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Original Article

Antioxidant Activity of Some Mongolian Plants

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Abstract

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Antioxidant agents reactive oxygen species can be used for several cosmetic and medical applications. The goal of our study was to evaluate the antioxidant activity of 69 plant samples of 68 species belonging to 55 genera and 25 families collected from Mongolia in August 2011. The antioxidant capacity of a methanolic extract of plants was evaluated by analyzing the scavenging capacities of free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and compared with the commercial standard, butylated hydraxyanisole (BHA). We compared our experimental data with the BHA and divided in 4 groups by the antioxidant activity of samples. There were 12 samples with very strong antioxidant activity (IC_{50} were < 4.4 µg/ml), 39 samples with strong antioxidant activity (IC_{50} were 4.4≤25.99 µg/ml), 10 samples with moderate antioxidant activity (IC_{50} were 26≤50.99 µg/ml), and 8 samples with weak antioxidant activity (IC_{50} were ≥ 51 µg/ml). All extracts of plant samples showed concentration dependent DPPH free radical scavenging activity indicating the presence of potent natural antioxidant compounds.

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Introduction

Types of reactive oxygen species (ROS) include the hydroxyl radical, hydrogen peroxide, the superoxide anion radical, nitric oxide radical, singlet oxygen, hypochlorite radical, and various lipid peroxides. These can react with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules (Simon *et al*., 2000). Oxidative stress can be due to several environmental factors, such as exposure to pollutants, alcohol, medications, infections, poor diet, toxins, radiation etc. Oxidative damage to DNA, proteins and other macromolecules may lead to a wide range of human diseases, most notably heart disease and cancer.

Everyday our bodies produce free radicals as a product of our natural processes. These free radicals are capable of attacking the healthy cells of the body. Cell damage caused by free radicals appears to be a major contributor to aging and diseases, like cancer, heart disease, decline in brain function, decline in immune system etc. (http://www.oxidativestressresource.org/). Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases (http://www.news-medical.net/health/What-are-Antioxidants.aspx).

Apart from diet, the body also has several antioxidant mechanisms that can protect itself from ROS mediated damage. The antioxidant enzymes – glutathione peroxidase, catalase, and superoxide dismutase (SOD) are such enzymes. They require micronutrient cofactors, such as selenium, iron, copper, zinc, and manganese for their activity. It has been suggested that an inadequate dietary

intake of these trace minerals may also lead to low antioxidant activity (Buyukokuroglu *et al*., 2001; Shahidi & Wanasundara, 1992; http://www.news-medical.net/health/What-are-Antioxidants.aspx).

Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are found in many foods, such as fruit and vegetables and are also synthesised in the body. Vitamin C, vitamin E, and beta carotene are among the most commonly studied dietary antioxidants. In addition to these uses of natural antioxidants in medicine, these compounds have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline (http://en.wikipedia.org/wiki/Antioxidant).

As antioxidants have been reported to prevent oxidative damage caused by free radicals, they can interfere with the oxidation process by reacting with free radicals, chelating, and catalytic metals and also by acting as oxygen scavengers.

During the many years, the medicinal plants have been investigated in the recent scientific food. Recently there has been an upsurge of interest in the therapeutic potentials of plants, as antioxidants in reducing free radical induced tissue injury. The medicinal application of specific plants for long periods in traditional medicines, suggests the presence of biologically active substances in plant species (Crista *et al*., 2008; Vinay *et al*., 2010).

Although several synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are commercially available, but are quite unsafe and their toxicity is a problem of concern. But natural antioxidants, especially phenolics and flavonoids from tea, wine, fruits, vegetables and spices are already exploited commercially either as antioxidant additives or as nutritional supplements. Also many other plant species have been investigated in the search for novel antioxidants (Koleva *et al*., 2002; Mantle *et al*., 2000; Oke & Hamburger, 2002; Bhattarai *et al*., 2008), but generally there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive.

Therefore, in our present study, considerable attention has been directed towards the identification of antioxidant activity of some selected medicinal plants using DPPH free radical scavenging assay. The samples of our study belong to plant families, which are locally known as medicinal plants, but the selected Mongolian species are, in general, scarcely investigated and only few studies exist about their efficacy.

