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Preliminarily Investigation on Antioxidant Phytochemical in Some Medicinal Plants of Kumaon Region

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ABSTRACT

While screening *Asparagus adscendens* Roxb, *Vitex negundo* Linn. and *Stephania glabra* Miers in search of natural antioxidant, we first time developed HPLC method for the determination of α-carotene, β-carotene, xanthophyll and DL-α-tocopherol in the said spices. These antioxidants were extracted in light petroleum ether/methanol/ethyl acetate (1:1:1 ratio) and separated by phase separation method. Chromatogram of carotenoids and DL-α-tocopherol showed characteristic absorbance at 450 and 291 nm, respectively. This method was successfully applied to the analysis of antioxidants of these plants. The retention time of xanthophyll, α-carotene and β-carotene were found 2.045, 10.947 and 11.495 min, respectively. The retention time of DL-α-tocopherol was found 11.780 min. The maximum content of xanhophyll (8.08±0.93 mg/100 g), α-carotene (3.89±0.53 mg/100 g), β-carotene (243.53±0.66 mg/100 g) and total phenolics (324.55±0.47 mg/100 g) was found in *V. negundo* under the three investigated plants. The content of α-tocopherol (11.32±0.19 mg/100 g) and vitamin-c (311.45±0.47 mg/100 g) were maximum in *A. adscendens*. In the present study, the plants are found good source of antioxidants.

Key words: Xanthophyll, α-carotene, β-carotene, α-tocopherol, HPLC, *Asparagus adscenden*, *Vitex negundo*, *Stephania glabra*

INTRODUCTION

Asparagus adscendens Roxb (Liliaceae), occurs throughout the sub Himalayan tract, the central and outer hill range up to 5000 feet. Roots have cooling, demulcent and diaphoretic property. The herb is a nutritive tonic useful in general debility, leucorrhea, sexual debility and spermatorrhea. This herb is also used during pregnancy and postpartum. It helps to nourish the foetus and increase the breast milk flow. Dried roots of *A. adscendens* Roxb are used as drugs (Osmaston, 1976). *Stephania glabra* Miers. (Menispermacae) occurs throughout the hills between 3000-6000 feet. Tubers are used in pulmonary tuberculosis, asthma and intestinal complaints (Osmaston, 1976).

Vitex negundo Linn. (family Verbenaceae) commonly known as Nirgundi or Shiwali occurs throughout the greater part of India up to an altitude of 1500 m in the outer western Himalayas (Anonymous, 1976; Chopra *et al*., 1956). The leaves are green in upper surface. Flowers are bluish purple, fruits are black when ripe. The plant can grow on nutritionally poor soil (Prasad and Wahi, 1965). Leaves are aromatic, bitter, acrid, astringent, anti-inflammatory, antipyretic or febrifuge,

tranquillizer, antihelmintic and vermifuge. Flowers are cool, astringent, carminative, hepatoprotective, digestive, febrifuge, vermifuge and are useful in haemorrhage and cardiac disorders. Fruits are nervine, cephalic, aphrodisiac and vermifuge (Chopra *et al.*, 1956).

Considering the importance of these plants in the traditional medicine and pharmaceutical industries, it is important to screen the species for its antioxidant contents. To the best of our knowledge on reference are available on the determination of xanthophyll, α-carotene, β-carotene, α-tocopherol. The objective of the present study is to provide database on HPLC estimation regarding the natural compounds and their quantification.

MATERIALS AND METHODS

Chemical and reagents: Standard of xanthophyll, α-carotene, β-carotene and DL-α-tocopherol were purchase from Sigma and each individual standard were accurately weighted, developed and diluted with HPLC grade ethanol. Petroleum ether, methanol, ethyl acetate and anhydrous sodium sulphate other chemicals and reagents purchase form Merck.

Plant material: *Asparagus adscendens* Roxb, *Vitex negundo* Linn and *Stephania glabra* Miers were collected in October 2006 from Harara (Distt. Almora, Uttarakhand) identified by Prof. Y.P.S. Pangtey, Department of Botany, Kumaun University, Nainital and also from Dr. H.C. Pandey, Botanical Survey of India, Dehradun and herbarium deposited at phytochemistry lab D.S.B. Campus, K.U. Nainital.

Isolation and extraction of carotenoids and tocopherol: The rhizomes root and leaves of each were dried in shade and powdered using electrical grinder. Dried plant material (1.0 g of each) was extracted with light petroleum ether/methanol/ethyl acetate (1:1:1, V/V/V, 4×30 mL) until the extracts were colorless. The extracts were mixed in a 250 mL separating funnel and shaken vigorously and allowed to stand for phase separation. Upper layer was collected and lower layer was shaken with 50 mL water and 50 mL petroleum ether for phase separation. Upper layers were mixed with the first extracts. The organic extract was dried over anhydrous sodium sulphate (10 g), filtered and evaporated to dryness in a Rotary Vacuum Evaporator under reduced pressure. The residue was dissolved in light petroleum ether (5 mL) and filtered by 0.2 μm membrane filter before HPLC analysis (Bernstein *et al.*, 2001).

