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Organic acid, phenolic content and antioxidant activity of wild yam (*Dioscorea* spp.) tubers of Nepal

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Abstract

Four wild yam (*Dioscorea* spp.) tubers available in Nepal were analyzed for their individual organic acids, total phenolic contents and antioxidant activities. Succinic acid was predominant with an average value of 1316 mg/100 g fresh weight (FW). Citric acid was the second most abundant, with an average value of 274 mg/100 g FW. Average values recorded for malic and oxalic acids were 147 and 110 mg/100 g FW, respectively. Total polyphenol content (as phenol) ranged from 13 to 166 mg/100 g FW. Wild yam tubers were found to have significant antioxidant activities, as evaluated by different methods such as DPPH free radical scavenging, ferrous ion chelating, reducing power and total antioxidant activity tests. Consumption of fresh yam tubers may thus serve as a good source of antioxidant in its natural form; and may have role in prevention of human diseases, in which free radicals are involved, such as cancer and cardiovascular diseases.

Keywords: Wild yam; *Dioscorea*; Organic acids; Phenolic content; Antioxidant activity

1. Introduction

Yam tubers of *Dioscorea* genus are major staple food crops in tropical and subtropical regions, annual world production being around 20 million tones (Ozo, Caygill, & Coursey, 1984). The global distribution of yam species varies greatly from Africa to Asian regions in genotype, wild and cultivated species (Coursey, 1967). Wild yams, known locally as "Githa-vyakur", are native wild plants in Nepal. Roots and tubers of yams have been used since pre-historic times by the aboriginal people, as a food, as well as traditional medicines (Singh, 1960). These tubers are found to be rich in essential dietary nutrients (Bhandari, Kasai, & Kawabata, 2003).

Numerous studies have shown that yams are sources of diverse nutrients and non-nutrient molecules, many of which display bioactive properties. Some examples of such non-nutrient molecules in yam tubers are organic acids and polyphenols. Organic acids are widely distributed in fruits and vegetables. The content of organic acids in food not only influences their flavor, but also their stability, nutrition, and acceptability (Poyrazoglu, Gokmen, & Artik, 2002). Holloway, Argall, Jealous, Lee, and Bradbury (1989) have reported the principal organic acids in some common root crops, including yams.

Phenolic compounds, widely existing in plants, are important for their contribution to colour, sensory attributes and nutritional and antioxidant properties of foods (Maga, 1978). Phenolic compounds are reported to have multiple biological effects, including antioxidant activity, antitumor, antimutagenic and antibacterial properties (Shui & Leong, 2002). Yam tubers show strong enzymatic browning reactions when cut and exposed to the air. This browning has been attributed to the oxidation of phenolic compounds (Ozo et al., 1984). Farombi, Britton, and Emerole (2000) reported that yam tubers contain polyphenols, such as catechins, chlorogenic acids, proanthocyanidinis, and anthocyanins. The total phenolic content and the presence of various phenolic compounds have been reported in some species of domesticated yam tubers (Martin & Ruberte, 1976; Muzac-Tucker, Asemota, & Ahmad, 1993; Ozo et al.,

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1984), but very scarce data on the quantity of phenolics and antioxidant activity have been reported.

To the authors' knowledge, there are no published reports on phenolic contents and antioxidant activities of wild yam tubers of Nepal. Similarly, no research has been done to understand the organic acid compositions of these tubers. Due to the lack of such information, we have investigated the levels of major organic acids, phenolic contents and antioxidant potentials of four wild yam tubers of Nepal.

2. Materials and method

2.1. Materials

The four species of wild yam (*Dioscorea bulbifera*, *D. versicolor*, *D. deltoidea* and *D. triphylla*) were collected from the central region (Narayani Zone) of Nepal. Yam tubers were weighed, peeled, fractionated into little pieces, and dried at 40°C in a hot air oven to constant weight. The dried samples were ground to fine powder by using an electric grinder, and chemical analyses were carried out on the flours.

2,2-Diphenyl-1-picryl-hydrazyl (DPPH) was purchased from Wako Pure Chemicals Industries Ltd. (Japan), ferrozine(3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine, monosodium salt), ABTS (2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid, diammonium salt), and peroxidase (Type II: from Horseradish) from Sigma-Aldrich Co., USA whereas $FeCl_2 \cdot 4H_2O$. Potassium hexacyanoferrate was purchased from Merck, Darmstadt, Germany. All other chemicals and reagents used were of analytical grade, and were purchased from Wako Pure Chemicals Industries Ltd. (Japan).

