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Deodorizing, antimicrobial and glucosyltransferase inhibitory activities of polyphenolics from biosource

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Abstract−We evaluated the deodorizing activities of polyphenolics against methyl mercaptan and their inhibitory activity on *Streptococcus mutans*, *Candida albicans*, and glucosyltransferase responsible for oral health. Polyphenolics including eckol, dieckol, catechol, catechin, phloroglucinol, chlorogenic acid, epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC), caffeic acid, and tannic acid were used to investigate their deodorizing, antimicrobial and enzyme activity. Among the polyphenolics, dieckol, eckol, and catechol showed higher deodorizing activity against methyl mercaptan than sodium copper chlorophyllin known as a commercial deodorant. Catechol, dieckol, and eckol exhibited a strong antimicrobial activity on *S. mutan*s, while cinnamic acid, caffeic acid, and catechol showed a strong antifungal activity on *C. albicans* compared to other polyphenolics. Tannic acid, ECG, and dieckol strongly inhibited the activity of the glucosyltransferase produced by *Streptococcus mutans*.

Keywords: Methyl Mercaptan, Polyphenolics, Deodorizing Activity, *Streptococcus mutans*, *Candida albicans*, Glucosyltransferase

INTRODUCTION

Methyl mercaptan is a volatile sulfide compound with a rotten cabbage smell and is one of the malodors with ammonia, hydrogen sulfide, and dimethyl sulfide [1]. Methyl mercaptan is the main unfavorable flavor ingredient of radish sprouts, garlic, and shiitake [2-4]. Methyl mercaptan generated by thermolysis of the sulfur-containing amino acids in meat processing is involved in the heated meat flavor [5]. Seaweed and alcohol beverages such as beer, whiskey and brandy produce methyl mercaptan by enzymatic reaction to give the resultant bad flavor [6-8]. Moreover, methyl mercaptan generated from the garbage, excrement and the sewage treatment plant causes environmental problems [9].

In addition, methyl mercaptan is known as a typical oral malodor compound to give bad breath [10-12]. In the mouth, methyl mercaptan is generated from the decomposition of the residual foods by the mouse bacteria and from tooth decay induced by cariogenic bacteria, *Streptococcus mutans* [13-16]. Particularly, the concentration of methyl mercaptan is high in the morning, because the growth of the causative microorganism is favorable by the stagnant saliva during the night [17]. Since there exists a higher correlation of concentration between methyl mercaptan and bad breath or malodor, reducing the level of methyl mercaptan is a favorable strategy to improve food quality, environmental condition as well as oral hygiene like halitosis.

Polyphenolics are widely distributed in fruits, vegetables and seaweeds. Eckol and dieckol are distributed in relatively abundant amounts in brown seaweed [18]. Phloroglucinol is a group unit of phlorotannins such as eckol and dieckol. Catechol, catechin, epigallocatechin gallate (EGCG), epicatechin gallate (ECG), and epigallocatechin (EGC) are rich in green tea [19,20]. Chlorogenic acid and caffeic acid are abundantly contained in coffee [21], and tannic acid is widely distributed in dicotyledonous forbs, shrubs, and trees [22].

Accumulated studies have been examined on the inhibition or reduction of methyl mercaptan using photocatalyst [23], bacteria [24,25], activated carbon [26], essential oil [27], ellagitannins from rose raspberry [28], thyme extracts [29], green tea extracts [30], phlorotannins from brown alga *Eisenia bicyclis* [31], and polyphenol oxidase from lettuce [32].

This study aims was to evaluate the deodorizing activities of polyphenolics against methyl mercaptan and their inhibitory activities on *Streptococcus mutans, Candida albicans* and glucosyltransferase.

