Comparative Evaluation of *in-Vitro* Effects of Brazilian Green Propolis and *Baccharis dracunculifolia* Extracts on Cariogenic Factors of *Streptococcus mutans*

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Streptococcus mutans triggers dental caries establishment by two major factors: synthesis of organic acids, which demineralize dental enamel, and synthesis of glucans, which mediate the attachment of bacteria to the tooth surface. Propolis is a natural product that may prevent dental caries. *Baccharis dracunculifolia* DC (Asteraceae), a native plant from Brazil, is the most important botanical origin for the production of green propolis (Brazilian propolis) by honeybees. However, whether *B. dracunculifolia* (*Bd*) has an anticariogenic effect, like green propolis, remains unknown. Herein, we have made a comparative evaluation of the effects of extracts from green propolis and *Bd* on the glucan synthesis and acidogenic potential of *S. mutans*. The inhibitory effects of the extracts on bacterial acid production were evaluated through the potentiometric measurement of pH from bacterial suspensions treated with serial concentrations of both extracts. Besides presenting close inhibitory values at the same concentration range, *Bd* leaf rinse and green propolis extracts had similar IC_{50} values (0.41 and 0.34 mg/ml, respectively). Both extracts produced a bacteriostatic effect on *S. mutans* cultures at a concentration of 0.40 mg/ml. Estimated inhibitory values of green propolis and *Bd* leaf rinse extracts on the synthesis of insoluble glucans (IC_{50}=12.9 and 25.0 μg/ml, respectively) and soluble glucans (IC_{50}=50.4 and 49.1 μg/ml, respectively) were not significantly different from each other at $p<0.05$. The results demonstrate that *Bd* leaf rinse and green propolis extracts have similar inhibitory effects on the *S. mutans* cariogenic factors evaluated herein, and allowed us to suggest that *Bd* leaves may be a potential source for pharmaceutical products employed for this purpose.

Key words   *Baccharis dracunculifolia*; green propolis; *Streptococcus mutans*; cariogenic factor

Streptococcus mutans is considered one of the primary causative agents of dental caries. The main virulence factors associated with cariogenicity include adhesion, acidogenicity, and acid tolerance. These bacteria produce glucosyltransferases and synthesize glucans from sucrose (in particular, water-insoluble glucans), which mediate the adherence of *S. mutans* and other oral bacterial flora on tooth surfaces and contribute to the formation of dental plaque. Selection for a cariogenic flora in dental plaque increases the magnitude of the drop in pH following the fermentation of available carbohydrates and increases the probability of enamel demineralization, which leads to dental caries establishment.[1]

According to many authors,[2—6] the inhibition of each step during this process would result in the prevention of dental caries. The use of natural products has been a successful strategy for the discovery of new medicines.[7] Propolis, a natural resinous substance collected by honey bees 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.[8] Moreover, propolis may also prevent dental caries.[9—18]

Baccharis dracunculifolia DC (Asteraceae), a native plant from Brazil, is the most important botanical source of Southeastern Brazilian propolis, known as green propolis for its color.[19—21] In recent years, many studies have examined green propolis because of its characteristic chemical composition and biological activities.[22] In addition, several current studies have been conducted to demonstrate Brazilian propolis anticariogenic effects. On the other hand, whether *B. dracunculifolia* has the same effects remains unknown. Therefore, the aim of this study was to determine and compare the effects of green propolis and *B. dracunculifolia* extracts on the acid production and synthesis of glucans by glucosyltransferases from *S. mutans*. Furthermore, the possibility of using *B. dracunculifolia* extract for caries prevention is discussed.

MATERIALS AND METHODS

Bacteria   *Streptococcus mutans* ATCC 25175 was kindly provided by Dr. Izabel Yoko Ito from the University of São Paulo, Faculty of Pharmaceutical Sciences, at Ribeirão Preto. The culture was stored at −70 °C in Brain Heart Infusion (Oxoid) containing 20% (w/v) glycerol.