Materials and Methods

Study area

For our investigations, we collected 68 plant species belonging to 55 genera 26 families, which are frequently used in the traditional therapy. Plant specimens were collected during the Mongolian and Korean joint expedition, from forest steppe, steppe and desert steppe ecosystems of Mongolia (N 44°05’/E 103°32’; N 45°08’/E 104°15’; N 44°10’/E 103°42’; N 44°18’/E 103°44’; N 45°30’/E 104°32’; N 47° 47’/E 107°19’; N 47°49’/E 107°22’; N 47°51’/E 107°24’; N 47°52’/E 107°22’; N 47°53’/E 107°23’; N 47°55’/E 107°27; N 47°56’/E 107°27’; N 48°06’/E 106°44’; N 48°11’/E 106°44’; N 48°14’/E 106°45’; N 48°15’/E 106°44’; N 48°17’/E 106°47’; N 48°19’/E 106°53’; N 48°20’/E 106°53’; N 48°21’/E 106°49’) in August 2011, and taken to the Korea Polar Research Institute in Incheon of Korea for further study.

Plant sampling

In the field we collected plants and kept these in mesh bags and transported to the laboratory without additional treatment. In the laboratory, we dried samples completely for maintaining their quality. When samples were dried completely, we grind them to fine powder with a power blender.

Extraction

Freeze-dried sample (20 g) was extracted in a methanol-water mixture (70:30) at room temperature. The solvent was evaporated under vacuum at 45°C and finally freeze dried. The test samples were stored at -20°C until further use.

DPPH free radical scavenging assay

The free-radical scavenging activity of the plant extract was estimated by using a previously described method (see Blois, 1958). One mL of DPPH solution (0.1 mM of DPPH in methanol) was mixed with 3 mL of various concentrations of the test sample. The mixture was incubated at room temperature for 30 min. and the quantity of reduced DPPH, which formed a yellow color was measured in term of absorbance at 517 nm in a UV-Visible spectrophotometer (SCINCO). A re-action mixture without the test sample was used

as a negative control and with BHA (butylated hydraxyanisole) as a positive control. The experiment was conducted in triplicate.

Results and Discussion

We evaluated the antioxidant activity of 69 plant samples of 68 species belonging to 55 genera 25 families using the DPPH free radical scavenging assay. Plant extracts were able to reduce the stable DPPH free radical to diphenyl-picrylhydrazine (visible, yellow) in a concentration dependent manner in an assay based on the reduction of DPPH in the presence of a hydrogen donating antioxidant. DPPH free radical scavenging activity of plants is shown in Table 1.

In the present experiment, the average IC_{50} for the commercial standard BHA was 4.4±0.41 µg/ml. We compared our experimental data with the BHA and divided in 4 groups by the antioxidant activity of samples. There were 12 samples with very strong antioxidant activity (IC_{50} were < 4.4 µg/ml), 39 samples with strong antioxidant activity (IC_{50} were 4.4≤25.99 µg/ml), 10 samples with moderate antioxidant activity (IC_{50} were 26≤50.99 µg/ml), and 8 samples with weak antioxidant activity (IC_{50} were ≥ 51 µg/ml).

DPPH free radical scavenging different activities of BHA and some selected plants in their various concentrations are shown in Figures 1-6. It has been shown that the scavenging effects on the DPPH radical increased sharply with the increasing concentration of the samples and standards to a certain extent and hence are said to be strongly dependent on the extract concentration.

