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Phenolic Acid Contents and ROS Scavenging Activity of Dandelion(*Taraxacum officinale*)

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민들레(*Taraxacum officinale*) 잎과 뿌리의 페놀산 조성 및 활성산소 소거활성

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Abstract

The purpose of this study was to investigate the antioxidant activity of 80% ethanol extracts and various solvent fractions of dandelion (Taraxacum officinale) leaves and roots. Total phenolics and phenolic acid contents were also examined. The total phenol content of leaves and roots were 7.9±0.4% and 9.4±0.3%, respectively. Eight phenolic acids were separated by GC, among which caffeic acid (113.7 mg%)and m-coumaric acid (152.6 mg) were the dominant phenolic acids in leaves and roots, respectively. Amongst solvent fractions of leaves and roots, the ethyl acetate fraction showed the strongest radical scavenging activity. A strong correlation was found between total phenol content and electron-donating ability, and ABTS radical scavenging activity showed a similar trend as electron-donating ability. Hydroxyl-radical-scavenging activity and lipid peroxidation were significantly higher in the ethyl acetate fraction than other factions. In particular, the SOD-like activity was highest (43.6%) in the ethyl acetate fraction of dandelion leaves, and was higher than that of trolox. Thus, the ethyl acetate fraction of dandelion leaves exhibited significant phenol content, antioxidant activity, and free-radical-scavenging effects.

Key words : dandelion, phenolic acid, ROS scavenging, antioxidant, solvent fraction

Introduction

Free radicals and other reactive oxygen species, collectively are known as reactive oxygen species (ROS) generated continuously via normal physiological processes, more so in pathological conditions(1). ROS can readily react with and oxidize biomolecules including carbohydrates, proteins, lipids and DNA. Although a living system possesses several natural defense mechanism, such as antioxidant enzyme and nutrients, which arrest the chain reaction of ROS initiation and production, continuous exposure of ROS for a long time may lead to irreversible oxidative damage(2). Antioxidants may directly react with and quench free oxygen radicals, form chelating complex with transition metals, act as reducing agents, induce the production of antioxidative enzyme, and/or suppress the generation of oxidative enzyme, such as cyclooxygenase, in the biological systems. Many medicinal plants contain large amounts of antioxidants such as polyphenols, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides(3-5).

It is believed that the higher intake of antioxidant rich food is associated with prevention of degenerative disease particularly cardiovascular disease and cancer(6). Several studies have shown that plant derived antioxidant nutraceuticals scavenge free radicals and modulate oxidative

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stress-related degenerative disease(7,8). Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in food, cosmetics and pharmaceutical materials(9,10).

Dandelion(*Taraxacum officinale*) has been widely used as a folk remedy for its choleretic, diuretic and anti-inflammatory properties. The anti-inflammatory activity of dandelion extracts was recently confirmed in animal studies and its aqueous extracts have been shown to exhibit anti-tumor activity(11). Dried dandelion leaves and roots are available today as herbal teas and the powdered roots is sold as a coffee substitute after roasting. Hagymasi et al.(12) reported that leaves and roots extracts of dandelion diminished the enzymatically-induced lipid peroxidation and reduced the cytochrome c with and without NADPH in a concentration dependent manner. Cho et al.(13) also reported that water extract supplement of dandelion leaves can improve the lipid metabolism and have a beneficial effect on diabetic complications prevention from lipid peroxidation and free radicals in diabetic rats. The anti-inflammatory and anti-tumor activities of dandelion extract have been confirmed in animal study(14). Antioxidant activities of aqueous extracts of dandelion have been determined *in vitro* and *in vivo*(15). Many researchers reported that quercetin and luteolin glycosides from leaf and flower, other phenolic compounds include caffeic and chlorogenic acid from the dandelion (16,17). Several active constituents such as sesquiterpenre lactones, taraxacoside, phytosterols from dandelion have also been elucidated their chemical structure(18). However, little information about composition and contents of phenolic acids in dandelion were available.

The aim of this study was to determine antioxidant activities of leaves and root of dandelion through a solvent fractionation against DPPH, ABTS, ·OH, and superoxide anion radicals. Lipid peroxidation inhibitory effect was also examined using phosphatidylcholin/ Fe^{2+}/ascorgate system. Furthermore, total phenolic compounds and phenolic acids were determined.

