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Preparation and Antimicrobial Activity of Gelatin Microparticles Containing Propolis Against Oral Pathogens

M. L. Bruschi and E. H. G. Lara
Programa de Pós-Graduação em Ciências Farmacêuticas, FCFRP–USP, Av. Zeferino Vaz, s/n, CEP 1404-0903, Ribeirão Preto, SP, Brazil

C. H. G. Martins and A. H. C. Vinholis
Laboratório de Pesquisa em Microbiologia Aplicada, Universidade de Franca, Av. Dr. Armando Salles Oliveira, 201, CEP 14404-600, Franca, SP, Brazil

L. A. Casemiro and H. Panzeri
Faculdade de Odontologia de Ribeirão Preto, Av. Zeferino Vaz, s/n, CEP 14040-903, Ribeirão Preto, SP, Brazil

M. P. D. Gremião
Departamento de Fármacos e Medicamentos, FCFAR- UNESP, Rod. Araraquara-Jaú, km 1, CEP 14802-902, Araraquara, SP, Brazil

Address correspondence to M. L. Bruschi, FCFRP–USP, Av. Zeferino Vaz, s/n CEP 14040-903, Ribeirão Preto, SP, Brazil; Tel: +55-16-602-4289; Fax: +55-16-602-4289; E-mail: bruschiml@yahoo.com.br

ABSTRACT Gelatin microparticles containing propolis ethanolic extractive solution were prepared by spray-drying technique. Particles with regular morphology, mean diameter ranging of 2.27 μm to 2.48 μm, and good entrapment efficiency for propolis were obtained. The in vitro antimicrobial activity of microparticles was evaluated against microorganisms of oral importance (*Enterococcus faecalis, Streptococcus salivarius, Streptococcus sanguinis, Streptococcus mitis, Streptococcus mutans, Streptococcus sobrinus, Candida albicans,* and *Lactobacillus casei*). The utilized techniques were diffusion in agar and determination of minimum inhibitory concentration. The choice of the method to evaluate the antimicrobial activity of microparticles showed be very important. The microparticles displayed activity against all tested strains of similar way to the propolis, showing greater activity against the strains of *E. salivarius, S. sanguinis, S. mitis,* and *C. albicans.*

KEYWORDS Propolis, Gelatin microparticles containing propolis, Antimicrobial activity, Oral pathogens

INTRODUCTION

Propolis (bee glue) is a strongly adhesive resinous bee hive product collected by honeybees from leaf buds and cracks in the bark of various plants (Burdock, 1998). Bees masticate this resin, salivary enzymes are added (mainly β-glucosidase), and the partially digested material is mixed with beeswax and used in the hives to exclude draught, to protect against external invaders, and to mummify their carcasses (Ghisalberti, 1979; Marcucci, 1995; Pietta et al., 2002). With complex chemical composition, 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).

Propolis has been used extensively in folk medicine for many years, at least 300 BC (Ghisalberti, 1979), and also has been reported to posses various

biological activities, namely antimicrobial (Tosi et al., 1996; Moreno et al., 1999; Koo et al., 2000; Sforcin et al., 2000; Banskota et al., 2001; Santos et al., 2002a; Kartal et al., 2003; Melliou & Chinou, 2004), fungicidal (Kujumgiev et al., 1999; Ota et al., 2001; Murad et al., 2002; Sawaya et al., 2002), antiviral (Ghisalberti, 1979; Marcucci, 1995; Kujumgiev et al., 1999), antiulcer (Burdock 1998), immunostimulating (Burdock, 1998), hypotensive (Marcucci, 1995; Burdock, 1998), anti-inflammatory (Burdock, 1998; Song et al., 2002a, b), antioxidant (Moreno et al., 2000; Isla et al., 2001; Nagai et al., 2001; Simões et al., 2004), and cytostatic (Banskota et al., 2001; 2002) activities.

Alone or incorporated in another dosage form propolis ethanolic extractive solution is commonly utilized on therapeutics, namely wound healing, tissue regeneration, treatment of burns, neurodermatitis, leg ulcers, psoriasis, herpes simplex and genitalis, rheumatism and sprains, candidiasis, cheilitis, stomatitis, influenza, and cold (Ghisalberti, 1979; Burdock, 1998). Propolis ethanolic extracts have demonstrated activity against oral pathogens (Park et al., 1998; Azevedo et al., 1999; Koo et al., 2000) and have been used in dentistry (Manara et al., 1999) mainly to treat periodontal diseases (Santos et al., 1999, 2002a,b; Gebara et al., 2002), and in mouthwashes and toothpaste to prevent caries and to treat stomatitis (Lu et al., 2004).