MATERIALS AND METHODS

1. Materials

Polyphenolics, including chlorogenic acid, cinnamic acid, catechin, catechol, caffeic acid, (-)-epigallocatechin gallate, (-)-epigallocatechin, (-)-epicatechin gallate, tannic acid and phloroglucinol, were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Eckol and dieckol were isolated and identified according to the method of Kim et al. (2013). Methyl mercaptan (1 μg/μL in benzene, Wako Pure Chem., Osaka, Japan) was used as the standard for malodor, and other chemicals were of analytical grade.

2. Strains

The strains *Streptococcus mutans* (KCTC 3300) and *Candida albicans* (KCTC 7122) were obtained from the Korean Collection for

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Type Cultures (KCTC; Daejeon, Korea).

3. Assay of the Deodorizing Activity

The deodorizing activity was measured by a method of Tokita et al. [33] with slight modifications. A sample solution was made with 99% ethanol solution. The sample solution (0.1 mL) was dissolved with 0.9 mL of 0.2 M potassium phosphate buffer (pH 7.5) in the glass bottle of 10 mL. Then, 1 mL of methyl mercaptan solution (made by 2 mL of authentic methyl mercaptan in 198 mL of 99% ethanol) was added to the bottle. The bottle was immediately stoppered with a silicone cap, and incubated at 36±1 °C for 6 min after stirring for 5 sec with Vortex mixer (GENIE 2; Scientific Industries Inc., Bohemia, USA). Afterwards 1 mL of the headspace gas in the bottle was taken with the gas syringe (SGE Int. Pty. LTD., Australia) and analyzed by injecting it into the gas chromatograph (Shimadzu, GC-17A, Japan) equipped with FPD (flame photometric detector). The glass column packed with β,β'-ODPN 25% Chromosorb (60/80 mesh, 3 m length, 2.6 mm ID) was used to analyze methyl mercaptan. The column pressure was set to 110 kPa. Carrier gas (N_2 with the purity of 99.999%) was maintained at a flow rate of 30 mL/min. The injector and detector temperatures were 200 °C and the column temperature was 85 °C. The deodorizing activity was calculated according to the following equation:

$$\text{Deodorizing activity (\%)} = \frac{C - S}{C} \times 100$$

S: Peak area of methyl mercaptan in the sample
C: Peak area of the methyl mercaptan in the control

4. Disk Diffusion Assay

A disk diffusion assay was performed according to the method of the National Committee for Clinical Laboratory Standards (NCCLS) [34] with slight modifications. For the assay, disc filter papers (6 mm, ADVANTEC, Toyo Roshi Kaisha, Japan) were sterilized for 15 min with UV. The sample (100 mg) was dissolved with 1 mL of dimethyl sulfoxide (DMSO). In the experiment group, 10 μL of each sample solution was absorbed in the paper discs, dried enough at 50 °C, and this process was repeated five times (5 mg/disc). In the control group, DMSO instead of sample was absorbed in the paper discs. After inoculating in Mueller-Hinton broth (MHB, Difco, USA), the strain of *S. mutans* was cultured at 36±1 °C for 24 h to obtain 10^6 CFU/mL bacterial counts. For *C. albicans*, the strain was cultured at 25±1 °C for 72 h after inoculating in yeast malt broth (YMB, Difco, USA). The culture solution (100 μL) of each strain was smeared on the Mueller-Hinton agar (MHA, Difco, USA) plate and then 6 mm paper discs containing the sample solution were placed on the MHA plate. The plate for *S. mutans* was incubated at 36±1 °C for 12 h in a 10% CO_2 incubator (NAPCO 5400; General Laboratory Supply, Pasadena, TX, USA). The plate for *C. albicans* was incubated at 25±1 °C for 24 h in an incubator (EYELA, WFO-700, Tokyo Rikakikai Co., LTD., Japan). The diameter of the inhibition zone was measured with the scale.

