Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2018, 10(2):40-48

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Amino Acid and Antioxidant Composition of Some Wild Edible Medicinal Plants of Uttarakhand Himalayas

Kundan Prasad[1*] and Deepak Chandra[2]

[1]*Department of Chemistry, Govt I C Garkha, Pithoragarh, Uttarakhand, India*
[2]*Department of Chemistry, Kumaun University, Nainital, Uttarakhand, India*

ABSTRACT

Wild plants play a vital role in the health security of Himalayan inhabitants and constitute significantly to their diet. In this report, we investigated the nutritional composition of Euphorbia hirta Linn growing at high altitudes in the Himalayas. The collected plant materials were first washed with cold water to remove the soil particles and then shade dried. The dried material was finely powdered in the grinding machine and weighed in an electrical balance. The dried material was finely powdered in the grinding machine and weighed in an electrical balance. Amino acid analysis was performed using the Waters Associates PICO-TAG method. The total amino acid content in P. indica was 58.80 mg amino acid/g sample (dry weight), E. thymifolia was 123.92 mg amino acid/g sample (dry weight) and P. hirta was 225.73 mg amino acid/g sample (dry weight). The total essential amino acids in P. indica, E. thymifolia and P. hirta were 33.58, 57.99 and 145.82 mg amino acid/g respectively. Among the three investigated plants, Carotenoids viz. xanthophyll content was found 0.13 to 151.01 mg/100g dry weight basis. The maximum xanthophyll content was found in P. indica leaves and minimum in P. hirta rhizomes. The α-carotene content in P. hirta and E. thymifolia was below detection limit (BDL), but in P. indica it was found 1.96 mg/100 g dry weight basis. The β-carotene content varies from 4.62 - 374.55 mg/100 g on dry weight basis. The phenolics content (336.73 mg/100 gm) was found higher in E. thymifolia leaves as compared to P. hirta rhizomes (230.59 mg/100g), while (251.52 mg/100 g) was found in P. indica leaves. DL-α-tocopherol in these medicinal plants was found 13.48 mg/100 g, 24.95 mg/100 g and 9.13 mg/100 g on dry weight basis in P. hirta, E. thymifolia and P. indica respectively. The maximum (24.95 mg/100 gm) in E. thymifolia and minimum (9.13 mg/100 gm) in the P. indica. The retention time of xanthophyll, α-carotene, β-carotene and DL-α- tocopherol were found to be 2.045, 10.947, 11.495 and 11.780 minutes respectively. Thus, it could be a better source of natural amino acids and antioxidants.

Keywords: Wild edible medicinal plant; Amino acid; Carotenoids; Vitamins and phenolics

---

INTRODUCTION

The medicinal value of the plants lies in their chemical substances that produce a definite physiological action on human body [1]. Therefore there is need to evaluate the local herbs for mineral and nutrient composition, so as to determine the potential of indigenous source of medicine [2]. *Pavetta indica* Linn (*Rubiaceae*) is a stout bushy shrub, found in Shri Lanka, South China and Northern India. The leaves of plant are used in the treatment of liver dysfunction, pile, urinary diseases and fever [3,4]. The root of *Pavetta* are bitter, frequently prescribed in visceral obstructions. The roots of plant and dried ginger is given in conjunction with water in the case of dropsy of renal [4]. Methanolic extract of leaves have been reported as antipyretic and anti-inflammatory [5]. *Euphorbia thymifolia* Linn (*Euphorbiaceae*) is traditionally used as blood purifier, cough, antiviral in brachial asthma and paronychia [6] and Water extract of this plants have antiviral activity [7-9]. *Pouzolzia hirta* Linn (*Urticaceae*) is a suberact herb found

in Kumaon region of India [10]. The powder of the plant rhizomes has been used as binder to flour of maize and wheat by the local population of Uttaranchal. The tuberous roots of plants are eaten raw or roasted. The rhizomes of plants are eaten as a vegetable to expel worms [11]. The rhizomes of plants have been reported to possess good antihelmintic activity [12].

MATERIALS AND METHODS

Chemicals

Standard of xanthophyll, α -carotene, β-carotene and DL-α-tocopherol 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 form Merck Chemical Co. Mumbai, India.

