Antioxidant and Antibacterial Activities of *Rumex japonicus* Houtt. Aerial Parts

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We evaluated total phenolic content, antioxidant activity, reducing power and antibacterial activity of ethanol, hexane, chloroform, ethyl acetate and aqueous extracts of aerial parts of *Rumex japonicus* Houtt. The ethyl acetate extract had the highest amount of phenolic compounds. It also exhibited the highest reducing power and antioxidant activity when assayed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH), β-carotene bleaching and superoxide radical methods. The ethyl acetate extract possessed the strongest antibacterial activity against *Bacillus subtilis*, *B. cereus* and *E. coli*. GC-MS analysis indicated that ethyl acetate extract contained a variety of phenolic compounds. HPLC analysis showed that pyrogallol was the predominant phenolic compound in this extract. Thus, our study verified that the ethyl acetate extract has strong antioxidant and antibacterial activities which are correlated with its high level of phenolic compounds, particularly pyrogallol and pyrocatechin. This extract of *R. japonicus* aerial parts can be utilized as an effective and safe source of antioxidants.

Key words *Rumex japonicus*; antioxidant activity; antibacterial activity; phenolic compound

Recently, there has been considerable medical interest in the association of free radicals and other reactive oxygen species (ROS) to the pathogenesis of various diseases such as atherosclerosis, cancer, hypertension, ischemia-reperfusion, aging and age-related diseases.[1—4] Antioxidants are believed to play an important role in preventing or alleviating chronic diseases by reducing the oxidative damage to cellular components caused by (ROS).[5] Naturally occurring flavonoids, tannins, anthocyanins and other phenolic constituents are potential antioxidants.[6] However, synthetic antioxidants such as *tert*-butyl hydroxyanisole (BHA) and *tert*-butyl hydroxytoluene (BHT) are known to have toxic and carcinogenic effects on human health.[7—9] Therefore, substitution of synthetic antioxidants by natural ones and the screening of plant species for identifying new antioxidants have become critically important in recent years.[10]

Rumex japonicus Houtt. (Family Polygonaceae) is a perennial herb with erect stems 40—100 cm long. Its leaves are long-petioled 10—25 cm long and 4—10 cm wide. It grows in wet meadows along ditches in the low lands of Japan, Korea and China.[11] It has traditionally been used by the local people in Okinawa, Japan for treatment of acute and chronic cutaneous diseases[12] and by Chinese as an effective drug for the treatment of constipation, jaundice, uterine hemorrhage and hematemesis.[13] In this respect, major anthraquinones, emodin and chrysophanol, have been isolated and identified from the roots of *R. japonicus*,[14,15] while the flavonoid quercitrin has been isolated from its leaves.[16]

Since the biological activities of extracts of *R. japonicus* aerial parts have not previously been reported, the objectives of this study were to (1) measure the antioxidant activities of different extracts of *R. japonicus* aerial parts, (2) measure the antibacterial activities of these extracts; and (3) identify the biologically active compounds of these extracts by GC-MS and HPLC.

MATERIALS AND METHODS

Chemicals Standard phenolic compounds: pyrogallol, pyrocatechin, *p*-hydroxybenzoic acid, syringic acid, *p*-coumaric acid and ferulic acid were purchased from Nacalai Tesque, Japan, Sigma, Germany and Wako, Japan. Folin-Ciocalteu's reagent was purchased from Kyowa Riken Co., Japan. β-Carotene, linoleic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), α-tocopherol, *tert*-butyl hydroxytoluene (BHT), polyoxyethylene sorbitan monopalmitate (Tween-40), nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), nicotinamide adenine dinucleotide-reduced (NADH) and all solvents used were of analytical grade and purchased from Wako Pure Chemical Industries, Japan.

Plant Material and Preparation of the Extracts *R. japonicus* plants were collected from ditches near to cultivated areas at Okinawa, Japan in May, 2002. The fresh aerial parts (1 kg) were cut into small parts and placed in ethanol. The extracts were filtered and concentrated under vacuum at 40 °C until 59 g were obtained. The dried ethanol extract (20 g) was dissolved in water and extracted successively with hexane, chloroform and ethyl acetate. The obtained extracts, in addition to the aqueous solution remaining after extraction, were filtered and concentrated under reduced pressure to get 4.9, 0.8, 1.1 and 4.4 g of hexane, chloroform, ethyl acetate and aqueous extracts, respectively.

