The single most important success factor in endodontic treatments is a bacteria-free root canal system. In the disinfection of an infected root canal system, chemomechanical preparation is the first step carried out to eliminate bacteria. However, it only limits but does not totally prevent regrowth of endodontic bacteria. Therefore, the use of intracanal medicaments has been advocated to further reduce the number of microorganisms.

Calcium hydroxide (Ca(OH)₂) is a widely used intracanal medicament because of its antimicrobial activity, organic tissue dissolution capability, and anti-inflammatory effects. However, clinical studies have shown that it is not possible to sterilize root canals in necrotic teeth, even with calcium hydroxide. Therefore, new therapeutic agents (such as chlorhexidine and antibiotics) and natural products (such as propolis) were suggested to be used as alternative intracanal medicaments.

For centuries, propolis has been known to be a natural antibiotic. It is a complex mixture of resinous and balsamic substances collected from plants by Apis mellifera bees, which transport them to their hives and modify them with the addition of their own secretions, pollen, and wax. Propolis contains flavonoids, which are considered as its main biologically active component responsible for a large proportion of its known therapeutic properties. Propolis has been proven to have antimicrobial activity against Streptococcus mutans and polymicrobial cultures collected from necrotic root canals. It was also shown to be useful as a root canal dressing because of its low toxicity and broad antibacterial spectrum.

Another prerequisite of intracanal medicaments is that they must be easily removed from root canal walls. When dentinal tubules are obstructed by either a smear layer or intracanal medicament residues, endodontic sealers will be prevented from penetrating the tubules. Sealer penetration into dentinal tubules also plays a highly critical role in the success of endodontic therapy. It increases the contact surface area between the sealer and root canal walls, thus improving the quality of the apical seal and mechanical retention of root canal fillings.

The aim of the present study was to investigate the effects of calcium hydroxide (Ca(OH)₂) and propolis intracanal medicaments on bond strength of AH Plus to root dentin. After chemomechanical instrumentation using Revo-S rotary system, three groups of root canal specimens were prepared: 10 root canals were left untreated as controls (G1), 10 received Ca(OH)₂ intracanal medicament (G2), and another 10 received propolis intracanal medicament (G3). Canals were obturated with AH Plus and gutta-percha. After bond strength evaluation using micro push-out test, data were analyzed using ANOVA and Tamhane’s test (p<0.05). At coronal and middle thirds, there were no significant differences in bond strength among the three groups (p>0.05). At apical third, G3 was significantly superior to G2 (p<0.05) and G1 (p<0.05), but there was no significant difference between G2 and G1 (p>0.05). Therefore, when AH Plus was used as the sealer in endodontic treatments, its combined use with propolis as an intracanal medicament seemed to result in favorable sealer-dentin interfacial bond strength.

Keywords: Propolis, AH Plus, Bond strength, Calcium hydroxide

INTRODUCTION

The single most important success factor in endodontic treatments is a bacteria-free root canal system. In the disinfection of an infected root canal system, chemomechanical preparation is the first step carried out to eliminate bacteria. However, it only limits but does not totally prevent regrowth of endodontic bacteria. Therefore, the use of intracanal medicaments has been advocated to further reduce the number of microorganisms.

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The aim of the present study was to investigate the effect of propolis paste, used as an intracanal medicament, on bond strength to root dentin of obturations performed with AH Plus (Dentsply DeTrey, Konstanz, Germany) root canal sealer and gutta-percha.

MATERIALS AND METHODS

Preparation and characterization of propolis
Propolis sample was hand-collected from Kayseri (Central Anatolia), Turkey, and kept desiccated in the dark until processing. Crude propolis of 30 g was dissolved in 100 mL of 70% ethanol by shaking for 3 days. Approximately 17.2 g of aqueous ethanol extract was filtered through Whatman No. 1 paper and evaporated at 50°C. The chemical composition of propolis prepared in the present study was analyzed using gas chromatography-mass spectrometry and listed in Table 1.
Table 1 Chemical composition of the ethanol extract of poplar propolis

