Natural Product Sciences
7(2) : 45~48 (2001)

Antioxidative Activity of the Extracts from the Leaves and Fruits of *Acer ginnala*

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Abstract – The antioxidative effect of the extracts from the leaves and fruits of *Acer ginnala* against free radicals was studied by two different methods using DPPH radical-generating system, and hydroxyl radical-generating system (Cu^{++}/H_2O_2 system) which induces DNA strand breaking. Compared with well known antioxidative plants, green tea, *Scutellaria baicalensis*, the *Acer ginnala* extracts showed excellent radical-scavenging activity in DPPH radical-generating system and inhibited effectively hydroxyl radical induced-DNA strand breaking in a concentration-dependent manner in Cu^{++}/H_2O_2 system whereas the green tea extract stimulated the strand breaking at a low concentration. These results suggest that the extracts from the leaves and fruits of *Acer ginnala* could be good antioxidative agents.

Key words – *Acer ginnala*, DPPH, hydroxyl radical, antioxidative

Introduction

Free radicals and ROS (reactive oxygen species) can be generated by exogenous factors, UV, pesticides, air pollution and anti-tumoral drugs as well as by endogenous factors, metabolic reactions such as oxidation reactions, excessive phagocytosis and arachidonic acid metabolism (Jay *et al.*, 1998). Biological systems have elaborate antioxidative system (SOD, catalase, peroxidase, vitamins, etc.) to prevent oxidative damage by free radicals and ROS. When the balance between antioxidants and free radicals, ROS is broken, the variety of pathological conditions such as cancer (Totter *et al.*, 1980; Ames *et al.*, 1989), diabetes (Baynes *et al.*, 1991) and aging (Harman, 1981) are induced. In particular, during oxidative stress, sensitive biomolecules such as proteins, lipids and DNA can be damaged. Of ROS, the hydroxyl radical that is produced when copper or iron (transition metal) reacts with H_2O_2 in a Fenton-type reaction is the most powerful oxidizing species. It denatures the proteins (collagen, elastin and glycosaminoglycans) of the connective tissue and reacts with fatty acids of membranes, which leads to membrane disorganization and causes strand breakage that induces irreparable DNA damage.

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Many plant extracts which are known to have various biological activities such as anti-inflammatory, anti-carcinogenic, antibacterial, anti-allergic, immune-stimulating and antioxidative effects, have been reported (Brown *et al.*, 1980; Middleton and Kandaswami, 1992). Recently, the antioxidative activity of phytochemicals widely occurring in plant kingdom has given rise to much attention. It has been proven that flavonoids, which are one of phytochemicals, are better antioxidants than the antioxidative nutrients vitamin C and vitamin E (Rice-Evans *et al.*, 1995; Castellucio *et al.*, 1995).

In this paper, the antioxidative activity of the extracts from the leaves and fruits of *Acer ginnala* was studied. To investigate radical-scavenging activity, we used DPPH radical-generating system, and chose Cu^{++}/H_2O_2 system to assess the inhibitory effect of the extracts on hydroxyl radical-induced DNA damage. For comparison, well known antioxidative plants, green tea (Matsuzaki *et al.*, 1985) and *Scutellaria baicalensis* (Gao *et al.*, 1999) were used.

Experimental

Materials – The fresh leaves and fruits of *Acer ginnala* were collected in June in Incheon, Korea. They were identified by botanical survey. The voucher specimen has been kept in our R&D center. The roots of *Scutellaria baicalensis* were offered

kindly from Bioland, Ltd., and the leaves of green tea were purchased from Po-Sung Co. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), $CuCl_2$ and H_2O_2 were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of reagent grade.

Preparation of extract – The air-dried leaves, fruits and roots of plants were frozen in liquid nitrogen, powdered in porcelain mortar and passed through 40-mesh sieve. Each one gram of the powdered leaves, fruits and roots was extracted with 20 gram of 50% methanol, stirred overnight at room temperature, respectively. The extract solution was centrifuged at 3,000 rpm for 10 min, and the supernatant was filtered (0.45 filter, Gelman Sciences, MI, USA). Final filtrate was used for the following study.

DPPH radical-scavenging activity – The modified method of Fujita *et al.* (1988) using a moderately stable free radical, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) was used to determine the free radical-scavenging activity of the *Acer ginnala* extracts and other extracts including green tea and *Scutellaria baicalensis*. Test sample was dissolved in absolute ethanol, and 1.0 ml of the ethanolic sample was added to 1.0 ml of 0.1 mM DPPH methanolic solution. The mixture was incubated at 37°C for 30 min, and the amount of free radical to be left was measured at 516 nm by a spectrophotometer.

Inhibitory effect on hydroxyl radical-induced DNA damage – pUC-19 plasmid DNA was reacted in the following sequence: 2 μl of DNA (20 μg/ml final concentration), 3 μl of 50 mM Na phosphate buffer, pH 7.4 (10 mM final concentration), 3 μl of 5 mM of H_2O_2 (1mM final concentration), 3 μl of sample and distilled water to a final volume of 12 μl. Lastly, 3 μl of 50 μM Cu^{++} solution (10 uM final concentration) was added to start the reaction. After 10 min at 37°C, the reaction was stopped by adding 3 μl of loading dye (6X, 0.03% bromophenol blue, 0.03% xylene cyanol, 15% Ficol l400 and 50 mM EDTA). Eighteen microliters of the reaction mixture was loaded on a 0.8% agarose gel containing 0.5 μg/ml ethidium bromide and electrophoresed in 0.5X TBE buffer, and directly visualized by UV fluorescence.

