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In vitro antimicrobial activities against cariogenic streptococci and their antioxidant capacities: A comparative study of green tea versus different herbs

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ABSTRACT

The antimicrobial activity against cariogenic bacteria, total antioxidant capacity and phenolic constituents of methanolic extracts from 11 herbs were investigated and compared with those of green tea (*Camellia sinensis*). Among the 12 tested herbs, eight herbal extracts could inhibit the growth of *Streptococcus sanguinis*. Jasmine, jiaogulan, and lemongrass were the most potent, with minimum inhibitory concentrations (MIC) of 1 mg/ml, while green tea was less effective, with a MIC of 4 mg/ml. Among them, only rosemary could inhibit the growth of *S. mutans* at a MIC of 4 mg/ml. Total antioxidant capacities of herbal extracts were analyzed by three different assays, including 2,2-diphenyl-1-picrylhydrazyl (DPPH·) radical scavenging activity, trolox equivalent antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC). Regardless of the assays used, green tea exhibited the highest antioxidant capacity, followed by osmanthus. Wide variations in total phenolics and total flavonoids of herbal tea extracts were observed. Chlorogenic acid was detected in high amount in honeysuckle and duzhong. These data suggest that rosemary is a potent inhibitor of oral streptococci, and green tea and osmanthus may be effective potential sources of natural antioxidants.

1. Introduction

Green tea (*Camellia sinensis*) has been considered, in traditional Chinese medicine, as a healthful beverage, and PET-bottled green tea has recently become popular with the Taiwanese. Recent human studies suggest that green tea may contribute to a reduction in the risk of cardiovascular disease and some forms of cancer, as well as to the promotion of oral health and other physiological functions, such as antioxidant and antibacterial activities, antihypertensive effect and body weight control (Cabrera, Artacho, & Gimenez, 2006). In Taiwan, green tea leaves are reprocessed and scented with osmanthus (*Osmanthus fragrans*), rose (*Rosa damascena*) or jasmine (*Jasminum sambac*) flowers, combining fragrance and sweet taste. Herbs have been used for many purposes, including medicine, nutrition, flavouring, beverages, and fragrance. Juhua (*Chrysanthemum morifolium*) and honeysuckle (*Lonicera japonica*) are used as Chinese natural medicine and possess antibacterial and anti-inflammatory activities (Miyazawa & Hisama, 2003; Park et al., 2005). Duzhong (*Eucommia ulmoides*) and jiaogulan (*Gynostemma pentaphyllum*) teas are commonly consumed and regarded as functional health foods in Taiwan. Duzhong exhibits antioxidant activity in various lipid peroxidation models (Yen & Hsieh, 2000) and anti-diabetic potential (Lee et al., 2005). Jiaogulan has a variety of biological activities, including anti-inflammatory, immunopotentiating, and antioxidant effects (Norberg et al., 2004).

In addition to the popular scented teas and traditional herbal teas in Taiwan, various herbal teas have become increasingly popular because of their fragrance and antioxidant activities. For example, lavender (*Lavandula angustifolia*) is a well-known aromatic herb and exhibits antibacterial and anti-inflammatory properties (Hajhashmi, Ghannadi, & Sharif, 2003; Rota, Carraminana, Burillo, & Herrera, 2004). Lemongrass (*Cymbopogon citratus*) has been found to possess antioxidant activity and is recommended to treat digestive disorders, diabetes, and inflammation (Cheel, Theoduloz, Rodrigues, & Schmeda-Hirschmann, 2005). Mate (mä_ta; *Ilex paraguariensis*) is a popular beverage of many South American countries; extracts contain polyphenols and flavonoids and exhibit strong antioxidant activity (Gugliucci, 1996).

