Asian Journal of Chemistry; Vol. 25, No. 3 (2013), 1761-1762

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.13688

NOTE

Determination of Total Antioxidant Capacity of Rosehip (*R. arvensis* Huds) Growing in Turkey and Rosehip Species by Using CUPRAC Spectrophotometric Method

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(*Received*: 7 March 2012; *Accepted*: 29 September 2012) AJC-12212

L-Ascorbic acid (vitamin C) is the most important vitamin for human nutrition supplied by fruits and vegetables, and is the main water-soluble antioxidant in human plasma. L-Ascorbic acid is reversibly oxidized to form dehydroascorbic acid, which also exhibits biological activity. As a potent antioxidant, L-ascorbic acid scavenges reactive oxygen species including superoxide, protects isolated low density lipoprotein against oxidative modification and plays an important role in the regulation of intracellular redox status. The aim of this study was to measure antioxidant capacity of *R. arvensis* Huds and rosehip species, by using CUPRAC (the cupric ion reducing antioxidant capacity) spectrophotometric method.

Key Words: *R. arvensis* Huds, Antioxidant, Ascorbic acid.

Natural antioxidants such as flavonoids, tannins, coumarins, xanthones, phenolics, terpenoids, ascorbic acid, and proanthocyanins are found in various plant products, including fruits, leaves, seeds, oils and juices[1-5]. The most direct, although empirical, approach for measuring an antioxidant effect, which accounts for both mechanisms, evolves from the simplest concept of antioxidant: *i.e.*, a molecule which delays, by a different mechanism, the onset of massive oxidative degradation of target molecules endowed in their specific matrix-from rubber polymers[6] to foods[7] to plasma lipoproteins[8]. To analyze antioxidant capacity, a sample, containing both the antioxidant and the molecule to be protected, is most frequently challenged with an accelerator of the free radical oxidation[7-9].

Ethyl alcohol (96% EtOH) from Riedel; ammonium acetate, copper(II) chloride and ascorbic acid, neocuprain hemi hydrate were purchased from Merck. Herbal tea and fruit used were supplied from the local supermarkets in Edirne and Tekirdag, west part of Turkey. 1.0×10^{-2} M copper(II) chloride solution was prepared from $CuCl_2 \cdot 2H_2O$ (0.4262 g) dissolved in H_2O and diluted to 250 mL with additional water. Ammonium acetate (NH_4OAc) buffer at pH = 7 was prepared by dissolving NH_4OAc (19.27 g) in water and diluting to 250 mL. Neocuproine (Nc) solution (7.5×10^{-3} M) was prepared by dissolving neocuproine (0.039 g) in 96 % EtOH and diluting to 25 mL with ethanol.

All absorbance measurements were made at the prespecified wavelength of the selected spectrophotometric method using a Shimadzu UV.1601 Vis spectrophotometer using a pair of matched quartz cuvettes of 1 cm thickness (light path).

Plant material and extraction: The fruit samples and herbal tea (weighing 1.00 g) was pounded into small parts with a porcelain mortar. The samples were extracted with distilled water and were mixed with magnetic stirrer for 0.5 h. The extract was filtered through a Whatman filter paper into a 25 mL flask, and diluted to the mark with distilled water. The aqueous extract of nettle was prepared just before the experiments so as to prevent any undesired degradation reactions.

CUPRAC spectrophotometric assay of total antioxidant capacity: CUPRAC method (cupric ion reducing antioxidant capacity) is based on the oxidation of ascorbic acid to dehydroascorbic acid with the cupric ion reducing antioxidant capacity reagent of total antioxidant capacity assay, *i.e.*, Cu(II)-neocuproine (Nc), in ammonium acetate-containing medium at pH 7, where the absorbance of the formed *bis*(Nc)-copper(I) chelate is measured at 450 nm. To a test tube were added 1 mL of $CuCl_2$ solution (1.0×10^{-2} M), 1 mL of neocuproine alcoholic solution (7.5×10^{-3} M) and 1 mL NH_4OAc buffer solution and mixed; (x) mL of herbal extract followed by (1.1 - x) mL of water were added (total volume = 4.1 mL) and mixed well Karasakal *et al.*[10]. Absorbance against a reagent blank was

measured at 450 nm after 0.5 h. Since the molar absorptivity of L-ascorbic acid in the CUPRAC method is $\varepsilon = 1.42 \times 10^4$ L mol^{-1} cm^{-1} and the calibration curve for L-ascorbic acid is a line passing through the origin, the L-ascorbic acid equivalent molar concentration of the herbal extract sample in final solution may be found by dividing the observed absorbance to the ε for L-ascorbic acid. The L-ascorbic acid equivalent antioxidant capacity may be traced back to the original extract considering all dilutions, and proportionated to the initial mass of herbal material taken to find a capacity in the units of mmol AA/g dry matter.