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Ethanol extract of propolis</th>
<th>RT</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-pentenoic acid</td>
<td></td>
<td>28.88</td>
<td>0.47</td>
</tr>
<tr>
<td>3-methoxy-cinnamic acid</td>
<td></td>
<td>34.56</td>
<td>0.32</td>
</tr>
<tr>
<td>2-propenoic acid</td>
<td></td>
<td>47.57</td>
<td>0.75</td>
</tr>
<tr>
<td>9-octadecanoic acid</td>
<td></td>
<td>48.34</td>
<td>4.42</td>
</tr>
<tr>
<td>Propenoic acid</td>
<td></td>
<td>51.24</td>
<td>2.56</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td></td>
<td>51.81</td>
<td>0.72</td>
</tr>
<tr>
<td>3-hydroxy-4-methoxycinnamic acid</td>
<td></td>
<td>41.14</td>
<td>2.12</td>
</tr>
<tr>
<td>Cinnamyl cinnamate</td>
<td></td>
<td>55.73</td>
<td>0.82</td>
</tr>
<tr>
<td>Aromatic and fatty acids and their esters</td>
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<td></td>
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<tr>
<td>Pinocembrin</td>
<td></td>
<td>57.60</td>
<td>13.5</td>
</tr>
<tr>
<td>Naringenin</td>
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<td>62.20</td>
<td>2.03</td>
</tr>
<tr>
<td>Chrysin</td>
<td></td>
<td>62.63</td>
<td>8.44</td>
</tr>
<tr>
<td>5-methyl-2,4-diisopropylphenol</td>
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<td>52.55</td>
<td>0.80</td>
</tr>
<tr>
<td>5-methoxy-3,7-dihydroxyflavonone</td>
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<td>60.04</td>
<td>3.08</td>
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<tr>
<td>Phenol</td>
<td></td>
<td>43.86</td>
<td>0.52</td>
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<tr>
<td>Alcohol, ketone and terpenes</td>
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<tr>
<td>Beta-eudesmol</td>
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<td>32.10</td>
<td>0.70</td>
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<tr>
<td>Alpha-eudesmol</td>
<td></td>
<td>32.22</td>
<td>0.80</td>
</tr>
<tr>
<td>4H-1-benzopyran-4-one</td>
<td></td>
<td>60.24</td>
<td>9.41</td>
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<td>Cryosphanol</td>
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<td>16.52</td>
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<tr>
<td>2-propen-1-one</td>
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<td>55.33</td>
<td>22.12</td>
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<tr>
<td>2-nonadecanone</td>
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<td>Ethyl oleate</td>
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<tr>
<td>E-7-tetradecene-1-ol</td>
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<td>63.18</td>
<td>1.43</td>
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<tr>
<td>1-ethanone</td>
<td></td>
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</tr>
<tr>
<td>Other compounds</td>
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<td>2-methyl-1-1-cyano-1-butene</td>
<td></td>
<td>37.84</td>
<td>0.53</td>
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<tr>
<td>Isocoumarin</td>
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<td>52.71</td>
<td>1.18</td>
</tr>
<tr>
<td>4-hydroxymethyl-3-2-methylindole</td>
<td></td>
<td>64.56</td>
<td>3.14</td>
</tr>
<tr>
<td>3-cyano-5,6-dimethoxy-2-methylthio-1-phenylindole</td>
<td></td>
<td>66.70</td>
<td>1.02</td>
</tr>
</tbody>
</table>

Root canal specimen groups
A total of 40 recently extracted, human maxillary central incisors with completely formed apices and straight canals were selected for this study. After the teeth were cleaned, standardized root lengths of 14 mm were obtained by sectioning the roots perpendicularly at the cementoenamel junction.

Root canals were debrided using 1% sodium hypochlorite (NaOCl) solution and instrumented using the Revo-S rotary system (Micro-Mega, Besançon, France). The last instrument used was #30/06. At every instrument change, the canals were irrigated with 0.5 mL of 1% sodium hypochlorite solution. Smear layer was removed by 3-min irrigation with 3 mL of 17% EDTA solution, followed by 3 mL of physiologic saline solution. Canals were sequentially dried using suction cannulas of decreasing diameters, followed by sterile absorbent paper points.

