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Antioxidant Activity of Medicinal Plant Extracts Used as Folk Remedies by Diabetic Patients

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Abstract

The aim of this study was to investigate the antioxidant effect of medicinal plants used by diabetic patients. Fourteen medicinal plants were selected and antioxidant activity such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, inhibition effect of linoleic acid autoxidation and low density lipoprotein (LDL) oxidation, thiobarbituric acid (TBA) value were measured. The *Cornus officinalis* had DPPH radical scavenging activity of 84.79%, which was higher than the 78.79% for α-tocopherol. *Rosa rugosa* Thunberg, *Pueraria thundergiana* Bentham, *Artemisia princeps var. orientalis* and *Sasamorpha purpurascens* Nakai also had high values. Extracts with higher DPPH radical scavenging activities had higher total phenol concentrations, and positive correlations between these parameters were found. Inhibitory activities of linoleic acid autoxidation, LDL oxidation and TBA value, used as indices of oxidative stress, were observed in most of the selected medicinal plants. The highest inhibitory activity for TBA value was observed in the extract of *Pueraria thundergiana* Bentham. *Rosa rugosa* Thunberg (75.50%), *Sasamorpha purpurascens* Nakai (74.00%), and *Cornus officinalis* (73.00%) all had high antioxidant activity against linoleic acid autoxidation, similar to that of α-tocopherol. The same 3 plants also had high in inhibitory activity against LDL oxidation. Therefore, we demonstrated that medicinal plants used as folk remedy by diabetic patients had antioxidant activity. Especially, *Rosa rugosa* Thunberg, *Cornus officinalis*, *Pueraria thundergiana* Bentham, *Artemisia princeps var. orientalis*, and *Sasamorpha purpurascens* Nakai had high phenol concentrations which resulted in high values for DPPH radical scavenging. These same plants exhibited high values for inhibitory activities for TBA value, linoleic acid autoxidation and LDL oxidation, indices associated with lipid peroxidation.

Key words: medicinal plants, antioxidant activity

INTRODUCTION

Antioxidants are important in diabetes, with low levels of plasma antioxidants implicated as a risk factor for the development of the disease (1-3) and circulating levels of radical scavengers impaired throughout the progression of diabetes (4,5). Therefore, antioxidants are important in diabetes to ameliorate diabetic oxidative stress (1,6,7). Many of the complications of diabetes, including microvascular complications (retinopathy, nephropathy and neuropathy) and macrovascular complications (arteriosclerotic vascular disorder and cardiac disorder), the leading causes of mortality in diabetics, have been linked to oxidative stress (8) and antioxidants have been considered as treatments of diabetes (6,9-13).

Oxidative stress has been mainly responsible for diabetes associated pathological conditions. Several lines of evidence have demonstrated that the diabetic oxidative stress, resulting from the generation of free radicals, is involved in the hyperglycemia and its related increased protein glycosylation (14). Plants often contain substantial amounts of antioxidants, including α-tocopherols, carotenoids, ascorbic acid, flavonoids and tannins (15, 16). We hypothesize that antioxidant action may be an important property of plant medicines used for the treatment and prevention of diabetes.

Diabetes can not be completely cured, and for that reason, many patients use plants as folk remedies to treat diabetes in Korea. Several medicinal plants have been used for the treatment of diabetic pathological conditions without any scientific confirmation. Cho and Choue (17) noted that 53.4% diabetic patients out of a total of 73 patients answered that they used folk remedies. There were 54 kinds of folk remedies for diabetes, most of which were plants. Many of these plants traditionally used for diabetes-related conditions had the ability to lower blood glucose and were screened for control of glucose metabolism, but there were few studies evaluated antioxidant activity. Nevertheless, antioxidants offer a promising avenue for preventing and treating diabetes

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and its complications since oxidative stress is a major factor in the etiology of diabetes.

Therefore, we studied the antioxidant activity of medicinal plant extracts used as folk remedy by diabetic patients without any scientific confirmation. In order to contribute further to the knowledge of the medicinal plants, we selected 14 medicinal plants and investigated antioxidant activity of their extracts on radical scavenging and their ability to inhibit lipid peroxidation. The results of this study can confirm the antioxidant capacity of these medicinal plants and assist diabetic patients in choosing traditional treatments with scientifically confirmed efficacy.