Some disadvantages of propolis ethanolic extracts are the strong and unpleasant taste, aromatic odor, and high ethanol concentration (Ghisalberti, 1979; Burdock, 1998). These disadvantages result in difficulties on packing, transport, and incorporation in oral dosage forms like lozenges, troches, gels, oral rinses, or mouthwashes. The patient compliance to the therapeutics is committed too. Bruschi et al. (2003a) showed that is possible, the production of gelatin microparticles containing propolis obtained by spray-drying technique using propolis ethanolic extractive solution. These microparticles do not have the propolis ethanolic extract strong and unpleasant taste, aromatic odor, and high ethanol concentration.

Despite these discoveries and the increasing use of propolis in dentistry (Manara et al., 1999; Bruschi et al., 2003a), no studies have been carried out to determine the inhibitory effect of gelatin microparticles containing propolis against oral pathogens. Within this context, the objective of this study was to evaluate in vitro the antimicrobial activity of the gelatin microparticles containing propolis against some microorganism of oral importance, namely *Enterococcus faecalis*, *Streptococcus salivarius*, *Streptococcus sanguinis*, *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus sobrinus*, *Candida albicans*, and *Lactobacillus casei*.

MATERIALS AND METHODS

Materials

Two samples of propolis were collected from an experimental apiary located on the farm of Universidade Estadual de Maringá (Paraná State, Brazil). The first propolis sample (P1) was collected in the dry season and another (P2) was collected in humid season. Type A gelatin, Royal (São Paulo, Brazil), was used without further purification. For the high-performance liquid chromatography (HPLC) mobile phase separation, HPLC grade methanol and acetonitrile were used (purchased from Mallinckrodt, Phillipsburg, NJ, USA), and water filtered through a Milli-Q apparatus, Millipore (Bedford, MA, USA). Pharmaceutical grade standard chrysin was purchased from Sigma (Saint Louis, MO, USA) and it was used as an external standard. Analytical grade acetone, ethyl acetate, methanol, acetic acid, dimethyl sulfoxide, and aluminium chloride were purchased from Merck (Darmstadt, Germany). Pharmaceutical grade mannitol and ethyl alcohol (96°GL), were also purchased from Merck (Darmstadt, Germany). Chlorhexidine gluconate aqueous solution (Periogard) was purchased from Colgate-Palmolive (São Paulo, Brazil). Penicillin, miconazole, resazurin, and tri-phenyl tetrazolium chloride were purchased from Sigma (Saint Louis, USA). Furthermore, Mueller Hinton culture medium (MH), Brain-Heart Infusion (BHI) medium, and Trypitic Soy Broth (TSB) medium were also purchased from Merck (Darmstadt, Germany).

Preparation and Characterization of the Propolis Extractive Solution (PES)

Propolis ethanolic extractive solution (PES) of each sample was prepared with propolis/ethanol ratio of 30/70 (w/w) by turbo extraction (List & Schmidt, 1989), at 3500 rpm for three times at 15 min with two intervals of 5 min. Each PES was filtered through Whatman grade 3 filter paper (Maidestone, UK) and was made up to the total initial weight with the ethanol, replacing the amount of solvent that evaporated

during the process. Exactly weighted 10 g of each PES were concentrated on water bath (100°C) with eventual shake. The concentrated material was dried on the Ohaus-MB 200 infrared analytical balance (Pine Brook, NJ, USA) at 110°C and the result was presented as "dryness residue" value (DR). Six replicates were carried out to estimate the inherent variability of each the determination.

The total flavonoids drift of each PES was obtained using the technique according Bruschi et al. (2003a). Three milliliters of distilled water, 3.0 mL of acetone, and 3.0 mL of PES were added in a separation funnel. This mixture was three times extracted with 15 mL of ethyl acetate. Ethyl acetate was added to 50.0 mL, rendering S1. Exactly 1.0 mL of aluminium chloride ethanolic solution (2%, w/v) and methanolic solution of acetic acid (5%, v/v) was added into 10.0 mL of S1 to 25.0 mL, rendering S2. At the time, methanolic solution of acetic acid (5%, v/v) was added into 10.0 mL of S1 to 25.0 mL, to be used as compensatory solution. After 30 min, S2 was analyzed by Beckman coulter DU-640 spectrophotometer (Fullerton, CA, USA) at wavelength (λ) of 425 nm. Six replicates were carried out to estimate the inherent variability of the determination and the total flavonoids drift of each PES was calculated in grams of quercetin (specific absortivity = 500) obtained in 100 g of dried propolis.