Plant Material

The plants were first identified in the Department of Botany, Kumaun University, Nainital and then at B.S.I., Dehradun. The voucher specimen was deposited in the Herbarium section at B.S.I., Dehradun. The voucher no. 112173 for *Pouzolzia hirta* (Blume) Hassk and 112173 for *Pavetta indica*. *Euphorbia thymifolia* was matched with specimen 17195 at B.S.I. Dehradun and herbarium was deposited at Phytochemisty Lab, Chemistry Department, DSB Campus, Nainital. The collected plant materials were first washed with cold water to remove the soil particles and then shade dried. The dried material was finely powdered in the grinding machine and weighed in an electrical balance.

Total Phenolic Content

The rhizomes of each source (wild and planted) were dried in shade and powdered using electrical grinder (Philip-HL1618). The amount of total phenolic content was estimated following [13], with minor modification. The reaction mixture contained 100 Dl of sample extract, 500 Dl 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 UV5704-SS). Results were expressed as mg of Catechol equivalent per 100 g of dry weight.

Ascorbic Acid Content

Ascorbic acid content was estimated by method [14], with modification. Dry leaves powder (2.0 g) was extracted with 4% oxalic acid and made up to 100 ml and centrifuged at 10,000 rpm for a 10 minute. 5 ml supernatant liquid was transferred in a conical flask, followed by addition of 10 ml 4% oxalic acid and titrated against standard dye solution (2, 6-dichlorophenol indophenol) to a pink end point. The procedure was repeated with a blank solution omitting the sample.

Amino Acid Analysis

Amino acid analysis was performed using the Waters Associates PICO-TAG method [15], an integrated technique for precolumn derivatization of amino acids using phenylisothiocyanate (PITC). The PICO-TAG technique comprises of three steps: (i) Hydrolysis of protein or peptide samples to yield free amino acids, (ii) pre-column derivatization of the samples with PITC and (iii) analysis by reverse phase HPLC. The chromatographic separation on the hydrolyzates was performed using a reverse phase Pico-Tag column (3.9 × 300 mm) C_{18} at 40°C and a UV detector at 254 nm. The solvent system consisted of two eluents, (A) an aqueous buffer and (B) 60% acetonitrile in water. Gradient elution were employed using two pumps, programmed to deliver the mobile phases eluents A and B. A gradient which was run for the separation consisted of 10% B traversing to 51% B in 10 min using a convex curve (number 5). A set of amino acid standards (Merck Germany) was analyzed with each set of three experimental samples. Identification of the amino acids in the samples was carried out by comparison with the retention times of the standards.

Extraction and Isolation of Carotenoids and Tocopherol

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 became colorless. The extract was mixed in a 250 ml separating funnel, shaken vigorously and allowed to stand for phase separation. Upper layer was collected in a 100 ml flask (Borosil India Co.

Ltd.) and lower layer was shaken with 50 ml water and 50 ml petroleum ether for phase separation. Upper layer was mixed with the first extract. 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 prior to HPLC analysis.

HPLC Analysis

All the samples were analyzed using Shimadzu HPLC interfaced with model SPD-10 AVP Variable wavelength (190-750 nm) UV- Vis detector, Column used was C_{18} Phenomenex® (150 × 4.60 nm), pore size 5 µm with solvent system 8:2:40:50 (methanol, ethyl acetate, acetonitrile and acetone), flow rate 0.7 ml/min, run time 20 minutes and detector wavelength was 450 nm. The HPLC condition for the estimation DL-α-tocopherol was adopted as described in [16].

RESULTS AND DISCUSSION

Amino Acid Composition

The amino acid content of each of the three plants *viz., P. hirta, E. thymifolia* and *P. indica* are summarized in Table 1. Quantitative determination of amino acid concentration was conducted by HPLC and the amino acid profile is shown in the chromatogram (Figures 1-4). Seventeen amino acids were detected and the separation of these amino acids in the sample is reasonably resolved. All the essential amino acids i.e., methionine, leucine, lysine, cysteine, phenylalanine, tyrosine, arginine, isoleucine, threonine and valine and seven non-essential amino acids were found to be present in the three plants. The total amino acid content in *P. indica* was 58.80 mg amino acid/g sample (dry weight), *E. thymifolia* was 123.92 mg amino acid/g sample (dry weight) and *P. hirta* was 225.73 mg amino acid/g sample (dry weight). The total essential amino acids in *P. indica*, *E. thymifolia* and *P. hirta* were 33.58, 57.99 and 145.82 mg amino acid/g respectively.

