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Antimicrobial and Anti-Biofilm Activities of the Methanol Extracts of Medicinal Plants against Dental Pathogens *Streptococcus mutans* and *Candida albicans*

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Several medicinal plants are ethnomedically used in Korea as agents for treating infection, anti-inflammation, and pain relief. However, beyond typical inhibitory effects on cell growth, little is known about the potential anti-biofilm activity of these herbs, which may help to prevent cavities and maintain good oral health. This study aimed to investigate the antimicrobial and anti-biofilm activities of the methanol extracts of 37 Korean medicinal plants against dental pathogens *Streptococcus mutans* and *Candida albicans,* which synergize their virulence so as to induce the formation of plaque biofilms in the oral cavity. The antimicrobial activities were investigated by broth dilution and disk diffusion assay. The anti-biofilm and antioxidant activities were evaluated based on the inhibitory effect against glucosyltransferase (GTase) and the DPPH assay, respectively. Among 37 herbs, eight plant extracts presented growth and biofilm inhibitory activities against both etiologic bacteria. Among them, the methanol extracts (1.0 mg/ml) from *Camellia japonica* and *Thuja orientalis* significantly inhibited the growth of both bacteria by over 76% and over 83% in liquid media, respectively. Minimum inhibitory concentration (MIC) values of these methanol extracts were determined to be 0.5 mg/ml using a disk diffusion assay on solid agar media. Biofilm formation was inhibited by more than 92.4% and 98.0%, respectively, using the same concentration of each extract. The present results demonstrate that the medicinal plants *C. japonica* and *T. orientalis* are potentially useful as antimicrobial and anti-biofilm agents in preventing dental diseases.

Keywords: *Camellia japonica, Thuja orientalis,* antimicrobial activity, anti-biofilm activity, *Streptococcus mutans, Candida albicans*

Introduction

According to a recent WHO report, dental caries prevail to a large extent among both children and adults [1]. Diet habits, bacterial adhesion to tooth surfaces, biofilm formation, and dentin mineralization play key roles in the formation of dental caries. In the early stages of dental caries formation, cariogenic plaque biofilms on tooth surfaces are synergistically constructed by the combined action of enzymes (glucosyltransferase and fructosyltransferase) released by *Streptococcus mutans* and the adhesive enhancing agents (glucans) secreted by *Candida albicans*, thereby providing habitats for the growth of etiologic bacteria [2].

The resulting biofilms not only contribute to the development of dental caries, but also support the growth of diverse microorganisms. Recently, a human oral microbiome project identified more than 764 kinds of bacteria and 102 kinds of fungi. Specifically, among 132 kinds of bacteria, 12 species were classified as pathogenic bacteria of the oral cavity [3, 4]. The identified pathogenic

bacteria were identical to or closely linked with opportunistic pathogens implicated in bacterial endocarditis, aspiration pneumonia, osteomyelitis, preterm low birth weight, coronary heart disease, and cerebral infarction [3, 5]. These reports showed that the dental microbiome not only was responsible for large outbreaks of diseases in the oral cavity, but could also be implicated in systemic diseases [6, 7]. These findings make the control of oral microorganisms an important issue. In these contexts, dental plaque biofilm is a major obstacle to such control owing to its ability to protect pathogenic bacteria from antibiotics [8]. Although chlorhexidine gluconate is widely used to reduce and inhibit biofilm formation, it has some disadvantages, including dental pigmentation, restoration by insensitive strains, burning sensation in the mouth, and alteration in the sensation of taste [9].

Increasing resistance among pathogens to conventional antibiotics and undesirable side effects of existing therapies have made traditional medicinal plants an attractive source to screen for antimicrobial agents. In line with this effort, the present study aimed at evaluating the antimicrobial and anti-biofilm activities of methanol extracts from 37 herbs, traditionally used to treat infection, inflammation, and pain in Korea, against two major dental pathogens, *S. mutans* and *C. albicans*.

