In vitro antimicrobial activity of propolis and *Arnica montana* against oral pathogens

H. Koo[a], B.P.F.A. Gomes[a,*], P.L. Rosalen[a], G.M.B. Ambrosano[a], Y.K. Park[b], J.A. Cury[a]

[a]*School of Dentistry of Piracicaba, State University of Campinas, Av. Limeira, 901, Areião, Piracicaba, S.P., 13414-018, Brazil*
[b]*School of Food Engineering, State University of Campinas, Campinas, S.P., Brazil*

Abstract

Arnica and propolis have been used for thousands of years in folk medicine for several purposes. They possess several biological activities such as anti-inflammatory, antifungal, antiviral and tissue regenerative, among others. Although the antibacterial activity of propolis has already been demonstrated, very few studies have been done on bacteria of clinical relevance in dentistry. Also, the antimicrobial activity of *Arnica* has not been extensively investigated. Therefore the aim here was to evaluate in vitro the antimicrobial activity, inhibition of adherence of mutans streptococci and inhibition of formation of water-insoluble glucan by *Arnica* and propolis extracts. *Arnica montana* (10%, w/v) and propolis (10%, w/v) extracts from Minas Gerais State were compared with controls. Fifteen microorganisms were used as follows: *Candida albicans* — NTCC 3736, F72; *Staphylococcus aureus* — ATCC 25923; *Enterococcus faecalis* — ATCC 29212; *Streptococcus sobrinus* 6715; *Strep. sanguis* — ATCC 10556; *Strep. cricetus* — HS-6; *Strep. mutans* — Ingbritt 1600; *Strep. mutans* — OMZ 175; *Actinomyces naeslundii* — ATCC 12104, W 1053; *Act. viscosus* OMZ 105; *Porphyromonas gingivalis*; *Porph. endodontalis* and *Prevotella denticola* (the last three were clinical isolates). Antimicrobial activity was determined by the agar diffusion method and the zones of growth inhibition were measured. To assess cell adherence to a glass surface, the organisms were grown for 18 h at 37°C in test-tubes at a 30° angle. To assay water-insoluble glucan formation, a mixture of crude glucosyltransferase and 0.125 M sucrose was incubated for 18 h at 37°C in test-tubes at a 30° angle. *Arnica* and propolis extracts (20 μl) were added to these tubes to evaluate the % of inhibition of cell adherence and water-insoluble glucan formation. The propolis extract significantly inhibited all the microorganisms tested ($p < 0.05$), showing the largest inhibitory zone for *Actinomyces* spp. The *Arnica* extract did not demonstrate significant antimicrobial activity. Cell adherence and water-insoluble glucan formation were almost completely inhibited by the propolis extract at a final concentration of 400 μg/ml and 500 μg/ml, respectively. The *Arnica* extract showed slight inhibition of the adherence of the growing cells (19% for *Strep. mutans* and 15% for *Strep. sobrinus*) and of water-insoluble glucan formation (29%) at these same concentrations. Thus, the propolis extract showed in vitro antibacterial activity, inhibition of cell adherence and inhibition of water-insoluble glucan formation, while the *Arnica* extract was only slightly active in those three conditions. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Propolis; *Arnica montana*; Microorganism; Dental plaque

---

1. Introduction

Dental plaque is a film of microorganisms on the tooth surface that plays an important part in the development of caries and periodontal diseases (Marsh, 1992). Mutans streptococci can colonize the tooth surface and initiate plaque formation by their ability to synthesize extracellular polysaccharides from sucrose, mainly water-insoluble glucan, using glucosyltransferase enzyme (Gibbons and Van Houte, 1975; Hamada and Slade, 1980; Tanzer et al. 1985). This sucrose-dependent adherence and accumulation of cariogenic streptococci is critical to the development of a pathogenic plaque. The further accumulation of plaque around the gingival margin and subgingival region may lead to a shift in its microbial composition from streptococcus-dominated to a larger number of *Actinomyces* spp. and an increased number of capnophilic and obligatory anaerobic bacteria, such as *Porphyromonas gingivalis* (Marsh, 1994). These microorganisms seem to be involved in root caries and periodontal disease, respectively (Schüpbach et al., 1995; Slots and Rams, 1992). Therefore, antimicrobial agents against these oral pathogens could play an important part in the prevention of dental caries and periodontal diseases, particularly those that can affect plaque formation.

