Available online at www.sciencedirect.com
ScienceDirect
Phytomedicine 15 (2008) 886–891
Phytomedicine
www.elsevier.de/phymed

Antimicrobial activity of *Rheedia brasiliensis* and 7-epiclusianone against *Streptococcus mutans*

L.S.B. Almeida[a], R.M. Murata[a], R. Yatsuda[b], M.H. Dos Santos[c], T.J. Nagem[d], S.M. Alencar[e], H. Koo[f], P.L. Rosalen[a,*]

[a]*Department of Physiological Sciences, Faculty of Dentistry of Piracicaba, State University of Campinas, SP, Brazil*
[b]*Center of Technology in Health, Federal University of Bahia, Vitória da Conquista, BA, Brazil*
[c]*Laboratory of Phytochemistry and Medicinal Chemistry, Federal University of Alfenas, MG, Brazil*
[d]*Department of Chemistry-ICEB, Federal University of Ouro Preto, MG, Brazil*
[e]*Department of Agri-Food Industry, Food and Nutrition, Escola Superior de Agricultura Luiz de Queiroz, University of São Paulo, Piracicaba, SP, Brazil*
[f]*Center for Oral Biology, Eastman Department of Dentistry, University of Rochester Medical Center, Rochester, NY, USA*

Abstract

This *in vitro* study evaluated the antimicrobial activity of extracts obtained from *Rheedia brasiliensis* fruit (bacupari) and its bioactive compound against *Streptococcus mutans*. Hexane, ethyl-acetate and ethanolic extracts obtained (concentrations ranging from 6.25 to 800 μg/ml) were tested against *S. mutans* UA159 through MIC/MBC assays. *S. mutans* 5-days-old biofilms were treated with the active extracts (100 × MIC) for 0, 1, 2, 3 and 4 h (time-kill) and plated for colony counting (CFU/ml). Active extracts were submitted to exploratory chemical analyses so as to isolate and identify the bioactive compound using spectroscopic methods. The bioactive compound (concentrations ranging from 0.625 to 80 μg/ml) was then tested through MIC/MBC assays. Peel and seed hexane extracts showed antimicrobial activity against planktonic cells at low concentrations and were thus selected for the time kill test. These hexane extracts reduced *S. mutans* biofilm viability after 4 h, certifying of the bioactive compound presence. The bioactive compound identified was the polyprenylated benzophenone 7-epiclusianone, which showed a good antimicrobial activity at low concentrations (MIC: 1.25–2.5 μg/ml; MBC: 10–20 μg/ml). The results indicated that 7-epiclusianone may be used as a new agent to control *S. mutans* biofilms; however, more studies are needed to further elucidate the mechanisms of action and the anticariogenic potential of such compound found in *R. brasiliensis*.

Keywords: *Rheedia brasiliensis*; Antimicrobial activity; 7-Epiclusianone; *Streptococcus mutans*

Introduction

Despite the advances concerning its prevention and control, dental caries is still considered a public health problem that affects many countries in the world (Bowen, 2002). Dental caries occurs by the action of acidogenic and aciduric bacteria (Hamada and Slade, 1980), which interact with other microorganisms in biofilms on dental surfaces (Marsh, 2003). Because of their role in the process of dental caries disease, some mutans streptococci have been extensively studied regarding their actions in dental biofilm and their

*Corresponding author. Tel.: +55 19 2106 5313; fax.: +55 19 2106 5218.
E-mail address: rosalen@fop.unicamp.br (P.L. Rosalen).

0944-7113/$ - see front matter © 2008 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2007.12.003

virulence factors, including their acid tolerance and synthesis of glucans (Duarte et al., 2003; Lemos et al., 2005). The *Streptococcus mutans* is an important pathogen related to the initiation of dental caries in animals and humans (Loesche, 1986). It occurs specially because of its capability of sucrose-dependent adhesion and acid production, which leads to the consequent enamel demineralization (Belli and Marquis, 1991; Paes Leme et al., 2006).

The reduction in bacterial biofilm or specific pathogens associated with caries lesion using therapeutic agents with action against *S. mutans* and its virulence factors is a very common, effective approach for the prevention and control of caries (Baehni and Takeuchi, 2003).