Dental caries, a common type of dental disease, is associated with microorganisms present on the tooth surface in dental plaque. Mutans streptococci, including *Streptococcus mutans*, *S. sobrinus*, *S. cricetus*, *S. rattus*, and *S. ferus*, participate in the formation of dental biofilm and play a significant role in the initiation of dental caries. *S. mutans* and *S. sobrinus* are regularly isolated from humans (Hardie, 1998). *S. sanguinis* (formerly *S. sanguis*) is colonized on the

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tooth surface during the early stage of dental plaque formation. Co-culture of transformed *S. mutans* with water-soluble glucan-synthesizing *S. sanguinis* yields firm adhesion (Tamesada, Kawabata, Fujiwara, & Hamada, 2004). There is increasing interest in the effect of natural compounds, especially food extracts, on resident oral bacteria. For example, cocoa bean husk extract (Ooshima et al., 2000), propolis (Koo, Rosalen, Cury, Park, & Bowen, 2002), and apple polyphenols (Yanagida, Kanda, Tanabe, Matsudaira, & Oliveria Cordeiro, 2000), have been shown to have potentially useful anticariogenic properties. We recently reported that aqueous and methanolic extracts of rosemary (*Rosmarinus officinalis*) inhibited *S. sobrinus* growth and its glucosyltransferase activity (Tsai, Tsai, & Ho, 2007). Numerous studies have demonstrated that spices and herbs possess antibacterial properties against food-borne bacteria and fungi (Roy & Lai, 2004), but there are few data or observations concerning the antimicrobial activity of herbal teas against oral pathogens.

Herbs and herbal extracts contain different phytochemicals with biological properties that promote human health and help reduce the risk of chronic disease (Craig, 1999; Curin & Andriantsitohaina, 2005; Larson, 1988). *C. sinensis* is a dietary source of antioxidant nutrients, such as carotenoids, tocopherols, ascorbic acid, and non-nutrient phytochemicals generally classified as flavonoids. Among these, the polyphenols and catechins constitute the most interesting group of tea leaf components: (−)-epigallocatechin gallate, (−)-epicatechin gallate, (−)-epigallocatechin, (−)-epicatechin, (−)-gallocatechin, and (−)-catechins (Graham, 1992). Phenolic acids and flavonoids, known as bioactive agents, frequently occur in herbal plants (Miean & Mohamed, 2001; Wen, Li, Di, Liao & Liu, 2005). Even though many herbs are known to be sources of phenolic compounds, their compositional data are still insufficient. Hence, one of the objectives of this study was to determine three major flavonols (kaempferol, quercetin, and myricetin) and two phenolic acids (chlorogenic acid and caffeic acid) in various herbs commonly consumed in Taiwan.

Several methods have been developed to measure the total antioxidant capacities of food and beverages; these assays differ in their chemistry, for example in the generation of different radicals and target molecules. In this study, three analyses were performed to determine the total antioxidant activities of herbs and green tea, including the 2,2-diphenyl-1-picrylhydrazyl (DPPH·) radical-scavenging analysis and trolox equivalent antioxidant capacity (TEAC) assay, based on electron transfer reaction, and oxygen radical absorbance capacity (ORAC) assay, based on hydrogen atom transfer reaction (Huang, Ou, & Prior, 2005).

The aim of this study was to characterize the antimicrobial activities against oral pathogens, antioxidant capacities, and selected phenolic components of herbs (juhua, honeysuckle, jasmine, lavender, rose, osmanthus, duzhong, jiaogulan, lemongrass, mate, and rosemary) commonly consumed in Taiwan, and to compare them with green tea for possible use as anticariogenic agents and natural antioxidants.

2. Materials and methods

2.1. Materials

Streptococcus sanguinis (BCRC15273) and *S. mutans* (BCRC10793) were obtained from the Bioresource Collection and Research Center, Food Industry Research and Development Institute (Hsinchu, Taiwan). *S. sanguinis* and *S. mutans* were, respectively, cultured in Brain Heart Infusion (BHI) broth and Tryptic Soy Broth (TSB) (Difco Laboratories, Detroit, MI, USA) containing 0.5% yeast extract, 0.05% L-cysteine, and 1% sucrose. These bacteria were cultured in an anaerobic atmosphere, using BBL GasPak systems (Becton Dickinson Microbiology Systems, Cockeysville, Md.). Chlorogenic acid, caffeic acid, rutin, quercetin, kapempferol, and myricetin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical-grade purity.

