

Trans-Himalayan *Rhodiola imbricata* Edgew. root: a novel source of dietary amino acids, fatty acids and minerals

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Abstract Roots of *Rhodiola imbricata* Edgew from Indian trans-Himalayan cold desert known for their nutritional and medicinal attributes were evaluated for the dietary amino acids, fatty acids and mineral composition. Nine essential and twelve non-essential amino acids were quantified. The contents ranged between 91.33 and 1640.67 µg/g. Histidine (1434.33 µg/g), lysine (1329.33 µg/g) and threonine (1015.67 µg/g) were dominant essential amino acids, while glycine (1640.67 µg/g), proline (1263.67 µg/g), alanine (1142.33 µg/g), cystine HCL (1136.33 µg/g) and nor leucine (1038.67 µg/g) were major non essential amino acids. The total lipid was found to be rich source of saturated fatty acids such as capric acid (19.91%), caproic acid (10.87%), palmitic acid (9.42%), lignoceric acid (6.16%) and behenic acid (5.71%), which together constituted 52% of the lipid content. Linoleic acid (15.06%), oleic acid (12.38%), arachidonic acid (8.38%), linolelaidic acid (6.11%) and docosadienoic acid (5.99%) were prominent unsaturated fatty acids (UFAs). Mono unsaturated fatty acids (MUFAs) and poly unsaturated fatty acids (PUFAs) were 35.64% and 12.33% of the lipid content respectively. Calcium

(11034.17 mg/kg), potassium (2143.25 mg/kg), iron (1441.17 mg/kg), magnesium (581.99 mg/kg), phosphorous (376.72 mg/kg) and sodium (109.75 mg/kg) were detected as the major dietary minerals.

Keywords *Rhodiola imbricata* root · Amino acids · Fatty acids · Minerals · Phytofoods

Introduction

The Indian trans-Himalayan cold desert region is a great repository of natural botanical resources having diverse utility towards development of phytofoods, health promoting natural products and other medicinal and therapeutic formulations (Ballabh et al. 2007; Dhar 2013; Tayade 2015). *Rhodiola imbricata* Edgew. (Family: Crasulaceae) is a trans-Himalayan high altitude medicinal herb widely used in the traditional system of medicine. It is also famous as Golden Root or Himalayan stone crop and locally known as Shrolo marpo in Ladakh. In India, it is mainly distributed at an altitude of 14,000–18,500 ft above mean sea level (AMSL) in the rocky slopes, wet places and higher passes of Ladakh region of trans-Himalayan cold desert (Tayade 2015). It is a source of various secondary metabolites like alkaloids, phenylpropanoids, phenylethanol derivatives, flavonoids, terpenoids, phenolic acids etc. with promising pharmacological properties and health promoting effects (Kumar et al. 2012). Using the root of this plant, our institute has developed a number of phyto-products having high nutritive attributes and antioxidant properties (Ballabh et al. 2007; Chaurasia et al. 2011; Dhar et al. 2012; Dhar et al. 2013a, b). Recently, Choudhary et al. (2015) isolated and characterized phenolic compounds from this plant root with antioxidant and anticancer

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potential. Avasthi et al. (2016) also performed the bioassay guided screening and characterization of secondary metabolites of *R. imbricata* collected from Himalayan high altitude region of Ladakh.

In our continuous efforts to delineate the antioxidant potential, vitamin content and composition of bioactive phytochemotypes in *R. imbricata* root, we reported the secondary metabolite profile of *R. imbricata* (Tayade et al. 2013a, b, c). However, the primary metabolites such as amino acids, fatty acids and mineral profile of this plant root have not been studied before. Amino acids, fatty acids and minerals derived from plant sources are also known as important dietary components for human and animal nutrition. Therefore, estimation of these nutritional attributes is essential for phytofood development. We report qualitative and quantitative analysis of amino acids, fatty acids and dietary minerals in *R. imbricata* root using pre column phenylisothiocyanate (PITC) derivatization following reverse phase-high performance liquid chromatography (RP-HPLC), fatty acid methyl esters (FAMES) derivatization with gas chromatography coupled with flame ionization detector (GC-FID) and inductively coupled plasma optical emission spectrometry (ICP-OES) analysis.