5. Measurement of Minimum Inhibitory Concentration (MIC)

After verifying the preliminary antibacterial activity with the paper disc, the minimum inhibitory concentration (MIC) was determined according to Grierson and Afolayan method [35] with a slight modification. To obtain the culture solution of strains, *S. mutans* was cultured in MHB until the bacterial count became 10^4 CFU/mL at 36±1 °C for 24 h in a 10% CO_2 incubator. On the other hand, *C. albicans* was cultured in YMB at 25±1 °C for 72 h. The culture solution of strains (180 μL) and the sample solution (20 μL) dissolved in 10% DMSO were added to the 96-well plate to make the initial concentration of 1 mg/mL of sample, and then diluted consecutively to obtain the next concentration, becoming half of the initial concentration. To determine MIC, the plate containing *S. mutans* was incubated at 36±1 °C for 12 h in a 10% CO_2 incubator, and the plate containing *C. albicans* was incubated at 25±1 °C for 24 h in an incubator.

6. Measurement of Glucosyltransferase Inhibitory Activity

Glucosyltransferase (GTase) inhibitory activity was determined according to Harlinda and Tohru method [36] with a slight modification. The crude GTase was prepared from the culture solution of *S. mutans*. For the preparation of crude GTase, 10 μL of the culture solution of *S. mutans* was added into 5 mL of brain heart infusion (BHI) broth (Difco, USA), and incubated at 36±1 °C for 48 h. The resultant culture solution was centrifuged (8,000 g, 30 min, 4 °C) and the supernatant was used for the crude GTase after filtration with 0.45 μm membrane filter (ADVANTEC, Toyo Roshi Kaisha, Japan). The substrate solution used was 1.25% sucrose solution dissolved in potassium phosphate buffer (6.35 mM, pH 6.5) containing 0.025% sodium azide. To measure the GTase inhibitory activity, 20 μL of the crude enzyme, 800 μL of substrate solution and 100 μL of the sample solution were mixed and then, 80 μL of distilled water was added to make a final volume of 1,000 μL. The resultant solution was incubated at 36 °C±1 for 16 h. The tube was gently rinsed with 3 mL of distilled water after decanting of the supernatant, and the tube incorporated with 3 mL of distilled water was ultra-sonicated (JAC-2010, KODO, Hwaseong, Korea) for 30 min to disperse the insoluble glucan adhered on inner wall of the tube. The absorbance was measured at 550 nm using UV-spectrophotometer (UV-1800, Shimadzu, Japan). The sample concentration for the assay was 0.1 mg/mL, and GTase inhibitory activity was calculated according to the following equation:

$$\text{GTase inhibitory activity (\%)} = \frac{AC - AS}{AC} \times 100$$

AC: Absorbance at the control
AS: Absorbance at the sample

7. Statistical Analysis

All the results were expressed in mean±S.E.M (standard error of the mean) (n=3). The difference in the results was analyzed by each group using IBM SPSS Statistic v. 21 (SPSS, Chicago, USA) and estimated at the level of $P<0.05$ by conducting Duncan's multiple range test.

RESULTS AND DISCUSSION

1. Deodorizing Activity of Polyphenolics Against Methyl Mercaptan

Methyl mercaptan is a causative compound of malodor from foods or bad breath caused by the oral cavity. Reducing methyl mercaptan level in foods, environmental fields, and mouse, is an

Table 1. Deodorizing activity (IC_{50}) of polyphenolics against methyl mercaptan

Polyphenolics	IC_{50} (mM)[1)]
Dieckol	72.2±9.9[a 2)]
Eckol	83.6±4.6[a 2)]
Catechol	300.0±1.8[a 2)]
Caffeic acid	13.9±16.4[e]
Catechin	23.5±8.0[g]
Chlorogenic acid	15.2±11.3[ef]
Cinnamic acid	33.3±5.1[h]
Epicatechin gallate	10.2±4.8[d]
Epigallocatechin	17.4±4.7[f]
Epigallocatechin gallate	5.6±7.6[c]
Phloroglucinol	4.9±2.4[bc]
Tannic acid	23.7±2.1[g]

[1)]The IC_{50} value indicated as the required concentration to inhibit 50% of deodorizing activity