2.2. Preparation of extracts

Twelve dried herbs, including juhua, honeysuckle, jasmine, lavender, rose, osmanthus, duzhong, green tea, jiaogulan, lemongrass, mate, and rosemary, were purchased from a local supermarket in Taipei City (Taipei, Taiwan). The dried herbs (10 g) were extracted with 50 ml of methanol at room temperature for 3 h. After extraction, the mixture was filtered and the residue was re-extracted with 50 ml of fresh methanol overnight. The combined methanolic solution was centrifuged at 12,000*g* for 10 min and evaporated on a rotary evaporator. The methanolic extract was reconstituted in dimethyl sulfoxide (DMSO) to a concentration of 400 mg/ml for subsequent experimentation. The extraction yield of methanolic extract was presented as % weight (gram of methanolic extract for each 100 g of dry herbs) and the results are shown in Table 1.

2.3. Determination of anti-streptococcal activity

The methanolic extracts were tested against oral pathogens by determining minimum inhibitory concentration (MIC) values obtained by a microdilution broth method, as previously described (Tsai, Tsai, & Ho, 2007). Briefly, *S. sanguis* and *S. mutans* from overnight cultures were adjusted to 1×10^6 colony-forming units (CFU)/ml. In sterile 96-well microtiter plates, 100 μl of herbal extract were diluted with broth and placed into the well containing 100 μl of bacterial suspension in broth. Twofold serial dilutions were made in broth over a range to give the final concentrations of 0.06–8 mg/ml of methanolic extracts. To adjust for the interference by plant pigments, a parallel series of mixtures with uninoculated broth was prepared. Triplicate samples were performed for each test concentration. After incubation for 48 h at 37 °C under anaerobic condition, the growth of streptococci was estimated spectrophotometrically at 610 nm using a microtitre plate reader. The MIC was defined as the minimum concentration of test compound limiting turbidity to <0.05 absorbance. The experiments were performed in duplicate.

2.4. Total phenolic compound analysis

The amount of total phenolics in herbal extracts was evaluated using spectrophotometric analysis with Folin-Ciocalteu reagent (Zheng & Wang, 2001). 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

Table 1
Characteristics of various herbal teas

Common name	Botanical name	Part examined	Extract yield (%, w/w)
Honeysuckle	*Lonicera japonica* Thunb.	Flowers	20.4
Jasmine	*Jasminum sambac*	Flowers	26.7
Juhua	*Chrysanthemum morifolium*	Flowers	26.7
Lavender	*Lavandula angustifolia*	Flowers	16.0
Osmanthus	*Osmanthus fragrans*	Flowers	24.5
Rose	*Rosa damascena*	Flowers	8.4
Duzhong	*Eucommia ulmoides* Oliv.	Leaves	10.3
Green tea	*Camellia sinensis*	Leaves	21.6
Jiaogulan	*Gynostemma pentaphyllum*	Leaves	13.7
Lemongrass	*Cymbopogon citratus*	Leaves	8.4
Mate	*Ilex paraguariensis*	Leaves	26.0
Rosemary	*Rosmarinus officinalis*	Leaves	16.4

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 milligrams of gallic acid equivalents (GAE) per gram of solid of extract.

2.5. Determination of total flavonoids

Total flavonoids were measured according to a colorimetric assay (Kim, Chun, Kim, Moon & Lee, 2003). Briefly, 0.25 ml of optimally diluted sample was put into the tube containing 1 ml of double-distilled water (dd H_2O). 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, 1.175 ml of dd H_2O was added to the above reacting mixture and thoroughly mixed. The absorbance at 510 nm was measured. The flavonoid content in each extract was then calculated by a standard curve prepared with catechin and expressed as milligrams of catechin equivalents (CE) per gram of solid of extract.

2.6. Scavenging of diphenyl-picrylhydrazyl (DPPH·) radicals

The 2,2-diphenyl-1-picrylhydrazyl (DPPH·) radical-scavenging capacity of each herbal methanolic extract was measured as described earlier (Tsai, Tsai, Yu, & Ho, 2007). Briefly, 20 μl of each sample or 100% DMSO (as a negative control) were allowed to react with 200 μl of freshly prepared 200 μM DPPH· ethanolic solution in a 96-well microplate. The reaction mixture was mixed and left to stand for 10 min. The absorbance at 540 nm was determined against a blank of ethanol. The antioxidant activity of the sample was calculated as follows: % DPPH· radical-scavenging activity = $(1-[A_{sample} - A_{blank\ of\ sample}/A_{DMSO} - A_{blank\ of\ DMSO}]) \times 100$.

