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# Antioxidative activities of water extract and ethanol extract from field horsetail (*tsukushi*) *Equisetum arvense* L.

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**Abstract**

Water extract and ethanol extract from top and body portions of field horsetail (*tsukushi*) were prepared, and the antioxidative activity was investigated using four different methods. The contents of total phenolic components were richer in the ethanol extract fractions of each portion than in the water extracts. On the other hand, protein contents were much lower in ethanol extract fractions than in water extract fractions. These fractions had remarkable antioxidative activities, similar to that of 5 mM ascorbic acid. Water extracts of both portions showed high superoxide anion radical-scavenging activities. Hydroxyl radicals were effectively scavenged by ethanol extracts. Field horsetail (*tsukushi*) is rich in vitamins C and E. Moreover, it contains high levels of copper and zinc. These are essential elements, for superoxide dismutase to act against active oxygen species. *Tsukushi* is not only a health food, but is also useful in preventing various degenerative diseases.


*Keywords:* Field horsetail; Water extract; Ethanol extract; Antioxidative activity; Scavenger; Vitamins; Trace element

## 1. Introduction

Vegetables contain many different phytonutrients, many of which have antioxidative properties. Research has shown that vegetables contain other antioxidatant nutrients, in addition to the well-known vitamins C and E, and carotenoids, which significantly contribute to their total antioxidant capacity (Cao, Sofic, & Prior, 1996; Wang, Cao, & Prior, 1996). For example, flavonoids, including compounds such as flavones, isoflavones, flavonones, anthocyanins and catechins, that are components of vegetables, have strong antioxidant capacity (Cao, Sofic, & Prior, 1997; Wang, Cao, & Prior, 1997). There is convincing evidence that vegetables are beneficial to health and contribute to the prevention of degenerative processes (Ames, Shigenaga, & Hagen, 1993; Bickford et al., 1997; Joseph et al., 1999). It is important to investigate the beneficial phytonutrients present in foods and the mechanisms responsible for these effects. The protection provided against diseases by vegetables has been attributed to the various antioxidants in foods (Ames et al., 1993; Gey, Puska, Jordap, & Moser, 1991). There is overwhelming evidence to indicate that free radicals cause oxidative damage to lipids, proteins, and nucleic acids. Free radicals may lie at the heart of the etiology or natural history of a number of diseases, including cancer, heart, vascular, diabetes and neurodegenerative diseases (Halliwell & Gutteridge, 1999; Yu, 1994). Therefore, antioxidants that can neutralize free radicals may be of central importance in the prevention of these disease states.

Field horsetail belongs to the order Sphenopsida and is a plant that grows naturally in early spring in green fields in Japan. Field horsetail is distinguished as a sporophyte (namely *tsukushi*) and a nutritive caulis (namely *sugina*). The latter is well known as *Sugina* tea in Japan and is drunk as a health drink. As it contains abundant silicic acid and saponin, it is thought that *Sugina* tea is efficacious as a diuretic, antipyretic, anticough and anti-inflammation agent. The former is consumed as food in sweetened vinegar, cooked food, and chopped fish (JAPAN association of training colleges

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for cooks, 1996). However, there are few reports about the functional properties of *tukushi*, to our knowledge. In the present study, we report the antioxidative properties of water extract and ethanol extract from field horsetail using four different methods, namely, auto-oxidation test, superoxide anion radical generated from the xanthine/xanthine oxidase system, DPPH free radical, and hydroxyl radical-scavenging tests. The data should be useful for preventing various diseases.

## 2. Materials and methods

### 2.1. Sample

Field horsetail (*tsukushi*), *Equisetum arvense* L., was collected in Shimonoseki City, Yamaguchi Prefecture, Japan. Field horsetail was separated into top portion and body portion, and then the sheath was removed from the body portion (Fig. 1). These portions were washed with distilled water and used as the sample for the following experiments.

### 2.2. Reagents

Linoleic acid, α-tocopherol, ascorbic acid, 2,2′-azobis-(2-amidinopropane) dihydrochloride (AAPH), nitroblue tetrazolium salt (NBT), xanthine, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2-deoxy-D-ribose, and 2-thiobarbituric acid (TBA) were purchased from Wako Chemicals Co., Ltd. (Osaka, Japan). Xanthine oxidase from butter milk (XOD; 0.34 U/mg powder) was obtained from Oriental yeast Co., Ltd. (Tokyo, Japan). Other reagents were of reagent grade.