Materials and Methods

Medicinal Plant Materials

The medicinal plants were selected in Korea based on their antibiotic activity and their traditionally known medical uses. Dried whole-plant materials of *Platycladus orientalis*, *Camellia japonica*, and *Sparganium erectum* (600 g of each sample) were purchased from the Gyeong-dong (Korea) oriental medicine market. The freeze-dried methanol extracts of other plants (20 mg of each sample) were obtained from the Korea Plant Extract Bank. The list of species used in this study is shown in Table 1.

Methanol Extraction from Plants

The dried ground materials of *P. orientalis*, *C. japonica*, and *S. erectum* (50 g) were extracted with 1.3 L of 95% methanol for 1 day at room temperature (25°C). Thereafter, it was centrifuged at 8,000 ×*g* for 30 min and then filtered. The residues were further extracted twice with methanol under the same conditions. The combined extracts were evaporated under reduced pressure at 45°C using a rotary vacuum evaporator (IKA RV 10 Digital, Germany). The concentrated and freeze-dried extracts (20 mg) were dissolved in 1 ml of methanol to assess the antibacterial and anti-biofilm activities.

Bacterial Strains and Culture Conditions

The strain *S. mutans* ATCC 25175 was purchased from the Korean Collection for Type Cultures in the Korea Research Institute of Bioscience and Biotechnology (Korea) and grown at 37°C for 24 h in Brain Heart Infusion (3.7% BHI; Difco, USA) medium. The strain *C. albicans* NUM961 was obtained from Dr. Suhn-Young Im's laboratory (Chonnam National University, Korea) and grown at 30°C for 18 h in YPD (1% yeast extract, 2% peptone, and 2% dextrose) broth.

Determination of Antimicrobial Activities and Minimum Inhibitory Concentration (MIC)

To preliminarily investigate the antimicrobial activity of the methanol extracts, 0.15 ml of each methanol extract was added into 3 ml of culture medium in which the specific amount (equivalent to the McFarland 0.5 standard) of pre-cultured *S. mutans* or *C. albicans* was inoculated. After the cells had been incubated for 24 h, the growth inhibition of etiologic pathogens was evaluated by measuring the absorbance at 600 nm using a spectrophotometer (UV/Vis Spectrophotometer UV-1700; Shimadzu Co., Japan) with methanol only as the negative control, and expressed as percent inhibition by each plant extract. Considering the physiological features of the oral cavity, MICs of the methanol extracts were determined using the disk diffusion method [10]. The cultured cells (5×10^6 cell/ml) were inoculated onto an appropriate solid medium using the pour plate method, and the medium was dried for 15 min at room temperature. The 6-mm filter paper disks were impregnated with 10 µl of varying concentrations of an extracted plant sample (100 µg/ml, 500 µg/ml, 1 mg/ml, and 2 mg/ml) and then placed on the inoculated agar plates. After the culture was performed, the diameter of the growth inhibition zone from the paper disk was measured for each sample concentration in duplicates. The MIC for each plant extract was defined as the lowest concentration of each tested methanol extract that prevented cell growth (diameter > 8 mm).

Recovery of Glucosyltransferase (GTase) from *S. mutans*

Secreted extracellular GTase was recovered by modifying the method from a previous report [11]. The strain *S. mutans* grown in 10 ml of BHI medium at 37°C for 24 h was removed by centrifugation. Culture supernatants were filtrated using a 0.2-µm membrane filter. The cell-free proteins in the supernatant were recovered by centrifugation using an Amicon ultra filter (MWCO 30 kDa; Millipore, USA) at 7,500 ×*g* and 4°C for 30 min. Subsequently, proteins were quantified using the Bradford assay and an aliquot of the same amount of protein solution (25–30 µg) was subjected to the inhibition assay against GTase activity.

Determination of GTase Inhibition

The GTase activity assay involves quantification of water-insoluble glucan using a spectrophotometer [12]. Briefly, 20 µl of recovered crude enzymes was mixed with 180 µl of diluted methanol extract and 800 µl of 62.5 mM potassium phosphate buffer (pH 6.5) containing 2% sucrose and 4 mM NaN_3, followed by incubation at 37°C for 16 h. As a control, the same volume of

Table 1. Oriental medicinal plants used in this study.