Many studies on caries-related microorganisms have shown that some natural products can interfere with survival and virulence factors of *S. mutans*. A remarkable anticariogenic potency was also observed for natural bioactive compounds tested *in vitro* and *in vivo* (Koo et al., 2002a, 2003; Yatsuda et al., 2005; Duarte et al., 2006). Thus, medicinal plants are a potential source of biomolecules that must be investigated as an adjunctive therapy to control cariogenic dental biofilms.

The great biodiversitiy of plants found in Brazil might serve as an important source of new pharmacological agents (Basso et al., 2005). The Guttiferae represents a large family of medicinal plants with approximately 1350 species, many of which are known for their fine-flavored fruits (Campbell, 1996). In addition, some genus, such as *Rheedia*, have many types of substances with various pharmacological properties (Bakana et al., 1987; Delle Monache et al., 1991; Gustafson et al., 1992; Dos Santos, 1996; Yamaguchi et al., 2000; Ito et al., 2003).

Previous studies have reported the presence of flavonoids, xanthones and polyprenylated benzophenones in some species of *Rheedia*, by analyzing their chemical constituents (Delle Monache et al., 1983; Dos Santos et al., 1999). One class of these compounds, the polyprenylated benzophenones, showed good antibacterial activity against important pathogens (Dos Santos, 1996; Alves et al., 1999), suggesting that *Rheedia* is a promising chemical medicinal genus.

Rheedia brasiliensis Planch. & Triana (Syn. *Garcinia brasiliensis* Mart.), considered a very known species of genus *Rheedia*, is a plant native to the Amazon region. Its fruit is popularly known as "bacupari" or "bacoparé" and is widely used in folk medicine and also by natives of that region to prepare candies and medicines (Corrêa, 1978; Morton, 1987).

Since no studies on *R. brasiliensis* activity against oral pathogens were found in the literature, the present study analyzed the activity of *R. brasiliensis* fruit extracts against *Streptococcus mutans* and also the isolation and identification of the bioactive compound obtained from the active fraction.

Materials and methods

Extracts and 7-epiclusianone

R. brasiliensis fruits were collected from trees grown under controlled conditions at the herbarium of the University of Viçosa (latitude 20°45′14″ south and longitude 42°52′55″ west), Minas Gerais, Brazil, where its voucher specimen is deposited (number VIC2604). To obtain the extracts, the peel and the seeds were dried and powdered, and then treated with *n*-hexane at room temperature to allow chemical fractioning based on the polarity gradient of such solvents as hexane, ethyl-acetate and ethanol (peel) and hexane (seed), using the Soxhlet equipment for 24 h (Dos Santos et al., 1999). Each extract concentration was obtained under reduced pressure to allow peel extracts from hexane, ethyl-acetate and ethanol and seed extract from hexane, which were finally chromatographed as described by Dos Santos et al. (1999). To obtain the bioactive compound, peel hexane extract (PHE) and seed hexane extract (SHE) were chromatographed on a silica gel column and eluted with crescent polarity mixtures of *n*-hexane/ethyl-acetate and ethyl-acetate/ethanol. Its structure was identified as the polyprenylated benzophenone 7-epiclusianone [m.p. 92–93 °C (MeOH); $[\alpha]_D^{25} + 77$ (*c* 0.1, $CHCl_3$)] (Fig. 1) using several spectroscopic techniques (IR, UV, MS and NMR). The data were compared with those verified in a previous study investigating the chemical structure of this compound (Dos Santos et al., 1998, 1999). The purity (99.85%) of the bioactive compound and the retention time (17.961 min in peel hexane extract and 17.959 min in seed hexane extract) were determined by HPLC (Shimadzu LC-10A), using a C18 column (150–4.6 mm) with a 5-μm particle size (Fig. 2). The suitable gradient was achieved using MeOH:acetic acid 5%, pH 3.84, (40:60 v/v) to MeOH 100% for 10 min, with a solvent flow rate of 1.2 ml/min, at 30 °C, $\lambda = 254$ nm. ClassVP-LC10 software was used for data collection.

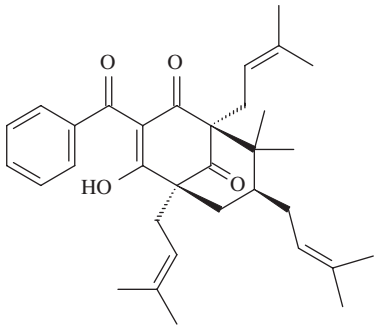

Fig. 1. Chemical structure of 7-epiclusianone, a polyprenylated benzophenone.

