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Nutrient Profiling, ICP-OES Mineral Analysis and UPLC Estimation of Amino Acids in Three Selected Ethnomedicinal Plants of North East India

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ABSTRACT

Background: Ethnomedicinal plants are rich traditional resources of Northeast India. Most of these plants are edible and are used as food by the tribal population of Northeast India. Quantitative nutritional analyses of three ethnomedicinal wild edible plant from Northeast India namely: *Zanthoxylum oxyphyllum* Edgew, *Rotheca serrata* (L.) Steane *and* Mabb. and *Blumea lanceolaria* (Roxb.) Druce were carried out to identify the occurrence and abundance of nutrients, minerals and amino acids.

Methods: Proximate nutrients were estimated by standard AOAC methods. ICP-OES mineral analyzer was used to screen and quantify individual mineral elements. By performing UPLC analysis the presence and abundance of amino acids were investigated.

Result: Proximate analysis revealed that the plant species contains adequate amounts of protein, fibre and ash. From nutritional perspectives, low fat and carbohydrate in these species specifies that they are desirable diet for human nutrition. In regard to mineral content, the plant species were rich in macro minerals K, Ca, Na and Mg. Heavy metals were found within the recommended dietary permissible limits for humans. All the essential and non essential amino acids excluding cysteine, methionine and histidine were present in reasonable quantities. The plant species may be exploited to provide quality diets to mitigate nutrient deficiency. Adding together its nutritional and medicinal properties it may potentially be utilized in therapeutics. The further scope of the research involves ascertaining their potentiality in food-based strategies to perk up nutritional health.

Key words: Dietary, Medicinal, Nutritional, Therapeutics, Traditional, Wild edible.

INTRODUCTION

According to FAO at least two billion people, on earth, have been estimated to suffer from micronutrient deficiencies making them more susceptible to disease. Currently attention is focused on wild and natural food resources which if exploited can likely be a sustainable solution to mitigate nutrient deficiency. A large variety of natural resources are already present in North East India. Wild edible plants form an important part of the food culture in this region. Most importantly they contribute to the food and medicine baskets as well as livelihoods of tribal communities of Northeast India.

Among these, *Zanthoxylum oxyphyllum* Edgew a wild shrub indigenous to Northeast India is widely utilized as food and ethnomedicine in Northeast India. Tribal people in this region use the young leaves of this plant as vegetable. Tonics are prepared from the aqueous extracts of young *Z. oxyphyllum* leaves to treat a variety of diseases. According to Buragohain *et al*. (2011), *Z. oxyphyllum* leaves have blood purifying properties, can reduce the risk of leucoderma and help in solving stomach issues. The bark is used to treat leg pain, varicose veins, ulcers, rheumatism, hypotension, fevers and inflammatory conditions (Arun and Paridhavi, 2012).

Another plant, *Rotheca serrata* (L.) Steane and Mabb. of Assam belongs to the family Lamiaceae. Locally, it is called as 'Nangal Bhanga' in Assamese. The use of *R. serrata* leaves has also been reported for the treatment of various diseases such as typhoid, cancer, jaundice and hypertension (Singh *et al*., 2012). The leaves are also informed to have analgesic and anti-diabetic activity (Saha *et al*., 2012; Kar *et al*., 2014).

The third plant species discussed here is *Blumea lanceolaria* (Roxb.) Druce. Locally known as "Jwglaori" in Assam is a tropical perennial herb belonging to the family Asteraceae. It is found in different parts of North-East India. In North East India, tribal people use this plant's young leaves as a vegetable. Traditionally, the leaves of the plant have also been used in the treatment of cough (Saikia *et al*., 2017). In Mizoram of North East India, a decoction of the leaves is taken orally for the treatment of stomach ulcers, dysentery and wounds (Rai and Lalramnghinglova, 2010). The utility of *B. lanceolaria* in the treatment of ulcer and

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cancer has also been mentioned by Sawmliana (2003) and Chawngkunga (2005).