Family name	Botanical name	Local name/tribe	Plant parts	Traditional use
Amaryllidaceae	*Lycoris radiate*	Spider lily, Red spider lily, cluster amayllis	Leaf	Tonsillitis, antitumor
Aquifoliaceae	*Ilex integra*	Machi tree	Heartwood	Wounds, simple fractures, toothache
Araliaceae	*Hedera rhombea*	Korean ivy	Whole plant	Anti-rheumatic, facial paralysis, antitumor
Araliaceae	*Aralia continentalis*	Aralia cordata	Whole plant	Analgesic, diuretic
Araliaceae	*Aralia elata*	Small-leaf Korean angelica	Leaf, stem	Anticancer, glycosuria, analgesic
Commelinaceae	*Pollia japonica*	Japanese pollia	Whole plant	Wounds, lower back pain, toothache
Compositae	*Ligularia fischeri*	Fischer ligularia	Whole plant	Asthma, analgesic, vulnerary, pertussis
Cruciferae	*Wasabia koreana*	Wasabi	Whole plant	Antibiotic, dental caries
Cupressaceae	*Thuja orientalis*	Oriental Arbor vitae, thuja	Leaf, stem	Bleeding, colitis, dysentery, insomnia
Cyperaceae	*Carex siderosticta*	Broadleaf sedge	Whole plant	Headache, toothache, dysmenorrhea
Geraniaceae	*Geranium sibiricum*	Siberian cranesbill	Whole plant	Dysentery, detoxification therapy, bleeding, antibiotic
Gramineae	*Eragrostis japonica*	Pond lovegrass	Whole plant	Wounds, analgesic
Hamamelidaceae	*Corylopsis coreana*	Korean winter hazel	Stem	Vomiting
Labiatae	*Prunella vulgaris* var. *lilacina*	Hagocho, selfheal	Whole plant	Fevers, diarrhea, internal bleeding, weaknesses of the liver and heart
Lauraceae	*Lindera glauca*	Greyblue spicebusi	Leaf	Stomach cancer, cancer of the esophagus, toothache
Leguminosae	*Robinia pseudo-acacia*	False acacia	Leaf, stem	Asthma, anti-inflammatory, diuretic
Liliaceae	*Hosta minor*	Minor hosta	Whole plant	Tympanitis, tuberculosis, toothache, gastralgia
Liliaceae	*Convallaria keiskei*	Lily of the valley, May lily	Whole plant	Diuretic, edema
Meliaceae	*Melia azedarach* var. *japonica*	Beed tree, white cedar	Fruit	Vermifuge, scaling
Oleaceae	*Osmanthus insularis*	Island devilwood	Bark	Tussis, toothache
Oleaceae	*Ligustrum obtusifolium*	Privet	Fruit	Gingival bleeding, halitosis
Onagraceae	*Oenothera laciniata*	Evening primrose	Whole plant	Anti-inflammatory, dysentery hyperlipidemia
Papaveraceae	*Chelidonium majus* var. *asiaticum*	Asian celandine	Whole plant	Wart, atopic dermatitis, anti-inflammatory
Phrymaceae	*Phryma leptostachya* var. *asiatica*	Asian lopseed	Whole plant	Detoxification therapy, scabies, anti-inflammatory
Polygonaceae	*Bistorta manshuriensis*	Bistort, snakeweed	Whole plant	Enteritis, bleeding, gingivitis
Polygonaceae	*Polygonum sagittatum* var. *sieboldii*	Arrow-leaf smartweed	Whole plant	Enteritis, antitumor, anti-inflammatory
Rhamnaceae	*Hovenia dulcis*	Oriental raisin tree	Heartwood	Vomiting, anti-rheumatic, anti-inflammatory
Rosaceae	*Rosa rugosa*	Turkestan rose	Leaf	Bleeding, analgesic
Saxifragaceae	*Kirengeshoma koreana*	Korean kirengeshoma	Root	Food poisoning, toothache, enteritis
Saxifragaceae	*Chrysosplenium flagelliferum*	Stolon golden saxifrage	Whole plant	Anti-inflammatory
Selaginellaceae	*Selaginella tamariscina*	Selaginella	Whole plant	Bleeding, asthma, nephritis
Sparganiaceae	*Sparganium stoloniferum*	Bur reed	Whole plant	Wounds, analgesic, melancholia
Sterculiaceae	*Firmiana simplex*	Chinese parasol tree	Bark	Gastralgia, toothache, alopecia
Theaceae	*Eurya emarginata*	Emarginate eurya	Stem	Gingival bleeding, diuretic
Theaceae	*Camellia japonica*	Camellia	Leaf	Anti-inflammatory, bleeding
Umbelliferae	*Centella asiatica*	Asiatic Pennywort	Whole plant	Anti-inflammatory, antitumor, ulcer
Valerianaceae	*Patrinia scabiosaefolia*	Patrinia	Whole plant	Dermatitis, detoxification therapy, dysentery