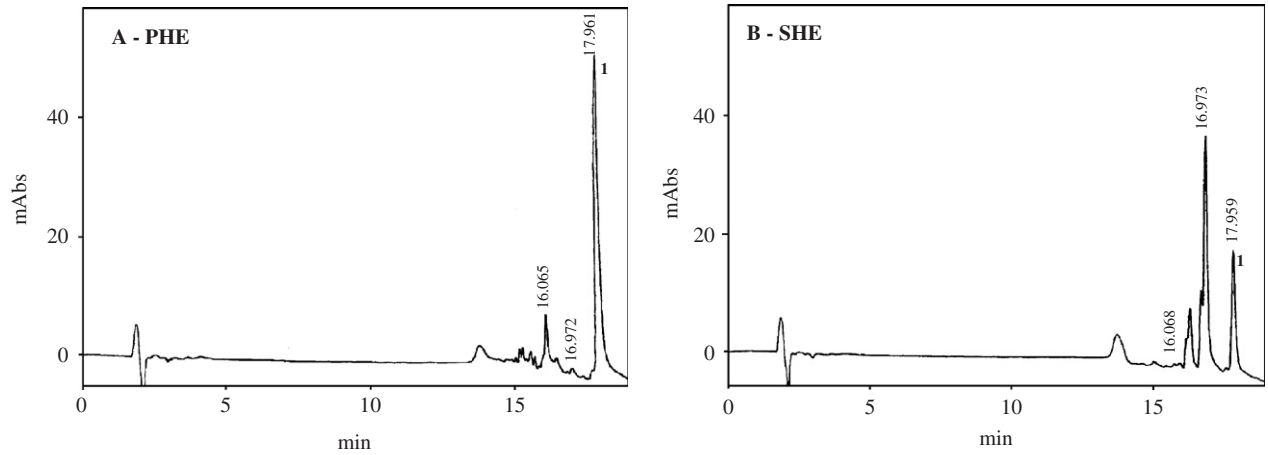

Fig. 2. HPLC chromatograms of hexane extracts from *Rheedia brasiliensis* fruit. A—PHE: peel hexane extract; B—SHE: seed hexane extract; 1 = 7-epiclusianone (retention time: 17.961 min in PHE and 17.959 min in SHE).

For the antimicrobial assays, the extracts and 7-epiclusianone were dissolved in 80% ethanol just prior to the microbiological tests, which were also used to monitor the biological activity of the extracts during the extraction process.

Bacterial strain

The bacterial strain used in this experiment was *Streptococcus mutans* UA159, a proven cariogenic dental pathogen (Ajdic et al., 2002). The cultures were stored at −80 °C in brain heart infusion (BHI) containing 20% glycerol (v/v).

Antimicrobial activity assays

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The antimicrobial activity of *R. brasiliensis* extracts was first evaluated by determining MIC and MBC, according to the methodology described by Piddock (1990) and Phillips (1991). Concentrations of the extracts tested ranged from 6.25 to 800 μg/ml. 7-epiclusianone was tested in concentrations ranging from 0.625 to 80 μg/ml. MIC was defined as the lowest concentration of the agents tested showing restricted growth at a lower level than 0.05 at 660 nm (no visible growth).

To determine MBC, an aliquot (50 μl) of each incubated tube containing suspension with extracts and compound at concentrations higher than the MIC was subcultured on BHI agar supplemented with 5% defibrinated sheep blood using a Spiral Plater (Whitley Automatic Spiral Plater). MBC was defined as the lowest concentration that allowed no visible growth on the agar (99.9% killed) (Koo et al., 2000). Three replicates were made for each agent tested and for all assays. Chlorhexidine digluconate 0.12% (v/v) (Sigma®) was used as positive control for determining MIC and MBC.

Bacterial viability in biofilm

Bacterial viability test was carried out before the isolation and identification of the bioactive compound. The peel hexane and seed hexane extracts showed antimicrobial activity against planktonic cells. Thus, the bacterial viability analysis of *S. mutans* biofilms, considering time-kill assay, yielded complementary data for MIC and MBC.