Natural products have been used for thousands of years in folk medicine for several purposes. Among them, propolis has attracted increased interest due to its antimicrobial activity against a wide range of pathogenic microorganisms. Propolis is a resinous hive product collected by *Apis mellifera* bees from tree buds and mixed with secreted beeswax. The ethanolic extract of propolis has some pharmacological activities, such as anti-inflammatory, anaesthetic and cytostatic, besides antimicrobial activity (Ghisalberti, 1979; Bankova et al., 1989; Grange and Davey, 1990). It also has antibacterial activity against *Streptococcus mutans* (Ikeno et al., 1991; Steinberg et al., 1996; Park et al., 1998). However, little is known about its antimicrobial activity against other oral pathogens or its effects on dental plaque formation in vitro. Another natural product that has many applications in phytotherapy is *Arnica montana*, a perennial herbaceous plant with creeping roots whose extracts possess anti-inflammatory and cytotoxic properties (Woerdenbag et al., 1994; Lyss et al., 1997). The antimicrobial activity of *Arnica montana* has not been thoroughly investigated.

Considering that only a few studies have been reported on the in vitro effect of propolis and *Arnica montana* extracts against oral pathogens and plaque formation, our aim now was to evaluate in vitro the antimicrobial activity of these extracts on some oral microorganisms, especially their effects on the adherence of mutans streptococci and the inhibition of water-insoluble glucan formation.

2. Materials and methods

2.1. Microorganisms

The following 15 microorganisms were used in this study: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Candida albicans* NTCC 3736, *C. albicans* F72, *Strep. sanguis* ATCC 10556, *Strep. mutans* Ingbritt 1600, *Strep. mutans* OMZ-175, *Strep. sobrinus* 6715, *Strep. cricetus* HS-6, *Actinomyces naeslundii* ATCC 12104, *Act. naeslundii* W1053, *Act. viscosus* OMZ 105, *Porphyromonas gingivalis*, *Porph. endodontalis* and *Prevotella denticola*. The last three microorganisms and *C. albicans* F72 were isolated from clinical trials. The facultative anaerobes were kindly donated by the Center for Oral Biology, University of Rochester, NY, USA.

2.2. Ethanolic extracts of propolis and Arnica montana

Ethanolic extracts of propolis and *Arnica montana* were used. The extract of propolis (10%, w/v) from Minas Gerais state, south-eastern Brazil, was prepared as described previously (Koo and Park, 1997; Park et al., 1997) and the *Arnica* extract (10%, w/v) was obtained from the flowerheads of *Arnica* and prepared according to the Brazilian Pharmacopoeia (Farmacopéia Homeopática Brasileira, 1977).

2.3. Antimicrobial activity assay

Antimicrobial activity was determined by agar diffusion using fastidious anaerobe agar (Lab. M, Burry, UK) plus 5% sheep blood, brain-heart infusion (Difco, MA, USA) and Mueller Hinton (Difco, MA, USA) agars. The microorganisms were seeded by pour plate, except the anaerobes.

Isolated 18–24 h colonies of aerobes and facultatives grown on brain–heart infusion agar were suspended in sterile 145 mM NaCl solution. The suspension was adjusted spectrophotometrically to match the turbidity of a McFarland 0.5 scale. A 400-μl portion of each test suspension was mixed with 40 ml brain-heart infusion agar at 45°C, and poured on to a previously set layer of Mueller Hinton agar (140 × 25 mm plate). For the anaerobes, isolated colonies were suspended to reach 1.0 on the McFarland scale. Sterile swabs were dipped into the bacterial suspension and inoculated on to pre-reduced fastidious anaerobe/sheep blood agar plates. The inoculum procedures were appropriate to

provide a semiconfluent growth of the microorganisms tested.

Six sterilized stainless-steel cylinders of 8.0×10 mm (inside dia. 6 mm) were placed on to each inoculated agar plate. The test extracts or control (80% aqueous ethanol, v/v) (40 μl) were applied inside the cylinders. The plates were kept for 2 h at room temperature to allow diffusion of the agents through the agar. Afterwards, the plates were incubated at $37°C$ in an appropriate gaseous condition and for an appropriate period of time: aerobes, 24 h; facultatives, 24–48 h in a CO_2 incubator (10% CO_2); and anaerobes in the anaerobic work station (Don Whitley Scientific, Bradford, UK) in an atmosphere of 5–10% H_2, 10% CO_2 and 80–85% N_2 for 7 days. Zones of inhibition of microbial growth around the cylinder containing the extracts were measured and recorded after the incubation time. The inhibitory zone was considered the shortest distance (mm) from the outside margin of the cylinder to the initial point of the microbial growth. Six replicates were made for each microorganism.

2.4. Inhibition of adherence of growing cells to a glass surface

To assess the adherence of growing cells of *Strep. mutans* Ingbritt 1600 and *Strep. sobrinus* 6715 to a glass surface, organisms were grown at $37°C$ at an angle of $30°$ for 18 h in test-tubes, as described by Hamada and Torii (1978). Individual 18–24 h colonies from brain-heart infusion agar plates were suspended in 5.0 ml of sterile 145 mM NaCl, and the suspension adjusted to 0.5 on the McFarland scale. A portion of the suspension was mixed with brain-heart infusion broth (1:100 dilution, v/v) containing 29 mM sucrose, and then 2.48 ml were transferred to a test-tube. Subsequently, 20 μl of a 2-fold dilution series of the test extracts (concentrations ranging from 6.2 to 400 μg/ml reaction) and their control (80% ethanol, v/v) were inoculated, gently stirred and then incubated. After incubation, the adherent cells were washed and suspended using the procedures outlined by Hamada and Torii (1978). The amount of adherent cells was measured at 550 nm, OD_{550} (Hamada and Torii, 1978). Six replicates were made for each concentration of the test extracts.

