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HPLC Analysis of α-tocopherol and Vitamin C in Important Medicinal Plants of Uttarakhand Himalayas

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ABSTRACT

Antioxidant phytochemicals such as Vitamin E and Vitamin C contents were estimated in fresh samples of three medicinal plants using a reverse-phase HPLC system. Maximum Vitamin E content (28.14 mg/100 g) was found in the leaves of *Osmanthus fragrans* and maximum Vitamin C content (746.79 mg/100 g) was found in the leaves of *Melia azedarach*. The range of Vitamin E and C in three medicinal plant was 3.53-28.14 mg/100 g and 183.13-746.79 mg/100 g, respectively.

Key words: Vitamin E, vitamin C, HPLC, antioxidants, medicinal plants

INTORDUCTION

Melia azedarach (Meliaceae) is a wild plant native to India, Southern state of America, Africa, South Europe and warmer parts of globe. *M. azedarach* has been widely used for its analgesic, emetic, antiseptic and anthelmintic properties (Chopra *et al*., 1956). *Asplenium dalhousiae* (Aspleniaceae) is a wild plant native to Western Himalayas of India. *A. dalhousiae* has been widely used in traditional medicine for spleen ailment, diuretic and jaundice (Dhar *el al*., 1968; Iwashina *et al*., 1990; Mizuno *et al*., 1990). *Osmanthus* a medicinally important plant, belonging to family Oleaceae is a wild species native to India, East Asia, China, Japan and Himalayas. *O. fragrans*, it has been used in the treatment of dysmenorrhaea, rheumatism and bruises. Flowers of plant are used by the Chinese to impart a pleasant flavour to tea, wine and sweet dishes such as lotus seed, soap, pastries and steamed pears (Srivastava and Kapoor, 1985; Van Poppel and van den Berg, 1997; Sun, 1990). Antioxidant vitamins have a number of biological activities such as immune stimulation, inhibition of nitrosamine formation and alteration of metabolic activation of carcinogens (Van Poppel and van den Berg, 1997; Finckh and Kunert, 1985; Naik, 2003).

There is a need for quantitative data on the antioxidant content of medicinal plants. Such information will not only increase the understanding of the function of these antioxidant phytochemicals in lowering incidence of aging and other chronic diseases, but this study related to qualitative and quantitative distribution of primary antioxidants in cruciferous vegetables may also help in breeding programmes to develop new germplasm with a high content of such phytochemicals. The objective of this study is to provide new data based on HPLC estimations regarding the antioxidant content of medicinal plants (Singh *et al*., 2007).

MATERIALS AND METHODS

Plant material sample were collected from Nainital District, Uttaranchal and authentic identification was done in Botany Department, Kumaun University, Nainital. The plant meterial was dried in shade after collection. The dried plant material was powdered separately in a electrical mill to 60 mesh size. The fine plant material powder so obtained was used for further vitamins analysis.

Extraction and analysis of ascorbic acid: For ascorbic acid extraction and analysis, the procedure of Kurilich *et al.* (1999) was followed. Twenty five gram of fresh tissue was homogenized in a waring blender with 100 mL of 1% m-phosphoric acid. The slurry was adjusted to 250 mL with 1% m-phosphoric acid and filtered using Whatman filter paper. Took 1.0 mL of this filtrate and added to it 1.0 mL of 5% dithiothreitol, the volume was made up to 10 mL with 1% m-phosphoric acid. The solution was filtered on a 0.2 μ nylon filter and injected 10 μL on reverse phase C-18 (150×4.60 mm 5 μ) HPLC column. The mobile phase consisted of Acetonitrile: 0.05 M KH_2PO_4 (pH 5.9) in the ratio of 75:25 with a flow rate of 1.5 mL min^{-1}. The sample was detected at 261 nm on a Shimadzu SPD-10AV, UV-Visible detector. The retention time for standard ascorbic acid was recorded as 2.565 min.

Extraction and analysis of β-carotene and lutein: The procedure described by Kurilich *et al.* (1999) and Torun *et al.* (1995) was followed for the analysis of β-carotene as well as lutein. The 300-500 mg of sample was taken in a test tube and added to it 10 mL of ethanol containing 0.1 g of BHT. The test tube along with the sample was placed in a water bath at 700C for 15 min. After removing the tubes from the water bath, added 180 μL of 80% KOH to each tube. The sample was vortexed and then saponified at 700C for 30 min. Saponification was essential for maximum extraction of carotene and their esters. The samples were placed directly on ice bath and 2.5 mL of de-ionized water and 2.5 mL Hexane/toluene mixture (10:8). Then the tubes were vortexed and then centrifuged at 2100 rpm for 5 min. The upper layer hexane/toluene fraction was then transferred to a separate test tube. The hexane/toluene extraction was repeated for two more times. The combined hexane/toluene fractions were dried using a Speed-vac concentrator. The residue was reconstituted in 200-400 μL THF. The solution was filtered on a 0.2 μ nylon filter and 20 μL of the filtered solution was injected in the Shimadzu High performance liquid chromatograph. The mobile phase consisted of Acetonitrile: Methanol: THF (52:40:8) (v/v/v) at a flow rate of 2.0 mL min^{-1}. The absorbance was recorded at 450 nm for β-carotene and lutein. The retention time for the standard β-carotene was recorded as 6.192 min for lutein at 2.350 min and for vitamin A at 4.400 min.