Materials and methods

Chemicals and reagents

HPLC grade acetonitrile, acetyl-chloride, 2-propanol, n-hexane, ethanol, methanol, sodium acetate trihydrate and glacial acetic acid were procured from Merck (Merck KGaA, Darmstadt, Germany). Triethylamine (TEA), phenylisothiocyanate (PITC) and amino acid standards, sodium hydrogen phosphate (Na_2HPO_4) and phosphoric acid (H_3PO_4) were obtained from Sigma–Aldrich (St. Louis, MO, USA). The FAMES standard mixture (Supelco 37-Component FAME Mix, 47885-U, Supelco, Bellefonte, PA, USA) was used. Industrial grade nitrogen was used (Sigma Gases & Services, Delhi, India). Rest of the chemicals and solvents were of analytical grade. High purity water prepared with Milli-R/Q water purification system (Millipore, Bedford, MA, USA) was used for chemical analysis.

Plant materials

R. imbricata roots were collected from the trans-Himalayan region (Chang-La Top, Changthag valley, altitude 17,500 ft AMSL, latitude 34°2'49.81"N, longitude 77°55'49.78"E) of India in the month of October 2011 after the period of senescence. For plant material collection, all

the necessary permits were acquired from the concerned authority Dr. B. Balaji (IFS), Divisional Forest Officer, Leh Forest Division, Jammu & Kashmir, India. The root samples were carefully washed, cut into small pieces and shade dried at room temperature for 15 days. Then, it was finely powdered and used for extraction. The matured root of *R. imbricata* having optimum bioactivities were collected after the period of senescence (Tayade et al. 2013a, b).

Amino acid analysis by RP-HPLC

The amino acid content of the root sample was determined using RP-HPLC with pre-column PITC derivatization according to our recent report with minor modifications (Dhar et al. 2013b).

Individual 21 amino acid standards were derivatized with PITC to a final concentration of 40 µg/ml. This solution was filtered and stored at –20 °C for further analysis. Acid hydrolysis was performed for extraction of amino acids from plant root (AOAC, 1990). Aliquots of prepared plant extract and working amino acid standard solutions were concentrated and dried under vacuum (37 °C, 20 mmHg), following addition of coupling reagent (methanol/water/TEA, 2:2:1, v/v). The solution was mixed and dried immediately under vacuum. Thereafter, the PITC reagent (methanol/TEA/water/PITC, 7:1:1:1, v/v) was mixed properly and kept at room temperature for 20 min prior to drying in vacuum.

Chromatographic analysis was performed by injecting 20 µl of the sample and standard in RP-HPLC system. Separation of amino acids was performed on reverse phase C-18 column (5 µm, 150 × 4.6 mm) (Pickering Laboratories, Inc., Mountain-View, California, USA) The chromatographic conditions have been depicted in Table 1. Windows® 98 2000 Data Station and CLASS-VP™ Version 6.13 software was used for data acquisition and analysis.

Fatty acid analysis by GC-FID

Fatty acids were analysed by GC-FID following FAMES derivatization similar to the method described in previous work (Dhar et al. 2013b). Fat was extracted into ether, methylated to FAMES using BF_3 in methanol, 14% (w/w). FAMES were then measured quantitatively by a GC-4000A system (East & West Analytical Instruments, Beijing, China) coupled with flame ionization detector, split/splitless mode injector (5 ml/min), capillary column (HP-88, 100 m × 0.25 mm × 0.20 µm film, Agilent Technologies Ltd., Santa Clara, CA, USA) and A5000 Chromatogram Data Processing Workstation Software, Version 1.6 (AOAC, 2002).