Amount of Total Phenolic Compounds The amounts of total phenolics in ethanol, hexane, chloroform, ethyl acetate extracts and aqueous solution of *R. japonicus* aerial parts were determined according to the Folin-Ciocalteu procedure.[17] Two hundred microliters of the ethanol solution of each extract (500 ppm) were introduced into test tubes to which 1.0 ml of Folin-Ciocalteu's reagent and 0.8 ml of sodium carbonate (7.5%) were added. The solutions were mixed and allowed to stand for 30 min. Absorption was measured at 765 nm using a Shimadzu UV-160A spectrometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram extract.

Antioxidant Assay Using the DPPH Radical Scavenging System The radical scavenging activity was evaluated as described previously.[18] Two milliliters of the ethanol solution of plant extracts and standard phenolic compounds were mixed with 1 ml of 0.5 mM DPPH ethanol solution and 2 ml of 0.1 M acetate buffer (pH 5.5). After shaking, the mixture

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was incubated at room temperature in the dark for 30 min, and then the absorbance was measured at 517 nm using a Shimadzu UV-160A spectrometer. α-Tocopherol and BHT were used as positive references while ethanol was used as negative one. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the formula of Son *et al.*[19]:

$$\% \text{ radical scavenging activity} = [(A_{control} - A_{test})/A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the control (DPPH solution without test sample) and A_{test} is the absorbance of the test sample (DPPH solution plus antioxidant). The EC_{50} value was determined as the concentration of each sample required to give 50% DPPH radical scavenging activity.[18]

Antioxidant Assay Using the β-Carotene Bleaching System Antioxidant activity was evaluated according to the β-carotene bleaching method.[20] β-Carotene (1.0 mg) was dissolved in 10 ml chloroform. One milliliter of the chloroform solution was mixed with 20 mg linoleic acid and 200 mg Tween-40. The chloroform was evaporated under vacuum at 45 °C, then 50 ml oxygenated water was added, and the mixture was vigorously shaken. The emulsion obtained was freshly prepared before each experiment. An aliquot (2 ml) of the β-carotene-linoleic acid emulsion was distributed in each of the 24-wells of the microtitre plates. Ethanolic solutions (80 μl) of the sample extracts, standard phenolic compounds and positive control standards (α-tocopherol and BHT) at 1000 ppm were added. An equal amount of ethanol was used for control. The microtitre plates were incubated at 50 °C, and the absorbance was measured using a model MTP-32 microplate reader (Corona Electric, Ibaraki, Japan) at 492 nm. Readings of all samples were performed immediately at zero time and every 15 min up to 180 min.

Antioxidant Assay Using Superoxide-Radical Scavenging Assay The superoxide scavenging activity was determined as described previously.[21] The reaction mixture containing equal volumes of test samples, 30 μM PMS, 338 μM NADH and 72 μM NBT in phosphate buffer (0.1 M, pH 7.4), was incubated at room temperature for 5 min and then the absorbance was read at 560 nm. The capability of scavenging superoxide radicals was calculated using the following formula:

$$\text{scavenging activity } \% = [1 - (A_{sample}/A_{control})] \times 100$$

Reducing Power Reducing power was determined as described previously.[22] One milliliter of each extract, standard phenolic compounds, α-tocopherol and BHT (25, 50, 100 and 250 ppm in ethanol) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [$K_3Fe(CN)_6$] (10 g/l), then the mixture was incubated at 50 °C for 30 min. Afterwards, 2.5 ml trichloroacetic acid (100 g/l) was added to the mixture, which was subsequently centrifuged at 4000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (1 g/l) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Antibacterial Activity Antibacterial activities of all extracts using ampicillin as positive control were tested against *Bacillus subtilis*, *Bacillus cereus* and *Escherichia coli*. Antibacterial activity was determined by using the disc diffusion method.[23] One hundred microliters of test organisms [10^6 colony forming units (CFU)/ml] grown in nutrient broth media for 24 h were spread over the surface of solid nutrient agar medium in 9 cm diameter Petri dishes. Filter paper discs (6 mm diameter) loaded with plant extracts or ampicillin were placed on the surface of the Petri dishes. Petri dishes were incubated at 37 °C for 24 h, and then the diameters of inhibition zones were measured in millimeters.

