Disease prevention is the ultimate goal in restorative dentistry (Featherstone, 2000; Pitts, 2003). Dental diseases are among the most prevalent and costly diseases affecting industrialised societies, yet they are highly preventable. Dental caries is the most prevalent disease affecting humans, and its incidence is particularly high during childhood (Araujo and Figueiredo, 1997). One of the primary causes of dental caries is Streptococcus mutans. There are several virulence factors that make these bacteria cariogenic, including adhesion, acidogenicity and acid tolerance. These bacteria produce glucosyltransferases and synthesise glucans from sucrose (in particular, water-insoluble glucans), which mediate the adherence of S. mutans and other oral bacteria to tooth surfaces and contribute to the formation of dental plaque. When a drop in the pH occurs after the fermentation of carbohydrates, the probability of enamel demineralisation increases, leading to cariogenesis (Banas, 2004). Success in inhibiting any of these previous steps would lead to caries prevention (James and Tagg, 1991; Matsumoto et al, 1999; Yanagida et al, 2000; Radcliffe et al, 2002; Matsumoto et al, 2003).

Both Streptococcus mutans and lactobacilli play an essential role in the pathogenesis of caries and consequently are a prime target for the prevention of this disease by antibiotics and vaccines (Loesche et al, 1989). More recently, several natural products have been introduced on the market with

Comparison of the Antimicrobial Effect of Egyptian Propolis vs New Zealand Propolis on Streptococcus mutans and Lactobacilli in Saliva

Ghada A. Elbaz\(^a\)/Iman I. Elsayad\(^b\)

**Purpose:** To evaluate the antimicrobial effect of Egyptian propolis vs New Zealand propolis on Streptococcus mutans and lactobacilli in saliva.

**Materials and Methods:** The strains used for the experiment were isolated from 12 patients having a high caries index. The ethanolic extract (EEP) of pure Egyptian propolis was obtained by dissolving 20 g of propolis in 70% aqueous ethanol to a final volume of 100 ml. The commercial New Zealand propolis, combined with antibacterial agents, was an ethanolic extract of propolis in lozenge form; this was dissolved in distilled water to obtain an EEP. The EEP was further fractioned using a liquid-liquid extraction technique with hexane and chloroform solvents. The antimicrobial properties of the two propolis types and their fractions on Streptococcus mutans and lactobacilli were examined separately by determining minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). Twelve clinical isolates were obtained from the collected saliva of all patients, one (Streptococcus mutans and Lactobacilli) from each patient, for susceptibility testing.

**Results:** The MIC values of the New Zealand propolis were lower than the MIC values of the Egyptian propolis, indicating that the New Zealand propolis and hexane fractions (H-fr) in general had stronger antimicrobial effects. In addition, its antimicrobial action was greater on S. mutans than on lactobacilli, except with H-fr they were the same.

**Conclusion:** The commercial New Zealand propolis hexane fraction had the strongest antimicrobial action. The EEP had a more potent antimicrobial effect on S. mutans than on lactobacilli.

**Key words:** bactericidal effect, lactobacilli, propolis, Streptococcus mutans

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\(^{a}\) Lecturer, Department of Pedodontics, Faculty of Dentistry, Suez Canal University, Egypt.

\(^{b}\) Associate Professor, Department of Operative Dentistry, Faculty of Oral and Dental Medicine, Cairo University, Egypt.

