Antioxidant Properties of Water Extract from Acorn

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Antioxidant and anti-diabetic activities of acorn were evaluated by its potential for scavenging stable DPPH free radical, inhibition of lipid peroxidation, reducing power, and inhibiton of α-glucosidase and α-amylase. The water extract of acorn exhibited strong antioxidant and anti-diabetic related activities in the tested model systems. Solvent fractionation of the water extract revealed that the water fraction and the EtOAc fraction had strong antioxidant activity, and inhibitory activity on α-glucosidase and α-amylase. The water fraction exhibited higher DPPH radical scavenging activity (EC_{50} = 7.19 μg/mL) than that of α-tocopherol (EC_{50} = 7.59 μg/mL). It is considered that water extract of acorn has the potential for natural antioxidant and anti-diabetic products.

Key words: *acorn, anti-diabetic activity, antioxidant activity, α-amylase, α-glucosidase*

Acorn has been used for local diet in Korea. It has been consumed in the form of bread cake and other types of foods. The beneficial use of acorn in the human diet has been reported in Serbia since the end of the 19th century (Pelagic, 1893), with recommendations about its application and beneficial effects. The preparation of drinks based on thermally treated acorn (dry roasting) was especially recommended for children. Antioxidative effects of some acorn components have been reported (Lee *et al.*, 1992; Chiou, 1989; Rakic *et al.*, 2006). It has been known that oxidative stress is closely related to various diseases such as artherosclerosis, cancer, brain disorder, and aging. Therefore, antioxidants are considered as protectants against oxidative stress related disease.

Here, we describe the solvent extracted fractions of water extract of acorn, and their antioxidant and anti-diabetic related activities.

Materials and Methods

Plant materials and solvent fractionation. Acorns were obtained from Chuncheon-si, Gangwon-do, Korea. Dried acorn powder (100 g) was refluxed with water for 3 days at room temperature, and the water extract (5 g) was suspended in distilled water and partitioned with hexane, CH_2Cl_2, EtOAc in sequence to afford the fractions of hexane (0.1 mg), CH_2Cl_2 (5 mg), EtOAc (45 mg) and H_2O (3185 mg)

DPPH radical scavenging activity. DPPH radical scavenging activity was assessed as described by Kilani *et al* (Kilani *et al.*, 2005) with some modification. Two milliliters of various concentrations of sample or water (control) was added to 2 mL DPPH solution (6.5×10^{-4} mol/mL in MeOH). Blanks contained each of 2 mL distilled water and sample solution. The mixture was shaken immediately after adding DPPH and allowed to stand at room temperature in the dark, and the decrease in absorbance at 517 nm was measured after 30 min until the reaction reached at steady state. All the experiments were run in triplicate. DPPH radical scavenging activity was calculated as follows : Scavenging effect (%) = $[1 - (A_i - A_j)/A_0] \times 100$; where A_0 is the A_{517} of DPPH without sample (control), A_i is the A_{517} of sample and DPPH, and A_j is the A_{517} of sample without DPPH (blank). The effective concentration required for 50% plaque reduction (EC_{50}) was determined from a curve relating plaque number to the concentration of a sample.

Reducing power assay. The determination of the reducing power was conducted according to the method developed (Oyaizu, 1986). Fractions were spiked with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was then placed in a 50°C water bath for 30 min. Then, samples

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Abbreviations: BHT, 2,6-di-tetra-butyl-4-methylphenol; DNS, 3,5-dinitrosalicylic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EtOAc, ethyl acetate; $FeCl_2$-AA; $FeCl_2$-ascorbic acid; MDA, malondialdehyde; SDS, sodium Dodecyl Sulfate; TBA, 4,6-dihydroxy-2-mercaptopyrimidine; TBA-RS, thiobarbituric acid-reactive substances.

were kept at room temperature and 2.5 mL of 10% trichloroacetic acid was added to the mixture. Then, the mixture was centrifuged at 3000 rpm for 10 min, and 2.5 mL of the upper layer solution as mixed with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance of the reaction mixture was measured at 700 nm with a UV-vis spectrophotometer.

Lipid peroxidation inhibitory activity in rat liver homogenate. Rat liver homogenate was prepared from young male rats according to the method as described by Masao *et al.* (Masao *et al.*, 1993). Rats were killed by decapitation and their liver tissues were quickly removed. A 2 g portion of liver tissue was sliced and then homogenized with 10 mL of 150 mM KCl-Tris-HCl buffer (pH 7.2). Protein content was determined by Lowery method (Lowery *et al.*, 1951). According to the method (Yoshiyuki *et al.*, 1981), the reaction mixture was composed of 0.1 mL of liver homogenate, 0.25 mL of Tris-HCl buffer (pH 7.2), 0.05 mL of 0.1 mM ascorbic acid, 0.05 mL of 4 mM $FeCl_2$ and 0.05 mL of various tests fraction. The mixture was incubated at 37°C for 1 h in a capped tube, then 0.5 mL of HCl (0.1 N), 0.2 mL of SDS (9.8%), 0.9 mL of distilled water and 2 mL of TBA (0.6%) were added to each tube and vigorously shaken. The tubes were placed in a boiling water bath (100°C) for 30 min. After cooling, the flocculent precipitate was removed by adding 5 mL of four fractions and centrifuged at 3000 rpm for 25 min, and then the absorbance of the supernatant was measured at 532 nm. MDA (nmol/mg protein) = [ODs · C standard/OD standard · Mp]; where ODs is the A_{532} of sample reactive solution, C standard is any concentration we selected, OD standard is A_{532} according to C standard in standard (TEP) curve and Mp is the mass of protein in the reactive solution.