GC-MS Analysis An aliquot of 1 μl of acetone solution of the sample was injected splitless into the GC-MS (QP-2010, Shimadzu Co., Japan). The data were obtained on a DB-5MS column, 30 m length, 0.25 mm i.d. and 0.25 μm thickness (Agilent Technologies, J&W Scientific Products, U.S.A.). Carrier gas was helium. GC oven temperature started at 50 °C and holding for 6 min to 280 °C and holding for 5 min with program rate 5 °C/min. The injector and detector temperatures were set at 250 °C and 280 °C, respectively. The mass range was scanned from 20 to 900 amu. The control of the GC-MS system and the data peak processing were carried out by means of the Shimadzu's GC-MS solution Software, version 2.1.

Quantification by HPLC Phenolic compounds were measured at 280 nm using a Shimadzu HPLC (SCL-10 A vp, Shimadzu Co., Japan). Separation was achieved with a TSK gel column ODS-80Tm (4.6 mmϕ×15 mm). A gradient elution was performed with solvent A (water : acetic acid, 99 : 1, v/v) and B (methanol : acetonitrile : acetic acid, 95 : 4 : 1, v/v/v) as follows: 0—2 min, 5% B; 2—10 min, 5—25% B; 10—20 min, 25—40% B; 20—30 min, 40—50% B; 30—40 min, 50—100% B; 40—45 min, 100% B; 45—55 min, 100—5% B. The flow rate was 0.8 ml/min and the injection volume was 5 μl. Identification of the phenolic compounds was carried out by comparing their retention times to those of standards. Content of phenolic compounds was expressed in mg/g extract.

Statistical Analysis Data were analyzed using SAS version 6.12 (SAS Institute, 1997)[24] using ANOVA with the least significant difference (LSD) at the 0.05 probability level.

RESULTS AND DISCUSSION

Amount of Total Phenolic Compounds Phenolic compounds are considered to be the most important antioxidative plant components.[25] The amounts of total phenolic compounds in the ethanol, hexane, chloroform, ethyl acetate and aqueous extracts of *R. japonicus* aerial parts are shown in Fig. 1. Among all extracts, the significantly highest amount was found in ethyl acetate (200.4 mg GAE/g extract). The Folin-Ciocalteu procedure is non-specific because it detects all phenolics (phenolic acids, flavonoids and tannins),[26] so it does not give details of the quantity and quality of the phenolic constituents of the extracts. Nevertheless, this widely used method provides a rapid and useful overall evaluation of the phenolic content of extracts.[27] It has been reported that the antioxidant activity of plant materials was well correlated with the content of their phenolic compounds.[28—30]

Antioxidant Activity Measured by the DPPH Radical Scavenging System DPPH is a free radical stable at room temperature which produces a violet solution in ethanol.[31] It can be reduced in the presence of an antioxidant molecule,

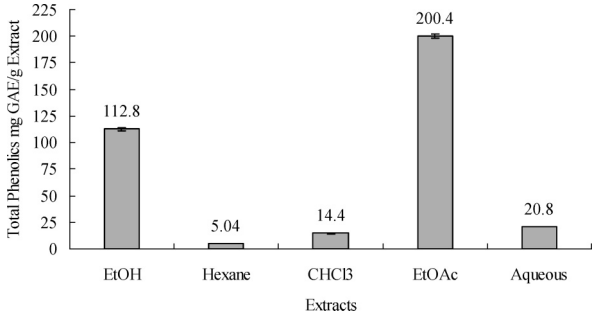

Fig. 1. Total Phenolic Compounds in Extracts of *R. japonicus* Aerial Parts

Each value represents the mean of 3 replicates±S.E.

