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Analysis of Flavonoid Composition of Korean Herbs in the Family of Compositae and their Utilization for Health

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Abstract – Compositional differences in flavonoids are varied in the big family of Compositae. By summarizing our previous analytical studies and other scientific evidences, new strategy will be possible to further analyze flavonoids and utilize them for human health. The HPLC analytical method has been established in terms of linearity, sensitivity, accuracy, and precision. Herbs of the family of Compositae have considerable amounts of peroxynitrite ($ONOO^-$)-scavenging effects and their phenolic substances. These effects may contribute to the prevention of disease associated with excess production of $ONOO^-$, depending on the high content of flavonoid substances.
Keywords – Compositae, Flavonoids, Phenolic substance, HPLC analysis, Peroxynitrite

Introduction

The beneficial effects of Compositae herbs on human health are partly due to their phytochemicals possessing antioxidant activity.[1] Of the phytochemicals that possess antioxidant capacity, flavonoids are one of the most important groups. The concentration of phenolic compounds in Compositae family plants is relatively higher than other families such as Labiatae, Umbelliferae, or Polygonaceae. In particular, mountainous vegetables belonging to Compositae are also generally rich mainly in flavonoids and caffeoylquinic acids.[2,3]

Peroxynitrite ($ONOO^-$) that can be excessively produced from the combination of nitric oxide radical and superoxide anion radical can cause diabetic complications including cardiovascular disease, atherosclerosis and neuropathy.[4] $ONOO^-$ scavengers are also usually found in flavonoids and caffeoylquinic acids. Flavonoids are a group of phenolic substances synthesized by condensation of three *p*-coumaroyl CoAs and a malonyl CoA. The reaction is catalyzed by chalcone synthase. These flavonoids are commonly found in the form of flavone- and flavonol-types in Compositae herbs. In particular, these substances are found in glycoside form rather than in algycone form.

In this review, we combined and summarized our scientific evidences on the traditional uses of five Compositae herbs: *Cirsium setidens*,[5] *Saussurea grandifolia*,[6] *Sonchus brachyotus*,[7] *Chrysanthemum boreale*,[8] and *Hemistepta lyrata*.[9] In addition, we summarized quantitative analysis as an important method to provide information of the composition and level of the active components in a plant material.

Morphology of Compositae and Traditional Uses

Compositae (Asteraceae) is the largest family of flowering plants (Angiospermae) containing the largest number of species of any plant families, with approximately 24,000 species spread across 1,620 genus and 12 subfamilies. Representatives of this family are found in most regions of the world. However, they are rare in tropical rain forests. They are mostly herbaceous plants, although a small proportion of them are trees. Only a few of them are climbers. Water and marsh plants as well as epiphytes are also found.[10] Compositae can be instantly recognized with characterics of florets arranged on a receptacle in centripetally developing heads surrounded by bracts, by anthers fused in a ring with the pollen pushed or brushed out by the style, and the presence of achenes (cypselas) usually with a pappus (Fig. 1).[11]

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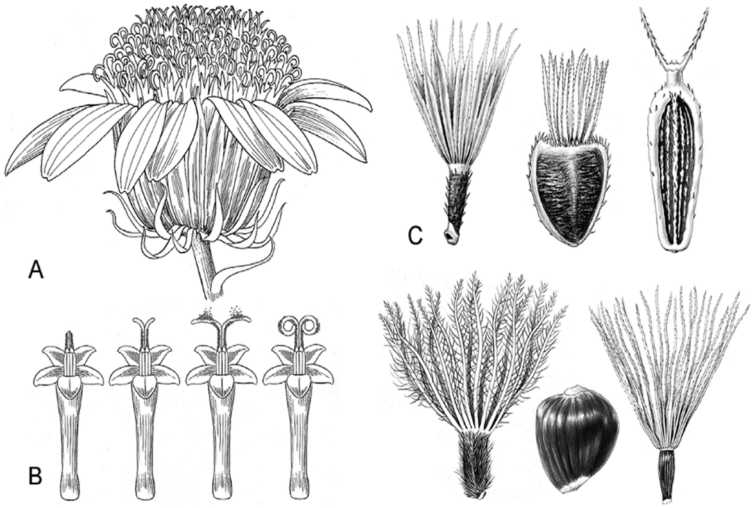


Fig. 1. Characteristics of Compositae.
The head with ray florets arranged around the perimeter, disc florets in the center, and an involucre with bracts (phyllaries) surrounding the outermost florets. B. The pollen is released via the style pushing out through the anthers, which are fused at the margins; sometimes the style branches are recurved and come in contact with the style shaft. C. Some of the achene (cypsela) and pappus types found in Compositae (Funk *et al*., 2009).

Scientific classification

Subkingdom: Tracheobionta (Vascular plant)
Superdivision: Spermatophyta (Seeding plant)
Division: Magnoliophyta (Angiospermae, flowering plant)
Class: Magnoliopsida (Dicotyledoneae)
Subclass: Asteridae
Order: Asterales
Family: Compositae (Asteraceae)

Compositae is an economically important family. Many species of this family are used as foods, herbal medicines, ornamental plants, insecticides, and timbers. Numerous species are consumed as vegetables including lettuce (*Lactuca sativa*), artichoke (*Cynara cardunculus*), topinambour (*Helianthus tuberosus*), and endive (*Cichorium endivia*). Some members provide products such as sweetening agents (*Stevia rebaudiana*), coffee substitutes (*Chicorium intybus*), and teas (*Chrysanthemum* flowers). Sunflower (*Helianthus annuus*) and safflower (*Carthamus tinctorius*) are also two of important oil crops in the world.[12]

Compositae contains the highest number of medicinal plants as important medicines. They are sources of many biologically active compounds, including essential oils, phenolic compounds, flavonoids, terpenoids, phenolic acids, alkaloids, lignans, saponins, stilbenes, sterols, and polysaccharides.[13] Some of them have been studied extensively for their antioxidant activity. Their antioxidative activities are usually attributed to the presence of phenolic