95% methanol was added in the reaction solution. After incubation, each reaction mixture was centrifuged to harvest water-insoluble glucans. The recovered water-insoluble glucans were washed with double-distilled water and dispersed by sonication (Vibra-cell VCX-750 Sonic & Materials, USA). Total amount of water-insoluble glucan was determined by measuring absorbance at 550 nm using a spectrophotometer (UV/Vis Spectrophotometer UV-1700). The GTase inhibition ratio (%) was calculated using the following equation:

$$\text{Glucosyltransferase inhibition ratio (\%)} = (A - B)/A \times 100$$

A and B refer to absorbance of the dispersed solution from the reaction containing 95% methanol and the plant extract, respectively.

Determination of Total Phenolic Compound Contents

The total phenolic content was determined by the method described in a previous report [13]. Briefly, 0.2 ml of the methanol extracts was mixed with 0.8 ml of distilled water, 0.5 ml of 2 N Folin-Ciocalteu reagent, and 2.5 ml of 10% $NaCO_3$. The resulting mixture was incubated in a water bath at 25°C for 20 min. To remove precipitates, the reaction mixture was centrifuged at 3,000 rpm for 20 min. After centrifugation, the absorbance of the supernatant was measured at 735 nm. The total phenolic content was calculated using a gallic acid calibration curve and expressed as gallic acid equivalents.

Determination of Flavonoid Content

The total flavonoid content in the extracts was determined using a colorimetric assay [13]. Briefly, 0.1 ml of methanol extract was diluted with 0.9 ml of 80% ethanol. Then, 0.5 ml of the resulting mixture was added to a 14 ml test tube containing 4.3 ml of 80% ethanol, 0.1 ml of 10% aluminum nitrate, and 0.1 ml of 1 M potassium acetate. The solution was left at room temperature for 40 min. The absorbance was measured at 415 nm using a spectrophotometer. The total flavonoid content was calculated using a quercetin calibration curve.

Determination of Antioxidant Activity

The antioxidant activity was measured by DPPH assay [14]. Briefly, 1,1-diphenyl-2-picrylhydrazyl (DPPH; Sigma, USA) was freshly dissolved in ethanol (95%) to obtain a 2 mM DPPH solution. Then, 1.0 ml of the plant extract (1 mg/ml) and 0.1 ml of DPPH solution were added in a 1.5-ml microtube. After incubation for 10 min in the dark, the changes in color were read at 517 nm using a spectrophotometer (UV/Vis Spectrophotometer UV-1700). A mixture of ethanol (0.1 ml) and plant extract (1 ml) was used as the blank. The control solution was prepared using ethanol and DPPH solution. The scavenging capacity of the antioxidant L-ascorbic acid was used for comparison of the radical scavenging activity of the plant extracts.

Statistical Analysis

All tests were independently performed in duplicates or triplicates. The results obtained were analyzed using a one-way ANOVA test and reported as the mean ± SD [15].

Table 2. Antimicrobial activity of methanol extracts (1 mg/ml) against oral pathogens in liquid culture.