Preparation of Green Propolis and *B. dracunculifolia* Extracts   Propolis samples were collected in Cajuru, São Paulo state, Brazil, in December 2001 from *Apis mellifera* hives. Leaves of *B. dracunculifolia* were collected from the same field and period from the neighboring hives. The plant material was authenticated by Jimi N. Nakagima, and a voucher specimen (SPFR 06143) was deposited in the herbarium of the Biology Department of the University of São Paulo (FFCLRP) at Ribeirão Preto, São Paulo state, Brazil. Both samples were air-dried at 40 °C for 48 h. The dried propolis sample was powdered (3.0 g), and exhaustively extracted with ethanol : H_2O, 9 : 1 (v/v) at room temperature by maceration. After filtration, the solvent was concentrated under vacuum below 40 °C to furnish the dried crude green

propolis extract (GPE), which contained 149.0 μmol/l equivalents of gallic acid (eq. G.A.) of total phenolic compounds, according to the Folin-Ciocalteu method.[23] The leaf rinse extract of *B. dracunculifolia* (Bd-LRE) was obtained by immersing the air-dried leaves (680.0 g) in dichlorometane for thirty seconds at room temperature, in order to extract the exudate from its glandular trichomes. The solvent was also removed under vacuum below 40 °C, affording 35.0 g of the Bd-LRE, which contained 116.0 μmol/l eq. G.A. of total phenolic compounds. The Bd-LRE was selected for this study as a result of our recent investigations, on the basis of its effect on growth and acid production of *S. mutans* when compared to extracts and fractions from different parts of *B. dracunculifolia*.[24]

In Vitro* Effect on Acid Production by *S. mutans Growth conditions for *S. mutans*, as well as the acidogenic activity assay, have been described in a previous work.[25] Briefly, *S. mutans* ATCC 25175 was grown in Trypticase Soy Broth (Oxoid) supplemented with 1.0% glucose (w/v), at 37 °C, and harvested in the exponential growth phase by centrifugation at 6000 g for 10 min. Bacterial cells were washed twice with sterile phosphate buffer saline solution (PBS), pH 7.4, containing Na_2HPO_4 1.15 g/l (w/v), NaCl 8.0 g/l (w/v) and KCl 0.2 g/l (w/v). Washed cells were then resuspended in sterile buffered solution containing 20.0% (w/v) glycerol to obtain a bacterial suspension of approximately 5.0 mg wet weight cells/ml, which was divided into 1.0 ml aliquots (*ca.* 5.0 mg wet weight cells) in sterile polyethylene tubes and stored at −70 °C. The strain glycolitic activity was checked regularly, as recommended by Dashper and Reynolds.[26]

For the acidogenic activity assay, aliquots of 1.0 ml of *S. mutans* suspensions were defrosted at 37 °C in a water-bath and centrifuged (15300 g for 2 min, room temperature) to remove the glycerol solution. Cell pellets were resuspended in 6.0 mmol/l phosphate buffer, pH 6.9, containing 0.865 g/l (w/v) NaCl, 0.625 g/l (w/v) KCl, 0.125 g/l (w/v) $MgCl_2 \cdot 6H_2O$, 0.072 g/l (w/v) $CaCl_2 \cdot 2H_2O$, 0.326 g/l (w/v) KH_2PO_4, and 0.803 g/l (w/v) K_2HPO_4 supplemented with 10.0 mmol/l glucose as a bacterial substrate and extract stock solution of green propolis extract (GPE) or *B. dracunculifolia* leaf rinse extract (Bd-LRE) (final concentrations ranged from 0.1 to 4.0 mg/ml). Both extracts were dissolved in dimethyl sulfoxide (DMSO)–ethanol (1 : 3, v/v) just prior to performance of the assays.