Wild edible plants including *Alternanthera sessilis, Amaranthus species, Enhydra fluctuans, Houttuynia cordata, Centella asiatica*, *Ipomoea aquatic* and *B. lanceolaria* are used as food and in ethnomedicine in Northeast India (Bhattacharya *et al*., 2001; Khakhalary *et al*., 2022). These plant species are harvested from their native environments and sold in the markets of North East India.

It is postulated that plants contain nearly all of the nutritionally desirable micro and macronutrients including amino acids (Indrayan *et al*., 2000). Onwordi *et al.* (2009) summarized that herbaceous vegetables contain good amounts of proteins, fats, carbohydrates, vitamins and minerals. The search for new natural therapeutic compounds which are less or non-toxic to humans, animals and the environment is a current research priority for scientists and it essentially begins with an emphasis on traditional, medical and veterinary procedures (Yeap *et al*., 2010).

Vegetables are preferably consumed due to hunger, medicinal requirements and perceived nutrient benefits (Shumsky *et al*., 2014). The tribal people in Northeast India claim that leaves of selected plant species holds medicinal properties as well as essential nutrients for human consumption. Therefore, this report provides a more comprehensive summary of the proximate, minerals and amino acid composition of an edible shrub of ethnomedical importance found in Northeast India. Studying their nutritional profiles will help in generating information about their potential nutritional benefits along with their medicinal properties.

MATERIALS AND METHODS

Collection of plant materials

Representative samples of *Z. oxyphyllum* Edgew*, R. serrata* (L.) Steane and Mabb and *B. lanceolaria* (Roxb.) Druce leaves were collected from Goalpara district of Assam, Northeast India. Herbarium of the collected samples were prepared and submitted to Gauhati University Botanical Herbarium (GUBH).

Sample preparation

Once in the laboratory the samples were cleaned and moisture content determined. The rest of the samples were shade dried, pulverized and stored in an airtight container for further analysis.

Estimation of proximate composition

Proximate composition analysis was performed in the Department of Biotechnology, Bodoland University, Kokrajhar in the year 2022. Moisture content was determined by oven-drying method, crude fat by extraction in Soxhlet apparatus, crude protein by micro Kjeldahl method, ash content by incineration in a muffle furnace, crude fibre content was estimated by treating the fat and moisture free sample with dilute acid and alkali and igniting the residue and carbohydrate content was calculated by subtracting the sum of the percentages of moisture, fat, protein *and* ash from 100 (AOAC, 2005). Total solid was calculated using the method of James (1995). The energy content of plant sample was calculated by the method of FAO (2003).

Estimation of minerals

For mineral analysis 0.2 g of pulverized sample was digested with a mixture of 5 ml of 65% HNO_3 and 2 ml of 37% HCL. The sample was completely dissolved after the mixture was vortexed and boiled in a water bath (Ang and Lee, 2005). After filtering, the mixture was examined for the presence of Na, K, Mg, Ca, Mn, Zn, Fe, Cu, Ni and Cr using an ICP-OES mineral analyzer (Model No. Thermo Scientific TM iCAPTM 7600). Mineral estimation was carried out in the Sophisticated Analytical Instrumentation Facility (SAIF), NEHU, Shillong in the year 2022.

Determination of amino acid

Amino acid composition was determined by WATERS Acquity (make) Ultra Performance Liquid Chromatography. 1 mg of sample was taken in a clean glass tube to which 3 ml of 6N HCL was added and covered with paraffin. The tube was placed in the dry bath at 60°C under N_2 gas for 15mins to maintain inertness. Then the temperature was increased at 110°C and incubated overnight. Derivatization was accomplished by combining 70 µl of Borate buffer and 20 µl of Accq Tag ultra-reagent to the sample and incubated for 10 mins at 55°C. After incubation 5 µl was loaded on to the instrument. Identification of amino acids was done by comparison to amino acid standard. Peaks were observed using 260 nm photodiode array detectors. Amino acid composition analysis was determined in Sandor Speciality Diagnostics Pvt. Ltd, Hyderabad in the year 2022.