MATERIALS AND METHODS

Plants material

Samples of medicinal plants were selected by reviewing scientific literature and Oriental medicine references (18-20). Medicinal plants selected for study were purchased at a local pharmacy in Busan, Korea (Table 1). Each dried plant was extracted in 100°C in distilled water for 4 hours. The extract was filtered and the filtrate was concentrated with a vacuum rotary evaporator under low pressure. The residue was freeze-dried in a freezing-dryer and diluted with distilled water to give a final extract concentration of 100 μg/mL (21).

Total phenol contents

The total phenol content was determined according to Folin-Denis method (22), using chlorogenic acid as the standard. A 0.3 mL sample of the extract and 2 mL of Na_2CO_3 were mixed, and after 2 mim 0.1 mL of 50% Folin-Ciocalteau reagent was added. Absorbance at 750 nm was measured after incubation for 30 min at room temperature.

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

A test sample solution (dissolved in ethanol, final extract concentration 100 μg/mL) 30 μL was added to 1.5×10^{-4} M DPPH ethanolic solution (2.97 mL). After vortexing, the mixture was incubated for 10 min at room temperature, and then the absorbance at 517 nm was measured (23).

Thiobarbituric acid (TBA) value

Sample extract (120 μL) was added to 9 mL of 0.04 M phosphate buffer and 2.88 mL of 2.51% linoleic acid and then incubated at 40°C. This solution (2 mL) was added to 0.75% TBA (2 mL) and 35% trichloroacetic acid (TCA, 1 mL) which was then added to the reaction solution (2 mL), heated for 15 min at 95~100°C in a water bath, and cooled immediately. The absorbance at 517 nm was measured (24).

Inhibitory activity of linoleic acid autoxidation

A linoleic acid autoxidation was estimated using the ferrithiocyanate method (25). A 120 μL aliquot of the sample solution and 0.04 M-phosphate buffer (9 mL) were mixed with 2.88 mL of 2.51% linoleic acid in ethanol and incubated at 40°C. This solution (0.1 mL), 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate were mixed with 0.02 M ferrous chloride in 3.5% HCl, and absorbance was measured at 500 nm after 3 min.

Inhibitory activity of LDL oxidation

Antioxidant protection of low density lipoprotein (LDL) was estimated using the method of Buege and Aust (26). LDL was purchased from Sigma (USA). 100 μL of the sample solution was mixed with the LDL solution in an isotonic salt solution, 7.5 μL of ethanol, 5.5 μL of Cu^{2+} solution, and fixed with phosphate-buffered saline (PBS) into 1,000 μL of final volume. 1 mL of this solution, 2 mL of TBARS solution in 0.4% TBA, 15% TCA and 2.5% HCl were mixed and heated for 20 min at 95~100°C in a water bath, and cooled immediately. The absorbance at 532 nm was measured. The antioxidant activity derived from a standard curve using

Table 1. Botanical species

General name	Scientific name	Organ
Seddugi	*Equisetum arvense* L. (EAL)	Whole
Hwasalnamoo	*Eunymus alatuas* Siebold (EAS)	Trunk
Garenamoo	*Juglans mandshurica* (JM)	Trunk
Chilg	*Pueraria thundergiana* Bentham (PTB)	Root
Hedangwa	*Rosa rugosa* Thunberg (RRT)	Root
Ogalpi	*Acanthopanax sessiliflorus* Seem (ASS)	Trunk
Hanultary	*Trichosanthes kirilowii* Max. (TKM)	Root
Igmocho	*Leonurus sibiricus* L. (LSL)	Leaf
Ousungcho	*Houttyunia cordata* (HC)	Leaf
Ssuk	*Artemisia princeps var. orientalis* (APO)	Leaf
Sansou	*Cornus officinalis* (CO)	Fruit
Dalgaebi	*Monochoria vaginalis var. plantaginea* (MVP)	Whole
Yunggi	*Ganoderma lucidum* (GL)	Whole
Choridea	*Sasamorpha purpurascens* Nakai (SPN)	Whole

malondialdehyde (MDA, 0~10 nmol/250 μL PBS).

Statistics

All measurements were replicated three times for each sample. Their means and standard deviations were reported, unless otherwise specified. A linear regression between the content of total phenolics and DPPH radical scavenging effect was assessed. The data were analyzed for statistical significance using Duncan's multiple range test (27).

