CHAPTER VI

Amino Acid Composition of Wild Edible Plants

Amino acids are the main components of proteins and enzymes which have important roles in human physiology. Deficiency of amino acids causes several disorders in human health such as insomnia, obesity, diabetes, arthritis, *etc.* due to metabolic disturbances [1, 2]. The essential amino acids (EAA) which are essential for human health are not synthesized in the human body and, therefore, have to be supplied from the diet. Amino acids can participate in gene expression, cell signalling, protein phosphorylation, homeostasis regulation and they also have antioxidant properties [3, 4]. Many amino acids have been discovered from the nature and most of them are α amino acids, and of these, only 20 amino acids are the components of proteins that code for the triplet codon of nucleotide [5, 6].

In this study, for the first time we are reporting the amino acid profiles of eight wild edible plants *viz. S. zeylanica*, *C. hirsuta*, *S. peguensis*, *M. perpusilla*, *C. sinensis*, *P. chinensis*, *L. javanica* and *P. perfoliatum* from Assam of North East India.

VI.1 Materials and Methods

VI.1.1 Materials

Eight wild edible plants viz. S. zeylanica, C. hirsuta, S. peguensis, M. perpusilla, C. sinensis, P. chinensis, L. javanica and P. perfoliatum were selected for amino acid analysis.

VI.1.2 Sample preparation

The plant samples (**Table II.1**) for determination of amino acid composition were prepared as per the procedure mentioned in the **Section II.2.3** (**Page No. 73**).

Six plant species viz. C. hirsuta, M. perpusilla, C. sinensis, P. chinensis, L. javanica and P. perfoliatum were analyzed with HPLC. The powdered sample (5 mg) was mixed with 5 mL of water and vortexed for 10 min. It was followed by addition of methanol (20 mL) which was then incubated at -20°C for overnight. The sample was again centrifuged and the supernatant was completely evaporated under nitrogen atmosphere at 60° C. Thereafter, 250 µL of PITC (Phenyl isothiocyanate) was added to the sample and it was vortexed for 1 h at 45°C followed by vacuum drying. It was again centrifuged by adding 1000 µL of buffer A (10 mM sodium acetate at pH 6.4 adjusted with 6% acetic acid) solution and the supernatant was filtered through syringe filter. Finally, 20 µL of the prepared solution was loaded into RP-HPLC (Zorbax 300 SB, Agilent 1200 series, C18 column: 4.6×250 mm, 254 nm) and allowed to run for 82 min at the flow rate of 1 mL/min. The buffer B solution used was combination of acetonitrile and buffer A in the ratio of 60:40 (v/v). The standard sample (acidic and basic amino acid mixture) was also allowed to run in the HPLC under same conditions. The identification and quantification of amino acid profiles were performed comparing the retention times of the individual peak with those of standard.

VI.1.4 Amino acid analysis by using ultra-performance liquid chromatography (UPLC)

Two plant species *viz. S. zeylanica* and *S. peguensis* were analyzed with UPLC. The sample (1 mg/mL) was prepared by dissolving powdered sample in methanol and it was completely dried under vacuum. To the pellet, 500 μ L of borate buffer was added. For derivatization, 10 μ L of the sample was mixed with 70 μ L of borate buffer and 20 μ L of AccQ.Tag Ultra reagent and then it was incubated at 55°C for 10 min. After incubation, 2 μ L of each sample was loaded into UPLC (Waters Acquity UPLC, Column temperature: 55°C, PDA Detector: 260 nm, Column: 2.1×30 mm, 1.7 μ m) at the flow rate of 0.7 mL/min and allowed to run for 15 min. The mobile phase A (AccQ.Tag Ultra eluent A1) and mobile phase B (AccQ.Tag Ultra eluent B) were used. The standard amino acid obtained from Sigma Aldrich was also allowed to run under the same conditions. The identification and quantification of amino acid profiles were performed comparing the retention times of the individual peak with those of standard.