The ratio of essential amino acids to total amino acid is 0.57 i.e., more then half of the amino acid in *P. indica*. The results also indicated that the ratio of essential amino acids to non-essential amino acids is 1.33. *P. indica* is rich in alanine, lysine, valine, arginine, alanine, glutamic acid, proline, and aspartic acid. Glycine, cysteine, methionine and phenylalanine are present in lower amount compared to the other amino acids. Data on threonine is not included in this work since this amino acid may be destroyed during acid hydrolysis.

The ratio of essential amino acids to total amino acid is 0.47 i.e., almost half of the amino acid in *E. thymifolia* consist of essential amino acids. The results also indicated that the ratio of essential amino acids to non-essential amino acids is 0.88.

Table 1: Amino acid content of three plants in mg/g dry weight basis

Amino	*P. indica*	% of total AA	*E.thymifolia*	% of total AA	*P. hirta*	% of total AA
Aspartic acid[n]	1.34 ± 0.15	2.28	5.86 ± 0.09	4.73	12.75 ± 0.60	5.65
Glutamic acid[n]	1.66 ± 0.06	2.82	7.31 ± 0.05	5.9	14.00 ± 0.09	6.2
Serine[n]	1.19 ± 0.08	2.02	3.47 ± 0.58	2.8	8.03 ± 0.06	3.56
Glycine[n]	0.58 ± 0.01	0.99	1.80 ± 0.04	1.45	8.16 ± 0.05	3.61
Histidine[n]	1.23 ± 0.01	2.09	3.65 ± 0.04	2.95	-	-
Alanine[n]	17.96 ± 0.01	30.54	43.84 ± 0.37	35.38	36.59 ± 0.05	16.21
Proline[n]	1.26 ± 0.07	2.14	-	-	0.38 ± 0.03	0.17
Lysine[a]	3.03 ± 1.34	5.15	-	-	1.44 ± 0.04	0.64
Threonine[a]	-	-	5.99 ± 0.04	4.83	40.74 ± 0.08	18.05
Tyrosine[a]	2.20 ± 0.06	3.74	4.51 ± 0.13	3.64	10.06 ± 0.10	4.46
Valine[a]	3.61 ± 0.07	6.14	11.69 ± 0.13	9.43	25.04 ± 0.03	11.09
Methionine[a]	0.55 ± 0.13	0.94	2.46 ± 0.07	1.99	4.24 ± 0.02	1.88
Cysteine[a]	0.60 ± 0.33	1.02	0.90 ± 0.10	0.73	1.95 ± 0.03	0.86
Isoleucine[a]	1.97 ± 0.33	3.35	8.66 ± 0.48	6.99	17.50 ± 0.08	7.75
Leucine[a]	1.93 ± 0.09	3.28	11.71 ± 0.14	9.45	27.90 ± 0.01	12.36
Phenylalanine[a]	0.62 ± 0.07	1.05	4.38 ± 0.01	3.53	12.06 ± 0.02	5.34
Arginine[a]	19.07 ± 0.09	32.43	7.69 ± 0.05	6.21	4.89 ± 0.04	2.17
TEAA	33.58	51.96	57.99	46.8	145.82	63.96
TNEAA	25.22	48.04	65.93	53.2	79.91	36.04
TAA	58.8		123.92		225.73	

All values are mean of triplicate determinations expressed on dry weight basis; ±, Denotes the standard error; [a]-TEAA, total essential amino acid; [b]TNEAA, total non-essential amino acid; AA, amino acid

Table 2: Comparison of the content of selected essential amino acid of 3 plants with that of the WHO Ideal pattern

Plant specimen	ILE	LEU	VAL	PHE+TYR	LYS	THR	MET+CYS	SCORE*
WHO standard	4	7	5	6	5.5	4	3.5	---
P. indica	3.35	3.28	6.14	4.79	5.15	-	1.96	01-Jul
E. thymifolia	6.99	9.45	9.43	7.17	-	4.83	2.72	05-Jul
P. hirta	7.75	12.36	11.09	9.8	0.64	18.05	2.74	05-Jul

*this pattern is based on the essential amino acid need for the preschool child; WHO/FAO. Energy and Protein Requirements. WHO Technical Report Series, No. 522. Geneva, World Health Organization, 1973

E. thymifolia is rich in alanine, methionine, phenylalanine, valine, glycine,arginine, alanine, glutamic acid, and aspartic acid. Cysteine is present in lower amount as compared to the other amino acids. Data on proline and lysine are not included in this work since this amino acid may be destroyed during acid hydrolysis.

