Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 105 (2007) 311–316
Food Chemistry
www.elsevier.com/locate/foodchem

In vitro inhibitory effects of rosemary extracts on growth and glucosyltransferase activity of *Streptococcus sobrinus*

Po-Jung Tsai [a,1], Tzung-Hsun Tsai [b,1], Su-Chen Ho [a,*]

[a] *Department of Food Science, Yuanpei University, No. 306, Yuanpei Street, Hsinchu 3000, Taiwan*
[b] *Department of Dentistry, Keelung Chang-Gung Memorial Hospital, Keelung, Taiwan*

Received 24 July 2006; received in revised form 24 October 2006; accepted 20 November 2006

Abstract

Rosemary (*Rosmarinus officinalis* L.) extract functions as an antioxidant and antimicrobial agent. In this study, we investigated *in vitro* effects of rosemary extracts on *Streptococcus sobrinus* growth and on its extracellular glucosyltransferase activity. The antibacterial activities of rosemary extracts were determined by the microdilution broth method. The minimum inhibitory concentrations of aqueous and methanolic rosemary extracts against *S. sobrinus* were 16 and 4 mg/ml, respectively. Glucosyltransferase activity was tested by incubating a crude enzyme preparation with sucrose and determining the amount of water-insoluble glucan formed. Both aqueous and methanolic extracts of rosemary markedly inhibited the formation of water-insoluble glucan. The 50% inhibitory doses of aqueous and methanolic extracts against the glucosyltransferases of *S. sobrinus* were 1.42 mg/ml and 0.34 mg/ml, respectively. Our results suggest that rosemary extract may prove effective for the inhibition of the growth of cariogenic oral streptococci.

Keywords: Glucan; Glucosyltransferase; Oral streptococci; Rosemary

1. Introduction

Oral disease, including dental caries, gingival inflammation, periodontal disease, and tooth loss, may significantly affect overall health. Among these, dental caries is a multifactorial infectious disease in which diet, nutrition, microbial infection, and host response all play important roles. Dental plaque is a complex but typical bacterial biofilm that contains mutans streptococci and other oral bacteria and their products. Dental plaque is formed through two different stages: initial and reversible cell-to-surface attachment and subsequent sucrose-dependent adhesion of the microorganisms, which is firm and irreversible. Glucosyltransferases (GTase) are strongly involved in the latter step. Dietary sucrose is essential for the accumulation of cariogenic mutans streptococci on teeth and for the initiation of carious lesions. Glucosyltransferase utilizes sucrose as a substrate and yields fructose and glucan with predominant $\alpha(1 \rightarrow 3)$ and $\alpha(1 \rightarrow 6)$ bonds as the products (Kawabata & Hamada, 1999). The glucosyltransferases from mutans streptococci cooperatively synthesize water-insoluble glucan from sucrose and facilitate their ability to colonize on the tooth surface and develop dental plaque (Ando, Tsumori, Shimamura, Sato, & Mukasa, 2003; Konishi et al., 1999). *Streptococcus sobrinus* is one of the most cariogenic bacteria of mutans streptococci and synthesizes extracellular glucan and fructan from sucrose which contribute to the pathogenicity of the dental plaque (Liljemark & Bloomquist, 1996). The elimination of cariogenic bacteria, such as *S. sobrinus*, is a fundamental step in preventing dental caries. Numerous chemicals, e.g. chlorhexidine, triclosan and delmopinol, are used as antibacterial agents against oral pathogens to reduce dental plaque-mediated diseases, including dental caries (Baehni & Takeuchi, 2003). Many plants and plant-derived antimicrobial components are

* Corresponding author. Tel.: +886 3 5381183x8496; fax: +886 3 5381183x8361.
E-mail address: sche@mail.ypu.edu.tw (S.-C. Ho).
[1] These authors contributed equally to this work.

0308-8146/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2006.11.051

used in folklore therapeutics for the treatment of periodontal disorders and for the purpose of oral hygiene. Sanguinarine, extracted from *Sanguinaria canadensis*, has a broad spectrum effect against a wide variety of oral bacteria (Joann & Sigmund, 1985). Kuwanon, derived from the root bark of mulberry (*Morus alba*), inhibits the growth of cariogenic bacteria, such as *S. sobrinus* and *S. sanguinis* (Park, You, Lee, Baek, & Hwang, 2003). Tichy and Novak (1998) reported that the ethanolic extracts of plants, including *Abies Canadensis*, *Ginkgo biloba*, *Sassafras albidium*, *Thuja plicata* and rosemary, exhibited antimicrobial activity against *Streptococcus sanguinis*.