α-Amylase inhibitory activity. Twenty μL of α-amylase (0.05 U/μL) was premixed with 20 μL of sample and 250 μL of 2% starch solution in 0.1 M sodium phosphate buffer (pH 6.9) was added as a substrate to start the reaction. The reaction was carried out at 37°C for 10 min and terminated by the addition of 200 μL of DNS reagent (1% 3,5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 M NaOH) (Maeda *et al.*, 1985). The reaction mixture was heated for 15 min at 100°C and then diluted with 5 mL of distilled. α-Amylase activity was determined by measuring absorbance at 540 nm. IR = $[1 - (A_i - A_{iB})/(A_0 - A_{0B})] \times 100$; A_i is the A_{540} of sample reactive solution, A_0 is the A_{540} of control reactive solution, A_{iB} is the blank of sample and A_{0B} is the blank of control.

α-Glucosidase inhibitory activity. One hundred microliters of 3 mM *p*-nitrophenyl α-D-glucopyranoside in 0.1 M sodium phosphate buffer (pH 6.9) was added as a substrate to the mixture of 50 μL of α-glucosidase (0.3 unit/mL) and 50 μL of sample to start the reaction. The reaction was conducted at 37°C for 15 min and stopped by the addition of 750 μL of 0.1 M Na_2CO_3. α-Glucosidase activity was assessed by measuring the release of *p*-nitrophenol from *p*-nitrophenyl α-D-glucopyranoside at 400 nm (Kim and Jeong, 2005).

Results and Discussion

Table 1 shows the DPPH radical scavenging ability of the acorn fractions and the commercial standards, α-tocopherol and vitamin C. H_2O fraction showed the highest DPPH radical scavenging activity (EC_{50} = 7.19 μg/mL) which was higher than that of α-tocopherol (EC_{50} = 7.59 μg/mL). EtOAc and CH_2Cl_2 fraction also showed DPPH radical scavenging activity with EC_{50} values of 10.89 μg/mL and 31.78 μg/mL, respectively. While, the EC_{50} values of α-tocopherol and vitamin C were 7.59 μg/mL and 3.42 μg/mL, respectively.

The reducing power ability of the fractions steadily increased by dose-dependent manner (Fig. 1). H_2O and EtOAc fractions showed higher activity than BHT.

Lipid peroxidation of rat liver homogenate was induced by non-enzymatically with ascorbic acid/Fe^{2+} ($FeCl_2$-AA). The H_2O and EtOAc fractions on formation of TBA-RS exhibited strong activity at a concentration of 0.5 mg/mL (Fig. 2). This is comparable to that of α-

Table 1. Antioxidant activity of solvent extracted fractions from acorns evaluated by DPPH radical scavenging ability

Samples	EC_{50} (μg/mL)
Hexane fraction	4599.02
CH_2Cl_2 fraction	31.78
EtOAc fraction	10.89
H_2O fraction	7.19
α-Tocopherol	7.59
Vitamin C	3.42

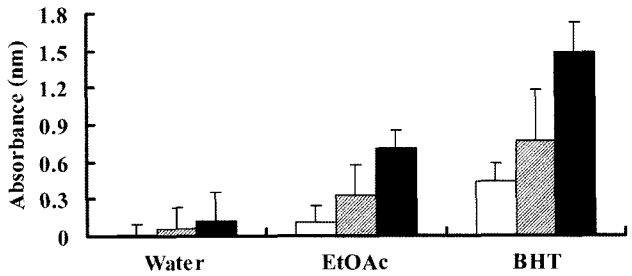

Fig. 1. Reducing power of water fraction and EtOAc fraction from acorn water extract. BHT was positive control (□: 5 μg/mL, ▨: 20 μg/mL, ■: 50 μg/mL).