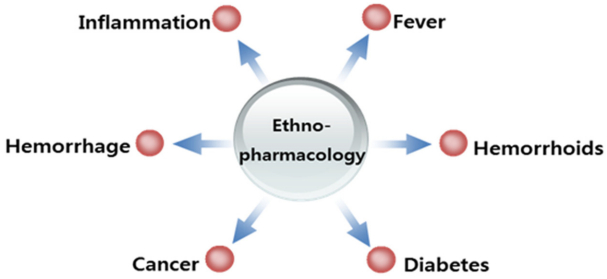


Fig. 2. Traditional use of *Hemitepta lyrata* as one of Compositae herbs.

substances.[1] Due to their bioactive properties, they are commonly used to treat various diseases, including inflammation, hypertension, atherosclerosis, diabetes mellitus, hypercholesterolemia, hepatic fibrosis, osteoporosis, asthma, and ulcers.[14] As a representative of Compositae, *Hemistepta lyrata,* are usually used to treat inflammation, hemorrhage, cancer, diabetes, hemorrohoids, and fever (Fig. 2).[15]

Several species of Compositae have also been investigated for their insecticidal activities. For example, pyrethrins isolated from dried flowers of *Chrysanthemum cinerariaefolium* possess insecticidal properties.[16] These compounds are also present in many Compositae species.

Chemistry and Biological Activities

Phenolics in Compositae herbs – Phenolics can be classified into diverse classes. They occur naturally in a range of plant foods. They are secondary metabolites that

are synthesized during normal development or in response to stress such as infection, wound, or UV radiation.[17] Phenolics are distributed in flowers, fruits, and seeds. They can attract pollinators and seed dispersers. The amounts of phenolics depend on biological factors (genotype, organ, and ontogeny) and environmental factors (temperature, salinity, water stress, and light intensity).[18]

Based on their structure, phenolic substances are classified into phenolic acids, flavonoids, stilbenes, and lignans. These are further sub-divided based on the hydroxylation of phenolic rings, glycosylation, acylation with phenolic acids, and the existence of stereoisomers.

These substances play important roles as antioxidants in several ways. They can act as radical scavengers due to the presence of phenolic group, as lipid peroxidation inhibitors, or breakers of oxidation reaction by binding free radicals, or as chelators of metal ions involved in oxidation.[19] Epidemiological studies have demonstrated that phenolic compounds can provide significant protection against several chronic diseases, including cardiovascular diseases, cancer, diabetes, infections, aging, and asthma. Consumption of red wine can prevent coronary heart disease because red wine contains polyphenols to provide protection against the oxidation of LDL-cholesterol.[28] The beneficial effects of dietary flavonoids on central nervous system (CNS) performance, with particular emphasis on cognitive performance and memory performance have been reviewed.[20]

Causal role of $ONOO^-$ in disease – $ONOO^-$ is one of the reactive nitrogen species (RNS) formed through the combination of superoxide anion radical ($\bullet O_2^-$) and nitric oxide radical (•NO). $ONOO^-$ is a highly reactive oxidant. It is not a radical but an ion. The existence of ONOO is necessary for cell. However, its excess production can cause a variety of metabolic diseases due to lipid peroxidation, cytotoxicity, and rapid neurotoxicity.[21] Furthermore it can cause hypercholesterolemia, atherosclerosis, obesity, diabetes mellitus, and Alzheimer disease.[22]

$ONOO^-$ generation also causes the mechanism for stroke, myocardial infarction, chronic heart failure, diabetes, circulatory shock, chronic inflammatory diseases, cancer, and neurodegenerative disorders.[6] A lot of natural phenolic substances can prevent obesity by inhibiting adipogenesis.[23] $ONOO^-$ scavengers are commonly found in flavonoids.[21] Furthermore, a causal role of ROS has been established in the development of diet-induced adiposity. In particular, neutralization of $ONOO^-$ can be a new strategy for preventing cardiovascular dysfunction, retinopathy, nephropathy, and neuropathy accompanied by diabetic complication. It has been established that neuropathy could be relieved by decomposition of $ONOO^-$ in streptozotocin-induced diabetic model.[21] Endothelial dysfunction caused by $ONOO^-$ production in a diabetic patients is associated with several vascular diseases. Therefore, $ONOO^-$ scavengers can prevent diabetic complication.

$ONOO^-$ and $\bullet CO_3^-$ are the most probable proximal biological mediators of *m*-aconitase inhibition that suppresses mitochondrial respiration.[24] $ONOO^-$ is also a generator of DNA single-strand breaks, and an inducer of the nitration of surfactant protein A.[25] Recently, it has been shown that the NO production in the gastric epithelial cells and *Helicobacter pylori*-induced NO production can result in gastrointestinal ulcer disease. $ONOO^-$ is protonated in very low pH condition of the stomach to yield peroxynitrous acid that can be readily cleaved into nitrite ion ($\bullet NO_2^-$) and hydroxyl radical (•OH). Hydroxyl radical is considered as an important factor for peptic ulcer due to its high capability inducing tissue injury.[26]

However, $ONOO^-$ scavengers prevent such diseases. Therefore, the development of $ONOO^-$ scavengers is required because human body is lack of endogenous enzymes to scavenge $ONOO^-$.[43] A great number of phenolic compounds with $ONOO^-$-scavenging activity have been reported, such as flavonoids, phenolic acids, stilbenes, and lignans.