2.2. Analysis of organic acids

The organic acids were determined by HPLC, using the extraction and analysis method developed by Holloway et al. (1989). A 7.8×300 mm ion exclusion column (HPX-87H, Bio-Rad) was used with 0.0125 M H_2SO_4 as mobile phase, at a flow rate of 0.5 ml/min, and the UV detector operating at 214 nm. Organic acids were quantified by an internal standard calibration method (Macrae, 1988) by using glutaric acid as the internal standard. The calculated results are expressed as milligrammes per 100 g FW.

2.3. Analysis of total polyphenolic content

The total polyphenolic compounds were extracted from yam powder as described by Julkunen-Tiitto (1985). Yam powder (5 g) was extracted with acetone (300 ml) using the Soxhlet apparatus for about 20 hours. The extract was concentrated to 30–40 ml under the reduced pressure. The concentrate was refluxed for a few seconds, filtered while hot, and diluted to 100 ml with distilled water. The total phenolics were determined spectrophotometrically in the cooled filtrate by Folin-Ciocalteu phenol method, as described by Muzac-Tucker et al. (1993) using phenol as a standard. Total polyphenolic contents were expressed as mg phenol per 100 g FW.

2.4. Preparation of yam extracts

Yam flour (1 g) and methanol (20 ml) were mixed and kept in a rotary shaker overnight, and then filtered (Whatman No. 1 filter paper). The filtrate was made to 25 ml with methanol, and stored at −20 °C until further use. This procedure was that of Hsu, Chen, Weng, and Tseng (2003). The methanolic yam extract (40 mg/ml) was diluted to suitable concentrations: 2, 4, 6, 8 and 10 mg/ml were used for the DPPH radical scavenging test, whereas 10, 20, 30 and 40 mg/ml were used for total antioxidant activity, ferrous ion chelating and reducing power test.

2.5. Test of DPPH free radical scavenging effect

The scavenging of DPPH radical was carried out according to the method described by Hsu et al. (2003). Aliquots of 1 ml methanolic yam flour extract and 5 ml of freshly prepared 0.1 mM DPPH methanolic solutions were thoroughly mixed, and kept for 50 min in the dark. The absorbance of the reaction mixture at 517 nm was read with a spectrophotometer (UV-1600, Shimazdu, Japan). Methanol (1 ml), replacing the extract, was used as the blank. The percentage of free radical scavenging effect was calculated as follows:

$$\text{Scavenging effect } (\%) = [1 - (A_{517\text{ nm, sample}}/A_{517\text{ nm, blank}})] \times 100.$$

2.6. Test of ferrous ion chelating capacity

The method described by Hsu et al. (2003) was used to determine the ferrous ion chelating activities of yam flours. One ml of methanolic yam flour extract, 0.1 ml of 2 mM $FeCl_2 \cdot 4H_2O$, 0.2 ml of 5 mM ferrozine (3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine) and 3.7 ml of methanol were mixed in a test tube, and were reacted for 10 min. The absorbance at 562 nm was measured; a lower absorbance indicated a higher ferrous ion chelating capacity, which was calculated as follows:

$$\text{Ferrous ion chelating capacity } (\%) = [1 - (A_{562\text{ nm, sample}}/A_{562\text{ nm, control}})] \times 100.$$

2.7. Test of reducing power

The reducing power of yam flours was measured according to the method described by Hsu et al. (2003).

One ml yam extract, 0.5 ml of phosphate buffer (0.2 M, pH 6.6), and 2.5 ml of potassium hexacyanoferrate solution (1% v/w) were placed in a test tube, and reacted for 20 min at 50 °C. The tubes were cooled immediately by using crushed ice and an aliquot of 0.5 ml trichloroacetic acid (10%) was added in. After centrifugation at 3000*g* for 10 min, an aliquot (1 ml) of supernatant was mixed with 1 ml distilled water and 0.1 ml ferric chloride (0.1%), and reacted for 10 min. Then, the absorbance at 700 nm was measured with a spectrophotometer. Increased absorbance of the reaction mixture indicated an increased reducing power.