RESULTS AND DISCUSSION

Total phenol contents

Total phenol concentrations of 14 medicinal plants are shown in Table 2. Phenolics are secondary compounds widely distributed in the plant kingdom. As the intake of vegetables and fruits that contain phenolic compounds increases, the risk of mortality from coronary heart disease decreases (28-30). The phenolic content is believed to be an important functional attribute of plants, contributing to the antioxidative and other beneficial effects, therefore we investigated the concentrations of phenolic compounds in 14 medicinal plants. The extract of *Rosa rugosa* Thunberg contained 6.91 g per 100 g total phenolics, one of the highest concentrations. Generally, green tea leaves have high concentrations of polyphenolic compounds, and the content of total phenol in green tea extract was recently determined to be 6.88 g per 100 g (31). The total phenol content of *Rosa rugosa* Thunberg, therefore, was higher than that of green tea. Phenolics in *Cornus officinalis*, *Artemisia princeps var. orientalis* and *Pueraria thundergiana* Bentham were 4.29 g, 4.26 g and 3.82 g, respectively. The *Sasamorpha purpurascens* Nakai and *Juglans mandshurica* also had high values, at 2.54 g and 1.96 g, respectively. In general, phenolic compounds were considered as antioxidants (32), and the phenol contents of these plant extracts are expected to contribute to antioxidant activity.

DPPH radical scavenging activity

The DPPH radical scavenging activities of the medicinal plant extracts (the final concentration of 100 μg/mL) are also shown in Fig. 1. A human body has several mechanisms of defense against free radical and other reactive oxygen species. The radical scavenging activity substantially contributes to the mechanisms of defense in the human body against free radicals. The *Cornus officinalis* had a DPPH radical scavenging activity of 84.79%. The scavenging activities of *Sasamorpha purpurascens* Nakai, *Rosa rugosa* Thunberg, *Artemisia princeps var. orientalis* and *Pueraria thundergiana* Bentham were also high. The radical scavenging activities of several samples such as *Trichosanthes kirilowii* Max., *Leonurus sibiricus* L., *Equisetum arvense* L., *Eunymus alatuas* Siebold, *Monochoria vaginalis var. plantaginea*, and *Ganoderma lucidum* were lower compared to the other samples. But many diabetic patients have used these as antidiabetic medicine, so that we could presume that other mechanisms or antidiabetic activity were involved in the improvement of diabetic conditions by mechanisms unrelated to their DPPH radical scavenging effects (20,33-36). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They may also have a metal chelating capacity (37). From these results, we could estimate antioxidant activity by phenolic compounds in *Cornus officinalis*, *Sasamorpha purpurascens* Nakai, *Rosa rugosa* Thunberg, *Artemisia princeps var. orientalis* and *Pueraria thundergiana* Bentham.

The regression analysis of radical scavenging effects

Table 2. Total phenol contents of medicinal plants (per 100 g)

General name	Scientific name	Total phenol (g)
Seddugi	*Equisetum arvense* L. (EAL)	1.21±0.06[1)]
Hwasalnamoo	*Eunymus alatuas* Siebold (EAS)	1.96±0.04
Garenamoo	*Juglans mandshurica* (JM)	2.26±0.13
Chilg	*Pueraria thundergiana* Bentham (PTB)	3.82±0.25
Hedangwa	*Rosa rugosa* Thunberg (RRT)	6.91±0.16
Ogalpi	*Acanthopanax sessiliflorus* Seem (ASS)	0.43±0.08
Hanultary	*Trichosanthes kirilowii* Max. (TKM)	0.15±0.04
Igmocho	*Leonurus sibiricus* L. (LSL)	0.59±0.02
Ousungcho	*Houttyunia cordata* (HC)	1.51±0.03
Ssuk	*Artemisia princeps var. orientalis* (APO)	4.26±0.04
Sansou	*Cornus officinalis* (CO)	4.29±0.10
Dalgaebi	*Monochoria vaginalis var. plantaginea* (MVP)	0.79±0.15
Yunggi	*Ganoderma lucidum* (GL)	0.65±0.03
Choridea	*Sasamorpha purpurascens* Nakai (SPN)	2.54±0.17

[1)]All determinations were done in triplicates.
[1)]Mean±SD.