Table 1 Gradient program employed for the separation of PITC derivatized amino acids^a

Run time ^b (min)	Flow rate (ml/min)	% Buffer A ^c	% Buffer B (60% acetonitrile in water)
0	1	100	0
0.1	1	95	5
5	1	90	10
14	1	90	10
25	1	60	40
30	1	50	50
35	1	40	60
40	1	10	90
52	1	10	90
62	1	95	5
65	1	100	0

^a Column temperature was maintained at 39 °C

^b Run time was 62 min with 3 min column regeneration time

^c Sodium acetate buffer [19 g of sodium acetate trihydrate was dissolved in 1 l of HPLC grade water. To this 0.5 ml of TEA was added and the contents were mixed properly. The pH of the solution was attuned to 6.4 with glacial acetic acid. To the filtrate (940 ml) acetonitrile (60 ml) was added, mixed and filtered through a 0.22 µm Millipore membrane]

Determination of minerals

The root sample was digested by hot block method with QBlock equipment (Questron Technologies Corp., Mississauga, Germany) (Dhar et al. 2013b). The digestion program was set for plant sample and reagents were mixed according to the manufacturer's directions. The mineral elements viz. calcium, chromium, copper, cobalt, iron, magnesium, manganese, molybdenum, nickel, phosphorous, potassium, sodium, and zinc were quantified by ICP-OES (Varian, VISTA-MPX, CCD Simultaneous ICP-OES, United States).

Results and discussion

The dearth of information regarding the amino acid composition, fatty acid profile and mineral content of *R. imbricata* root which could play a vital role not only for the bio-activity and pharmacological properties but also provide new insights into the physiological adaptation aspects of the plant in the severe stressful environment of trans-Himalaya, motivated us towards the analysis of amino acid composition, fatty acid profiling and mineral content analysis in this plant root.

Amino acid content in *R. imbricata* root determined by RP-HPLC has been depicted in Table 2. The peaks were identified by comparing the retention time of amino acid standards and those in the sample (Fig. 1). Quantitation was performed using the external standard method by preparing calibration curves derived from linear regression analysis (Statistica 5.1, StatSoft, Tulsa, OK). There were

21 amino acids including 9 essential and 12 non-essential amino acids determined in the root sample of *R. imbricata* (Table 2). The content ranged between 91.33 ± 7.77 and 1640.67 ± 11.85 µg/g. Gly (1640.67 µg/g) was found to exhibit the highest content followed by His (1434.33 µg/g), Lys (1329.33 µg/g), Pro (1263.67 µg/g), Ala (1142.33 µg/g), Cys HCL (1136.33 µg/g), and Nor Leu (1038.67 µg/g). His and Lys were found to be the dominant contributors of essential amino acids, while the major non-essential amino acids were Gly, Pro, Ala, Cys HCL and Leu. L-2-amino-n-butyric acid, Trp and Orn were not detected. Val was found to be in the below detection limit (BDL) range and hence its content was not quantified. In agricultural and food sciences, precise identification of edible plants and cataloguing of food products and nutraceutical botanicals have become important issues with the ever increasing alertness in the consumers (Donno et al. 2016). Free amino acids correspond to a constant fraction of numerous natural foods, and on occasion, their quantity has been exploited for differentiation rationales (Maro et al. 2011; Bharate and Bharate 2014). The recent advances in amino acid profiling techniques provide a good alternative approach by converting enantiomers to diastereomers through chemical reactions with chiral derivatizing reagents, and by this strategy chromatographic separation can be easily achieved (Chandrul and Srivastava 2010). Pre-column derivatization and separation of amino acids using RP-HPLC is a well versed method for its cost-effectiveness, simplicity, sensitivity and speed of separation (Ilisz et al. 2012). We utilized the Pico-Tag technique with PITC derivatizing reagent that has the advantage of being highly perceptive and proficient of detecting nanogram (ng) quantities of

Table 2 Content, type of amino acid, retention time (RT) and peak area as quantified by RP-HPLC (n = 3)