Rosemary (*Rosmarinus officinalis* L.) is a spice and medicinal herb widely used around the world. The aqueous infusion of dried rosemary leaves has gained popularity in Taiwan for its claimed healthy benefit in recent years. Rosemary contains several antioxidant compounds and exhibits different protective effects, such as hepatoprotective (Fahim, Esmat, Fadel, & Hassan, 1999; Sotelo-Felix, Martinez-Fong, & Mureil De la Torre, 2002), anti-hyperglycemic (Al-Hader, Hasan, & Aqel, 1994) and anti-ulcerogenic actions (Dias, Foglio, Possenti, & Ernesto de Carvahlo, 2000). Rosemary extract has been widely used as a preservative in the food industry, due to its antioxidant activity (Frankel, Huang, Prior, & Aeschbach, 1996). The antifungal and antimicrobial effects of essential oil of rosemary were extensively reported (Angioni et al., 2004; Santoyo et al., 2005). Pandit and Shelef (1994) reported that the anti-listerial activity of the aqueous extract of rosemary was lower than that of the freshly ground herb or the ethanolic extract of rosemary. Although the antimicrobial activity of rosemary has been reported for various food-spoilage and/or poisoning pathogens, little information is available on the antibacterial activity of rosemary against the oral pathogen, *S. sobrinus*. In most studies performed to explore antioxidant and antimicrobial properties of rosemary, the extraction procedures are based on the use of organic solvents, most often either methanol or acetone. Therefore, we investigated the effects of crude aqueous and methanolic extracts of rosemary on the growth of *Streptococcus sobrinus* and on the synthesis of water-insoluble glucans by its extracellular glucosyltransferase.

2. Materials and methods

2.1. Materials

Streptococcus sobrinus (BCRC14757) was obtained from the Bio-resource Collection and Research Center, Food Industry Research and Development Institute (Hsinchu, Taiwan) and cultured in Brain Heart Infusion (BHI) broth (Difco Laboratories, Detroit, MI, USA) containing 0.5% yeast extract, 0.05% l-cysteine, and 1% sucrose in an anaerobic atmosphere, using BBL GasPak systems (Becton Dickinson Microbiology Systems, Cockeysville, Md.). Folin-Ciocalteu reagent, gallic acid, catechin, dimethyl sulfoxide (DMSO) and periodic acid-Schiff's reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA). A full-range Rainbow molecular weight protein marker was purchased from Amersham Bioscience (Piscataway, NJ, USA). The BCA protein assay reagent kit was purchased from Pierce (Rockford, IL, USA). All other chemicals were of analytical-grade purity.

2.2. Preparation of extracts

The dried rosemary (*Rosmarinus officinalis* L.) leaves were purchased from a local supermarket in Taipei City (Taipei, Taiwan). The dried ground leaves of rosemary were soaked in boiling deionized water (20 ml/g) for 5 min. The aqueous infusion was cooled to room temperature, then centrifuged (12,000*g* for 10 min) and lyophilized. The lyophilized aqueous extract was kept at −20 °C. The weighed aqueous extract was dissolved in phosphate buffer solution (PBS) and used at a concentration of 500 mg/ml for subsequent experimentation.

The dried ground leaves of rosemary were extracted with methanol (5 ml/g), using a magnetic mixer at room temperature for 3 h. After extraction, the mixture was filtered and the residue was re-extracted with fresh methanol (5 ml/g) overnight. The combined methanolic solution was centrifuged at 12,000*g* for 10 min and evaporated on a rotary evaporator. Methanolic extract was reconstituted in dimethyl sulfoxide (DMSO) to a concentration of 400 mg/ml for subsequent experimentation.

The extraction yields of solids from aqueous and methanolic extracts of rosemary were 16.3% and 23.3%, respectively.

2.3. Determination of total phenolic compounds

The total phenolic contents of aqueous and methanolic extracts were determined according to the Folin–Ciocalteu method. Briefly, Folin–Ciocalteu phenol reagent was added to the reconstituted samples and held for 3 min. Then 2 ml of 10% (w/v) sodium carbonate solution were added and the mixture allowed to stand at room temperature for 30 min. The absorbance at 765 nm was measured. The total phenolic content was calculated by a standard curve prepared with gallic acid and expressed as milligrammes of gallic acid equivalents (GAE) per gramme of solid of extract.