The ratio of essential amino acids to total amino acid is 0.65 i.e., more than half of the amino acid in *P. hirta* consist of essential amino acids. The results also indicated that the ratio of essential amino acids to non-essential amino acids is 1.82. *P. hirta* is rich in alanine, glycine, phenylalanine, threonine, valine, methionine,arginine, alanine, glutamic acid, proline, and aspartic acid. Lysine and cysteine are present in lower amount as compared to the other amino acids. Data on histidine is not included in this work since this amino acid may be destroyed during acid hydrolysis.

In this study, we compared the amino acid composition of each of three specimens to that of a World Health Organization standard protein [17]. According to the WHO reference protein, the highest quality plant proteins were found in *Euphorbia thymifolia*, and *Pouzolzia hirta* (Table 2) each of these scored at or above the score of the WHO standard for 5 of 7 amino acids or amino acid pairs.

The nutritional analysis of the indigenous edible and fodder plants of the Uttarakhand region by chemical means gives the potential values of these foods to those populations who rely upon them as staples or supplements to their diet. The next step is to assess the bioavailability of the essential nutrients in these plants, such studies must be contemplate. These studies will focus on the composition of the biochemical, mineral, amino acid present in these plants and on the possible presence of antinutrients, such as metal chelators (e.g., phytates, oxalates) and protease inhibitors.