Peak no.	Amino acid	Abbreviation	Type	RT (min)	Peak area	Content ($\mu\text{g/g}$)
1.	L-Arginine	Arg	Non essential	3.992	216,927	214.67 \pm 7.09
2.	L-Aspartic Acid	Asp	Non essential	4.475	324,956	434.67 \pm 8.74
3.	L-Glutamic Acid	Glu	Non essential	6.317	273,840	320.67 \pm 7.77
4.	L-Serine	Ser	Non essential	8.500	1,210,077	839.67 \pm 10.97
5.	L-Glycine	Gly	Non essential	9.250	3,064,675	1640.67 \pm 11.85
6.	L-Histidine	His	Essential	10.442	1,154,401	1434.33 \pm 10.02
7.	L-Threonine	Thr	Essential	12.042	1,232,241	1015.67 \pm 8.02
8.	L-Alanine	Ala	Non essential	12.850	1,517,814	1142.33 \pm 11.02
9.	L-Proline	Pro	Non essential	13.367	1,269,920	1263.67 \pm 10.50
10.	L-Methionine	Met	Essential	24.642	434,764	736.67 \pm 8.02
11.	L-Cystine HCL	Cys HCl	Non essential	26.383	1,450,843	1136.33 \pm 11.72
12.	L-Cystine	Cys	Non essential	27.500	267,474	239.33 \pm 8.39
13.	L-Isoleucine	Ile	Essential	28.600	66,781	91.33 \pm 7.77
14.	L-Leucine	Leu	Essential	29.842	1,084,489	928.67 \pm 10.79
15.	L-Nor Leucine	Nor Leu	Non essential	30.158	1,569,530	1038.67 \pm 10.21
16.	L-Phenylalanine	Phe	Essential	31.767	689,754	855.33 \pm 9.02
17.	L-Lysine	Lys	Essential	33.550	2,147,649	1329.33 \pm 11.55
18.	L-2-amino-n-butyric acid	Abu	Non essential	ND	ND	ND
19.	L-Valine	Val	Essential	21.192	27,197	BDL
20.	L-Tryptophan	Trp	Essential	ND	ND	ND
21.	L-Ornithine	Orn	Non essential	ND	ND	ND

ND not detectable; BDL below detection limit

amino acids (Dhar et al. 2013b). Plants subjected to different environmental and physiological stresses can accumulate amino acids in their system that play pivotal role in combating the stress. Amino acids are osmolyte that can regulate ion transport, stomatal opening, activation of phytohormones and growth substances, chelating effect on micronutrients and play vital role in detoxification of heavy metals. They are also responsible for the synthesis and functional properties of specific enzymes, gene expression, and redox-homeostasis (Rai 2002). Most importantly, in higher plants the amino acids serve as precursors for secondary metabolism (Zhao et al. 1998). Thus, the amino acids are directly related to plant stress physiology and have diverse preventive and recovery effects. In general, the major amino acids are present in elevated concentrations and typically associated with the primary carbon metabolism and nitrogen assimilation. Whereas, minor amino acids that comprise essential amino acids of human diet is generally less abundant (Noctor et al. 2002). The present analysis revealed a higher concentration of essential amino acids especially His and Lys than non-essential amino acids in *R. imbricata* root. Research investigations on the availability of amino acids in different *Rhodiola* sp. are very limited. In the last decade only, six types of *Rhodiola* L. from Xingjiang region of China were studied to estimate the amino acids in the root and rootstalk and

they were reported to contain 8–18 amino acids including 3–7 essential type. Out of the six *Rhodiola* sp. grown in Xingjiang region, *Rhodiola rosea* L. is licensed in the traditional medicinal system of China as to herbal drug owing to the presence of most classes of amino acids (Ruan et al. 2001). These results also revealed that *R. imbricata* will be an excellent source of the natural amino acids that are useful to human consumption. This is the first ever study on the amino acid profiling of *R. imbricata* root that would help research efforts on metabolomics.