2.5. Preparation of crude extracellular glucosyltransferase

Strep. sobrinus 6715 was grown in a medium containing 2.5% tryptone, 1.5% yeast extract, 17 mM glucose and 1.0% sorbitol, which was ultrafiltered through a 5.0-kDa mol. wt cut-off membrane (low molecular-weight medium) as described by Schilling and Bowen (1988).

Actively growing *Strep. sobrinus* 6715 was incubated in 200 ml of low molecular-weight medium at $37°C$ in 10% CO_2 for 18 h. After incubation, the cells were removed by refrigerated centrifugation at 12,000 ***g*** and the protease inhibitor PMSF, at a final concentration of 1.0 mmol/l, and the preservative NaN_3 (at a final concentration of 3 mM) were added to the culture supernatant fluid. None of the reagents has any adverse effects on enzyme activity or stability (D. Wunder and W.H. Bowen, unpublished data). The pH of the culture supernatant fluid was adjusted to 6.8 by the addition of 2 M KOH. The supernatant fluid was treated with ammonium sulphate at 55% saturation and then centrifuged (Chludzinski et al., 1974). The precipitate was resuspended in potassium phosphate buffer, pH 6.8, containing PMSF (1.0 mmol/l) and NaN_3 (3 mM), and extensively dialysed against the same buffer using a 12.0–14.0-kDa mol. wt cut-off membrane. The dialysed preparation was used as crude extracellular glucosyltransferase.

2.6. Inhibition of water-insoluble glucan formation

To assay the formation of water-insoluble glucan, we used a reaction mixture consisting of 1000 μl of 0.25 M of sucrose, 100 μl of crude glucosyltransferase and 20 μl of a 2-fold dilution series of test extracts (concentrations ranging from 7.8 to 500 μg/ml of reaction) in a total volume of 2 ml of potassium phosphate buffer containing NaN_3 and PMSF to a final concentration of 0.02% and 1.0 mmol/l, respectively. For the control, the same reaction was done to which 80% ethanol (v/v) was added instead of test extracts. The mixture was incubated at $37°C$ for 18 h at an angle of $30°$. After incubation, the fluid was carefully removed and the tube content was washed with sterile water as described by Takada et al. (1985). The water-insoluble glucan was determined by the phenol–sulphuric method (Dubois et al., 1956). Six replicates were made for each concentration of the test extracts.

2.7. Statistical analysis

An exploratory data analysis was performed to determine the most appropriate statistical test. The data from zones of inhibition of growth of each microorganism related to the treatments were compared by non-parametric Kruskal–Wallis and multiple-comparison tests. The values obtained for inhibition of cell adherence and inhibition of water-insoluble glucan formation were tested by ANOVA considering a factorial 2×7 (treatments × concentration) and an additional treatment (control). The Dunnett test was applied for comparison between each treatment concentration and the respective control. The chosen level of significance for all statistical tests was $p < 0.05$.

Table 1
Mean area of the zones of microbial growth inhibition[c] in mm ($n = 6$) provided by the ethanolic extracts of propolis and *Arnica montana*

Microorganisms	Treatments		
	Propolis	*Arnica montana*	Control (80% ethanol)
Staphylococcus aureus ATCC 25923	2.33[a]	0.00[ab]	0.00[ab]
Enterococcus faecalis ATCC 29212	2.17[a]	0.00[a]	0.00[a]
Candida albicans NTCC 3736	0.83[a]	0.00[ab]	0.00[ab]
Candida albicans F72	0.83[a]	0.00[ab]	0.00[ab]
Streptococcus mutans OMZ 175	2.00[a]	0.00[ab]	0.00[ab]
Streptococcus mutans Ingbritt 1600	2.17[a]	0.00[ab]	0.00[ab]
Streptococcus cricetus HS-6	1.83[a]	0.00[ab]	0.00[ab]
Streptococcus sobrinus 6715	2.25[a]	0.00[a]	0.00[a]
Streptococcus sanguis ATCC 10556	4.42[a]	0.00[ab]	0.00[ab]
Actinomyces naeslundii ATCC 12104	9.25[a]	0.33[a]	0.00[ab]
Actinomyces naeslundii W1053	8.17[a]	0.67[a]	0.00[ab]
Actinomyces viscosus OMZ 105	9.50[a]	0.00[ab]	0.00[ab]
Porphyromonas gingivalis	3.42[a]	0.48[a]	0.00[ab]
Porphyromonas endodontalis	2.25[a]	0.00[ab]	0.00[ab]
Prevotella denticola	2.58[a]	0.00[ab]	0.00[ab]

[a] Means followed by the same bold letters on a line are not significantly different from each other, $p > 0.05$, non-parametric multiple-comparison tests.
[b] Direct contact inhibition only.
[c] The inhibitory zone was considered the shortest distance (mm) from the outside margin of the cylinder to the initial point of the microbial growth.