Each PES was also analyzed by HPLC, according to Bruschi et al. (2003b). An analytical aliquot of 1.0 mL of PES was extracted with 25 mL of ethyl acetate. The acetate fraction was dried using a water bath (40°C) and the residue was dissolved in 10.0 mL of methanol. This solution was filtered through a modified PTFE membrane filter with pore size of 0.45 μm (Millipore, Bedford, MA, USA) and an aliquot (50 μl) of the filtrate was diluted with 10.0 mL of methanol, obtaining the solution for analysis. A 100 μl aliquot of this solution was injected in a fixed loop injector (Rheodyne VS 7125, 100 μl), which was utilized to carry the sample into the reversed phase Chromsep RP C18 column (250 mm × 4.6 mm i.d., particle size 5 μm, Varian). The column temperature was maintained at 30 ± 0.1°C. Furthermore, the HPLC system consisted of LC-10AD two pumps, an automatic controller of flow, a SPD-M10A Ultraviolet-Visible Photo Diode Array spectrophotometric detector module, column oven, and an integrator system (Shimadzu, Kyoto, Japan). The mobile phase was methanol (A) and acetonitrile/water (2.5/97.5, v/v) (B), previously filtrated through a 0.45 μm PALL-Gelman membrane filter (East Hills, NY, USA) and degassed by ultrasound. The gradient elution system changed as the time, thus, at 0 min, 50% A and 50% B; 47 min, 80% A and 20% B; 50 min, 100% A and 0% B; 58 min, 100% A and 0% B; 62 min, 47% A and 53% B; 67 min, 47% A and 53% B. The flow-rate was 1.0 mL/min and the absorbance of the eluate at 310 nm was monitored. The propolis concentration in the solution was determined quantifying the three major peaks (markers) of the obtained chromatogram in relation to chrysin.

Preparation and Characterization of Propolis Microparticles (PM)

Each propolis extractive solution was dispersed in a gelatin solution at 25°C through the dripping technique using a syringe of 10 mL, and with magnetic agitation by 30 min (Bruschi et al., 2003a). The quantity of gelatin utilized was a function of the PES DR (Table 1). The final dispersions were spray-dried in a BÜCHI Mini Spray Dryer model B-191 (Büchi, Flawil, Switzerland) through the nozzle, using the conditions illustrated in Table 1. The resultant dried products were collected and kept away from rehydration until further tests.

The spray-dried products were coated under argon atmosphere with gold/palladium and examined under a Jeol JSM-T330A scanning electron microscope (Tokyo, Japan). The scanning electron photomicrographs (SEM) were evaluated. Moreover, the samples of PM were subjected to particle analysis by a Leica DMRXA optical microscopy (Wetzlan, Germany) with Leica *Qwin Image Analysis System*. Particles were placed on a glass slide and the size measurements of

TABLE 1 **Microparticles Spray-Drying Conditions**

Inlet temperature (°C)	Gelatin/PESDR % ratio (w/w)	Feed rate (%)	Aspiration (%)	Pressure (%)	Mannitol (%)
160	6/1	6	80	3	20

microparticles were performed using Feret's diameter as parameter. One thousand microparticles were measured and the particle size distribution was estimated.

The amount of propolis in the microparticles was determined by spectrophotometric and HPLC techniques (Bruschi et al, 2003a,b). For the spectrophotometric determination, 3.0 g of PM, 3.0 mL of acetone, and 3.0 mL of distilled water were added in a separation funnel. This mixture was extracted three times with 15 mL of ethyl acetate. Ethyl acetate was added to 50.0 mL, rendering M1. Exactly 1.0 mL of aluminium chloride ethanolic solution (2%, w/v) and methanolic solution of acetic acid (5%, v/v) was added into 10.0 mL of M1 to 25.0 mL, rendering M2. At the time, methanolic solution of acetic acid (5%, v/v) was added into 10.0 mL of M1 to 25.0 mL, to be used as compensatory solution. After 30 min, M2 was analyzed by Beckman coulter DU-640 spectrophotometer (Fullerton, CA, USA) at wavelength (λ) of 425 nm. Six replicates were carried out to estimate the inherent variability of the determination and the total flavonoids drift of each PES was calculated in grams of quercetin (specific absortivity = 500) obtained in 100 g of dried PM. For the HPLC, a 15 mg sample of each PM was added in acetonitrile to 25.0 mL and sonicated for 5 min. The dispersion was filtered through a modified PTFE membrane filter with pore size of 0.45 μm (Millipore, Bedford, USA) and the propolis concentration in the filtered dispersion was determined by quantifying the three major peaks (markers) of the obtained chromatogram. The HPLC analysis conditions and equipments were the same than those utilized to HPLC analyses of PES. The entrapment efficiency for propolis of the PM was calculated comparing the results obtained from the PES with the PM.

