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Evaluation of Antioxidant Potential of *Aloe vera* (*Aloe barbadensis* Miller) Extracts

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The polysaccharide and flavonoid concentrations of two-, three-, and four-year-old *Aloe vera* were determined, and their antioxidant activities were evaluated compared to BHT and α-tocopherol by the DPPH radical scavenging method and the linoleic acid system at 100 μg of soluble solids per mL of ethanol. The results showed that three-year-old *Aloe vera* contained significantly higher levels of polysaccharides and flavonoids than two- and four-year-old *Aloe vera*, and no significant differences in flavonoid levels were found between three- and four-year-old *Aloe vera*. All the aloe extracts showed significant antioxidant activity. The antioxidant activity of *Aloe vera* extracts and reference compounds followed the order: three-year-old *Aloe vera* > BHT > four-year-old *Aloe vera* > α-tocopherol > two-year-old *Aloe vera*. The three-year-old extract exhibited the strongest radical scavenging activity of 72.19%, which is significantly higher than that of BHT at 70.52% and α-tocopherol at 65.20%. These data suggest that the growth stage plays a vital role in the composition and antioxidant activity of *Aloe vera*.

KEYWORDS: ***Aloe vera*; growth period; extract; antioxidant activity**

INTRODUCTION

There is growing interest in the use of natural antioxidants for expanding the shelf life of food without the need for synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butyhydroquinone (TBHQ). These food additives used by the food industry to prevent lipid peroxidation have been reported to possess possible toxic and carcinogenic effects on health (*1*). Thus, efforts have been made to search for novel natural antioxidants from tea, fruits, vegetables, herbs, and spices. *Aloe vera* products have long been used in health foods for medical and preservative purposes (*2*). There are more than 360 different species of aloes grown in the dry regions of North American, Europe, and Asia. *A. barbadensis* Miller (*A. vera* Linne) is the most widely used both commercially and for its therapeutic properties. Studies on the physiological function of the extracts of *Aloe vera* have been widely reported. The positive influence of *Aloe vera* aqueous extracts has been reported on the healing of full thickness wounds in diabetic rats (*3*). The organic extract of *Aloe vera* leaves provided antiinflammatory activity in the experimental rat (*4*). The medicinal properties were attributed to the active components in *Aloe vera* and its extracts, such as anthrone, chromone, aloe verasin, and hydroxyaloin separated and identified by high-performance liquid chromatography (*5*). Glycoprotein aloctin A is reported to have antitumor and antiulcer effects (*6*). Glucmannan and acemannan can accelerate wound healing, activate macrophages, and demonstrate antineoplastic, antiviral effects (*7*−*9*). It was of interest to determine effects of antioxidant activity in vitro and in vivo in whole *Aloe vera* leaf extracts and their physiological effects in biological systems. A potent antioxidative compound was isolated from a methanolic extract of *A. vera barbardenis* Miller (*10*). The substance demonstrated equal antioxidant activity to that of α-tocopherol assessed in vitro using rat brain homogenate. *Aloe vera* also protected against prooxidant-induced membrane and cellular damage by a significant reduction in the levels of cytochrome P_{450} and cytochrome b_5 (*11*). Ethanolic extracts of fresh *Aloe vera* juice possessed stronger radical scavenging activity than that of *Aloe vera* powder, and the antioxidative effect of *Aloe vera* extracts was correlated to its development stage (*12*). The objective of this study was to determine and compare the antioxidant activity of *Aloe vera* of different growth periods and their extracts with BHT and α-tocopherol.

MATERIALS AND METHODS

Chemicals and Materials. The following chemicals were used: Linoleic acid (ca. 99%) (Wako Chemical Pure Chemical Industries Ltd., Osaka, Japan); α,α-diphenyl-β-picrylhydrazyl (DPPH); α-tocopherol (Sigma Chemicals Co., St. Louis, MO); butylated hydroxytoluene (BHT); ammonium thiocyanate; and ascorbic acid (Nanjing Chemical Industry, Nanjing, China). Other reagents were of analytic reagent grade.

Aloe Vera. The whole fresh leaves of *Aloe vera* L. (*A. barbadensis* Miller) of two-, three-, and four-year-old plants were collected from Institute of New Century Horticulture of Nanjing, Jiangsu Province.

