Inhibitory Effects of Extracts from Plant Materials on In Vitro Glycation and Oxidation

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Abstract

Extracts from *Aloe vera* leaves, *Aloe arborescens* leaves, *Aloe vera* callus, *Portulaca oleracea* and cacao (*Theobroma cacao* L.) bean husk (CBH) were prepared using acetone, chloroform, ethanol, hexane, and water. Solvent extracts of *Aloe vera* leaf had very high antioxidant activities showing IC$_{50}$ values in the ranges of 0.02-0.17 mg/mL, and had the highest total phenolic and flavonoid content among the tested samples. We hypothesized that *Aloe vera* leaf and CBH extracts might possess considerable in vitro anti-glycation activities. Indeed, these extracts strongly inhibited the formation of advanced glycation end-products from RNase in the presence of ribose. The chloroform extract of *Aloe vera* leaf showed the strongest inhibition of AGE formation (99.9%), followed by the 95% acetone extract (92.8%) at a concentration of 1 mg/mL, exhibiting higher anti-glycation activities than those of AG and rutin (73.4% and 96.1% at 1 mg/mL, respectively). The anti-glycation activity of all extracts was correlated positively with their total contents of phenolics and flavonoids. We conclude that *Aloe vera* leaf extracts and their constituents may be used as anti-glycation agents.

Key words: *Aloe vera* leaf, cacao bean husk, in-vitro anti-glycation activity, antioxidant activity, advanced glycation end-products

Introduction

Glycation is a non enzymatic reaction or Maillard reaction between reducing sugars and proteins (McCance et al., 1993). Advanced glycation end-products (AGEs) from non-enzymatic glycation are believed to participate in the pathogenesis of such microvascular complications as nephropathy, arteriosclerosis, retinopathy, neuropathy, and cataracts in the aged population and people with diabetes mellitus (Vlassara et al., 1994; Brownlee, 2001; Ahmad et al., 2007).

The non-enzymatic glycation can be separated into three main stages: early, middle and late stage (Lapolla et al., 2005). The early stage leads to the formation of reversibly glycosylated proteins (Schiff base) that subsequently rearrange into stable Amadori products. This reaction is non-enzymatic. Formation of the Schiff base occurs over a period of several hours, whereas formation of Amadori products takes days (Brownlee et al., 1988; Lapolla et al., 2005). These reactions are reversible, and the equilibrium is highly dependent on substrate concentrations and incubation time (Bonnefont-Rousselot, 2002). At the middle stage, the Amadori product degrades through an oxidation and dehydration reaction into a variety of dicarbonyl intermediates such as glyoxal, methylglyoxal and deoxyglucosones, which are much more reactive than sugars and react again with free amino groups of proteins (Thornalley, 1996; Lapolla et al., 2005). Methylglyoxal (MGO) is a very reactive intermediate, formed by anaerobic decomposition of triose phosphate intermediates in glycolysis. At the late stage, the products undergo conversion to dicarbonyl intermediates to form advanced glycation end-products (AGEs) (Brownlee et al., 1988).

Aminoguanidine (AG) is a well-known inhibitor of the formation of AGEs. Among various inhibitors of advanced protein glycation, AG is one of the most promising compounds (Bucala et al., 1995). AG, a nucleophilic hydrazine, reacts with Amadori fragmentation products (Edelstein & Brownlee, 1992). Its beneficial effect has been demonstrated in vitro and in vivo in animal models, with a significant reduction in the AGE levels in blood and tissues (Souli et al., 1996; Kelly et al., 2001). AG significantly has decreased the formation of AGE-hemoglobin, and was effective in inhibition of the inducible form of NO-synthase. However, administration of AG in therapy is necessary to consider carefully because of its possible toxic and prooxidative effects (Ou and Wolff, 1993).
Therefore, screening of novel AGE inhibitors from natural sources seems feasible. Diet rich in fruit and vegetables protects against degenerative diseases due to the presence of bioactive substances that exert specific actions on biological targets, including anti-glycation activity (Bousova et al., 2005; Ardestani & Yazdanparast, 2007a; Hsieh et al., 2007). Compounds with antioxidant capacity such as polyphenols have been proven to exert anti-glycation effects at physiological concentrations (Kim & Kim, 2003; Lunceford & Gugliucci, 2005; Rudnicki et al., 2007).