Fatty acids influence a broad range of cellular processes such as functional ingredients of food and dietary supplements and provide numerous health benefits that include growth promoting effect, nutrition, metabolic functions, and many more (Huang 2007; Abuajah et al. 2015). A variety of techniques have been employed for fatty acid profiling in biological materials. Of these, gas chromatography coupled with flame ionization detection (GC-FID) is a widely used rapid and efficient method for the analysis of complex mixtures of biological samples with compounds of diverse molecular weights (Seppänen-Laakso et al. 2002). Separation, identification and quantification of long-chain fatty acid mixtures by GC-FID have been extensively used to acquire information about the less volatile fatty acids are converted into more volatile derivatives [such as fatty acid methyl esters (FAMES)] prior to GC analysis. A

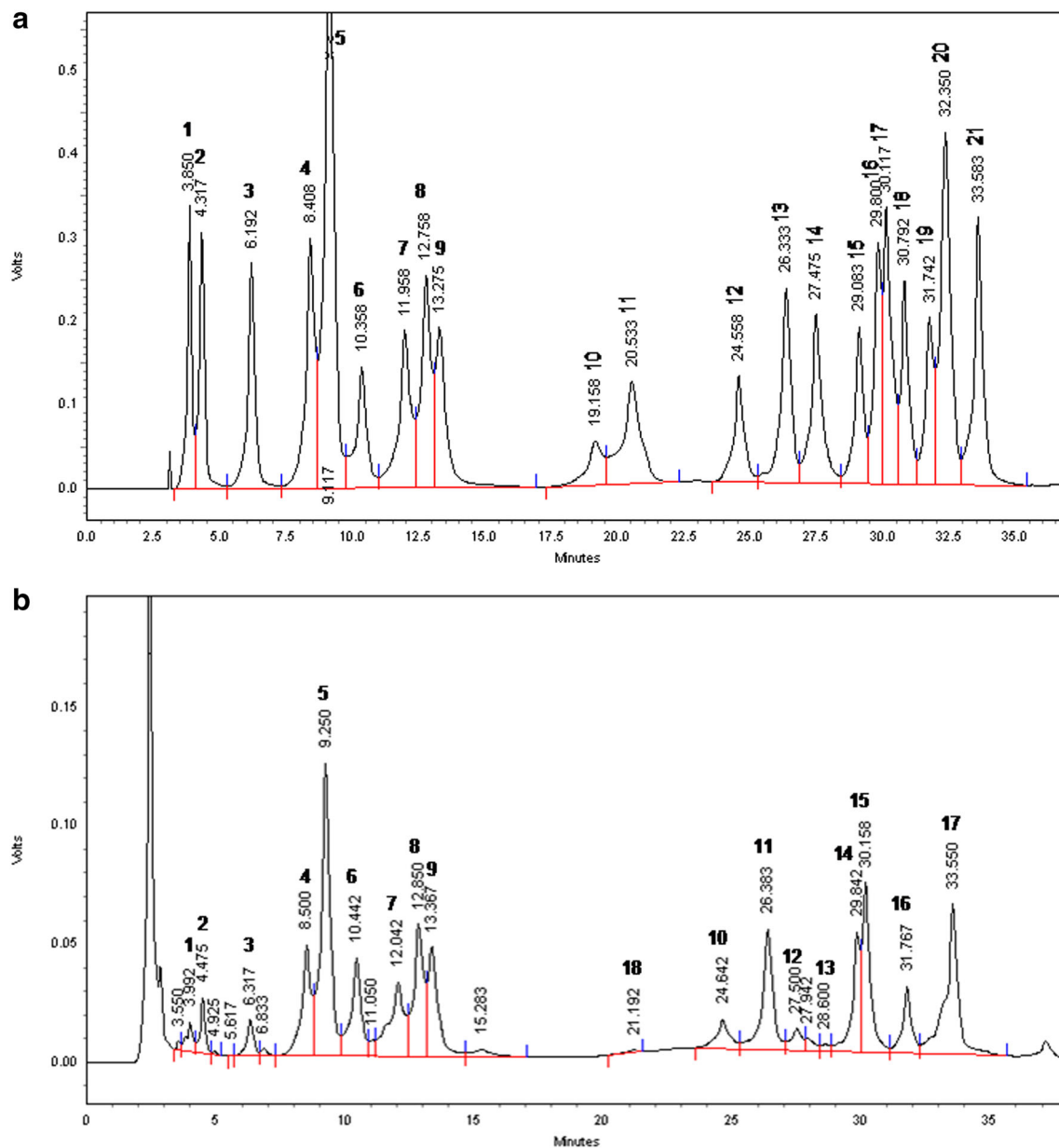


Fig. 1 RP-HPLC chromatogram of **a** 21 amino acid standards, **b** amino acid profile of *R. imbricata* root. 1 L-Arginine; 2 L-Aspartic Acid; 3 L-Glutamic Acid; 4 L-Serine; 5 Glycine; 6 L-Histidine; 7 L-Threonine; 8 L-Alanine; 9 L-Proline; 10 L-2-amino-n-butyric acid; 11

L-Valine; 12 L-Methionine; 13 L-Cystine HCl; 14 L-Cystine; 15 L-Isoleucine; 16 L-Leucine; 17 L-Nor Leucine; 18 L-Tryptophan; 19 L-Phenylalanine; 20 L-Ornithine; 21 L-Lysine

number of studies were conducted to demonstrate the essential oil composition of *Rhodiola* sp. (Bai et al. 2005). Research investigation on *Rhodiola rosea* was also carried out with an objective to determine the phytochemical composition and antioxidant potential (Pooja et al. 2006). However, fatty acid profile of *R. imbricata* root from Indian trans-Himalayan region has not been investigated till date. In this present report, analysis of fatty acids in *R. imbricata* root showed the occurrence of 10 fatty acids contributing 99.99% of the total lipid (Table 3). The GC-FID chromatographic separation of 37 FAMES standards