3. Results

3.1. Antimicrobial activity

The means of the zones of microbial growth inhibition by propolis and *Arnica montana* extracts are shown in Table 1. Only the ethanolic extract of propolis produced inhibitory zones against all the microorganisms tested ($p < 0.05$), demonstrating strong inhibition of *Actinomyces* spp. growth, mainly *Act. viscosus*. *Strep. sanguis* and the mutans group of streptococci were also significantly inhibited by propolis. However, propolis extract showed only a slight inhibitory zone against *Candida albicans* spp. It is important to mention that the ethanolic extract of propolis also affected the growth of anaerobes, mainly *Porph. gingivalis*. On the other hand, the ethanolic extract of *Arnica montana* demonstrated only slight activity, in which 12 out of 15 strains presented no zone of growth inhibition. The *Arnica* extract showed only a slight inhibitory action on the growth of *Porph. gingivalis* and *Act. naeslundii* (ATCC 12104 and W1053), in which the values for the inhibitory zones were not statistically different from control ($p > 0.05$). The control (80% aqueous ethanol, v/v) did not form an inhibitory zone with any of the microorganisms tested, although it had shown inhibition in direct contact with some of them.

3.2. Inhibition of adherence of Strep. mutans and Strep. sobrinus to a glass surface

Fig. 1 shows the effects of the ethanolic extracts of propolis and *Arnica* on the adherence of growing cells of *Strep. mutans* and *Strep. sobrinus*. Inhibition of the in vitro adherence of these microorganisms was evident when they were grown in broth containing sucrose and different concentrations of propolis extract. The rate of inhibition was 98% for *Strep. mutans* and 90% for *Strep. sobrinus*, at a concentration of 400 µg/ml of reaction. Even at a concentration of 50 and 100 µg/ml, the rate of inhibition for both microorganisms was about 35 and 55%, respectively. However, the *Arnica* extract demonstrated only slight inhibition of *Strep. mutans* (19%) and *Strep. sobrinus* (15%) adherence at a concentration of 400 µg/ml. In addition, it showed a significant inhibition only at concentration of 400 and 200 µg/ml ($p < 0.05$), while the propolis inhibited the adherence of both microorganisms at all concentrations tested ($p < 0.05$) when compared with its control (80% ethanol).

3.3. Inhibition of water-insoluble glucan synthesis

Ethanolic extract of propolis clearly inhibited the synthesis of water-insoluble glucan by crude glucosyltransferase. As shown in Fig. 2, the synthesis was
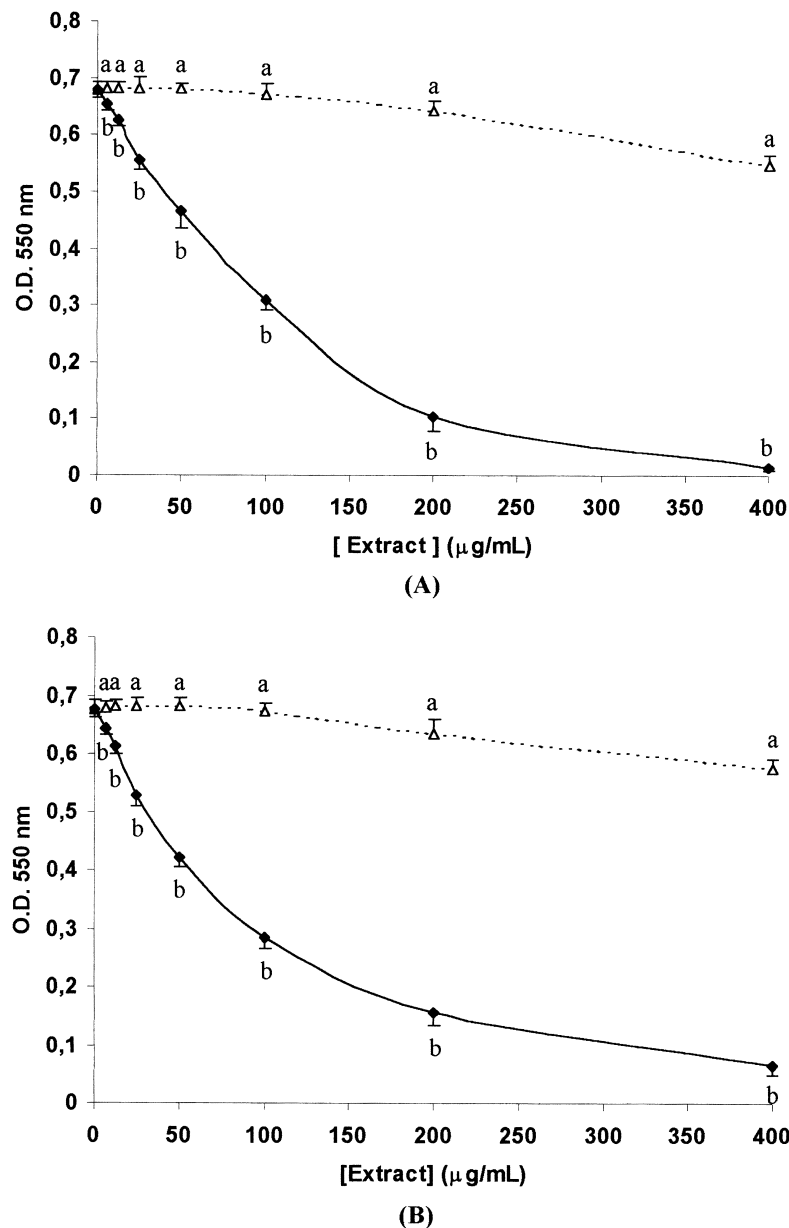
Fig. 1. Effect of ethanolic extracts of propolis (◆) and *Arnica montana* (△) on the adherence of growing *Strep. mutans* Ingbritt 1600 (A) and *Strep. sobrinus* 6715 (B) cells. Means (OD_{550}, $n = 6$) marked by different letters between treatments, in each concentration, differ significantly from each other, $p < 0.05$, *F*-test.