Therefore, the plants might offer a new source of glycation inhibition agents. So far, there have been no reports on the inhibition of protein glycation by the extracts from Aloe vera leaf, Aloe arborescens leaf, Aloe vera callus, Portulaca oleracea and cacao (Theobroma cacao L.) bean husk. This study aimed to evaluate and compare the anti-glycation capacities of extracts from these five materials with that of AG as a positive control. Correlation between the contents of phenolics and flavonoids and anti-glycation activity was also analysed to identify the components responsible for the activity.

Materials and Methods

Chemicals and materials

Aluminum chloride was purchased from Jusei Chemical Co., Ltd. (Tokyo, Japan). All reagents, unless otherwise stated, were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Cacao bean husk (CBH) was supplied by Lotte Groups R&D Center (Seoul, Korea); Aloe vera and Aloe arborescens leaves were supplied by KJM Aloe Co. Ltd. (Seoul, Korea). Aloe vera callus at maintained at New Biomaterials Lab of Kangwon National University (Chuncheon, Korea). Portulaca oleracea was purchased from a local market (Herbal Love, Seoul, Korea). CBH was supplied by Lotte Group R&D Center (Seoul, Korea) and defatted by extraction with ethyl ether in a Soxhlet apparatus for 12 h. All raw materials were dried and grinded to 80 mesh.

Preparation of samples

Samples were extracted with hot water, cold water or 60% EtOH solution. Additionally, Aloe vera leaves were extracted with 60% acetone (v/v), 95% acetone (v/v), hexane, chloroform, chloroform : EtOH (3:1, v/v) or 95% EtOH.

Briefly, powdered samples (30 g) were extracted twice for 2 h with 300 mL of cold (30°C) or hot water (95°C), and centrifuged (5000 x g, 15 min). The supernatants were concentrated in a rotary vacuum evaporator (N-N Series, EYELA Co., Tokyo, Japan) and evaporated to dryness under vacuum. Powdered samples were extracted with solvents at a ratio of 60 mL/g for 3 h at room temperature three times, followed by filtration through filter paper (Whatman No. 4, Springfield Mill, Huddersfield, UK). The filtrates were combined and concentrated in a rotary vacuum evaporator until dryness. Samples were kept in a desiccator until use. Depending on the assay, the extracts that were insoluble in appropriate buffer were first dissolved in methanol and then diluted to different concentrations needed for a particular assay.

Dot-blot assay using DPPH staining

Dot-blot DPPH staining was used to assess rapid screening of antioxidation, and was determined according to the method described by Soler-Rivas et al. (2000). Extracts were dissolved in water or methanol; 5-µL aliquots were applied on Merck Silica gel F254 plates and allowed to dry for a few minutes. Ascorbic acid was used as a positive control. A 0.4 mM DPPH solution in methanol was sprayed on the plates until they were evenly covered. The excess solution was removed with tissue paper. Stained silica layer revealed a purple background with spots indicating radical scavenger activity. The intensity of the yellow color depends on the amount and nature of the radical scavenger present in the sample.

Radical scavenging activity

DPPH radical scavenging activity of the extracts was determined using the method described by Choi et al. (2002). Test solutions were prepared by adding 1 mL of a 0.3 mM DPPH solution in ethanol to samples (2.5 mL) diluted to different concentrations. To prepare blank solutions, 1 mL of ethanol was used instead DPPH. To prepare a negative control, ethanol was used instead of samples. As DPPH is sensitive to light, solutions were protected from light as much as possible. The reactions were allowed to proceed at room temperature for 30 min. The absorbance was measured at 518 nm (Spectronic Genesys-5TM spectrophotometer, NY, USA) and converted into the percentage antioxidant activity using the following equation:

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\text{Scavenging activity (\%)} = 100 - (\text{sample blank/control}) \times 100
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Inhibition of lipid peroxidation

TBA-RS (thiobarbituric acid-reactive species) assay was used to assess lipid peroxidation of the extracts and was