2.4. Determination of total flavonoids content

Total flavonoids contents of aqueous and methanolic extracts were determined by the following procedure. Briefly, 0.25 ml of optimally diluted reconstituted sample was added into the tube containing 1 ml of double-distilled water. Then, 0.75 ml of 5% $NaNO_2$, 0.075 ml of 10% $AlCl_3$, and 0.5 ml of 1 M NaOH were sequentially added at 0, 5 and 6 min. Finally, the volume of reacting solution was adjusted to 2.5 ml with double-distilled water. The absorbance at 510 nm was measured. The flavonoid content in each extract was then calculated by a standard curve pre-

pared with catechin and expressed as milligrammes of catechin equivalents (CE) per gramme solid of extract.

2.5. Determination of minimum inhibitory concentration (MIC)

The anti-streptococcal activity of extract was assessed by determining MIC values obtained by a modified microdilution broth method described by Cai and Wu (1996). Briefly, *Streptococcus sobrinus*, from overnight cultures, was adjusted to 1×10^6 colony-forming units (CFU)/ml. The rosemary extracts were serially diluted with broth to give the concentrations of 0.5, 1, 2, 4, 8, 16, 32, and 64 mg/ml of aqueous extracts and the concentrations of 0.125, 0.25, 0.5, 1, 2, 4, 8, and 16 mg/ml of methanolic extracts. In sterile 96-well microtitre plates, 100 μl of diluted rosemary sample were added into wells containing 100 μl of bacterial suspension. Two percent of DMSO solvent was tested and had no significantly inhibitory effect on *S. sobrinus* growth. To adjust the interference of colour due to extracts themselves, a parallel series of mixtures, with uninoculated broth, was prepared. Triplicate samples were taken for each test concentration. After incubation for 48 hours at 37 °C under anaerobic conditions, *S. sobrinus* growth was estimated spectrophotometrically, at 630 nm, using a microtitre plate reader. The MIC was defined as the minimum concentration of test compound limiting turbidity to <0.05 absorbance units. The experiments were performed in triplicate.

2.6. Preparation of extracellular glucosyltransferase

A crude glucosyltransferase (GTase) preparation was extracted according to the method described by Koo et al. (2000) with some modifications. Actively growing *Streptococcus sobrinus* was incubated in 6 l of low molecular-weight medium which was composed of 2.5% tryptone, 1.5% yeast extract, and 1% glucose. After incubation for 18 hours at 37 °C under anaerobic conditions, the bacteria were removed by refrigerated centrifugation. The pH of culture supernatant was adjusted to 6.8 by addition of 2 M NaOH. The supernatant fluid was treated with ammonium sulfate at 50% saturation and then centrifuged. The precipitate was dialyzed against PBS, pH 6.8, containing 1 mM phenylmethylsulfonyl fluoride (PMSF) as a protease inhibitor. The dialyzed preparation was concentrated using ultrafiltration with a 30 kDa cut-off membrane (YM-30, Amicon) at 4 °C. The retentate preparation was used as crude extracellular glucosyltransferase and then stored at −20 °C.

In order to confirm the presence of glucosyltransferase, native polyacrylamide gel electrophoresis and activity staining were performed. Crude glucosyltransferase sample was separated on a 7.5% native polyacrylamide gel (PAGE) at 4 °C. Proteins were stained with Coomassie brilliant blue R-250. Immediately following electrophoresis, the gel was incubated in a 1% sucrose-containing PBS buffer (pH 6.0) at 37 °C for 18 h. The glucan formed in the gel was stained with periodic acid-Schiff's reagent (Ando et al., 2003). The gel was rinsed in slowly running tap water for 1 min and then washed with distilled water. The gel was treated with 1% periodic acid for 10 min, washed with distilled water, and stained with Schiff's reagent for 25 min at room temperature. For gel destaining, the 7% acetic acid solution was changed three times over a 24-h period. The destaining container was always covered with aluminium foil to prevent excessive exposure of gel to light.