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Extraction and Preparation for Antioxidant Activity Assay. The whole leaves of *Aloe vera* were cut into thin pieces, put onto a glass plate, lyophilized in vacuo for 2 days, and then ground into a fine powder for further use. Fifty milliliters of 80% ethanol (v/v) was added to 1 g of lyophilized *Aloe vera* powder in a round flask, the sample was sonificated for 30 min and then filtered, and the residue was washed twice with 10 mL of ethanol. The filtrate was combined onto a flask that was weighed previously with a constant weight and concentrated to dryness at 30 °C by rotary evaporation in vacuo. Then, the flask containing the dried ingredients was weighed, and the weight difference between the empty and sample flask was recorded as the mass of solid in the *Aloe vera* extracts.

Preparation of Standard Solutions of Aloe Extracts and Other Reference Antioixidants. Dehydrated *Aloe vera* extracts were dissolved in 75% ethanol (v/v) with a final concentration of 100 mg of solid L^{-1} and stored in a refrigerator for further use. BHT and α-tocopherol were prepared at the same concentrations (100 mg L^{-1}) as the aloe standard solution.

Determination of Polysaccharide in Lyophilized *Aloe vera* Powder. The carbohydrate composition of polysaccharide in the extracts was estimated using a colorimetric assay. One gram of *Aloe vera* powder was extracted with 80 mL of water by vortex stirring at 100 °C for 2 h and then the sample was filtered in vacuo. The filtrate was diluted to a 100 mL volume in a volumetric flask. Two milliliters of solution and 10 mL of ethanol were added into a plastic tube, the sample was centrifuged at 2500 r min^{-1} for 30 min, and then the supernatant was removed; the precipitate was dissolved in a final volume of 100 mL in water. One milliter of water, 1 mL of 4% (w/v) phenol, and 1.0 mL of concentrated sulfuric acid were added into a screw-capped test tube and the sample was kept in a constant temperature water bath at 40 °C for 30 min. After the samples were frozen, the absorbance of thawed samples was determined at 490 nm (*13*). The content of polysaccharide was estimated by comparison with a standard curve generated from the analysis of glucose.

Determination of Flavonoid Content in the Extracts of Lyophilized *Aloe vera* Powder. The flavonoid content of aloe extracts was measured on the basis of an aluminum trichloride colorimetric assay (*14*). The absorbance recorded at 420 nm was used to calculate the content of flavonoid, and the results were expressed as flavonoid glycoside equivalents per kilogram of ethanol soluble solids (1.00 absorbance equivalents, 320 μg of flavonoid glycoside).

Assay of DPPH Radical Scavenging Activity. The antioxidant activities of *Aloe vera* extract solutions, BHT, α-tocopherol were measured in terms of their hydrogen donating or radical scavenging ability, using the stable radical DPPH (*14*). A total of 2 mL of 100 μg mL^{-1} BHT or α-tocopherol, or an 80% ethanol (v/v) solution of *Aloe vera* extract was placed in a cuvette, respectively, and 2 mL of a 2×10^{-4} mol L^{-1} ethanol solution of DPPH• was added. Absorbance measurements commenced immediately. The decrease in absorbance at 517 nm was determined continuously at every 5-min intervals using a spectrophotometer until the absorbance reached a steady state (after nearly 120 min). All the determinations were performed in triplicate. The inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (*15*).

Determination of Antioxidant Activity with the Ferric Thiocyanate (FTC) Method. Two milliters of 100 mg L^{-1} aloe extract solution, 2 mL of 2.51% (w/v) linoleic acid in ethanol, 4 mL of 0.05 mol L^{-1} of phosphate buffer (pH 7.0), and 2 mL of distilled water were mixed in a 10-mL vial with a screw cap and then kept in a 40 °C water bath in the dark. A blank sample was prepared using 2 mL of 75% ethanol added to linoleic acid in ethanol, phosphate buffer (pH 7.0), and distilled water. A total of 0.1 mL of the above mixture was added to 9.7 mL of 75% (v/v) ethanol and 0.1 mL of 30% (w/v) ammonium thiocyanate. After 5 min, 0.1 mL of 0.02 mol L^{-1} ferrous chloride in 3.5% (v/v) hydrochloric acid was added to the above mixture and mixed. The absorbance of the mixture was measured at 500 nm every 24 h for one week. The ferric thiocyanate (FTC) method was described in detail by Kikuzaki (*16*).

Statistics Analysis. The data were presented as mean ± standard deviation of three determinations. Statistical analyses were performed using a one-way analysis of variance. Multiple comparisons of means were done by the least significant difference (LSD) test. A probability value of <0.05 was considered significant. All computations were made by employing the statistical software (SPSS, version 11.0).