Table 1. DPPH Radical Scavenging Activity and EC_{50} Values of (A) Extracts of *R. japonicus* Aerial Parts and (B) Standard Phenolic Compounds

(A)

Samples	Concentrations (ppm)		EC_{50} (mg/ml)[a)]
	50[a)]	100[a)]	
Ethanol	76.2±1.26[b]	86.1±0.20[a]	0.045±0.0008[c]
Hexane	5.5±0.40[d]	7.4±0.37[d]	>1
Chloroform	6.8±0.43[d]	11.6±0.11[c]	0.653±0.0043[a]
Ethyl acetate	86.0±0.20[a]	86.7±0.17[a]	0.041±0.0001[c]
Water	20.3±1.29[c]	28.1±0.86[b]	0.252±0.0043[b]
α-Tocopherol	78.0±0.88[b]	86.0±0.38[a]	0.044±0.0003[c]
BHT	85.3±0.08[a]	86.0±0.24[a]	0.042±0.0001[c]
LSD (0.05)	2.4	1.2	0.007

(B)

Samples	Concentrations (50 ppm)[a)]	EC_{50} (mg/ml)[a)]
Pyrogallol	91.3±0.115[a]	0.038±0.00005[b]
Pyrocatechin	89.9±0.088[b]	0.038±0.00003[b]
Ferulic	90.2±0.057[b]	0.038±0.00003[b]
Syringic	90.0±0.057[b]	0.038±0.00003[b]
p-Coumaric	30.1±0.519[c]	0.238±0.0069[a]
LSD (0.05)	0.769	0.009

a) Each value represents the mean of three replicates±S.E. Means with the same letter are not significantly different at $p=0.05$.

giving rise to uncolored ethanol solutions. The DPPH reagent evidently offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants.[32)] The percent DPPH radical scavenging activity of *R. japonicus* extracts, standard phenolic compounds, α-tocopherol and BHT is shown in Table 1. Similar to the total phenolics, ethyl acetate extract exhibited a very strong DPPH scavenging activity. Ethyl acetate extract showed a significantly similar DPPH activity to that of BHT and higher than that of α-tocopherol at 50 ppm. However, no significant difference was observed among ethyl acetate extract and commercial antioxidants (α-tocopherol and BHT) at 100 ppm. Furthermore, standard phenolic compounds showed very strong DPPH scavenging activity. These results suggest that the highest DPPH activity of ethyl acetate extract is strongly related to the high levels of phenolic compounds and due to scavenging of the radical by hydrogen donation.[33)] To compare the DPPH scavenging activity of *R. japonicus* extracts to the commercial antioxidants, Table 1 shows the concentrations needed to obtain 50% DPPH radical scavenging activity

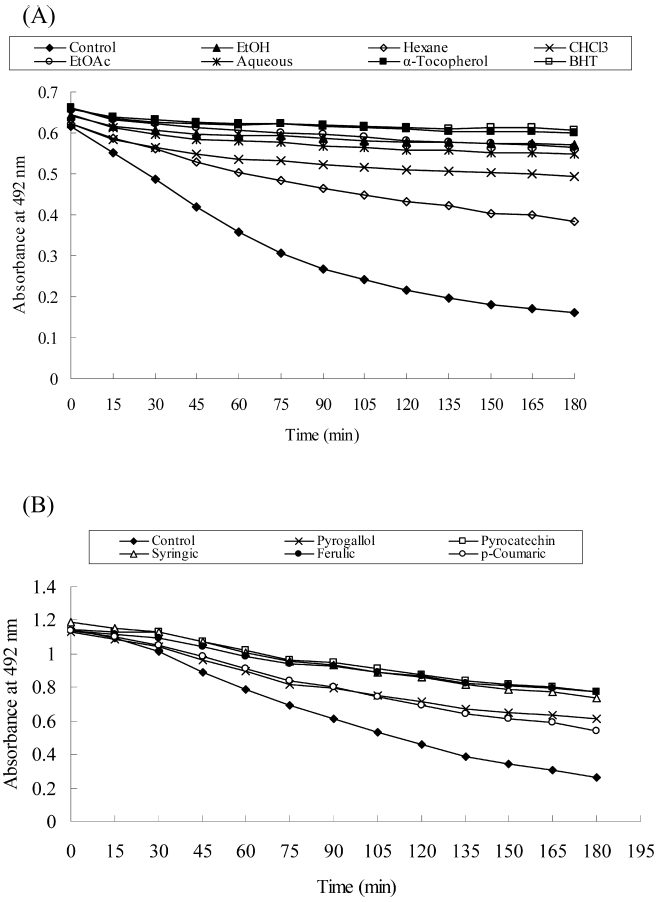

Fig. 2. Antioxidant Activity of (A) Extracts of *R. japonicus* Aerial Parts and (B) Standard Phenolic Compounds Measured by β-Carotene Bleaching Method