Microorganisms

A total of eight microbial strains, obtained from American Type Culture Collection (ATCC, Manassas, Virginia, USA), were tested: *Enterococcus faecalis* ATCC 4082, *Streptococcus salivarius* ATCC 25975, *Streptococcus sanguinis* ATCC 10556, *Streptococcus mitis* ATCC 49456, *Streptococcus mutans* ATCC 25175, *Streptococcus sobrinus* ATCC 33478, *Candida albicans* ATCC 28366, and *Lactobacillus casei* ATCC 11578. All strains were kept in laboratory and cryopreserved at −86°C. Before the experiments, the cultures were Gram-stained to determine purity.

Antimicrobial Activity Assay by Agar Diffusion

The antimicrobial activity of the samples was determined by agar diffusion (AD) by the well technique using the double layer agar system (Grove & Randall, 1955). The strains were suspended in 2.5 mL of the sterile Brain-Heart Infusion (BHI) medium. The standardization of each microorganism suspension was carried out using spectrophotometer (Femto, São Paulo, Brazil) at wavelength (λ) of 800 nm to match the transmittance of 90, equivalent to 0.5 McFarland scale (1.5×10^8 CFU/mL), except for the *C. albicans* strain suspension which was adjusted to have transmittance of 80, equivalent to 1.0 McFarland scale (3.0×10^8 CFU/mL). A 12.5 mL portion of the BHI agar (50°C) and 2.5 mL of each test suspension was gently mixed and poured on to a previously set layer of 25 mL BHI agar (125 × 25 mm Petri plate). After the solidification, the seed layer was perforated with a sterilized stainless-steel cylinder (inside diameter 4 mm) to form the wells, which were located 25 mm of the plate border and 40 mm halfway to each other. The PM, propolis (dissolved in dimethyl sulfoxide to 300 mg/mL), PES, ethanolic alcohol or dimethyl sulfoxide (negative controls), and aqueous solution of chlorhexidine gluconate (0.12%, w/w) as positive control, were applied inside the wells. The plates were kept for 2 h at room temperature to allow the diffusion of the agents through the agar. Afterwards, all the plates were incubated at 37°C in an appropriate gaseous condition and for 24–48 h in a CO_2 incubator (10% CO_2). Zones of inhibition of microbial growth around the wells containing the test samples were measured (mm) and recorded after the incubation time (Möller, 1966; Koo et al., 2000). Three replicates were made for each microorganism.

Determination of the Minimum Inhibitory Concentration (MIC)

The MIC determination of the propolis, PES, and PM were performed by the microplate dilution method (Andrews, 2001). Individual 24 h colonies from BHI agar plates were suspended in 10.0 mL of sterile TSB. The standardization of each microorganism suspension was carried out using spectrophotometer (Femto, São Paulo, Brazil) at wavelength (λ) of 800 nm to match the transmittance of 90, equivalent to

0.5 McFarland scale (1.5×10^8 CFU/mL), except for the *C. albicans* strain suspension which was adjusted to have transmittance of 80, equivalent to 1.0 McFarland scale (3.0×10^8 CFU/mL). In a sterilized microplate were poured 100 µl of TSB, microbial suspension, and the test sample (concentrations ranging from 0.078 to 300 µg/mL). Penicillin or miconazole were used as positive control and, without antibiotic addition, wells were used as negative control. After 37°C/24 h incubation 30 µL of 0.01% resazurin aqueous solution was poured in each microplate reservoir (for bacteria). The red color presence was interpreted as positive growth and the blue color presence as negative growth. In each microplate reservoir for the yeast, 40 µL of 0.5% tri-phenyl tetrazolium aqueous solution was poured. After 30 min more of incubation, the red color presence was interpreted as positive growth. The results were expressed as MIC, i.e., the minimum concentration which prevented visible growth of microorganisms (blue color or color absence). Three replicates were made for each microorganism.