Protein concentration was determined by the BCA method. Glucosyltransferase activity was estimated by measuring reducing sugar release from sucrose, as described previously (Kawai & Tsuchitani, 2000). Reducing sugar was measured by the 3,5-dinitrosalicylic acid (DNSA) method. One unit of enzyme activity was defined as the amount of GTase which released 1.0 μmol of reducing sugar per minute from sucrose. From our results, GTase activity of crude enzyme preparation was 0.14 U/mg protein.

2.7. Water-insoluble glucan formation by glusosyltransferase

Quantization of glucan synthesis was carried out according to a previous method (Ooshima et al., 2000) with some modification. The reaction mixtures contained sterile 0.1 M sucrose as a substrate and 50 μl of crude GTase were buffered in PBS, pH 6.0. Different final concentrations of aqueous rosemary extract (0.001, 0.01, 0.1, 1, 2, 4, 8 and 16 mg/ml) and methanolic extract (0.001, 0.01, 0.1, 0.25, 0.5,1, 2 and 4 mg/ml) were tested to see if they inhibited water-insoluble glucan synthesis. Following incubation at 37 °C for 18 h, the water-insoluble glucan produced by enzyme reaction was suspended by sonication, and the turbidity of the suspension was measured at 550 nm. To adjust for quantification errors due to extracts themselves, a parallel series of mixtures without crude enzyme was prepared. The formation of water-insoluble glucan formation was expressed as percent of control (i.e., $100 \times OD_{550\,nm}$ of test sample/$OD_{550\,nm}$ of control sample without adding rosemary extract). All reactions were carried out in triplicate.

2.8. Statistical analysis

All data were presented as means ± SD. Statistical analysis was performed using the SPSS 12.0 statistical package (Chicago, IL, USA). The Mann–Whitney *U* test was used to compare the differences between aqueous and methanolic extracts. A *P* value of less than 0.05 was considered statistically significant.

3. Results and discussion

Dental caries is a multifactorial disease associated with the presence of cariogenic bacteria which are embedded in the dental plaque biofilm. To prevent the dental caries caused by cariogenic bacteria, different strategies, such as

inhibition of bacterial growth of mutans streptococci, decreasing of glucosyltransferase activity, prevention of streptococcal colonization, and hydrolysis of glucan by enzymes, were developed. In recent years, the general population has demonstrated increased awareness and interest in functional foods with positive human health benefits. The components with antimicrobial properties against dental pathogens derived from food and common beverages, such as black tea, green tea, oolong tea and oligomeric catechins from such preparations (Hamada et al., 1996; Hamilton-Miller, 2001; Nakahara et al., 1993), high-molecular-weight components of cranberry (Steinberg, Feldman, Ofek, & Weiss, 2005), cocoa bean husk extract (Ooshima et al., 2000), water-soluble extract of cacao (Ito, Nakamura, Tokunaga, Iijima, & Fukushima, 2003), propolis (Koo et al., 2000) and apple polyphenols (Yanagida, Kanda, Tanabe, Matsudaira, & Oliveria Cordeiro, 2000) were previously reported. Some compounds with anti-caries activity from propolis have already been characterized, including flavonoids, pinocembrin, galangin, pinobanksin-3-acetate and a mixture of *p*-coumaric acid benzyl ester and caffeic ester (Tichy & Novak, 1998) and sesquiterpene *tt*-farnesol (Koo, Rosalen, Cury, Park, & Bowen, 2002).

Thousands of other potentially useful plants have not been tested. In our preliminary study, aqueous and methanolic extracts from different herbal teas, common beverages consumed in Taiwan, including sweet osmanthus (*Osmanthus fragrans Lour*), rose (*Rosa damascena*), lavender (*Lavandula officinalis*), jasmine (*Jasminum officinale*), lemongrass (*Cymbopogon citrates*), daisy (*Chrysanthemum morifolium*), honeysuckle (*Lonicera japonica* Thunb.), mate (*Ilex paraguariensis*), Jiaogulan (*Gynostemma pentaphyllum*), and rosemary, were evaluated for their antimicrobial activity against *Streptococcus sobrinus*. Of the herbs tested, rosemary extract was the most effective antibacterial agent. These aqueous (32 mg/ml) and methanolic (8 mg/ml) extracts of herbal teas, except those of rosemary, showed no apparently inhibitory effect on *Streptococcus sobrinus* growth. To our knowledge, the present work is one of the first to examine the inhibitory effect of rosemary extracts on *Streptococcus sobrinus* and its related enzyme.