(EC_{50}) for the *R. japonicus* extracts as well as α-tocopherol and BHT. Lower EC_{50} value indicates greater antioxidant activity. Only 0.041 mg/ml of ethyl acetate extract was required to reduce the DPPH radicals by 50%. This was significantly similar to the concentration needed from commercial antioxidants (α-tocopherol and BHT) to give the same activity. By contrast, the EC_{50} of chloroform and aqueous extracts were 0.653 and 0.252 mg/ml, respectively. On the other hand, more than 1 mg/ml of hexane extract was necessary to achieve the same results.

Antioxidant Activity Assayed by the β-Carotene Bleaching Method The antioxidant assay using the discoloration of β-carotene is widely used to measure the antioxidant activity of plant extracts, because β-carotene is extremely susceptible to free-radical-mediated oxidation of linoleic acid.[34)] All the extracts of *R. japonicus* inhibited β-carotene oxidation (Fig. 2A). The polar extracts (ethanol, ethyl acetate and aqueous solution) exhibited a superior inhibition than non-polar extracts (hexane and chloroform). Standard phenolic compounds (Fig. 2B) also inhibited β-carotene oxidation suggesting that the antioxidant activity of polar extracts of *R. japonicus* could be related to their high levels of phenolic compounds. The linoleic acid free radical, formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups, attacks the highly unsaturated β-carotene molecules. As β-carotene molecules lose their double bonds by oxidation, the compound loses its orange color.

Table 2. Superoxide Radical Scavenging Activity of (A) Extracts of *R. japonicus* Aerial Parts and (B) Standard Phenolic Compounds

(A)

Samples	Concentrations (ppm)	
	50[a)]	500[a)]
Ethanol	4.4±0.11[b]	44.6±2.52[b]
Hexane	—	—
Chloroform	—	—
Ethyl acetate	16.4±1.44[a]	81.2±1.32[a]
Water	6.7±0.55[b]	40.3±1.97[b]
α-Tocopherol	—	—
BHT	—	—
LSD (0.05)	3.09	6.93

(B)

Samples	Concentrations (ppm)	
	10[a)]	100[a)]
Pyrogallol	21.3±1.47[a]	46.4±1.65[b]
Pyrocatechin	21.1±2.22[a]	63.2±1.46[a]
Ferulic	13.9±1.50[b]	39.9±0.43[c]
Syringic	11.3±1.87[b]	36.3±0.49[d]
p-Coumaric	14.7±1.53[b]	39.0±0.41[cd]
LSD (0.05)	5.50	3.30

a) Each value represents the mean of three replicates±S.E. —, no activity measured up to these concentrations. Means with the same letter are not significantly different at $p=0.05$.