Statistical analysis

Mean values and standard deviations were calculated using SPSS statistical software version 26.0 and the data were expressed as mean±standard deviation.

RESULTS AND DISCUSSION

Proximate composition

Results of proximate analysis of the three plant species are shown in Table 1. An attempt was made to compare the nutritional content of three most commonly consumed wild species. Findings showed that *B. lanceolaria* had the highest moisture (59.4±0.50 g/100 g) and fat (4±0.10 g/100 g) content among the samples analyzed. While analyzing the protein content of selected plants, it showed that *Z. oxyphyllum* had the highest protein content (24.30±0.34g/100g) followed by *R. serrata* (15.73±0.05 g/100 g) and *B. lanceolaria* (10.75±0.11 g/100 g). Among the proximate composition analyzed, *R. serrata* had the maximum amount of ash (12.02±0.06 g/100 g), fibre (17.43±0.05 g/100 g), carbohydrate (23.22±0.45 g/100 g), energy (189.1±1.04 kcal/100 g) as compared to *Z. oxyphyllum* and *B. lanceolaria*.

It has been reported by Ogie *et al.* (2001) that high moisture content promotes microorganism growth and enzyme activity. Data shows that the moisture content was moderate and nearly identical in all the studied plant species (the values ranged from 44.97 g/100 g to 59.4 g/100 g). Thus, the presence of moderate moisture indicates its reasonable shelf life. Proteins are necessary for the formation of body tissues and regulating of compounds like hormones and enzymes (Akindahunsi and Salawu, 2005). Among the wild plants, the highest value of crude protein was found in *Z. oxyphyllum* (24.30 g/100 g). The protein content found in the samples exceeded the protein value reported by Tharmabalan (2021) in wild edible plants. The recommended dietary allowances (RDA) for protein for adult male and female set by ICMR-NIN (Indian Council of Medical Research-National Institute of Nutrition) (2020) is 54 g/d and 46 g/d respectively. The protein content in the studied sample can fulfill around 44% and 52% of daily requirement of protein in adult male and female at the maximum. Fats in foods are regarded as the primary source of energy but total fats also contains saturated and trans fatty acids which are undesirable for nutrition. Low fat foods are therefore preferred for human consumption. Fat content in the investigated samples is lower than that of other wild edible plants studied by Ullah *et al.* (2017). *Z. oxyphyllum* leaves has fat content of 2 g/100 g on dry weight because of which it can be marked as a low fat food and recommended to overweight or obese people as health food (Brahma *et al*., 2014). According to Tuncturk and Ozgokce (2015), a plant's ash level is a good predictor of its overall mineral richness. Total ash content observed in this study corresponds to the findings of Aletan and Kwazo (2019) for wild edible plants. The data represented in Table 1 shows high amounts of ash indicating the the presence of rich minerals desirable to our diet. In its 2002 report, the Institute of Medicine (IOM) set the recommended dietary allowances (RDA) for dietary fibre for adult to 25 gm per day. The recommended dietary allowances (RDA) set by ICMR-NIN (2020) for dietary fibre for adult male and female are 30 g/d and 25 g/d respectively. Fibre contents of all studied samples were high as compared to the report of Aletan and Kwazo (2019) and Tharmabalan (2021). The presence of high fibre lowers cholesterol level in the blood, reduces the risk of various cancers, bowel disease and improves general health and well being (Haub and Lattimer, 2010). Crude fibre content (14.43 g/100 g) in *Z. oxyphyllum* can fulfill around half of the daily requirements for dietary fibre if consumed with other foods. The carbohydrate detected in all the samples was below the RDA values established by IOM (Institute of Medicines, 2002) in its reports. Food with low carbohydrate content is considered ideal for diabetic and hypertensive patients requiring low-sugar diets (Singha and Hassan, 2017). Results of the current study revealed that the selected plant species *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* could be used for nutritional purpose of human being due to their good nutritional qualities and adequate protection may be obtained against diseases arising from malnutrition. It also highlights the significance of wild vegetable species as cheap sources of nutrient for rural tribals.