Biofilms were formed on standard glass microscope slides in batch cultures for 5 days (Koo et al., 2002a). The 5-days-old biofilms were exposed to the extracts tested (final concentrations of 100 × MIC) for 0, 1, 2, 3 and 4 h. Every one hour starting at baseline, the biofilms were removed, suspended in salt solution (50 mM KCl and 1 mM $MgCl_2$, pH 7.0) and subjected to sonication twice, each consisting of three 10-s pulses, at 5-s intervals, at 50 W. Then, the homogenized suspension was serially diluted (10^1, 10^2, 10^3 and 10^4) and plated on tryptic soy agar (TSA) using a Spiral Plater (Whitley Automatic Spiral Plater). The plates were incubated in 5% CO_2, at 37 °C, for 48 h, and then colony forming units per ml (CFU/ml) were quantified.

Killing curves were constructed by plotting values in the ordinate label: N_0 stands for the original number of CFU/ml; N stands for the number after the times of exposure. The assays were made in duplicate on at least three different occasions. A bactericidal effect was defined as a decrease in the CFU/ml ($\log N/N_0 > 3$) from initial viable counts at baseline (Koo et al., 2002b). Chlorhexidine digluconate 0.12% (v/v) (Sigma®) was used as positive control for the time-kill assay.

Results

Effect of agents on planktonic cells

The effect of the *R. brasiliensis* fruit extracts on *S. mutans* planktonic cells is shown in Table 1. Among the extracts tested, the peel hexane and seed hexane extracts presented a potential antibacterial activity against *S. mutans* at low concentrations (seed hexane extract—MIC: 12.5–25 μg/ml/ MBC: 50–100 μg/ml; peel hexane extract—MIC: 12.5–25 μg/ml/MBC: 25–50 μg/ml). The ethanol and ethyl-acetate peel extracts displayed no inhibitory activity against the microorganism tested.

The isolated bioactive compound 7-epiclusianone presented lower MIC/MBC values (1.25–2.5 and 10–20 μg/ml, respectively) than those found for the active extracts (Table 1). Chlorhexidine showed MIC/MBC values of 1–2 and 8 μg/ml, respectively.

Effect of extracts on biofilm viability

Among the extracts tested for antimicrobial activity, only the bioactive extracts, such as peel hexane and seed hexane extracts, were tested in a biofilm model using the time-kill analysis (Fig. 3). These extracts, at a concentration of 100 × MIC (2500 μg/ml or 2.5 mg/ml), reduced viable counts of *S. mutans* UA159. However, only the seed hexane extract was able to decrease the log $N/N_0 > 3$ units after 4 h exposure. Chlorhexidine 0.12%, showed total bactericidal effect after 2 h exposure (Fig. 3).

Discussion

It is well known that most of the new drugs discovered in the last few decades have originated from the nature (Newman and Cragg, 2007). Chemical constituents obtained from medicinal plants and other natural products have been increasingly used to treat many infectious diseases. Since dental caries is considered one of the most common infectious disease of mankind (Smith, 2002), many studies have been aimed at identifying compounds from natural products against mutans streptococci, especially *Streptococcus mutans* (Tichy and Novak, 1998; Badria and Zidan, 2004; Yatsuda et al., 2005; Duarte et al., 2006).

The present study showed that hexane extracts obtained from peel and seeds of *Rheedia brasiliensis* fruit, a member from Guttiferae family, showed a potential activity against *S. mutans* at low concentrations, showing the same MIC values. According to Rios et al. (1988), natural extracts that exhibit activity at concentrations lower than 100 μg/ml could have great antimicrobial potential, since the active compounds can be isolated and used at lower concentrations.

Of all the species identified in Brazil, the Guttiferae family is outstanding for its great number of pharmacological properties and bioactive compounds from its species. Some polyprenylated benzophenone derivatives obtained from Guttiferae have been reported as having potential antimicrobial activity against Gram-positive and Gram-negative cocci, mycobacteria and fungi (Bakana et al., 1987), *Staphylococcus aureus* (Iinuma et al., 1996; Dos Santos et al., 1996), *Trypanossoma cruzi* (Alves et al., 1999) and *Helicobacter pylori* (Chatterjee et al., 2003). However, Guttiferae antimicrobial activity against oral microorganisms had never been investigated.