2.7. Trolox equivalent antioxidant capacity (TEAC) assay

The radical-scavenging capacity of the methanolic extracts of selected herbs and green tea leaves was measured against 2,2′-azinobis (3-ethylbenzothiazoline sulfonate ($ABTS^+$) according to the manufacturer's instruction (Randox Laboratories Ltd., Crumlin, UK) This assay was based on $ABTS^+$ incubated with metmyoglobin and hydrogen peroxide to generate the radical cation $ABTS^+$. $ABTS^+$ has a stable blue-green colour, which is measured at 600 nm. Antioxidants in the sample suppress the development of colour to an extent that is proportional to their concentration. Results were expressed in millimoles of trolox equivalents (TE) per gram of solid of herbal tea extract.

2.8. Oxygen radical absorbing capacity (ORAC) assay

The ORAC assay was performed as described by Ou, Hampsch-Woodill, and Prior (2001). Briefly, 25 μl of sample dissolved in 100 μl of 75 mM phosphate buffer (pH 7.0) were pipetted into a 96-well flat-bottomed plate. Trolox was used as a control antioxidant standard. Following this, 150 μl of fluorescein (3′,6′-dihydroxyspiro [isobenzofuran-1[3H], 9′[9H]-xanthen]-3-one) solution were put into each well and the plate was incubated at 37 °C for 30 min in the dark. Then, 25 μl of the 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH) solution was added as a peroxyl radical generator, and the fluorescence readings were taken immediately by a Zenyth 3100 microplate multimode detector (Anthos Labtec Instruments Inc, Lagerhausstr, Wals, Austria) every 5 min for 120 min. The final ORAC values were calculated using the differences of area under the quenching curves of fluorescein between a blank and a sample. The unit was expressed as millimoles of trolox equivalents (TE) per gram of methanolic herbal extracts.

2.9. Analysis of selected phenolic compounds in herbs

The selected phenolics in methanolic herbal extracts, namely, caffeoyl derivatives (chlorogenic acid and caffeic acid) and flavonoids (rutin, quercetin, kapempferol, and myricetin), were identified by using a reverse-phase high-performance liquid chromatography (HPLC) method (Filip, Lopez, Giberti, Coussio, & Ferraro, 2001). The phenolic compounds were expressed as mg/g of methanolic extract. The HPLC analysis was performed using a Hewlett-Packard HPLC system (HP 1100 series, Waldbronn, Germany), consisting of binary pump and UV photodiode array detector and equipped with a ODS Hypersil-C18 (5 mm, 250 × 4.6 mm) column (Thermo Electron Corporation, Waltham, MA, USA). The HPLC method used the following gradient elution programme: (solvent A; water/acetic acid 98:2 (v/v) and solvent B; methanol/acetic acid 98:2 (v/v)): 15% B to 40% B, 30 min; 40% B to 75% B, 10 min; and 75% B to 85% B, 5 min. The flow rate was 1 ml/min and the injection volume was 20 μl. Detection was at 325 nm for caffeoyl derivatives, at 254 nm for rutin, myricetin, and quercetin, and at 263 nm for kaempferol. Identification of individual phenolics was based on the comparison of the retention time and UV spectrum of unknown peaks with those of reference authentic standards.