**Correspondence:** Ghada AbdelHameed Elbaz, 16 Sybawaye Elmsary Street, Nasr City, Cairo, Egypt. Tel: +2-022-261-6747, Fax: +2-064-322-4471. Email: Dr.G.baz@live.co.uk
claims of being anticariogenic. Some natural products have been suggested for use in developing countries as an economic, safe and probably effective alternative for prevention and treatment of caries. Propolis, a natural resinous substance collected by honeybees from buds and exudates of plants to be used as a protective barrier in the beehive, is currently incorporated in food and beverages to improve health and to prevent several diseases. Besides its use in folk medicines, it possesses various corroborative pharmacological activities, such as antibacterial, antiviral, antifungal, anaesthetic, anti-inflammatory, hypotensive, immunostimulatory and cytostatic properties (Bankova et al, 2000). Moreover, propolis may also prevent dental caries (Ikeno et al, 1991; Park et al, 1998; Koo et al, 1999; Koo et al, 2000a; Koo et al, 2000b; Koo et al, 2000c; Koo et al, 2002a; Koo et al, 2002b; Koo et al, 2002c; Duarte et al, 2003), as previous in vitro and in vivo studies have shown that propolis inhibits the growth of mutans streptococci in vitro (Ikeno et al, 1991; Park et al, 1998) and reduces the incidence of caries and dental plaque accumulation in vivo (Koo et al, 1999; Koo et al, 2002a; Koo et al, 2002b). Two mechanisms of action have been associated with the anti-caries/anti-plaque properties of propolis: the first is the antimicrobial activity against cariogenic bacteria and the second is the inhibition of glucosyltransferase enzyme activity (Koo et al, 2000a).

The chemical composition of propolis is complex, typically consisting of waxes, resins, water, inorganics, phenolics and essential oils; the exact composition of propolis is dependent upon the source plant(s) (Bankova et al, 1992; Marcucci, 1995; Markham et al, 1996; Burdock, 1998). Flavonoids and (hydroxyl) cinnamic acid derivatives have been considered the primary biologically active compounds of propolis (Burdock, 1998). Nevertheless, its composition is highly variable, depending on its geographical origin (Greenaway et al, 1990; Bankova et al, 1992; Park et al, 1997). Analyses of the chemical composition of propolis have identified at least 300 compounds (Castro, 2001). Abd El Hady and Hegazi (2002) found 104 different chemicals in three samples of propolis collected from different areas in Egypt, which were grouped into seven categories: aliphatic acids, aromatic acids, esters, di- and triterpenes, flavonoids, sugars and miscellaneous. Walker and Crane (1987) listed 149 compounds isolated from samples collected from around the world, and again, acids and flavonoids were well represented.

This study was conducted to evaluate the antimicrobial effect of Egyptian propolis vs New Zealand propolis on Streptococcus mutans and lactobacilli in saliva. The null hypothesis is that there is no difference between the antimicrobial effect of Egyptian and New Zealand propolis.

**MATERIALS AND METHODS**

**Propolis samples and fractionation**

The propolis samples were obtained from two different sources, an ethanolic extract of pure crude Egyptian bee propolis and commercial New Zealand bee propolis lozenges (International Medical Guide, Egypt).

**Egyptian bee propolis**

The Egyptian crude samples were taken from hybrid Carniolan honeybee (Apis mellifera) colonies to obtain honeybee propolis. Honeybee propolis was collected from camphor (Eucalyptus spp.) trees. Three beehives were located at the Beekeeping Department, Plant Protection Research Institute, Dokki, Giza, Egypt. Propolis samples were dehydrated, ground and extracted in absence of bright light at room temperature, (20 g of propolis and 70% ethanol to a final volume of 100 ml). After a week, extracts were filtered to obtain an ethanolic extract of propolis EEP (Sforcin et al, 2000). The EEP was serially fractioned with hexane and chloroform solvents (Duarte et al, 2003). The ethanolic extracts of the isolated fractions were obtained by dissolving 1 g of these fractions in 10 ml of 80% (v/v) ethanol.

**New Zealand bee propolis**

Each propolis lozenge contains 93 mg ethanolic extract of New Zealand bee propolis in an all-natural base of raw sugar, glucose, water, honey, oil of lemon, menthol and peppermint oil with no artificial flavours or preservatives. The lozenges were dissolved in distilled water to form an aqueous ethanolic extract solution (90% v/v). To obtain the hexane and chloroform fractions, the same technique was used as described above.

