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*Note*

# Antioxidant Activity of Some Edible Yemeni Plants Evaluated by Ferrylmyoglobin/ ABTS$^{\cdot+}$ Assay

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**The antioxidant activity, expressed in mM Trolox equivalent antioxidant capacity (TEAC), of some edible Yemeni plants belonging to different families was evaluated using the ferrylmyoglobin/ABTS$^{\cdot+}$ method. Methanolic (80%) extracts of tested plants exhibited higher ($p$<0.05) TEAC than extracts of other solvents. Extracts of coffee beans (*Coffea arabica*), sweet basil (*Ocimum basilicum*), wild thyme (*Thymus serpyllum*) and sorrel (*Rumex acetosa*) had antioxidant activities equivalent to 8.7–47.1 mM Trolox or 7.9–42.8 mM BHA per g dry weight of plant. The TEAC of methanolic extracts for sorrel was not significantly different ($p$>0.05) from extracts of *Leucaena leucocephala* (Thailand) and Japanese green tea (*Camellia sinensis*). A good correlation ($R^2$=0.937) was observed between TEAC and total phenol contents in 80% methanol extracts of tested plants.**

Keywords: antioxidant activity, Trolox equivalent antioxidant capacity(TEAC), *Rumex acetosa*, ferrylmyoglobin/ABTS$^{\cdot+}$, Yemeni plants

There is increasing epidemiological and pharmacological evidence that plants contain biologically active components (*e.g.*, free radical scavengers) offering health benefits and protection against degenerative diseases. In fact, oxygen radicals and lipid peroxides have been known for their alleged role in the etiology of many *in vivo* pathological reactions such as aging and cancer (Nakatani, 1992; Ferguson, 1994; Rice-Evans *et al*., 1995; Rapisarda *et al*., 1999). Synthetic antioxidants like BHA have been developed to retard lipid peroxidation, but their use as food additives and therapeutic chemicals has been hindered due to their possible toxicity to human health (Nakatani; 1992, Haraguchi *et al*., 1998; Jimenez & Garcia-Carmona, 1999). Therefore, effective antioxidants with less toxicity, especially those originating from natural plants used in folk medicine and food, are attracting the attention of medical and food scientists alike. Flavonoids (*e.g*., catechins and myricetin) of dietary plants and spices exhibited anti-oxidative, anti-mutagenic, anti-allergic and anti-carcinogenic activities (Jimenez & Garcia-Carmona, 1999; Asai *et al*., 1999; Hashimoto *et al*., 1999). Murakami *et al*. (1995) reported on the strong anti-tumor activity of several edible plants of Southeast Asia. Extracts of Thai fingerroots (*Boesenbergia panndurata*) and galanga (*Languas galanga*) contained potent antimutagens against Trp-P-1 in the Ames *Salmonella* test (Trakoontivakorn *et al*., 1999). Recently, another biological activity (insulin-like functions) was shown by extracts of many herbs such as tea and basil (Broadhurst *et al*., 2000).

In many regions of the world, certain edible plants and spices have been used in folk medicine as remedies of various diseases. For example, turmeric and *Pulicaria crispa* were used in treating inflammation (Al-Yahya *et al*., 1988; Asai *et al*., 1999) whereas licorice from *Glycyrrhiza* spp. relieved rheumatic pain and ulcers (Haraguchi *et al*., 1998). In Yemen and other Islamic countries, many herbs and spices (*e.g*. thyme, basil and black cumin) have been used in folk medicine and as condiments. In spite of the increasing interest in the chemopreventive potential of dietary phytochemicals worldwide, no data have referred to the biological functions of Yemeni plants. Based on the compelling evidence of health benefits of edible plants, especially those commonly consumed in many Asian countries, the present study was the first conducted on the antioxidant activity of some edible plants from Yemen.