Estimation of DL-α-tocopherol: The extraction method used for DL-α-tocopherol was similar to the method as described for carotenoids, however the absorbance was measured at 295 nm for DL-α-tocopherol estimation. The retention time for standard DL-α-tocopherol was 9.067 min. The concentration was calculated from the peak area and was corrected for % recovery.

Recovery experiments were performed in triplicate from plant homogenates of each replication, spiked with known concentrations of standard solutions were performed during the analyses and the mean values for the recoveries were 92, 87, 96 and 81% for vitamin C, DL (α) tocopherol, β-carotene and lutein respectively. The values reported were accordingly modified based on the recovery percentage.

RESULTS AND DISCUSSION

In this study, we have observed that among three medicinal plants the range of α-tocopherol in leaves varied from 3.53-28.14 mg/100 g (Table 1) and vitamin C in leaves varied from 183.13-746.79 mg/100 g (Table 2) on dry weight basis. α-Tocopherol content was observed maximum (28.14 mg/100 g) in *O. fragrans* leaves and minimum (3.53 mg/100 g) in the leaves of *A. dalhousiae*. Vitamin C content was observed maximum (746.79 mg/100 g) in the leaves of *M. azedarach* and minimum (183.13 mg/100 g) in the leaves of *O. fragrans* (Jaffe, 1984).

The study concludes that *M. azedarach*, *A. dalhousiae* and *O. fragrans* are good source of antioxidant especially vitamin E and vitamin C (London *et al*., 1985) (Fig. 1 and 2).

Table 1: α-Tocopherol content in medicinal plants (mg/100 g) dry weight basis

Plant species	Leaves (mg/100 g)	Roots (mg/100 g)
M. azedarach	4.17	0.396
A. dalhousiae	3.53	-
O. fragrans	28.14	-

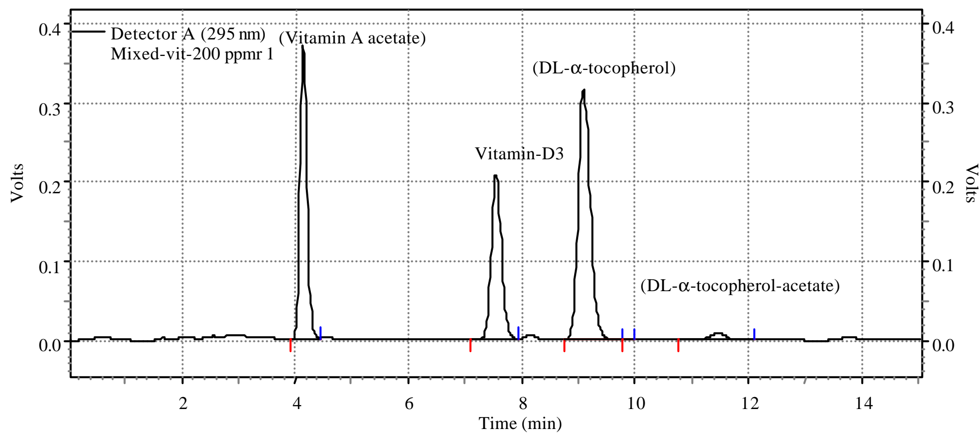

Fig. 1: Standard peak of vitamin E

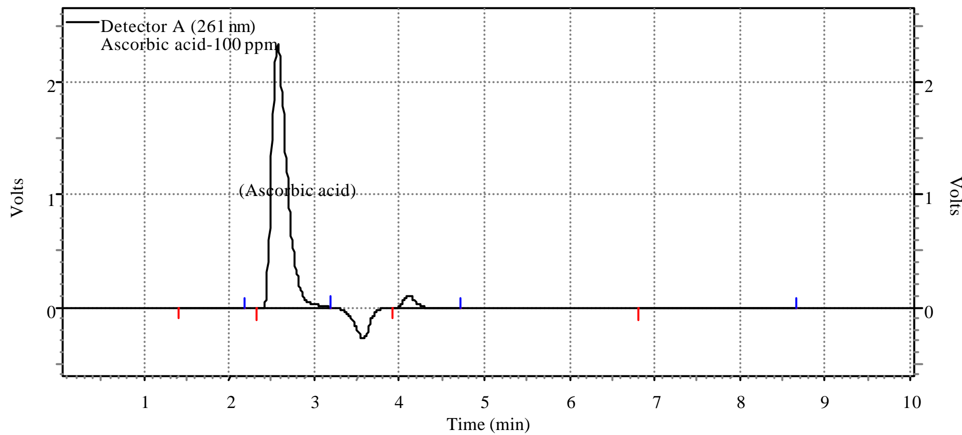

Fig. 2: Standard peak of vitamin C

Table 2: Vitamin C content in medicinal plants (mg/100 g) dry weight basis

Plant species	Leaves (mg/100 g)	Roots (mg/100 g)
M. azedarach	746.79	95.38
A. dalhousiae	485.47	-
O. fragrans	183.13	-

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