Materials and methods

Materials

Dandelion(*Taraxacum officinale*) leaves and root were obtained from Mindulae Food Company located Kyungnam, South Korea. The leaves and root were freeze-dried and powdered. Dried and powdered leaves and root of dandelion (100 g) were extracted with 80% methanol (2×1 L) and filtered with Whatman filter paper No 4. Total crude extract was collected by a rotary under 40°C. The concentrate was extracted with n-hexane, diethyl ether, ethyl acetate and *n*-butanol, in successive order. Each extract was concentrated and tested for radical scavenging activities.

Determination of total phenolic compounds and phenolic acids

One milligram of each sample was dissolved in 1 mL methanol and mixed with 2% Na_2CO_3, and then, added with Folin-Ciocalteu reagent. The mixture was kept for 30 min at room temperature. The absorbance was measured three times at 750 nm using UV-visible spectrophotometer (UV-1601 PC, Shimadzu, Kyoto, Japan). Total phenolic contents were exhibited as a gallic acid equivalent. A HP-6890 gas chromatography equipped with ZB-50 capillary column (0.25 mm × 30 cm, Phenomex Co., U.S.A.) was used for determination of phenolic acids. Nitrogen was used as a carrier gas on the flow rate 1 mL/min. The injector and FID detector temperature were 230℃ and 260℃, respectively. Oven temperature was 120℃ held for 3 min and programmed at 6℃/min to 250℃ held for 3 min.

DPPH radical: DPPH radical scavenging activity was determined according to the method of Blois(19). Briefly, 0.2 mL of sample was added to an ethanol solution of 0.4 mM DPPH. The mixture was shaken vigorously and then left to stand for 10 min in ambient temperature. Then, the absorbance of the mixture was measured at 525 nm. Trolox, water-soluble derivative of tocopherol, was used as a positive control.

ABTS radical: The ABTS radical scavenging activity was determined by a slightly modified method of Van den Berg et al.(20). In brief, 1.0 mM AAPH(radical initiator) was mixed with 2.5 mM ABTS in phosphate buffer(100 mM, pH 7.4). The mixed solution was heated at 68℃ for 1 hr in a water bath. Twenty microliters of sample was added to 980 μL ABTS radical solution, and the mixture was incubated at 37℃ for 10 min in a water bath under dark conditions. The absorbance was measured at 734 nm against appropriate blanks.

Hydroxyl radical: Hydroxyl radical scavenging activity was determined by the 2-deoxyribose oxidation method(21). Hydroxyl radical was generated by the Fenton reaction in the presence of $FeSO_4 \cdot 7H_2O$. The reaction mixture consisted of 200 μL of $FeSO_4 \cdot 7H_2O$(10 mM), EDTA(10 mM) and

2-deoxyribose(100 mM). The sample solution and a phosphate buffer(pH 7.4, 0.1 M) were then added to generate a total volume of 1.8 mL. Finally, 200 μL of a H_2O_2(10 mM) was added to the reaction mixture and incubated at 37℃ for 4 hr. After incubation, 1 mL of trichloroacetic acid(2.8%) and thiobarbituric acid(1.0%) were added to the reaction mixture. After boiling for 10 min, its absorbance was measured at 532 nm.

Superoxide anion: The superoxide anion radical scavenging capacity was determined by the method of Marklund et al.(22). A 50 μl sample solution and Tris-HCl buffer(pH 8.5) were mixed with 50 μL of 24 mM pyrogallol. The absorbance of the reaction mixture was measured for 2 min at 420 nm.

Hydrogen peroxide: Hydrogen peroxide scavenging activity was determined according to method of Muller et al.(23). The test sample, dissolved in methanol, was mixed with 100 μL of PBS buffer(0.1 M, pH 5) and 20 μL of hydrogen peroxide(10 mM) in a 96 microwell-plate and then incubated at 37℃ for 5 min. ABTS(30 μL, 125 mM) and peroxidase(30 μL, 1 unit/mL) were added to the mixture, which was incubated at 37℃ for 10 min. The absorbance was read with ELISA reader(ELISA Processor Ⅱ, Behring Co., Germany) at 405 nm.