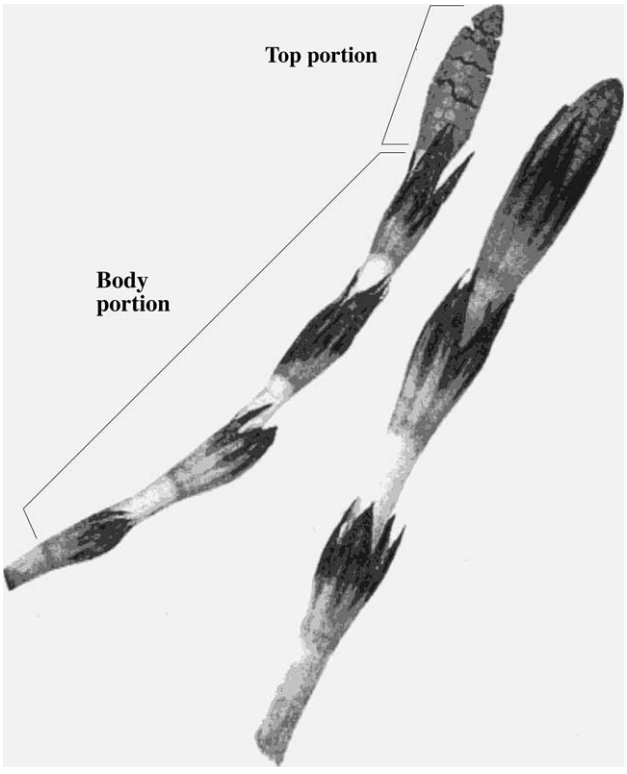


Fig. 1. Field horsetail (*tsukushi*).

### 2.3. Preparation of water extract and ethanol extract from field horsetail

Five grammes of top portion in field horsetail were suspended and extracted with 5 volumes of cold distilled water with shaking at 4 °C for 1 day. The extracts were centrifuged at 58,300*g* for 30 min at 4 °C, and the supernatants were pooled, evaporated, and then dissolved in 1.0 ml of cold distilled water. Similarly, five grammes of the top portion were suspended and extracted with 5 volumes of cold ethanol with shaking at 4 °C for 1 day. The extracts were centrifuged at 58,300*g* for 30 min at 4 °C, and the supernatants were pooled, evaporated, and then dissolved in 1.0 ml of cold ethanol. Water extract and ethanol extract of body portion were prepared in the same manner as the top portion. Each sample solution was diluted and 0.1 and 1.0% (w/w) sample solutions were used for the following tests.

### 2.4. Determination of total phenolic compounds

The total phenolic compounds were measured spectrophotometrically at 760 nm (Slinkard & Singleton, 1997).

### 2.5. Determination of protein concentration

The protein concentration was measured by the method of Lowry, Rosebrough, Farr, and Randall (1951).

### 2.6. Auto-oxidation test

Antioxidative activity was assayed by using a linoleic acid model system. 83.3 μl of sample solution and 208 μl of 0.2 M sodium phosphate buffer (pH 7.0) were mixed with 208 μl of 2.5% (w/v) linoleic acid in ethanol. The preoxidation was initiated by the addition of 20.8 μl of 0.1 M AAPH and carried out at 37 °C for 200 min in the dark. The degree of oxidization was measured according to the thiocyanate method (Mitsuda, Yasumoto, & Iwai, 1966) for measuring peroxides by reading the absorbance at 500 nm after colouring with $FeCl_2$ and ammonium thiocyanate. A control was performed with linoleic acid but without sample solution. Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive controls.

### 2.7. Effect of superoxide anion radical

The effect of superoxide anion radical was evaluated by the method of Nagai and Inoue (2003). This system

contained 480 μl of 0.05 M sodium carbonate buffer (pH 10.5), 20 μl of 3 mM xanthine, 20 μl of 3 mM ethylenediaminetetraacetic acid disodium salt (EDTA), 20 μl of 0.15% bovine serum albumin, 20 μl of 0.75 mM NBT, and 20 μl of sample solution. After preincubation at 25 °C for 10 min, the reaction was started by adding 6 mU XOD and carried out at 25 °C for 20 min. After 20 min the reaction was stopped by adding 20 μl of 6 mM CuCl. The absorbance of the reaction mixture was measured at 560 nm and the inhibition rate was calculated by measuring the amount of the formazan that was reduced from NBT by superoxide. Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive controls.