Very strong radical scavenging activity (IC_{50} were ≤4.4 µg/ml) than that of BHA was observed in *Rosa acicularis* Lindl.*,* *Potentilla bifurca* L.*,* *Rumex acetosa* L.*,* *Campanula glomerata* L.*,* *Geum aleppicum* Jacq.*,* *Dasiphora fruticosa*

Table 1. DPPH free scavenging capacity (IC_{50}, µg/ml) of the Mongolian plants

№	Families	№	Genera	№	Species	IC_{50}, mg/ml
1	Asteraceae	1	*Achillea*	1	*A. asiatica* Serg.	22.8±0.19
		2	*Artemisia*	2	*A. lacinata* Willd.	2.7±0.07
				3	*A. sericea* Web. Ex Stechm	5.49±0.19
				4	*A. Adamsii* Bess.	6.5±0.19
				5	*A. scorparia* Waldst. Et Kit	11.84±0.34
				6	*A. frigida* Willd.	16.82±1.05
				7	*A. pectinata*Pall.	23.37±0.4
				8	*A. Sieversiana* Willd.	28.4±0.31
				9	*A. macrocephala* Jacquem	43.56±0.68
		3	*Asterothamnus*	10	*A. molliusculus* Novopokr.	13.87±0.39
		4	*Galatella*	11	*G. dahurica* DC.	6.0±0.15
		5	*Heteropappus*	12	*H. hispidus* (Thunbd.) Less.	13.44±0.25
		6	*Inula*	13	*I. britannica* L.	18.86±0.26
		7	*Saussurea*	14	*S. amara* (L.) DC.	346±6.03
2	Alliaceae	8	*Allium*	15	*A. mongolicum* Rgl.	75.15±0.53
				16	*A. polyrrhizum* Turcz. Ex Rgl.	74.15±0.43
3	Boraginaceae	9	*Lappula*	17	*L. intermedia* (Ldb.) M. Pop.	17.7±0.12
4	Campanulaceae	10	*Campanula*	18	*C. glomerata* L.	1.96±0.03
5	Caryophyllaceae	11	*Stellaria*	19	*S. media* (L.) Cyr.	50.46±0.71
6	Chenopodiaceae	12	*Chenopodium*	20	*C. aristatum* L.	49.99±0.57
		13	*Haloxylon*	21	*H. ammodendron* (C. A. Mey.)	27.28±0.46
		14	*Salsola*	22	*S. passerinia* Bge.	30.07±0.57
				23	*S. collina* Pall.	99.5±3.78
7	Convolvulaceae	15	*Convolvulus*	24	*C. arvensis* L.	24.45±0.37