**Isolation of bacterial strains**

The strains used for the experiment were isolated from 12 patients having a high caries index.
(DMFT ≥ 7) after they had given informed consent. The patients, all with permanent dentition, were selected from the outpatient clinic of the Operative Dentistry Department, Faculty of Oral and Dental Medicine, Cairo University and the Pedodontic Department, Faculty of Dentistry, Suez Canal University. The protocol of the research project follows the guidelines of the human ethics committee at Suez Canal University, Faculty of Dentistry, which approved this project. Each patient’s medical history was recorded to make sure that no antibiotics had been used recently prior to sampling. Before examination, the teeth were cleaned using cotton pellets and air from the triple syringe. Patients were examined while sitting in an upright position on the dental chair, and a plane dental mirror and explorer No. 23 (HU-Freidy; Chicago, IL, USA) were used to detect dental caries. The criteria used for the dental caries examination were published by the WHO (1971). In permanent teeth, the DMF index was used (D = decayed tooth, filling indicated; M = missing tooth due to caries; F = filled tooth).

From each patient, fresh whole saliva was collected in a graduated plastic tube under masticatory stimulation by having the subject chew sugar-free gum. The freshly collected saliva samples were cultured on plates of selective media: mitis salivarius bactiracin agar and Rogosa agar for the primary isolation of \textit{Streptococcus mutans} and lactobacilli, respectively. The cultured mitis salivarius agar plates were incubated aerobically in a candle jar (5% to 10% CO$_2$), whereas the cultured Rogosa agar plates were incubated anaerobically. All plates were kept at 37°C for 48 h. The suspected bacterial growth was identified according to Ruoff et al (2003). Twelve clinical isolates were obtained from the saliva collected from all patients, one (\textit{Streptococcus mutans} and lactobacilli) per patient for the susceptibility testing.

**Susceptibility testing**

The antimicrobial activity of the two types of propolis extracts on the isolated caries-associated strains (\textit{Streptococcus mutans} and lactobacilli) was examined separately by determining the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) in accordance with the Clinical and Laboratory Standard Institute guidelines (CLSI, 2005) and Koo et al (2000b). To determine MIC, the starting inoculum of the test strains was 3 x 10$^8$ CFU/ml in Mueller Hinton broth, and the test extract concentrations ranged from 3200 mg/ml (first concentration) to 12.5 mg/ml for EEP and from 1600 mg/ml (first concentration) to 6.25 mg/ml for the other fractions of propolis (in a series of 2-fold dilutions); the control was ethanol 0.6% v/v. MIC was determined as the lowest concentration causing inhibition of bacterial growth.

To determine MBC, an aliquot (50 μl) of all incubated tubes with concentrations higher than MIC was subcultured on BHI agar. The agar was supplemented with 5% of defibrinated sheep blood. MBC was determined as the lowest concentration that causes the killing of bacteria and prevents visible growth on the agar (Koo et al, 2000b). Three separate experiments were conducted for each concentration of the extracts tested.

**RESULTS**

The MIC and MBC values at which \textit{S. mutans} growth was inhibited and killed, respectively, are shown in Table 1. The MIC and MBC values at which lactobacilli growth was inhibited and killed, respectively, are shown in Table 2. The MIC values of EEPs and their hexane (H-fr) and chloroform (Chlo-fr) fractions for the New Zealand propolis lozenges were similar for \textit{S. mutans} and lactobacilli were similar, with the exception of H-fr for lactobacilli, which was less. The MIC values of the Egyptian propolis were the lowest with H-fr for both \textit{S. mutans} and lactobacilli. The MIC values of the New Zealand propolis were lower than the MIC values of the Egyptian propolis, indicating that the New Zealand propolis had a more potent antimicrobial effect. For the New Zealand propolis, the MBC values were from 2 to 12 times greater than the MIC values. In addition, its antimicrobial action was greater on \textit{S. mutans} than on lactobacilli, except with H-fr, they were the same. The EEP of the pure Egyptian propolis had a stronger antimicrobial effect with a lower MIC on \textit{S. mutans} than on lactobacilli. Most of the pure Egyptian propolis extracts did not show any antimicrobial action except for the H-fr.

**DISCUSSION**

The results allow partial confirmation of the null hypothesis, because although for Egyptian propolis, there is no MBC except for H-fr, some of the MIC values between Egyptian and New Zealand propolis are similar. Thus, both Egyptian and New Zealand
propolis have an antimicrobial action, albeit of different potencies.