### 2.8. Effect of hydroxyl radical

The effect of hydroxyl radical was assayed by using the deoxyribose method. The reaction mixture contained 450 μl of 0.2 M sodium phosphate buffer (pH 7.0), 150 μl of 10 mM 2-deoxyribose, 150 μl of 10 mM $FeSO_4$–EDTA, 150 μl of 10 mM $H_2O_2$, 525 μl of $H_2O$, and 75 μl of sample solution in an Eppendorf tube. The reaction was started by the addition of $H_2O_2$. After incubation at 37 °C for 4 h, the reaction was stopped by adding 750 μl of 2.8% trichloroacetic acid and 750 μl of 1.0% of TBA in 50 mM NaOH, the solution was boiled for 10 min, and then cooled in water. The absorbance of the solution was measured at 520 nm. Hydroxyl radical-scavenging activity was evaluated as the inhibition rate of 2-deoxyribose oxidation by hydroxyl radical (Chung, Osawa, & Kawakishi, 1997). Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive controls.

### 2.9. Effect of DPPH radical

The effect of DPPH radical was evaluated by the method of Okada and Okada (1998) with a slight modification. The assay mixture contained 300 μl of 1.0 mM DPPH radical solution, 2.4 ml of 99% ethanol, and 300 μl of sample solution. The solution was rapidly mixed and scavenging capacity was measured spectrophotometrically by monitoring the decrease in absorbance at 517 nm. Ascorbic acid (0.1 and 1.0 mM) and α-tocopherol (1 mM) were used as positive controls.

## 3. Results and discussion

### 3.1. Preparation of water extract and ethanol extract from field horsetail

Water extract (TWEF) and ethanol extract (TEEF) in top portion and water extract (BWEF) and ethanol extract (BEEF) in body portion were successfully extracted from field horsetail, and the contents of total phenolic components and proteins of these fractions were measured. The total phenolic components of these fractions were as follows: 12.8 mg/g sample tissue (TWEF), 12.3 mg/g sample tissue (TEEF), 7.98 mg/g sample tissue (BWEF), and 23.9 mg/g sample tissue (BEEF), respectively (Table 1). The protein contents of these fractions were 919 mg/g sample tissue (TWEF), 476 mg/g sample tissue (TEEF), 729 mg/g sample tissue (BWEF), and 200 mg/g sample tissue (BEEF), respectively (Table 1). The contents of total phenolic components were richer in the ethanol extract fractions of each portion than in the water extract ones. On the other hand, protein contents were much lower in ethanol extract fractions than in water extract fractions.

Table 1
The contents of total phenolic components and protein in the extracts from field horsetail

| Sample species | Total phenlic components (mg/g sample tissue) | Protein (mg/g sample tissue) |
|---|---|---|
| TWEF | 12.8 | 919 |
| TEEF | 12.3 | 476 |
| BWEF | 7.98 | 729 |
| BEEF | 23.9 | 200 |

### 3.2. Antioxidant activities of water extract and ethanol extract from field horsetail

Antioxidative activity on the peroxidation of linoleic acid was investigated to evaluate *in vitro* effects of the extracts from field horsetail at the initiation stage of lipid peroxidation. As shown in Table 2, each fraction showed the antioxidative effect and the activity of each fraction decreased with passage of time to 200 min. However, the activity increased with increasing of the sample concentration except for those of TEEF. The activity in 0.1% concentrations in water extracts of both portion was lower than that in the ethanol extract, but the activity in 1.0% concentration in water extract of both portions was the same as that in the ethanol extract. The activities of TWEF and BWEF of 0.1% concentration were higher than that of 1 mM ascorbic acid and were much lower than that of 5 mM ascorbic acid. The activities of TEEF and BEEF, at each concentration, were high and were similar to that of 5 mM ascorbic acid, except for that of 0.1% BEEF. The activities of all fractions were lower than that of 1 mM α-tocopherol.