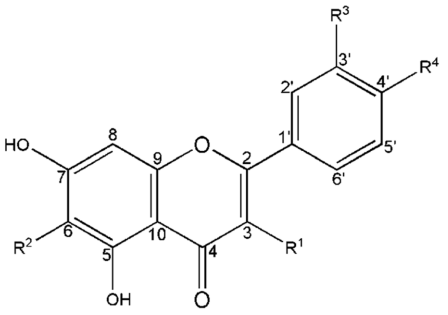
An additional hydroxyl group at the C-2 of phenol (catechol), as in caffeic acid, drastically can increase the $ONOO^-$-scavenging activity. Meanwhile, the hydroxyl group of C-3 in the C-ring of flavonoid is a key of its activity. The activity can be enhanced by the hydroxyl groups at other positions. The increase of the number of phenol groups can increase the activity.

Composition and biosynthetic pathway of flavonoids in five Compositae herbs – Many flavonols, flavones, and caffeoylquinic acid derivatives have been identified from hundreds of species of Compositae family. In our investigations, twenty four phenolic compounds, consisting of three caffeoylquinic acids, two caffeic acids, six flavonols, and thirteen flavones, have been isolated from five Compositae herbs (*C. setidens, S. grandifolia, S. brachyotus*, *C. boreale,* and *H. lyrata*) by column chromatography. They are identified by HPLC and TLC as well as spectroscopic methods. Their chemical structures are shown in Fig. 3.

The five caffeoyl types are caffeic acid, caffeic acid methyl ester, chlorogenic acid (5-caffeoylquinic acid), 3, 4-dicaffeoylquinic acid (3, 4-DQ), and 3, 5-dicaffeoylquinic acid. Chlorogenic acid is found in all plant materials. Chlorogenic acid is present in all Compositae plants (18

Compositae plants) studied.[1]

As are presentatives of the flavonol type, quercetin and its glycosides such as quercetin 3-*O*-glucoside (isoquercitrin), quercetin 5-*O*-glucoside (saxifragin), quercetin 3-*O*-galactoside (hyperoside), quercetin 3-*O*-rutinoside (rutin), and a kaempferol derivatives (kaempferol 3-*O*-rutinoside) have been identified. Among those compounds, saxifragin, with its bright yellow fluorescent color under UV light and the structure of a flavonol 5-*O*-glycoside, is uncommon in natural sources. This compound was first isolated from *Saxifraga stolonifera*.[54] It was then isolated from silkworm (*Bombyxmori*).[27]



Flavonoid	R_1 (C-3)	R_2 (C-6)	R_3 (C-3')	R_4 (C-4')
Quercetin	OH	H	OH	OH
Kaempferol	OH	H	H	OH
Luteolin	H	H	OH	OH
Apigenin	H	H	H	OH
Acacetin	H	H	H	OCH_3
Pectolinarigenin	H	OCH_3	H	OCH_3
Diosmetin	H	H	OH	OCH_3

Fig. 3. Chemical structures of several flavonols and flavones.

All those flavones are observed together with their 7-rutinoside, i.e.,luteolin 7-rutinoside, apigenin 7-rutinoside (isorhoifolin), acacetin 7-rutinoside (linarin), pectolinarigenin 7-rutinoside (pectolinarin), and diosmetin 7-rutinoside (diosmin). Of these substances, linarin is the most common one; it is present in three plants (*C. setidens*, *C. boreale*, and *H. lyrata*). Linarin, chlorogenic acid, 3, 4-DQ, caffeic acid methyl ester, and hyperoside are present in *C. setidens*. In addition, luteolin and its 7-rutinoside, luteolin 7-glucoside (cynaroside), and luteolin 7-glucuronide are also present in *S. brachyotus*.

The most common flavonoids are the glycosides of

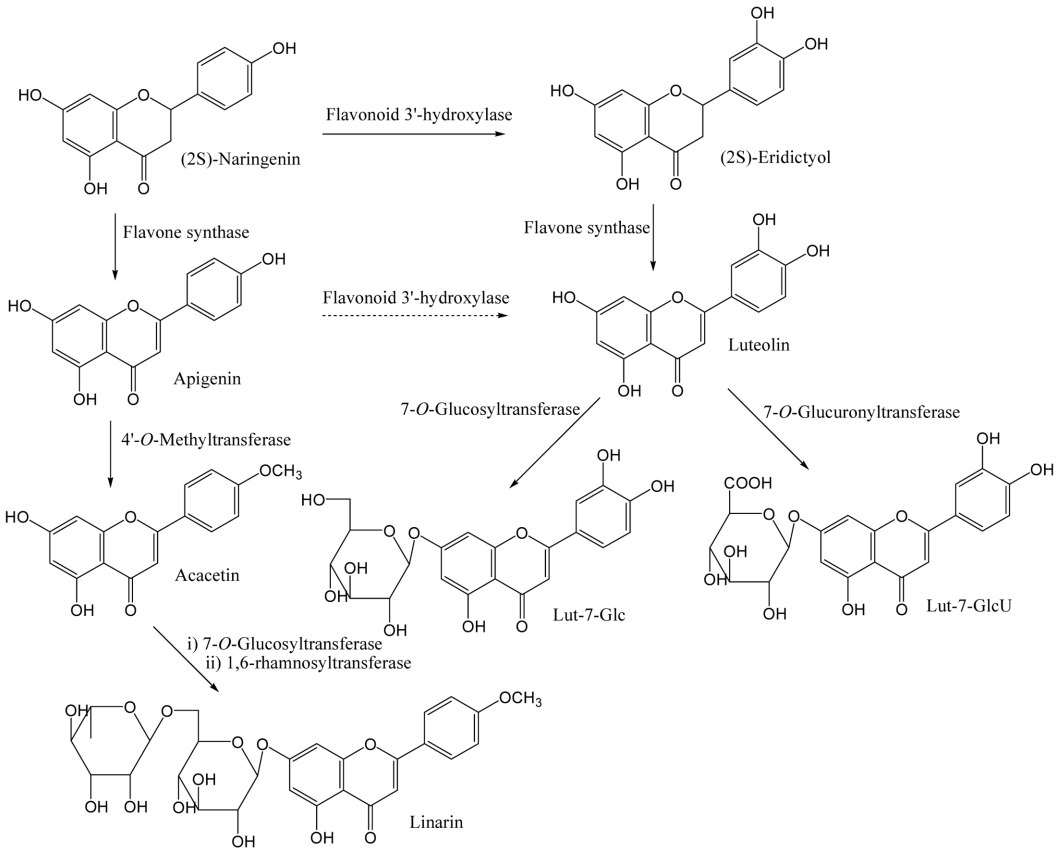


Fig. 4. Structure of luteolin, acacetin, Lut-7-Glc, Lut-7-GlcU and acacetin identified from *Y. japonica* and their presumed pathway. A dotted arrow represents a less favored pathway.