Here, we describe the antimicrobial activities of aqueous and methanolic extracts of rosemary (Table 1). Methanolic extract exhibits an inhibitory effect against *Streptococcus sobrinus* with a MIC value of 4 mg/ml. Aqueous extract is also effective against *Streptococcus sobrinus* with a MIC value of 16 mg/ml. This indicates that methanolic extract of rosemary extract confers stronger antibacterial activity than does its aqueous extract.

Streptococcus sobrinus produces three kinds of GTase: one GTase synthesizes water-insoluble glucan and the other two GTases make water-soluble glucan from sucrose (Ando et al., 2003). The presence of glucosyltransferase is confirmed by native PAGE and direct activity staining (Fig. 1). The aqueous (Fig. 2) and methanolic (Fig. 3) extracts of rosemary inhibit water-insoluble glucan synthesis by glucoslytransferase in a dose-dependent manner. The

Table 1
Contents of total phenolics and flavonoids and antimicrobial activity of rosemary extracts

	Total phenolics (mg GAE/g extract)	Total flavonoids (mg CE/g extract)	MIC[a] (mg/ml)
Rosemary extracts			
Aqueous	98.7 ± 5.9*	128 ± 0.8*	16*
Methanolic	58.1 ± 0.9	60.7 ± 1.1	4

The values are expressed as means ± SD. Asterisk indicates a significant difference at level $p < 0.05$ between aqueous and methanolic extract of rosemary.

[a] Minimum inhibitory concentration against *S. sobrinus*.

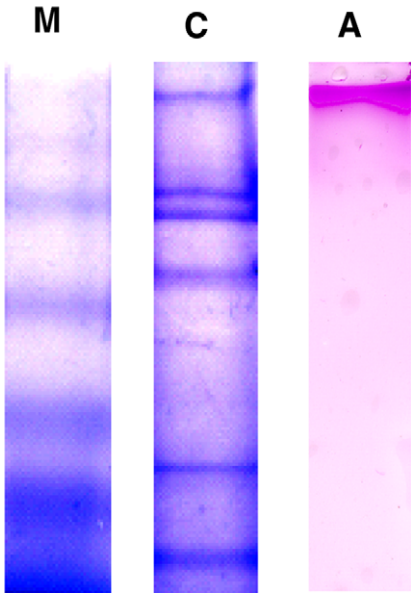

Fig. 1. Polyacrylamide gel electrophoresis of crude glucosyltransferase preparation. Glucosyltransferase was visualized by Coomassie blue staining (C) and periodic acid-Schiff's staining of glucan after incubation of the gel in sucrose-containing buffer (activity staining, A). M represents protein markers.

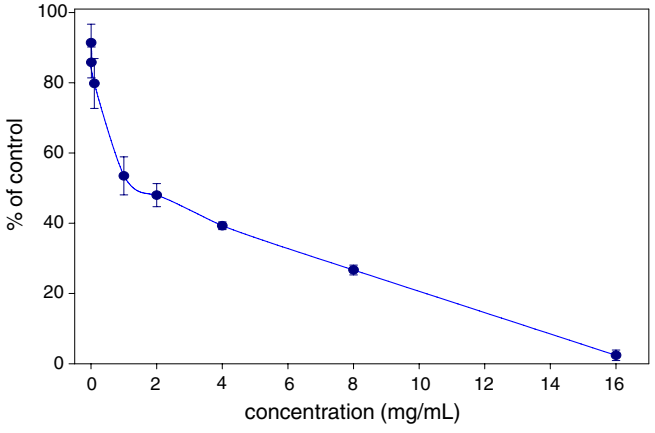

Fig. 2. Effect of rosemary aqueous extract on water-insoluble glucan formation by glucosyltransferase of *S. sobrinus* from sucrose. The formation of water-insoluble glucan was expressed as percent of control (without test samples).

