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Study on the Inhibitory Effect of Tannins and Flavonoids against the 1,1-Diphenyl-2-picrylhydrazyl Radical

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ABSTRACT. Fifty-one tannins and forty-one flavonoids isolated from Oriental medicinal herbs were evaluated for their antioxidant ability with a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-generating system. The results showed that tannins and certain flavonoids are potential free-radical scavengers, and that their activity against the DPPH radical is closely associated with their chemical structure. A comparison of the two classes of compounds showed that tannins have more potential than flavonoids because almost all the tannins demonstrated significant scavenging action within a low concentration range, whereas the activity of flavonoids varied distinctively among the different compounds. An increase of galloyl groups, molecular weight, and ortho-hydroxyl structure enhanced the activity of tannins, whereas the number and position of hydroxyl groups were important features for the scavenging of free radicals by flavonoids. Moreover, it appeared that when the free hydroxyl group was methoxylated or glycosylated, the inhibitory activity was obviously decreased or even abolished. BIOCHEM PHARMACOL **56**;2:213–222, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. tannin; flavonoid; 1,1-diphenyl-2-picrylhydrazyl; radical; structure–activity relationship

Since ancient times, humans have received many benefits from natural plants and compounds. It has been generally recognized that traditional Oriental medicine has a unique therapeutic role in the treatment of many human diseases, especially some chronic conditions. To investigate the therapeutic mechanisms of Oriental medicines, a number of principles and compounds have been isolated and studied. Among them, polyphenols including tannins and flavonoids, which are known to demonstrate a variety of biological activities in both experimental and clinical settings, are considered to be important active components of medicinal plants [1, 2].

Up to now, although the etiology of many diseases has remained unclear, various lines of evidence have confirmed that toxic free radicals play a role in a variety of pathological conditions [3], and interest has focused on the development of safe and effective antioxidants. As a result of numerous investigations, tannins and flavonoids have been shown to have such potential [4–8]. However, some recent studies have shown that structure–activity relationships play an extremely important role in determining whether compounds will exert an antioxidant or free-radical scavenging effect [9, 10], and thus it is necessary to screen a number of individual compounds of different chemical classes in various systems for their activity.

The DPPH‖ system is a stable radical-generating procedure [11]. Because it can accommodate a large number of samples in a short period, and is sensitive enough to detect active principles at low concentrations, it was used in the present study for primary screening of the antiradical activities of 51 tannins and 41 flavonoids as well as their derivatives; the relationship between the activity and the chemical structure of these compounds was also addressed.

MATERIALS AND METHODS

Compounds

Forty-nine tannins and two flavonoids used in this experiment were isolated from plant materials as reported previously [12–33]. Tannic acid and caffeic acid were reagent grade. Other flavonoids were a gift from Professor M. Shimizu of the Toyama Medical and Pharmaceutical University. Their identification and purity were determined according to UV, IR, ^{1}H NMR, ^{13}C NMR, and melting point data. The structural formulae are given in Fig. 1.

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‖ *Abbreviations:* DHHDP, dehydrohexahydroxydiphenoyl; and DPPH, 1,1-diphenyl-2-picrylhydrazyl.

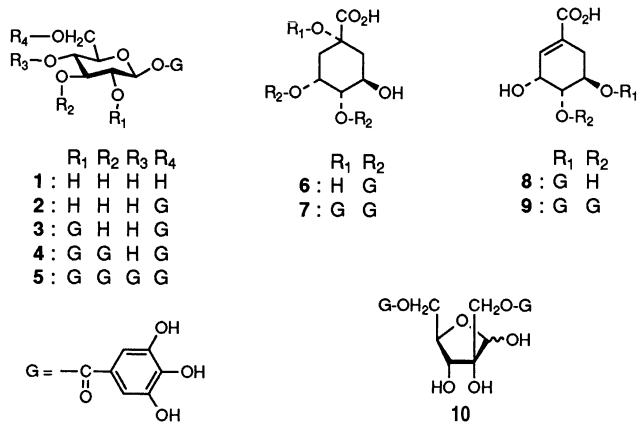


FIG. 1-1. Gallotannin.