lueolin or apigenin in Compositae.[1] The glycosides of acacetin, pectolinarigenin, and diosmetin are also found. The chemical structures of these five flavones are shown in Fig. 4. Structurally, luteolin and apigenin are only different in the B-ring: luteolin with a catechol, and apigenin with a phenol. Acacetin has the structure of 4'-*O*-methylated apigenin. The enzyme apigenin 4'-*O*-methyltransferase uses *S*-adenosyl methionine and apigenin to produce *S*-adenosylhomocysteine and acacetin,[28] as shown in Fig. 4. The acacetin with an additional methoxy group at C-6 position is called pectolinarigenin. Diosmetin is structurally different from luteolinonly at the C-4' position.

Production of the luteolin 7-glucuronide is caused by enzyme UDP-glucuronide: luteolin 7-*O*-glucuronyltransferase. This enzyme catalyzes the attachment of D-glucuronic acid to luteolin to produce luteolin 7-glucuronide.[29] Luteolin 7-*O*-glucosyltransferase catalyzes the attachment of D-glucose to luteolin to produce luteolin 7-glucoside. The 1, 6-rhamnosyltransferase catalyzes the linkage of L-rhamnose to 6-*O*-position of D-glucose to produce rutinose. The biosynthesis of luteolin 7-rutinoside iscurrently unknown. A presumed pathway for biosynthesis of the luteolin glycosides is shown in Fig. 4.

Based on the phenolics composition (Table 1), pattern of the HPLC chromatograms and the yields of the isolated compounds, each plant has its unique phytochemical characteristics. *C. setidens* is rich in pectolinarin, whereas *S. brachyotus* is abundant in luteolin 7-glucuronide. Another flavone type, linarin, is very abundant in *C. boreale* and *H. lyrata*. On the contrary, saxifragin (quercetin 5-*O*-glucoside) belonging to the flavonol type has been isolated in a high amount from *S. grandifolia*. Of the five Compositae herbs studied, flavone types are more common as the main constituents than flavonol- or caffeoyl types.

Pharmacological actions of luteolin, its 7-*O*-glucoside and 7-*O*-glucuronide – Light/water stressor salinity stress[30] can increase the biosynthesis of luteolin and its 7-glucoside in certain plants. Three Compositae plants, *Ixeris dentata*, *I. dentata* var. *albiflora*, and *I. sonchifolia* have been reported to possess antiinflammatory effects in lipopolysaccharide-stimulated macrophage cells based on the high content of luteolin 7-glucoside or luteolin 7-glucuronide.[31] *Lactuca raddeana* containing the phenolic substances has been reported to be able to

Table 1. Recapitulation of the phenolics identified in the five Compositae herbs

Type	Compounds	*C. setidens*	*S. grandifolia*	*S. brachyotus*	*C. boreale*	*H. lyrata*
Caffeoyl type	Caffeic acid					●
	CAME	●				
	Chlorogenic acid	●	●	●	●	●
	3,4-DQ	●				
	3,5-DQ				●	●
Flavonol type	Quercetin		●			
	Isoquercitrin		●			
	Saxifragin		●			
	Hyperoside	●				
	Rutin		●			●
	Kp 3-rut					●
Flavone type	Luteolin			●		
	Luteolin 7-glc			●		
	Luteolin 7-glcU			●		
	Luteolin 7-rut			●		
	Acacetin				●	●
	Linarin	●			●	●
	Acacetintrisaccharide				●	
	Pectolinarigenin					●
	Pectolinarin	●				●
	Apigenin					●
	Isorhoifolin					●
	Diosmetin					●
	Diosmin					●

suppress fat accumulation in 3T3-L1 adipocytes.[32]

Luteolin and its 7-glucoside are absorbed after being converted into luteolin-glucuronide and luteolin during passage through the intestinal mucosa. They are present in the serum in free form or as the conjugate (monoglucuronide).[33] Luteolin 7-glucoside can be hydrolyzed by an anaerobic bacterium, *Eubacterium ramulus*.[34] Luteolin 7-*O*-glucoside has several pharmacological actions, including inhibiting superoxide generation in neutrophiles,[35] inhibiting protein expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX) in lipopolysaccharide-induced macrophage RAW 264.7 cells,[36] anti-asthmatic activity through down-regulation of helper 2 cytokine[37] and hepatoprotective activity.[38] Significant pharmacological activities of luteolin are known, including inhibition of iNOS and COX expression;[36] anxiolytic activity;[39] gastroprotective activity due to inhibition of H^+-K^+-ATPase;[40] inhibitory activities on α-glucosidase,[41] monoamine oxidase,[42] and low density lipoprotein oxidation.[43] Luteolin 7-glucuronide, has preventive effects against reflux esophagitis and gastritis,[44] antimutagenic activity[45] and antidepressant activity.[46] Based on the literatures cited above, luteolin and its 7-*O*-glucuronide may represent the pharmacological actions of luteolin glycosides *in vivo*.

Pharmacological actions of apigenin, acacetin, and pectolinarigenin – Less amounts of apigenin or its glycosides have been found in the Compositae herbs compared to luteolin or its glycoside. However, acacetin with the structure of 4'-*O*-methylated apigenin exists in its glycoside linarin. This flavonoid is abundant in *Hemistepta lyrata*. Pectolinarin with the structure of 6-methoxylated linarin has relatively high quantity in *Cirsium setidens*.