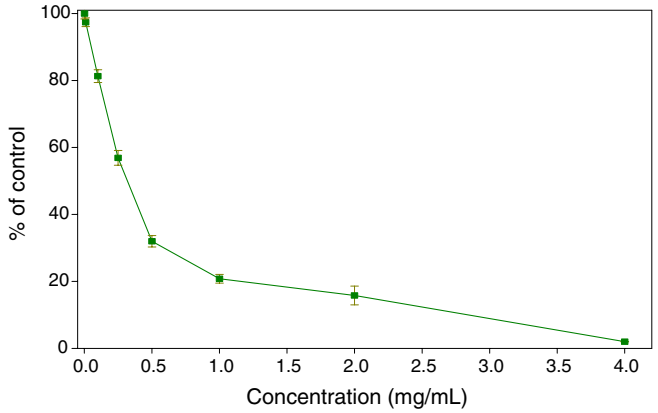

Fig. 3. Effect of rosemary methanolic extract on water-insoluble glucan synthesis by glucosyltransferase of *S. sobrinus* from sucrose. The formation of water-insoluble glucan was expressed as percent of control (without test samples).

aqueous and methanolic extracts completely inhibit GTase activity at 16 and 4 mg/ml, respectively (Figs. 2 and 3). The 50% inhibitory doses (ID_{50}) of aqueous and methanolic extracts of rosemary against glucosyltransferase of *S. sobrinus* are 1.42 mg/ml and 0.34 mg/ml, respectively. It is also noteworthy that, at lower concentrations, aqueous extract, as compared with methanolic extract, appears to be an effective inhibitor of GTase ($91.4 \pm 5.3\%$ vs $99.2 \pm 1.6\%$ of control at 0.001 mg/ml, and $85.8 \pm 4.4\%$ vs $94.7 \pm 1.4\%$ of control at 0.01 mg/ml) (Figs. 2 and 3). However, according to their ID_{50} values, the methanolic extract still shows remarkable inhibition of GTase activity. Numerous attempts to find natural substances with GTase-inhibitory activity have been made to prevent the colonization of *S. sobrinus* (Hamada et al., 1996; Nakahara et al., 1993; Yanagida et al., 2000). Flavanones and some dihydroflavonols inhibit the growth of *S. mutans* and *S. sobrinus* (Koo et al., 2002). Apigenin is a novel and potent inhibitor of GTase activity from *S. mutans* and *S. sanguinis* (Koo et al., 2002). Our results indicate that aqueous and methanolic extracts of rosemary are potential inhibitors of glucoslytransferase from *S. sobrinus*.

There is not much knowledge concerning anti-streptococcal activity of rosemary; the mechanism underlying this effect is not known. Table 1 shows that total polyphenols of aqueous and methanolic extracts were 98.7 ± 5.9 and 58.1 ± 0.9 mg GAE/g extract, respectively. Total flavonoid levels of aqueous and methanolic extracts are 128 ± 0.8 and 60.7 ± 1.1 mg CE/g extract, respectively (Table 1). The non-nutrient phytochemicals of rosemary are complex: carnosic acid, rosmarinic acid, chlorogenic acid and caffeic acid. These polyphenols contribute to antioxidant and antimicrobial properties of rosemary (Moreno, Scheyer, Romano, & Vojnov, 2006; Petersen & Simmonds, 2003). The most active compounds in water extract of rosemary are carnosol, rosmanol, carnosic acid, methylcarnosate and some flavonoids, such as cirsimaritin and genkwanin (Ibanez et al., 2003). The main phenolic compounds in organic extract of rosemary are the diterpene derivatives, carnosic acid and rosmanol, followed by rosemarinic acid (Zheng & Wang, 2001). It was reported that both water and organic rosemary extracts had antimicrobial properties linked to their different polyphenol compositions (Moreno et al., 2006). The present study demonstrates that methanolic extract, as compared with aqueous extract, exhibits a higher inhibitory effect on *S. sobrinus* growth, despite a lower content of phenolic compounds. This may in part be due to different chemical compositions between aqueous and methanolic rosemary extracts. Further studies are required to understand the rosemary components that contribute to the anticariogenic potential.

Rosemary is an available herb, inexpensive, and shown to be relatively non-toxic in animal models (Lemonica, Demasceno, & Di-Stasi, 1996). Rosemary leaf extracts are proposed as important human dietary factors and have been investigated as potential therapeutic agents against several diseases (Moreno et al., 2006). However, very little is known regarding their application in human oral health. The estimated concentration of "a cup" of rosemary infusion (3 g of leaves/200 ml of boiling water) was 2.45 mg of water-soluble extract per ml of infusion, and this was higher than the ID_{50} value of the GTase-inhibitory dose. A large proportion of the components in rosemary plant were extractable with water and the obtained extract was found to be rich in phenolic compounds. From the practical point of view, rosemary infusion, as gargle, may be recommended to prevent dental caries. However, further studies are required to investigate the effects of rosemary on different species of cariogenic bacteria and to verify the potential application of rosemary as a functional food for human oral health.