Botanical name[a]	Growth inhibition (%)	
	S. mutans	*C. albicans*
Sparganium stoloniferum	98 ± 0.7	22 ± 0.7
Carex siderosticta	94 ± 0.5	-
Pollia japonica	91 ± 1.3	15 ± 0.9
Aralia continentalis	89 ± 1.2	11 ± 0.9
Firmiana simplex	84 ± 0.8	40 ± 0.9
Thuja orientalis	83 ± 0.5	93 ± 1.1
Chelidonium majus var. *asiaticum*	80 ± 1.2	52 ± 0.9
Hovenia dulcis	79 ± 0.2	-
Convallaria keiskei	78 ± 1.8	14 ± 1.4
Polygonum sagittatum var. *sieboldii*	77 ± 1.2	21 ± 0.1
Camellia japonica	76 ± 0.9	76 ± 0.1
Wasabia koreana	73 ± 0.3	10 ± 0.1
Chrysosplenium flagelliferum	71 ± 2.5	49 ± 0.1
Bistorta manshuriensis	7 ± 1.2	-
Prunella vulgaris var. *lilacina*	65 ± 0.2	-
Rosa rugosa	54 ± 0.8	45 ± 0.1
Phryma leptostachya var. *asiatica*	49 ± 1.1	-
Oenothera laciniata	47 ± 0.7	29 ± 0.6
Kirengeshoma koreana	47 ± 0.2	14 ± 0.5
Geranium sibiricum	46 ± 0.9	58 ± 1.0
Robinia pseudo-acacia	42 ± 0.2	14 ± 0.4
Lycoris radiata	41 ± 0.9	52 ± 0.8
Centella asiatica	41 ± 0.7	-
Lindera glauca	40 ± 1.1	96 ± 0.9
Aralia elata	40 ± 0.6	-
Hedera rhombea	36 ± 0.9	8 ± 1.1
Eragrostis japonica	29 ± 0.6	7 ± 1.1
Osmanthus inularis	21 ± 1.3	-
Corylopsis coreana	19 ± 0.6	8 ± 0.9
Ligustrum obtusifolium	17 ± 1.1	-
Ilex integra	16 ± 0.3	76 ± 0.1
Eurya emarginata	-	51 ± 0.2
Melia azedarach var. *japonica*	-	-
Hosta minor	-	-
Patrinia scabiosaefolia	-	-
Selaginella tamariscina	-	-
Ligularia fischeri	-	-

Values are the mean ± SD (n = 5).

[a]The Korean name is given in Table 1.

Results and Discussion

Antimicrobial Activity of Plant Extracts

We selected the target strains with the aim of studying the suppression or inhibition of pathogenic growth in the oral cavity by the extracts of medicinal plants. In the oral cavity of Korean patients with periodontal disease, *S. mutans*, *S. mitis*, *S. intermedius*, and the fungus *C. albicans* were observed to be dominant and frequently found, as observed in another report [16]. Recently, it was reported that exopolysaccharides produced by GTase from *S. mutans* and β-1,3-glucan from *C. albicans* contribute to the formation of biofilms. The coexistence of these two species within plaque biofilms induces *S. mutans* to produce virulence factors [2]. Therefore, we screened for medicinal plants with anti-biofilm and antibacterial activities against *S. mutans* and *C. albicans*.