Measurement of lipid peroxidation inhibitory activity: Phosphatidylcholin in a chloroform solution was dried under nitrogen gas, and dried lipid film was dispersed in a 10 mM Tris-HCl buffer(pH 7.4) by vigorous shaking on a vortex mixer. Transition metal ion-dependent peroxidation was induced by the addition of 2 mM $FeSO_4$ and 2 mM ascorbic acid. Incubation was carried out for 2 hr in 37℃ water bath with constant shaking. Lipid peroxidation products were analyzed by the thiobarbituric method(24), their absorbance was measured at 532 nm.

Results and Discussion

Total phenolics and phenolic acid content

The contents of phenolics, as gallic acid equivalents, and phenolic acid contents in each of the methanol extract and five fractions of leaves and root is presented in Table 1. Total phenolics contents in the methanol extract of dandelion leaves were higher than that of dandelion root(9.4±3% and 7.9±0.4%, respectively). Phenolic substances have been shown to be responsible for the antioxidant activity of plant materials(25), and a good correlation between the concentrations of plant phenolics and the total antioxidant capacities has been reported(26). Hagymasi et al.(12) reported that polyphenol content of lyophilized extract(9.9%) of dandelion leaves were higher than the root extract(0.086%), and the flavonoid content was relatively low in both leaves and root. Among the fractions, ethyl acetate fractions of leaves and root showed the most effective antioxidant activity. Hexane fractions contained significantly less polyphenols than other fractions. Among the various solvents used for the extraction of total phenols, ether and ethyl acetate gave high total phenol content of the extracts.

Table 1. Total phenol contents of extract and fractions isolated from root and leaf of dandelion (*Taraxacum officinale*)

(%)

	Leaf	Root
MeOH ex.	9.4±0.3[1)c]	7.9±0.4[c2)]
Hexane fr.	2.6±0.1[d]	3.0±0.1[d]
Et_2O fr.	11.7±1.1[b]	22.1±0.6[a]
EtOAc fr.	21.1±0.3[a]	22.0±0.7[a]
n-BuOH fr.	9.0±0.4[c]	11.6±0.5[b]
H_2O fr.	9.1±0.2[c]	4.0±0.2[d]

[1)]All value are mean±SD(n=3).
[2)]Values within a column with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range test.

Six phenolic acids, catechol, gentisic acid, *p*-courmaric acid, caffeic acid, ferulic acid, and *m*-coumaric acid were identified from dandelion leaves, and catechol, vanillic acid, syringic acid, caffeic acid, ferulic acid, and *m*-coumaric acid were identified from root of dandelion by GC(Table 2). Total contents of phenolic acids of leaves and root were 158.6 mg% and 303.8 mg%, respectively. Caffeic acid(113.7 mg%) was a dominant phenolic acid in leaves and *m*-courmaric acid(152.6 mg%) was in root. The most abundant phenolic compounds in leaves and flowers are hydroxycinnamic acid derivatives, chicoric acid and monocaffeyltataric acid(27). In general, dandelion leaves have a higher polyphenolic compounds contents compared to root, in particular, cinnamic acid content showed more than 10 fold in leaves. Luteolin and its glycoside, chlorogenic acid and caffeic acid were identified and quantified from dandelion flower by HPLC(16). Some flavonoids, type of aglycone, luteolin, quercetin, and glycoside of luteolin, quercetin, isorahmnetin, combined with glucose, rutinose, also were isolated from dandelion tissues(17). Sesquiterpene lactone glucoside, combined with phenolic acid, a major component of root has been reported as a taraxacoside(18).

Table 2. Phenolic acids contents of dandelion leaf and root

(mg%)

	Catechol	Caffeic acid	Ferulic acid	m-coumaric acid	p-coumaric acid	Gentisic acid	Vanillic acid	Syringic acid
Leaf	15.44±3.3	113.7±12.4	7.5±2.1	15.5±3.2	3.9±0.6	2.6±0.4	N.D.[1)]	N.D.
Root	41.7±5.7	49.7±6.5	40.1±3.9	152.6±21.5	N.D.	N.D.	7.66±2.1	12.1±3.3

[1)]N.D.: Not detected.