## Materials and Methods

*Chemicals* Unless stated otherwise, all chemicals were purchased from Wako Pure Chemicals Industries, Ltd. (Osaka). Water was purified through a Milli Q Millipore System. Stock solutions of 500 μM ABTS diammonium salt (2.2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), 400 μM myoglobin, 740 μM potassium ferricyanide and 450 μM $H_2O_2$ were freshly prepared in 5 mM phosphate buffered saline, PBS (155 mM NaCl, pH 7.4). Metmyoglobin (MbIII) was made by mixing equal volumes of myoglobin and potassium ferricyanide solutions (Rice-Evans & Miller, 1994). Trolox (6-Hydroxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid) obtained from Aldrich Chemical Company Inc. (Milwaukee, WI) was dissolved in ethanol (Rice-Evans *et al*., 1995). Similarly, catechol (Extrasynthese, Genay, France) and BHA were prepared in ethanol.

*Plants and solvent extractions* Plants (14 species) were collected from natural sources and retail markets in Yemen: *Ocimum kilimandscharicum, Salvadora persica., Trigonella foenumgraecum, Nigella sativa, Zizyphus spina-christi, Cissus* spp. *Cissus rotundifolia, Ruta graveolens, Coffea arabica* (green beans and hulls), *Pulicaria crispa, Ceratonia siliqua, Ocimum*

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*basilicum, Thymus serpyllum,* and *Rumex acetosa*.

For comparative purposes, other edible plants (6 species) were obtained from marketplaces in Thailand and Japan; these were *Leucaena leucocephala* and *Glochidion perakense* (syn. *Glochidion wallichianum*) from Thailand, *Chrysanthemum coronarium* and green tea (*Camellia sinensis*) from Japan, *Ilex paraguariensis* originated from Argentina, and Ceylon black tea (*Camellia sinensis*).

Plants were extracted by mixing 500 mg of lyophilized samples with 20 ml solvent for 1 min using a polytron type homogenizer. Extracts were then centrifuged (9390×*g*, 10 min) at 5°C and clear supernatants were used for the antioxidant assay described below. Depending on the plant, extracts were further diluted prior to assays.

*Solvent soluble antioxidants* To investigate the effects of solvent on antioxidant activity, lyophilized samples of *Thymus serpyllum, Ocimum basilicum, Nigella sativa* and *Pulicaria crispa* were separately extracted with each of the following solvents of different polarity: hexane, ethyl acetate, methanol (80%) or water. Solvents were evaporated at 35°C *in vacuo* using an Eyela CVE-200D centrifugal vaporizer (Tokyo Rikakikai Co., Tokyo), and then solids were weighed before dissolving in 80% methanol for determination of the total antioxidant activity as outlined below.

*Total antioxidant activity* The radical-scavenging activity of extracts, defined as the total antioxidant activity, was determined using the ferrylmyoglobin/ABTS$^{\cdot+}$ protocol of Rice-Evans and Miller (1994). Briefly, the reaction mixture (2 ml total volume) contained the following substances (final concentration in a mixture); ABTS (150 μM), MbIII (2.5 μM), 16.8 μl sample extract (0.84%) or a Trolox standard, and 978 μl PBS (to make 2 ml). The reaction was initiated by the addition of $H_2O_2$ (75 μM) and the lag time in seconds before absorbance of ABTS$^{\cdot+}$ at 734 nm began to increase was recorded. The reaction was conducted at 30°C using a Shimadzu UV-1200 spectrophotometer fitted with a temperature control (Shimadzu Co., Kyoto). The total antioxidant activity (TAA) of extracts was determined as Trolox equivalent antioxidant capacity (mM TEAC) from the linear regression of Trolox standards (0–4 mM) and their lag times. Results were calculated as TEAC in mM per g dry weight of original samples.