RESULTS AND DISCUSSION

Characteristics of the PES and the PM

The two samples of propolis were collected in different seasons and resulted extractive solutions with different characteristics (Table 2).

The propolis extractive solution prepared from propolis P1 (PES1) displayed greater dryness residue than propolis extractive solution prepared from propolis P2 (PES2). In addition, the total flavonoids drift of PES1 was greater than PES2. Then, P1 had a greater amount of substances to be extracted by the ethanol, including flavonoids, than P2. The flavonoids constitute a very important class of polyphenols, widely present in propolis (Bankova et al., 1992), to which are attributed the great part of propolis biological activities (Burdock et al, 1998). Thus, the propolis collected in the dry season originated a PES with better qualities.

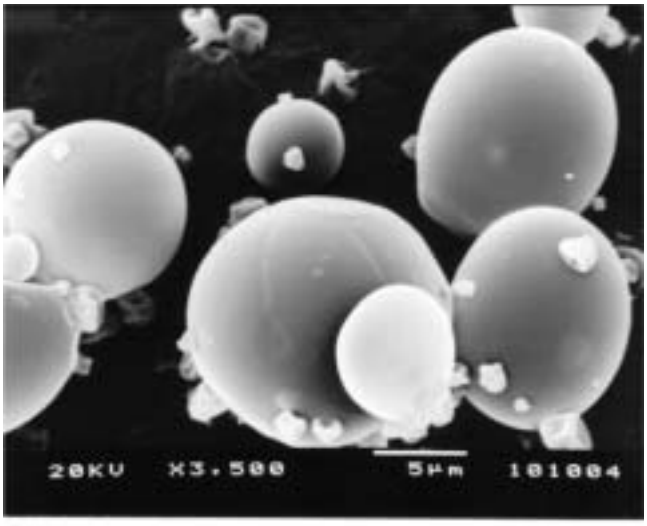
TABLE 2 **Characteristics of Propolis Ethanolic Extractive Solution**

Parameters	PES1[a]	PES2[b]
Dryness residue (%)	18.32 ± 0.41	17.57 ± 0.16
Total flavonoids drift (%)	2.53 ± 0.04	1.83 ± 0.02

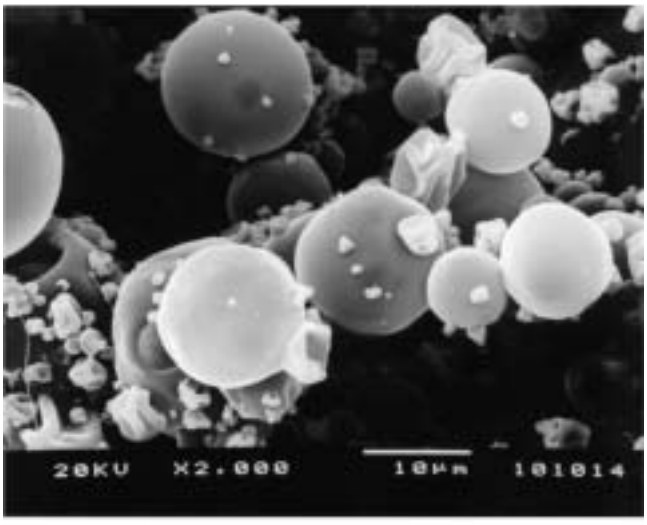
[a]From propolis sample P1 (dry season).
[b]From propolis sample P2 (humid season).

Propolis extraction solution (PES) characteristics, composition of dispersion, and spray-drying conditions gave propolis microparticles with a very smooth and uniform surface, and spherical shape (Fig. 1). A low number of coalesced microparticles and low agglomeration were observed both in propolis microparticles prepared from PES1 (PM1) and propolis microparticles prepared from PES2 (PM2), according to Bruschi et al. (2003a, 2004).