2.10. Statistical analysis

Data were presented as means + SD. Statistical analysis was conducted using the SPSS 12.0 statistical package (Chicago, IL, USA). Data were subjected to analysis of variance, and means were separated using Fisher's least significant difference (LSD) test at $P = 0.05$. Pearson correlation analysis was used to evaluate the relationships among the variables of interest. A P-value of less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. Anti-streptococcal activity

The elimination of cariogenic bacteria from the oral cavity using antibacterial agents is one of the primary strategies for the prevention of dental caries. As shown in Table 2, the crude methanolic extracts from herbs and green tea leaves were evaluated for growth inhibitory activity against *S. mutans* and *S. sanguinis*. Among the 12 tested herbs, only rosemary could inhibit the growth of *S. mutans*. We previously demonstrated that aqueous and methanolic extracts of rosemary exhibit antimicrobial activity against oral bac-

Table 2
The minimal inhibition concentration (MIC) of methanolic extracts of various herbs against oral pathogens

Herbs	MIC (mg/ml)		
	S. mutans	*S. sanguinis*	*S. sobrinus*
Honeysuckle	>8	4	>8
Jasmine	>8	1	>8
Juhua	>8	>8	>8
Lavender	>8	>8	>8
Osmanthus	>8	>8	>8
Rose	>8	>8	>8
Duzhong	>8	4	>8
Green tea	>8	4	>8
Jiaogulan	>8	1	>8
Lemongrass	>8	1	>8
Mate	>8	4	>8
Rosemary	4	2	4*

* Data for *S. sobrinus* were reported previously (Tsai, Tsai, & Ho, 2007).

teria *S. sobrinus* at MICs of 16 and 4 mg/ml, respectively (Tsai, Tsai, & Ho, 2007). This study demonstrated that methanolic extract from rosemary also possessed antimicrobial activity against *S. mutans* and *S. sanguinis* at MIC values of 4 and 2 mg/ml, respectively (Table 2). Although the results herein indicate that a crude methanolic extract of rosemary suppresses the growth of cariogenic streptococci, relatively little is known about its activity against periodontal pathogens such as *Porphyromonas gingivalis* and *Prevotella intermedia*. Further studies are needed to explore the potential values of rosemary or its components as natural antiplaque or antigingivitis agents.

The methanolic extracts from jasmine, jiaogulan, and lemongrass demonstrated preferential antimicrobial activity against *S. sanguinis* at a MIC of 1 mg/ml. Honeysuckle, duzhong, green tea, and mate showed less inhibitory activity against *S. sanguinis* with MIC values of 4 mg/ml. In contrast, juhua, lavender, rose, and osmanthus had no effect on the growth of *S. sanguinis* (Table 2). The mechanism of antimicrobial action of these herbs has not been clearly elucidated. Numerous studies have demonstrated that green tea, oolong tea (semi-fermented tea leaves of *C. sinensis*), black tea, and oligomeric catechins, from these preparations, exhibit bactericidal activity against *S. mutans* and *S. sobrinus* (Hamilton-Miller, 2001; Rasheed & Haider, 1998).Green tea extract was reported to be effective in the prevention of dental caries because of its antimicrobial and anti-plaque activity (Hamilton-Miller, 2001). However, as shown in Table 2, methanolic extract from green tea possessed antimicrobial activity against *S. sanguinis*, but no appreciable activity against *S. mutans* and *S. sobrinus*. The yield of methanolic extract from green tea leaves was 21.6% (w/w) (Table 1). Theoretically, a cup of green tea prepared with 2 g of tea leaves (the usual amount of a commercial tea bag) in 100 ml of infusion contains a total of 4.32 mg/ml of methanolic extract. Our results showed that methanolic extract from green tea did not completely inhibit the growth of S. *mutans* up to 8 mg/ml. Kubo, Muroi, and Himejima (1992) demonstrated that volatile flavour compounds in green tea had inhibitory activity against *S. mutans* (Kubo et al., 1992; Muroi & Kubo, 1993). However, MICs were in excess of concentrations found in tea infusion. Furthermore, it is difficult to directly compare the results from different studies due to difference in analytical methods, e.g., serotypes of *S. mutans*, the presence of sucrose in culture broth, sources and manufacturing methods of tea, and procedures for extraction. The anti-plaque activity of herbs was not determined in this study; further studies are needed to investigate the anticariogenic properties of herbs.