Our study showed that, in addition to their ability to inhibit planktonic cells, the hexane extracts tested reduced the colony forming units of *S. mutans* biofilms, with the seed hexane showing bactericidal activity. Microorganisms in biofilms are known to be more resistant to antimicrobial agents than are cells in

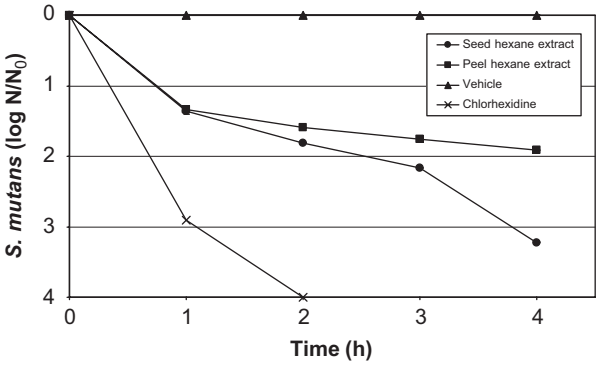

Fig. 3. Results from time-kill study with the hexane extracts and negative (vehicle) and positive (chlorhexidine) controls.

Table 1. MIC and MBC results from *Rheedia brasiliensis* fruit extracts and 7-epiclusianone

Microorganism	Extracts (μg/ml)								Compound (μg/ml)	
	Seed hexane		Peel hexane		Peel ethanol		Peel ethyl-acetate		7-epiclusianone	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. mutans UA159	12.5–25	50–100	12.5–25	25–50	>800	>800	>800	>800	1.25–2.5	10–20

MIC and MBC values for chlorhexidine (positive control) were 1–2 and 8 μg/ml, respectively.

suspension (planktonic state), and are more complex and similar to the biofilms in the conditions of oral cavity (Lewis, 2001; Bowen, 2002; Marsh, 2003), showing that these results might be of great importance for anticaries studies. However, none of the extracts was as effective in reducing colony forming units as chlorhexidine 0.12%, a clinically proven antimicrobial agent (Marsh, 1992).

Since the other extracts tested displayed no antibacterial activity and the hexane extracts could reduce the viability of *S. mutans* biofilms in time-kill assay, the bioactive compound present in both peel and seed hexane extracts was isolated and identified in our study. Delle Monache et al. (1983) were the first to investigate the genus *Rheedia* (Guttiferae) and its chemical composition. Dos Santos et al. (1999) have isolated the new polyprenylated benzophenone, denominated 7-epiclusianone. This compound has been reported as having good antimicrobial activity against *Staphylococcus aureus* and phytobacteria (Dos Santos, 1996).

The present study identified 7-epiclusianone as the bioactive compound, with MIC values approximately 10 times lower than those observed for the active hexane extracts, having more complexes in their composition (7-epiclusianone present in a diluted form) and similar to those observed for chlorhexidine. 7-epiclusianone is not a synthetic substance, with its origin exclusively from the nature. This new biological property of 7-epiclusianone is of great relevance in dentistry due to its antibacterial activity against *S. mutans*.

In conclusion, our results showed that the polyprenylated benzophenone 7-epiclusianone, obtained from the *R. brasiliensis* fruit, could be used as an agent to control cariogenic microorganisms and prevent dental caries. However, further studies are needed to investigate the underlying mechanisms of action and the anticariogenic potential of 7-epiclusianone.

Acknowledgements

The authors thank FAPESP (#06/56379-4), FINEP (2006) and CNPq (#304803/2005-7 and #131751/2006-9) for finacial support. This publication is part of the thesis submitted by the first author to the Faculty of Dentistry of Piracicaba.

References

Ajdic, D., McShan, W.M., McLaughlin, R.E., Savic, G., Chang, J., Carson, M.B., Primeaux, C., Tian, R., Kenton, S., Jia, H., Lin, S., Qian, Y., Li, S., Zhu, H., Najar, F., Lai, H., White, J., Roe, B.A., Ferretti, J.J., 2002. Genome sequence of *Streptococcus mutans* UA159, a cariogenic dental pathogen. Proc. Natl. Acad. Sci. USA. 99, 14434–14439.

Alves, T.M., Alves, R., Romanha, A.J., Zani, C.L., dos Santos, M.H., Nagem, T.J., 1999. Biological activities of 7-epiclusianone. J. Nat. Prod. 62, 369–371.

Badria, F.A., Zidan, O.A., 2004. Natural products for dental caries prevention. J. Med. Food. 7, 381–384.

Baehni, P.C., Takeuchi, Y., 2003. Anti-plaque agents in the prevention of biofilm-associated oral diseases. Oral diseases 9, 23–29.