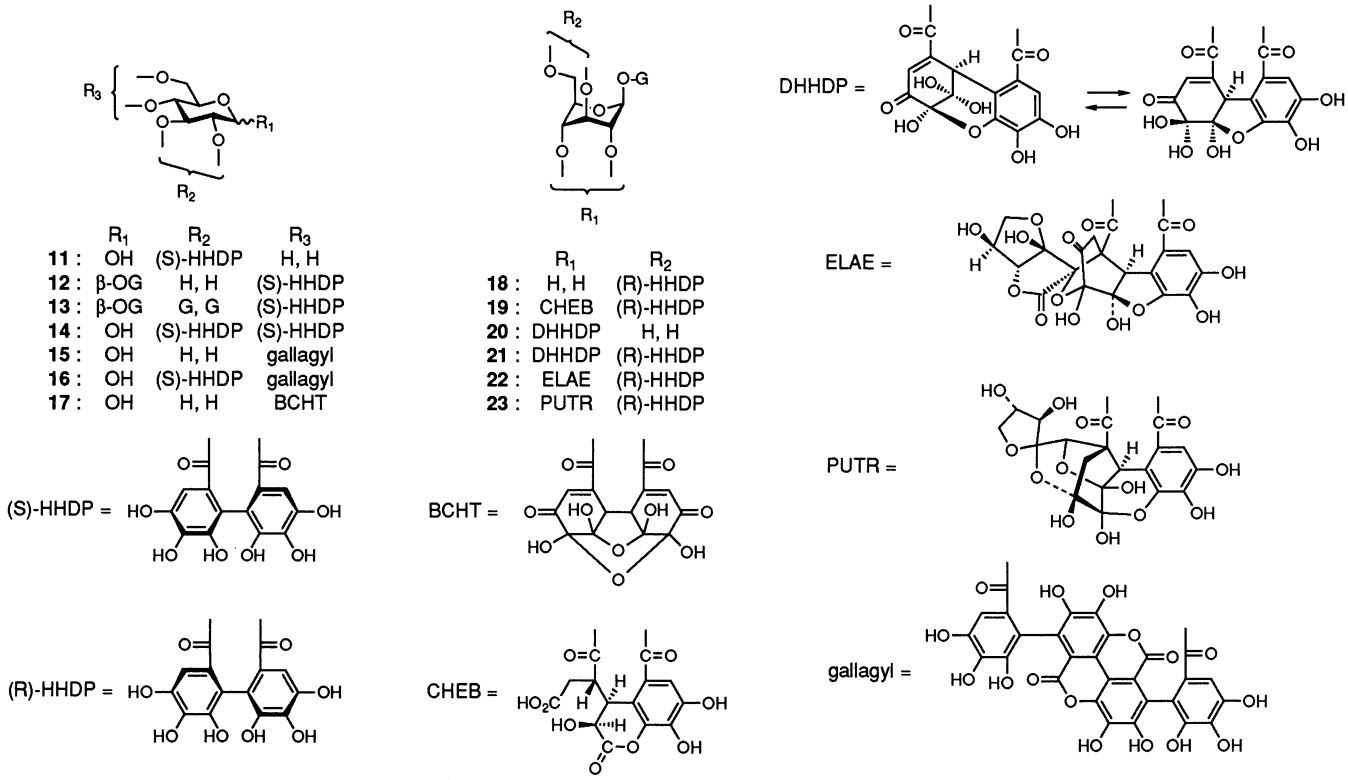


FIG. 1-2. Ellagitannin.

Determination of DPPH Radical

In microwells, 100 μL of an aqueous solution of the sample (control: 100 μL of distilled water) was added to an ethanolic solution of DPPH (60 μM) according to the method of Hatano *et al.* [34]. Seven concentrations were prepared for each sample. After mixing gently and leaving to stand for 30 min at room temperature, the optical density was determined using a Microplate Reader, model 3550-UV (BIO-RAD). The antioxidant activity of each sample was expressed in terms of IC_{50} (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from the log-dose inhibition curve.

RESULTS

Tannins and Related Compounds

As shown in Table 1, all the tannins tested except for carpinin D and bergenin demonstrated significant inhibitory activity against the DPPH radical. Among hydrolyzable gallotannins, 1-*O*-galloyl-β-D-glucose showed 50% inhibition at a concentration of 8.00 μM, while the same action was shown by di-, tri-, tetra-, and penta-galloylglucose at concentrations of 4.06, 3.51, 2.62, and 1.68 μM. 4,5-Di-*O*-galloyl quinic acid and 3-*O*-galloyl shikimic acid may be regarded as potential free-radical scavengers because of their low IC_{50} values (4.14 and 4.91 μM, respectively).