Table 1. Polysaccharide and Flavonoids Content of Different Growth Stages of *Aloe vera* L.[a]

Aloe vera L. samples	polysaccharide (g kg^{-1})	flavonoids (g kg^{-1})
two-year-old	3.82 ± 0.15^{a}	3.63 ± 0.38^{a}
three-year-old	6.55 ± 0.75^{b}	4.70 ± 0.48^{b}
four-year-old	4.10 ± 0.11^{c}	4.26 ± 0.18^{b}

[a] Values are means of three determinations ± standard deviation. Values followed by different letters are different ($P < 0.05$) from one another.

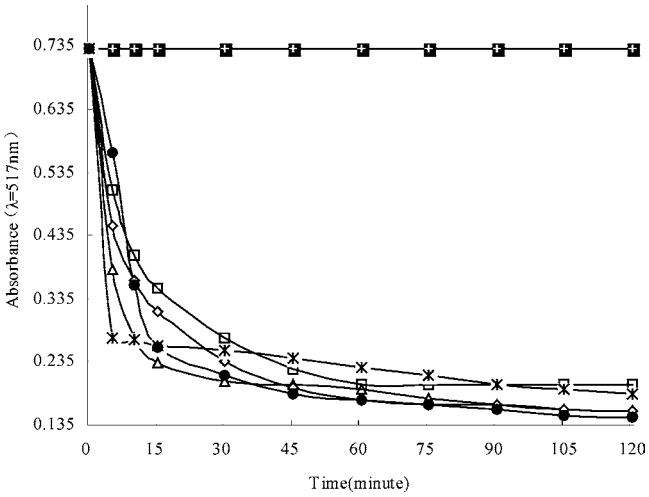

Figure 1. Kinetics behavior of radical scavenging activity of aloe extracts of different growing stages as assessed by the DPPH• method compared to BHT and α-tocopherol at 100 μg of soluble solids per mL of ethanol. Solid square with white cross, blank; □, two-year-old *Aloe vera*; △, three-year-old *Aloe vera*; ◇, four-year-old *Aloe vera*; ●, BHT; ∗, α-tocopherol.

RESULTS AND DISCUSSION

Determination of the Contents of Polysaccharide in *Aloe vera* L. The contents of active constituents in *Aloe vera* are shown in **Table 1**. The three-year-old *Aloe vera* had a 4.70 g kg^{-1} flavonoid content, which is significantly higher than that of two-year-old *Aloe vera* at 3.63 g kg^{-1}. The three-year-old *Aloe vera* possessed significant higher contents of polysaccharide and flavonoids than those of two- and four-year-old *Aloe vera*.

DPPH Radical Scavenging Activity. The decrease in absorbance of the DPPH radical due to the scavenging capability of the 80% ethanol (v/v) extracts of *Aloe vera* at different development stages at 100 mg soluble solids L^{-1} is illustrated (**Figure 1**). All the extracts showed a rapid decrease in absorbance, with extracts of three-year-old *Aloe vera* exhibiting the fastest scavenging rate, while the extracts of two-year-old samples showed the slowest rate over the first 10 min.

The radical scavenging activity of *Aloe vera* extracts and other antioxidants followed the order over the first 10 min of the assay: two-year-old < four-year-old < BHT < three-year-old < α-tocopherol. Compared to the selected antioxidants and *Aloe vera* in other growth phases, two-year-old *Aloe vera* exhibited the slowest scavenging effect on DPPH. There was a little change in the order of antioxidant activity of *Aloe vera* extracts and their contrasts during the last 110 min: two-year-old < α-tocopherol < four-year-old < BHT < three-year-old.

Table 2. Antioxidant Activities of Different Growth Stages of *Aloe vera* L. as Assessed by the DPPH Radical Scavenging Method[a]

samples	inhibition^A (%)[b]	inhibition^B (%)
two-year-old	51.92 ± 0.45[a]	62.70 ± 0.44[a]
three-year-old	67.95 ± 0.99[b]	72.19 ± 0.98[b]
four-year-old	56.85 ± 3.42[c]	67.64 ± 2.99[c]
BHT	64.60 ± 1.12[d]	70.52 ± 0.89[d]
α-tocopherol	64.20 ± 1.55[d,e]	65.20 ± 1.32[c,e]

[a] Values are means of three determinations ± standard deviation. Values followed by different letters are different ($P < 0.05$) from one another. [b] Inhibition^A and inhibition^B represent the inhibition percentage counted at the 16th and 30th min, respectively.