2.8. Test of total antioxidant activity

Total antioxidant activity was measured according to the method described by Hsu et al. (2003). Exactly 0.2 ml of peroxidase (4.4 units/ml), 0.2 ml of H_2O_2 (50 μM), 0.2 ml of ABTS (2,2-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid, diammonium salt, 100 μM) and 1.0 ml distilled water were mixed, and were kept in the dark for 1 h to form a bluish-green complex. After adding 1.0 ml of methanolic yam flour extract, the absorbance at 734 nm was measured to represent the total antioxidant activity. The total antioxidant activity was calculated as follows:

$$\text{Total antioxidant activity } (\%) = [1 - (A_{734\text{ nm, sample}}/A_{734\text{ nm, control}})] \times 100.$$

3. Results and discussion

3.1. Organic acids

Organic acids are important for their contribution to sensory attributes in fruits and vegetables. Among the organic acids, oxalic, citric, malic and succinic acids are considered to be major organic acids in yam tubers (Holloway et al., 1989). The concentrations of these individual organic acids in wild yam tubers are given in Table 1. Succinic acid was determined to be a prominent organic acid in all four species of wild yam. Its concentrations ranged between 119 and 2510 mg/100 g FW, with an overall mean concentration of 1316 mg/100 g FW. Citric acid was determined to be the second most abundant organic acid in these samples. Citric acid concentration ranged between 222 and 337 mg/100 g FW, with an overall mean concentration of 274 mg/100 g FW. Both malic and oxalic acids were determined in considerable amounts in all yam species. The overall mean concentrations of malic and oxalic acids were found to be 147 and 110 mg/100 g FW, respectively. Our results clearly showed a wide variation in concentrations of individual organic acid in different wild yam species. It is very difficult to compare these results, because very scarce data on quantities of organic acids in yam tubers have been reported. However, these results are in agreement with that of the study conducted by Holloway et al. (1989), who reported that the amounts of organic acids were variable from one cultivar to another, of the same root crop, and from one root crop to another. The organic acid composition of fruits and vegetables is not easily predictable and is largely dependent on growing regions, climates and the varieties (Poyrazoglu et al., 2002).

Table 1
Organic acids composition of yam tubers (mg/100 g FW)[a]

Yam species	Organic acids			
	Oxalic	Citric	Malic	Succinic
D. bulbifera	67 ± 9	282 ± 24	266 ± 20	2510 ± 108
D. versicolor	70 ± 15	256 ± 26	115 ± 18	330 ± 7
D. deltoidea	197 ± 25	222 ± 31	139 ± 10	2305 ± 88
D. triphylla	104 ± 24	337 ± 19	69 ± 20	119 ± 5
Overall mean	110	274	147	1316

[a] Values are mean ± SD ($n = 6$).

3.2. Polyphenol content

Phenolics are aromatic secondary plant metabolites, and are widely spread throughout the plant kingdom. Phenolics have been associated with colour, sensory qualities, and nutritional and antioxidant properties of food (Robbins, 2003). The total polyphenolic contents in four species of wild yam tubers are given in Table 2. The wild yam tubers were found to have widely varying levels of phenolics, ranging from 13 to 166 mg phenol/100 g FW. Our results are consistent with the reports of Muzac-Tucker et al. (1993) and Kaur and Kapoor (2002). *Dioscorea bulbifera* had the highest (166 mg/100 g FW) content of total polyphenols, followed by *D. versicolor* (41 mg/100 g FW) and *D. triphylla*, which had the lowest level (13 mg/100 g FW). The presence of various phenolic compounds has been reported in some species of yam tubers (Ozo et al., 1984; Martin et al., 1976). However, in this study, only total polyphenolic content has been analyzed and the various kinds of polyphenol have not been examined. Kirakosyan et al. (2003) reported that phenolic compounds in plants

Table 2
Total polyphenol content of yam tubers (as phenol, mg/100 g FW)[a]

Yam species	Browning nature[b]	Polyphenol content
D. bulbifera	+ + +	166 ± 10
D. versicolor	+ +	41 ± 5
D. deltoidea	+	15 ± 2
D. triphylla	+	13 ± 1
Overall mean		58

[a] Values are means ± SD, ($n = 4$).
[b] Browning nature, +++: very fast; ++: medium; +: slow.

possess antioxidant activity, and may help protect cells against the oxidative damage caused by free radicals. The present investigation shows that wild yam tubers contain considerable amount of phenolics, and this implies that these tubers may be useful in relation to diseases involving free radical reactions.

The phenolic contents of yam are the substrates responsible for the browning reaction, which occur when the tubers are cut or damaged (Muzac-Tucker et al., 1993). The phenolic levels in these wild yam tubers are proportional to the rates of browning; those were observed when the tubers were cut during handling (Table 2); *D. bulbifera*, with a high level of phenolics, showed fast and strong browning compared to other species.