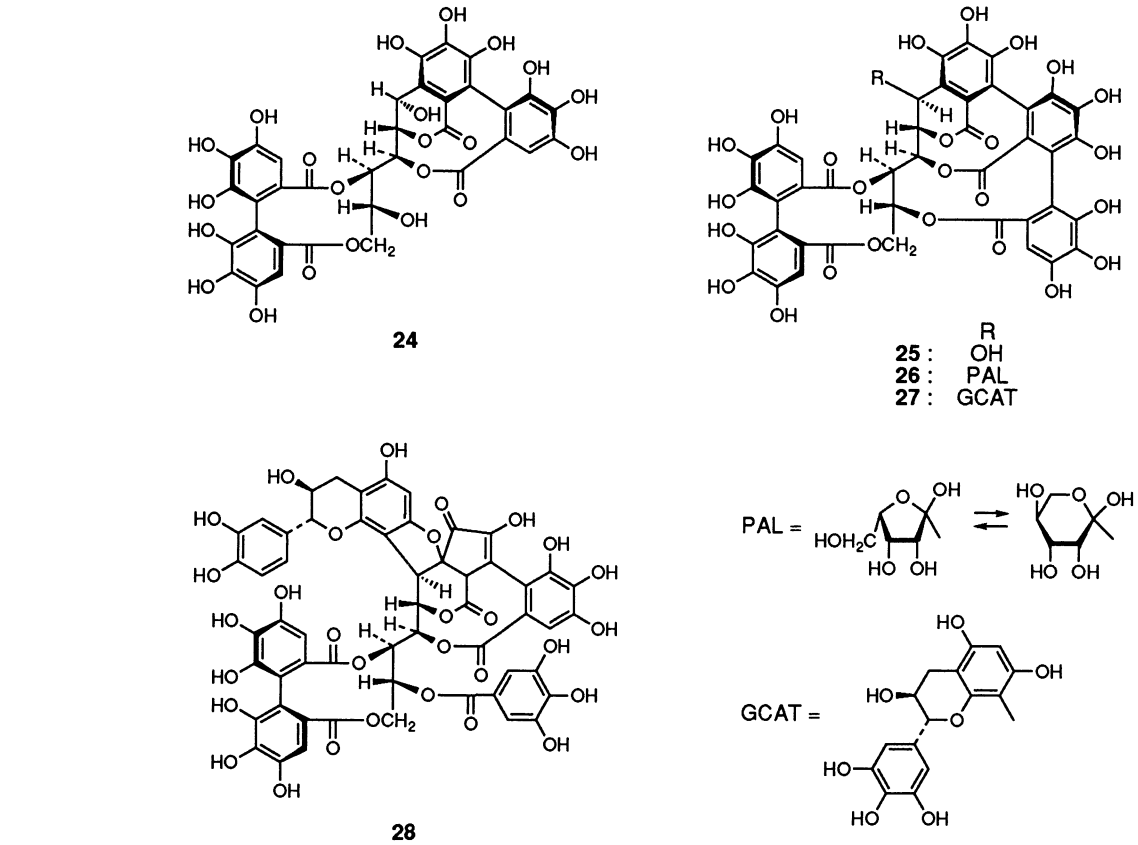


FIG. 1-3. Ellagitannin and complex tannin.

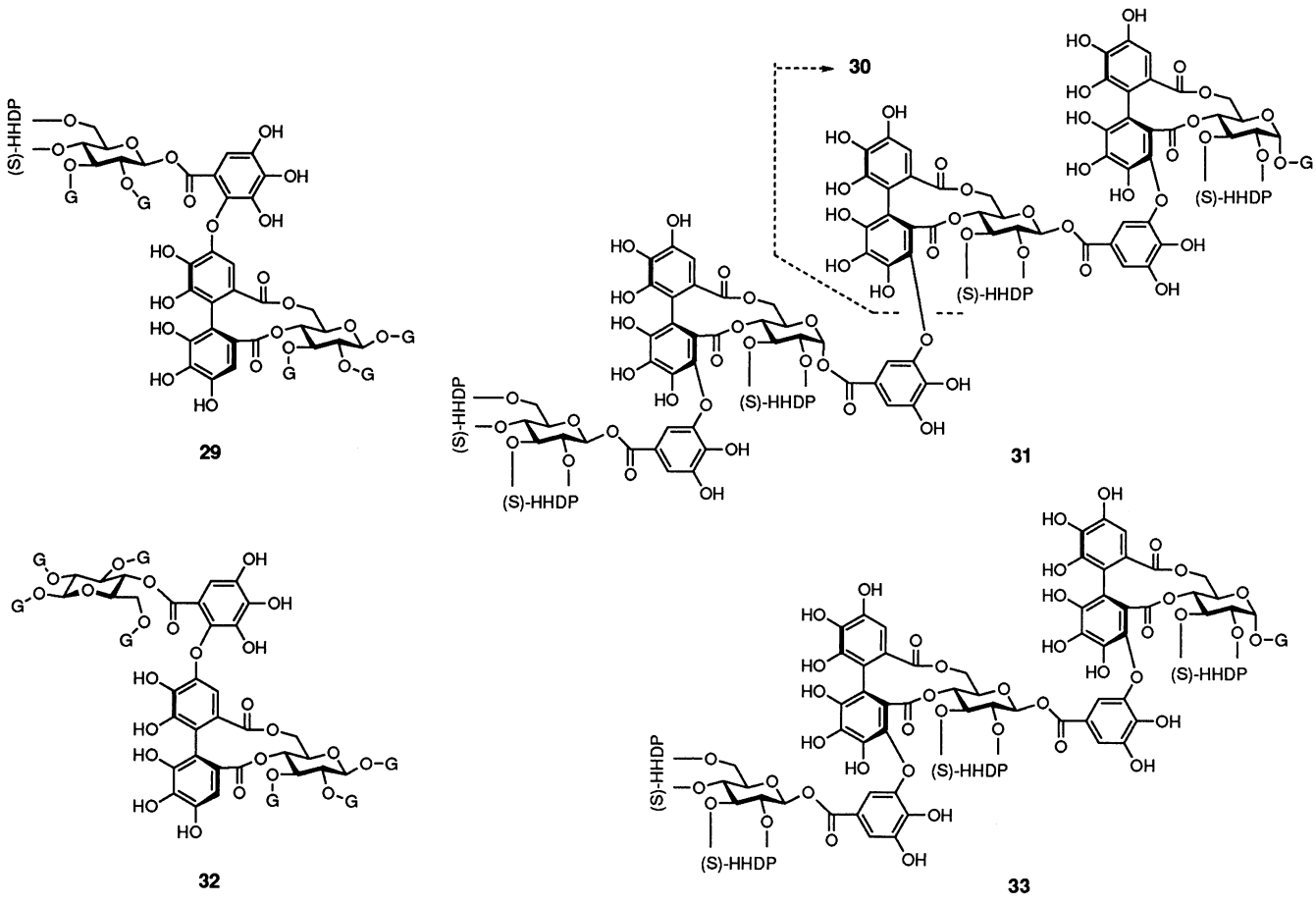


FIG. 1-4. Ellagitannin.