The pharmacological activities of apigenin on CNS are widely studied, including inhibition of β-amyloid-induced neuronal death,[47] anxiolytic activity on central benzodiazepine receptors,[48] and neuroprotective activity.[49] The pharmacological actions of acacetin have been known, including inhibition of neuroinflammation,[50] apoptosis-inducing action,[51] anti-inflammation,[52] vasorelaxation,[53] and anti-cancer action.[54] A few pharmacological action of pectolinarigenin have been also reported, including anti-inflammation,[55] hepatoprotective action,[56] and anti-allergy.[57] However, the $ONOO^-$-scavenging activity of apigenin, acacetin and pectolinarigenin is weak.

Analysis of Flavonoids

Optimization of the HPLC method – Compared to other methods, the HPLC method is preferred because it offers higher sensitivity and greater efficiency than thin-layer chromatography. In addition, it enables us to analyze phenolic compounds without derivatization necessary for gas chromatography. Reversed-phase HPLC columns are the most appropriate for separating phenolic compounds with the C18 column being frequently used. For our study, a C18 column (Shiseido Capcell PAK C18 column, 5 μm, 4.6 mm × 250 mm) was used together with a Varian HPLC system consisting of two Prostar 210 pumps and a Prostar 325 UV-vis detector.

The HPLC method was optimized by investigating four parameters influencing the quality of chromatogram, i.e., mobile phase composition, gradient elution, column temperature, and UV wavelength. The mobile phase system, consisting of H_2O as solvent A, and MeOH-CH_3CN (60:40) as solvent B, was selected because it produced a better separation. This composition also showed an improved resolution together with acceptable peak shapes. In addition, these solvents are environment-friendly. Addition of 0.05% TFA to both solvents improved the resolution of target compounds probably by deionization of phenolic substances. TFA can inhibit the ionization of acids.

Various gradient elution programs were tested to obtain a gradient that could cover a wide range of polarity of phenolic compounds (from simple and high polar substances to complex and less polar substances) with good separation. This gradient elution, (A)/(B) = 85/15 (0 min) → 35/65 (35 min, hold for 5 min) → 0/100 (42 min; hold for 4 min to wash the column)→85/15 (49 min; hold for 6 min to equilibrate the column condition) with a flow rate of 1.0 ml/min, and column temperature of 40 ºC demonstrated the best performance of separation with more constant retention times.

A wavelength 254 nm for detection was selected during our consideration of the following four 254, 280, 300 and 360 nm. The 254 nm demonstrated a good sensitivity with low noise for detection of several flavonols, flavones, and caffeoylquinic acids as common phenolic constituents in some Compositae plants. The LOD and LOQ of the analyzed compound could be achieved at a smaller concentration. Therefore, low concentration of phenolic constituents in the extract can be detected and quantified accurately in our method. As shown in Table 2, LODs and LOQs of twenty four phenolic compounds were observed over 0.01 - 0.85 μg/ml and 0.04 - 2.83 μg/ml, respectively, suggesting that the present HPLC method is adequate for simultaneous detection and quantification of flavonoids and some phenolic acids including caffeoylquinic acids.

The linearity of an analytical method is its ability (within a given range) to obtain test results which are

Table 2. Linearities and detection/quantification limits of the standard compounds

Standard compound	t_R (min)	Linear regression[a]	R^2 [b]	LOD[c] (μg/ml)	LOQ[d] (μg/ml)
Chlorogenic acid	8.32	$y = 166.80x + 58.46$	0.9998	0.30	1.01
Caffeic acid	10.36	$y = 253.42x + 50.84$	0.9999	0.22	0.72
Saxifragin	14.78	$y = 414.62x + 65.93$	0.9998	0.12	0.40
Luteolin 7-rutinoside	15.63	$y = 175.25x + 34.55$	0.9999	0.38	1.26
Rutin	16.13	$y = 143.53x + 15.18$	0.9999	0.42	1.39
Luteolin 7-glucoside	16.29	$y = 200.82x + 40.73$	0.9999	0.34	1.13
Hyperoside	16.37	$y = 217.04x + 46.33$	0.9999	0.14	0.46
Luteolin 7-glucuronide	16.63	$y = 48.813x + 95.76$	0.9997	0.85	2.83
Isoquercitrin	16.76	$y = 472.75x + 75.62$	0.9999	0.11	0.36
3,5-dicaffeoylquinic acid	16.95	$y = 157.87x + 75.02$	0.9999	0.27	0.90
Isorhoifolin	18.09	$y = 232.85x + 65.10$	0.9999	0.17	0.57
Acacetintrisaccharide	18.42	$y = 104.76x + 81.54$	0.9999	0.30	1.01
Kaempferol 3-rutinoside	18.71	$y = 236.19x + 54.25$	0.9998	0.22	0.72
3,4-dicaffeoylquinic acid	18.95	$y = 133.62x + 45.50$	0.9999	0.23	0.76
Diosmin	19.10	$y = 244.78x + 53.54$	0.9999	0.21	0.71
Caffeic acid methyl ester	19.68	$y = 172.39x + 44.58$	0.9999	0.12	0.40
Quercetin	24.79	$y = 659.28x + 87.33$	0.9999	0.09	0.23
Linarin	24.85	$y = 309.18x + 30.86$	0.9999	0.23	0.76
Luteolin	25.26	$y = 399.22x + 164.9$	0.9997	0.25	0.83
Pectolinarin	25.58	$y = 103.76x + 60.10$	0.9997	0.43	1.44
Apigenin	28.67	$y = 529.46x + 49.28$	0.9999	0.11	0.35
Diosmetin	29.53	$y = 741.76x + 96.14$	0.9999	0.01	0.04
Acacetin	36.88	$y = 369.27x + 45.71$	0.9999	0.15	0.50
Pectolinarigenin	37.73	$y = 199.06x + 33.37$	0.9999	0.20	0.67