A thin-bulb analytical electrode (model 8103, Orion) was placed in the cell suspension, which was warmed to 37 °C, and the glycolitic production of acid was monitored during a 30-min time period on the basis of pH decay measured with a pH meter (model 710 A, Orion). The negative control test (no inhibition) consisted of *S. mutans* aliquots resuspended in the same buffer solution supplemented with 10.0 mmol/l glucose. The positive control test (no acid production) consisted of *S. mutans* aliquots resuspended in the same buffer solution supplemented with 10.0 mmol/l glucose and Periogard® (Colgate), a commercially available mouthrinse containing the antimicrobian chlorexidine digluconate 0.06% (final concentration). Appropriate solvent controls were also included.

The pH curves (pH *versus* time) were built from a plot of the average values of triplicate and/or duplicate assays, and their integrated area values were used to estimate the in-

hibitory activity of both extracts at each concentration tested, considering that no acid production was observed in the positive control (*i.e.*, abscence of pH decay), and the negative control pH curve represented no inhibition of the acid production. Inhibition curves (log concentration *versus* activity) were plotted, and 50% inhibitory concentrations (IC$_{50}$; the concentration of extract required to inhibit acidogenic activity by 50%) were calculated from non-linear regression sigmoidal curves using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, California, U.S.A.).

Effect on Growth of *S. mutans* The effect of a sublethal concentration of GPE and Bd-LRE on the growth of *S. mutans* was estimated by measuring kinetically the development of turbidity (*i.e.* growth) in extract-treated cultures.

For the growth assay, each extract was dissolved in DMSO and diluted in Brain Heart Infusion broth (BHI, Oxoid) to a final concentration of 0.4 mg/ml (w/v). Overnight cultures of *S. mutans* grown in BHI broth at 37 °C were then added (1% v/v) to a final volume of 2.0 ml of the extract-treated broth, which was placed in sterile glass cuvettes and incubated in a spectrophotometer model CPS-240A (Shimadzu) with continuous agitation at 37 °C for 9 h. The absorbance (660 nm) of each culture medium was measured at 30-min intervals throughout the incubation period. Controls grown in BHI broth alone were set up in parallel. All assays were performed in triplicate.

Preparation of Glucosyltransferases *S. mutans* ATCC 25175 was grown in a shaker (100 rpm) for 24 h at 37 °C in 6.0 l of low-molecular-weight broth containing 2.5% (w/v) Tryptone, 1.5% (w/v) yeast extract, 0.3% (w/v) glucose, 0.1% (w/v) fructose, 0.1% (w/v) sorbitol, 0.1% (w/v) $MgSO_4$ and 0.5% (w/v) K_2HPO_4.[15] Cell pellets were harvested in the exponential growth phase by centrifugation at 6000 g for 15 min at 4 °C, and were washed twice with 10.0 mmol/l sodium phosphate buffer pH 6.5. The cells were then resuspended in 30 ml of 8.0 mol/l urea and incubated at 4 °C for 1 h with gentle agitation.[4] The cell suspension was centrifuged as previously described, and the supernatant was ultrafiltered through a membrane with a 100 kDa molecular size cut-off coupled to a Pellicon® ultrafiltration system. The retained fluid (molecular size>100 kDa), which was used as the source of glucosyltransferases (GTFs), was carefully collected, concentrated in polyethyleneglycol 20000 (Merck) using a 12.0—14.0 kDa molecular weight cut-off membrane (Sigma), and extensively dialysed against 10.0 mmol/l sodium phosphate buffer pH 6.5. The dialyzed preparation was designated as the crude cell-associated GTFs solution, and its total protein content was estimated according to the method of Bradford,[27] using bovine serum albumin (Sigma) as a standard.