8	Crassulaceae	16	*Sedum*	25	*S. aizoon* L.	2.94±0.05
				26	*S. purpureum* (L.) Schult.	5.5±0.29
		17	*Orostachys*	27	*O. malacophylla* (Pall.) Fisch.	8.44±0.08
9	Ericaceae	18	*Vaccinum*	28	*V. vitis-idaea* L.	2.6±0.03
		19	*Pyrola*	29	*P. incarnata* (DC.) Freyn.	2.9±0.14
10	Equisetaceae	20	*Equisetum*	30	*E. pratense* Ehrh.	88.65±0.62
11	Fabaceae	21	*Hedysarum*	31	*H. inundatum* Turcz.	8.27±0.31
		22	*Thermopsis*	32	*T. dahurica* Czefr.	41.17±1.17
		23	*Astragalus*	33	*A. propinguus* Schischk	80.91±1.04
12	Gentianaceae	24	*Gentiana*	34	*G. barbata* Froel.	20.95±0.52
13	Geraniaceae	25	*Geranium*	35	*G. pretense* L.	18.26±0.19
14	Iridaceae	26	*Iris*	36	*I. lactea* Pall.	4.79±0.11
		27	*Thymus*	37	*T. gobicus* Tschern.	4.12±0.1
		28	*Schizonepeta*	38	*S. multifida* (L.) Briq.	7.62±0.15
15	Lamiaceae	29	*Phlomis*	39	*P. tuberosea* L.	9.89±0.14
		30	*Leonurus*	40	*L. sibiricus* L.	9.96±0.12
		31	*Dracocephalium*	41	*D. foetidium* Bunge.	10.37±0.25
		32	*Scutelleria*	42	*S. scordifolia* Fisch ex Schran	12.06±0.13
16	Nitrariaceae	33	*Peganum*	43	*P. nigellastrum* Bge.	101.74±4.29
17	Onagraceae	34	*Chamaenerion*	44	*C. angustifolium* (L.) Scop.	3.18±0.19
		35	*Veronica*	45	*V. incana* L.	4.81±0.06
18	Plantaginaceae	36	*Plantago*	46	*P. major* L.	6.14±0.22
		37	*Rumex*	47	*R. acetosa* L.	1.86±0.06
19	Polygonaceae	38	*Polygonum*	48	*P. aviculare* L.	5.42±0.22
		39	*Rheum*	49	*R. undulatum L.*	22.81±0.08
		40	*Anemonia*	50	*A. sylvestris* L.	6.14±0.08
		41	*Delphinum*	51	*D. grandiflorum* L.	18.05±0.18
		42	*Aconitium*	52	*A. barbatum* Pers.	21.12±0.46
20	Ranunculaceae	43	*Atragene*	53	*A. sibirica* L.	25.5±0.75
		44	*Pulsatilla*	54	*P. ambigua* (Turcz.) Juz	35.01±0.48
		45	*Thalichtrum*	55	*T. foetidium* L.	10.31±0.39
				56	*T. simplex* L.	13.38±0.08
				57	*T. minus* L.	18.94±0.26
21	Rosaceae	46	*Rosa*	58	*R. acicularis* Lindl.	0.81±0.02
		47	*Geum*	59	*G. aleppicum* Jacq.	1.96±0.06
		48	*Dasiphora*	60	*D. fruticosa* (L.) Rydb.	2.31±0.05
		49	*Sanguisorba*	61	*S. officinalis* L.	10.61±0.22
		50	*Padus*	62	*P. asiatica* Kom.	21.29±0.49
		51	*Potentilla*	63	*P. bifurca* L. (sample 1)	1.07±0.07
					P. bifurca L. (sample 2)	7.39±0.09
				64	*P. anserina* L.	8.42±0.2
22	Rubiaceae	52	*Galium*	65	*G. verum* L.	10.54±0.13
23	Solanaceae	53	*Hyoscyamus*	66	*H. niger* L.	39.69±2.02
24	Scrophulariaceae	54	*Scropularia*	67	*S. gracilis incise* Weinm	54.35±1.77
25	Thymelaeaceae	55	*Stellera*	68	*S. chamaejasme* L.	30.39±0.54