The typical size distribution graphs are presented in Fig. 2. The microparticles had a mean diameter of



(a)



(b)

FIGURE 1 **SEM Micrographs of Spray-dried Propolis Microparticles Showing the Outer Topology: (a) PM1 (Original Magnification × 3500); (b) PM2 (Original Magnification × 2000).**

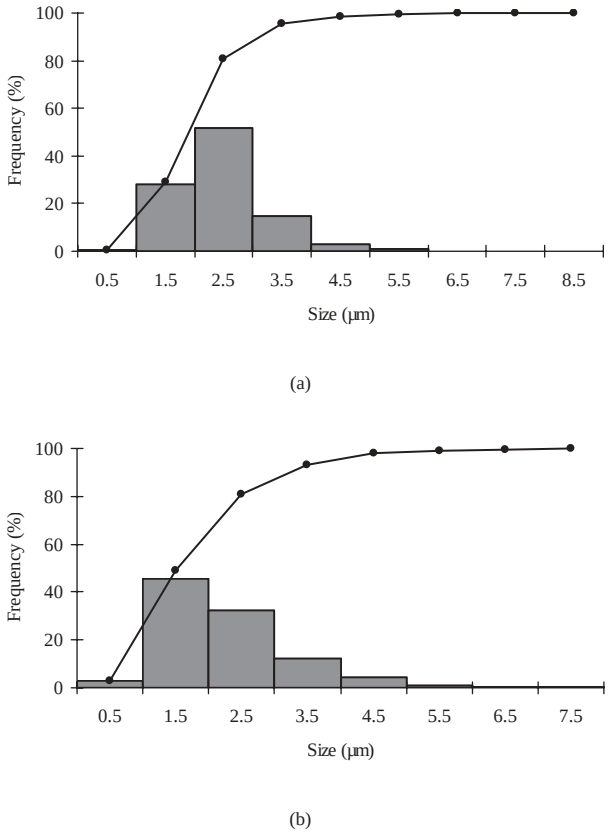


FIGURE 2 **Size Distribution of PM1 (a) and PM2 (b) Propolis Microparticles: Size Frequency Distribution (Bars) and Size Cumulative Frequency Distribution (Line). The Particle Size Class Interval Is 1.0 μm.**

2.27 μm (PM1) and 2.48 μm (PM2). These results confirm that obtained structures are microparticles.

To calculate the trapping efficiency for propolis of the microparticles, PES and PM were quantified by spectrophotometric and HPLC methods (Table 3).

The chromatograms of PES showed three major peaks that were used as markers (Fig. 3). These peaks are substances that correspond to the flavonoids (Bruschi et al., 2003b) and for each one, the propolis trapping efficiency was calculated (Table 3).

These PM characteristics are useful for development of propolis oral dosage form without the PES strong and unpleasant taste, aromatic odor, and high ethanol concentration.

Antimicrobial Activity Evaluation

The combination of temperature, small space, and humidity provide the beehives with good conditions for microbial growth. Nevertheless, this does not occur because of the antimicrobial activity of propolis (Ota et al., 2001). The antibacterial and antifungal activities are the most popular of the propolis, being between its biological actions more extensively investigated (Marcucci, 1995). Thus, antimicrobial activity of propolis, PES, and PM were evaluated by two different techniques (AD and MIC) against microorganism strains of American Type Culture Collection (ATCC) that have importance in oral diseases (Gibbons, 1984; Loesche, 1986; Seymour & Heasman, 1992; Azevedo et al., 1999; Koo et al., 2000).

The agar diffusion (AD) technique is very similar to that used to determine the sensitivity of bacteria to the drugs. Both the propolis and PES showed activity against the tested strains (Table 4). The PES obtained from P1 (PES1) and the PES obtained from P2 (PES2) showed inhibition zones from 2.74% to 46.15% and from 16.64% to 56.77% bigger than P1 and P2, respectively. However, the inhibition zones of propolis and their respective PES were similar against *C. albicans* and *L. casei*. The PES inhibition zones were more similar than those of chlorhexidine to some strains (*S. mitis*, *E. faecalis*, *S. sanguinis*, and *S. sobrinus*), indicating a good antimicrobial activity against these microorganisms. In addition, ethanolic alcohol (used to prepare PES) and dimethyl sulfoxide (used to dissolve the propolis) did not display a zone of microbial growth inhibition, indicating that they are not responsible for the propolis and PES antimicrobial activity observed.