3.2. Contents of total phenolic compounds and flavonoids

Total phenolics and total flavonoids in 12 herbal teas are shown in Fig. 1. Total phenolics of herbal teas varied from 33.9 to 149 mg GAE/g extract. Green tea was found to have the highest total phenolics among the 12 herbs, whereas lavender had the lowest. The level of total phenolics in green teas was ~4.4-fold higher than in lavender, showing the wide variance of total phenolic concentrations in tested herbal teas. Total flavonoid levels ranged between 23.7 and 225 mg CE/g of extract. Sweet osmanthus had the highest total flavonoid content, whereas jasmine had the lowest. The total flavonoid content of sweet osmanthus was ~9.5 times higher than that of jasmine. As with total phenolics in herbal teas tested, the levels of total flavonoids among the herbal teas resulted in significant differences.

3.3. Total antioxidant capacity

Herbs may be good sources of natural antioxidants. The high level of polyphenolics and antioxidant activity in green tea has been

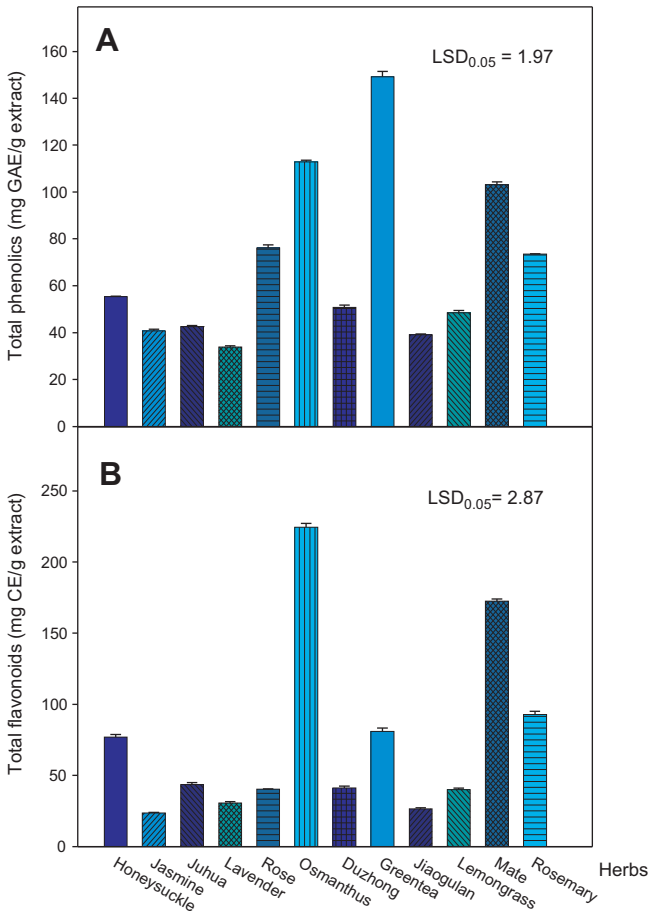

Fig. 1. Total phenolics and flavonoid contents of methanolic herbal extracts.

reported (Cabrera et al., 2006). Several studies also showed that juhua (Kim & Lee, 2005), jiaogulan (Norberg et al., 2004), rosemary (Tsai, Tsai, Yu, et al., 2007), lemongrass (Cheel et al., 2005), mate (Bixby, Spieler, Menini, & Gugliucci, 2005; Gugliucci, 1996), and duzhong (Yen & Hsieh, 2000) exhibited antioxidant properties.

The total antioxidant capacities of the herbal methanolic extracts and the ranking order for each assay are shown in Table 3. Green tea had the greatest antioxidant capacity of all the herbs used in each assay, followed by osmanthus. Notably, there was no significant difference in DPPH·-scavenging capacity between green tea and osmanthus. After green tea leaves and osmanthus, the DPPH radical- scavenging capacity, in decreasing order, for the remaining herbs analyzed was: rose > mate > rosemary > honeysuckle > juhua > duzhong > lemongrass > jasmine > jiaogulan > lavender. After green tea leaves and osmanthus, the TEAC values in decreasing order for the remaining herbs analyzed were mate > rosemary > rose > honeysuckle > jiaogulan > duzhong > juhua > lemongrass > lavender > jasmine. When the ORAC assay was used to provide a ranking order of antioxidant activity, the result, in decreasing order, was: green tea > osmanthus > mate > rosemary > rose > honeysuckle > duzhong > jiaogulan > lemongrass > jasmine > lavender > juhua (Table 3). The herbal extracts exhibited different antioxidant capacities in relation to the method applied; thus the same material ranked differently, depending on the assay. The data obtained using the DPPH·-scavenging capacity and ORAC values had the best correlation coefficients ($r = 0.934$, $p < 0.001$), whereas the TEAC values and ORAC values ($r = 0.856$, $p < 0.001$) and the DPPH·-scavenging capacity and TEAC values ($r = 0.734$, $p < 0.001$) were less well correlated. The antioxidant capacities of herbal extracts ranked differently in