After chemomechanical preparation, the roots were randomly divided into three groups according to the intracanal medicament received:

Group 1: Ten root canals did not receive any intracanal medicament. Coronal access was sealed with a temporary filling material (Cavit G, 3M ESPE, Seefeld, Germany).

Group 2: Fifteen root canals received Ca(OH)₂ intracanal medicament, which was manipulated using distilled water until a creamy consistency (1:1.5, powder to liquid ratio) was reached. Paste was inserted into each root canal with the aid of a lentulo spiral, and coronal access was sealed with a temporary filling material.

Group 3: Fifteen root canals received propolis intracanal medicament, which was manipulated using glycerin until a creamy consistency was reached. Paste was inserted into each root canal with the aid of a lentulo spiral, and coronal access was sealed with a temporary filling material.

All 40 roots were stored at 37°C and 100% relative humidity for 14 days. For the 30 canals which received Ca(OH)₂ or propolis, intracanal dressings were removed with #15 K-file (Mani Inc., Japan) for 30 s at low power (mode 1) and 3 mL of 1% sodium hypochlorite irrigant under ultrasonic agitation (EMS, Nyon, Switzerland).
Using flexible 30-G needle tips (NaviTip, Ultradent, South Jordan, UT), final irrigation was completed using 3 mL of 17% EDTA for 3 min followed by 3 mL of physiologic saline solution.

**Scanning electron microscopy evaluation**

After final irrigation, five root canal specimens each from Groups 2 and 3 were randomly selected for scanning electron microscopy (SEM) evaluation (Model S440, Leica-Leo, Cambridge, UK) to observe the residues of Ca(OH)₂ and propolis intracanal medicaments on root canal walls. Grooves were prepared on the buccal and lingual surfaces with a diamond bur used with a high-speed water-cooled handpiece. Teeth were split along their long axis in a buccolingual direction using a hammer and chisel. All SEM specimens were dehydrated and coated with 20-nm gold-palladium particles, and ×1,000 magnification was used to observe the root canal walls at apical, middle, and coronal thirds.

**Micro push-out test**

Remaining 10 root canals each from Groups 1 to 3 were filled with gutta-percha (Diadent Group International, Chongchong Buk Do, Korea) and AH Plus sealer by cold lateral condensation technique. AH Plus sealer was prepared according to manufacturer’s instructions, and 75 mg of sealer was placed into each root canal using a lentulo spiral filler. All filled root canals were sealed with a temporary filling material. Teeth were stored in 37°C and 100% humidity for 7 days to allow the sealer to set. After sealer setting, each specimen was sectioned perpendicular to the longitudinal axis of the root by using a low-speed diamond saw (Minitom, Struer, Denmark) under water cooling. Three slices, of 1±0.1 mm thickness, were prepared along the apical, middle, and coronal regions of each root. Coronal-third slices were cut at 1 mm from the coronal surface of the root, middle-third slices at 7 mm from the coronal surface, and apical-third slices at 10 mm from the coronal surface. Thickness of each slice was carefully monitored using a digital caliper to exclude the influence of specimen thickness variation.

Each slice was loaded using a universal testing machine. For the coronal-third slices, load was applied using a 1-mm-diameter cylindrical plunger; for middle-third slices, it was a 0.50-mm-diameter cylindrical plunger; and for apical-third slices, it was a 0.30-mm-diameter cylindrical plunger. The plunger was positioned such that it contacted only the root filling material on loading (Fig. 1). Loading was carried out at a speed of 1 mm/min until dislodgement of the filling material occurred. Load applied at the time of dislodgement was recorded in Newtons for each slice.

The force applied to dislodge the filling material (in kN) was converted to shear stress (in MPa) using the formula, MPa=F/SL¹³, for both the upper and lower surfaces of each slice. SL was calculated using the formula below:

\[
SL = \pi (R + r) g
\]

where \(SL=\)sealer adhesion area, \(p=3.14, R=\)mean radius of coronal aspect of canal (mm), \(r=\)mean radius of apical aspect of canal (mm), and \(g=\)height relative to the tapered inverted cone (mm).