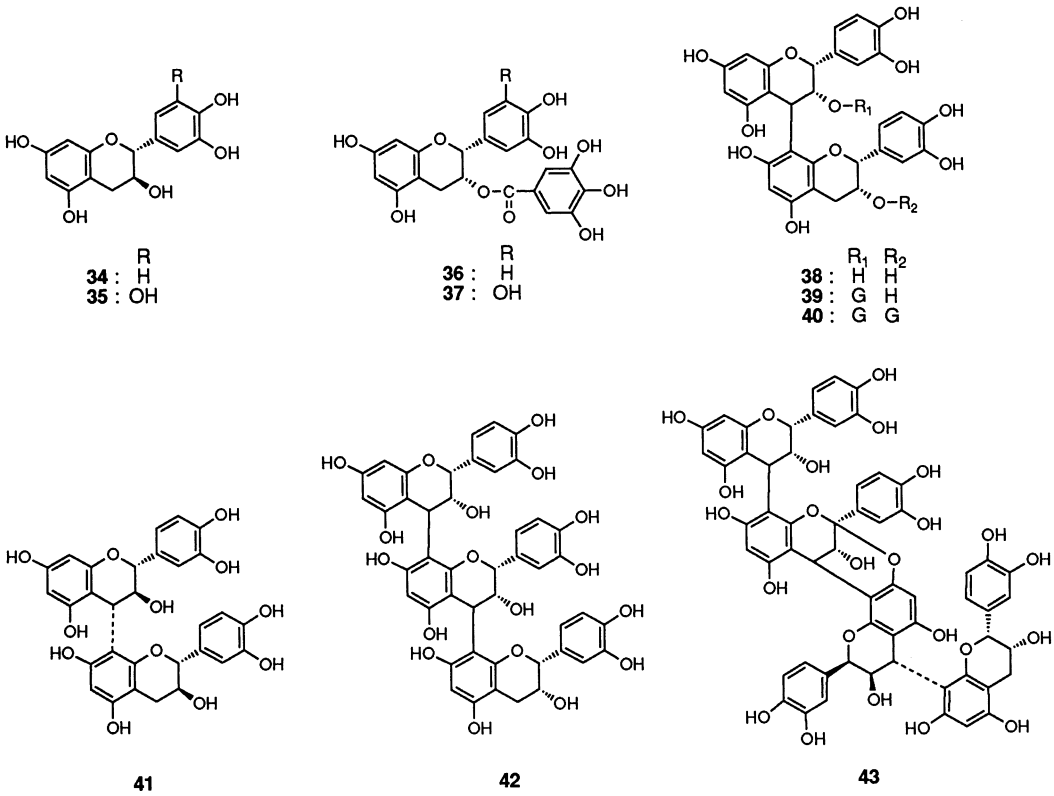


FIG. 1-5. Condensed tannin.

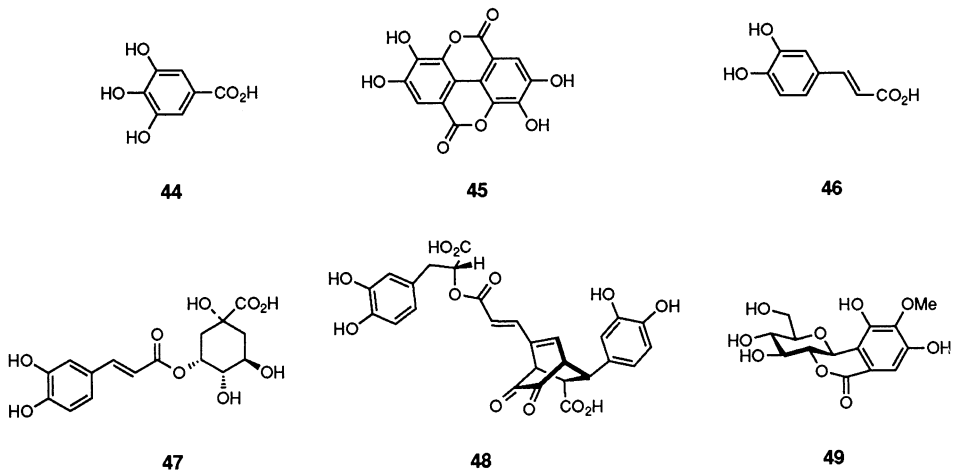
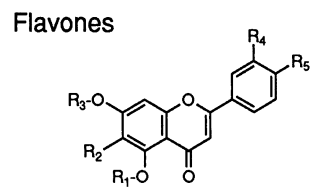
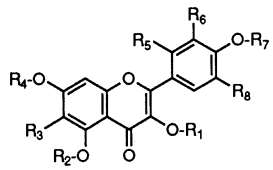


FIG. 1-6. Phenolcarboxylic acid and related compounds.