along with fatty acid composition of root sample has been shown in Supplementary Fig. 2 The total lipid was found to be a rich source of SFAs viz. capric acid (19.91%), caproic acid (10.87%), palmitic acid (9.42%), lignoceric acid (6.16%) and behenic acid (5.71%), which together constituted 52% of the total lipid (Table 3). Among the UFAs, linoleic acid (15.06%), oleic acid (12.38%), arachidonic acid (8.38%), linolelaidic acid (6.11%) and docosadienoic acid (5.99%) were prominent. MUFA and PUFA were 35.54% and 12.38% of the total lipid content respectively in the root sample. In general, the nutritional value of fat is

Table 3 Fatty acid composition of *R. imbricata* root ($n = 3$)

Type of fatty acid	IUPAC name	CAS number	Retention time (min)	Peak area	Peak height	Peak width	% Content in total lipid	Content in root (mg/g)
Saturated fatty acid (SFA)								
Caproic acid (C6:0)	Hexanoic acid	142-62-1	13.58	49,613	11,702	0.247	10.87 ± 0.27	8.8 ± 0.22
Capric acid (C10:0)	Decanoic acid	334-48-5	22.60	90,878	13,856	0.392	19.91 ± 0.49	16.2 ± 0.41
Lignoceric acid (C24:0)	Tetracosanoic acid	557-59-5	59.30	28,127	6418	0.742	6.16 ± 0.15	5.0 ± 0.13
Palmitic acid (C16:0)	Hexadecanoic acid, (9Z)-	57-10-3	40.70	42,991	9095	0.348	9.42 ± 0.23	7.6 ± 0.19
Behenic acid (C22:0)	Docosanoic acid	112-85-6	55.25	26,055	955	1.164	5.71 ± 0.14	4.6 ± 0.12
Unsaturated fatty acid (UFA)								
<i>Monounsaturated fatty acid (MUFA)</i>								
Oleic acid (C18:1 n9c)	(9Z)-Octadec-9-enoic acid	112-80-1	47.46	56,480	11,686	0.39	12.38 ± 0.31	10.0 ± 0.25
<i>Poly unsaturated fatty acid (PUFA)</i>								
Linolelaidic acid (C18:2 n6t)	(9E,12E)-Octadeca-9,12-dienoic acid	506-21-8	48.86	27,903	4221	0.383	6.11 ± 0.15	5.0 ± 0.14
Linoleic acid (C18:2 n6c)	<i>cis, cis</i> -9,12-Octadecadienoic acid	60-33-3	49.51	68,738	14,473	0.342	15.06 ± 0.38	12.2 ± 0.31
Arachidonic acid (C20:4 n6)	(5Z,8Z,11Z,14Z)-Eicosatetraenoic acid	506-32-1	57.33	38,262	2985	0.424	8.38 ± 0.21	6.8 ± 0.17
<i>cis</i> -13,16-Docosadienoic acid (C22:2)	<i>cis</i> -13,16-Docosadienoic acid	7370-49-2	59.05	27,314	5478	0.265	5.99 ± 0.15	4.9 ± 0.12
∑ SFA							52.07 ± 1.28	
∑ UFA							47.22 ± 1.2	
∑ MUFA							12.38 ± 0.31	
∑ PUFA							35.54 ± 0.89	