Mineral composition

The plant species were analyzed for nine minerals of which one is heavy metals. Table 2, 3 and 4 represent the results of mineral composition of *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* (mg/100 g) dry matter. A comparison of mineral content in plants and their (%) fulfillment of recommended intake for particular nutrient as per the recommended dietary allowance (RDA) set by ICMR-NIN (2020) and Food Safety and Standards Authority of India, New Delhi is also shown in Table 2, 3 and 4. Results showed that selected plant species contain minerals like Na, K, Mg, Ca, Mn, Zn, Cu and Ni in varying amount. The plant species under investigation contained high amount of K and Ca with highest value recorded in *Z. oxyphyllum* and *B. lanceolaria* respectively. Na content was found equally in all the samples. Mg, Mn and Fe were of higher range in *R. serrata*, higher amount of Zn was detected in *Z. oxyphyllum* and Cu and Ni were highest in *B. lanceolaria*.

Macro minerals in *Z. oxyphyllum* including K, Ca, Na and Mg can fulfill about 0.12% to 4.13% of the RDA requirement in both human male and female (Table 2). On the contrary, micro nutrients such as Mn, Zn, Fe and Cu can mitigate nearly about 0.15% to 53.05% in male and 0.17% to 68.33% in female of the daily RDA. Heavy metals such as Ni can fulfil the RDA of 5% in both human male and female (Table 2). In *R. serrata*, macro minerals including K, Ca, Na and Mg can fulfill about 0.14% to 6.27% of the RDA in both human male and female. On the other hand, micro nutrients such as Mn, Zn, Fe and Cu can meet RDA of around

Table 1: Proximate composition of three ethnomedicinal plants (g/100 g) dry weight.

Parameters	*Z. oxyphyllum*	*R. serrata*	*B. lanceolaria*
Moisture	50.94±1.59	44.97±0.06	59.4±0.50
Total solid content	47.62±0.57	47.60±0.01	43.33±2.08
Protein	24.30±0.34	15.73 ±0.05	10.75±0.11
Fat	2.00 ±0.10	3.3 ±0.26	4±0.10
Ash	10.37±0.41	12.02 ±0.06	10.43±0.40
Fibre	14.43±0.52	17.43 ±0.05	15.46±0.35
Carbohydrate	10.98±0.12	23.22±0.45	15.61±0.32
Energy (kcal/100 g)	160.01±0.09	189.1 ±1.04	140.33±1.15

Note: Values are in triplicate mean±SD.

0.37% to 18.05% in male and 0.44% to 16.66% in female (Table 3). In *B. lanceolaria*, macro minerals including K, Ca, Na and Mg can fulfill about 0.14% to 6.63% of the RDA in both male and female. Among micro nutrients Mn, Zn, Fe and Cu can meet RDA of around 0.32% to 78.50% in male and 0.38 % to 78.50 % in female. Heavy metals such as Ni can fulfil the RDA requirement of 47% in both male and female (Table 4).

Looking to the permissible levels set by the ICMR-NIN, 2020, the data obtained in the present study were within the specified limits demonstrating that a positive contribution of the mineral elements to the diet of consumers is provided

Table 2: Mineral profile of *Z. oxyphyllum* and recommended intake of essential minerals per day compared with *Z. oxyphyllum*.