[2)]IC_{50} values presented as nM

*Values are mean±S.D (n=3), and superscripts of different letters in the same column are significantly different by Duncan's multiple range test (P<0.05)

Table 2. Antimicrobial activity of polyphenolics against *Streptococcus mutans* and *Candida albicans*

Polyphenolics	Clear zone on plate (mm)	
	S. mutans	*C. albicans*
Caffeic acid	10.5±3.5[c]	16.3±0.6[ab]
Catechin	11.5±3.5[bc]	15.3±0.6[b]
Catechol	30.0±0.0[a]	15.7±0.6[b]
Chlorogenic acid	12.0±0.0[bc]	16.3±0.6[ab]
Dieckol	18.3±0.6[bc]	-
Cinnamic acid	-	20.7±0.6[a]
Epicatechin gallate	16.0±0.0[bc]	4.0±1.0[c]
Eckol	18.0±1.0[bc]	-
Epigallocatechin	22.0±0.0[ab]	6.3±1.5[c]
Epigallocatechin gallate	18.0±2.8[bc]	13.3±0.6[b]
Phloroglucinol	17.3±1.2[bc]	13.7±0.6[b]
Tannic acid	19.5±2.1[abc]	13.0±1.0[b]

[1)]-: No inhibition

*Values are mean±S.D (n=3) and superscripts of different letters in the same column are significantly different by Duncan's multiple range test (P<0.05)

important approach to improving food quality, environmental soundness, and oral hygiene, respectively. As shown in Table 1, dieckol (72.2±9.9 nM), eckol (83.6±4.6 nM), and catechol (300±1.8 nM) showed stronger deodorizing activity against methyl mercaptan than other polyphenolics. While, phloroglucinol (4.9±2.4 mM), EGCG (5.6±7.6 mM), ECG (10.2±4.8 mM), caffeic acid (13.9±16.4 mM), chlorogenic acid (15.2±11.3 mM), EGC (17.4±4.7 mM), catechin (23.5±8.0 mM), tannic acid (23.7±2.1 mM), and cinnamic acid (33.3±5.1 mM) exhibited relatively high IC_{50} values. Especially, dieckol and eckol showed stronger deodorizing activity than sodium copper chlorophyllin (605.1±17.6 nM), which is known as a commercial deodorant used as a positive control in this study [31]. Using sodium copper chlorophyllin as an additive of mouthwash to remove malodor or to inhibit halitosis did not cause any serious side effects [37]. However, the oral administration of sodium copper chlorophyll has occasionally been reported to be causing diarrhea [38]. On the other hand, Yasuda and Arakawa [39] and Zeng et al. [30] reported that the reduction of bad breath by green tea polyphenolics resulted from conversion of the volatile methyl mercaptan into nonvolatile compounds by the actions of the epigallocatechin gallate. Kita et al. [40] reported phlorotannins had higher reducing effect against methyl mercaptan than conventional natural deodorant, sodium copper chlorophyllin. Among the eckol, dixinodehydroeckol, and dieckol isolated from brown seaweed *Eisenia bicyclis* [31], dieckol showed the highest deodorizing activity against methyl mercaptan. Also, Hiramoto et al. [41] suggested that the deodorizing effect of polyphenolics against methyl mercaptan might be related to the conversion of phenolic hydroxyl group into quinone and the formation of thiol ether with benzene ring caused by the nucleophilic addition of methylthiol group. Thus, the deodorizing ability of the polyphenolics is due to their direct actions or interactions with quinones produced from the oxidation of phenolic hydroxyl group.