evaluated by PUFA/SFA ratio. According to Chang and Huang (1998), MUFA content and ratio of sum of PUFA and MUFA to SFA (PUFA + MUFA/SFA) have potential influence on lipid concentrations in rat plasma and liver. Low MUFA/SFA ratio, low PUFA/SFA ratio, high PUFA/MUFA ratio, and PUFA + MUFA/SFA ratio <2 are the requisite factors for maintenance of low plasma and liver lipid concentration. In the present investigation, MUFA/SFA value was 0.24. In addition, lower PUFA/SFA value (0.68), high PUFA/MUFA value (2.87) was also determined. The estimated value of PUFA + MUFA/SFA was 0.92 due to the occurrence of very low MUFA in *R. imbricata* root. Studies by previous investigators showed that even though virgin coconut oil contains <90% saturated fats and very low MUFA and PUFA, it has cholesterol and triacylglycerol lowering properties which may be accredited to small chain fatty acids (Mensink et al. 2003) and polyphenolic antioxidants (Nevin and Rajamohan

2004). In contrast, high MUFA containing diets were also reported to lower cholesterol concentration (Jenkins et al. 2010). It was also reported that the palm oil with elevated saturated fats (50%) is suitable for development of food and food products, soap manufacture and production of bio-diesel (Soh et al. 2006). Therefore, the high yields of lipid from *R. imbricata* root deserve scientific attention towards its utilization for health promoting medicinal foods, nutraceuticals, dietary botanical supplements, and other natural products. In this investigation, PUFAs were found to be the major contributor of fatty acids which is an important health-promoting nutrient having strong influence in alleviating cardiovascular, inflammatory, and heart diseases, atherosclerosis, autoimmune disorder, diabetes and many other health complains (Finley and Shahidi 2001). Especially, the essential amino acid linoleic acid possesses antioxidant (Peyrat-Maillard et al. 2003), acne reductive, (Latawe et al. 1998) anti-inflammatory, and

moisture retentive properties (Darmstadt et al. 2002). Arachidonic acid, oleic acid, linoleic acid, and docosadienoic acid were also present in considerable amount in *R. imbricata* root which have great importance for human health improvement (Harris et al. 2009; Terés et al. 2008). Therefore, further research should be performed on the isolation and biological efficacy evaluation of these bio-active components that may have potential applications towards human health promotion.