*Total polyphenols* The method of Singleton and Rossi

**Table 1.** Trolox equivalent antioxidant capacity (TEAC) and solvent soluble solids of some edible plants from Yemen.

| Extraction solvent | TEAC, mM $g^{-1}$ dry weight | | | |
|---|---|---|---|---|
| | *Thymus serpyllum* | *Ocimum basilicum* | *Pulicaria crispa* | *Nigella sativa* |
| Hexane | 0.7$^{a}$ (40)* | 0.5$^{a}$ (34) | 0.4$^{a}$ (120) | 0.4$^{a}$ (340) |
| Ethyl acetate | 0.8$^{a}$ (37) | 0.7$^{a}$ (10) | 0.7$^{a}$ (41) | 0.5$^{ab}$ (260) |
| Methanol (80%) | 14.7$^{c}$ (200) | 12.7$^{c}$ (180) | 5.6$^{b}$ (160) | 1.1$^{c}$ (80) |
| Water | 7.4$^{b}$ (380) | 5.8$^{b}$ (306) | 0.8$^{a}$ (420) | 0.7$^{bc}$ (240) |

*values in parenthesis are mg solvent soluble $g^{-1}$ dry weight of plants.
$^{a\text{-}c}$means in a column without common superscripts are significantly different ($p$<0.05).

**Table 2.** Trolox equivalent antioxidant capacity (TEAC) and total phenolics of edible plants(mean±sd)[1]

| Botanical name | English name | Edible part | Origin | TEAC[2] | Total phenolics[3] |
|---|---|---|---|---|---|
| *Ocimum kilimandscharicum* | camphor basil | leaf | Yemen | 0.6$^{a}$ ±0.2 | 10.8$^{abc}$±1.1 |
| *Salvadora persica* | — | stem | Yemen | 0.7$^{a}$ ±0.3 | 1.6$^{a}$ ±0.6 |
| *Trigonella foenumgraecum* | fenugreek | seeds | Yemen | 0.9$^{a}$ ±0.3 | 7.3$^{ab}$ ±0.5 |
| *Nigella sativa* | black cumin | seeds | Yemen | 1.1$^{a}$ ±0.1 | 1.9$^{ab}$ ±0.1 |
| *Zizyphus spina-christi* | Christ's thorn | fruits | Yemen | 2.0$^{a}$ ±0.5 | 11.9$^{abc}$±2.9 |
| *Cissus* spp. | — | leaf | Yemen | 3.1$^{a}$ ±0.2 | 24.8$^{abc}$±0.5 |
| *Cissus rotundifolia* | — | leaf | Yemen | 3.2$^{a}$ ±0.1 | 26.8$^{b}$ ±1.1 |
| *Ruta graveolens* | rue | leaf/stem | Yemen | 3.7$^{a}$ ±0.1 | 28.4$^{bc}$ ±1.1 |
| *Chrysanthemum coronarium* | garland | leaf | Japan | 4.0$^{a}$ ±0.3 | 23.2$^{abc}$±0.6 |
| *Coffea arabica* (hulls) | coffee | hulls | Yemen | 4.5$^{a}$ ±1.4 | 16.8$^{abc}$±5.2 |
| *Pulicaria crispa* | — | leaf/stem | Yemen | 5.6$^{a}$ ±1.6 | 22.5$^{abc}$±0.5 |
| *Ceratonia siliqua* | carob | pods | Yemen | 6.0$^{a}$ ±1.8 | 16.5$^{abc}$±3.4 |
| *Coffea arabica* (green beans) | coffee | beans | Yemen | 8.7$^{a}$ ±0.1 | 34.2$^{c}$ ±2.1 |
| *Ocimum basilicum* | sweet basil | leaf | Yemen | 12.7$^{a}$ ±0.9 | 54.6$^{cd}$ ±3.0 |
| *Thymus serpyllum* | thyme (wild) | leaf | Yemen | 14.7$^{a}$ ±2.8 | 61.1$^{d}$ ±1.6 |
| *Ilex paraguariensis* | maté | leaf | Argentina | 28.9$^{b}$ ±1.4 | 90.4$^{e}$ ±1.1 |
| *Leucaena leucocephala* | — | leaf | Thailand | 38.2$^{c}$ ±13.8 | 146.3$^{f}$ ±5.2 |
| *Camellia sinensis* | black tea | leaf | Sri Lanka | 42.2$^{cd}$ ±11.5 | 113.1$^{ef}$ ±6.8 |
| *Rumex acetosa* | sorrel | leaf | Yemen | 47.1$^{cd}$ ±3.3 | 134.2$^{f}$ ±1.1 |
| *Camellia sinensis* | green tea | leaf | Japan | 52.2$^{d}$ ±8.5 | 109.4$^{ef}$ ±15 |
| *Glochidion perakense* | — | leaf | Thailand | 70.6$^{e}$ ±15.0 | 183.3$^{g}$ ±6.4 |
| Antioxidants (1 mM): | | | mM Trolox equivalent | | |
| Sodium ascorbate | | | 1.2±0.02 | | |
| Catechol | | | 1.4±0.01 | | |
| BHA | | | 1.1±0.22 | | |

