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Quantitative Evaluation of β -Carotene and Xanthophyll in Some Medicinal Plants from Kumaon Himalayas

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ABSTRACT

Antioxidant phytochemicals such as β -carotene and xanthophyll contents were estimated in three different medicinal plants, widely distributed in Kumaon Himalayas using a reverse-phase HPLC system. Maximum β -carotene content (481.57 mg/100 g) was found in the leaves of *Asplenium dalhousiae* and maximum xanthophyll content (678.61 mg/100 g) was found in the leaves of *Osmanthus fragrans*. The aim of this study was to characterize the antioxidant value of the medicinal plants with particular attention to carotenoids and xanthophyll.

Key words: β -carotene, xanthophyll, HPLC, antioxidants, medicinal plants

INTRODUCTION

Melia azedarach (Meliaceae) is a wild plant native to India, southern states of America, Africa, South Europe and warmer parts of the globe. It has been widely used for its analgesic, emetic, antiseptic and anthelmintic properties. The plant resembles neem in having the medicinal properties (Rastogi and Mehrotra, 1995; Chopra *et al.*, 1956).

Asplenium dalhousiae (Aspleniaceae) is a wild plant native to India, Mule, Hauchuca and Baboquivari mountains of southern Arizona. It has been widely used in traditional medicines for spleen ailments jaundice and diuretic. Rhizomes of the plant are used in abscesses (Dhar *et al.*, 1974). *Osmanthus fragrans* (Oleaceae) is a wild plant native to India, E. Asia, China, Japan and Himalayas. *O. fragrans*, has been used in the treatment of dysmenorrhoea, 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).

Carotenoids are important nutritionally as antioxidants in the prevention of atherosclerosis and in the prevention of age related macular degeneration (AMD) (Palozza and Krinsky, 1992; Dwyer *et al.*, 2001; Moeller *et al.*, 2000). These natural pigments are more acceptable to consumers as they have always been present in natural foods and are readily metabolized. The metabolites are good for human health. The hydrocarbon carotenoids have provitamin A. Activity and the oxygenated carotenoids or xanthophyll are possibly linked to a lower risk of cancer (Beecher and Khachik, 1984). Some workers have reported the possibility that certain carotenes or their isomers have anticancer potential (Beems, 1987; Hennekens, 1986). β -Carotene has been proved to prevent

peroxidation caused by singlet oxygen and also by scavenging free radicals (Di Mascio *et al.*, 1991; Krinsky, 1989). Carotenoids are mainly responsible for the prevention of the deleterious effect of singlet oxygen (Cadenas, 1989).

MATERIALS AND METHODS

Chemicals: Standard of xanthophyll and β -carotene were procured from Sigma Chemical Co. St Louis, USA. Individual standard was accurately weighed, developed and diluted with HPLC grade ethanol. Petroleum ether, methanol, ethyl acetate and anhydrous sodium sulphate and other chemicals and reagents used in this study were purchased from Merck Chemical Co. Mumbai, India.

Plant material: Plant material (sample) was collected from Nainital District, Uttaranchal and authentic identification was done in Botany Department, Kumaun University, Nainital. The plant material 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 β -carotene and xanthophyll: The procedure described by Kurilich *et al.* (1999) was followed for the analysis of β -carotene as well as xanthophyll. 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 70°C for 15 min. After removing the tubes from the water bath, 180 μ L of 80% KOH was added to each tube. The sample was vortexed and then saponified at 70°C 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 μ L THF. The solution was filtered on a 0.2 μ nylon filter and 20 μ L 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 xanthophyll at 2.350 min and for vitamin A at 4.400 min.

RESULTS AND DISCUSSION

In this study, we have observed that amongst three medicinal plants the range of β -carotene in leaves varied from 110.68-481.57 mg/100 g (Table 1) and xanthophyll in leaves varied from 124.58-678.61 mg/100 g (Table 2) at dry weight basis. In the leaves of *M. azedarach*, both

Table 1: β -carotene content in some important medicinal plants

Species	Leaves (mg/100 g)	Roots (mg/100 g)
<i>Melia azedarach</i>	110.68	8.31
<i>Asplenium dalhousiae</i>	481.57	-
<i>Osmanthus fragrans</i>	163.26	-

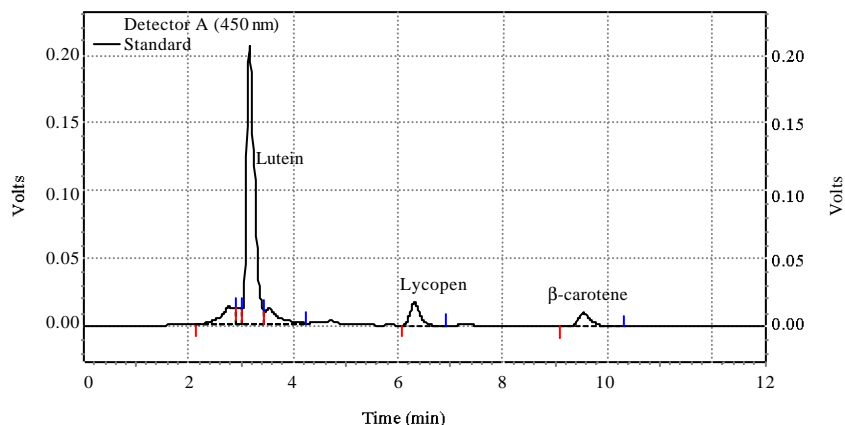
Fig. 1: Standard peaks of β -carotene and xanthophyll

Table 2: Xanthophyll content in some important medicinal plants

Species	Leaves (mg/100 g)	Roots (mg/100 g)
<i>Melia azedarach</i>	124.58	0.56
<i>Asplenium dalhousiae</i>	136.57	-
<i>Osmanthus fragrans</i>	678.61	-

β -carotene and xanthophyll content was much higher than the roots of the same plant. The maximum β -carotene was observed in *A. dalhousiae* and minimum in *M. azedarach* leaves. The maximum xanthophyll content was observed in *O. fragrans* and minimum in *M. azedarach* leaves.