almost completely abolished at concentrations between 125 and 500 μg/ml of reaction. Propolis also showed marked inhibition at low concentrations, e.g. 31.3 μg/ml of reaction ($> 80\%$ of inhibition). The extract of *Arnica* only demonstrated significant inhibition at 250 and 500 μg/ml of reaction ($p < 0.05$); only a slight reduction was observed at these concentrations (18 and 29%, respectively).

4. Discussion

Natural products have been used for folk medicine purposes throughout the world for thousand of years. Many of them have demonstrable pharmacological properties, such as antimicrobial, anti-inflammatory and cytostatic, among others (Wu-yuan et al., 1990).
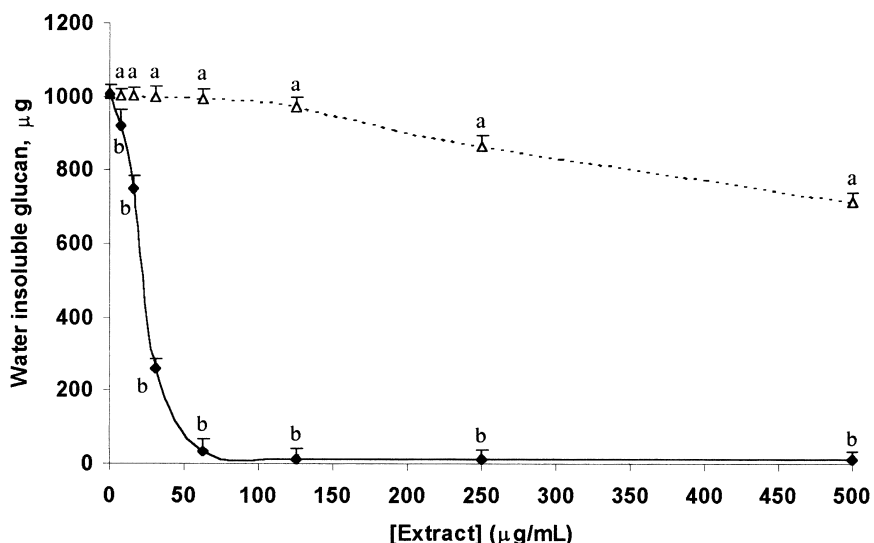
The main aetiological factor of dental caries and

Fig. 2. Inhibition of water-insoluble glucan (WIG) synthesis by ethanolic extracts of propolis (♦) and *Arnica montana* (△). Means (*n* = 6) marked by different letters between treatments, in each concentration, differ significantly from each other, *p* < 0.05, *F*-test.

periodontal disease is dental plaque. Therefore, it is reasonable to search for natural products that have antiplaque properties and antimicrobial activity against oral pathogens. Propolis has been extensively studied for its biological properties, mainly antimicrobial activity (Lindenfelser, 1967; Metzner et al., 1979; Grange and Davey, 1990; Bonheví et al., 1994). Although the antimicrobial activity of the ethanolic extract of propolis has already been reported for various pathogenic microorganisms, few studies have been conducted on oral pathogens (Ikeno et al., 1991; Steinberg et al., 1996; Park et al., 1998). *Arnica montana* seems to have anti-inflammatory and cytotoxic properties (Woerdenbag et al., 1994; Lyss et al., 1997), but little is known about its antimicrobial properties. Since mutans streptococci are involved in caries and plaque formation, we chose them as the main test microorganisms. *Actinomyces* spp. and three anaerobic bacteria, such as *Porph. gingivalis*, were also selected because of their relation to root caries and periodontal disease, respectively. In addition, antimicrobial activity against *Staph. aureus*, *C. albicans* and *Ent. faecalis*, which are important in other oral diseases, was also tested.