Effect on Insoluble and Soluble Glucan Synthesis by Glucosyltransferases The GTFs activity assay was performed according to Sakanaka *et al.*[28] and Yanagida *et al.*[4] Both extracts to be tested (GPE and Bd-LRE) were dissolved in DMSO just prior to performance of the assays. A 0.1 mol/l sodium phosphate buffer, pH 6.5, containing 42.0 mmol/l sucrose, 40.0 mmol/l dextran T-10 (Amersham Biosciences) and 0.01% (w/v) thimerosal, was prepared as the substrate solution to which the crude cell-associated GTFs preparation was added (30.0 μg/ml of total protein; final concentration). To measure GTFs activity and its inhibition by GPE and Bd-

LRE, the reaction mixture was incubated with stock solutions of extracts (1.0 to 100.0 µg/ml; final concentration) at 37 °C for 15 h, in a total volume of 3.0 ml. The reaction was terminated by placing the reaction tubes in a boiling water bath for 5 min.

The water-insoluble glucan formed was sedimented at 5000 *g* for 15 min at 4 °C and washed three times, each with 5.0 ml of distilled water. The sediment was smoothly suspended in 1.0 ml of distilled water, and a sample of 0.1 ml of the sediment was assayed for total carbohydrate determination, at 490 nm by the phenol–sulphuric acid method,[29] using glucose as a standard. For the analysis of water-soluble glucan, 1.0 ml of the supernatant was precipitated with four volumes of absolute ethanol. The precipitate was washed three times with 5.0 ml of 75.0% (v/v) ethanol, dissolved in 1.0 ml of distilled water, and assayed by the phenol–sulfuric acid method.

Statistical Analyses Intergroup differences of various factors were estimated by statistical one-way analysis of variance (ANOVA). The Dunnett's post test was applied for comparison between each treatment concentration and the respective control. Student's *t* test was applied to compare two paired groups of treatments. The chosen level of significance for all statistical tests was $p < 0.05$. Statistical computation was performed using GraphPad Prism version 3.00 for Windows.

RESULTS

In Vitro* Effect on Acid Production by *S. mutans The glycolytic production of acids by *S. mutans* ATCC 25175 in the presence of green propolis extract (GPE; 0.06—4.0 mg/ml final concentration) and *B. dracunculifolia* extract leaf rinse extract (Bd-LRE; 0.1—1.0 mg/ml final concentration) was evaluated during 30 min of incubation, as illustrated in Fig. 1. The inhibitory effects of GPE and Bd-LRE on acid production by *S. mutans* were examined as a function of their concentrations. Both GPE and Bd-LRE reduced the rate of acid production by *S. mutans*, and the effectiveness of inhibition was dependent on the extract concentrations in the reaction mixture. A significant reduction of bacterial acid production was found at concentrations of more than 0.1 mg/ml for GPE and 0.2 mg/ml for Bd-LRE ($p < 0.05$), when compared to the negative control by Dunnett's statistical test of variance. Although the dose–response relation of the two extracts was represented by curves with different slopes, GPE and Bd-LRE exhibited close IC_{50} values, which were estimated at 0.34 and 0.41 mg/ml, respectively (Fig. 2). GPE suppressed bacterial acid production by 92.1% at a concentration of 4.0 mg/ml while Bd-LRE presented a maximum inhibitory activity of 82.5% at 1.0 mg/ml.

Effect on Growth of *S. mutans* The growth profiles of *S. mutans* ATCC 25175 exposed to sub-lethal concentrations of GPE and Bd-LRE were established by the plot of absorbance (660 nm) of the cultures *versus* incubation time, as illustrated in Fig. 3. A concentration of 0.4 mg/ml (w/v) (an approximate estimate of acidogenic potential IC_{50}) was fixed for both extracts.