Rosemary is an antibacterial agent that has received little attention in the dental field. The results of this study suggest that *Rosmarinus officinalis* may prevent carious lesions by inhibiting the growth of *S. sobrinus* and may eliminate dental plaque through the suppressing activity of glucosyltransferase. An extension of this work in the future will be to investigate components present in crude rosemary extracts and to evaluate their contribution to the antibacterial activity against *S. sobrinus* and other *Streptococcus* sp.

Acknowledgements

The authors would like to thank You-Chia Chien and Chi-Wei Lee for technical assistance. This study was supported by a grant from the Keelung Chang Gung Memorial Hospital, Keelung, Taiwan (CMRPG 240241).

References

Al-Hader, A., Hasan, Z., & Aqel, M. (1994). Hyperglycemic and insulin release inhibitory effects of *Rosmarinus officinalis*. *Journal of Ethnopharmacology, 43*, 217–221.

Ando, T., Tsumori, H., Shimamura, A., Sato, Y., & Mukasa, H. (2003). Classification of oral streptococci by two-dimensional gel electrophoresis with direct activity stain for glucosyltransferases. *Oral Microbiology and Immunology, 18*, 171–175.

Angioni, A., Barra, A., Cereti, E., Barile, D., Coisson, J. D., Arlorio, M., et al. (2004). Chemical composition, plant genetic difference, antimicrobial and antifungal activity investigation of the essential oil of *Rosmarinus officinalis* L. *Journal of Agriculture and Food Chemistry, 52*(11), 3530–3535.

Baehni, P. C., & Takeuchi, Y. (2003). Anti-plaque agents in the prevention of biofilm-associated oral disease. *Oral Disease, 9*(suppl. 1), 23–29.

Cai, L., & Wu, C. D. (1996). Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens. *Journal of Natural Products, 59*(10), 987–990.

Dias, P., Foglio, M., Possenti, A., & Ernesto de Carvahlo, J. (2000). Antiulcerogenic activity of crude hydroalcoholic extract of *Rosmarinus officinalis*. *Journal of Ethnopharmacology, 69*, 57–62.

Fahim, F., Esmat, A., Fadel, H., & Hassan, K. (1999). Allied studies on the effect of *Rosemarinus officinalis* L. on experimental hepatotoxicity and mutagenesis. *International Journal of Food Sciences and Nutrition, 50*, 413–427.

Frankel, E. N., Huang, S., Prior, E., & Aeschbach, R. (1996). Evaluation of antioxidant activity of Rosemary extracts, carnosol and carnosic acid in bulk vegetable oils and fish oil and their emulsions. *Journal of the Science of Food and Agriculture, 72*, 201–208.

Hamada, S., Kontani, M., Hosono, H., Ono, H., Tanaka, T., Ooshima, T., et al. (1996). Peroxidase-catalyzed generation of catechin oligomers that inhibit glucosyltransferase from *Streptococcus sobrinus*. *FEMS Microbiology Letters, 143*, 35–40.

Hamilton-Miller, J. M. T. (2001). Anti-cariogenic properities of tea (*Camellia sinensis*). *Journal of Medical Microbiology, 50*, 299–302.

Ibanez, E., Kubatova, A., Senorans, F. J., Cavero, S., Reglero, G., & Hawthorne, S. B. (2003). Subcritical water extraction of antioxidant compounds from rosemary plants. *Journal of Agriculture and Food Chemistry, 51*(2), 375–382.

Ito, K., Nakamura, Y., Tokunaga, T., Iijima, D., & Fukushima, K. (2003). Anti-cariogenic properties of a water-soluble extract from cacao. *Bioscience, Biotechnology, and Biochemistry, 67*(12), 2567–2573.

Joann, L. D., & Sigmund, S. S. (1985). Comparative in vitro activity of sanguinarine against oral microbial isolates. *Antimicrobial Agents and Chemotherapy, 27*(4), 663–665.

Kawabata, S., & Hamada, S. (1999). Studying biofilm formation of mutans streptococci. *Methods in Enzymology, 30*, 513–523.