HPLC analysis: The samples were analyzed by Shimadzu HPLC system, column used was C_{18} phenomenex® (5 μ, 150×4.6 mm analytical column) with solvent system 8:2:40:50 (methanol, ethyl acetate, acetonitrile and acetone) flow rate 0.7 mL min^{-1}, run time 20 min and detector wavelength was 450 nm. The HPLC condition of the estimation DL-α-tocopherol by as described by Kurilich *et al.* (1999) methods at 291 nm.

Total phenolic compound analysis: The rhizomes root and leaves of each were dried in shade and powdered using electrical grinder. The amount of total phenolic content was estimated following Singleton *et al.* (1999) with minor modification. The reaction mixture contained 100 dL of sample extract, 500 μL Folins-Ciocalteu's reagent (freshly prepared), 2 mL of 20% sodium carbonate and 5 mL of distilled water. After 15 min reaction at 45°C, the absorbance at 650 nm was measured using spectrophotometer (HITACHI, Model U 2001). Results were expressed as mg of catechol equivalent per 100 g of dry weight.

Vitamin-C (ascorbic acid) analysis: Ascorbic acid content was estimated by (Ranganna, 1976) method with little modification. Dried leaves powder (2.0 g) was extracted with 4% oxalic acid, made upto 100 mL and centrifuged at 10,000 rpm for 10 min. Five milliliter supernatant liquid was transferred in a conical flask, 10 mL of 4% oxalic acid was added and finally titrated against standard dye solution (2, 6-dichlorophenol indophenol). The procedure was repeated with a blank solution omitting the sample. Five milliliter ascorbic acid with concentration (100 ppm) was used as standard.

RESULTS AND DISCUSSION

The aim of this study was to characterize the antioxidant value of these medicinal plants with particular attention to carotenoids, vitamins and phenolics. In this study, we have observed that xanthophyll, α-carotene, β-carotene and DL-α-tocopherol contents are present in these plants. The composition of these compounds is presented in Table 1. The retention time of xanthophyll, α-carotene and β-carotene were found 2.045, 10.947 and 11.495 min, respectively. The retention time of DL-α-tocopherol was found 11.780 min. The available percentage of compounds i.e., xanthophyll, α-carotene, β-carotene and DL-a-tocopherol content is presented (Table 1). The chromatographic separation of xanthophylls, β-carotene, α-carotene and DL-α-tocopherol standard is presented in Fig. 1-3.

In the present study we have observed that β-carotene in root *Asparagus adscendens* Roxb were 15.47-15.54 mg/100 g and xanthophylls in roots varied from 0.09-0.11 mg/100 g on dry weight basis. The range of α-tocopherol was 11.19-11.46 mg/100 g, total phenolics were 234.12-234.9 and vitamin C was 310.63-311.81 mg/100 g but α-carotene was not determined in the roots of plants. The *Vitex negundo* Linn. leaves contain the range of β-carotene was 243.51-243.96 mg/100 g, α-carotene was 3.52-4.27 and xanthophyll was 8.08-8.73 mg/100 g on the dry weight basis. The range α-tocopherol were 9.84-10.31 mg/100 g, total phenolics were 324.21-324.88 mg/100 g and vitamin C was 264.99±0.58 mg/100 g on dry weight basis. Carotenoids are a group of natural pigments, widely acceptable to consumers being present in natural foods and are readily metabolized. The hydrocarbon carotenoids have provitamin-A activity and the oxygenated carotenoids or xanthophylls are possibly linked to a lower risk of cancer (Beecher and Khachik, 1984). Studies have shown that carotenoids may play an important role in the prevention of age related macular degeneration (AMD) (Landrum *et al*., 1997). β-Carotene has proved to prevent peroxidation caused by singlet oxygen and also by scavenging free radicals. The rhizomes

Table 1: Antioxidant content in the selected medicinal plants of Kunaon region

	Asparagus adscendens		*Vitex negundo*		*Stephania glabra*	
Antioxidants	mg/100 g	Range	mg/100 g	Range	mg/100 g	Range
Xanthophyll	0.10±0.01	0.09-0.11	8.08±0.93	7.42-8.73	3.33±0.11	3.24-3.38
α-carotene	-	-	3.89±0.53	3.52-4.27	1.66±0.47	1.33-2.00
β-carotene	15.51±0.05	15.47-15.54	243.51±0.66	243.03-243.96	15.51±0.05	15.47-15.54
DL-α-tocopherol	11.32±0.19	11.19-11.46	10.08±0.33	9.84-10.31	10.52±0.44	10.09-10.98
Vitamin C	311.45±0.96	310.63-311.81	166.17±0.95	165.78-166.94	264.99±0.58	264.58-265.4
Total phenolics	234.58±0.58	234.12-234.9	324.55±0.47	324.21-324.88	183.61±0.55	183.19-183.95