For the control cultures (not exposed to extracts), a simple sigmoid growth curve was obtained, and it can be divided into two parts: the lag phase (no growth) and the exponential
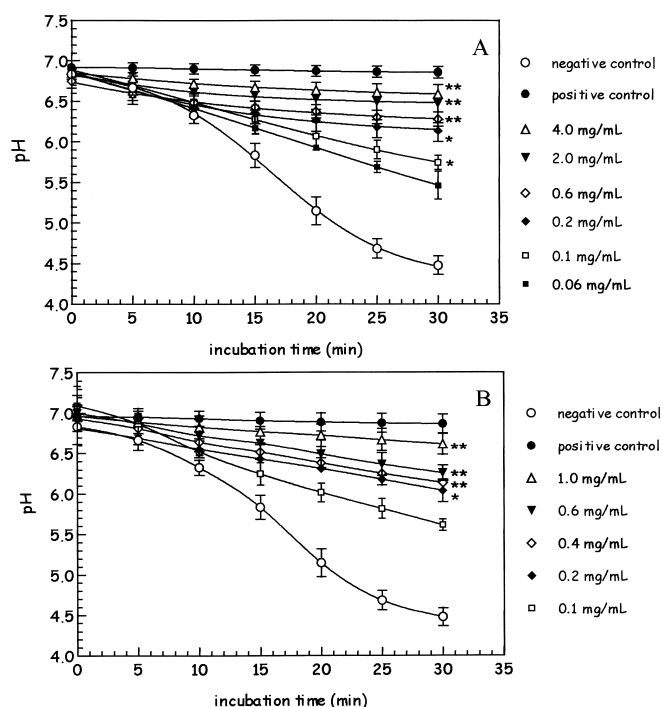

Fig. 1. Inhibitory Effects of (A) Green Propolis Extract, GPE and (B) *B. dracunculifolia* Leaf Rinse Extract, Bd-LRE on the Acid Production by *S. mutans* ATCC 25175

The pH decay of bacterial medium results from the glycolitic production of acid by *S. mutans* in the presence of glucose. Statistically significant difference between control (no inhibition) and treatment with extract (∗$p < 0.05$, ∗∗$p < 0.01$).
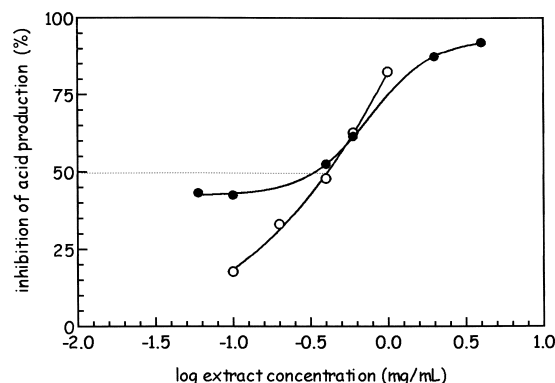

Fig. 2. Dose–Effect Curves of Log Concentration *versus* Inhibition of Bacterial Acid Production (%) Provoked by the Incubation of *S. mutans* ATCC 25175 with (●) Green Propolis Extract, GPE and (○) *B. dracunculifolia* Leaf Rinse Extract, Bd-LRE

Final concentrations of extracts in reaction mixture ranged from 0.06 to 4.0 mg/ml.

phase (maximum growth). The control culture lag phase lasted approximately 4 h when the exponential phase took place to reach absorbance values as high as 1.2326±0.0690. The mean exponential phase slope was estimated at 0.3342± 0.0053.