### 3.3. Superoxide anion radical-scavenging activities of water extract and ethanol extract from field horsetail

Superoxide anion radical-scavenging activities of the extracts from field horsetail were measured using the xanthine–xanthine oxidase system. The results indicated the superoxide productivity. Each fraction showed the

Table 2
Antioxidative activities of water extract and ethanol extract from field horsetail

| Time (min) | OD (500 nm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sample | | | | | | | | | | | |
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 50 | 0.102 | 0.010 | 0.020 | 0.038 | 0.071 | 0.026 | 0.038 | 0.033 | 0.022 | 0.016 | 0.006 | 0.232 |
| 100 | 0.185 | 0.059 | 0.061 | 0.060 | 0.146 | 0.057 | 0.088 | 0.050 | 0.135 | 0.032 | 0.025 | 0.642 |
| 200 | 0.396 | 0.120 | 0.113 | 0.123 | 0.316 | 0.106 | 0.173 | 0.101 | 0.469 | 0.090 | 0.028 | 1.435 |

(A) 0.1% TWEF; (B) 1.0% TWEF; (C) 0.1% TEEF; (D) 1.0% TEEF; (E) 0.1% BWEF; (F) 1.0% BWEF; (G) 0.1% BEEF; (H) 1.0% BEEF; (I) 1 mM ascorbic acid; (J) 5 mM ascorbic acid; (K) 1 mM α-tocopherol; (L) control. Values are averages of three replicates. Significant difference ($p < 0.05$).

Table 3
Superoxide anion radical and hydroxyl radical-scavenging activities of water extract and ethanol extract from field horsetail

| Sample | Superoxide productivity (%)[a] | Inhibition rate (%)[b] |
|---|---|---|
| A | 67.8 | 2.36 |
| B | 7.33 | 46.4 |
| C | 60.0 | 94.6 |
| D | 54.2 | 100 |
| E | 68.6 | 0.04 |
| F | 12.8 | 19.1 |
| G | 93.5 | 87.1 |
| H | 80.6 | 100 |
| I | 86.7 | 13.2 |
| J | 10.0 | 17.6 |
| K | 47.5 | 67.6 |

(A) 0.1% TWEF; (B) 1.0% TWEF; (C) 0.1% TEEF; (D) 1.0% TEEF; (E) 0.1% BWEF; (F) 1.0% BWEF; (G) 0.1% BEEF; (H) 1.0% BEEF; (I) 1 mM ascorbic acid; (J) 5 mM ascorbic acid; (K) 1 mM α-tocopherol. Values are averages of three replicates. Significant difference ($p < 0.05$).

[a] Superoxide anion radical-scavenging activity.

[b] Hydroxyl radical-scavenging activity.

superoxide anion radical-scavenging activity, and the activity increased with increasing sample concentration (Table 3). Water extracts of both portions showed high activities in comparison with ethanol extracts. The activities of TEEF and BEEF were higher than that of 1 mM ascorbic acid, except for that of 0.1% BEEF. On the other hand, TWEF and BWEF, at 0.1% concentration, showed intermediate activities between those of 1 mM ascorbic acid and 1 mM α-tocopherol. Moreover, BWEF, at 1.0% concentration, possessed the same activity as 5 mM ascorbic acid. Among these extracts, the activity of 1.0% TWEF was highest and its fraction scavenged superoxide anion radical about 93%.

### *3.4. Hydroxyl radical-scavenging activities of water extract and ethanol extract from field horsetail*

Hydroxyl radical-scavenging activity was investigated on the extracts from field horsetail using the Fenton reaction mechanism; the results indicated the inhibition rate. Many extracts showed hydroxyl radical-scavenging activity, and its activity tended to increase with increasing sample concentration (Table 3). TWEF, at 1.0% concentration, showed intermediate activity (between 5 mM ascorbic acid and 1 mM α-tocopherol) and it scavenged this radical about 46%. BWEF, at 1.0% concentration, possessed low activity, about 19% against hydroxyl radical, but showed of slightly higher activity than that of 5 mM ascorbic acid. At 0.1% concentration, TWEF and BWEF hardly scavenged hydroxyl radical. On the other hand, TEEF and BEEF exhibited much higher hydroxyl radical-scavenging activities than 1 mM α-tocopherol. These extracts at 0.1% concentration, scavenged about 87–95% and, furthermore, the extracts at 1.0% concentration completely scavenged hydroxyl radical (Table 3). Hydroxyl radicals are known to be capable of abstracting hydrogen atoms from membranes and bring about peroxidic reactions of lipids (Kitada, Igarashi, Hirose, & Kitagawa, 1979). From this it was expected that the extracts from field horsetail would show antioxidant effects against lipid peroxidation on biomembranes and scavenge the hydroxyl radicals and superoxide anions at the stage of initiation and termination of peroxy radicals.

