# Comparison of Relative Antioxidant Capacity and Antiperoxidation Activity of Traditional Medicines in Vitro

Gee Bok Yeom, Hyung Cheol Lee, Sung Min Ju, Kun Jung Kim, Won Sin Kim[1], Chae Ho Lee[1], Ho Yeon Jang, Jeong Ho Kang, Shin Ki Park, Key Sang Lee, Byung Hun Jeon*

*Department of Oriental Pathology, College of Oriental Medicine,*
*1:Division of Life science College of Natural Sciences and Institute of Basic Natural Sciences, Wonkwang University*

To investigate the antioxidant capacity of traditional Korean medicines, water extracts from 57 species were tested on their antioxidant activity using radical scavenging effects against ABTS · +. Of which, Rhus javanica, Caesalpinia sappan, Rosa rugosa, Spatholobus suberectus, and Magnolia obovata showed strong antioxidant capacities at 10μg concentration. Therefore, antioxidant capacities of 5 traditional medicine extracts in the different concentration (1 μg, 5 μg, and 10 μg) were determined. The 5 traditional medicine extracts was detected in antioxidant capacity dose dependently. R. javanica was showed the highest antioxidant capacity, the antioxidant activity at 1 μg of herbal extract being 0.85 mM TE. At the same time, the antiperoxidation effects of these 5 medicines were determined. Lipid peroxidation in brain homogenates induced by NADPH and ADP-$Fe^{2+}$ was strong inhibited by S, suberectus, R. rugosa, and M. obovata. Extract of C. sappan was showed the highest inhibition against lipid peroxidation compared with 4 herbal extracts in vitro.

These traditional medicines are a potent antioxidant capacity and antiperoxidation activity, further investigation into the in vivo antioxidant therapeutic potential for treatment of human disorders such as Alzheimer's disease.

Key words : Caesalpinia sappan, Antioxidant capacity, Antiperoxidation activity, Traditional medicine

## Introdution

Reactive oxygen species (ROS) such as superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), and hydroxyl (·OH) is potentially transient chemical species and generated in all aerobic metabolism cells. Oxidative damage resulting from an inbalance between production and elimination of ROS has been implicated in the pathogenesis of a variety of human diseases, including cancer, diabetes, cataractogenesis, rheumatoid arthritis, reperfusion injury, as well as degenerative disorders of the neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease[1,3,6,21]. The cells have biological system to protect from ROS induced damage by a variety of endogenous ROS scavenging enzymes and chemical compounds[8]. Lipid peroxidation is a complex process occurring in all aerobic organisms and reflects the interactions between molecular oxygen and unsaturated fatty acids. Antioxidants, which act as radical scavengers, inhibit lipid peroxidation and other free radical-mediated processes. Therefore, they are able to protect the human body from several diseases. attributed to the reactions of radicals[14,22]

Medicinal herbs are the source of healthcare and disease management for a natural antioxidants in various medicinal plants has been reported as therapeutic drugs for free radical pathologies[2,7,12]. Recently, natural products acts as potent scavengers have been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical-induced tissue injury. Therefore, the present study was undertaken to evaluate the antioxidant capacities and antiperoxidation activities of 57 traditional medicine.

* To whom correspondence should be addressed at : Byung Hun Jeon, Department of Oriental Pathology, College of Oriental Medicine, Wonkwang University, Iksan, JeonBuk-Do, 570-749, South Korea
· E-mail : omdjbh@wonkwang.ac.kr, · Tel : 063-850-6843
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## Materials and methods

1. Chemicals

Ferrous sulfate, 2-thiobarbituric acid (TBA), ADP and NADPH were purchased from Sigma-Aldrich Chem. Co. (St. Louis, USA), and total antioxidant status (TAS) kit was obtained from Randox Lab. (Crumlin, UK). All other chemicals and reagents were the highest grade of commercially available.

2. Preparation of medicinal plant extracts

The air-dried plants of medicinal herbs were obtained from Medicinal Resources Research Center of Wonkwang University. Fifty gram of each herb was extracted with 500ml distilled water for 2hr at 100℃, respectively, and then centrifuged at 3,000 rpm for 20 min. The supernatant was filtered, dried using freeze dryer, stored at -70℃ until used.