A significant reduction of *S. mutans* growth was found in cultures exposed to 0.4 mg/ml of GPE or Bd-LRE ($p < 0.01$), when compared to the control cultures by Dunnett's statistical test of variance. On the other hand, for both treatments, bacterial growth was not completely supressed, as a slight development of turbidity was observed during the first hours of incubation, followed by the establishment of a stationary
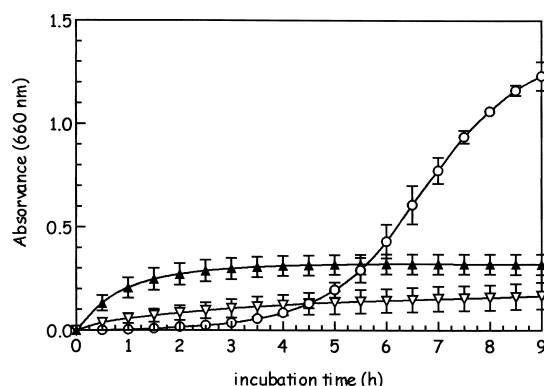
Fig. 3. Growth of *S. mutans* ATCC 25175 Exposed to (▲) Green Propolis Extract, GPE and (▽) *B. dracunculifolia* Leaf Rinse Extract, Bd-LRE in BHI at 37 °C was Monitored for 9 h by the Absorbance Measurement (660 nm) of the Cultures

Final concentration of extracts in reaction mixture was 0.4 mg/ml (approximate value of acid production IC_{50} estimated for both extracts). Control cultures (○) were not exposed to extracts. Absorbance *versus* time graph of triplicates form one growth curve.

phase (growth slow-down and stop). No lag phase was observed in either of the extract-treated culture profiles. The exponential phase slopes estimated for GPE and Bd-LRE treatments were 0.2651 ± 0.0121 and 0.0699 ± 0.0077, respectively. The highest absorbance values reached by the extract-treated cultures ranged from 0.3190 ± 0.0502 for GPE to 0.1679 ± 0.0896 for Bd-LRE, and treatments with both extracts were found to be significantly different from each other at the tested concentration ($p < 0.01$).

Effect on Insoluble and Soluble Glucan Synthesis by Glucosyltransferases The inhibitory effects of GPE and Bd-LRE on water-insoluble and soluble glucan synthesis by cell-associated glucosyltransferases (GTFs) from *S. mutans* were also examined as a function of their concentrations, and are illustrated in Fig. 4. The inhibitory effects of the extracts were graphically expressed as the relative amount (%) of glucans produced at a certain extract concentration as compared to the amount produced in the absence of any extract (*i.e.*, the control test where no inhibitory effect is observed).

As shown in Fig. 4A, both GPE and Bd-LRE concentrations higher than 25.0 μg/ml suppressed insoluble glucan synthesis by GTFs from *S. mutans* by 67.3% and 50.0%, respectively. Furthermore, a significant reduction of insoluble glucan synthesis by GTFs was found at concentrations of more than 10 μg/ml, for both extracts ($p < 0.001$), when compared to the negative control by Dunnett's test for analysis of variance. However, the IC_{50} value (concentration needed for 50% inhibition of insoluble glucan synthesis) was slightly different between GPE and Bd-LRE, that is, the IC_{50} of GPE and Bd-LRE were estimated at 12.9 and 25.0 μg/ml, respectively, yet these values were not significantly different from each other at $p < 0.05$ when treatments with both extracts were compared by Student's *t* test.

Figure 4B shows the inhibition of soluble glucan synthesis with increasing concentrations of GPE and Bd-LRE. Similarly, both GPE and Bd-LRE at a concentration of 100.0 μg/ml suppressed soluble glucan synthesis by GTFs from *S. mutans* by 63.0% and 83.7%, respectively. A significant reduction of soluble glucan synthesis by GTFs was found at concentrations higher than 20.0 μg/ml for both extracts ($p < 0.05$). Moreover, both extracts exhibited close IC_{50} val-
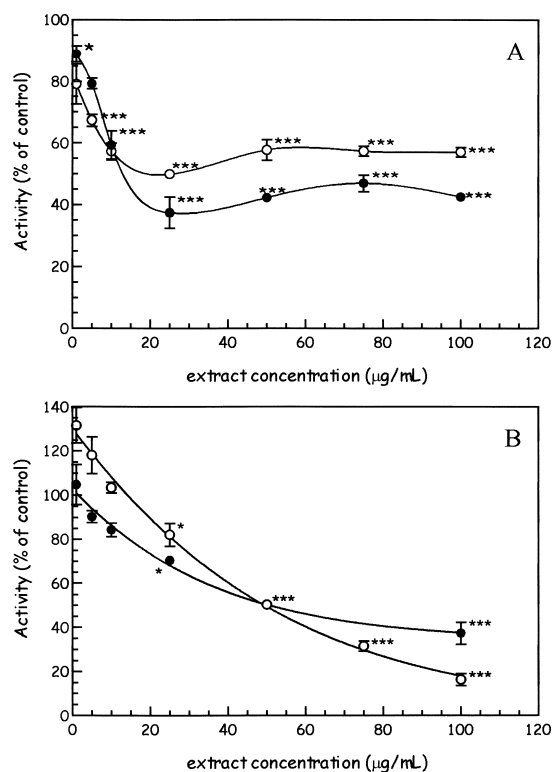

