<td>22&lt;sup&gt;h&lt;/sup&gt;</td>
<td>22&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Different superscripts showed statistical significance between groups (p<0.05). Lower and upper cases are used for different bacteria.

It is shown on the table that positive control groups demonstrated the widest inhibitory zones. All of the propolis groups showed lower inhibition of bacteria than positive control groups. Besides, CHX had the highest inhibition and this result is statistically significantly different than all of the groups including chloramphenicol (p=0.000). The first experimental group (Umay Organic) presented no growth of either S. mutans or E. faecalis (Figure 1). Similarly, experimental groups 4 and 5 showed no inhibitory zone against E. faecalis.

All experimental propolis drops formed statistically significantly different inhibition zones against S. mutans (p<0.05). Propolis drops tested against E. faecalis showed significantly different results except for the groups 2 and 6 (p=0.362) and 4 and 5 (p=0.186). Reactions of groups 2 and 4 to E. faecalis are shown in Figure 2.

DISCUSSION

Oral diseases are both the most common and also easily preventable pathologies amongst non-communicable diseases. Dental caries constitute a great part of these diseases and there are many methods for preventing caries and protecting risky teeth such as fluoride intakes, diet analysis, gels and mousses, oral hygiene motivation and others. On the other hand, other oral diseases such as periodontitis or endodontic problems usually need treatment or anti-microbial agents. Nowadays, herbal extracts and natural products are on great growth inhibition by propolis drops and antibacterial agents. *

![Example of bone level measurements on ImageJ programme.](ImageJ programme)

Propolis extracts have numerous benefits such as antibacterial, antifungal, antitumoral and antioxidative. Its direct mechanism is not known however it showed that propolis' could directly damage the cell membrane and the main active ingredients, phenolic compounds. These compounds are responsible for the inhibition of glucosyltransferase enzyme which creates the sticky layer to initiate biofilm formation. The ingredients of propolis products depend on both its water or alcohol content and also its year of collection. Although differences from the collection year distinctively inhibited the biofilm formation of S. Mutans, it did not influence bacterial growth.

![Cultivation of S. mutans and E. faecalis in the same medium with experimental group 1 and positive control groups.](medium with experimental group 1 and positive control groups.)
in terms of inhibition zone so, phenolic contents of the groups had not evaluated. Generally, propolis extracts are solved in alcoholic contents but except for the alcohol-based drops, water-based propolis (Group 1) had included in the present study to distinguish the effect of alcohol.

Similar to our study, Agüero et al. found that alcohol-based propolis was more effective. In the present study, only water-based propolis could not inhibit any of the oral pathogens. Even though that group had the highest propolis ratio among all groups, it showed no effect. That result could also support the outcomes from some studies evaluating water and alcohol-based propolis.

According to results, group 5 (Eğriçayır) formed the largest inhibition zone against S. mutans compared to positive control groups. That group contains not only one of the highest propolis ratios (30%), but it also had the highest alcohol content (67%) among others. One of the limitations of the study is that there is not an alcohol group (i.e. ethanol) used as negative control so, the individual contribution of propolis extract and alcohol toward inhibition of bacteria cannot be determined. Although group 5 showed great inhibition to S. mutans, it could not affect E. faecalis. Besides, the same reaction was also seen in group 4 (Brazilian Green Liquid) as well. Propolis extracts showed superior inhibitory effects against E. faecalis in many studies. However, the contra-results on inhibition of propolis drops could be attributed to the content of groups. There are conflicting results over Brazilian green propolis against E. faecalis. Although Brazilian Green propolis contains astaxanthin, which is 10 times effective than strong antioxidants, carotenoids found in many propolis formulae, inferior results were found in the present study. Vasconcelos et al. showed a 16±0.5 mm inhibition zone against E. faecalis and a 22±0.5 mm zone against S. mutans with Brazilian green propolis. The propolis extract used in that study consisted of 40% crude propolis so, the difference between the ratios of the content may lead to higher inhibition zones for both bacteria. However, a study verified that Brazilian green propolis was only susceptible to E. faecalis and another recent study showed that green propolis was effective on E. faecalis as irrigant solution, but not able to inhibit bacteria. 10% propolis ratio may not form an inhibitory zone so, the present study is in accordance with those researches. Low propolis level in the drop used in the study could be the reason for inferior results on E. faecalis.

The main factors of propolis that serve as antioxidants and show antibacterial and antiinflammatory properties are mainly flavonoids. Ketones, acids, esters and other phenolics may contribute to those actions. The more propolis extract involves in solutions, the more beneficial activities reveals. Therefore it is obvious that group 7 with 10% propolis extract had the least inhibition against S. mutans growth and lower inhibition against E. faecalis. Group 3 had also 20% of propolis extract however with the addition of L-ascorbic acid which is an antioxidant as well. Results of group 3 on both bacteria may appear from the synergic effect of ascorbic acid and propolis itself. The importance of the propolis ratio was revealed between the similar results of groups 2 and 6. All the ingredients were the same except for half of the propolis ratio of group 2 (15%) and it showed statistically significantly lower inhibition than group 6 (30%).

CONCLUSION

The present study determined the effect of various types of propolis drops, which are found in the Turkish market, against the growth of common oral pathogens by measuring inhibition zones. Drops with higher propolis ratios could able to inhibit S. mutans and E. faecalis more. Instead of water-based propolis drops, alcohol-based groups showed higher inhibition of the growth of oral pathogens.

The molecular level of propolis was not investigated in the present study even so, instead of dental forms such as kinds of toothpaste and mouthwashes, oral drops that were highly retained to hard and soft tissues were found effective on especially S. mutans. Within the limitations of the present study, propolis extracts could be used as a more natural and easily accessible prevention method to dental caries specifically. Further investigations are needed on improving the standardization of intensity and type of propolis formulas that can be used in dentistry areas.

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REFERENCES


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