3. Brain homogenate

Adult male Sprague-Dawley rats were obtained from Samtako Hi-Quality Laboratory Animal Inc. (Osan city, Kyungki Province, Korea). After decapitation of rat, brain was rapidly dissociated and placed on chilled surface. The superficial blood vessels were removed, and washed with ice-cold 50mM Tris-HCl contained 32 mM sucrose buffer (pH 7.4). The brain was homogenized (200 mg tissue/ml buffer) in ice-cold 50mM Tris-HCl buffer (pH 7.4) using a Potter Elvehjehm Teflon homogenizer. These homogenates were used for the determination of antioxidant and lipid peroxidation.

4. Determination of antioxidant activity in vitro

The antioxidant activity was determined in vitro by means of scavenging of the ABTS·+ (2,2-azino-bis-3-ethyl-benzthiazoline-6-sulphuric acid) radical generated by a metamyoglobin/hydrogen peroxide system as described previously[17]. The test sample (10 $\mu\ell$) was added to a 1cm pathlength spectrophotometer cuvette (1 ml capacity) containing 20 mM phosphate buffer (pH 7.4), 2.5 μM metamyoglobin. The reaction initiated by addition of 75 μM hydrogen peroxide and the absorbance change at 734 nm monitored at 30℃. The antioxidant status of the plant extract, determined relative to Trolox (a water soluble vitamin E analogue) antioxidant standards, was expressed in terms of mM Trolox equivalent (mM TE). Corresponding samples of medicinal plant extracts for antioxidant activity as above were dried, and the final antioxidant activity was expressed in terms of mmole TE/mg dried extracts.

5. Measurement of thiobarbituric acid reactive substances (TBARS)

Induced lipid peroxidation in brain homogenates was examined by the detection of malondialdehyde (MDA) production in a medium of 1 ml total volume with a protein content of 0.5 mg ml-1. The medium consisted of 25 mM Tris-HCl buffer (pH 7.4), 5 mM ADP, 0.2 mM $FeSO_4$, 1 mM NADPH, plus various concentrations of medicinal plant lyophilizates. The reaction mixture was incubated at 37℃ for 30min, and reaction was terminated by adding 1 ml of thiobarbituric acid reagent (0.375% thiobarbituric acid and 10% acetic acid). The samples were heated in boiling water bath of 98℃. The malondialdehyde (MDA) and related materials formed were measured at 535 nm and quantitated using an extinction coefficient of $1.56\times10^{-5}$ M-1 cm-1. For inhibition studies, the media were preincubated with different concentrations of medicinal plant extracts for 5 min before the initiation of the reaction with ADP-$Fe^{2+}$ and NADPH.

# Results and Discussion

The formation of reactive oxygen species from different sources, such as enzymatic reaction and xenobiotic metabolism, may lead to lipid peroxidation, subsequent cell injury and toxicity[19]. Oxidative stress is considered to be of major pathophysiological relevance for a variety of pathological processes, such as cancer, Parkinson's disease, and Alzheimer disease[6,26]. This hypothesis has prompted research efforts to identify compounds that might act as antioxidants. The effect of antioxidants in decreasing oxidative damage is believed that contribute to lower the cancer incidence and neurodegenerative diseases[26]. with the increasing acceptance of traditional medicines as an alternative form of healthcare, the screening of medicinal plants for biological active compounds is very important. Therefore, this study was designated to investigate Oriental folk medicines for potential antioxidants and antiperoxidants by preliminary in vitro assay screening.

In the present study, we have indentified traditional medicines, and water extracts of 57 different plants species belonging to 33 families were tested for antioxidant capacity and antiperoxidation activity by in vitro assay. The antioxidant capacity in such extracts was determined using the total antioxidant status kit (Randox Labs) against ABTS·+ radical was determined in biological fluid and human plasma/serum for routine use in clinical chemistry analysis has been proposed[18,21,25], and recently, the assessment of antioxidant capacity in plant extracts and medicinal drugs using this assay method has been applied in recently[12]. The antioxidant levels of traditional medicines are shown in Table 1. Of the medicinal plants investigated, the highest levels of antioxidant activity were obtained from the S. suberectus

(2.63 mM TE), M. obovata (2.46 mM TE), R. javanica (2.44 mM TE), C. sappan (2.43 mM TE), R. rugosa (2.28 mM TE), H, syriacus (2.25 mM TE), E. pekinensis (2.20 mM TE), E. caryophyllata (2.20 mM TE), R. coreanus (2.18 mM TE), respectively, On the other hand, several medicinal plants ( e.g. E. ulmoides, K. scoparia, B. falcatum, and C. officinalis) appeared to contain little antioxidant capacity.