When an additional galloyl group was linked to their structures, as in the case of 1,4,5-trigalloyl quinic acid and 3,4-di-*O*-galloyl shikimic acid, stronger inhibition was exhibited. The ellagitannin compounds, some of which contain DHHDP groups, presented relatively weak activity. The IC_{50} of putranjivain A was 11.23 μM, while carpinin D appeared to have no activity (i.e., the IC_{50} exceeded 500 μM). Dimeric, trimeric, and tetrameric ellagitannins, except for lambertianin B, were observed to have more potential as free-radical scavengers than monomeric ellagitannins, their IC_{50} values ranging from 0.61 to 1.16 μM. In the condensed tannins, on the other hand, the influence of the galloyl group and molecular weight on the activity was found to be similar. They showed effective inhibition from 0.76 to 12.90 μM. Moreover, some derivatives of tannin such as gallic acid, caffeic acid, and 5-*O*-caffeoyl quinic acid, as well as yunnaneic acid C, showed less potential than most of the other tannins, their IC_{50} values being 8.14, 13.56, 12.38, and 13.69 μM, respectively. Bergenin, a methyl ether of a gallic acid derivative, was inactive in



	R_1	R_2	R_3	R_4	R_5
50 :	H	OMe	Me	OH	OH
51 :	H	OH	H	OH	OH
52 :	H	H	glc	OH	OH
53 :	H	OH	H	H	H
54 :	H	OH	H	H	OH
55 :	H	OH	glcA	H	H
56 :	H	OH	glc	H	OH
57 :	H	H	H	OH	OH
58 :	H	OH	rha	H	OH
59 :	H	H	glc^2-api	H	OH
60 :	H	H	glc	H	OH
61 :	H	H	H	H	OMe
62 :	H	H	H	H	OH
63 :	H	H	H	OMe	OH
64 :	H	OMe	Me	OMe	OH
65 :	H	OMe	Me	H	O-glc
66 :	H	OMe	Me	H	OH
67 :	H	H	glc^6-rha	H	OMe
68 :	H	OMe	H	H	OMe
69 :	H	OMe	glc^6-rha	H	OMe
70 :	H	H	glc^2-rha	H	OH
71 :	H	H	Me	H	H
72 :	glc	H	H	H	H



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
73 :	rha(2-gallyol)	H	H	H	H	OH	H	OH
74 :	H	H	H	glc	H	OH	H	H
75 :	H	H	H	Me	H	OH	H	H
76 :	H	H	H	H	H	OH	H	H
77 :	glc^6-rha	H	H	H	H	OH	H	H
78 :	Me	H	OMe	Me	H	OH	H	H
79 :	gal	H	H	H	H	OH	H	H
80 :	rha	H	H	H	H	OH	H	H
81 :	Me	H	OH	Me	H	OMe	H	H
82 :	rha	H	H	H	H	OH	H	OH
83 :	glc	H	H	H	H	OH	H	H
84 :	H	H	H	H	H	OMe	H	H
85 :	Me	H	H	Me	OH	H	Me	OH
86 :	H	H	H	H	H	H	H	H
87 :	H	Me	H	Me	H	OMe	Me	H
88 :	rha	H	H	H	H	H	H	H
89 :	rha	H	H	rha	H	H	H	H
90 :	H	H	H	glc	H	H	H	H

FIG. 1-7. Flavonoids.

FIG. 1. Structural formulae of compounds used in this study. Tannic acid and rhatannin are not shown.

scavenging the DPPH radical (i.e., its IC_{50} exceeded 500 μM), suggesting the possible influence of methylation on inhibitory activity.

Flavonoids

As shown in Table 2, about half of the flavone compounds tested demonstrated significant inhibitory activity against the DPPH radical. Nine compounds showing strong activity ($IC_{50} < 20$ μM) were found to be 3′,4′-dihydroxyl, 5,6-dihydroxyl- or 5,6,7-trihydroxylflavone-type compounds such as cirsiliol, 6-hydroxyluteolin, baicalein, scutellarein, luteolin, luteolin-7-glucoside, baicalin, and plantaginin, as well as sorbarin. Among these, the former five are flavongenins, while the rest are glycosylated at the C-7 position with glucose, rhamnose, or glucuronic acid. Compared with the above compounds, apiin and cosmosiin presented moderate and weak inhibition, with IC_{50} values of 64.75 and 232.51 μM, respectively. In contrast, the other flavones, i.e. acacetin, apigenin, chrysoeriol, cirsilineol, cirsimarin, cirsimaritin, linarin, pectolinarigenin, pectolinarin, rhoifolin, tectochrysin and toringin, appeared to have no action because their IC_{50} values exceeded 500 μM; these flavones included five glycosides. In contrast to flavones, more flavonol compounds showed effective radical-scavenging activity, as shown in Table 2. Among the 18 flavonols tested, 13 showed 50% inhibition at very low concentrations of less than 20 μM. Similar to the flavones, most of the flavonols such as quercimeritrin, rhamnetin, quercetin, rutin, chrysosplenol D, hyperin, quercitrin, myricitrin, 2″-galloyl myricitrin and isoquercitrin were observed to possess ortho-hydroxyl groups in the B-ring, while chrysosplenol C has the same functional group in the A-ring. Besides these, isorhamnetin, oxyayanin A, and kaempferol, as well as tetramethylquercetin, showed appreciable effects against free radicals, even though there are no ortho-hydroxyl groups in their structure. On the other hand, the flavonol compounds considered to have no inhibition ($IC_{50} > 500$ μM) included the three glycosides of kaempferol (afzelin, kaempferitrin and kaempferol-7-glucoside), suggesting the possible influence of glycosylation on their activity.