[a] y, peak area at 254 nm; x, concentration of the standard (μg/ml); [b] R^2, correlation coefficient for 6 data points in the calibration curves (n = 4); [c] LOD, limit of detection (S/N = 3); [d] LOQ, limit of quantification (S/N = 10).

directly proportional to the concentration (amount) of analyte in the sample.[91] The linearities of twenty four analytes (Table 2), represented by the values of R^2 (correlation coefficient), were more than 0.999, indicating that the present HPLC method was sufficient to generate linear regressions with nice linearities. In addition, the HPLC method also exhibited a good performance in the chromatographic separation of many flavonoids and several phenolic acids as demonstrated by resulting different retention times (t_R) of twenty four compounds. The data on accuracy and precision in validation experiments can be referred from literatures.[8-12]

Contents of the flavonoids and caffeoylquinic acids of the five Compositae – Quantitative analysis is commonly used to provide information about the composition of active components contained in a plant material. HPLC analysis is most frequently employed for simultaneous quantification because it offers higher sensitivity and greater efficiency. HPLC quantitative analyses were performed to determine the contents of flavonoids and caffeoylquinic acids identified in the MeOH extracts of five Compositae herbs (*C. setidens, S. grandifolia, S. brachyotus, C. boreale,* and *H. lyrta*) (Fig. 5). Twenty-four compounds consisting of three caffeoylquinic acids, two caffeic acids, six flavonols, and thirteen flavones were used as standards for quantitative analysis. The isolated compounds with high purity (> 98%) were used as reference standards.

In general, the main compounds play a principal role in some particular pharmacological effects including antioxidant effect.[1] Contents of the flavonoids and caffeoylquinic acids in the five Compositae herbs are presented in Table 3 (extracts) and Table 4 (dry material). The main substances are distinct and comparable among the five plant species (Fig. 6).

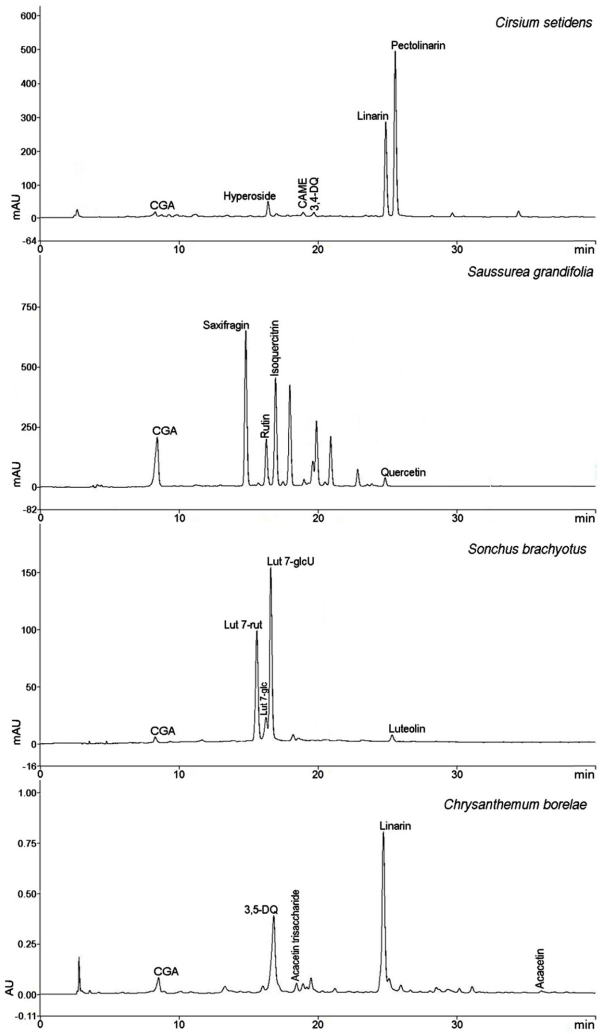


Fig. 5. HPLC chromatograms of MeOH extracts of the four Compositae herbs (Compound names are abbreviated).

ONOO$^-$-scavenging Activities of the Flavonoids

ONOO$^-$-scavenging activities of twenty-two phenolics were shown in Table 5. These substances are classified into three groups: caffeic acid type, flavonol type, and flavone type. The IC_{50} values determined by peroxynitrite-scavenging assay of at least three different concentrations over 0.08 - 50.0 μg/mlare shown in μg/ml unit and μM. The activities can be compared more easily from the illustrating chart (Fig. 7). The IC_{50} value of the positive control, L-penicillamine is 3.86 μM. Only a few compounds belonging to the flavone type, acacetin, pectolinarigenin, and apigenin, displayed relatively weak activities, whereas their glycosides, linarin and pectolinarin, exhibited moderate activities (7.77 μM and 6.47 μM, respectively).

Luteolin and its two glycosides (luteolin 7-*O*-glucoside and luteolin 7-*O*-glucuronide) have strong activities (IC_{50}: 0.81 μM, 0.86 μM, and 1.12 μM, respectively). Their IC_{50} levels are comparatively similar to those of quercetin and its derivatives such as isoquercitrin, saxifragin, hyperoside, and rutin with IC_{50}s at less than 2.0 μM. Meanwhile, the flavonols, kaempferol and its two glycosides, astragalin and kaempferol 3-rutinoside, showed weaker activities (with IC_{50} of 2.48 μM, 4.02 μM, and 4.15 μM, respectively).

Quercetin displayed a powerful antioxidant capacity, depending on the unique structure of a catechol in its B-ring and 2, 3-double bond in conjunction with a 4-carbonyl group in C-ring. This structure can allow for delocalization of the phenoxy radical electron to the flavonoid nucleus. The combined presence of a 3-hydroxy group with a 2, 3-double bond can additionally increase the resonance stabilization for electron delocalization. Hence, it has a higher antioxidant value.