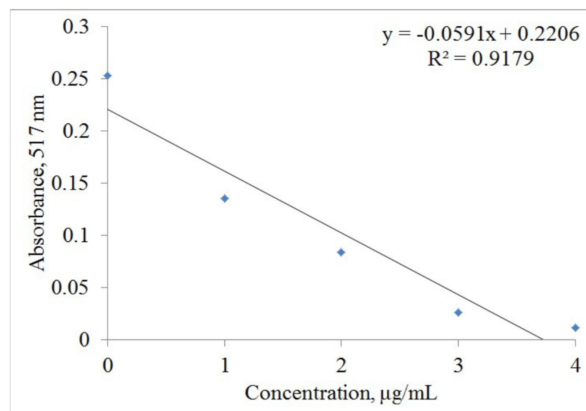

Figure 1. Very strong activity of *Rumex acetosa*

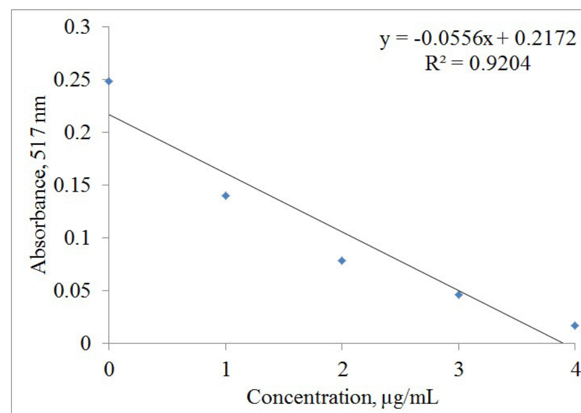

Figure 2. Very strong activity of *Geum aleppicum*

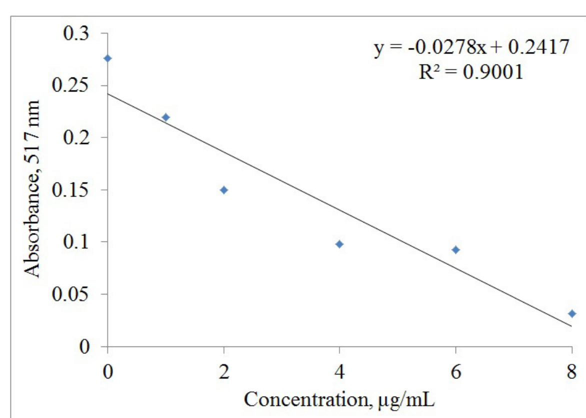

Figure 3. Strong activity of BHA

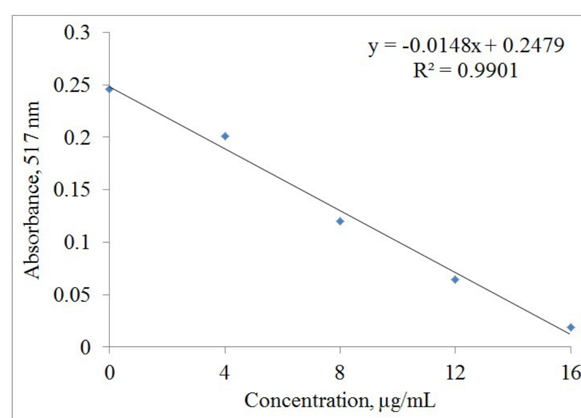

Figure 4. Strong activity of *Potentilla anserina*

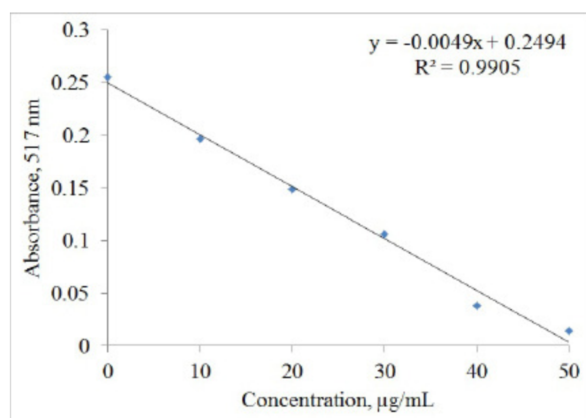

Figure 5. Moderate activity of *Atragene sibirica*

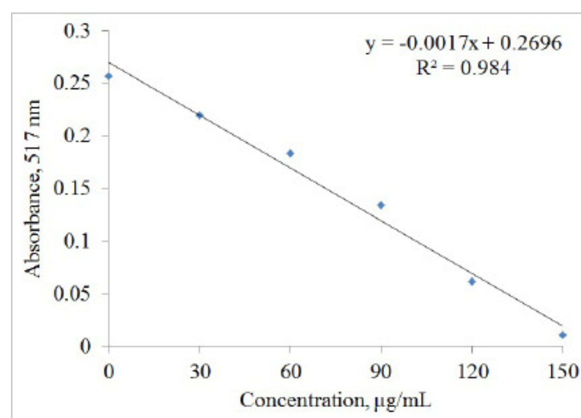

Figure 6. Weak activity of *Astragalus propinguus*

(L.) Rydb., *Vaccinum vitis-idaea* L., *Artemisia lacinata* Willd., *Pyrola incarnata* (DC.) Freyn., *Sedum aizoon* L., *Chamaenerion angustifolium* (L.) Scop., *Thymus gobicus* Tschern. This result showed that, above mentioned plants must be potential sources of natural compounds.

Therefore, our future study would be the confirmation experiments of antioxidant activity by the other methods and the screening of these stronger antioxidant agents from these plants, which had very strong antioxidant activity.

Other remaining plant extracts are still less effective than the commercial available synthetic compound BHA. As the plant extracts are quite safe and their toxicity is a not a problem of concern unlike those of BHA, they could be exploited as antioxidant additives or as nutritional supplements.

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