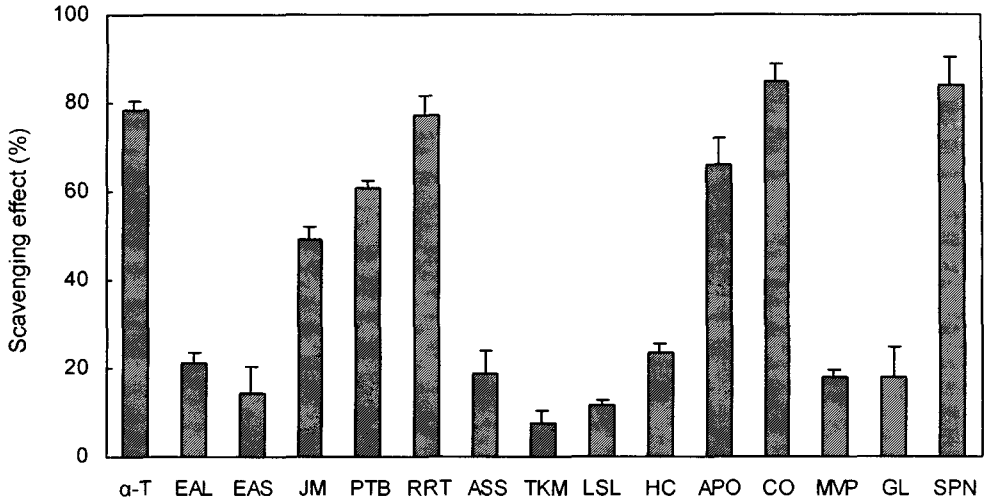

Fig. 1. Scavenging effect of water extracts prepared from medicinal plants on DPPH. Concentrations of α-tocopherol each sample in reaction mixture were 100 μg/mL. α-T : α-Tocopherol, EAL: *Equisetum arvense* L., EAS: *Eunymus alatuas* Siebold, JM: *Juglans mandshurica*, PTB: *Pueraria thundergiana* Bentham, RRT: *Rosa rugosa* Thunberg, ASS: *Acanthopanax sessiliflorus* Seem, TKM: *Trichosanthes kirilowii* Max., LSL: *Leonurus sibiricus* L., HC: *Houttyunia cordata*, APO: *Artemisia princeps var. orientalis*, CO: *Cornus officinalis*, MVP: *Monochoria vaginalis var. plantaginea*, GL: *Ganoderma lucidum*, SPN: *Sasamorpha purpurascens* Nakai.

on DPPH and total phenols is shown in Fig. 2. In general, the extracts with higher DPPH radical scavenging activities showed a higher total phenol contents, and positive correlations between these parameters were found (38).

TBA value

TBA values of water extracts of 14 medicinal plants are shown in Fig. 3. Most of the 14 selected medicinal plants were observed to be effective inhibitors of TBA value. Especially, the highest activity was observed in the extract of *Pueraria thundergiana* Bentham, which is of interest considering that it has been used to prevent diabetes, hypertension, coronary arteriosclerosis, and stenocardia (39,40). Inhibitory activities on TBA values of

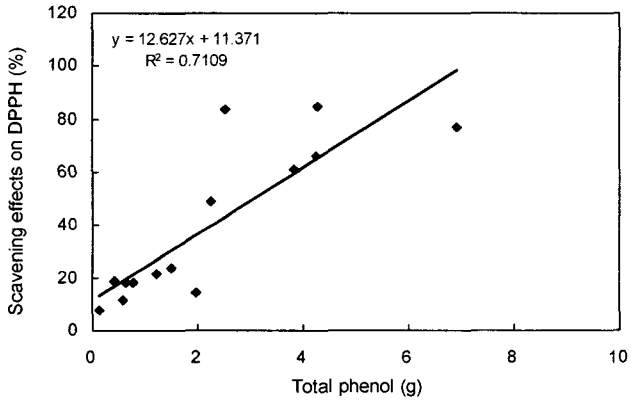

Fig. 2. Correlation between total phenol content and scavenging effect on DPPH.