Table 3
In vitro free radical-scavenging activities of methanolic herbal extracts[a]

Herbs	DPPH·– scavenging		TEAC		ORAC	
	Value (% quenched*)	Rank	Value (mmole TE/ g extract)	Rank	Value (mmole TE/ g extract)	Rank
Honeysuckle	45.2 ± 1.65	6	0.595 ± 0.011	6	1.66 ± 0.01	6
Jasmine	13.0 ± 1.07	10	0.240 ± 0.009	12	0.92 ± 0.23	10
Juhua	26.4 ± 0.90	7	0.316 ± 0.002	9	0.84 ± 0.04	12
Lavender	6.15 ± 1.79	12	0.274 ± 0.017	11	0.90 ± 0.05	11
Osmanthus	93.2 ± 0.20	2	1.64 ± 0.045	2	4.18 ± 0.32	2
Rose	89.3 ± 0.34	3	1.12 ± 0.048	5	2.77 ± 0.28	5
Duzhong	25.4 ± 1.90	8	0.341 ± 0.006	8	1.48 ± 0.12	7
Green tea	94.5 ± 0.15	1	4.60 ± 0.089	1	4.63 ± 0.37	1
Jiaogulan	7.48 ± 1.31	11	0.425 ± 0.007	7	1.16 ± 0.18	8
Lemongrass	23.5 ± 0.97	9	0.313 ± 0.009	10	0.99 ± 0.11	9
Mate	86.9 ± 1.72	4	1.54 ± 0.045	3	3.34 ± 0.13	3
Rosemary	70.1 ± 1.04	5	1.28 ± 0.002	4	2.80 ± 0.06	4
$LSD_{0.05}$	2.31		0.076		0.24	

*The concentration was 1 mg of methanolic extract per ml in DPPH radical reaction mixtures.
[a] Data are expressed as means ± SD.

TEAC and DPPH· assays, although both of them were based on the measurement of electron transfer propensity of antioxidant (Table 3). The TEAC values were not strongly related to chemical structures or the number of electrons that an antioxidant can give away (Huang et al., 2005). Hence, these phenomena may be among reasons for the different observations using TEAC and DPPH assays.

Since the methanolic extracts from green tea leaves and osmanthus possess high antioxidant activity, the osmanthus-scented green tea, which is familiar in Taiwan and served with tea bags or in restaurants, may be used as a good source of natural antioxidants. Osmanthus extract has been approved as a food additive and confirmed as GRAS in the USA (Oser, Weil, Woods, & Bernard, 1985). In Chinese cuisine, osmanthus flowers are also used to make osmanthus-scented jam, sweet cake, and soups. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials. There is little information concerning the antioxidant activity of osmanthus; future studies are needed to explore the role of active phytochemicals with respect to antioxidant activity.

The antioxidant values correlated well with both total phenolics and total flavonoids contents. A positive relationship was presented between total phenolics and total antioxidant capacity in 12 herbs, expressed as DPPH· ($r = 0.893$, $p < 0.001$), TEAC ($r = 0.928$, $p < 0.001$), and ORAC ($r = 0.968$, $p < 0.001$). We previously demonstrated that there was a good correlation between total phenolic and TEAC values in aqueous extracts from herbs, such as green tea, jasmine, juhua, lavender, lemongrass, rose, and rosemary (Tsai, Tsai, Yu, et al., 2007). A significant relationship was also displayed between total flavonoids and total antioxidant capacity, expressed as DPPH· ($r = 0.711$, $p < 0.001$), TEAC ($r = 0.393$, $p = 0.018$), and ORAC ($r = 0.736$, $p < 0.001$).