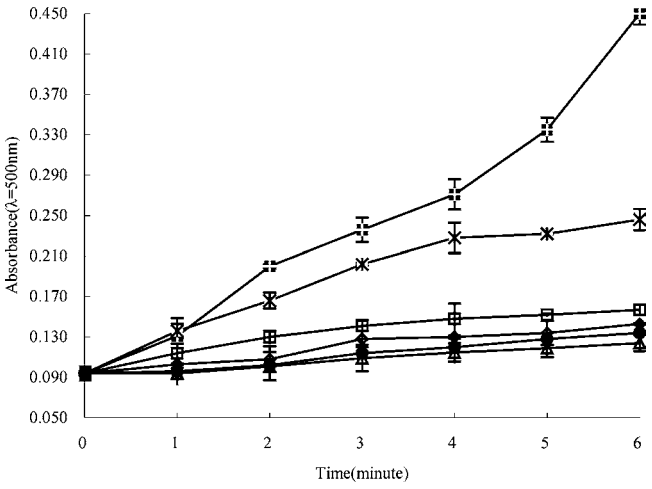

Figure 2. Antioxidant activity of different *Aloe vera* extracts compared to BHT and α-tocopherol at 100 μg of soluble solids per mL of ethanol as assessed by linoleic acid. Solid square with white cross, blank; □, two-year-old *Aloe vera*; △, three-year-old *Aloe vera*; ◇, four-year-old *Aloe vera*; ●, BHT; ∗, α-tocopherol.

The same trends were also present in the percentage of inhibition of the extracts of *Aloe vera* and other antioxidants in the DPPH assay (see **Table 2**). At the 16th min, the inhibition percentage followed the order: three-year-old (67.95%) > BHT (64.60%) > α-tocopherol (64.20%) > four-year-old (56.85%) > two-year-old (51.92%). Significance differences were observed among the values of the percentage of inhibition of all the samples.

Antioxidant Activity of Aloe Vera Ethanolic Extracts Assessed by the Linoleic Acid System Method. To evaluate the antioxidant potential of *Aloe vera* extracts, their lipid inhibitory activities were compared with selected standard antioxidants BHT and α-tocopherol by using the ferric thiocyanate method of measuring the amount of peroxides formed in emulsion during incubation. High absorbance is an indication of a high concentration of formed peroxides. Similar results were obtained from this method as compared to the DPPH radical scavenging method (**Figure 2**). The absorbance of linoleic acid emulsion without the addition of *Aloe vera* extracts or antioxidants increased rapidly, and there was a significant difference between the blank and antioxidants at the $P < 0.011$ level (data not shown). As can be seen in this figure, *Aloe vera* extracts and antioxidants in the linoleic acid emulsion were able to reduce the formation of peroxides. The lipid inhibition capability of three-year-old *Aloe vera* extracts was significantly higher than the other two *Aloe vera* extracts and α-tocopherol, whereas no notable difference was present between three-year-old *Aloe vera* extracts and BHT ($P < 0.05$).

On the basis of the similar results from two assays, three-year-old *Aloe vera* provided a higher antioxidant effect than that of two- and four-year-old *Aloe vera*. It suggests that *Aloe vera* of various development stages contains different active components and possesses antioxidant activity to different degrees. However, few studies report on the components in *Aloe vera* extract responsible for the antioxidant activity. Polysaccharides from *Asparagus cochinchinensis*, *Sedum telephium l.* leaves, Tongcao were reported to exhibit strong scavenging activity and inhibition activity on lipid oxidation by in vitro and in vivo assay of antioxidant activity (*18*−*21*). Flavonoids from *Salvia officinalis l.*, *Thymus vulgaris l.*, and *Licania licaniaeflora* were also reported to be potent antioxidants (*22*, *23*), and their antioxidant effect was dose-dependent. In our studies, three-year-old *Aloe vera* contained significantly higher levels of polysaccharides and flavonoids (**Table 1**) than the other aloe extracts. It was postulated that unequal contents of active components in *Aloe vera* of different growing stages resulted in their varying antioxidant activities. More studies are needed to clarify the antioxidant mechanisms of the *Aloe vera* constituents and the effect of the growth period on *Aloe vera* antioxidant activity. On the basis of the above results, it was observed that *Aloe vera* extracts provided equivalent or higher antioxidant activity as compared to BHT and α-tocopherol, which provides a way to screen antioxidants from *Aloe vera* for foods, cosmetics, and medicine.

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