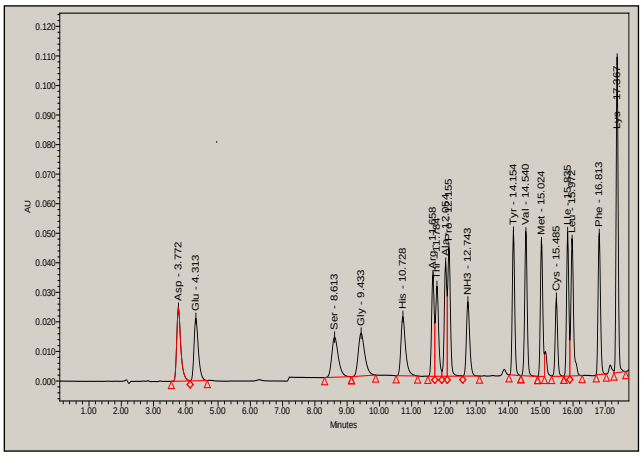

Figure 1: Amino acid profile of Standard

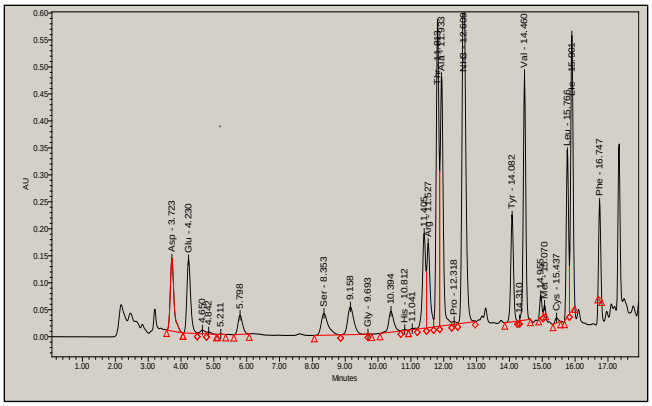

Figure 2: Amino acid profile of *Pavetta indica*

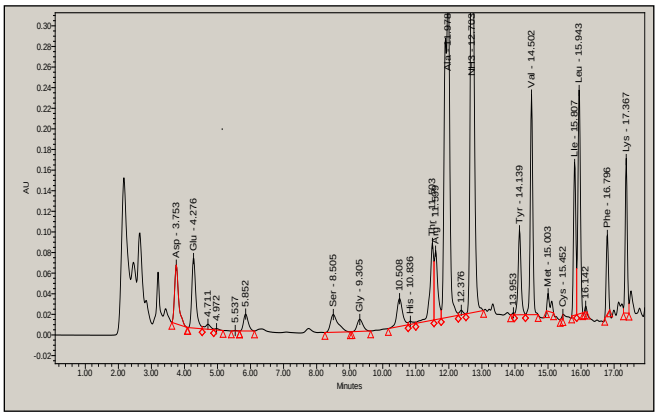

Figure 3: Amino acid profile of *Euphorbia thymifolia*

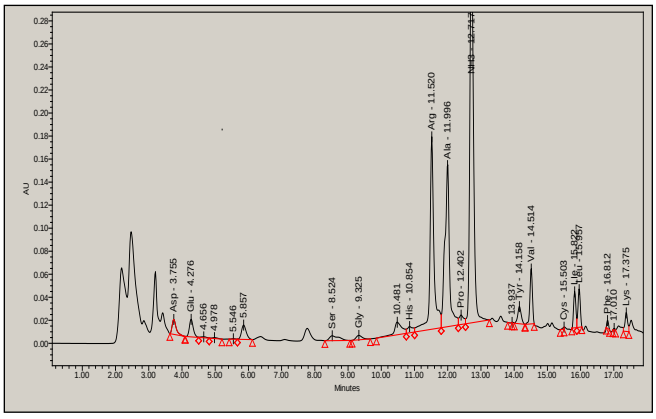

Figure 4: Amino acid profile of *Pouzolzia hirta*

Antioxidant Analysis

The aim of this work was to characterize the antioxidant value of the medicinal plants with particular attention to carotenoids, phenolics and vitamins. In this study, we observed that xanthophyll, α-carotene, β-carotene, vitamin C, and DL-α-tocopherol contents are present in theses medicinal plants (Table 3). The retention time of xanthophyll, α-carotene, β-carotene and DL-α- tocopherol were found to be 2.045, 10.947, 11.495 and 11.780 minutes respectively (Figures 5-13).

Among the three investigated plants, Carotenoids *viz.* xanthophyll content was found 0.13 to 151.01 mg/100 g dry weight basis (Table 3). The maximum xanthophyll content was found in *P. indica* leaves and minimum in *P. hirta* rhizomes. The α-carotene content in *P. hirta* and *E. thymifolia* was below detection limit (BDL), but in *P. indica* it

was found 1.96 mg/100 g dry weight basis. The β-carotene content varies from 4.62 - 374.55 mg/100 g on dry weight basis. *P. indica* contains more β-carotene content than *P. hirta* rhizomes. α-Carotene and β- carotene were found more in the leaves of *P. indica* as compared to other two plants, but DL-α-tocopherol was found more in *E. thymifolia* and the range was 3.48 to 24.14 mg/100 g on the dry weight basis. This is the first study for quantitative variation of antioxidant in these three medicinal plants, so we could not correlate above data with earlier workers.

The amount of total phenolics content varies between three plants rhizomes/leaves (Table 3). The phenolics content (336.73 mg/100 gm) was found higher in *E. thymifolia* leaves as compared to *P. hirta* rhizomes (230.59 mg/100 g), while (251.52 mg/100 g) was found in *P. indica* leaves. As such phenolics are known for their antioxidant activity. The phenolic acids have repeatedly been implicated 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, resmarinic acid, an important phytochemical has been found to be potent active substances against human immunodeficiency virus type1 (HIV-1). DL-α-tocopherol in these medicinal plants was found 13.48 mg/100 g, 24.95 mg/100 g and 9.13 mg/100 g on dry weight basis in *P. hirta*, *E. thymifolia* and *P. indica* respectively. The maximum (24.95 mg/100 gm) in *E. thymifolia* and minimum (9.13 mg/100 gm) in the *P. indica*. DL-α-tocopherol is essential for the human body because it halts lipid oxidation and counteract the prooxidative effect of other compounds like ascorbate and combination of ascorbate and β-carotene. The amount of vitamin C content varied between three plants rhizomes/ leaves (Table 3). The vitamin C contents (108.40 mg/100 gm) was found higher in *P. hirta* rhizomes as compared to (77.49 mg/100 g) *P. indica* leaves, while (88.48 mg/100 g) was found in *E. thymifolia* leaves.