Khachik and Beecher (1987) have reported carotene 137.60-179.82 mg/100 g in carrots, 47.38-53.33 mg/100 g in sweet potatoes and 208.00-232.01 mg/100 g in pumpkin at dry weight basis. Plants of *A. dalhousiae* have more carotene level than carrot, sweet potatoes and pumpkin.

The chromatographic separation of β -carotene and xanthophyll standard is presented in Fig. 1. Typical chromatograms of β -carotene and xanthophyll extracted from medicinal plants are shown in Fig. 2. Standards of β -carotene and xanthophyll were purchased from sigma and each individual standard was accurately weighed, dissolved and diluted with ethanol to obtain concentration about $100 \mu\text{g mL}^{-1}$.

Identification of the β -carotene and xanthophyll was performed with reference to standard. The quantification of these carotenoids in samples were compared with known quantity of standard peak area.

Thus, the study concludes that *M. azedarach*, *A. dalhousiae* and *O. fragrans* are excellent sources of antioxidants especially β -carotene and xanthophyll. The distribution of carotenoids and their related geometrical isomers in common medicinal plants has important application for the health of Asian people in addition to the basic needs of developing countries.

The significance of xanthophyll and E/Z isomers is becoming increasingly evident in eye health and more specifically in relation to the prevention of AMD and other blinding disorders.

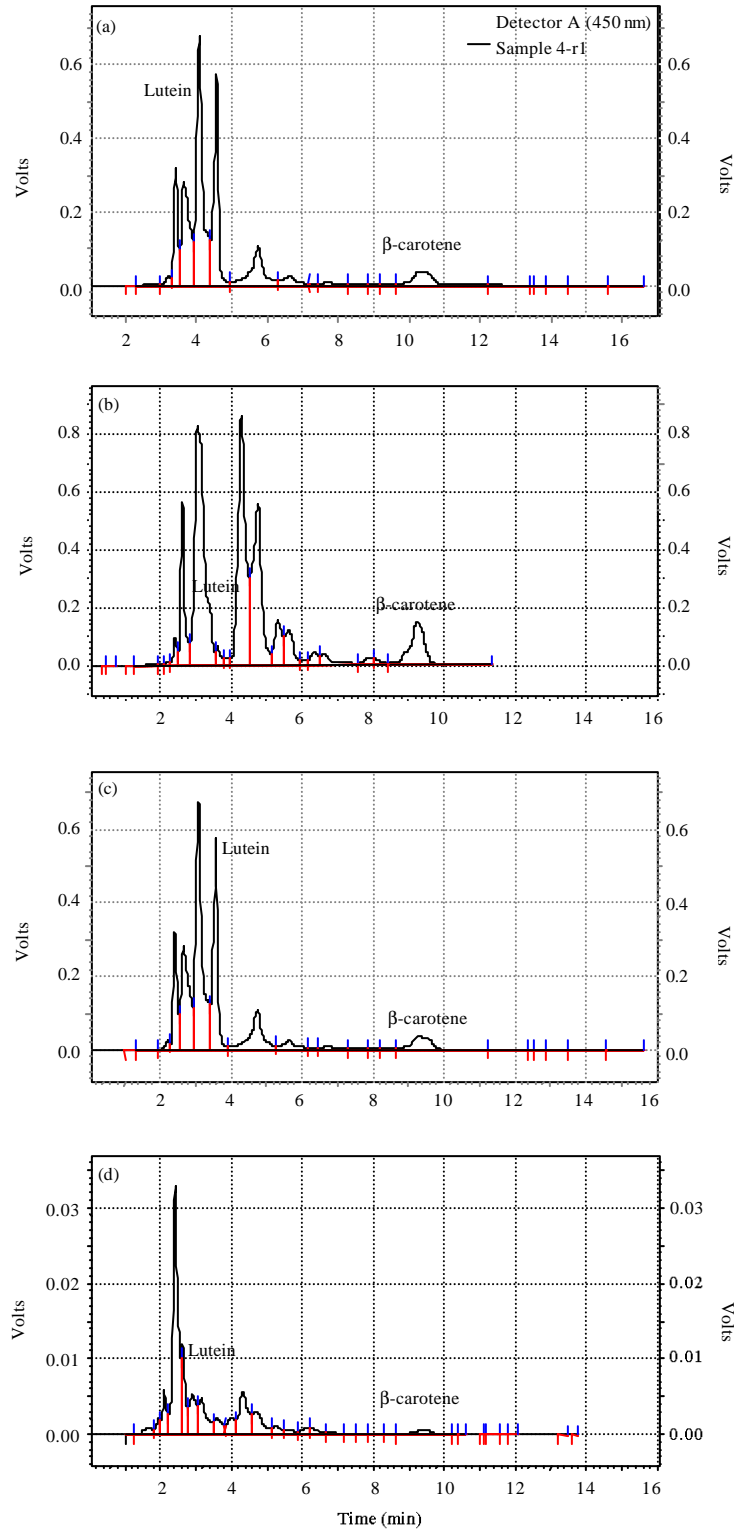


Fig. 2(a-d): Standard peaks of β -Carotene and xanthophyll extracted from (a) *O. fragrans*, (b) *A. dalhousiae*, (c) *M. azedarach* (leaves) and (d) *M. azedarach* (roots)

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