The antimicrobial assay showed that only the ethanolic extract of propolis has significant activity against microbial growth in vitro when compared to its control (80% ethanol, v/v). Propolis showed an inhibitory zone for every group of microorganisms tested, including the periodontopathogenic anaerobes. Growth of the mutans streptococci and *Staph. aureus* was also significantly inhibited, confirming previously results (Ikeno et al., 1991; Steinberg et al., 1996; Park et al., 1998; Lindenfelser, 1967; Grange and Davey, 1990;

Bonheví et al., 1994). The size of the zones of growth inhibition was variable, depending on the strain tested, although this does not necessarily mean that one microorganism is more susceptible than another. In addition, some strains, e.g. *C. albicans*, showed very small inhibitory zones. The biological relevance of these zones of inhibition needs to be evaluated using appropriate in vivo models. The present data suggest that propolis has bioactive compounds that possess antimicrobial activity in vitro.

The main biologically active compounds in propolis are polyphenolic, chiefly flavonoids (Ghisalberti, 1979; Bankova et al., 1989, 1992). Some of these flavonoids are considered antimicrobial, such as pinocembrin, galangin and sakuranetin (Villanueva et al., 1964; Villanueva et al., 1970; Metzner et al., 1979; Ghisalberti, 1979). In addition, Osawa et al. (1992) reported that kaempferol had antimicrobial activity against *Strep. mutans* and *Act. viscosus*. Recently, Cai and Wu (1996) showed that kaempferol, isolated from *Syzygium aromaticum*, also inhibited the growth of *Porph. gingivalis* and *Prev. intermedia*. Park et al. (1997; 1998) report the composition of flavonoid aglycones in Brazilian propolis, in which galangin, pinocembrin, kaempferol and sakuranetin were identified. In addition, other phenolic compounds could be involved. Ikeno et al. (1991) relate that cinnamic acid, identified from Chinese and Japanese propolis, demonstrated antimicrobial activity (*Strep. mutans*). Aga et al. (1994) showed that some hydroxycinnamic acid derivatives, e.g. 3,5-diprenyl-4-hydroxycinnamic acid, isolated and identified in Brazilian propolis showed antimicrobial activity against *Bacillus cereus*,

Enterobacter aerogenes, and *Arthroderma benhamiae*. The mechanism of antimicrobial action of propolis seems to be complex and is not completely understood. According to Amoros et al. (1994) and Bonhevì et al. (1994), its activity against microorganisms is more related to the synergistic effect of flavonoids (and other phenolics) than to the individual compounds. These findings are in agreement with those of Takaisikikuni and Schilcher (1994), who observed that the antibacterial action against *Strep. agalactiae* was complex, involving several mechanisms such as the formation of pseudomulticellular streptococci; disorganization of the cytoplasm, the cytoplasmatic membrane, and the cell wall; partial bacteriolysis; and inhibition of protein synthesis. They concluded that a simple analogy cannot be made with the mode of action of any classic antibiotics.

On the other hand, the ethanolic extract of *Arnica* did not demonstrate significant antimicrobial activity when compared to its control (80% ethanol), although slight inhibition was noted against *Porph. gingivalis* and *Act. naeslundii* (ATCC 12104 and W1053). The pharmacological effect of *Arnica montana* preparations has been attributed mainly to sesquiterpene lactones (Woerdenbag et al. 1994). Although these compounds demonstrate cytotoxic activity, in our study they seemed to have no effect on the growth of oral pathogens.

The propolis extract showed in vitro inhibition of the adherence of growing *Strep. mutans* and *Strep. sobrinus* cells to a glass surface at all concentrations tested. Furthermore, it was a potent inhibitor of water-insoluble glucan synthesis. Again, the *Arnica* extract presented only slight activity in these two conditions. The glucosyltransferase-catalysed synthesis of water-insoluble glucan from dietary sucrose is known to enhance the pathogenic potential of dental plaque by promoting the adherence and accumulation of large numbers of *Strep. mutans* and *Strep. sobrinus* on the teeth of animals and humans (Frostell et al., 1967; Hamada and Slade, 1980). It is well known that *Strep. sobrinus* produces at least two separate types of glucosyltransferase: an enzyme (glucosyltransferase-I) that produces water-insoluble glucan, and another (glucosyltransferase-S) that produces water-soluble glucan. Apparently propolis strongly inhibited the glucosyltransferase-I activity of *Strep. sobrinus*. Studies are in progress to determine the effect of propolis on activity of individual and purified glucosyltransferases.