Bakana, P., Claeys, M., Totte, J., Pieters, L.A., Van Hoof, L., Tamba-Vemba, Van den Berghe, D.A., Vlietinck, A.J., 1987. Structure and chemotherapeutical activity of a polyisoprenylated benzophenone from the stem bark of *Garcinia huillensis*. J. Ethnopharmacol. 21, 75–84.

Basso, L.A., da Silva, L.H., Fett-Neto, A.G., de Azevedo Jr., W.F., Moreira Ide, S., Palma, M.S., Calixto, J.B., Astolfi Filho, S., dos Santos, R.R., Soares, M.B., Santos, D.S., 2005. The use of biodiversity as source of new chemical entities against defined molecular targets for treatment of malaria, tuberculosis, and T-cell mediated diseases—a review. Mem. Inst. Oswaldo Cruz. 100, 475–506.

Belli, W.A., Marquis, R.E., 1991. Adaptation of *Streptococcus mutans* and *Entercoccus hirae* to acid stress in continuous culture. Appl. Environ. Microbiol. 57, 239–246.

Bowen, W.H., 2002. Do we need to be concerned about dental caries in the coming millennium? Crit. Rev. Oral Biol. Med. 13, 126–131.

Campbell, R.J., 1996. South American fruits deserving further attention. In: Janick, J. (Ed.), Progress in new crops. ASHS Press, Arlington, pp. 431–439.

Chatterjee, A., Yasmin, T., Bagchi, D., Stohs, S.J., 2003. The bactericidal effects of *Lactobacillus acidophilus*, garcinol and Protykin compared to clarithromycin, on *Helicobacter pylori*. Mol. Cell. Biochem. 243, 29–35.

Corrêa, M.P., 1978. Dicionário de plantas úteis do Brasil e das exóticas cultivadas. Rio de Janeiro: Ministério da Agricultura 3, 517–520.

Delle Monache, G., Delle Monache, F., Bettolo, G.B.M., Lima, R.A., 1983. Chemical investigation of the genus *Rheedia*. II Prenylated xanthones from *Rheedia gardneriana*. J. Nat. Prod. 46, 655–659.

Delle Monache, F., Delle Monache, G., Gacs-Baitz, E., 1991. Chemistry of the *Clusia* genus 6. Prenylated benzophenone from *Clusia sandiensis*. Phytochemistry 30, 703.

Dos Santos, M.H., 1996. Estudo químico dos frutos de *Rheedia gardneriana* e efeito biológico de seus constituintes. Thesis, Universidade Federal de Viçosa, Minas Gerais, Brazil.

Dos Santos, M.H., Speziali, N.L., Nagem, T., Oliveira, T.T., 1998. Epiclusianone: a new natural product derivative of bicyclo [3.3.1] nonane-2,4,9-trione. Acta Crystallogr. C 54, 1990–1992.

Dos Santos, M.H., Nagem, T.J., Oliveira, T.T., Braz-Filho, R., 1999. 7-epiclusianona: a nova benzofenona tetraprenilada e outros constituintes químicos dos frutos de *Rheedia gardneriana*. Quim. Nova. 22, 654–660.

Duarte, S., Koo, H., Bowen, W.H., Hayacibara, M.F., Cury, J.A., Ikegaki, M., Rosalen, P.L., 2003. Effect of a novel type of propolis and its chemical fractions on glucosyltransferases and on growth and adherence of mutans streptococci. Biol. Pharm. Bull. 26, 527–531.

Duarte, S., Rosalen, P.L., Hayacibara, M.F., Cury, J.A., Bowen, W.H., Marquis, R.E., Rehder, V.L., Sartoratto, A., Ikegaki, M., Koo, H., 2006. The influence of a novel propolis on mutans streptococci biofilms and caries development in rats. Arch. Oral Biol. 51, 15–22.

Gustafson, K.R., Blunt, J.W., Munro, M.H.G., Fuller, R.W., Mckee, T.C., Cardellina II, J.H., McMahon, J.B., Cragg, G.M., Boyd, M.R., 1992. The guttiferones, HIV-inhibitory benzophenones from Symphonia globulifera, Garcinia livingstonei, Garcinia ovalifolia and Clusia rosea. Tetrahedron 48, 10093–10102.