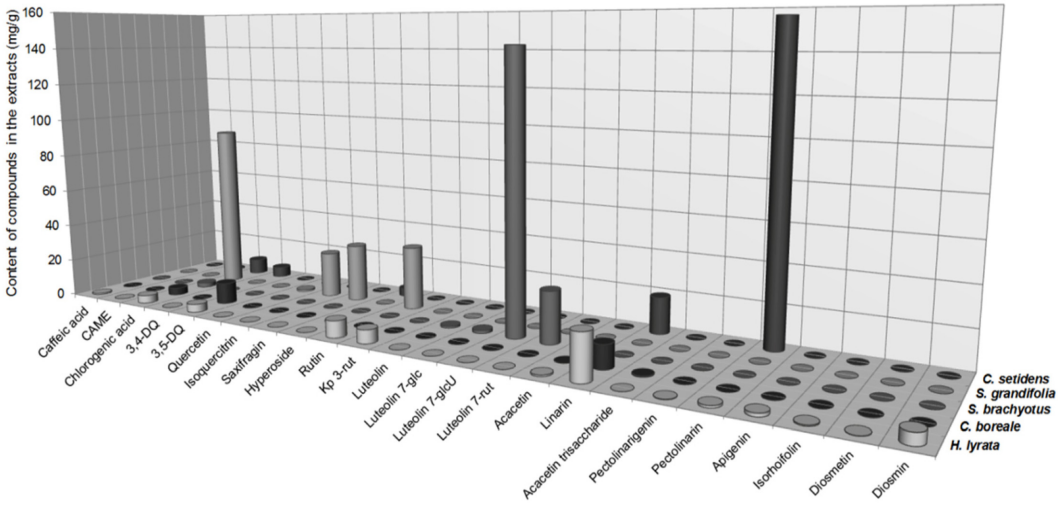


Fig. 6. Composition of flavonoids and caffeoylquinic acids in the five Compositae herbs.

Table 3. Contents of the flavonoids and caffeoylquinic acids in MeOH extracts of the five Compositae herbs (mg/g)

Type	Compounds	*C. setidens*	*S. grandifolia*	*S. brachyotus*	*C. boreale*		*H. lyrata*
					Leaf	Flower	
Caffeic acid-type	Caffeic acid						0.69
	CAME	0.51					
	Chlorogenic acid	8.41	89.87	2.53	20.86	13.02	4.22
	3,4-DQ	5.74					
	3,5-DQ				60.43	47.28	4.32
Flavonol-type	Quercetin		0.99				
	Isoquercitrin		24.46				
	Saxifragin		30.76				
	Hyperoside	4.33					
	Rutin		33.74				8.81
	Kp 3-rut						7.03
Flavone-type	Luteolin			1.05			
	Luteolin 7-glc			1.64			
	Luteolin 7-glcU			143.01			
	Luteolin 7-rut			26.7			
	Acacetin				0.86	0.46	0.74
	Linarin	18.99			63.80	43.59	23.2
	Acacetintrisaccharide				2.99	6.97	
	Pectolinarigenin						0.24
	Pectolinarin	156.48					1.10
	Apigenin						1.59
	Isorhoifolin						0.49
	Diosmetin						0.16
	Diosmin						5.40
Total amount		194.46	179.82	174.93	148.94	111.32	57.99

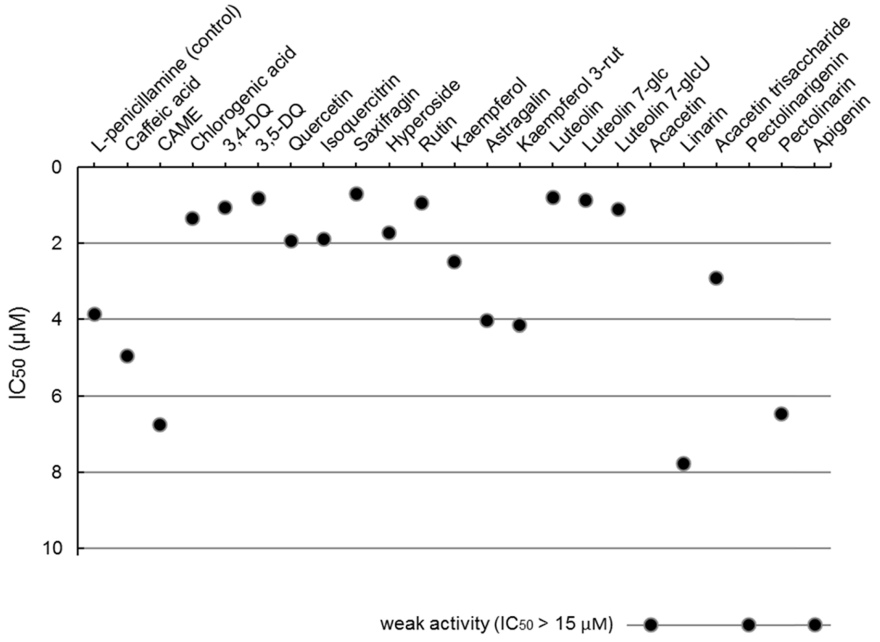


Fig. 7. Peroxynitrite-scavenging activities (IC_{50}s) of the flavonoids and caffeoylquinic acids identified in the five Compositae herbs.