2. Antimicrobial Activity of Polyphenolics

2-1. Antibacterial Activity against *Streptococcus mutans*

Dental decay is one of the most widely common oral diseases. Halitosis originating from the oral cavity is mainly associated with dental plaque and dental caries induced by *S. mutans* [15,42]. Methyl mercaptan as a causative compound of a bad breath largely results from the degradation of sulfur-containing amino acids by *S. mutans* [12]. In this regard, inhibition of *S. mutans* growth is a critical approach for improving oral hygiene as well as for reducing bad breath. Table 2 shows the antimicrobial activity of polyphenolics against *S. mutans* using disk diffusion assay. Disk diffusion assay tells of potential antimicrobial activity when the clear zone is larger than 13 mm. As the result, catechol (30.0±0.0 mm) represented the highest antibacterial activity followed by EGC (22.0±0.0 mm), tannic acid (19.5±2.1 mm), dieckol (18.3±0.6 mm), and EGCG (18.0±2.8 mm). Cinnamic acid did not form the clear zone.

Table 3 shows the MICs of polyphenolics against *S. mutans*. Among polyphenolics tested, catechol, dieckol, eckol, and EGC showed a higher antibacterial activity against *S. mutans* than others, and their MICs were 0.3 mg/mL. The MICs of ECG and EGCG showed 0.5 mg/mL, and catechin and chlorogenic acid exhibited the highest MIC of 5 mg/mL. Sakanaka et al. [43] reported that the MICs of EGC isolated from green tea were 0.25-0.5 mg/mL. The results showed a good agreement with a study reported by Sakanaka et al. Hirasawa et al. [44] reported that catechol and pyrogallol containing the galloyl radical showed higher antibacterial activity than other compounds without this radical. Catechol can more easily form galloyl radical than polyphenolics examined in this study. The galloyl radical in catechins is responsible for the inhibition of enzyme activity producing lactic acid, which causes dental plaque. Thus, catechol has the highest antimicrobial activity against *S. mutans*. On the other hand, the extract of *Scutellaria baical-*

Table 3. Minimum inhibitory concentration (MIC) of polyphenolics against *Streptococcus mutans* and *Candida albicans*

Polyphenolics	MIC (mg/mL)	
	S. mutans	*C. albicans*
Caffeic acid	2.2±0.3[c]	0.5±0.0[b]
Catechin	5.0±0.0[d]	0.5±0.0[b]
Catechol	0.3±0.0[a]	0.5±0.0[b]
Chlorogenic acid	5.0±0.0[d]	0.5±0.0[b]
Cinnamic acid	-[1)]	0.1±0.0[c]
Dieckol	0.3±0.0[a]	-
Epicatechin gallate	0.5±0.0[ab]	-
Eckol	0.3±0.0[a]	-
Epigallocatechin	0.3±0.0[a]	-
Epigallocatechin gallate	0.5±0.0[ab]	1.0±0.0[c]
Phloroglucinol	2.0±0.0[c]	1.0±0.0[c]
Tannic acid	0.8±0.2[b]	1.0±0.0[c]

[1)]-: No inhibition

*Values are means±S.D (n=3) and superscripts of different letters in the same column are significantly different by Duncan's multiple range test (P<0.05)

ensis, which is a medicinal herb, indicated the MIC of 125 mg/mL against *S. mutans* [45]. Moreover, the Iranian green and black tea extracts showed the MICs of 150 mg/mL and 50 mg/mL, respectively, against *S. mutans* [46]. However, polyphenolics in this study showed much lower MICs compared to reports of [46]. The discrepancy of the results would come from the concentration of polyphenolics in the extracts. Therefore, herb and tea extracts containing a considerable amount of polyphenolic compounds have a protective effect on the formation of dental plaque and caries via inhibition of *S. mutans*.

2-2. Antifungal Activity Against *Candida albicans*

The opportunistic fungus *C. albicans* is the commonest Candida species found in the oral cavity [47]. When the host's defense system is impaired as in medically compromised or immune suppressed conditions, *C. albicans* infection can lead to oral candidiasis and severe periodontitis [48,49]. Inhibiting *C. albicans* growth is an important strategy in preventing oral candidiasis and periodontitis, and reduces bad breath. Table 2 shows the antifungal activity of polyphenolics against *C. albicans* using disk diffusion assay. Cinnamic acid (20.7±0.6 mm) represented the highest antifungal activity followed by caffeic acid (16.3±0.6 mm), chlorogenic acid (16.3±0.6 mm), catechol (15.7±0.6 mm), and catechin (15.3±0.6 mm). Dieckol and eckol did not form clear zones.