VI.2 Results and Discussion

In this study, six plant species viz. C. hirsuta, M. perpusilla, C. sinensis, P. chinensis, L. javanica and P. perfoliatum were analyzed with HPLC and their amino acid profiles were identified and quantified. However, the amino acid profiles of the two plant species viz. S. zeylanica and S. peguensis were analyzed with UPLC. The HPLC chromatogram and amino acid profiles of standard are shown in the Fig.VI.1 and Table VI.1, respectively. Similarly, the UPLC chromatogram and amino acid profiles of standard are presented in the Fig.VI.2 and Table VI.2, respectively. The chromatograms of the plant species viz. S. zeylanica, S. peguensis, C. hirsuta, M. perpusilla, C. sinensis, P. chinensis, L. javanica and P. perfoliatum are shown in Fig.VI.3 to Fig.VI.10, respectively. The amino acid profiles in mg/g of dry weight (DW) in the selected wild edible plants are presented in Table VI.3. It was observed that a total of sixteen essential and non-essential amino acids were detected in S. zeylanica and S. peguensis along with some other amino acids. In this study, the total amino acid content was found to be the highest in S. zeylanica (42.87 mg/g) followed by S. peguensis (32.65 mg/g) and the lowest amino acid content was detected in L. *javanica* (0.62 mg/g).

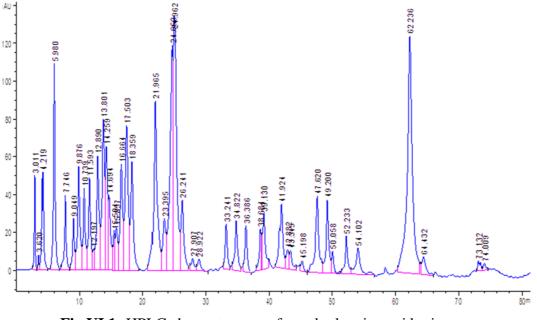


Fig.VI.1: HPLC chromatogram of standard amino acid mixture.

Sl. No.	RT	Amino acids	Sl. No.	RT	Amino acids
1	2.76	Phosphoserine	17	19.58	Arginine
2	3.01	Aspartic acid	18	21.96	3-Methyl histidine
3	3.62	Glutamic acid	19	23.39	1-Methyl histidine
4	4.21	Amino adipic acid	20	24.65	Anserine
5	5.98	OH-Proline	21	31.53	Tyrosine
6	7.75	Phosphoenolamine	22	35.14	Valine
7	9.87	Serine	23	36.38	Methionine
8	10.51	Glycine	24	38.68	Cystathionine
9	10.74	Asparagine	25	39.13	Cysteine
10	11.59	Taurine	26	42.09	Isoleucine
11	12.89	Threonine	27	42.88	Leucine
12	13.80	Histidine	28	44.23	OH Lysine
13	14.26	Alanine	29	46.53	Tryptophan
14	15.86	β-amino butyric acid	30	47.62	Phenylalanine
15	17.50	Carnosine	31	49.20	Ornithine
16	18.36	Proline	32	50.06	Lysine

Table VI.1: Amino acid profiles of amino acid standard (mixture) detected in HPLC

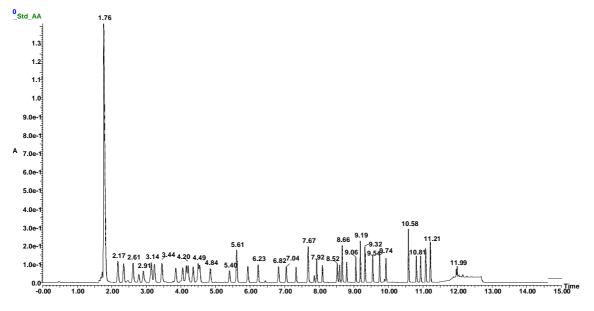
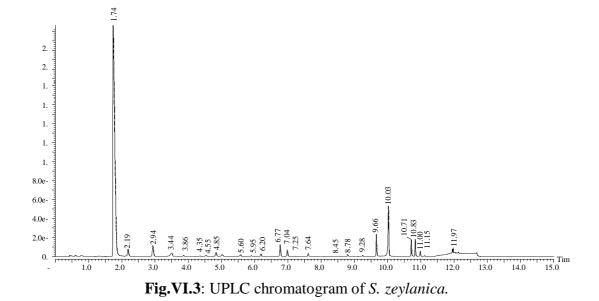


Fig.VI.2: UPLC chromatogram of standard amino acid mixture.