Based on a variety of literature, including traditional Korean knowledge of folk remedies, 37 plant species were selected to investigate the antimicrobial activity against *S. mutans* ATCC25175 and *C. albicans* NUM961 as described in Table 2. The methanol extract from *Sparganium stoloniferum*, *Pollia japonica*, *Carex siderosticta*, *Chelidonium majus* var. *asiaticum*, *Firmiana simplex*, *Thuja orientalis*, and *Aralia continentalis* showed high antibacterial activity (>80% growth inhibition) against *S. mutans*. *Lindera glauca* and *Thuja orientalis* extracts revealed high antifungal activity (>93% growth inhibition) against *C. albicans*. *Camellia japonica* and *T. orientalis* extracts inhibited cell growth by more than 70% against both pathogenic microorganisms in the liquid broth. Otherwise, the MIC test using the disk diffusion assay showed that eight plant extracts distinctly inhibited cell growth of both etiologic microorganisms in the preliminary test. These extracts were further quantitatively analyzed to determine their MIC values at concentrations of 0.1, 0.5, 1, and 2 mg/disk and the results are summarized in Table 3. *Camellia japonica, Geranium sibiricum*, and *T. orientalis* showed distinct growth inhibition zones at 0.5 mg/disk against *S. mutans* and *C. albicans*. The particular, *T. orientalis* revealed a growth inhibition zone at 0.1 mg/disk against *S. mutans*. As shown in Tables 2 and 3, the measured antimicrobial activities of selected methanol extracts fluctuated with variation of assay methods. For example, the methanol extract from *Chrysosplenium flagelliferum* in liquid culture showed growth inhibition of more than 50% against *S. mutans* and *C. albicans*, whereas this extract on solid medium inhibited only the growth of *S. mutans*. These features might be attributed to the difference in solubility of the effective ingredient, thereby resulting in differences in diffusion rates of the antibacterial agents of plant extracts between a liquid and a water-limited solid medium. Considering the physicochemical conditions of the oral

Table 3. MICs (mg/ml) of methanol extracts with antibacterial activity in liquid media.

Botanical name[a]	Strain	Diameter of clear zone (mm)				MIC (mg/disk)
		Concentration of plant extract (mg/disk)				
		0.1	0.5	1	2	
Camellia japonica	*S. mutans*	-	8	10	12	0.5
	C. albicans	-	9	10	14	0.5
Chelidonium majus var. *asiaticum*	*S. mutans*	-	-	8	8	1
	C. albicans	-	-	-	-	-
Chrysosplenium flagelliferum	*S. mutans*	-	-	9	12	1
	C. albicans	-	-	10	10	0.5
Firmiana simplex	*S. mutans*	-	-	9	9	1
	C. albicans	-	-	9	10	1
Geranium sibiricum	*S. mutans*	-	10	13	15	0.5
	C. albicans	-	8	9	10	0.5
Lindera glauca	*S. mutans*	-	-	8	8	1
	C. albicans	-	-	9	12	1
Rosa rugosa	*S. mutans*	-	-	9	12	1
	C. albicans	-	-	9	10	1
Thuja orientalis	*S. mutans*	8	10	12	13	0.1
	C. albicans	-	9	12	12	0.5

[a]The Korean name is given in Table 1.

cavity, we selected eight plants extracts effective on solid media for further analysis.

Anti-GTase Activity of the Plant Extracts

To determine anti-biofilm activity, crude GTase secreted from *S. mutans* was treated with various concentrations (0.1, 0.3, 0.5, and 1 mg/ml) of eight plant extracts that had shown antibacterial activity (Table 4). As a result, four plant extracts (*C. japonica*, *C. flagelliferum*, *C. majus* var. *asiaticum*, and *T. orientalis*), all of which showed more than 70% inhibition against *S. mutans* in liquid media, also inhibited GTase activity by more than 99% at 1.0 mg plant extract/ml. In samples treated with these extracts, insoluble glucan could not be observed. Among them, *C. japonica* and *T. orientalis* at MIC (0.5 mg/ml) showed an enzyme inhibitory activity of 92.4% and 98.0%, respectively. Although the anti-GTase activity of each plant extract did not entirely correlate with the antibacterial activity, these results demonstrated that a higher anti-GTase activity could be advantageous in enhancing the antibacterial activity.

Phenolic Compound Content, Flavonoid Content, and Antioxidant Activity of Plant Extracts

As is generally well known, the antimicrobial effect of plant extracts is closely linked with the content of secondary metabolites, such as phenolic and/or flavonoid compounds [17, 18]. The phenolic compound content is also deeply associated with the antioxidant activity of plant extracts [19]. Therefore, we determined the content of phenolic compounds and flavonoids in the methanol extracts (1 mg/ml), and then the antioxidant activity was analyzed. For these purposes, we arbitrarily selected six plant species based on their distinct antimicrobial activity and/or anti-biofilm activity (see Tables 3 and 4). As shown in Table 5, although the phenolic compound content did not have a linear relationship with antioxidant activity, most plant

Table 4. Glucosyltransferase inhibitory activities of crude extracts with antibacterial activity.