Table 3: Antioxidant content in medicinal plants

S.N.	Antioxidants	*P. hirta*		*E. thymifolia*		*P. indica*	
		mg/100g	**Range**	**mg/100g**	**Range**	**mg/100g**	**Range**
1	Total phenolics	230.59 ± 0.33	230.15-230.95	336.73 ± 0.55	336.25 - 337.50	251.52 ± 1.00	250.23 – 252.68
2	Xanthophyll	0.13 ± 0.01	0.12 - 0.13	0.51 ± 0.04	0.48 - 0.55	151.01 ± 2.16	149.34 - 152.34
3	α–Χαροτενε	-	-	-	-	1.96 ± 0.07	1.96 - 2.01
4	β–Χαροτενε	4.62 ± 0.68	4.14 - 5.10	178.98 ± 4.62	175.45 - 181.90	374.55 ± 1.40	373.77 - 375.66
5	DL-α-tocopherol	13.48 ± 0.83	12.81 - 13.92	24.95 ± 1.10	24.14 - 25.69	9.13 ± 0.28	8.94 - 9.33
6	Vitamin-C	108.40 ± 0.32	108.05-108.83	88.48 ± 0.95	87.37 - 89.68	77.49 ± 1.83	74.90 – 78.90

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

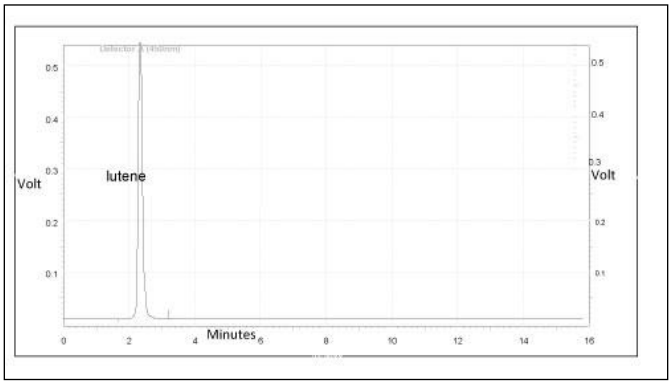

Figure 5: Chromatogram of standard peak of xanthophyll

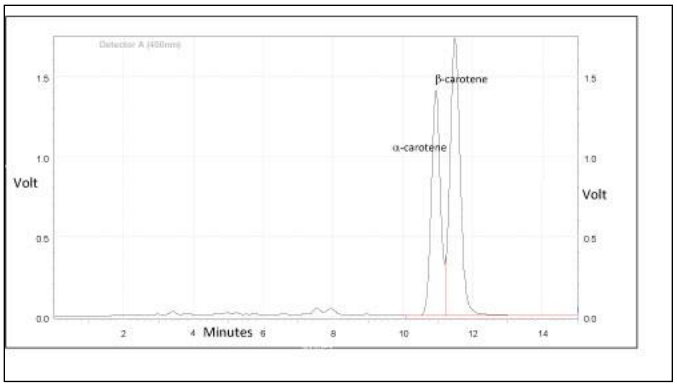

Figure 6: Chromatogram of standard peak of α-carotene and β-carotene

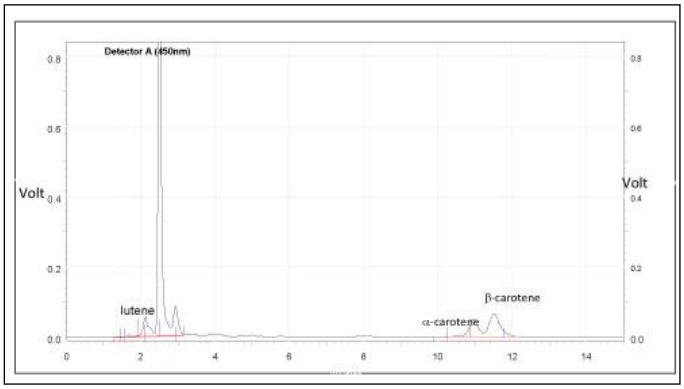

Figure 7: Chromatogram of *Pavetta indica* leaves

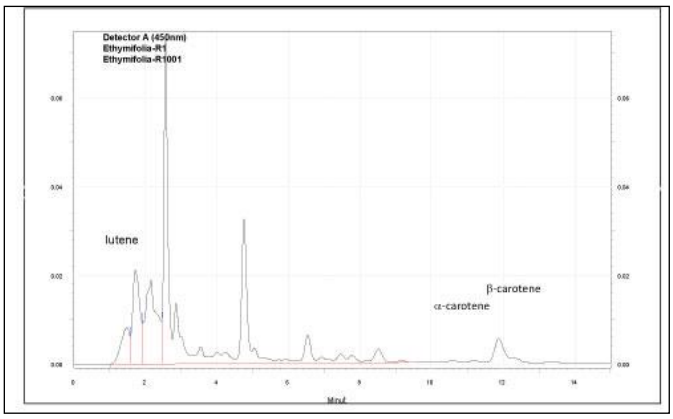

Figure 8: Chromatogram of *Euphorbia thymifolia* aerial parts

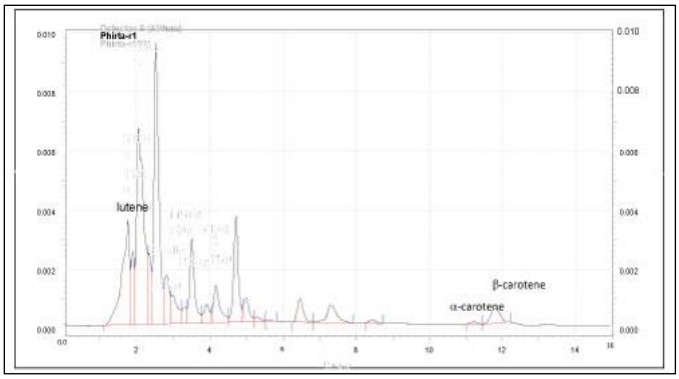

Figure 9: Chromatogram of *Pouzolzia hirta* rhizomes

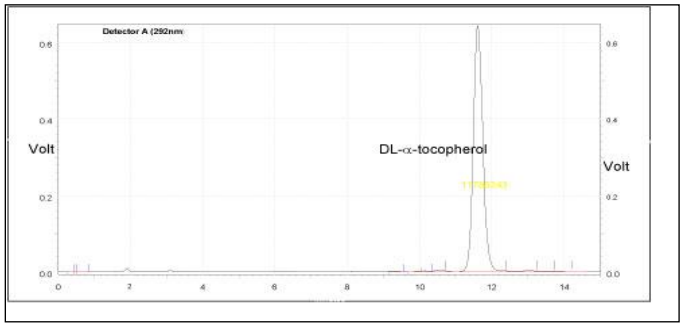

Figure 10: Chromatogram of standard peak of DL-α-tocopherol

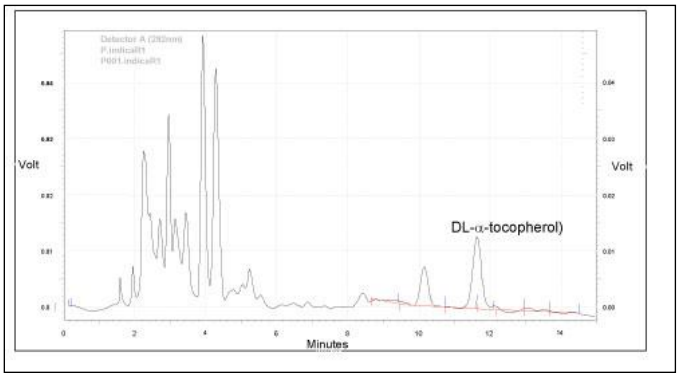