[1]80% methanolic extracts of plants.
[2]mM TEAC $g^{-1}$ dry weight.
[3]mg gallic acid equivalent $g^{-1}$ dry weight.
$^{a\text{-}g}$means in a column without common superscripts are significantly different ($p$<0.05).

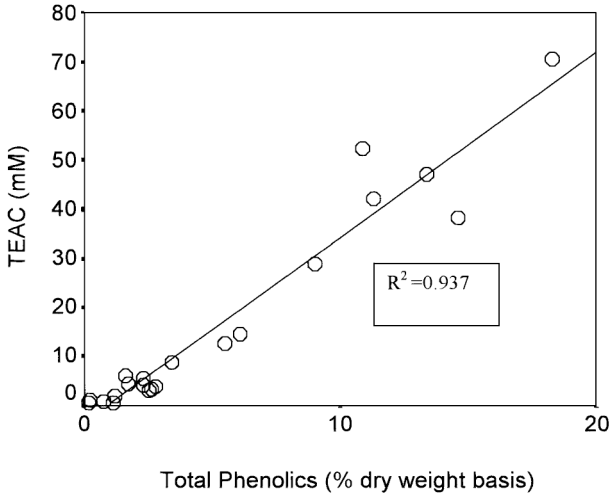


**Fig. 1.** Relationship between antioxidant activity (mM TEAC $g^{-1}$ dry weight) and % total polyphenols in methanolic extracts of tested edible plants.

(1965) downscaled to 2 ml (final volume in a cuvette) was followed to determine amounts of total polyphenols in extracts; 200 μl of methanolic extract of a tested plant was mixed with 1000 μl of 1:10 Folin-Ciocalteu's reagent (Nacalai Tesque Inc., Kyoto); 30 s later and just prior to 8 min later, 800 μl of $Na_2CO_3$ (7.5%) was added. The reaction mixture was incubated at 24°C for 2 h before absorbance values (λ 765 nm) were recorded. Gallic acid monohydrate was used as a standard (0–0.07 mg/ml). Total polyphenols were expressed as mg gallic acid equivalents per g dry weight of original samples.

*Statistical analysis* The analysis of variance, ANOVA (SPSS 9.05 for Windows, 1999), was used to evaluate data ($\alpha$=0.05). Means were separated by Tukey's HSD multiple comparisons.

## Results and Discussion

*Effect of solvent on antioxidant activity* As shown in Table 1, 80% methanol extracts had the highest TEAC. Depending on the plant, TEAC values of water extracts of plants represented 14.2% to 65.8% of the corresponding values determined in methanolic extracts. However, a correlation between amount of solvent soluble solids and TEAC was not recognized ($R^2$=0.055). Methanolic extracts of plants presumably contained higher concentrations of antioxidants, *i.e.* polyphenols, compared to those in other solvents. Miller *et al.* (1995) indicated that water soluble substances (*e.g.*, sugars and organic acids) did not contribute to the antioxidant activity of fruits. In fact, Azuma *et al.* (1999) indicated that prooxidative activity of water and ethanolic extracts of vegetables was reversed by a metal chelator (diethylenetriaminepentaacetic acid, DTPA). Moreover, polyphenols (*e.g.*, flavonoids) could be oxidized by polyphenol oxidases forming the corresponding quinones and thus losing their antioxidant capacity (Jimenez & Garcia-Carmona, 1999).