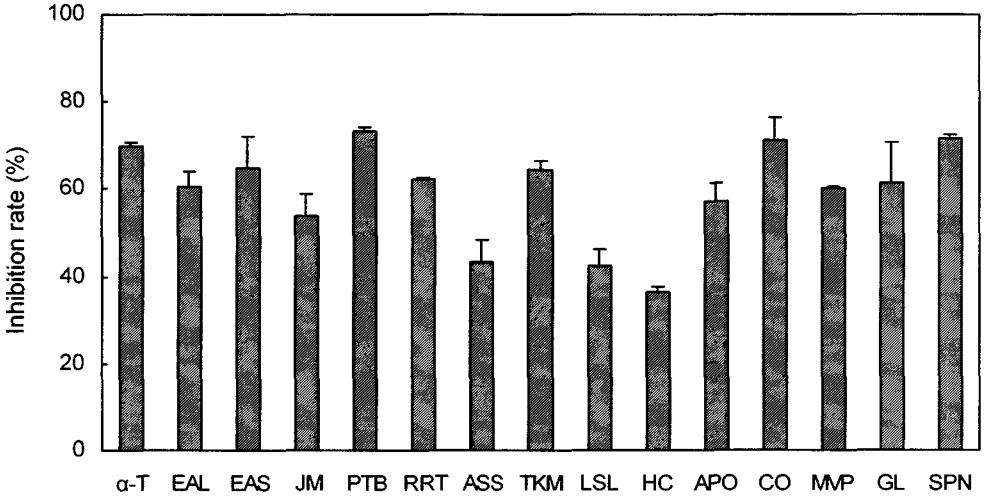

Fig. 3. Inhibitory activity of water extracts prepared from medicinal plants on TBA value.
See the abbreviations in Fig. 1. Concentrations of α-tocopherol each sample reaction mixture were 100 μg/mL.

the extracts of *Trichosanthes kirilowii* Max., *Leonurus sibiricus* L., *Equisetum arvense* L., *Eunymus alatuas* Siebold, *Monochoria vaginalis var. plantaginea*, and *Ganoderma lucidum* were also high, even though these had low levels of radical scavenging effects on DPPH. The DPPH assay does not discount possible reactions with free radical intermediates and the production of oxidative chain reaction. Therefore, the inhibitory activity on TBA values of these plants seemed to be associated with lipid peroxidation and hydrogen peroxide production (41,42). The amelioration of oxidative stress through radical scavenging effects and the inhibition of lipid peroxidation might be responsible for their efficacy for treatment or prevention of several disorders.

Inhibitory activity of linoleic acid autoxidation

Fig. 4 shows the inhibitory activity of water extracts prepared from medicinal plants on linoleic acid autoxidation. *Rosa rugosa* Thunberg (75.50%), *Sasamorpha purpurascens* Nakai (74.00%), *Cornus officinalis* (73.00%) had as high an antioxidant activity for protecting linoleic acid against autoxidation as α-tocopherol. Most of the selected medicinal plants were effective also. The extracts of *Trichosanthes kirilowii* Max., *Leonurus sibiricus* L., *Equisetum arvense* L., *Eunymus alatuas* Siebold, *Monochoria vaginalis var. plantaginea*, and *Ganoderma lucidum* were also effective inhibitors of linoleic acid autoxidation, like TBA values. Therefore, these plants had antioxidant activity against lipid peroxidation, therefore we can infer that these plants effectively protect against oxidative stress.