DISCUSSION

Since modern chemical and pharmacological methods were first used to investigate traditional medicinal materials, there has been a rapid increase in the number of known natural principles and compounds. Among these compounds, polyphenols including tannins and flavonoids have received increasing attention recently because of some interesting new findings regarding their biological activi-

TABLE 1. IC_{50} Values of tannins tested against the DPPH radical

Compound	IC_{50} (μM)
Gallotannin	
Galloylglucose	
1-*O*-Galloyl-β-D-glucose [12] (1)*	8.00 ± 0.41
1,6-Di-*O*-galloyl-β-D-glucose [13] (2)	4.06 ± 0.13
1,2,6-Tri-*O*-galloyl-β-D-glucose [14] (3)	3.51 ± 0.10
1,2,3,6-Tetra-*O*-galloyl-β-D-glucose [15] (4)	2.62 ± 0.11
1,2,3,4,6-Penta-*O*-galloyl-β-D-glucose [15] (5)	1.68 ± 0.04
Tannic acid	1.29 ± 0.03
Galloylquinic acid	
4,5-Di-*O*-galloyl quinic acid [16] (6)	4.14 ± 0.08
1,4,5-Trigalloyl quinic acid [17] (7)	2.66 ± 0.09
Galloylshikimic acid	
3-*O*-Galloyl shikimic acid [18] (8)	4.91 ± 0.14
3,4-Di-*O*-galloyl shikimic acid [18] (9)	3.52 ± 0.12
Galloylhamamelose	
Hamamelitannin [19] (10)	3.78 ± 0.07
Ellagitannin	
Monomer-I (pyranose type)	
2,3-(*S*)-HHDP-D-glucose [20] (11)	4.31 ± 0.12
Strictinin [20] (12)	2.86 ± 0.13
Corilagin [21] (18)	2.89 ± 0.05
Eugeniin [15] (13)	1.78 ± 0.07
Pedunculagin [20] (14)	2.50 ± 0.07
Monomer-II (pyranose type)	
Chebulinic acid [21] (19)	3.06 ± 0.11
Punicalin [20] (15)	2.82 ± 0.08
Punicalagin [20] (16)	1.85 ± 0.04
Monomer-III (dehydroellagitannin and related compounds)	
Furosin [13] (20)	3.70 ± 0.14
Geraniin [12] (21)	2.50 ± 0.07
Carpinin D [22] (17)	> 500
Elaeocarpusin [12] (22)	2.16 ± 0.07
Putranjivain A [23] (23)	11.23 ± 0.31
Monomer-IV (open-chain type)	
Casuariin [24] (24)	2.14 ± 0.05
Vescalagin [25] (25)	2.15 ± 0.41
Grandinin [25] (26)	2.09 ± 0.05
Dimeric ellagitannin (MW about 1900)	
Sanguiin H-6 [26] (30)	1.16 ± 0.03
Rugosin D [15] (29)	1.15 ± 0.03
Phillyraeoidin A [24] (32)	1.02 ± 0.02
Trimeric ellagitannin (MW about 2700)	
Lambertianin B [27] (33)	3.18 ± 0.12
Tetrameric ellagitannin (MW about 3600)	
Sanguiin H-11 [26] (31)	0.61 ± 0.02
Complex tannin	
Stenophyllinin A [24] (28)	2.02 ± 0.09
Eugenigrandin A [25] (27)	1.94 ± 0.05
Condensed tannin	
Monomer	
Catechin [28] (34)	12.90 ± 0.35
Gallocatechin [25] (35)	4.76 ± 0.07
Epicatechin 3-*O*-gallate [28] (36)	3.92 ± 0.26
Epigallocatechin 3-*O*-gallate [28] (37)	3.95 ± 0.13
Dimer	
Procyanidin B-2 [29] (38)	3.42 ± 0.09
Procyanidin B-3 [29] (41)	4.85 ± 0.15
Procyanidin B-2 3′-gallate [29] (39)	3.25 ± 0.08
Procyanidin B-2 3,3′-digallate [29] (40)	1.66 ± 0.11
Trimer	
Procyanidin C-1 [29] (42)	2.35 ± 0.10
Tetramer	
Cinnamtannin B2 [30] (43)	2.31 ± 0.13
Polymer	
Rhatannin [31]	0.76 ± 0.05
Phenolcarboxylic acid and related compounds	
Gallic acid [26] (44)	8.14 ± 0.26
Ellagic acid [26] (45)	4.60 ± 0.15
Caffeic acid (46)	13.56 ± 0.47
5-*O*-Caffeoyl quinic acid [22] (47)	12.38 ± 0.32
Yunnaneic acid C [32] (48)	13.69 ± 0.78
Bergenin [13] (49)	> 500

Results are presented as means ± SEM of 5 determinations.
*[ ] Reference number; ( ) compound number.

ties. The biological activities of tannins include marked anti-tumor, anti-viral, and anti-HIV activities, inhibition of lipid peroxidation and plasmin activity, mediation of DNA nicking, amelioration of renal failure, and several others [35–41], whereas flavonoids exhibit anti-inflammatory, anti-spasmodic, and anti-allergic activities as well as protective effects against hepatic and vascular disorders [42]. Investigations of the mechanisms of these effects have revealed that antioxidant and free-radical scavenging actions are involved. However, the antiradical and antioxidant activities vary greatly among different classes of compounds, even in those of the same type. Therefore, in order to search for potent free-radical scavengers and clarify the correlation between the structure of compounds and their antioxidant activities, 51 tannins including hydrolyzable and condensed types, and 41 flavonoids substituted at the C-3, C-5, C-7, C-2′, C-3′, C-4′, and C-5′ positions were investigated, including flavongenins and their glycosides.