3.3. Antioxidant activities of yam extracts

The importance of antioxidant constituents of plant materials in maintaining health and in protecting against coronary heart diseases and cancer is raising interest among scientists, food manufacturers, and consumers, as the trend of the future is moving towards functional food with specific health effects (Kahkonen et al., 1999). The phenolic compounds have been reported to have multiple biological effects, including antioxidant activity. The presence of different antioxidant components in plant tissues, especially fruits and vegetables, makes it relatively difficult to measure each antioxidant component separately. Therefore, several methods have been developed, in recent years, to evaluate the antioxidant activity of biological samples (Kaur & Kapoor, 2002). We evaluated our samples by DPPH radical scavenging, ferrous ion chelating, reducing power, and total antioxidant activity tests.

The DPPH radical scavenging effects of yam extracts are presented in Fig. 1. All the studied yam species showed appreciable free radical scavenging activities. *D. bulbifera* had the strongest radical scavenging activity, compared to other yam species. However, *D. triphylla* showed the lowest radical scavenging activity. A dose–response relationship was found in the DPPH radical scavenging activity; the activity increased as the concentration increased for each individual yam species. The involvement of free radicals, especially their increased production, appears to be a feature of most, if not all human disease, including cardiovascular disease and cancer (Deighton, Brennan, Finn, & Davies, 2000). Therefore, such dietary antioxidants from wild yams may be particularly important in fighting these diseases by conferring protection against free radical damage to cellular DNA, lipids and proteins.

Ferrous ion, commonly found in food systems, is well known as an effective pro-oxidant (Hsu et al., 2003). Polyphenols can chelate pro-oxidant metal ions, such as iron and copper, thus preventing free radical formation from these pro-oxidants (Kris-Etherton et al., 2002). The purpose of the test of ferrous ion chelating activity was to determine the capacity of yam tubers to bind the ferrous ion catalyzing oxidation. The ferrous ion chelating effect of yam extract is presented in Fig. 2. *Dioscorea triphylla* had the highest ferrous ion chelating capability, compared to other yam species. A dose–response relationship was also found in the ferrous ion chelating activity.

The reducing power has been used as one of the antioxidant capability indicators of medicinal herbs (Duh & Yen, 1997). Fig. 3 shows the reducing power of the yam extracts by using the potassium hexacyanoferrate reduction method. All yam extracts exhibited a high reducing activity. However, *D. deltoidea* had a higher reducing power than other wild yam species. Although *D. triphylla* showed a higher ferrous ion chelating activity, it was weaker in reducing power among the yam species studied. Interestingly, *D. bulbifera* with the highest free radical scavenging activity (Fig. 1) had a

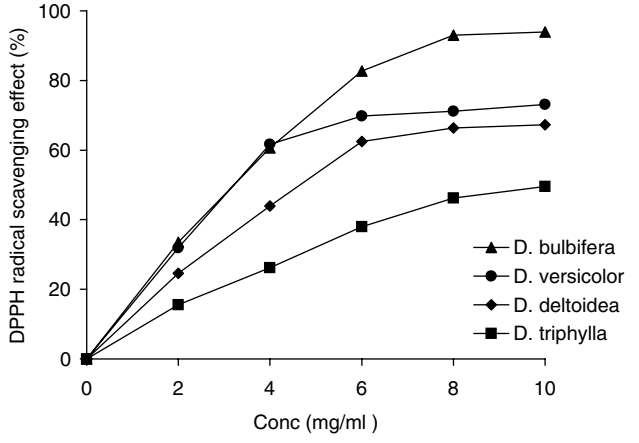

Fig. 1. DPPH free radical scavenging effect of yam extract. Values are mean of triplicates.

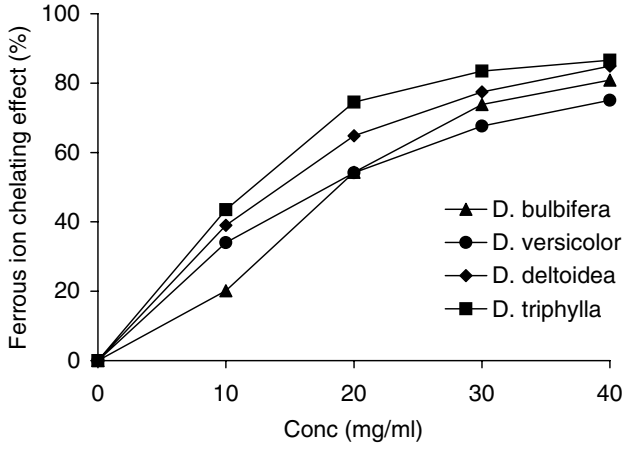

Fig. 2. Ferrous ion chelating effect of wild yam extract. Values are mean of triplicates.