Sl. No.	RT	Amino acids	Sl. No.	RT	Amino acids		
1	1.72	Phosphoserine	22	5.93	Sarcosine		
2	2.34	OH-Proline	23	6.23	Threonine		
3	2.61	Histidine	24	6.82	Alanine		
4	2.78	Phosphoenolamine	25	7.04	gABA		
5	2.91	Asparagine	26	7.33	aAAA		
6	3.14	3-Methyl-histidine	27	7.67	bAIBA		
7	3.23	Taurine	28	7.67	Proline		
8	3.44	1-Methyl-histidine	29	7.92	OH-Lysine-1		
9	3.77	Cystathionine-1	30	8.09	OH-Lysine-2		
10	3.85	Serine	31	8.52	aABA/bAIBA		
11	3.99	Cystathionine-2	32	8.66	Ornithine		
12	4.05	Glutamine	33	9.06	Cysteine		
13	4.15	Carnosine	34	9.19	Lysine		
14	4.20	Arginine	35	9.32	Tyrosine		
15	4.35	Glycine	36	9.54	Methionine		
16	4.49	Anserine	37	9.74	Valine		
17	4.54	Ethanolamine	38	9.93	Norvaline		
18	4.84	Aspartic acid	39	10.81	Isoleucine		
19	5.4	b-Alanine	40	10.93	Leucine		
20	5.61	Citrulline	41	11.08	Phenylalanine		
21	5.61	Glutamic acid	42	11.21	Tryptophan		

Table VI.2: Amino acid profiles of amino acid standard (mixture) detected in UPLC

 $aAAA = \alpha$ -amino adipic acid, $gABA = \gamma$ -amino butyric acid, $bAIBA = \beta$ -amino isobutyric acid.



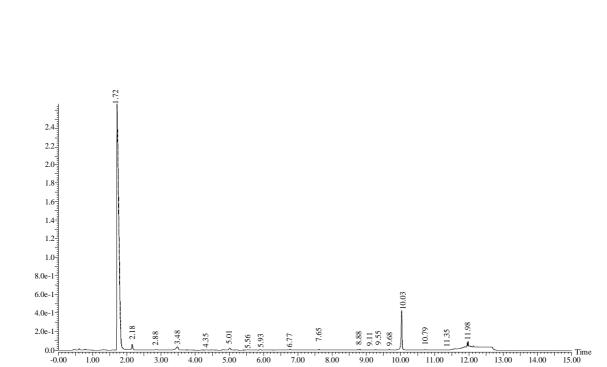
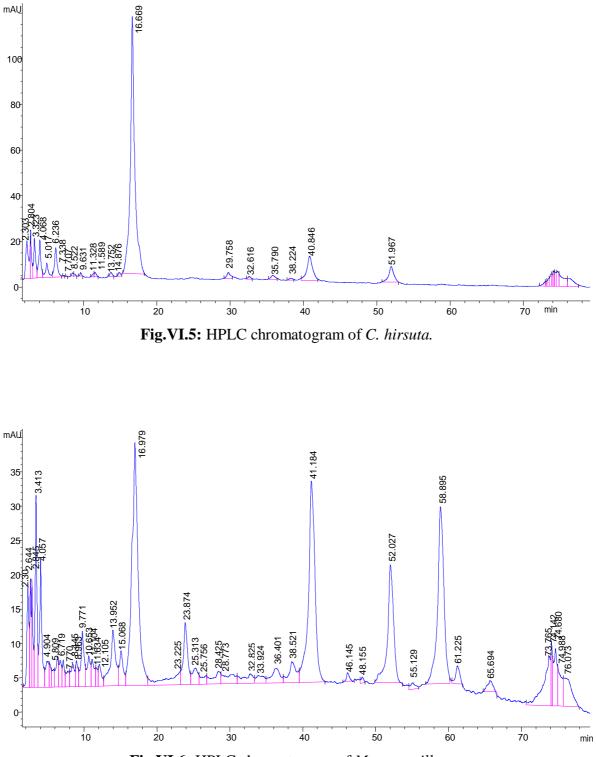
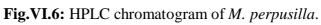
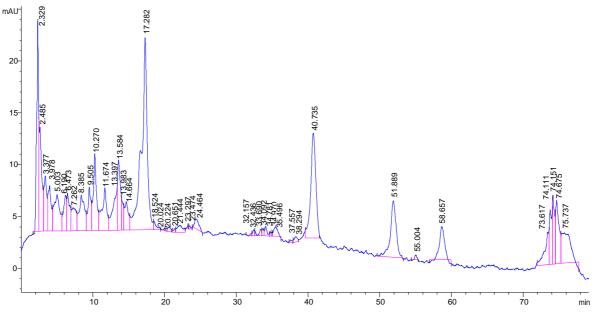