Almost all the tannins and related compounds tested in this study, except for carpinin D and bergenin, showed strong inhibitory activity to the DPPH radical within a very low concentration range. In comparison, the activities of flavonoids showed a much wider variation, some presenting effective inhibition, while others appeared to have no activity. Among the various tannin compounds tested, there was an obvious increase in the influence of galloyl groups on inhibitory activity. Myricitrin, which is structurally classed as a flavonoid, showed effective inhibition at 12.74 μM, but when the 2″-core on the rhamnose was galloylated, the scavenging activity was increased markedly, so that 2″-*O*-galloyl myricitrin was able to produce the same effect at a much lower concentration of 3.83 μM. A similar influence was also found upon comparison of activity between compounds such as 4,5-di-*O*-galloyl quinic acid and 1,4,5-trigalloyl quinic acid, and 3-*O*-galloyl shikimic acid and 3,4-di-*O*-galloyl shikimic acid, where the activity of the latter compound of each pair was higher than that of the former because of the extra galloyl group, and more obviously, the activity of galloylglucoses increased in accordance with the increase of galloyl groups. Some previous

TABLE 2. IC_{50} Values of flavonoids tested against the DPPH radical

Compound	IC_{50} (μM)
Flavones	
Cirsiliol (50)*	7.08 ± 0.57
6-Hydroxyluteolin (51)	9.13 ± 0.40
Luteolin-7-glucoside (52)	9.90 ± 0.36
Baicalein (53)	10.81 ± 0.33
Scutellarein (54)	11.60 ± 0.80
Baicalin (55)	12.52 ± 0.54
Plantaginin (56)	13.81 ± 0.80
Luteolin (57)	17.78 ± 1.01
Sorbarin (58)	18.57 ± 0.81
Apiin (59)	64.75 ± 1.82
Cosmosiin (60)	232.51 ± 2.78
Acacetin (61)	> 500
Apigenin (62)	> 500
Chrysoeriol (63)	> 500
Cirsilineol (64)	> 500
Cirsimarin (65)	> 500
Cirsimaritin (66)	> 500
Linarin (67)	> 500
Pectolinarigenin (68)	> 500
Pectolinarin (69)	> 500
Rhoifolin (70)	> 500
Tectochrysin (71)	> 500
Toringin (72)	> 500
Flavonols	
2″-*O*-Galloyl myricitrin [33] (73)	3.83 ± 0.06
Quercimeritrin (74)	7.43 ± 0.30
Rhamnetin (75)	7.72 ± 0.82
Quercetin (76)	7.94 ± 0.16
Rutin (77)	9.07 ± 0.41
Chrysosplenol D (78)	9.63 ± 0.36
Hyperin (79)	9.86 ± 0.17
Quercitrin (80)	11.13 ± 0.58
Chrysosplenol C (81)	11.35 ± 0.58
Myricitrin [33] (82)	12.74 ± 0.42
Isoquercitrin (83)	13.28 ± 0.79
Isorhamnetin (84)	16.47 ± 0.73
Oxyayanin A (85)	19.40 ± 0.42
Kaempferol (86)	22.81 ± 1.47
Tetramethylquercetin (87)	50.99 ± 3.15
Afzelin (88)	> 500
Kaempferitrin (89)	> 500
Kaempferol-7-glucoside (90)	> 500

Results are presented as means ± SEM of 5 determinations.
*[ ] Reference number; ( ) compound number.

studies have indicated that the galloyl radical, which was formed during reaction with DPPH, is a highly reactive species that can easily participate in a variety of reactions to give dimers through C—C and C—O coupling, thus halting the chain reaction of the radicals [43]. In addition, among hydrolyzable ellagitannins, compounds with large molecules showed a stronger free-radical scavenging action than those with small molecules. Sanguiin H-11, a tetrameric ellagitannin, showed the strongest inhibitory activity among all the compounds tested, and other dimeric and trimeric ellagitannins such as sanguiin H-6, rugosin D, phillyraeoidin A, and lambertianin B also inhibited free radicals more markedly than monomeric ellagitannins. This result corresponds to that of tannins scavenging the superoxide anion radical [34]. Such a trend was also found in condensed tannins. The IC_{50} values of monomeric, dimeric, trimeric, tetrameric and polymeric tannins decreased in order with the increase in molecular weight, although procyanidin B-2 3,3′-digallate (a dimer) showed stronger activity than procyanidin C-1 (a trimer) due to galloylation. Different from most tannins, because of methylation of the C4-hydroxyl group that obstructs the formation of an ortho-trihydroxyl structure on the gallic acid ring, bergenin showed barely any activity, confirming the previous finding that ortho-trihydroxyl groups act as an active scavenger of the DPPH radical [34].