Minerals	*Z. oxyphyllum* (mg/100 g)	Intake recommended for adults (mg per day)		% fulfilment of recommended intake	
		Male	Female	Male	Female
Na	2.57±0.02	2000	2000	0.12	0.12
K	104.7±0.20	3500	3500	2.99	2.99
Mg	4.39±0.09	400	310	1.09	1.41
Ca	41.38±0.01	1000	1000	4.13	4.13
Mn	0.66±0.01	440	370	0.15	0.17
Zn	9.02±0.02	17	13.2	53.05	68.33
Fe	3.08±0.02	19	29	16.21	10.62
Cu	0.06±0.02	2	2	3	3
Ni	0.05±0.03	1.0	1.0	5	5

Note: Values are in triplicate mean±SD. The data for recommended dietary allowances and estimated average requirements for Indians are as provided by Indian Council of Medical Research (ICMR).

Table 3: Mineral profile of *R. serrate* and recommended intake of essential minerals per day compared with *R. serrata.*

Minerals	*R. serrata* (mg/100 g)	Intake recommended for adults (mg per day)		% fulfilment of recommended intake	
		Male	Female	Male	Female
Na	2.90±0.1	2000	2000	0.14	0.14
K	47.76±0.04	3500	3500	1.36	1.36
Mg	6.53±0.32	400	310	1.63	2.10
Ca	62.73±0.20	1000	1000	6.27	6.27
Mn	1.65±0.05	440	370	0.37	0.44
Zn	2.20±0.02	17	13.2	12.94	16.66
Fe	3.43±0.40	19	29	18.05	11.82
Cu	0.09±0.01	2	2	4.5	4.5
Ni	0.01±0.02	1.0	1.0	1	1

Note: Values are in triplicate mean±SD. The data for recommended Dietary Allowances and estimated average requirements for Indians are as provided by Indian Council of Medical Research (ICMR).

Table 4: Mineral profile of *B. lanceolaria* and recommended intake of essential minerals per day compared with *B. lanceolaria.*

Minerals	*B. lanceolaria* (mg/100 g)	Intake recommended for adults (mg per day)		% fulfilment of recommended intake	
		Male	Female	Male	Female
Na	2.92±0.05	2000	2000	0.14	0.14
K	44.80±0.18	3500	3500	1.28	1.28
Mg	5.30±0.04	400	310	1.32	1.70
Ca	66.37±0.06	1000	1000	6.63	6.63
Mn	1.43±0.04	440	370	0.32	0.38
Zn	5.17±0.03	17	13.2	30.41	39.16
Fe	1.39±0.02	19	29	7.31	4.79
Cu	1.57±0.05	2	2	78.50	78.50
Ni	0.47±0.03	1.0	1.0	47	47

Note: Values are in triplicate mean±SD. The data for recommended dietary allowances and estimated average requirements for Indians are as provided by Indian Council of Medical Research (ICMR).

by these ethno medicinally important plants. Na and K are two essential macro minerals required by the body to maintain cellular homeostasis, metabolism and many other functions. The Na/K ratio in our body is very important to control high blood pressure and the ratio should be less than one (Akubugwo *et al*., 2007). In the present study, all the samples have the Na/K ratio less than one that indicate the consumption of these vegetables are helpful for human and might be able to control the high blood pressure of our body. Low Na and high K intake also aid in the prevention of hypertension and atherosclerosis (Saupi *et al*., 2009). Na and K of *Clerodendrum colebrookianum* recorded by Payum, (2020) is lower as compared to this study. The role of Mg and Ca in maintenance of heart function has been well pointed out by Insel *et al.* (2010). Mg content detected in the samples is high than Payum, (2020) in *Clerodendrum colebrookianum*. An analysis showed the percentage fulfillment of Ca is found high among macro minerals in all samples with the highest value recorded in *B. lanceolaria*. Ca content recorded by Njoku *et al.* (2021) from *Urena lobata* stems is comparable to the Ca content of *R. serrata* and *B. lanceolaria.* The data indicates that these wild vegetables could be a good source of Ca to our diet and provide health benefits by lowering the risk of such diseases. Through analysis, Mn and Zn content found in all the samples is lower than the recommended limit. Mn and Zn content studied by Payum (2020) in *Clerodendrum colebrookianum* is lower than the present study. Similarly, Zn content detected by Islary *et al.* (2016) in *Grewia sapida* fruit is also low. Demirezen and Ahmet (2006) analyzed various vegetables and reported that the Zn concentrations in vegetables are within the recommended international standards. All analyzed samples possess an adequate amount of Fe and is higher than the study conducted by Payum, (2020) in *Clerodendrum colebrookianum*. Fe content of *Z. oxyphyllum* and *R. serrata* is comparable to Njoku *et al.* (2021) detected in *Urena lobata* stems. The percentage of RDA for Fe recorded shows that *R. serrata* has the highest percent fulfilment in comparison to *Z. oxyphyllum* and *B. lanceolaria* in both male and female. Cu an essential trace element in human body has an important role in oxidation reduction reactions and in scavenging of free radicals (Linder and Azam, 1996). When its concentration exceeds the safe limit, it can be toxic in some cases (Ogwok *et al*., 2014). Among the analyzed samples, the per cent fulfillment of recommended intake for Cu was found high in *B. lanceolaria.* A trace quantity of Ni may promote healthy skin, iron metabolism and optimal growth in humans, but it can be toxic when its concentration exceeds the safe limit (Satter *et al*., 2016). The percentage of RDA recorded for Ni was found high in *B. lanceolaria.*