Calculation of CUPRAC spectrophotometric method: The molar absorptivity of L-ascorbic acid in the above reference methods were as follows:

$$\varepsilon_{AA} = 1.42 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1} \text{ (CUPRAC method)}$$

If a herbal infusion (initial volume = V_{cup}) prepared from (m) grams of dried plant was diluted (r) times prior to analysis and a sample volume of V_s was taken for analysis from the diluted extract and colour development (after addition of reagents) was made in a final volume of (V_f) to yield an absorbance of (A_f), then the L-ascorbic acid equivalent antioxidant capacity of the herb (in mmol AA per gram of dried plant or simply mmol AA/g) was found using the equation[10]:

$$\text{Capacity (in mmol AA/g)} = (A_f/\varepsilon_{AA})(V_f/V_s)r(V_{cup}/m)$$

Rosehip samples were measured twice. Total antioxidant capacity of samples were calculated from average absorbance values Table-1 shows total antioxidant capacity of different rosehip species (in the units of ascorbic acid equivalent antioxidant capacity) as measured by CUPRAC Method.

TABLE-1
TOTAL ANTIOXIDANT CAPACITY OF DIFFERENT ROSEHIP SPECIES

	Extraction with Pure water (mmol AA/g)	Extraction with 50 % ethanol-water (mmol AA/g)	Extraction with ethanol (mmol AA/g)
Sample	CUPRIC	CUPRIC	CUPRIC
Rosehip tea	0.420 ± 0.2	0.325 ± 0.1	0.120 ± 0.3
Rosehip juice	0.034 ± 0.4	0.026 ± 0.1	0.008 ± 0.4
Fresh Rosehip seed	0.174 ± 0.5	0.150 ± 0.1	0.012 ± 0.6
Fresh Rosehip fruit	0.658 ± 0.1	0.643 ± 0.2	0.019 ± 0.1
Dry Rosehip seed	0.245 ± 0.3	0.220 ± 0.5	0.050 ± 0.2
Dry Rosehip fruit	0.582 ± 0.7	0.380 ± 0.1	0.047 ± 0.1
Rosehip marmalade	0.089 ± 0.9	0.050 ± 0.2	0.025 ± 0.3
Orange colour rosehip	0.049 ± 0.8	0.060 ± 0.1	0.009 ± 0.3
Red colour rosehip	0.107 ± 0.1	0.033 ± 0.6	0.005 ± 0.1
Bold red colour rosehip	0.136 ± 0.2	0.098 ± 0.3	0.013 ± 0.2

AA = L-Ascorbic acid

Table-2 shows total antioxidant capacity of fresh rosehip fruit (in the units of ascorbic acid equivalent antioxidant capacity) by drying at room temprature, 50 and 85 ºC.

TABLE-2
TOTAL ANTIOXIDANT CAPACITY OF FRESH ROSEHIP FRUITS BY DRYING IN DIFFERENT PERIOD TIMES

	Fresh rosehip dried at room Temperature (mmol AA/g)	Fresh rosehip dried in oven at 50 ºC (mmol AA/g)	Fresh rosehip dried in oven at 85 ºC (mmol AA/g)
Time period	CUPRIC	CUPRIC	CUPRIC
5 h	0.080 ± 0.3	0.076 ± 0.1	0.059 ± 0.9
24 h	0.042 ± 0.4	0.067 ± 0.5	0.045 ± 0.7
48 h	0.034 ± 0.1	0.051 ± 0.2	0.038 ± 0.4

AA = L-Ascorbic acid

Table-3 shows statistics graphic. The antioxidant capacity (AC) of rosehips was tested by CUPRAC assays and evaluated against the standard TR (L-ascorbic acid).

TABLE-3
STATISTICS GRAPHIC

	CUPRIC
X Average	0.249 ± 0.1
Standard deviation (s)	0.433 ± 0.4
Variation (s^2)	0.187 ± 0.6
Test of t (95 %)	-0.91 < 2.57
	$t_d < t_t$
Test of F	1.02 < 4.28
	$F_d < F_t$

In general, the results pointed out that all rosehips displayed antioxidant capacity properties.Fresh rosehip fruit antioxidant capacity was the highest in extraction with pure water and rosehip juice antioxidant capacity was the lowest. Fresh rosehip fruit antioxidant capacity was the highest in extraction with % 50 ethanol-water and rosehip juice antioxidant capacity was the lowest.Rosehip tea antioxidant capacity was the highest in extraction with ethanol and red colour rosehip antioxidant capacity was the lowest. We observed that while the drying periods increase total antioxidant capacity of fresh rosehip fruits decrease.

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