Fig. 4. Inhibitory Effects of Green Propolis Extract, GPE and *B. dracunculifolia* Leaf Rinse Extract, Bd-LRE on (A) Insoluble Glucan and (B) Soluble Glucan Synthesis by Cell-Associated Glucosyltransferases of *S. mutans* ATCC 25175

The % glucan synthesis means the relative amount (%) of glucan produced at a certain extract concentration as compared to the amount produced in the control test. Each value indicates mean±S.E. ($n=3$). Statistically significant difference between control (no extract) and treatment with extract (*$p < 0.05$, ***$p < 0.001$). Symbols: (●) GPE; (○) Bd-LRE treatment.

ues for soluble glucan synthesis, which were estimated at 50.4 μg/ml (GPE) and 49.1 μg/ml (Bd-LRE). It is worth noting that at concentrations of more than 50.0 μg/ml, the inhibitory values of soluble glucan synthesis obtained with Bd-LRE were slightly higher than those obtained with GPE, although treatments with both extracts were not significantly different from each other at $p < 0.05$.

DISCUSSION

Over the past six years, many studies showing the inhibitory effects of propolis extracts from various regions of Brazil on the synthesis of water-insoluble glucans by GTFs of *S. mutans* were examined *in vitro*. Park *et al.*[10] have demonstrated that crude ethanolic extracts of propolis samples from Southeastern, Southern and Northeastern Brazil presented inhibitory values ranging from 8.0 to 30.5%. Furthermore, Koo *et al.*[13] found that crude ethanolic extracts of propolis from Southeastern (Minas Gerais state) and Northeastern Brazil (Bahia state) showed inhibitory doses against the GTFs of *S. mutans* Ingbritt 1600, ranging from 6.2 to 200.0 μg/ml, while propolis extract samples from Southern Brazil (Rio Grande do Sul state) provoked the same effect within a lower range of concentrations (6.2 to 50.0 μg/ml). Recently, Duarte *et al.*[18] evaluated the effects of the crude ethanolic extract of propolis from Northeastern Brazil, which effectively inhibited the activity of *S. mutans* GTFs in solu-

tion by more than 80.0% at 500.0 μg/ml. Our results on propolis effects are in agreement with data previously published. Notably, similar inhibitory effects on glucan synthesis (50.0 to 83.7%) were obtained herein, with lower concentrations of green propolis extract, GPE and *B. dracunculifolia* leaf rinse extract, Bd-LRE (25.0 to 100.0 μg/ml), although differences among experimental protocols and bacterial strains should be taken into account.

The glucans synthesized by GTFs not only promote the accumulation of cariogenic streptococci on the tooth surface, but also contribute significantly to the bulk of dental plaque,[30] and therefore the inhibition of GTF activity is one of the important factors in the inhibition of bacterial cellular adherence in caries prevention.