**Table 1. Comparison of antioxidant activity of Korean medicinal plants invitro**

| Species (family) | Use of herb extract | Antioxidant activity (mM TE) |
|---|---|---|
| Spatholobus suberectus (Leguminosae) | Rhizome | 2.63±0.25 |
| Magnolia obovata (Magnoliaceae) | Cortex | 2.46±0.32 |
| Rhus javanica (Anacardiaceae) | Gall | 2.44±0.23 |
| Caesalpinia sappan (Leguminosae) | Lignum | 2.43±0.34 |
| Rosa rugosa (Rosaceae) | Radix | 2.28±0.27 |
| Hibiscus syriacus (Malvaceae) | Cortex | 2.25±0.31 |
| Euphorbia pekinensis (Euphorbiaceae) | Radix | 2.20±0.21 |
| Eugenia caryophyllata (Myrtaceae) | Flower | 2.20±0.17 |
| Rubus coreanus (Rosaceae) | Fruit | 2.18±0.15 |
| Rheum palmatus (Polygonaceae) | Rhizome | 2.11±0.24 |
| Gleditsia Japonica var. koraiensis (Leguminosae) | spine | 2.04±0.14 |
| Euodia officinalis (Rutaceae) | Fruit | 1.67±0.08 |
| Uncaria sinensis (Rubiaceae) | Stem | 1.65±0.11 |
| Cynomorium songaricum (Cynomoriaceae) | Herb | 1.52±0.09 |
| Zanthoxylum bungeanum (Rutaceae) | Fruit | 1.42±0.07 |
| Euphorbia lathyris (Euphorbiaceae) | Semen | 1.35±0.06 |
| Ampelopsis japonica (Vitaceae) | Radix | 1.34±0.08 |
| Salvia miltiorrhiza (Labiatae) | Radix | 1.31±0.13 |
| Artemisia capillaries (Compositae) | Herb | 1.29±0.12 |
| Arctium lappa (Compositae) | Fruit | 1.24±0.13 |
| Drynaria fortunei (Polypodiaceae) | Rhizome | 1.22±0.12 |
| Morus alba (Moraceae) | Cortex | 1.19±0.14 |
| Cibotium barometz (Dieksoniaceae) | Rhizome | 1.11±0.11 |
| Artemisia asiatica (Compositae) | Herb | 1.07±0.07 |
| Scutellaria baicalensis (Labiatae) | Radix | 1.02±0.09 |
| Phlomis umbrosa (Labiatae) | Radix | 0.90±0.11 |
| Epimidium koreanum (Berberidaceae) | Herb | 0.71±0.08 |
| Rehmannia glutinosa var. purpurea | Rhizome | 0.69±0.05 |
| Mentha arvensis var. piperascens (Labiatae) | Herb | 0.68±0.06 |
| Santalum album (Santalaceae) | Lignum | 0.67±0.07 |
| Ostericum koreanum (Umbelliferae) | Radix | 0.66±0.09 |
| Sophora flavescens (Leguminosae) | Radix | 0.66±0.07 |
| Prunus persica (Rosaceae) | Flower | 0.66±0.09 |
| Forsythia virdissima (Oleaceae) | Fruit | 0.62±0.06 |
| Euonymus alatus (Celastraceae) | Lignum | 0.60±0.05 |
| Phellodendron amurense (Rutaceae) | Cortex | 0.55±0.07 |
| Lycium chinense (Solanaceae) | Fruit | 0.55±0.04 |
| Ligusticum tenuissima (Umbelliferae) | Radix | 0.52±0.09 |
| Dianthus chinensis (Caryophyllaceae) | Herb | 0.44±0.07 |
| Leonurus sibiricus (Labiatae) | Herb | 0.44±0.02 |
| Fritillaria thunbergii (Liliaceae) | Bulb | 0.43±0.04 |
| Lonicera japonica (Caprifoliaceae) | Flower | 0.42±0.03 |
| Ophiopogonis japonicus (Liliaceae) | Radix | 0.39±0.05 |
| Akebia quinata (Lardizabalaceae) | Stem | 0.35±0.06 |
| Schizonepeta tenuifolia var. japonica (Labiatae) | Herb | 0.34±0.03 |
| Cornus officinalis (Cornaceae) | Fruit | 0.33±0.03 |
| Pueraria thunbergiana (Leguminosae) | Radix | 0.30±0.04 |
| Taraxacum platycarpum (Compositae) | Herb | 0.27±0.04 |
| Bletilla striata (Orchidaceae) | Rhizome | 0.24±0.05 |
| Solanum nigrum (Solanaceae) | Herb | 0.22±0.05 |
| Hodyotis diffusa (Rubiaceae) | Herb | 0.22±0.02 |
| Fraxinus rhynchophylla (Oleaceae) | Cortex | 0.20±0.03 |
| Prunus persica (Rosaceae) | Semen | 0.19±0.05 |
| Trichosanthes kirilowii (Cucurbitaceae) | Semen | 0.18±0.01 |
| Bupleurum falcatum (Umbelliferae) | Radix | 0.17±0.02 |
| Kochia scoparia (Leguminosae) | Lignum | 0.12±0.01 |
| Eucommia ulmoides (Eucommiaceae) | Cortex | 0.11±0.03 |