Table 3 shows the MICs of polyphenolics except for dieckol and eckol against *C. albicans*. Among tested samples, cinnamic acid exhibited the lowest MIC (0.1 mg/mL). Caffeic acid, catechin, catechol, and chlorogenic acid showed MIC of 0.5 mg/mL against *C. albicans*. On the other hand, EGCG, phloroglucinol and tannic acid exhibited MIC of 1.0 mg/mL with a low antifungal activity against *C. albicans*. Papadopoulou et al. [50] reported that white wine containing cinnamic acid possessed antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *C. albicans*. The wine, largely containing cinnamic acid, formed about 30 mm inhibition zone on a plate spread *C. albicans*. Han [51] reported that catechin and EGCG represented the antifungal activities on the growth of *C. albicans*. Moreover, EGCG synergistically enhanced the antifungal effect of amphotericin B or fluconazole on antimycotic-susceptible and -resistant *C. albicans* [52]. Thus, polyphenolics can prevent oral candidiasis and periodontitis by inhibiting *C. albicans*.

Table 4. Inhibitory activity of glucosyltransferase by polyphenolics

Polyphenolics	Inhibition rate (%)
Caffeic acid	53.1±0.5[bcd]
Catechin	45.8±4.3[d]
Catechol	67.6±8.6[abc]
Chlorogenic acid	54.0±0.8[bcd]
Dieckol	81.9±2.4[a]
Epicatechin gallate	82.6±0.8[a]
Eckol	67.8±0.3[abc]
Epigallocatechin	68.5±2.4[ab]
Epigallocatechin gallate	73.7±0.5[a]
Phloroglucinol	50.8±0.0[cd]
Tannic acid	83.2±0.5[a]

*Values are mean±S.D (n=3) and superscripts of different letters in the same column are significantly different by Duncan's multiple range test (P<0.05)

3. Glucosyltransferase Inhibitory Activity

Glucosyltransferase (GTase) secreted by *S. mutans* is a key enzyme for the production of glucan, and the adhesion of resultant glucan on teeth induces dental plaque and caries [53]. The GTase inhibitory activity can lead to reducing halitosis and dental caries. Table 4 shows the GTase inhibitory activity of polyphenolic compounds. As shown in Table 4, EGCG (73.7%), EGC (68.5%), tannic acid (68.0%), and catechol (67.6%) exhibited higher GTase inhibitory activities. Other polyphenol compounds showed lower GTase inhibitory activity. Hattori et al. [54] and Matsumoto et al. [55] reported that the condensed tannin prepared with green tea showed strong GTase inhibitory activity with non-competitive inhibition of glucan production. From these results, polyphenolics have a potential to reduce the plaques by inhibiting GTase activity secreted by *S. mutans* and may inhibit the bad breath produced by oral microorganisms.

CONCLUSIONS

Methyl mercaptan is a causative material of malodor from foods and sewage treatment or bad breath from the oral cavity. *S. mutans*, *C. albicans* and GTase play an important role in the formation of methyl mercaptan and dental caries. The present study elucidated the deodorizing activities of polyphenolics against methyl mercaptan and their inhibitory activities on *S. mutans*, *C. albicans* and GTase. Dieckol, eckol, and catechol showed strong deodorizing activity against methyl mercaptan. Catechol, dieckol, eckol, and EGC showed a higher antibacterial activity against *S. mutans*. Cinnamic acid, caffeic acid, and catechol also possessed a high antifungal activity against *C. albicans*. Furthermore, EGCG, EGC, tannic acid, and catechol exhibited high GTase inhibitory activity. The

results provide information of polyphenolics for developing beneficial products for oral health such as toothpaste, chewing gum, and mouthwash.

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