Amino acid composition

Amino acid composition of the plant species and its percent fulfillment of the RDA requirement in adults as given by ICMR-NIN (2020) are presented in Table 5. UPLC chromatograms of standard and samples are shown in Fig 1, 2, 3 and 4. Both essential and non essential amino acids were found in the samples in varying quantities. Cysteine, among the nonessential amino acid was not detected in any of the plants. Among essential amino acids, histidine was not found in both *Z. oxyphyllum* and *R. serrata*. In *R. serrata*, methionine was not detected. Of the essential

Table 5: Amino acid composition of studied plants (mg/100 g) dry weight.

Amino acid	*Z. oxyphyllum*	*R. serrata*	*B. lanceolaria*	RDA (mg/100 g)
Essential amino acid				
Thr	2.90±0.02	ND	0.64±0.01	1.5
Val	2.57±0.02	0.19±0.01	0.66±0.02	2.6
Met	0.59±0.01	ND	0.70 ± 0.01	1
IIle	1.28±0.005	0.17±0.005	0.47±0.02	2
Leu	4.51±0.03	0.84±0.001	1.12±0.02	3.9
Phe	7.08±0.02	1.76±0.03	5.77±0.03	NL
His	ND	ND	0.28±0.005	1
Lys	3.92±0.06	0.39±0.02	0.90±0.01	3
Arg	7.47±0.03	0.30±0.01	1.21±0.02	NL
Non-essential amino acid				
Asp	2.37±0.02	0.28±0.01	2.21±0.03	NL
Ser	3.02±0.01	0.83±0.02	0.28±0.005	NL
Glu	1.07±0.01	1.50±0.01	1.83±0.02	NL
Pro	6.63±0.02	0.91±0.01	0.70±0.01	NL
Gly	5.37±0.03	1.09±0.01	0.68±0.02	NL
Cys	ND	ND	ND	4
Ala	3.28±0.02	0.53±0.05	0.89±0.01	NL
Tyr	4.49±0.01	0.64±0.02	1.85±0.03	NL

Note: Values are in triplicate mean±SD. Recommended dietary allowances and estimated average requirements for Indians-2020 for age group of more than 24 months (2 years). “NL” indicates “not listed”.