All values are mean of triplicate determinations expressed on dry weight basis, ± is the standard error

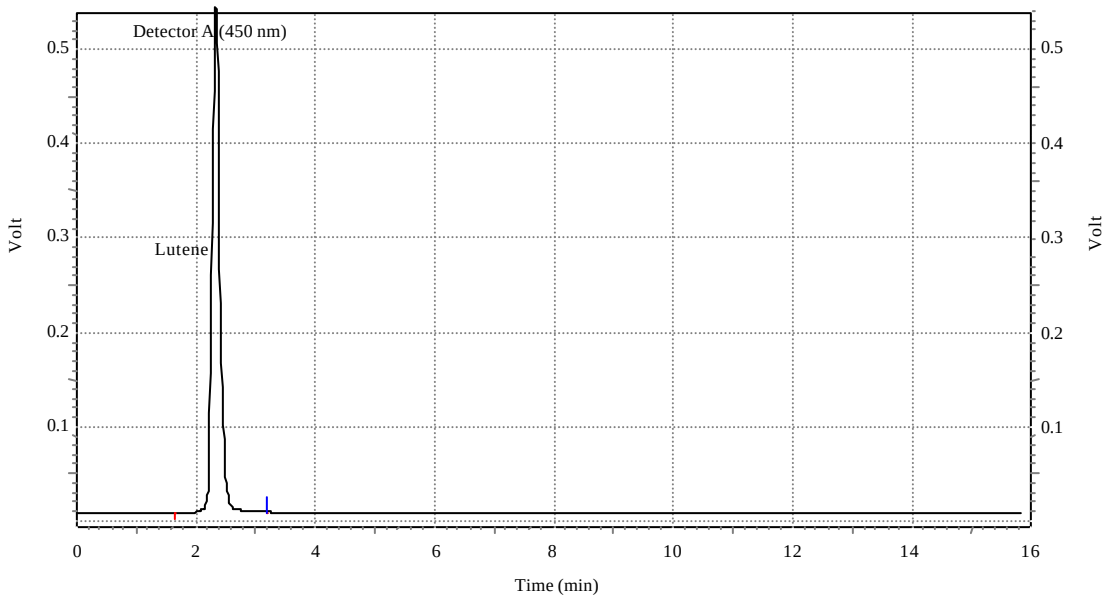

Fig. 1: Chromatogram of standard peak of xanthophyll

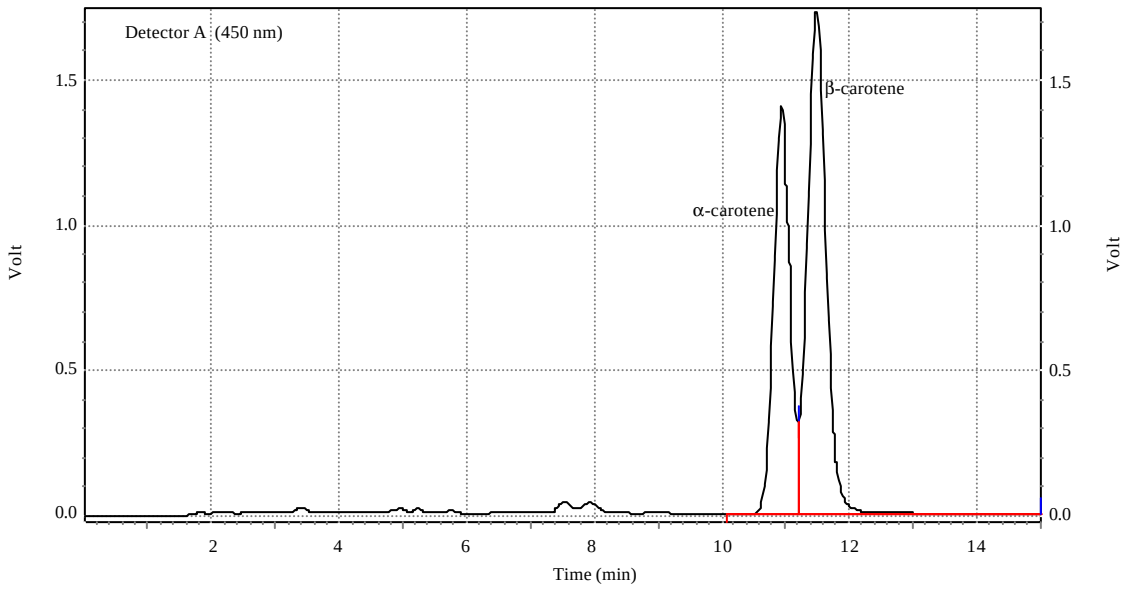

Fig. 2: Chromatogram of standard peak of α-carotene and β-carotene

of *Stephania glabra* were contain 1.33-2.00 mg/100 g α-carotene, 15.51-15.54 mg/100 g β-carotene and 3.33-3.38 mg/100 g xanthophyll on dry weight basis. The range of α-tocopherol in *Stephania glabra* were 10.09-10.98 mg/100 g on dry weight basis. Vitamin C in this plant was 264.99±0.58 mg/100 g and total phenolics were 183.19-183.95 mg/100 g on dry weight basis. The result shows that this plant is very powerful antioxidant.

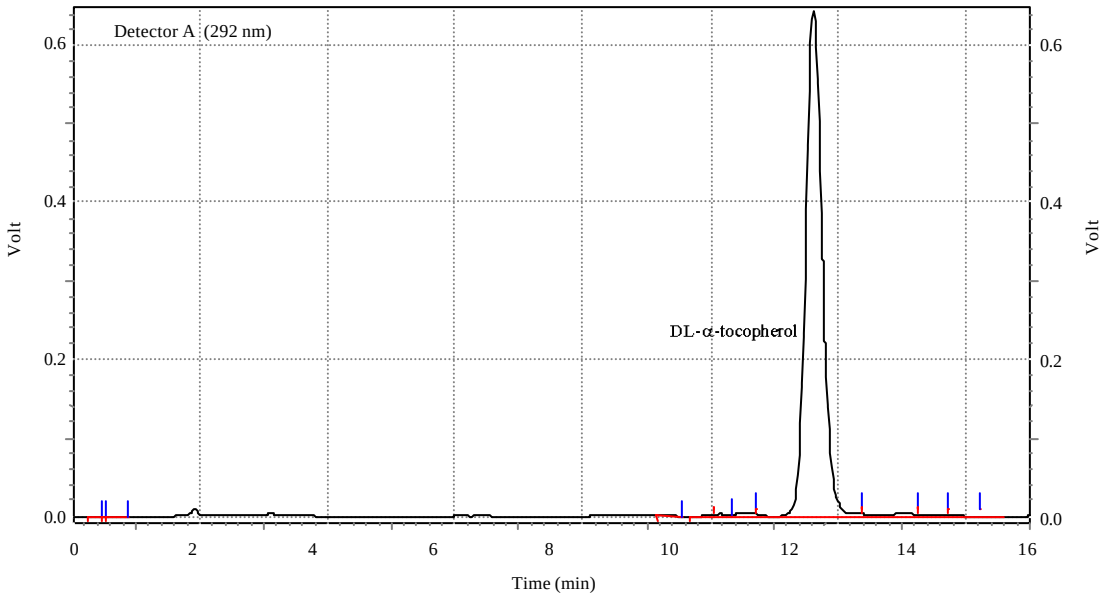

Fig. 3: Chromatogram of standard peak of DL-α-tocopherol

The maximum content of xanhophyll (8.08±0.93 mg/100 g), α-carotene (3.89±0.53 mg/100 g), β-carotene (243.53±0.66 mg/100 g) and total phenolics (324.55±0.47 mg/100 g) were found in *V. negundo* under the three investigated plants. Phenolics, carotenoids and vitamins are well known for its antioxidant activity (Kahkonen *et al*., 1999; Javanmardi *et al*., 2002) and repeatedly been used as natural antioxidants in fruits, vegetables and other plants. For example, caffeic acid, ferulic acid and vanillic acid are widely distributed in the plant kingdom (Larson, 1988). Rosamarinic acid, an important phytochemical has been found to be a potent active substances against Human Immunodeficiency Virus type1 (HIV-1) (Mazumder *et al*., 1997). The content of α-tocopherol (11.32±0.19 mg/100 g) and Vitamin C (311.45±0.47 mg/100 g) were maximum in *A. adscendens*. In the present study, the plants are found good source of antioxidants. Likewise, α-Tocopherol is known to have a number of biological activities such as immune stimulation, inhibition of nitrosamine formation and alteration of metabolic activation of carcinogens (Sun, 1990). The major protective function of the vitamins against cancer is the lowering the lipid oxidation in human body and counteract the prooxidative effect with other compound like ascorbate and combination of ascorbate and β-carotene (Skibsted *et al*., 2005).

CONCLUSION

This study concludes that the rhizomes and leaves of each are excellent source of natural antioxidants. There is a great need to further study in which the plants rhizomes and leaves easily available in the local markets for preparation of drugs.

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