TABLE 3 **Propolis Trapping Efficiency**

Propolis microparticles	Drug trapping efficiency (%)			
	HPLC method			Spectrophotometric method
	Marker 1	Marker 2	Marker 3	
PM1	32.46	61.67	41.87	86.44
PM2	27.03	67.23	67.85	73.90

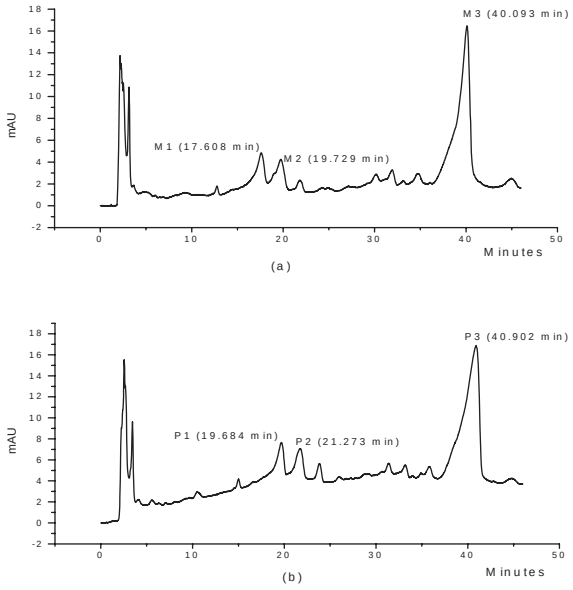


FIGURE 3 **HPLC Chromatograms of the Extractive Solutions: (a) PES2 and (b) PES1.**

In contrast, the PM didnot show activity against the tested strains (Table 4). The use of microparticles could improve or enhance the therapeutic effect of biomedical materials or drugs (Baras et al., 2000). Thus, the expected result would be a major size of PM inhibition zones than its respective PES, but inhibition was not observed when PM was tested. The inhibition zone size is the consequence of the microbial sensibility, solubility, and delivered amount of drug in the first hours after its use. The PM possesses substances with varied polarities (Kosonocka, 1991; Sforcin et al., 2000), many of which have little or no solubility in hydrophilic medium (agar), committing the liberation and diffusion of the extract substances with antimicrobial properties. In addition, Bruschi et al. (2004) showed that less polar compounds, as the marker 3, possess slower in water release and that the PM structure could hinder the exit of the markers, increasing the release time of them. In an agar medium, with low amount of free water, the PM substances release was probably prejudiced.

Those deductions were confirmed by the results obtained with the determination of the MIC (Table 5) where the propolis, PES, and PM showed activity against all the tested strains.

This result can be explained by the fact of the MIC technique to use TSB medium (and not agar), providing a larger amount of free water, facilitating the delivery and diffusion of the active substances. Considering that the propolis always presented activity, even with the variability of chemical composition in the different geographical areas, owed the different vegetables sources (Markham et al., 1996; Kujumgiev et al., 1999), similar problems obtained in this study were observed when it was verified that some authors reported propolis activity against *E. coli* and *P. aeruginosa* (Grange & Davey, 1990; Woisky et al., 1994; Tosi et al., 1996; Sforcin et al., 2000; De Vargas et al., 2004) and others did not (Kujumgiev et al., 1999; Nieva-Moreno et al., 1999). Bruschi et al. (2003a) reported the antibacterial activity of gelatin microparticles containing propolis against *S. aureus* using Mueller Hinton medium (broth). Analyzing the methodologies used in all those studies, it is observed that the techniques that facilitated the dissolution and/or diffusion of the extract and/or active substances in the medium were more adequate.

The MICs of the propolis samples were equal or lower than 200 μg/mL, showing good antimicrobial activity against the tested strains. The propolis were

TABLE 4 **Mean Diameter (in mm) of the Zones of Microbial Growth Inhibition Provided by Propolis, PES, Gelatin Microparticles Containing Propolis, and Chlorhexidine Gluconate**