The assay of antioxidant activity against ABTS · + was described in Materials and Methods. Determination of antioxidant activity was expressed in terms of mmole Trolox equivalent. Data are shown as mean±SD from three independent experiments, each herbal extract was tested (10 μl/assay) at 1 mg/ml of the extracts concentration.

The comparative antioxidant results shown in Fig. 1 were determined after treatment of the various concentrations of S. suberectus, M. obovata, R. javanica, C. sappan, and R. rugosa extracts. Among these 5 herb extracts, R. javanica and C. sappan extracts showed the highest antioxidant activity in rat brain homogenates, the antioxidant activity at 1 μg of herbal extract being 0.85 mM TE and 0.24 mM TE, respectively(Fig. 1).

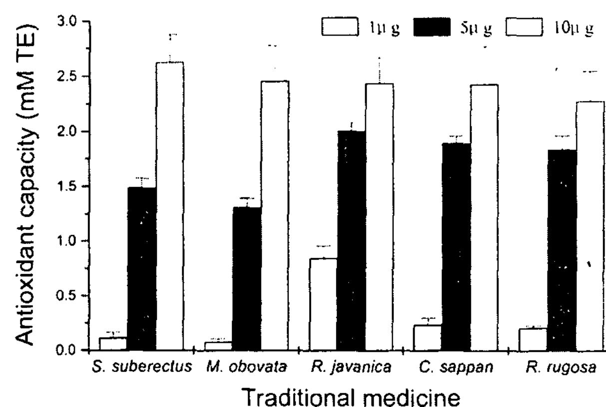


**Fig. 1. Comparison of antioxidant capacities in the various concentrations of traditional medicines against ABTS · + in vitro.** The assay of antioxidant activity against ABTS · + was described in Materials and Methods. The values are expressed as mean±SD of three independent experiments, each medicinal extracts was tested at the various concentrations (1, 5, and 10 μg).

Malondialdehyde (MDA) is the major oxidative degradation product of membrane unsaturated fatty acid, and has been shown to be biologically active with cytotoxic and genotoxic properties (Husain et al., 1987). Quantitation of MDA, one of the product of lipid peroxidation, with thiobarbituric acid reactive substance (TBARS) is the most common assay used for determination of the lipid peroxidation. In the present study, figure 2 shows $NADPH^{+}$-$Fe^{2+}$ induced lipid peroxidation in brain homogenates.