Botanical name[a]	Inhibitory activity (%)			
	Concentration of plant extract (mg/ml)			
	0.1	0.3	0.5	1.0
Camellia japonica	56.5 ± 0.2	75.7 ± 0.1	92.4 ± 0.3	99.0 ± 0.1
Chelidonium majus var. *asiaticum*	56.5 ± 0.8	75.7 ± 0.1	98.4 ± 0.4	99.0 ± 0.1
Chrysosplenium flagelliferum	45.6 ± 0.5	47.9 ± 0.6	65.9 ± 0.2	99.0 ± 0.1
Firmiana simplex	24.1 ± 0.2	25.5 ± 0.4	28.9 ± 0.2	35.7 ± 0.5
Geranium sibiricum	36.5 ± 0.3	36.3 ± 0.3	40.9 ± 0.3	69.3 ± 0.2
Lindera glauca	22.9 ± 0.2	42.8 ± 0.1	58.9 ± 0.1	85.9 ± 0.2
Rosa rugosa	50.1 ± 0.3	50.3 ± 0.3	54.9 ± 0.1	64.9 ± 0.2
Thuja orientalis	42.7 ± 0.4	83.9 ± 0.3	98.0 ± 0.4	99.0 ± 0.1

Values are the mean ± SD (n = 5).

[a]The Korean name is given in Table 1.

Table 5. Antioxidant activity, total phenolic compound content, and flavonoid content of methanol extracts screened.

Botanical name[a]	Free radical scavenging activity[b] (% inhibition)	Total phenolic compound[c] (μg/ml)	Flavonoid compound[d] (μg/ml)
Camellia japonica	92.8 ± 0.3	150.5 ± 1.1	35.2 ± 0.8
Chelidonium majus var. *asiaticum*	70.6 ± 3.1	57.8 ± 1.7	30.0 ± 1.4
Chrysosplenium flagelliferum	68.5 ± 0.9	88.5 ± 1.4	39.7 ± 0.6
Geranium sibiricum	92.9 ± 0.3	124.2 ± 0.3	21.2 ± 1.7
Lindera glauca	81.1 ± 0.8	108.7 ± 0.3	27.5 ± 1.5
Thuja orientalis	96.8 ± 0.6	197.0 ± 1.4	88.0 ± 0.6

Values are the mean ± SD (n = 5).

[a]The Korean name is given in Table 1.

[b]Free radical scavenging activity of each sample was determined according to the procedure described in the methods section.

[c]Phenolic compounds were expressed as gallic acid equivalents.

[d]Flavonoid compounds were expressed as quercetin equivalents.

extracts showed good correlation between them. Notably, among the selected plant extracts, *C. japonica* and *T. orientalis* had the highest phenolic compound content and antioxidant activity. In addition, except for *C. majus* var. *asiaticum*, plant extracts with a high content of phenolic compounds also had higher antibacterial activity against *S. mutans*, *C. albicans*, or both strains. These results were consistent with those obtained in previous studies [20, 21]. The flavonoid contents of the plant extracts were relatively low and seemed to be irrelevant in relation to the antibacterial and anti-GTase activities in this study.

To the best of our knowledge, our study is the first report of the simultaneous antibacterial, anti-biofilm, and antioxidant activities of the crude extracts from *C. japonica* and *T. orientalis*. The high antibacterial and anti-biofilm activities of the crude extracts suggest that *C. japonica* and *T. orientalis* are potential sources of dental care products.

In summary, the results of this study show that the methanol extracts of *C. japonica* and *T. orientalis* not only have a distinctive antibacterial activity against *S. mutans* and *C. albicans* in liquid and solid agar media, but also possess anti-biofilm activity through their GTase inhibitory activity. These data provide evidence that both plants could be potentially used as natural additives in the prevention of oral diseases, including dental caries.

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