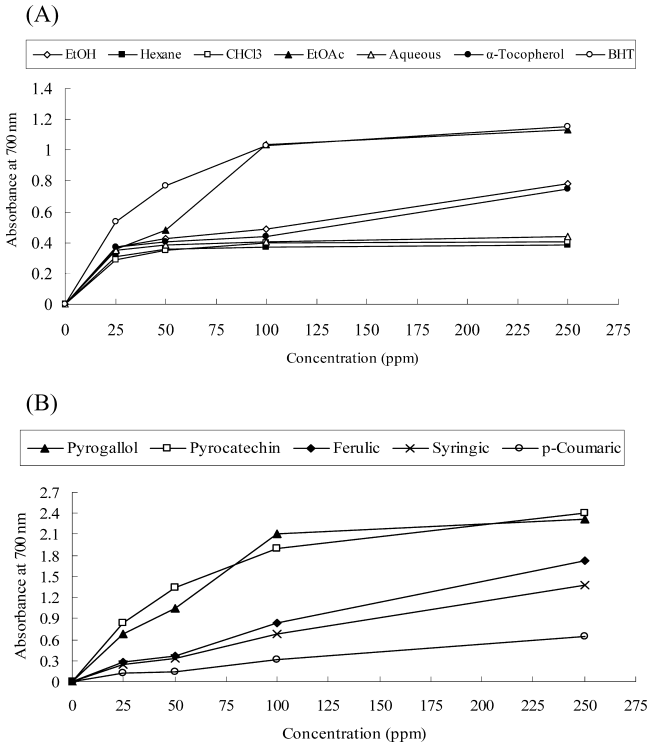

Fig. 3. Reducing Powers of (A) Extracts of *R. japonicus* Aerial Parts and (B) Standard Phenolic Compounds

The presence of different extracts that have antioxidant activity, can hinder the extent of β-carotene-bleaching by neutralizing the free radicals formed in the system.[35)]

Antioxidant Activity Assayed by the Superoxide Radicals Method The superoxide radicals ($O_2^{\cdot}$) are precursor of many other toxic ROS such as hydroxyl radical ($HO^{\cdot}$), hypochlorous acid (HOCl), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2).[36)] These radicals can easily initiate the peroxidation of membrane lipids leading to the accumulation of lipid peroxides and damaging a wide range of essential molecules.[21)] In the reaction mixture, superoxide radicals are generated from the reaction of PMS and NADH. These superoxide radicals, unless scavenged by the test samples, would readily reduce the electrophilic NBT to blue formazan that can be detected at 560 nm.[37)] The inhibitory effects of polar extracts of *R. japonicus* (ethanol, ethyl acetate and aqueous solution) on superoxide radicals were superior to non polar extracts (hexane and chloroform) and commercial antioxidants (α-tocopherol and BHT) which did not show any scavenging activity up to an inclusion level of 500 ppm (Table 2A). The results further indicated that ethyl acetate extract had the highest activity upon the elimination of superoxide radicals. Moreover, all standard phenolic compounds effectively scavenged superoxide radicals (Table 2B). It has been reported that phenolic compounds had superoxide radical scavenging activity.[36,38)] Thus, our results verified that the ethyl acetate extract effectively inhibited the generation of superoxide radicals and this activity could be related to its high level of phenolic compounds.

Reducing Power The reducing powers of *R. japonicus* extracts, standard phenolic compounds, α-tocopherol and BHT increased with the concentration of tested samples (Fig. 3). The reducing power of ethyl acetate extract was more pronounced than those of other extracts, and it was not only higher than that of α-tocopherol but also similar to that of BHT. The reducing power of a compound is related to its electron transfer ability and may, therefore, serve as an indicator of its potential antioxidant activity.[22)] Moreover, extracts with phenolic substance-mediated antioxidant activity were shown to be concomitant with the development of reducing power,[20)] thus, the ethyl acetate extract contains higher amounts of reducing compounds which are electron donors and can react with free radicals and convert them to more stable products and terminate radical chain reaction.[39)]

Table 3. Antibacterial Activity of Extracts of *R. japonicus* Aerial Parts Using Disc Diffusion Method

Extracts (800 μg/disc)	Zone of inhibition (mm)[a)]		
	Bacillus subtilis[b)]	*B. cereus*[b)]	*E. coli*[b)]
Ethanol	12±0.50[c]	13±1.73[c]	16±0.88[c]
Hexane	11±0.33[c]	—	13±0.33[d]
Chloroform	12±0.88[c]	11±0.33[c]	—
Ethyl acetate	15±0.33[b]	17±0.33[b]	20±0.88[b]
Water	8±0.88[d]	16±0.88[b]	18±1.15[bc]
Ampicillin (30 μg/disc)	35±0.50[a]	22±0.88[a]	33±1.45[a]
LSD (0.05)	1.8	2.7	2.7

a) —, inhibition zone was not >6 mm (diameter of disc). *b*) Each value represents the mean of three replicates±S.E. Means with the same letter are not significantly different at $p=0.05$.