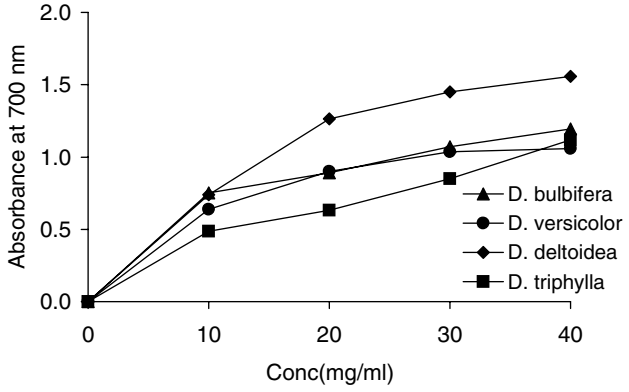

Fig. 3. Reducing power of wild yam extracts. Values are mean of triplicates.

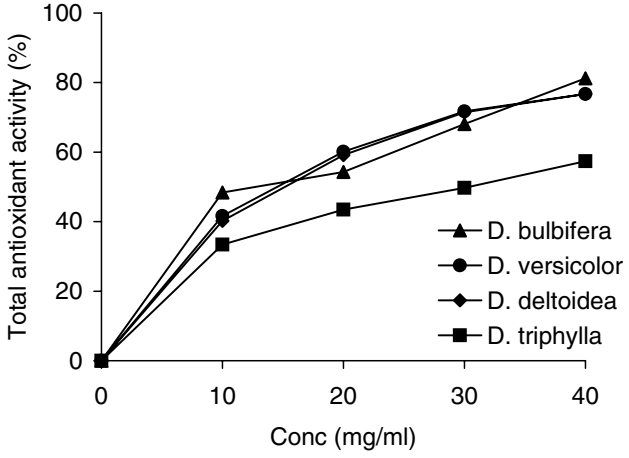

Fig. 4. Total antioxidant activity (%) of wild yam extracts. Values are mean of triplicates.

reducing power lower than *D. triphylla*, which had the lowest radical scavenging activity.

The ABTS/H_2O_2/HRP discoloration method is reported to represent the total antioxidant activity of methanolic yam extracts (Hsu et al., 2003). The total antioxidant activity of yam extract is presented in Fig. 4. The methanolic extract of wild yam tubers showed a remarkable antioxidant activity with a dose-dependent response. In contrast to our anticipation, no significant correlations could be found between the total polyphenolic contents (Table 2) and the total antioxidant activity of yam extracts. Our results are consistent with the findings of Gazzani, Papetti, Massolini, and Daglia (1998), Heinonen, Lehtonen, and Hopia (1998), and Kahkonen et al. (1999).

Yam (*Dioscorea* spp.) extracts have been reported to have more than 70% antioxidant activity, as evaluated by using a model system consisting of β-carotene and linoleic acid, and are placed in the vegetable group with a high antioxidant activity (Kaur & Kapoor, 2002). Farombi et al. (2000) reported a high level of antioxidant activity, as evaluated by the ABTS method, in some domesticated yam species from Nigeria. Recently, Hsu et al. (2003) reported remarkable antioxidant activities, as evaluated by DPPH radical scavenging, ferrous ion chelating, reducing power and ABTS tests, in some cultivated yam species from Taiwan. However, reports on the antioxidant activity of wild yam species are very rare in the literature. Therefore, it is very difficult to compare our results with that of previous studies. However, our results show higher antioxidant activities than the reported values for some cultivated yam species (Hsu et al., 2003). This could partly be due to the differences in species, maturity and other growing conditions. On the other hand, the observed data showed no positive correlations between total phenolic contents and antioxidant activities. This implies that the antioxidant activity observed in yam extracts was not solely from phenolic contents of yam, but could possibly be due to the presence of some other phytochemicals such as ascorbic acid, tocopherol and pigments, which also contribute to the total antioxidant activity (Kaur & Kapoor, 2002).

The high antioxidant activity of yams enhanced the potential interest in these under-exploited wild tubers for improving the efficacy of different products as nutraceutical and pharmacological agents. The consumption of these wild yams may play a role in preventing human diseases in which free radicals are involved, such as cancer, cardiovascular disease, and aging. However, further investigations on individual phenolic compounds, their in vivo antioxidant activity, and the different antioxidant mechanisms are warranted.

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