ROS scavenging activity

ROS scavenging activities of dandelion were compared with those of commercial antioxidant trolox, α-tocopherol analogue, as described in Table 3. DPPH radical is a stable nitrogen-centered free radical, and the color of which changes from violet to yellow depends upon reduction degree by the process of hydrogen of electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavenger(28). DPPH scavenging activity in 80% methanol extracts of dandelion leaves and root were determined to show 44.6±2% and 32.7±0.6%, respectively. Methanol extract of leaves showed greater antioxidant activity than that of root. Kang et al.(29) also reported a similar antioxidant activity of dandelion leaves and roots extracted with water. Among the solvent fractions, ethyl acetate fraction of dandelion leaves and root showed the strongest radical scavenging activity with 70.2% and 67.1%, respectively. Hu et al.(30) reported that ethyl acetate fraction of dandelion flower had a greater free radical scavenging activity compared to the water fraction, but less activity than trolox. ABTS radical scavenging activity of 80% methanol extract of leaves was showed a higher activity than that of root. However, in the solvent fractions of root, diethyl ether, *n*-butanol fraction showed a higher ABTS scavenging activity than that of leaves, which exhibited marked inhibition around 76.0% and 93.9%, respectively.

Deoxyribose degradation occures in hydroxyl radicals generated by a fenton reaction. The antioxidant effect of several polyphenols that acts as inhibitors of hydroxy radical formation and lipid peroxidation has been correlated with iron chelating properties(31). Generally molecules that inhibit deoxyribose degradation are those that can chelate the iron ions and thereby prevent them from complex with the deoxyribose and render them inactive in a fenton reaction(32). Hydroxyl radical scavenging activities of methanol extract and fraction of dandelion leaves and root exhibited around 70-80% except 0.1 mg/mL *n*-hexane fraction . There was no significant difference in hydroxyl radical scavenging activity according to the solvent fractions between leaves and root.

Superoxide anion scavenging activity of the methanol extract and the fraction of dandelion leaves and root was measured in using the pyrogallol autoxidation system and the results were expressed as a inhibitory rate of the superoxide productivity. Superoxide anion radical, as the precursor of the more reactive oxygen species including hydroxyl and peroxynitrite radicals, is very harmful to the cellular components in biological system. SOD-like activities of methanol extract and fractions of dandelion leaves of were 28.8, 31.1, 26.6, 43.6, 19.7 and 20.1% for methanol extract, hexane fraction, ether fraction, ethyl acetate fraction, butanol fraction and water fraction, respectively. And antioxidant activity of methanol extract and fraction of dandelion root were 8.5, 10.7, 7.4, 23.5, 7.2 and 17.5% for methanol extract, hexane fraction, ether fraction, ethyl acetate fraction, butanol fraction and water fraction. Ethyl acetate fraction of dandelion leaves showed high scavenging ability of 43.6% on hydroxyl radicals at 20 mg/mL. However at 20 mg/ml, trolox scavenged hydroxyl radicals by 29.7%. Overall, ethyl acetate fraction of dandelion leaves showed better activity than other fraction. Trolox, however, showed less scavenging property on superoxide compared with other free radicals. A negative effect of lipophillic antioxidant such as α-tocopherol has been reported in a pyrogallol autooxidation system(33).

Table 3. Oxygen radical scavenging activities of dandelion leaf and root

(unit: inhibition, %)