Antibacterial Activity Higher plants represent a potential source of new anti-infective agents.[40)] *B. subtilis* and *B. cereus* which are Gram-positive bacteria were inhibited by all extracts except for hexane extract which was inactive against *B. cereus* (Table 3). Although chloroform extract had no ef-

fect on *E. coli* (a Gram-negative bacterium), other extracts exhibited strong antibacterial activity against this bacterial strain. The antibacterial activity of non-polar extracts (hexane and chloroform) can be attributed to the presence of several compounds including oleoresins and sterols.[41] Phenolic compounds such as pyrogallol were toxic to microorganisms as they inhibit the enzymes through reaction with sulfhydryl group.[42] Moreover, tea catechins have antibacterial activity against various pathogenic bacteria through damaging bacterial membranes of *Staphylococcus aureus* and *E. coli*.[43] Our study indicated that ethyl acetate extract was a strong antibacterial inhibitor and was significantly superior to other extracts. This activity may be due to the presence of phenolic compounds especially pyrogallol and pyrocatechin.

Analysis of Ethyl Acetate Extract by GC-MS and HPLC Since the ethyl acetate extract exhibited the strongest antioxidant and antimicrobial activity among all extracts, it was subjected to further analysis by GC-MS and HPLC. Ethyl acetate extract contained a variety of phenolic compounds (Fig. 4). By comparing the MS of these compounds with those of standards and MS library, ten phenolic compounds were identified (Table 4). Furthermore, the HPLC results (Fig. 5, Table 5) indicated that pyrogallol was the predominant compound in this extract, followed by pyrocatechin. Flavonoids, phenylpropanoids and phenolic acids are important contributing factors to the antioxidant activity of the human diet.[44] Gallic acid and pyrogallol have three hydrogen groups bonded to the aromatic ring in an *ortho* position in relation to each other and this model of substitution seems to be the most important factor associated with a strong H_2O_2 and DPPH scavenging activity of these phenolic compounds.[45] Furthermore, compounds with pyrogallol or catechol moieties were revealed as the most rapid superoxide scavengers.[36] Similarly, the antibacterial activity of tea catechins was related to the gallic acid moiety and the hydroxyl group.[45] Based on the results for the phenolic composition of ethyl acetate extract of *R. japonicus* aerial parts, we can concluded that these compounds (particularly pyrogallol and

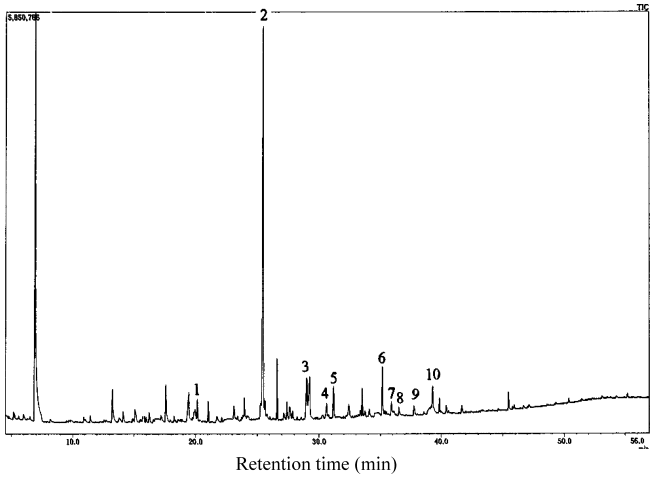

Fig. 4. GC-MS Chromatogram of Ethyl Acetate Extract of *R. japonicus* Aerial Parts

Peaks 1: pyrocatechin; 2: pyrogallol; 3: *p*-hydroxybenzoic acid; 4: vanillic acid; 5: 3,4-dihydroxybenzaldehyde; 6: ethyl 3,4-dihydroxybenzoate; 7: *p*-coumaric acid; 8: syringic acid; 9: ferulic acid; 10: ethyl gallate.