Table 4. Contents of the flavonoids and caffeoylquinic acids in the dry plant material (DM) of the five Compositae herbs (mg/g of dry weight)

Type	Compounds	*C. setidens*	*S. grandifolia*	*S. brachyotus*	*C. boreale*		*H. lyrata*
					Leaf	Flower	
Caffeic acid-type	Caffeic acid						0.13
	CAME	0.08					
	Chlorogenic acid	1.35	12.35	0.32	3.90	2.54	0.78
	3,4-DQ	0.92					
	3,5-DQ				11.30	9.22	0.80
Flavonol-type	Quercetin		0.14				
	Isoquercitrin		3.37				
	Saxifragin		4.24				
	Hyperoside	0.69					
	Rutin		4.65				1.62
	Kp 3-rut						1.29
Flavone-type	Luteolin			0.13			
	Luteolin 7-glc			0.21			
	Luteolin 7-glcU			18.30			
	Luteolin 7-rut			3.40			
	Acacetin				0.16	0.09	0.14
	Linarin	3.06			11.93	8.50	4.26
	Acacetintrisaccharide				0.56	1.36	
	Pectolinarigenin						0.05
	Pectolinarin	25.19					0.20
	Apigenin						0.29
	Isorhoifolin						0.09
	Diosmetin						0.03
	Diosmin						0.99
Total amount		31.29	24.75	22.36	27.85	21.71	10.67

Table 5. Peroxynitrite-scavenging activities (IC_{50}s) of the flavonoids and caffeoylquinic acids identified in the five Compositae herbs

Type	Compound	Concentration (μg/ml)					IC_{50}	
		50.00	10.00	2.00	0.40	0.08	(μg/ml)	μM
Caffeic acid-type	Caffeic acid	–	97.34±0.23	73.95±0.84	30.65±3.21	–	0.89	4.94
	CAME	–	93.32±0.30	67.33±1.02	18.67±1.90	–	1.31	6.76
	Chlorogenic ac.	–	94.34±0.58	78.70±1.85	56.56±4.38	12.67±8.55	0.47	1.34
	3,4-DQ	–	93.05±0.68	75.31±1.18	47.92±0.65	15.32±4.65	0.55	1.06
	3,5-DQ	–	94.26±1.11	86.03±2.31	50.53±3.18	17.72±4.90	0.43	0.83
Flavonol-type	Quercetin	–	94.82±1.49	82.68±3.19	47.44±3.93	6.88±4.22	0.58	1.93
	Isoquercitrin	–	94.58±1.69	76.71±3.35	25.17±3.11	3.97±2.57	0.88	1.90
	Saxifragin	–	95.45±1.84	86.40±0.56	61.29±1.51	19.59±8.79	0.33	0.71
	Hyperoside	–	95.70±0.27	75.62±4.21	26.94±3.22	9.97±4.58	0.80	1.73
	Rutin	–	97.30±0.21	81.06±1.02	48.89±1.10	6.34±3.43	0.57	0.94
	Kaempferol	–	94.41±0.11	92.50±1.45	28.95±4.18	3.58±3.88	0.71	2.48
	Astragalin	–	74.38±1.18	42.50±2.02	36.66±4.64	13.77±4.29	1.60	4.02
	Kp 3-rut	–	71.34±1.71	38.97±2.34	25.93±3.41	19.32±5.64	2.47	4.15
Flavone-type	Luteolin	–	97.56±5.71	96.15±0.27	85.85±3.70	10.80±9.65	0.23	0.81
	Luteolin 7-glc	–	97.16±0.71	88.80±1.27	54.34±3.41	17.01±3.50	0.39	0.86
	Luteolin 7-glcU	–	96.19±2.17	83.36±0.31	48.39±1.57	11.48±4.33	0.52	1.12
	Acacetin	82.33±0.45	59.54±1.22	21.66±1.34	–	–	7.87	27.72
	Linarin	97.45±0.24	66.89±0.98	32.56±1.21	–	–	4.60	7.77
	Acacetintrisaccharide	97.57±0.31	81.44±1.39	45.14±1.24	–	–	2.19	2.91
	Pectolinarigenin	88.69±0.39	66.00±1.46	30.46±1.13	–	–	5.23	16.99
	Pectolinarin	90.65±0.59	69.94±0.68	35.86±1.36	–	–	4.03	6.47
	Apigenin	54.49±1.85	36.73±1.45	18.22±2.21	–	–	33.19	122.9
Control	L-penicillamine	–	91.76±1.05	83.55±1.51	47.28±2.89	8.75±5.87	0.57	3.86

Data represent mean±SD (n = 2).

Luteolin also demonstrated a potent antioxidant activity.[58] Quercetin as a representative flavonoid may directly capture ROS, such as $\cdot O_2^-$, H_2O_2, or HClO.[59] Quercetin, myricetin, and kaempferol are flavonoids with greatest free radical neutralizing activity. These substances can act as antioxidants by directly capturing the unpaired ROS electron.

As shown in Table 5, the IC_{50} levels of caffeic acid and caffeic acid methyl ester are 4.94 μM and 6.76 μM, respectively. These values are much higher than the IC_{50}s of caffeoylquinic acids (less than 2 μM). However, in terms of the μg/ml unit, the values are similarto each other.

The antioxidant activity of caffeic acid with *o*-dihydroxyl group is stronger than *p*-coumaric acid with mono-hydroxyl. Caffeic acid has a higher antioxidant activity because of additional conjugation in the propenoic side chain. The powerful antioxidant activities of caffeic acid and its conjugates such as chlorogenic acid and dicaffeoylqunic acids were evident.[60] In particular, among caffeoylquinic acids, 3,4-DQ and 3,5-DQ as representatives of dicaffeoylquinic acids showed stronger activities (IC_{50}: 1.06 μM and 0.83 μM, respectively) than the monocaffeoylquinic acid (IC_{50} of chlorogenic acid, 1.34 μM), probably due to the number of caffeoyl groups.

Conclusion

Since $ONOO^-$-scavengers of flavonoids and caffeoylquinic acids are abundant in Compositae herbs, they could prevent diabetic complication caused by excess production of $ONOO^-$. In addition, our HPLC method could be used to reliably analyze flavonoids in other Compositae herbs. Flavonoids mainly exist in the form of glycosides in those herbs.

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