	Propolis		PES		PM		
Microorganism	1	2	1	2	1	2	Chlorhexidine
E. faecalis (ATCC 4082)	9.68 ± 1.16	10.00 ± 1.00	13.00 ± 0.00	13.67 ± 1.53	0	0	14.33 ± 0.58
E. salivarius (ATCC 25975)	11.68 ± 2.31	11.33 ± 2.31	12.00 ± 1.00	16.33 ± 0.58	0	0	19.00 ± 1.00
S. sanguinis (ATCC 10556)	12.00 ± 0.00	14.00 ± 1.00	16.68 ± 1.16	16.33 ± 1.16	0	0	17.68 ± 0.58
S. mitis (ATCC 494560	16.33 ± 1.16	15.33 ± 0.58	18.00 ± 2.00	17.68 ± 1.16	0	0	16.67 ± 1.52
S. mutans (ATCC 25175)	13.68 ± 2.10	13.68 ± 1.16	15.00 ± 1.00	16.33 ± 1.53	0	0	24.67 ± 1.53
S. sobrinus (ATCC 33478)	13.00 ± 2.00	12.33 ± 1.16	19.00 ± 1.00	19.33 ± 1.53	0	0	21.00 ± 0.00
C. albicans (ATCC 28366)	10.00 ± 0.00	10.33 ± 0.58	9.00 ± 1.00	9.00 ± 0.00	0	0	16.00 ± 0.00
L. casei (ATCC 11578)	12.00 ± 2.65	10.67 ± 2.89	11.00 ± 2.00	11.33 ± 1.16	0	0	22.33 ± 2.52

TABLE 5 **Minimum Inhibitory Concentration (MIC) of Propolis (P), PES, Gelatin Microparticles Containing Propolis (PM)**

	MIC μg/mL						
Microorganism	P1	P2	PES1	PES2	PM1	PM2	Controls[a]
E. faecalis (ATCC 4082)	200	200	200	200	200	200	2.95 (P)
E. salivarius (ATCC 25975)	100	90	200	200	90	90	0.3688 (P)
S. sanguinis (ATCC 10556)	30	30	100	80	50	50	0.1844 (P)
S. mitis (ATCC 49456)	20	20	20	20	50	50	0.0461 (P)
S. mutans (ATCC 25175)	200	200	200	200	200	200	0.0922 (P)
S. sobrinus (ATCC 33478)	200	200	200	200	200	200	0.0922 (P)
C. albicans (ATCC 28366)	90	90	200	200	80	90	5.9 (M)
L. casei (ATCC 11578)	200	200	300	200	200	200	5.9 (P)

[a]P = penicillin and M = miconazole.

more effective against *S. sanguinis* and *S. mitis*, this late one being more susceptible to them. Similar results were observed to the extractive solutions, but showed bigger MICs against *E. salivarius*, *S. sanguinis*, *C. albicans*, and *L. casei* than its respective propolis. The lowest susceptibility to the PES was showed by *L. casei* strain, heading 300 μg/mL of PES1 to inhibit its growth. Moreover, the PES2 showed better results for *S. sanguinis* and *L. casei* than PES1.

Similar results were observed to the MIC of microparticles. But, considering the PES dryness residue (Table 2), the PES amount added to prepare the microparticles (Table 1), and the drug trapping efficiency of PM, that was not greater than 86.44% (Table 3), the PM activity was greater than the respective PES to some microorganisms. The MIC of microparticles was from 37.5% to 60% lower than the MIC of the respective PES, to *E. salivarius*, *S. sanguinis*, and *C. albicans*. In addition, the MIC results of PM were very similar to the propolis MIC values, except to *S. mitis* which was more susceptible to the propolis than to the PM.

Controls (penicillin and miconazole) displayed MIC values much lower than those of propolis, PES, and PM. However, the antimicrobial activity of extracts of natural products is very interesting at concentrations below 100 μg/mL (Ríos & Recio, 2005), as was obtained in this study against *E. salivarius* (propolis and PM), *S. sanguinis* (propolis, PES, and PM), *S. mitis* (propolis, PES, and PM), and *C. albicans* (propolis and PM). Moreover, the propolis can display antimicrobial synergism when administrated together with some antimycotic drugs (Holderna & Kedzia, 1987).

CONCLUSIONS

Propolis and PES showed in vitro antimicrobial activity against all the tested microorganism strains. Regarding the necessity of free water presence and the slow release of substances from gelatin microparticles containing propolis, the choice of the method to evaluate their antimicrobial activity is very important. In addition, considering that the applied technology can modify the biological activity of the drugs (Baras et al., 2000; Santoyo et al., 2002), the results indicated that the tested strains of different microorganisms of oral importance could be inhibited by gelatin microparticles containing propolis of similar way to the propolis and PES. The determination of the MIC results showed that the strains of *E. salivarius*, *S. sanguinis*, *S. mitis*, and *C. albicans* were more susceptible to the propolis. Otherwise, considering that in vitro cannot be directly extrapolated to in vivo effects (Azevedo et al., 1999), further studies are necessary to evaluate the in vivo antimicrobial activity of the gelatin microparticles containing propolis against oral pathogens.

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