### *3.5. DPPH radical-scavenging activities of water extract and ethanol extract from field horsetail*

DPPH is a free radical compound and has been widely used to test the free radical-scavenging ability of various samples (Hatano, 1995; Hatano, Takagi, Ito, & Yoshida, 1997; Yoshida et al., 1989). To evaluate the scavenging effect of DPPH on the extracts from field horsetail, DPPH inhibition was investigated and these results are shown as relative activities against control. Each extract showed DPPH radical-scavenging activity, but the activities were very low (Table 4). The activities of any of these extracts were similar to or slightly higher than that of 0.1 mM ascorbic acid. On the other hand, the activity of 1.0% TEEF was the highest among these extracts. It was lower than that of 1 mM ascorbic acid and α-tocopherol, and scavenged DPPH radical about 78% over 10 min.

Table 4
DPPH radical-scavenging activities of water extract and ethanol extract from field horsetail

| Time (min) | Relative activity (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sample | | | | | | | | | | | |
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 1 | 97.0 | 93.5 | 93.5 | 47.7 | 97.1 | 91.4 | 98.8 | 95.7 | 94.6 | 5.3 | 9.5 | 99.9 |
| 2 | 96.3 | 90.7 | 92.4 | 39.0 | 96.6 | 88.9 | 98.6 | 94.0 | 94.4 | 5.3 | 5.3 | 98.5 |
| 3 | 95.8 | 89.0 | 91.1 | 33.6 | 96.2 | 87.1 | 98.7 | 93.0 | 94.4 | 5.3 | 5.3 | 98.8 |
| 5 | 94.9 | 86.5 | 89.7 | 27.7 | 95.7 | 84.5 | 98.4 | 91.7 | 94.4 | 5.3 | 5.3 | 99.1 |
| 10 | 93.8 | 82.4 | 87.3 | 22.4 | 94.7 | 80.1 | 98.3 | 90.1 | 94.4 | 5.3 | 5.3 | 98.7 |

(A) 0.1% TWEF; (B)% TWEF; (C) 0.1% TEEF; (D) 1.0% TEEF; (E) 1.0% BWEF;(F) 1.0% BWEF; (G) 0.1% BEEF; (H) 1.0% BEEF; (I) 0.1 mM ascorbic acid; (J) 1 mM ascorbic acid; (K) 1 mM α-tocopherol; (L) control. Values are averages of three replicates. Significant difference ($p < 0.05$).

Field horsetail is rich in many kinds of vitamins, such as carotein, vitamin $B_1$, $B_2$, $B_6$, nicotinic acid, folic acid, pantothenic acid and vitamins C, E, and K (Science & Technology Agency, 2002). Particularly, it contains large amounts of vitamins C (33 mg) and E (4.9 mg) per 100 g edible portion (fresh weight). These reports confirm that field horsetail has good antioxidative acitivity. It also contains, not only large amounts of alkaloid and saponin, but also great amounts of flavonoids, such as flavone, isoflavone, flavonol and flavanol. It is known that flavonoids play an important role in the high antioxidative activity in plants. Moreover, field horsetail is rich in many kinds of trace elements such as Na, K, Ca, Mg, P, Fe, Zn, Cu, Mn, Si, Sr, Ti. Among these, potassium (640 mg per 100 g edible portion) is an element that is essential for the regulation of osmotic pressure in cells of humans. Moreover, copper (0.22 mg per 100 g edible portion) and zinc (1.1 mg per 100 g edible portion) are essential elements, needed for superoxide dismutase to act against active oxygen species. High contents of these elements agree with the high antioxidative activity and the high scavenging activities against superoxide anion radical, hydroxyl radical, and DPPH radical.

In conclusion field horsetail is not only a health food, but also useful to protect against the various diseases. Research is underway to analyze the several constituents of *tsukushi* in relation to its antioxidant properties, in detail.

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