Therefore, only propolis demonstrated effectiveness in reducing both cell adherence and the formation of water-insoluble glucan by crude glucosyltransferase in vitro, which are critical factors in plaque formation. Iio et al. (1984) demonstrated that quercetin and chrysin inhibited in vitro glucosyltransferase activity and glucan formation. Quercetin and chrysin were also found in significant amounts in Brazilian propolis (Park et al., 1997, 1998). Cai and Wu (1996) relate that some flavonoids, e.g. kaempferol, inhibited glucosyltransferase activity and adherence of *Strep. mutans* cells to a glass surface. In addition, cinnamic acid inhibited crude glucosyltransferase (Ikeno et al., 1991).

We demonstrate that *Arnica montana* extract has no or little antimicrobial activity effect on the adherence of *Strep. mutans* and *Strep. sobrinus*, or effect on the synthesis of water-insoluble glucan. On the other hand, the ethanolic extract of propolis was effective not only in inhibiting the growth of oral pathogens, but also in reducing plaque formation in vitro. Probably, the bioactive flavonoids, e.g. galangin, pinocembrin, kaempferol, sakuranetin and quercetin, and other phenolic compounds, e.g. cinnamic acid and its derivatives, present in propolis are involved in its biological activity against oral pathogens. Nevertheless, its phenolic composition is qualitatively and quantitatively variable depending on the region and season of propolis collection (König, 1985; Greenaway et al., 1990; Bankova et al., 1992; Park et al., 1997). It is clear that it is highly desirable to identify and isolate the active compounds of propolis responsible for the inhibitory effects.

In conclusion, the results of the present study suggest that propolis can prevent dental caries and periodontal disease, since it demonstrated significant antimicrobial activity against the microorganisms involved in such diseases and, especially, inhibition of dental plaque formation in vitro. However, *Arnica montana* extract showed only slight effects, although it has proved to have a strong anti-inflammatory and cytotoxic activity. Research is in progress to evaluate these biological effects of propolis using in vivo models.

Acknowledgements

References

Aga, H., Shibuya, T., Sugimoto, T., Kurimoto, M., Nakajima, S., 1994. Isolation and identification of antimicrobial compounds in Brazilian propolis. Biosci. Biotech. Biochem. 58, 945–946.

Amoros, M., Lurton, E., Boustie, J., Girre, L., Sauvager, F., Cormier, M., 1994. Comparison of the anti-herpes simplex virus activities of propolis and 3-methyl-but-2-enyl caffeate. J. Nat. Prod. 57, 644–647.

Bankova, V.S., Popov, S.S., Marekov, N.L., 1989. Isopentenyl cinnamates from poplar buds and propolis. Phytochemistry 28, 871–873.

Bankova, V., Christov, R., Stoev, G., Popov, S., 1992. Determination of phenolics from propolis by capillary gas chromatography. J. Chromatogr. 607, 150–153.

Bonhevì, J.S., Coll, F.V., Jordà, R.E., 1994. The composition, active components and bacteriostatic activity of propolis in dietetics. J. Am. Oil Chem. Soc. 71, 529–532.

Cai, L., Wu, C.D., 1996. Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens. J. Nat. Prod. 59, 987–990.

Chludzinski, A.M., Germaine, G.R., Schachtele, C.F., 1974. Purification and properties of dextransucrase from *Streptococcus mutans*. J. Bacteriol. 118, 1–7.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.

Farmacopéia Homeopática Brasileira, 1977. 1a. edn. Andrei Editora SA, São Paulo, Brazil, pp. 79–80.

Frostell, G., Keyes, P.H., Larson, R.H., 1967. The effect of various sugars and sugar substitutes on dental caries in hamsters and rats. J. Nutri. 93, 65–76.

Ghisalberti, E.L., 1979. Propolis: a review. Bee World 60, 59–84.

Gibbons, R.J., van Houte, J., 1975. Bacterial adherence in oral microbial ecology. Annu. Rev. Microbiol. 29, 19–44.

Grange, J.M., Davey, R.W., 1990. Antibacterial properties of propolis (bee glue). J. R. Soc. Med. 83, 159–160.

Greenaway, W., Scaysbrook, T., Whatley, F.R., 1990. The composition and plant origins of propolis: A report of work at Oxford. Bee World 71, 107–118.

Hamada, S., Slade, H.D., 1980. Biology, immunology and cariogenicity of *Streptococcus mutans*. Microbiol. Rev. 44, 331–384.

Hamada, S., Torii, M., 1978. Effect of sucrose in culture media on the location of glucosyltransferase of *Streptococcus mutans* and cell adherence to glass surfaces. Infect. Immun. 20, 592–599.

Iio, M., Uyeda, M., Iwanami, T., Nakagawa, Y., 1984. Flavonoids as a possible preventive of dental caries. Agric. Biol. Chem. 48, 2143–2145.