*Antioxidant activity and total phenolics of plants* The TEAC and total polyphenolic values of plants extracted with 80% methanol are summarized in Table 2. It is noteworthy that some of the tested plants which have no English or common names may not be widely distributed in the world. Apparently, plants varied in their antioxidant capacity, ranging from <1 to 71 mM TEAC per g dry weight of plant. Extracts of sorrel, thyme (wild), sweet basil and *Pulicaria crispa* exhibited antioxidant activity corresponding to the antioxidant capacity of 5.6–47.1 mM Trolox (a water soluble analogue of α-tochopherol) or 5.1–42.8 mM BHA per g dry weight of the tested plants (Table 2). A striking difference in TEAC was found within species of the genus *Ocimum,viz. O. basilicum* and *O. kilimandscharicum* (Table 2).

In addition to sorrel, plants belonging to the *Labiatae (Lamiaceae), i.e.* thyme and basil, had higher TEACs than other Yemeni plants (Table 2). Among the Yemeni plants tested, sorrel did not exhibit a significant difference ($p$>0.05) in TEAC from either *Leucaena leucocephala* (Thailand) or Japanese green tea. A study on Finnish plants indicated that sorrel had much less antiperoxidant activity than other species of thyme (*T. vulgaris*) using the methyl linoleate (MeLo) oxidation system (Kahkonen *et al.*, 1999). However, Liebert *et al.* (1999) mentioned that the MeLo test was less reliable in measuring the antioxidant capacity of tea compared to the method used in this study, the ferrylmyoglobin/$ABTS^{\cdot+}$ system. It should be noted that TEAC of plant extracts containing iron chelating antioxidants may be underestimated when the ferrylmyoglobin/$ABTS^{\cdot+}$ method is used (Yu & Ong, 1999). Common world beverages (tea, coffee and maté) were significantly different ($p$<0.05) in their antioxidative capacity or TEAC. Presumably, that is related to their total phenolic content (Table 2). In this regard, it was reported that tea and coffee contained higher concentrations of antioxidant phenolics (*e.g.*, epigallocatechin gallate ) which reduce lipid hydroperoxidation in human plasma (Chuda *et al.*, 1996; Liebert *et al.*, 1999; Nakagawa *et al.*, 1999). It is worthwhile to mention that TEAC was well correlated ($R^2$=0.937) with total phenolics in methanolic extracts of plants (Fig. 1). Earlier findings indicated that amounts of phenolic compounds markedly influenced the antioxidative capacity of fruits, grains, tea and other edible plants (Liebert *et al.*, 1999; Rapisarda *et al.*, 1999).

Thai plants were distinct in their total phenolic content (Table 2), ranging from 15%–18.3% (dry weight basis). It was mentioned by Liebert *et al.* (1999) that polyphenols could be as high as 35% of the dry weight of tea. The environmental conditions (*e.g.*, higher temperatures and humidity) contributed to the stronger biological functions of Thai plants compared to those grown in other Asian countries (Murakami *et al.*, 1995; Murakami *et al.*, 2000).

In summary, commonly consumed plants contain antioxidants which are potential prophylactic agents and natural food additives. Recognition of the biological functions of phytochemicals would prompt the expansion in cultivation and market potential of these edible plants.

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### References

Al-Yahya, M., El-Sayed, A., Mossa, J., Kozlowski, J., Antoun, M., Ferin, M., Baird, W. and Cassady, J. (1988). Potential cancer chemopreventive and cytotoxic agents from *Pulicaria crispa*. *J. Natl. Prod.*, **51**, 621–624.

Asai, A., Nakagawa, K. and Miyazawa, T. (1999). Antioxidative effects of turmeric, rosemary and capsicum extracts on membrane

phospholipid peroxidation and liver lipid metabolism in mice. *Biosci. Biotechnol. Biochem.*, **63**, 2118–2122.

Azuma, K., Ippoushi, K., Ito, H., Higashio, H. and Terao, J. (1999). Evaluation of antioxidative activity of vegetable extracts in linoleic acid emulsion and phospholipid bilayers. *J. Sci. Food Agric.*, **79**, 2010–2016.

Broadhurst, C., Polansky, M. and Anderson, R. (2000). Insulin-like biological activity of culinary and medicinal plant aqueous extracts in vitro. *J. Agric. Food Chem.*, **48**, 849–852.