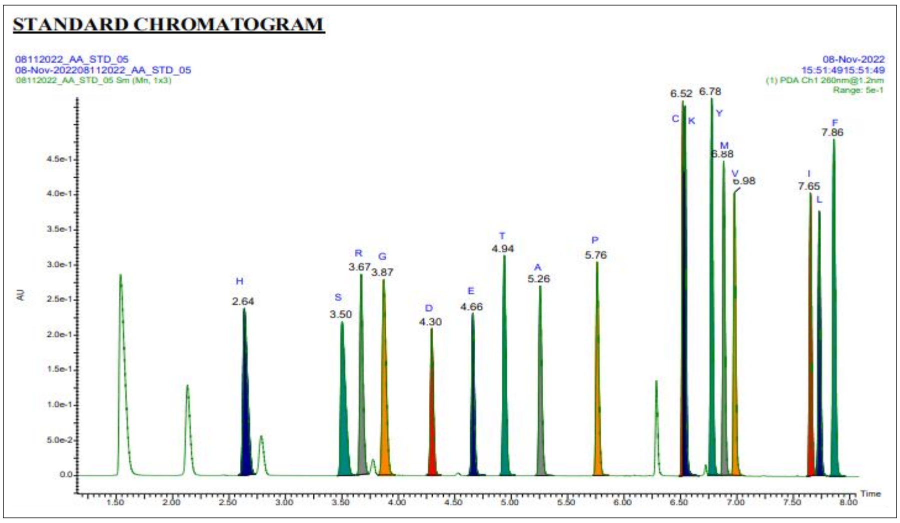

Fig 1: UPLC Chromatogram of amino acid of standard.

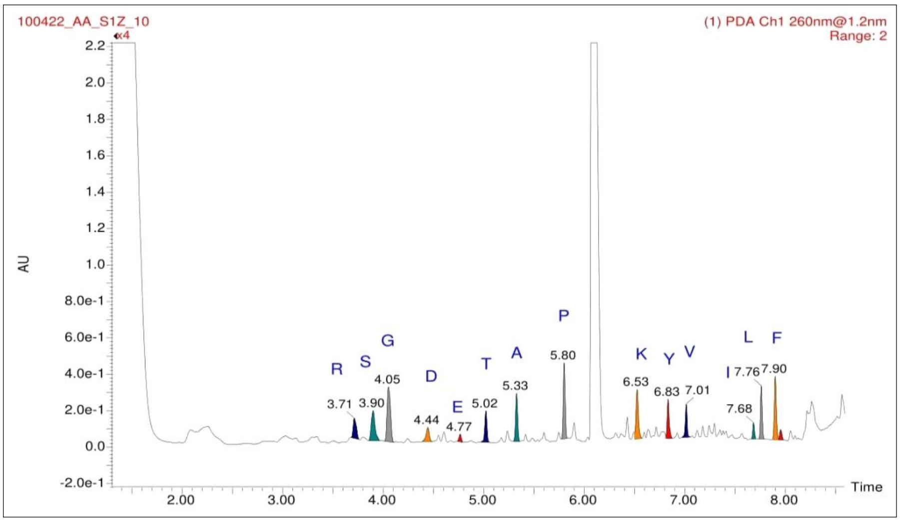

Fig 2: UPLC Chromatogram of amino acid present in *Z. oxyphyllum*.

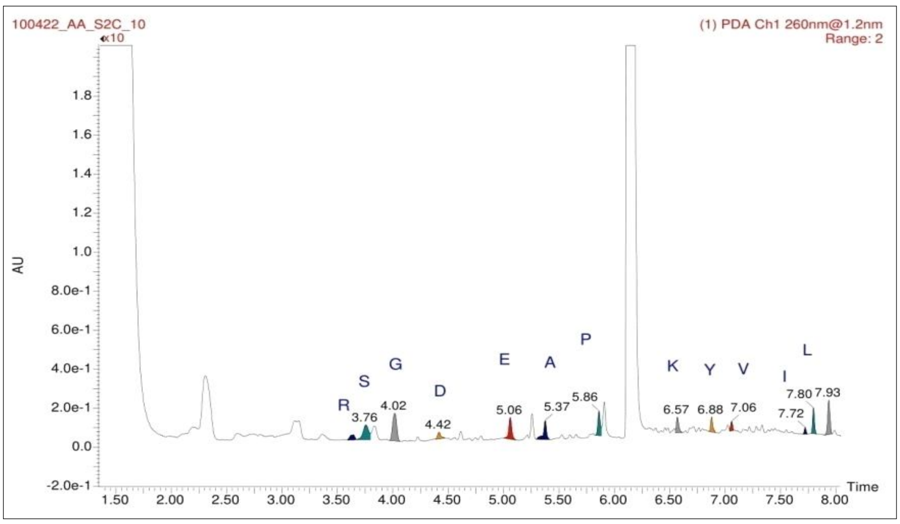

Fig 3: UPLC Chromatogram of amino acid present in *R. serrata*.