Hamada, S., Slade, H.D., 1980. Biology, immunology and cariogenicity of *Streptococcus mutans* and dental caries prevention. J. Dent. Res. 63, 407–411.

Ito, C., Itoigawa, M., Miyamoto, Y., Onoda, S., Rao, K.S., Mukainaka, T., Tokuda, H., Nishino, H., Furukawa, H., 2003. Polyprenylated benzophenones from *Garcinia assigu* and their potential cancer chemopreventive activities. J. Nat. Prod. 66, 206–209.

Koo, H., Rosalen, P.L., Cury, J.A., Ambrosano, G.M., Murata, R.M., Yatsuda, R., Ikegaki, M., Alencar, S.M., Park, Y.K., 2000. Effect of a new variety of *Apis mellifera* propolis on mutans streptococci. Curr. Microbiol. 41, 192–196.

Koo, H., Pearson, S.K., Scott-Anne, K., Abranches, J., Cury, J.A., Rosalen, P.L., Park, Y.K., Marquis, R.E., Bowen, W.H., 2002a. Effects of apigenin and tt-farnesol on glucosyltransferase activity, biofilm viability and caries development in rats. Oral Microbiol. Immunol. 17, 337–343.

Koo, H., Rosalen, P.L., Cury, J.A., Park, Y.K., Bowen, W.H., 2002b. Effects of compounds found in propolis on Streptococcus mutans growth and on glucosyltransferase activity. Antimicrob. Agents Chemother. 46, 1302–1309.

Koo, H., Hayacibara, M.F., Schobel, B.D., Cury, J.A., Rosalen, P.L., Park, Y.K., Vacca-Smith, A.M., Bowen, W.H., 2003. Inhibition of *Streptococcus mutans* biofilm accumulation and polysaccharide production by apigenin and tt-farnesol. J. Antimicrob. Chemother. 52, 782–789.

Lemos, J.A., Abranches, J., Burne, R.A., 2005. Responses of cariogenic streptococci to environmental stresses. Curr. Issues Mol. Biol. 7, 95–107.

Lewis, K., 2001. Riddle of biofilm resistance. Antimicrob. Agents Chemother. 45, 999–1007.

Loesche, W.J., 1986. Role of Streptococcus mutans in human dental decay. Microbiol. Rev. 50, 353–380.

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

Marsh, P.D., 2003. Plaque as a biofilm: pharmacological principles of drug delivery and action in the sub- and supragingival environment. Oral diseases 9, 16–22.

Morton, J., 1987. Bakupari. In: Morton, J.F. (Ed.), Fruits of warm climates. Miami, FL, pp. 309–310.

Newman, D.J., Cragg, G.M., 2007. Natural products as sources of new drugs over the last 25 years. J. Nat. Prod. 70, 461–477.

Paes Leme, A.F., Koo, H., Bellato, C.M., Bedi, G., Cury, J.A., 2006. The role of sucrose in cariogenic dental biofilm formation—new insight. J. Dent. Res. 85, 878–887.

Phillips, I., 1991. A guide to sensitivity testing. J. Antimicrob. Chemother. 27, 1–50.

Piddock, L.J.V., 1990. Techniques used for the determination of antimicrobial resistance and sensitivity in bacteria. J. Appl. Bacteriol. 68, 307–318.

Rios, J.L., Recio, M.C., Villar, A., 1988. Screening methods for natural products with antimicrobial activity: a review of the literature. J. Ethnopharmacol. 23, 127–149.

Smith, D.J., 2002. Dental caries vaccines: prospects and concerns. Crit. Rev. Oral Biol. Med. 13, 335–349.

Tichy, J., Novak, J., 1998. Extraction, assay, and analysis of antimicrobials from plants with activity against dental pathogens (Streptococcus sp.). J. Altern. Complement. Med. 4, 39–45.

Yamaguchi, F., Saito, M., Ariga, T., Yoshimura, Y., Nakazawa, H., 2000. Free radical scavenging activity and antiulcer activity of garcinol from *Garcinia* indica fruit rind. J. Agric. Food Chem. 48, 2320–2325.

Yatsuda, R., Rosalen, P.L., Cury, J.A., Murata, R.M., Rehder, V.L., Melo, L.V., Koo, H., 2005. Effects of *Mikania* genus plants on growth and cell adherence of mutans streptococci. J. Ethnopharmacol. 97, 183–189.