Table 4. Retention Time, Molecular Weight and Major Peaks of Phenolic Compounds Detected by GC-MS in Ethyl Acetate Extract of *R. japonicus* Aerial Parts

Compound	Retention time (min)	Molecular weight	Major peaks
Pyrocatechin	20.150	110.10	110, 64, 81
Pyrogallol	25.467	126.10	126, 52, 80
p-Hydroxybenzoic acid	29.267	138.10	121, 138, 93
Vanillic acid	30.625	168.10	168, 153, 97
3,4-Dihydroxybenzaldehyde	31.183	138.10	137, 138, 109
Ethyl 3,4-dihydroxybenzoate	35.167	182.20	137, 182, 154
p-Coumaric acid	35.908	164.20	164, 147, 163
Syringic acid	36.508	198.20	198, 183, 127
Ferulic acid	37.750	194.19	194, 179, 133
Ethyl gallate	39.292	198.20	153, 198, 170

Table 5. Phenolic Compounds Content (mg/g Extract) of Ethyl Acetate Extract of *R. japonicus* Aerial Parts Measured by HPLC

Compound	Content mg/g extract[a)]
Pyrogallol	291.40±5.66[a]
Pyrocatechin	43.20±0.23[b]
p-Hydroxybenzoic acid	4.04±0.04[c]
Syringic acid	1.80±0.08[c]
p-Coumaric acid	7.50±0.17[c]
Ferulic acid	8.70±0.34[c]
LSD (0.05)	7.1

a) Each value represents the mean of three replicates±S.E. Means with the same letter are not significantly different at $p=0.05$.

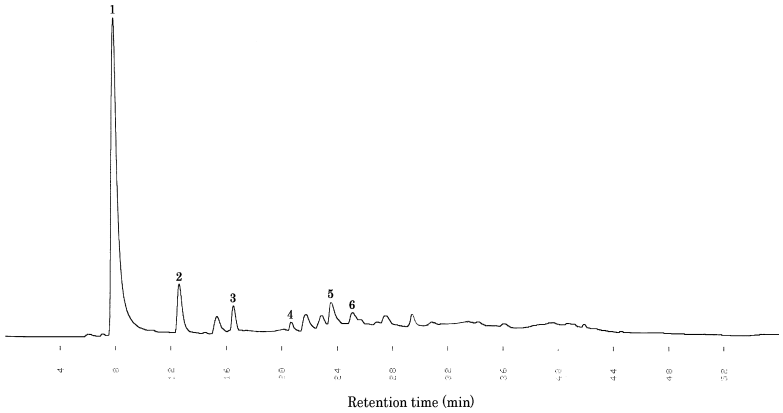

Fig. 5. HPLC Chromatogram of Ethyl Acetate Extract of *R. japonicus* Aerial Parts

Peaks 1: pyrogallol; 2: pyrocatechin; 3: *p*-hydroxybenzoic acid; 4: syringic acid; 5: *p*-coumaric acid; 6: ferulic acid.

pyrocatechin) contribute to the antioxidant and antimicrobial activities of this extract.

CONCLUSION

Free radicals are well known to cause chronic diseases such as heart disease and rheumatism.[46] Since synthetic antioxidants such as BHA and BHT have some toxicity,[47] interest in the search for new natural antioxidants has grown dramatically over the past years.[48] The results obtained in this work are noteworthy, not only with respect to the antioxidant and antibacterial activity of ethyl acetate extract of *R. japonicus* aerial parts, but also with respect to its content of a variety of phenolic compounds. The activity of this extract is attributed to these phenolic compounds and in particular to pyrogallol and pyrocatechin. Safety is an essential consideration for antioxidants as they may be utilized in the manufacture of foods and pharmaceuticals. Since pyrogallol is the predominant phenolic compound detected in ethyl acetate extract, the safety of pyrogallol for using as antioxidant agent is very important. Pyrogallol did not promote carcinogenic potential in skin tumors,[49] bladder cancer[50] and mouse skin cancer.[51] Furthermore, the US Food and Drug Administration (FDA) has reported the safe use of pyrogallol as a color additive in hair dyes. Pyrogallol is an antioxidant which exerts its protective effect against cancer by inhibiting the formation of carcinogenic metabolites.[52]

Finally, our results verified that ethyl acetate extract of *Rumex japonicus* aerial parts has a very strong antioxidant activity and can be utilized as an effective and safe antioxidant source.

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