In spite of the fact that GPE and Bd-LRE inhibitory values obtained for soluble glucan synthesis were more expressive than those obtained for insoluble glucan synthesis at the highest concentration tested (100.0 μg/ml), the 50% inhibitory doses (IC$_{50}$) of both GPE and Bd-LRE against insoluble glucan synthesis were significantly lower (12.9 μg/ml and 25.0 μg/ml, respectively) than those IC$_{50}$ values estimated for soluble glucan synthesis by the same extracts (50.4 μg/ml and 49.1 μg/ml, respectively), which means that lower amounts of both extracts were required to provoke 50% inhibition of insoluble glucan synthesis. This may be a desirable finding, considering that water-insoluble glucans, which are synthesized by GTFB and GTFC, are practically responsible for the attachment of *S. mutans* to the tooth surface,[31] and for that reason constitute one of the main targets for interfering with *S. mutans* cariogenicity.

It can be observed from Fig. 4A that GPE and Bd-LRE presented similar inhibitory profiles for the synthesis of insoluble glucans (the same observation can be made for soluble glucan synthesis, Fig. 4B), which suggests that both GPE and Bd-LRE interacts with glucosyltransferases by analogous mechanisms.

The production of acids from fermentable carbohydrates is another cariogenic factor of *S. mutans* that deserves attention during the investigation of new medicines to be employed in dental care. The effect of GPE and Bd-LRE on the acidogenic potential of *S. mutans* was evaluated, and significant reductions in bacterial acid production were achieved with concentrations in the range of 0.1—0.2 mg/ml (w/v). Both extracts also presented close IC$_{50}$ values, which were estimated at about 0.4 mg/ml (w/v). Notwithstanding, it would be premature to suppose that this inhibitory activity might be due to a direct effect on the bacterial glycolytic pathway, but then, it is important to suggest that the inhibitory effect on acid production may be due to the antibacterial activity of both extracts. In a recent investigation,[24] we briefly screened GPE and Bd-LRE for their antibacterial activity on *S. mutans* using an agar-well diffusion method with positive results, although the study lacked quantitative estimations. Herein, the effect of GPE and Bd-LRE on the growth of *S. mutans* was assessed at a fixed concentration of 0.4 mg/ml (w/v) using an absorbance method in order to evaluate whether both extracts significantly inhibited *S. mutans* acidogenic potential due to an antibacterial activity. In both treatments, slight development of turbidity in the first hours of incubation, followed by a long stationary phase, suggests a bacteriostatic effect of GPE and Bd-LRE at the tested concentration. Interestingly,

no lag phase was observed in either of the extract-treated culture profiles. In the lag phase, the initial number of cells does not increase, because in a new environment cells take time to adjust as they may need to synthesise new enzymes, repair any cell damage and initiate plasmid or chromosomal replication.[32] Therefore, the experimental observation suggests that GPE and Bd-LRE may inhibit bacterial metabolic reactions that precede cell doubling, or may trigger precocious bacterial cell multiplication as an answer to the aggressive agent.

A great variety of phenolic compounds have been identified from Brazilian propolis samples, such as flavonoids, diterpenes, triterpenic, lignans, and acetophenones, as well as prenylated *p*-coumaric acids. Further, prenylated phenylpropanoids, which are particularly found in green propolis, were also identified in *B. dracunculifolia* and other South American *Baccharis* species as secondary metabolite phenolic compounds.[33,34]

In spite of its biological activities, the chemical composition of propolis can change depending on several factors, including the site of collection and the plant sources of propolis.[8] This leads to difficulty in standardizing raw material and commercial products for medicinal purposes.[8] For this reason, we understand that if *B. dracunculifolia* and green propolis present comparable anticariogenic activity, *B. dracunculifolia* extracts could be successfully incorporated into pharmaceutical products employed in dental care.

This is the first time that this plant has been bioassayed and compared to green propolis. Thus, more biochemical and phytochemical investigations are necessary in order to investigate the biological activities of secondary metabolites of *B. dracunculifolia* on cariogenic factors of *S. mutans*.

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