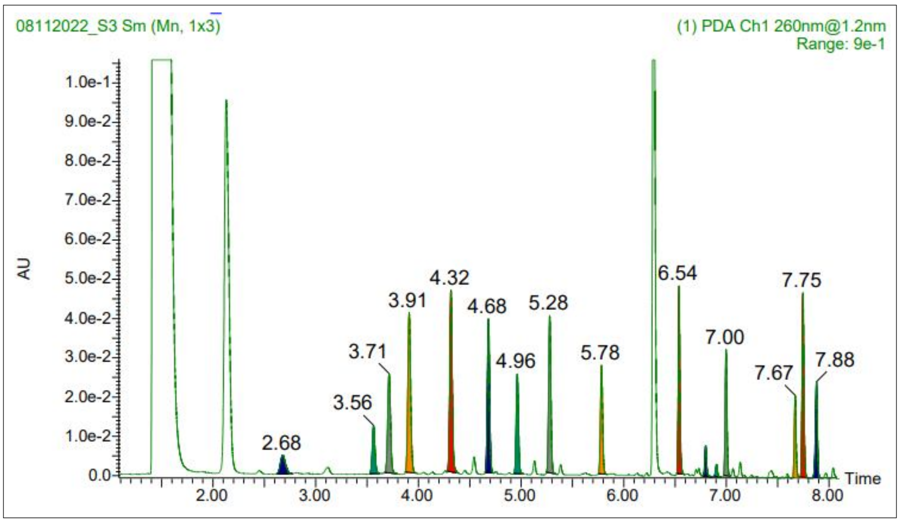

Fig 4: UPLC Chromatogram of amino acid present in *B. lanceolaria*.

amino acid, leucine, threonine and lysine were detected slightly higher than RDA value. The UL (Upper limit) sets the experimentally determined safe upper limits for the highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population as mentioned by National Academy of Medicine (2005). Elango (2023) reported that in some situations, amino acids above normal consumption amounts are considered beneficial such as exercise performance, recovery from injury, *etc*. The UL for the current adult leucine intake recommendations kept RDA of 2.9 g/d (Elango, 2023). RDA recommendations are "minimum" dietary intakes to prevent deficiency and considered adequate to meet 50% and 97.5% of the needs, respectively in a population. With nutrients being consumed in excess of the body's needs, there is a possibility of reaching intake levels where adverse effects can occur. Amino acids are precursors for the synthesis of secondary metabolites that have physiological beneficial effects in our bodies. They are essential components for healing processes and a lack of these components impedes recovery. Aside the structural functions, amino acids could also serve as valuable sources of energy especially in the absence of carbohydrate and fats in the body (Olusanya, 2008). Amino acids composition reveals information about the quality of food proteins. Collectively the present findings have clearly shown the presence of most essential and non essential amino acids indicating the studied plants can potentially contribute in mitigating protein deficiency. It also implicates the possibility of incorporating the studied plant material into the modern health care system through providing useful information for further application of these plants.

CONCLUSION

Findings of the current study provide an overview of nutrient composition of three wild edible ethnomedicinal plants of Northeast India. It contains significant proportions of proximate nutrients, minerals and amino acids required for human nutrition and health in addition to its medicinal properties. Both essential and non-essential amino acids were found. Result availed from this study may be incorporated into the National Food Composition Database of India to enhance its food value.

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Conflict of interest

All authors declare that they have no conflict of interest to disclose.

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