Figure 11: Chromatogram of *Pavetta indi*ca leaves

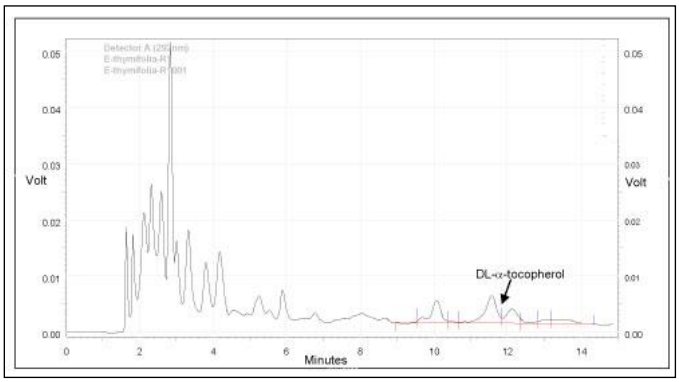

Figure 12: Chromatogram of *Euphorbia thymifolia* aerial parts

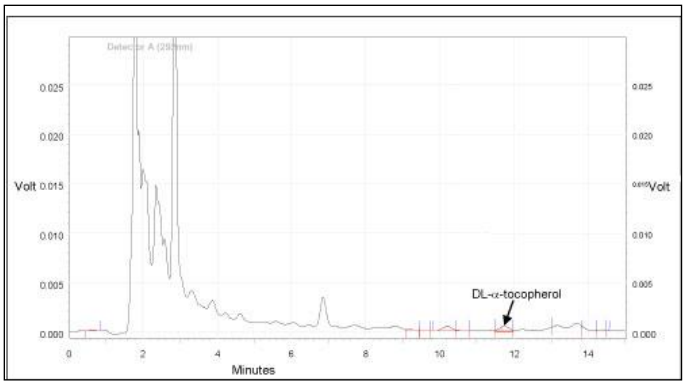

Figure 13: Chromatogram of *Pouzolzia hirta* rhizomes

The analytical data on crude protein, crude fat, gross energy and amino acid profiles of *Pavetta*, *Euphorbia* and *Pouzolzia* clearly suggested their high potentials as cheap source of alternative proteins for humans and animals. Because of the simplicity of technology involved in leaf protein concentrate production, its incorporation into local food production systems is recommended as a practicable, sustainable and ameliorative intervention strategy for the endemic protein under-nutrition in this region.

CONCLUSION

The leaves/ rhizomes of the plant from the data, reveals that it contains an appreciable amount of proteins, minerals, fats, fibres, amino acids, antioxidants, carbohydrates, caloric value and low levels of toxicants whose value can be reduced by cooking. Since it contains substantial amount of nutrients, it can therefore be concluded that these plant leaves/ rhizomes can contribute significantly to the nutrient requirements of man and should be recommended.

REFERENCES

[1] AF Hill. McGraw-Hill Book Company, New York, **1937**.
[2] TN Rahila; A Rukhsandra; A Zaidi; R Shamishila. *Pak Vert J*. **1994**, 14, 160-162.