In the case of flavonoids, the most important active substitution was the 3′,4′-dihydroxyl group. All compounds possessing this substitution, as reported by Heilmann *et al.* [44], showed excellent inhibitory activity against the DPPH radical. In addition, some flavonoids with a 5,6-dihydroxyl or 5,6,7-trihydroxyl group such as chrysosplenol C, baicalein, scutellarein, baicalin, plantaginin, and sorbarin were also found to have inhibitory activity similar to that of 3′,4′-dihydroxylflavone. This indicates that ortho-hydroxyl substitutions, whether on the B-ring or the A-ring, are the most important feature for the antiradical activity of flavonoids, while additional substitution seems to have no obvious influence, because all the ortho-hydroxylflavones had IC_{50} values that fell within a narrow range. On the other hand, some compounds such as isorhamnetin and kaempferol also showed strong inhibitory action against the DPPH radical. In their structure, there are four non-ortho-hydroxyl groups, suggesting that the activity may be enhanced with increasing hydroxyl substitution, and that these four hydroxyl groups could confer strong activity. However, this influence becomes less clear when the number of hydroxyl groups is less than three. In the present study, most trihydroxylflavones such as apigenin, chrysoeriol and others, acted weakly, but oxyayanin A showed extensive inhibition (IC_{50} = 19.40 μM). One possible explanation is that the two para-phenol hydroxyl groups on the B-ring (2′,5′-dihydroxy) may have the same action as the ortho-hydroxyl group, because the phenyl radical reaction may occur exclusively at the exocyclic position, and the ortho and para positions have the same odd-electron character [45].

When the two types of flavonoids were compared, a large percentage of flavonol compounds displayed strong inhibitory activity. As shown in Table 2, almost all flavonols showed clear inhibitory activity at concentrations below 50 μM, except for afzelin, kaempferitrin and kaempferol-7-glucoside. In comparison, only about half of the flavones (9 out of 23) showed the same inhibitory activity. This implied that the oxo- group at the C-3 position seems to be involved to a certain extent in the antiradical activity of flavonoids. From chemical and kinetic viewpoints, it should be reasonable to assume that the free hydroxyl group at the C-3 position would enhance the free-radical scavenging ability, since according to the reduction mechanism of free

radicals, a scavenger should be capable of providing electrons or hydrogen, or of receiving electron pairs, while the hydrogen bond between the 3-hydroxyl group and the 4-keto group would increase this ability [46]. This is reflected in the IC_{50} (50.99 μM) of tetramethylquercetin (5,7,3′,4′-tetramethoxylflavonol), which has only one free hydroxyl group at the C-3 position.

In addition, glycosylation of the hydroxyl group and substitution of the methoxyl group have been shown to exert a varied influence due to differences in structure. The flavongenins and their glycosides, as well as methoxylflavones, showed similar efficacy in scavenging free radicals when an ortho-hydroxyl group was present. However, when such a group was lacking or substituted, a sharp decrease in activity occurred by glycosylation or methylation. For instance, kaempferol, which contains four free hydroxyl groups at positions C-3, C-5, C-7, and C-4′, showed a relatively high inhibition, whereas hardly any effect was shown by its three glycosides, whose IC_{50} values all exceeded 500 μM. To clarify the contribution and influence of glycoside glycosylation on the scavenging of free radicals, we examined 6 ginseng saponins and 5 saikosaponins as well as 4 sugars, including ginsenoside-Rb_1, -Rb_2, -Rc, -Rd, -Re, -Rg_1; saikosaponin-a, -b_1, -b_2, -c; glucose, rhamnose, galactose, and arabinose using the same system (findings to be published separately). The results showed that these saponins and sugars failed to inhibit the DPPH radical, suggesting that the free-radical scavenging activity of glycosides is due mainly to active glycogenins. However, the activity may be influenced greatly by glycosylation, perhaps because this reduces the number of free hydroxyl groups or destroys the ortho-hydroxyl structure. On the other hand, the linkage of sugar may hinder access of the free radical scavengers to the radical center of DPPH. However, apiin and cosmosiin were exceptions in this respect. They displayed effective inhibition, whereas their glycogenin apigenins had no action, suggesting that sugar also may increase the activity of glycosides, perhaps by changing the electron distribution of flavone glycogenin or by another, unclear, pathway, even though sugars do not show any activity themselves. Similarly, chrysoeriol, cirsimarin, cirsimaritin, pectolinarigenin and pectolinarin also acted weakly because the ortho-hydroxyl group is substituted by a methoxyl group in their molecular skeleton.

Moreover, it was noticed that all the compounds used in this experiment, except for toringin and tetramethylquercetin, have a free hydroxyl group at the C-5 position, although they displayed great differences in their inhibitory activities against free radicals. Therefore, it is likely that the 5-hydroxyl group has little influence on activity.

Based on the above results, the ortho-hydroxyl group can be considered the most important structural feature of both tannins and flavonoids for the inhibitory activity against free radicals. The activity of tannins increases with an increase in the number of galloyl groups and molecular weight. When galloyl groups are replaced by DHHDP, the activity may be somewhat decreased, due possibly to the lack of a hydroxyl group or spatial hindrance. The dimeric, trimeric, or polymeric tannins are generally stronger against the DPPH radical than the monomeric tannins, and some derivatives of small molecules. In contrast to tannins, fewer flavonoids showed the same action. In general, flavonoids that have ortho-hydroxyl groups or four free hydroxyl groups exhibit strong inhibitory activity, and glycosylation and methylation may reduce their activity markedly. Compared with other positions, the 3-oxo group on the flavone skeleton seems to have more influence, while the 5-hydroxyl group appears to have no action. On the other hand, it is noteworthy that all the flavonoids tested in this study have an unsaturated C2—C3 bond. Krol *et al.* [47] reported that the C2—C3 double bond is essential for the antioxidant effect of flavonols. Hu *et al.* [48] also reported that a saturated C2—C3 bond shows higher antioxidant ability than an unsaturated bond. Further investigation of these features is needed.

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