In terms of health promotion, propolis extract may represent a new option, as this substance is easily obtained and inexpensive, in addition to showing long-term beneficial effects (Oliveira, 2004). The chemical composition of propolis is complex; it depends on the flora in the areas where it is collected (Bankova et al, 2000). It is generally recognised that Gram-positive bacteria are more susceptible to the antibacterial action of propolis than Gram-negative bacteria (Mirzoeva et al, 1997; Drago et al, 2000; Sforcin et al, 2000). Although the antimicrobial properties of propolis have been evaluated in several investigations, it is difficult to compare the results of different studies due to the different methods used (Drago et al, 2000). However, the results of this study reveal that the New Zealand propolis lozenges had a potent antimicrobial activity. This may also be attributed to the action of other ingredients, such as honey, menthol and other essential oils present in the New Zealand propolis lozenges. It is well established that the hydrogen peroxide activity in honey correlates with floral sources, and the antibacterial activity of honey is mainly due to the enzymatic formation of hydrogen peroxide (Molan, 1992; Baltrusaityte et al, 2007). Menthol, which is a compound obtained from peppermint oil, also has a proven antibacterial action (Saeed et al, 2006). While essential oils derive their antibacterial effect from their unique chemical makeup, each single, pure essential oil consists of several, sometimes hundreds of distinct natural chemicals. Many of these have antimicrobial activity and show synergistic effects (Ncube et al, 2008).

Several compounds have been identified in propolis, falling into three distinct chemical groups: 1. flavonoid aglycones, 2. cinnamic acid derivatives and 3. terpenoids (Bankova et al, 1995; Park et al, 1998). The antibacterial activity of propolis has been attributed to the phenolic compounds, especially flavonoids, phenolic acids and their esters (Loesche et al, 1989). Some prenylated p-coumaric acids isolated from Brazilian propolis have been shown to possess antibacterial activity (Aga et al, 1994). Bankova et al (1995; 1996; 2000) reported the antibacterial activity of volatile compounds and diterpenic acids from Brazilian propolis. Park et al (1998) showed the presence of different flavonoids and the inhibitory effects of propolis on cariogenic bacteria. Hegazi and Abd El Hady (2002) investigated the chemical composition of two types of Egyptian propolis collected from two different areas; they identified 75 compounds, with 22 being new for propolis. The new esters belonged to 4-methoxyhydrocinnamic acid, hydroferulic acid, ferulic acid and 2,6-bis-(pentanyloxy)-4-pentanylphenethanol. They also found that one type had higher antioxidant, antibacterial and antifungal activity than the other type.

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<th>Table 1</th>
<th>MIC and MBC values in μg/ml of propolis and their fractions for Streptococcus mutans</th>
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*No bactericidal action was observed at the starting inoculum.*

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<th>Table 2</th>
<th>MIC and MBC values in μg/ml of propolis and their fractions for lactobacilli</th>
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*No bactericidal action was observed at the starting inoculum.*
The findings of this study confirm and extend previous observations on the anti-caries properties of propolis (Hayacibara et al, 2005). Here, it was shown that H-fr, followed by Chlo-fr were the most effective extracts, indicating that the putative biologically active compounds are mostly apolar. In general, H-fr from both propolis samples showed the most potent antibacterial effect in vitro. Although the Egyptian propolis did not show any antimicrobial action with EEP and Chlo-fr at the starting concentration of inoculum, it might have shown such an effect at a higher concentration of the starting inoculum.

However, using propolis from two different geographic origins was a major limitation in this study. This was unavoidable due to difficulties in importing fresh crude New Zealand propolis; thus, commercial New Zealand propolis lozenges were used. Furthermore, some of the antimicrobial action of the New Zealand propolis may be due to the added antibacterial substances in the lozenges. Because well-characterised reference bacterial strains were not available, many clinical isolates were tested to overcome the variation in the susceptibility pattern among different bacterial strains.

CONCLUSIONS

Under the conditions of this study, it can be concluded that both propolis types can inhibit *S. mutans* and lactobacilli growth. The hexane fractions contain most of the active compounds of propolis and should be the fractions of choice for further chemical characterisation and isolation. The EEP had a more potent antimicrobial effect on *S. mutans* than on lactobacilli.

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REFERENCES