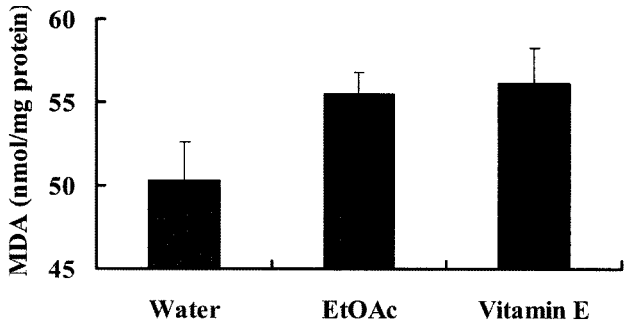

Fig. 2. MDA contents after lipid peroxidation inhibition assay. The concentration of all the fractions is 0.5 mg/mL, α-tocopherol (0.5 mg/mL) was used as a control. Hexane fraction and CH_2Cl_2 fraction have no inhibition effect.

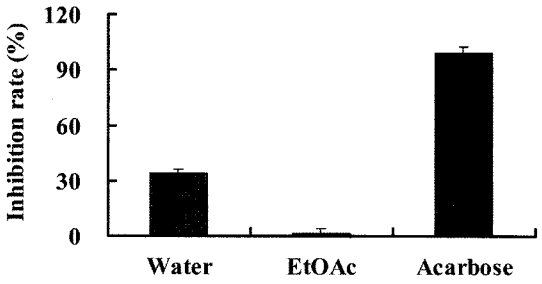

Fig. 3. α-Glucosidase inhibition rate of fractions of acorn. The concentration of all the fractions is 0.5 mg/mL, acarbose (0.5 mg/mL) was used as a positive control. Hexane fraction and CH_2Cl_2 fraction have no inhibition effect.

tocopherol at a concentration of 0.5 mg/mL, which was used as the positive control. These results, the H_2O and EtOAc fractions showed significant anti-lipid peroxidation activities.

H_2O fraction and EtOAc fraction had α-glucosidase and α-amylase inhibitory activity at the concentration of 0.5 mg/mL (Fig. 3; Fig. 4). H_2O fraction inhibited α-glucosidase and α-amylase 33.82% and 10.59% at the concentration of 0.5 mg/mL, respectively. Many drugs have been developed for diabetes, and the best way to control postprandial plasma glucose levels was with medication combined with dietary restrictions and exercise programs (Yki-Jarvinen, 1997). One therapeutic approach to decreasing postprandial hyperglycemia was to retard the absorption of glucose by inhibiting carbohydrate hydrolysing enzymes, for example α-glucosidase, in the digestive organs (Toeller, 1997). Inhibitors of intestinal α-glucosidases (AGIs) have used in the treatment of non-insulin-dependent diabetes mellitus (NIDDM) and represented at the huge proportion of antidiabetic drug market (Inzucchi, 2002).

The total phenolic content of various fractions of acorn was measured by the Folin-Denis method. The phenolic

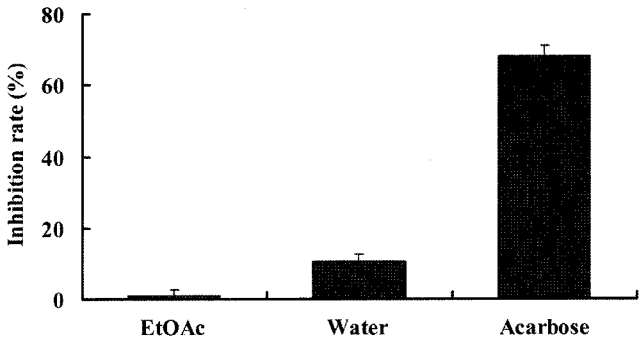

Fig. 4. α-Amylase inhibition rate of fractions from acorn water crude extract. The concentration of all the fractions is 10 mg/mL, acarbose (0.5 mg/mL) was used as a positive control. Hexane fraction and CH_2Cl_2 fraction have no inhibition effect.

Table 2. The yield and total phenolic content of the solvent extracted fractions from water extract of acorn

Samples	Yield (%)	Total phenolic content (μg TA[a]/mg)
Hexane fraction	0.0021	1.21
CH_2Cl_2 fraction	0.1	8.90
EtOAc fraction	0.9	28.40
H_2O fraction	63.7	6.31

[a]Tannic acid (TA) was used as a standard for measuring the total phenolic content.

contents of EtOAc, CH_2Cl_2, H_2O and hexane fractions were 28.41, 8.90, 6.31 and 1.21 TA μg/mg (Table 2). Previously a strong positive correlation has been observed between total polyphenolic content and DPPH radical scavenging activity (Siriwardhana and Shahidi, 2002; Oki *et al.*, 2002). But, here it didn't match between total polyphenolic content and DPPH radical scavenging activity. It may be suggested that some other undiscovered compounds are still remaining in H_2O fraction of acorns.

The results of the various inhibitor assays (including DPPH free radical scavenging, lipid peroxidation scavenging and reducing power assay) revealed that the various fractions from crude H_2O extract of acorn have shown significant antioxidant activity. The H_2O fraction has represented the highest levels of antioxidant activity in different assays. From the results, acorn could be considered as an ingredient of functional foods and pharmaceutical purposes. Furthermore, the identification of biologically active compounds containing antioxidant properties *in vivo* experiments is needed to understand their own actions.

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