3.4. Characterization of phenolic constituents

The selected phenolics in the 12 tested herbal extracts were identified and are presented in Table 4. Considerable variation was found in selected phenolic compounds of different herbs. Chlorogenic acid was the predominant phenolic constituent in the extracts of honeysuckle, duzhong and mate. Rutin was a major component in the extracts of rose, mate and duzhong. For lemongrass extract, all kinds of analyzed phenolic compounds were trace constituents.

Many substances in green tea exhibit antibacterial activity against *S. mutans*, including several polar polyphenolic compounds (Sakanaka, Kim, Taniguchi, & Yamamoto, 1989) and nonpolar volatile flavour compounds (Kubo et al., 1992). However, the polyphenolic compounds identified in green tea, such as (−)-epicatechin and (−)-epigallocatechin, did not show any antibacterial activity against S. *mutans* up to 500 μg/ml (Muroi & Kubo, 1993). Flavanones and some dihydroflavonols inhibit the growth of *S. mutans* and *S. sobrinus* (Koo et al., 2002). Chlorogenic and caffeic acid inhibit the growth of enterobacteria and *S. mutans* (Almeida, Farah, Silva, Numan, & Gloria, 2006). Cai and Wu (1996) reported that kaempferol

Table 4
Phenolic compounds of methanolic herbal extracts

Herbs	Chlorogenic acid	Caffeic acid	Rutin	Quercetin (mg/g of methanolic extract)	Kaempferol	Myricetin
Honeysuckle	153	1.79	13.0	2.54	nd*	1.64
Jasmine	1.80	1.01	10.4	4.05	1.01	nd
Juhua	3.98	0.27	nd	1.15	nd	0.44
Lavender	nd	10.4	nd	nd	1.11	2.83
Osmanthus	6.13	0.25	1.00	1.10	0.45	0.70
Rose	nd	nd	27.8	1.16	0.58	2.33
Duzhong	60.3	0.98	21.9	nd	nd	nd
Green tea	0.12	2.30	6.65	nd	0.97	nd
Jiaogulan	0.07	nd	12.8	1.59	nd	nd
Lemongrass	3.00	0.35	nd	nd	nd	2.65
Mate	22.1	0.44	23.4	0.88	0.27	3.66
Rosemary	2.44	0.81	1.90	2.81	0.90	5.16

Each value is the mean (mg/g extract) of two replications.
*nd, not detectable.

and myricetin had antimicrobial activity against *S. mutans* and the periodontal pathogens, *P. gingivalis*, and *P. intermedia*.

In many herbs, the main flavonoid constituents are flavonol aglycones, such as quercetin, myricetin, kaempferol and their glycosides. These phenolic acids and flavonoids possess antioxidant activity (Kähkönen et al., 1999). The dicaffeoylquinic acids derived from juhua showed potent DPPH· radical-scavenging activity (Kim & Lee, 2005). Chlorogenic acid and caffeic acid are well-known plant antioxidants and show a strong effect toward DPPH· (Cheel et al., 2005).

The phenolic compounds in herbs are diverse and complex. Although honeysuckle had the highest amount of chlorogenic acid among 12 tested herbs herein, it possessed moderate antioxidant activity and no inhibitory effect on the growth of *S. mutans*. The results suggested that other unidentified phenolic components herein contributed to antioxidant capacities of herbal extracts and antimicrobial activity of rosemary against *S. mutans*.

In summary, the present study shows that crude methanolic extract of rosemary can inhibit the growth of the cariogenic bacteria, *S. mutans* and *S. sanguinis*. The crude methanolic extracts from jasmine, jiaogulan, lemongrass, rosemary, honeysuckle, duzhong, green tea, and mate possess antimicrobial activity against *S. sanguinis* alone. Future investigations should examine the role of herbal teas in a wider range of bacteria, including Gram-negative species associated with gingivitis and other forms of periodontal diseases. This study also reveals that osmanthus may be an effective potential source of natural antioxidants. In conclusion, these results suggest that herbs may have potential application in preventing dental caries and other health problems associated with free radical-mediated damage.

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