Kawai, K., & Tsuchitani, Y. (2000). Effects of resin composite components on glucosyltransferase of cariogenic bacterium. *Journal of Biomedical Materials Research, 51*(1), 123–127.

Konishi, N., Torii, Y., Yamamoto, T., Miyagi, A., Ohta, H., Fukui, K., et al. (1999). Structure and enzymatic properties of genetically truncated forms of the water-insoluble glucan-synthesizing glucosyltransferase from *Streptococcus sobrinus*. *Journal of Biochemistry, 126*(2), 287–295.

Koo, H., Gomes, B. P. F. A., Rosalen, P. L., Ambrosano, G. M. B., Park, Y. K., & Cury, J. A. (2000). In vitro antimicrobial activity of propolis and *Arnica montana* against oral pathogens. *Archives of Oral Biology, 45*, 141–148.

Koo, H., Rosalen, PL., Cury, JA., Park, YK., & Bowen, WH. (2002). Effects of compounds found in propolis on streptococcus mutans growth and on glucosyltransferase activity. *Antimicrobial Agents and Chemotherapy, 46*(5), 1302–1309.

Lemonica, I. P., Demasceno, D. C., & Di-Stasi, L. C. (1996). Study of the embryonic effects of an extract of rosemary (*Rosmarinus officinalis* L.). *Brazilian Journal of Medical and Biological Research, 29*, 223–227.

Liljemark, W. F., & Bloomquist, C. (1996). Human oral microbial ecology and dental caries and periodontal disease. *Critical Reviews in Oral Biology and Medicine, 7*(2), 180–198.

Moreno, S., Scheyer, T., Romano, C. S., & Vojnov, A. A. (2006). Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radical Research, 40*(2), 223–231.

Nakahara, K., Kawabata, S., Ono, H., Ogura, K., Tanaka, T., Ooshima, T., et al. (1993). Inhibitory effect of oolong tea polyphenols on glucosyltransferase of mutans streptococci. *Applied and Environmental Microbiology, 59*(4), 968–973.

Ooshima, T., Osaka, Y., Sasaki, H., Osawa, K., Yasuda, H., Matsumura, M., et al. (2000). Caries inhibitory activity of cocao bean husk extract in in-vitro and animal experiments. *Archives of Oral Biology, 45*, 639–645.

Pandit, V. A., & Shelef, L. A. (1994). Sensitivity of *Listeria monocytogenes* to rosemary (*Rosmarinus officinalis* L.). *Food Microbiology, 11*(1), 57–63.

Park, K. M., You, J. S., Lee, H. Y., Baek, N. I., & Hwang, J. K. (2003). Kuwanon G: an antibacterial agent from the root bark of *Morus alba* against oral pathogens. *Journal of Ethnopharmacology, 84*, 181–185.

Petersen, M., & Simmonds, M. S. (2003). Rosmaric acid. *Phytochemistry, 62*(2), 121–125.

Santoyo, S., Cavero, S., Jaime, L., Ibanez, E., Senorans, F. J., & Reglero, G. (2005). Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. essential oil obtained via supercritical fluid extraction. *Journal of Food Protection, 68*(4), 790–795.

Sotelo-Felix, J., Martinez-Fong, D., & Mureil De la Torre, P. (2002). Protective effect of carnosol on CCl_4-induced acute liver damage in rats. *European Journal of Gastroenterology and Hepatology, 14*, 1001–1006.

Steinberg, D., Feldman, M., Ofek, I., & Weiss, E. I. (2005). Cranberry high molecular weight constituents promote *Streptococcus sobrinus* desorption from artificial bioflim. *International Journal of Antimicrobial Agents, 25*, 247–251.

Tichy, J., & Novak, J. (1998). Extraction, assay, and analysis of antimicrobials from plants with activity against dental pathogens (*Streptococcus* sp.). *The Journal of Alternative and Complementary Medicine, 4*(1), 39–45.

Yanagida, A., Kanda, T., Tanabe, M., Matsudaira, F., & Oliveria Cordeiro, J. G. (2000). Inhibitory effects of apple polyphenols and related compounds on cariogenic factors of mutans streptococci. *Journal of Agriculture and Food Chemistry, 48*(11), 5666–5671.

Zheng, W., & Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agriculture and Food Chemistry, 49*(11), 5165–5170.