Inhibitory activity against LDL oxidation

Recently, it has been clearly shown that the oxidation

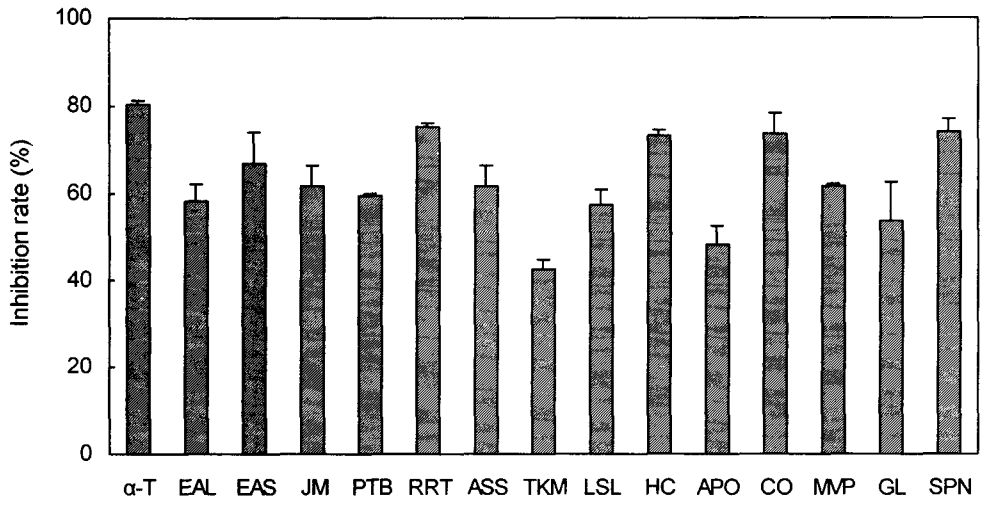

Fig. 4. Inhibitory activity of water extracts prepared from medicinal plants on linoleic acid autoxidation.
See the abbreviations in Fig. 1. Concentrations of α-tocopherol and each sample in reaction mixture were 100 μg/mL.

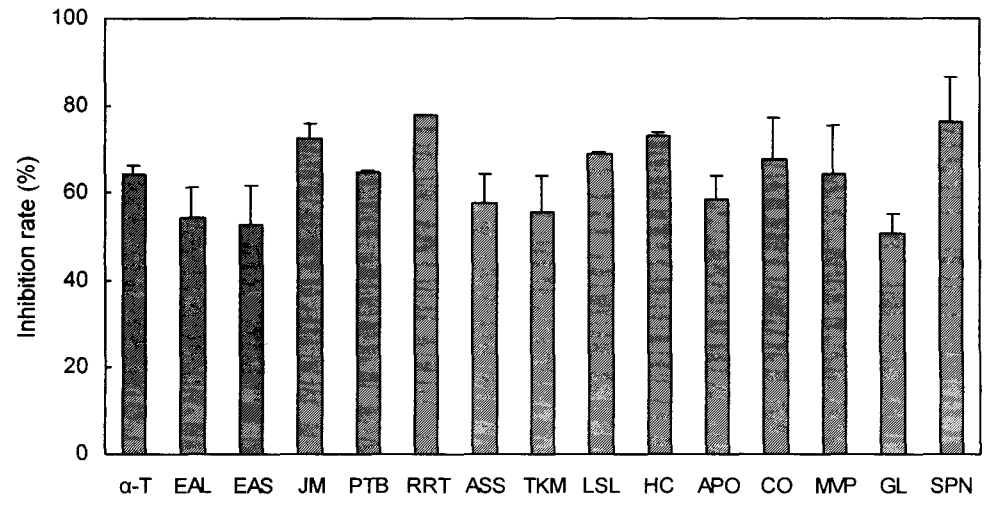

Fig. 5. Inhibitory activity of water extracts prepared from medicinal plants on LDL oxidation.
See the abbreviations in Fig. 1. Concentrations of α-tocopherol and each sample in reaction mixture were 100 μg/mL.

of LDL plays a primary role in the initiation of arteriosclerosis, so that LDL oxidation has important attribute of antioxidant activity. The antioxidant activity on LDL oxidation of the medicinal plant extracts are shown in Fig. 5. The inhibitory activity of LDL oxidation by the extracts of *Rosa rugosa* Thunberg, *Sasamorpha purpurascens* Nakai, *Houttyunia cordata*, *Juglans mandshurica*, *Cornus officinalis* and *Leonurus sibiricus* L. were high, and higher than that of α-tocopherol. Increased oxidative stress in diabetes mellitus results in increased lipid peroxidtion. Oxidized LDL and other lipid peroxidation products have been implicated in the complications of diabetes (43). This suggests that these plants may prevent complications from oxidation of LDL.

Therefore, most of the 14 selected medicinal plants had antioxidant activity. Especially, *Rosa rugosa* Thunberg, *Cornus officinalis*, *Pueraria thundergiana* Bentham, *Artemisia princeps var. orientalis*, and *Sasamorpha purpurascens* Nakai had high concentrations of phenolic compounds and were effective DPPH radical scavengers, and were positively correlated. The extracts of *Trichosanthes kirilowii* Max., *Leonurus sibiricus* L., *Equisetum arvense* L., *Eunymus alatuas* Siebold, *Monochoria vaginalis var. plantaginea*, and *Ganoderma lucidum* were observed to have high inhibitory activity on TBA value, linoleic acid autoxidation and LDL oxidation, all of which are associated with lipid peroxidation, even though they had low level of radical scavenging effect on DPPH. Therefore, we suggest that these medicinal plants have efficacy for the amelioration of oxidative stress in diabetic patients.

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