Apical and coronal aspects of each slice were scanned with a digital scanner. Scanned images were transferred to the Photoshop software, where \(R\) and \(r\) values of each slice were measured (Fig. 1).

**Failure mode analysis**

After micro push-out test, fractured specimens were examined under a surgical microscope (OPMI Pico, Carl Zeiss, Germany) at ×10 magnification to evaluate their failure modes: adhesive, cohesive, or mixed failure. Failure mode was considered “adhesive failure” if sealer was totally separated from dentin (i.e., dentin surface without sealer); considered “cohesive failure” if fracture occurred within the sealer (i.e., dentin surface was totally covered by sealer); or “mixed failure” when a mixture of adhesive and cohesive failure modes occurred (i.e., dentin surface partially covered by sealer)¹⁰. The failure modes were not statistically analyzed.

**Statistical analysis**

Power analysis was performed with MiniTab release 14.1 (Minitab Inc., PA, USA) to determine the sample size (\(n=10\)). All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 13.0 (SPSS for Windows, SPSS Inc., Chicago, IL, USA).
SEM observation

Figures 2(a)–(c) and 2(d)–(f) show the representative SEM images of root canal walls after the removal of propolis and Ca(OH)₂ intracanal medicaments respectively. Figure 3 is a higher-magnification SEM image of a root canal wall after the removal of Ca(OH)₂ medicament.
image of the dentinal tubules and propolis residues. After propolis was removed, Figs. 2(a) and (b) show open dentinal tubules in coronal and middle thirds. In contrast, Fig. 2(c) shows that propolis residues occluded the dentinal tubules. After Ca(OH)₂ paste was removed, Figs. 2(d) and (e) also show open dentinal tubules in coronal and middle thirds. In contrast, Fig. 2(f) shows that Ca(OH)₂ residues occluded the dentinal tubules.

**Failure modes**

Table 3 shows the failure mode results of the three experimental groups according to root canal region. Adhesive failure was the least observed failure mode for all the three groups. Cohesive and mixed failure modes accounted for most of the failures which occurred.

**DISCUSSION**

Endodontic treatment success depends on what is removed during cleaning and disinfection as well as what is placed during obturation. To render the root canal system optimally bacteria-free, intracanal medicaments have been highly advocated for root canal disinfection¹⁵-¹⁷.

Calcium hydroxide (Ca(OH)₂) is not an effective intracanal medicament against all types of bacterial species found in endodontic infections⁹. For example, Ca(OH)₂ could not efficiently eliminate *enterococci*, which were isolated in one third of the patients in whom endodontic treatment had failed. Their resistance to Ca(OH)₂ reportedly stemmed from their abilities to survive in a high-pH environment⁶,¹⁸ and to invade dentinal tubules and adhere to collagen in the presence of human serum¹⁹. Propolis, a centuries-old natural antibiotic, has also been used as an intracanal medicament because of its good antimicrobial activity against a wide range of bacterial species²⁰-²³. Therefore, propolis could be used as an alternative intracanal medicament in cases of persistent endodontic infections.

Several authors have evaluated the difficulty of removing intracanal medicaments from root canal walls, especially in the apical part of the root canal¹⁰,²⁴-²⁶. Presence of their residues could adversely affect dentinal bond strength and compromise endodontic sealing. Victorino et al.¹⁰ evaluated the removal efficiency of inter-appointment endodontic dressing materials (propolis paste and Ca(OH)₂ paste) using 1% NaOCl, 17% EDTA, and saline as final irrigation solutions. They found no significant differences in root canal cleanliness for the removal of propolis or Ca(OH)₂ root canal dressings with these irrigants. Based on the findings of Victorino et al.¹⁰, 1% NaOCl, 17% EDTA, and saline were therefore used in this study to remove intracanal dressing materials before obturation. Apart from irrigants, other irrigation methodologies were explored and passive ultrasonic agitation of irrigation solutions was found to be more effective than irrigant-only techniques²⁵,²⁷-²⁹. Therefore, passive ultrasonic irrigation was also used in this study in the removal of intracanal medicaments.