Results and Discussion

In this study, we have focused our research on antioxidative activity of the extracts from the leaves and fruits of *Acer ginnala*. To investigate antioxidative activity, DPPH radical-generating system and Cu^{++}/H_2O_2 system generating hydroxyl radical were used. First, in DPPH radical-scavenging assay, both leaves and fruits extracts of *Acer ginnala* showed very strong radical-scavenging activity when compared with other plant extracts including green tea and *Scutellaria baicalensis* which had been demonstrated to be effective antioxidative plants, and the order of DPPH radical-scavenging activity was *Acer ginnala*> green tea> *Scutellaria baicalensis* at tested concentrations (Fig. 1). In case of the extracts from *Acer ginnala*, the leaves extract was more effective than the fruits extract.

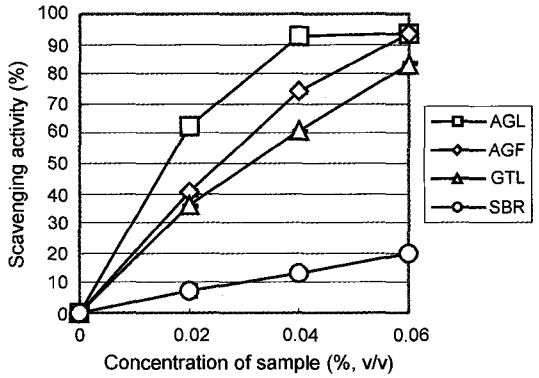

Fig. 1. DPPH radical-scavenging activity. The extracts from the leaves (□, AGL) and fruits (◇, AGF) of *Acer ginnala* scavenged DPPH radical effectively in comparison with those of the leaves (△, GTL) of green tea and the roots (○, SBR) of *Scutellaria baicalensis*. The percent inhibition value is the average of the duplicate assays, and error bar represents the difference between duplicates.

It has been well known that hydroxyl radical induces DNA base modification and strand breaking, which may cause serious diseases in connection with carcinogenesis (Hochstein *et al.*, 1988). In this assay, we evaluated the antioxidative activity of the plant extracts by measuring hydroxyl radical-induced DNA damage. The inhibitory effect on DNA strand breaking was assayed by measuring the conversion of supercoiled circular double-stranded DNA (form I) into nick open circular DNA (form II) and linear DNA (form III). All of the plant extracts that were tested in this assay, by themselves were inactive in breaking DNA strand at tested concentrations (data not shown). Both leaves and fruits extracts of *Acer ginnala* inhibited effectively hydroxyl radical-induced DNA damage, and form II and form III DNA disappeared in a dose-dependent manner at final

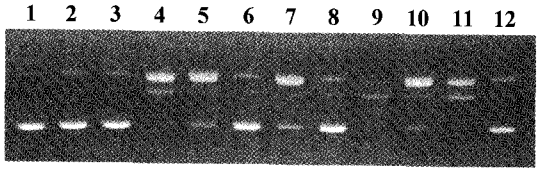

Fig. 2. Inhibitory effect on hydroxyl radical-induced DNA damage. Lane 1, pUC19 DNA only; lane 2, pUC19 DNA plus final 10uM Cu^{++}; lane 3, pUC19 DNA plus final 1 mM H_2O_2; lane 4, pUC19 DNA, 10 μM Cu^{++} and 1 mM H_2O_2. The plant extracts were added to the mixture of pUC19 DNA, 10 μM Cu^{++} and 1 mM H_2O_2 at final concentrations of 1% and 5%. The leaves extract of *Acer ginnala* (lane 5, 1%; lane 6, 5%), fruits extract of *Acer ginnala* (lane 7, 1%; lane 8, 5%). The green tea extract and *Scutellaria baicalensis* extract were added to the reaction mixture at 1% (lane 9, 11) and 5% (lane 10, 12), respectively.

concentrations of 1% and 5% (Fig. 2). In case of the roots extract of *Scutellaria baicalensis* which was used as a comparative plant, it showed similar preventive pattern against DNA breaking like the *Acer ginnala* extracts, but it was less effective on inhibition of DNA damage than the *Acer ginnala* extracts. On the other hand, the leaves extract of green tea apparently enhanced DNA strand breaking at a concentration of 1%, whereas it inhibited the strand breaking slightly at 5%, where the band of form I DNA was observed. This result indicates that the principles of the green tea extract could act as pro-oxidants or generators of hydroxyl radical at a low concentration. There have been reports that hydrolyzable tannins within plants in the presence of Cu(II) induce DNA strand breakage via the generation of hydroxyl radical during oxidation (Bhat *et al.*, 1994), and the polyphenolic principles of green tea have an enhancing effect on the DNA strand breaking by hydroxyl radical. (−)-epigallocatechin gallate (EGCG), the principal polyphenol of green tea stimulated DNA strand breaking at a low concentration, whereas it inhibited DNA strand breaking at a high concentration in Fe^{++}/H_2O_2 system generating hydroxyl radical (Hiramoto *et al.*, 1996). However, the mechanism of the generation of hydroxyl radical from phenolic compounds is still unknown. All of the plant extracts that show good radical-scavenging effect on DPPH radical could not be regarded as antioxidants for hydroxyl radical, and many of them could be actually regarded as pro-oxidants or generators of hydroxyl radical under certain conditions where hydroxyl radical is generated.

In this study, we have confirmed that the extracts from the leaves and fruits of *Acer ginnala* have powerful antioxidative activity in DPPH radical-generating system as well as hydroxyl radical-generating system that induces DNA strand breaking. It is evident that *Acer ginnala* extracts possess excellent antioxidative principles. The separation of main antioxidative principles from *Acer ginnala* needs to be studied further.

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(Accepted May 19, 2001)