Minerals are also essential micronutrients that are needed in small amounts to maintain proper health and optimum physical performance (Soetan et al. 2010). There are a number of methods to analyze the mineral content in plant sample and among these techniques inductively coupled plasma optical emission spectrometer (ICP-OES) has become one of the most convenient analytical tools for the determination of trace elements in a wide variety of sample types (Hou and Jones 2000; Rodrigues et al. 2015). The mineral composition of *R. imbricata* has been depicted in Table 4. It was found to contain thirteen important mineral elements required for human nutrition. Calcium (11034.17 mg/kg) was quantified in highest amount tracking down to potassium (2143.25 mg/kg), iron (1441.17 mg/kg), magnesium (581.99 mg/kg), phosphorous (376.72 mg/kg), sodium (109.75 mg/kg), manganese (75.78 mg/kg), zinc (16.27 mg/kg), chromium (7.27 mg/kg), nickel (4.89 mg/kg), copper (3.49 mg/kg), cobalt (2.98 mg/kg) and molybdenum (2.65 mg/kg). These micronutrients could be beneficial for normal growth and development for human and other animals.

The cold-arid region of Indian trans-Himalaya possesses unique ecology and environment that have endowed with a mosaic of sparse vegetation pattern. Plants of this high altitude cold desert areas therefore show stress tolerant

properties with the capacity of producing certain bioactive metabolites such as mineral ions, amino acids, fatty acids and other bioactive secondary metabolites which are acting as osmolyte, ion transport regulator, modulating stomatal opening, detoxification of heavy metals, modification of membrane fluidity, synthesis and activity of some enzymes, gene expression, and redox-homeostasis (Rai 2002; Upchurch 2008). Therefore, various amino acids, fatty acids and mineral elements found in *R. imbricata* may reflect stress adaptation of this plant in the harsh terrain of trans-Himalayan cold desert. Although the root of *R. imbricata* had been extensively studied for its pharmacological and therapeutic potentials, data on its free amino acid, fatty acid and mineral content is very scarce and therefore limits the scope for comparison of present analysis with other reports on this plant. In this report, we are primarily focusing on quantification of amino acids, fatty acids and mineral elements in *R. imbricata* root because these components are the basic nutritional source for human. Other compounds like sugars, sugar alcohols, low-complexity carbohydrates, tertiary amines, sulfonium compounds, starch etc. may also be interesting to quantify and future studies would be performed to explore the role of these metabolites in stress adaptation of this plant. In future, research efforts should also be concentrated on the effect of seasonal, geographical and altitudinal variations on the bioactive composition of *R. imbricata*. In addition, changes in bioactive metabolites with respect to the course of development will also provide new insights for isolation of novel compounds from this plant.

Conclusion

To the best of our knowledge, this is the first document reporting the use of RP-HPLC with pre-column derivatization for amino acid analysis in roots of *R. imbricata* from trans-Himalayan cold desert. The present study revealed the presence of high concentration of essential amino acids in the plant root. Fatty acid analysis carried out using GC-FID with FAMES derivatization also showed the occurrence of vital fatty acids in the plant root for the first time. A good number of dietary minerals were also determined by ICP-OES technique. Therefore, it can be concluded that *R. imbricata* root contains different health promoting bioactive nutritional attributes like essential amino acids, fatty acids and dietary mineral elements. The plant root could therefore be utilized for development of herbal supplements, nutraceutical products and functional foods to combat health issues associated with nutrition deficiency.

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Table 4 Mineral content of *R. imbricata* root estimated by ICP-OES ($n = 3$)

Sr. no.	Minerals	Symbols	Content (mg/kg)
1.	Calcium	Ca	11034.17 ± 332.04
2.	Potassium	K	2143.25 ± 65.37
3.	Iron	Fe	1441.17 ± 27.98
4.	Magnesium	Mg	581.99 ± 17.40
5.	Phosphorous	P	376.72 ± 11.87
6.	Sodium	Na	109.75 ± 3.32
7.	Manganese	Mn	75.78 ± 2.21
8.	Zinc	Zn	16.27 ± 0.54
9.	Chromium	Cr	7.27 ± 0.32
10.	Nickel	Ni	4.89 ± 0.21
11.	Copper	Cu	3.49 ± 0.12
12.	Cobalt	Co	2.98 ± 0.06
13.	Molybdenum	Mo	2.65 ± 0.05

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Conflict of interest The authors declare no conflict of interest for this work.

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