The antiperoxidative effect was expressed as MDA formation, setting the values obtained without inhibitor, as 100% activation. Our experiments proved that NADPH and ADP-$Fe^{2+}$ induced lipid peroxidation was inhibited dose-dependent manner in water extracts of C. sappan, R. javanica, R. rugosa, M. obovata, and S. suberectus. Water extract of C. sappan proved to be more effective (C. sappan extract, 1 μg = 68%, 10 μg = 23%; 100 μg = 2%) compared with 4 herbal extracts in this system. Among 6 herb extracts, C. sappan and R. javanica extracts were found to highest inhibition TBARS generated (lipid peroxidation) by the induction of NADPH and ADP-$Fe^{2+}$ in rat brain homogenates, IC50 values being about 4.6 μg and 5.8 μg, respectively (Fig. 2).

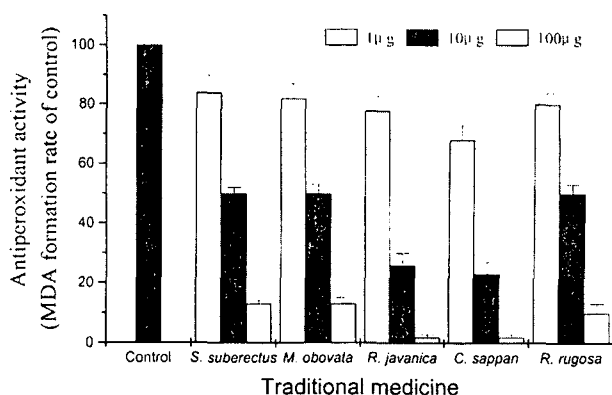


**Fig. 2. Antiperoxidant activity of traditional medicines lyophilized water extracts on NADPH and ADP-$Fe^{2+}$ induced lipid peroxidation in rat brain homogenates.** Data are extract was tested (10 μl) at the various concentrations (1, 10, 100 μg). The antiperoxidation activity was expressed as MDA formation and the value was expressed percentage of control without inhibitor, as 100% activation.

So the hot water extract of these medicinal plants, which might act as scavengers of reactive oxygen species because of confirmed with antioxidant effect, could inhibit lipid peroxidation. Prevention of the TBARS formation and action of antioxidant in the extracts is good health importance in decreasing the risk of neurodegenerative diseases.

Reactive oxygen species (ROS) including superoxide anion radical ($O_2$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical (·OH) formed in cells, can oxidize a number of cellular constituents like lipids, proteins, and DNA. The excess production of ROS can easily initiate lipid oxidation in the cell membranes, especially in the intracellular organelles, resulting in the formation lipid peroxides. ROS and lipid peroxides are destruction of the membrane function and cellular components, which can result in mutagenicity and carcinogenicity, and also accelerate aging[1,4]. A number of vitamins such as C and E as well as beta-carotene are excellent antioxidant, and they also contribute to good health in aging and longevity through other mechanisms such as being co-factors for certain enzymes, involved in oxidation-reduction reactions in the body[23]. Recent publications indicate that there is much evidence that plant antioxidative defense. Antioxidant compounds have already been found in numerous plant materials such as crops, fruits, vegetables, herba and crude plant drugs[15,16,23].

Recently extracts of C. sappan have been show to strong inhibition the enzyme acetylcholinesterase in brain tissue in vitro[11]. and flavonoids extracted from R. rugosa were protected the erythrocyte membrane against lipid peroxidation [10]. Data obtained in the present investigation show extracts of R. jabanica and C. sappan have appreciable levels of antioxidant capacity and antiperoxidation activity in vitro (Table 1, Fig. 1, 2). On the basis of the above, it may be worthwhile undertaking clinical trials of these plant extracts in Alzheimer's disease patients, where there may be a synergistic effect, since free radical induced tissue damage has been implicated in disorder. The body, however, possesses defense mechanisms to reduce the oxidative damage and such mechanisms include using both enzymes and antioxidant nutrients or medicine to arrest the damaging properties of exited oxygen species. A great number of studies have suggested that antioxidant nutrient and/or medicines play a protective role in human health[23].

In conclusion, our study provides evidence that R. javanica and C. sappan water extracts exhibit interesting antioxidant and antiperoxidation properties for scavenging of reactive oxygen species. These effects may be useful in the treatment of pathologies in which free radical oxidation plays a fundamental role. At the same time a great deal of work remains to be carried out in order to confirm more completely the biological activity of extracts in various animal models.

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