Chuda, Y., Ono, H., Ohnishi-Kameyama, M., Nagata, T. and Tsushida, T. (1996). Structural identification of two antioxidant Quinic acid derivatives from Garland (*Chrysanthemum coronarium* L.). *J. Agric. Food Chem.*, **44**, 2037–2039.

Ferguson, L. (1994). Antimutagens as cancer chemopreventive agents in the diet. *Mutat. Res.*, **307**, 395–410.

Haraguchi, H., Ishikawa, H., Mizutani, K., Tamura, Y. and Kinoshita, T. (1998). Antioxidative and superoxide scavenging activities of retrachalcones in *Glycyrrhiza inflata*. *Bio. Med. Chem.*, **6**, 339–374.

Hashimoto, T., Kumazawa, S., Nanjo, F., Hara, Y. and Nakayama, T. (1999). Interaction of tea catechin with lipid bilayers investigated with liposome system. *Biosci. Biotechnol. Biochem.*, **63**, 2252–2255.

Jimenez, M. and Garcia-Carmona, F. (1999). Myricetin, an antioxidant flavonol, is a substrate of polyphenol oxidase. *J. Sci. Food Agric.*, **79**, 1993–2000.

Kahkonen, M., Hopia, A., Vuorela, H., Rauha, J., Pihlaja, K., Kujala, T. and Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.*, **47**, 3954–3962.

Liebert, M., Licht, U., Bohm, V. and Bitsch, R. (1999). Antioxidant properties and total phenolics content of green and black tea under different brewing conditions. *Z. Lebensm. Unters. Forsch. A.*, **208**, 217–220.

Miller, N., Diplock, A. and Rice-Evans, C. (1995). Evaluation of the total antioxidant activity as a marker of the deterioration of apple juice on storage. *J. Agric. Food Chem.*, **43**, 1794–1801.

Murakami, A., Ali, A., Mat-Salleh, K., Koshimizu, K. and Ohigashi, H. (2000). Screening for the *in vitro* anti-tumor-promoting activities of edible plants from Malaysia. *Biosci. Biotechnol. Biochem.*, **64**, 9–16.

Murakami, A., Jiwajinda, S., Koshimizu, K. and Ohigashi, H. (1995). Screening for in vitro anti-tumor promoting activities of edible plants from Thailand. *Can. Lett.*, **95**, 139–146.

Nakagawa, K., Ninomiya, M., Okubo, T., Aoi, N., Juneja, L., Kim, M., Yamanaka, K. and Miyazawa, T. (1999). Tea catechin supplementation increases antioxidant capacity and prevents phospholipid hydroperoxidation in plasma of humans. *J. Agric. Food Chem.*, **47**, 3967–3974.

Nakatani, N., Natural antioxidants from species. (1992). "Phenolic Compounds in Food and Their Effects on Human Health II, Antioxidants and Cancer Prevention," ed. by M.-T. Huang, C.-T. Ho, C.-Y. Lee. American Chemical Society, Washington, DC, pp. 72–86.

Rapisarda, P., Tomaino, A., Cascio, R., Bonina, F., Pasquale, A. and Saija, A. (1999). Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. *J. Agric. Food Chem.*, **47**, 4718–4723.

Rice-Evans, C. and Miller, N. (1994). Total antioxidant status in plasma and body fluids. *Methods Enzymol.*, **234**, 279–293.

Rice-Evans, C., Miller, N., Bolwell, P., Bramley, P. and Pridham, J. (1995). The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Rad. Res.*, **22**, 375–383.

Singleton, V. and Rossi, J. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.*, **16**, 144–158.

Trakoontivakorn, G., Nakahara, K., Shinmoto, H. and Tsushida, T. (1999). Identification of antimutagenic substances (Ames Test) from *Boesenbergia pandurata* Schl. (fingerroot) and *Languas galanga* (Galanga). *JIRCAS J.*, **7**, 105–116.

Yu, T. and Ong, C. (1999). Lag-time measurement of antioxidant capacity using myoglobin and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid): Rationale, application, and limitation. *Anal. Biochem.*, **275**, 217–223.