The sealing ability of endodontic sealers was reported to be affected by the physical and chemical properties of root filling materials and by the presence of smear layer or intracanal medicament residues²²,³⁰,³¹. However, there were contradicting reports. Amin et al.²² found that placement of Ca(OH)₂ intracanal medicament did not improve, but neither did degrade, the bond strength of AH Plus. On the other hand, Carvalho et al.²⁴ found that Ca(OH)₂ had positive influence on the bond strength of AH Plus to root dentin. The effects of different cavity disinfection agents —such
as chlorhexidine, propolis, NaOCl, ozone, Er,Cr:YSGG laser—on shear bond strength of composites were also investigated, and it was shown that propolis paste did not affect the dentin bond strength of composite resin fillings when used as a cavity disinfectant franch.

Sagsen et al. evaluated the push-out bond strengths of root fillings obturated with different root canal sealers (AH Plus, I Root SP, and MTA Fillapex), and a bond strength of about 2.9 MPa was obtained at apical third without prior intracanal medicament placement. In the present study, the micro push-out bond strength of propolis paste was significantly superior to those of Ca(OH)$_2$ and Control groups ($p<0.05$) at apical third. Bond strength of Control group at apical third was about 2.6 MPa, while that of Propolis group was 6.0 MPa.

At coronal and middle thirds, there were no significant differences in micro push-out bond strength among the three experimental groups in this study ($p>0.05$). This result agreed with the findings of Amin et al., in that Ca(OH)$_2$ placement did not affect the bond strength of AH Plus and two other calcium silicate-based sealers.

Root canal space at coronal and middle thirds is anatomically larger than that at apical third. Therefore, with a higher circulation volume of irrigation solutions at the coronal and middle thirds of root canal space than at apical third, it facilitates the removal of smear layers and intracanal medicaments. Moreover, according to Victorino et al., there was no significant difference in the removal efficiency of either propolis or Ca(OH)$_2$ from root canal walls, which thus explained the statistically similar push-out bond strength results between propolis and Ca(OH)$_2$ at coronal and middle thirds in this study.

No adhesive failures were observed for Propolis group at apical third in the present study, but not so for the Control and Ca(OH)$_2$ groups (Table 3). This result could be explained by the high bond strength of Propolis group at apical third. The predominant occurrence of cohesive and mixed failures in this study could be explained by the size similarity between the diameter of cylindrical plunger and that of prepared root canals. Therefore, the plunger was in full contact with the root filling material on loading, consequently leaving only root canal sealer on dentin.

Based on the results of the present study, the use of propolis as an inter-appointment dressing material improved the push-out bond strength at apical third. Ingredient list in Table 1 revealed that propolis contained hydrophilic components such as aromatic and fatty acids. It was highly likely that these hydrophilic components of propolis paste were preferably and tightly bound to the hydrophilic dentin surface. Moreover, since the circulation volume of irrigation solutions at apical third was lower than at coronal and middle thirds, removal of propolis paste which had a resinous sticky form from the root dentin walls became more difficult. On the same note, the resin ingredients of propolis paste could be responsible for binding to AH Plus root canal sealer, thus accounting for the higher push-out bond strength at apical third. The chemical reaction between the resin ingredients of propolis and epoxy resin should be further investigated.

CONCLUSION

While no significant differences in bond strength were observed at coronal and middle thirds, Propolis group showed significantly superior push-out bond strength than Ca(OH)$_2$ and Control groups at apical third. Propolis is not a mainstream intracanal medicament material yet, but it had shown promising results when used with an epoxy resin-based sealer such as AH Plus. More studies are needed to prove that propolis could be a better intracanal medicament than Ca(OH)$_2$ as it also improved dentin bond strength.

REFERENCES

13) Costea JA, Rached-Junior FA, Souza-Gabriel AE, Silva-Sousa