Ikeno, K., Ikeno, T., Miyazawa, C., 1991. Effects of propolis on dental caries in rats. Caries Res. 25, 347–351.

König, B., 1985. Plant sources of propolis. Bee World 66, 136–139.

Koo, M.H., Park, Y.K., 1997. Investigation of flavonoid aglycones in propolis collected by two different varieties of bees in the same region. Biosci. Biotech. Biochem. 61, 367–369.

Lindenfelser, L.A., 1967. Antimicrobial activity of propolis. Am. Bee. J. 107, 90–92.

Lyss, G., Schmidt, T.J., Merfort, I., Pahl, H.L., 1997. Helenalin, an anti-inflammatory sesquiterpene lactone from *Arnica*, selectively inhibits transcription factor NF-kappaB. Biol. Chem. 378, 951–961.

Marsh, P.D., 1992. Microbiological aspects of the chemical control of plaque and gingivitis. J. Dent. Res. 71, 1431–1438.

Marsh, P.D., 1994. Microbial ecology of dental plaque and its significance in health and disease. Adv. Dent. Res. 8, 263–271.

Metzner, J., Bekemeier, H., Paintz, E., Schneidewind, E.,

1979. Zur antimicrobiellen Wirksamkeit von Propolis und Propolisnhaltstoffen. Pharmazie 34, 97–102.

Osawa, K., Yasuda, H., Maruyama, T., Morita, H., Takeya, K., Itokawa, H., 1992. Isoflavanones from the heartwood of *Swartzia polyphylla* and their antibacterial activity against cariogenic bacteria. Chem. Pharm. Bull. 40, 2970–2974.

Park, Y.K., Koo, M.H., Ikegaki, M., Contado, J.L., 1997. Comparison of the flavonoid aglycone contents of *Apis mellifera* propolis from various regions of Brazil. Arq. Biol. Tecnol. 40, 97–106.

Park, Y.K., Koo, M.H., Abreu, J.A.S., Ikegaki, M., Cury, J.A., Rosalen, P.L., 1998. Antimicrobial activity of propolis on oral microorganisms. Current Microbiol. 36, 24–28.

Schilling, K.M., Bowen, W.H., 1988. The activity of glucosyltransferase adsorbed onto saliva-coated hydroxyapatite. J. Dent. Res. 67, 2–8.

Schüpbach, P., Osterwalder, V., Guggenheim, B., 1995. Human root caries: Microbiota in plaque covering sound, carious and arrested carious root surfaces. Caries Res. 29, 382–395.

Slots, J., Rams, T.E., 1992. Microbiology of periodontal disease. In: Slots, J., Taubman, M.A. (Eds.), Contemporary Oral Microbiology and Immunology. Mosby Year Book, St. Louis, USA, pp. 425–443.

Steinberg, D., Kaine, G., Gedalia, I., 1996. Antibacterial effect of propolis and honey on oral bacteria. Am. J. Dent. 9, 236–239.

Takada, K., Shiota, T., Curtiss, R., Michalek, S.M., 1985. Inhibition of plaque and caries formation by a glucan produced by *Streptococcus mutans* mutant UAB108. Infect. Immun. 50, 833–843.

Takaisikikuni, N.B., Schilcher, H., 1994. Electron-microscopic and microcalorimetric investigations of the possible mechanism of the antibacterial action of a defined propolis provenance. Planta Med. 60, 222–227.

Tanzer, J.M., Freedman, M.L., Fitzgerald, R.J., 1985. Virulence of mutants defective in glucosyltransferase, dextran mediated aggregation, or dextranase activity. In: Mergenhagen, S.E., Rosan, B. (Eds.), Molecular Basis of Oral Microbial Adhesion. ASM, Washington, USA, pp. 204–211.

Villanueva, V.R., Bogdanovsky, D., Barbier, M., Gonnet, M., Lavie, P., 1964. Sur l'identification de la 3,5,7-trihydroxy flavon (galangine) à partir de la propolis. Ann. Inst. Pasteur (Paris) 106, 292–302.

Villanueva, V.R., Barbier, M., Gonnet, M., Lavie, P., 1970. Les flavonoides de la propolis. Isolament d'une nouvelle substance bacteriostatique: la pinocembrine. Ann. Inst. Pasteur (Paris) 118, 84–87.

Woerdenbag, H.J., Merfort, I., Paßreiter, C.M., Schmidt, T.J., Willuhn, G., van Uden, W., Pras, N., Kampinga, H.H., Konings, W.T., 1994. Cytotoxicity of flavonoids and sesquiterpenes lactones from Arnica species against the GLC4 and the COLO 320 cell lines. Planta Med. 60, 434–437.

Wu-yuan, C.D., Green, L., Birch, W.X., 1990. In vitro screening of Chinese medicinal toothpastes: Their effects on growth and plaque formation on mutans streptococci. Caries Res. 24, 198–202.