[3] KR Kritikar, BD Busu. Indian Medicoanl Plants, Vol. III, LM Basu, Allahabad, India, **1933**, 1786-1789.
[4] MI Thabrew; PD Joice; W Rajatissa. *Plant Medica*. **1987**, 53(3), 239-241.
[5] SC Mandal; S Mohana Lakshmi; CK Ashok Kumar; TK Sur; R Boominathan. *Phytother Res*. **2003**, 17, 817-820.
[6] K Manickam; K Rajappan. *Ann Plant Protect Sci*. **1998**, 6(2), 127-130.
[7] The Wealth of Indian Raw Materials, Volume 3, CSIR, New Delhi, India, **1952**, 3, 224-230.
[8] CC Lin; HY Cheng; CM Yang; TC Lin. *J Biomed Sci*. **2002**, 9, 656-664.
[9] NH Khan; M Rahman; MS Nur-e-Kamal. *IJMR*. **1988**, 87, 395-397.
[10] SK Singh; GS Rawat. Flora of Great Himalayan National Park, Himachal Pradesh. Bishen Singh Mahendra Pal Singh, Dehradun, India, **2000**, 304.
[11] SP Ambasta. Publication and Information Directorate, CSIR, New Delhi, India **1994**.
[12] HC Ong; M Nordiana. *Fitoterapia*. **1999**, 70(5), 502-513.
[13] VL Singleton; R Orthofer; RM Lamuela Raventos. *Enzymology*. **1999**, 299, 152-178.
[14] FH Witham, DF Blaydes, RM Devlin. Experiments in plant physiology. Van Nostrad, New York. **1971**, 245.
[15] BA Bidlingmeyer; SA Cohen; TL Tarvin. *J Chromatogr*. **1984**, 336, 93-104.
[16] AC Kurilich; GJ Tsau; A Brown; L Howard; BP Klein; EH Jeffery; M Kushad; MA Walig; JA Javik. *J Agri Food Chem*. **1999**, 47, 1576-1581.
[17] WHO/FAO. WHO Technical Report Series, no. 522. Geneva, World Health Organization. **1973**.