		DPPH	ABTS	$\cdot O_2^-$	·OH
Leaf	MeOH ex.	44.6±2.8[1)c]	53.7±5.8[c2)]	28.8±8.9[bc]	80.9±1.5[ab]
	Hexane fr.	4.0±2.8[f]	2.4±1.6[f]	31.3±0.7[b]	39.2±5.2[c]
	Et_2O fr.	43.3±3.3[cd]	50.5±1.1[cd]	26.6±3.7[bc]	74.6±6.5[b]
	EtOAc fr.	70.2±1.8[b]	98.9±0.3[b]	43.6±0.5[a]	82.7±0.0[a]
	n-BuOH fr.	22.5±3.0[e]	33.3±0.5[e]	19.7±0.3[c]	78.0±0.9[ab]
	H_2O fr.	37.9±5.4[d]	49.1±2.1[d]	20.1±0.3[c]	80.5±0.3[ab]
Root	MeOH ex.	32.7±0.6[e]	33.8±2.7[c]	8.5±1.6[cd]	81.0±1.2[a]
	Hexane fr.	5.9±1.0[g]	3.2±0.3[e]	10.7±6.3[cd]	43.5±12.1[c]
	Et_2O fr.	57.6±0.4[c]	76.0±1.8[b]	7.4±2.1[cd]	80.2±0.2[a]
	EtOAc fr.	67.1±0.7[b]	92.6±3.9[a]	23.5±2.1[ab]	82.1±0.9[a]
	n-BuOH fr.	44.2±1.7[d]	92.6±3.9[a]	7.2±4.4[d]	82.1±0.9[a]
	H_2O fr.	11.6±2.8[f]	15.6±2.2[d]	17.5±6.5[bc]	71.0±2.6[b]
Trolox		97.4±1.0[a]	96.7±2.1[a]	29.7±1.4[b]	76.7±1.8[ab]

[1)]All value are mean±SD(n=3).
[2)]Values within a column with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range test.

Lipid peroxidation inhibitory activity

The antioxidant effects of the methanol extract and fractions of dandelion leaves and root on the peroxidation of phosphatidylcholine were investigated and the results are presented in Table 4. Lipid peroxidation leads to rapid development of rancid and stale flavors and it is considered as a primary mechanism of quality deterioration in lipid foods and oils(34). It not only causes a loss of food quality but is also strongly associated with carcinogenesis, mutagenesis, aging, and atherosclerosis(35). The phenolic compounds and other chemical components present in the extract may suppress lipid peroxidation through different chemical mechanism, including free radical quenching, electron transfer, radical addition or radical recombination. In this study, peroxyl radical generated through thermolysis of Fe^{3+} and ascorbic acid at 37°C, and efforts were made to evaluate the affinity of methanol extract and fractions of dandelion leaves and root to prevent peroxyl radical-induced damage in lipophilic model system. Ethyl acetate fraction of dandelion leaves exhibited antioxidant activity with 56.5% inhibition of phosphatidylcholin peroxidation at a 0.5 mg/mL concentration. Trolox showed 58.2% of inhibitory activity at a same concentration. The protective effect of ether and ethyl acetate fraction of dandelion leaves was more significant than other fractions at the same concentration. Among the fraction of dandelion root, ether, ethyl acetate, butanol, and water fraction were exhibited similar affinities to prevent peroxyl radical-induced phosphatidylcholin peroxidation at 0.5 mg/mL. Although, generation of peroxyl radicals precedes lipid oxidation, it is also common that carbon centered radical are produced when a hydrogen atom is abstracted by a hydroxyl radical. This result indicate that the ethyl acetate fraction of dandelion leaves exhibited effective chain-breaking antioxidant toward the free radical chain reaction, reported earlier for many polyphenolic flavonoids(36).

The ability of the extract to retard lipid oxidation is attributable to the ability of its phenolic constituents to quench ROS. There are, however, reports of phytophenolics exhibiting antioxidant/prooxidant activities, which depend on factors like metal reducing potential, chelating behavior, pH, solubility etc. and therefore further detailed studies on fraction of dandelion and its phenolic fraction with regard to antioxidant activity *in vivo* are needed.

Table 4. Lipid peroxidation inhibitory activity of dandelion leaf and root

(%)

	Leaf	Root
MeOH ex.	9.4±0.3[1)c]	7.9±0.4[c2)]
Hexane fr.	2.6±0.1[d]	3.0±0.1[d]
Et_2O fr.	11.7±1.1[b]	22.1±0.6[a]
EtOAc fr.	21.1±0.3[a]	22.0±0.7[a]
n-BuOH fr.	9.0±0.4[c]	11.6±0.5[b]
H_2O fr.	9.1±0.2[c]	4.0±0.2[d]
Trolox	58.2±5.7[a]	

[1)]All value are mean±SD(n=3).
[2)]Values within a column with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range test.

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