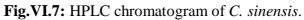
Fig.VI.4: UPLC chromatogram of S. peguensis.

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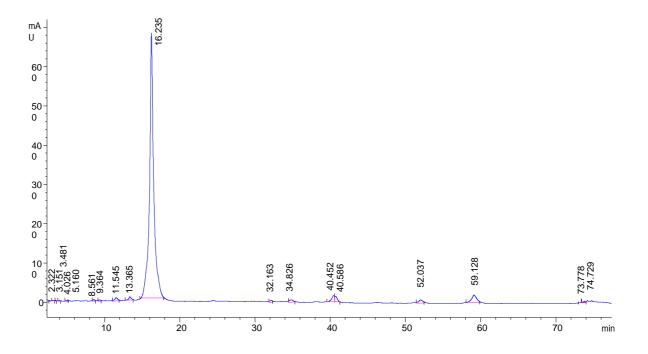
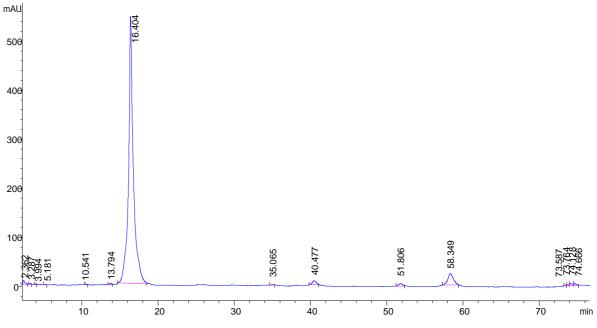
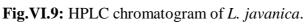
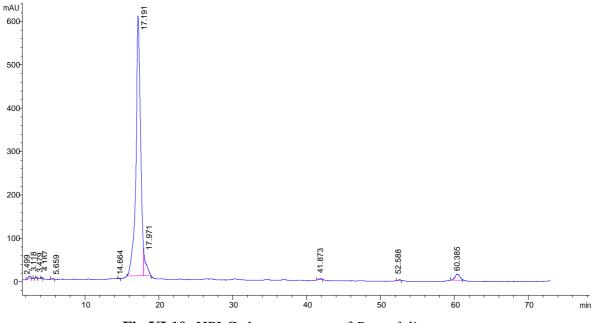
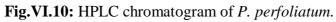


Fig.VI.8: HPLC chromatogram of *P. chinensis*.









Amino Acids	Sz	Ch	Sp	Мр	Cs	Pc	Lj	Рр
Non-essential amin	o acids (NEAA)						
Aspartic acid	0.90	0.28	0.13	0.20	0.33	0.01	0.03	0.01
Glutamic acid	0.39	0.53	0.02	0.75	0.23	0.03	0.02	0.05
Serine	0.18		0.03	0.27	0.13	0.05		
Glycine	0.06		0.02		0.18		0.02	
Asparagine	2.86		0.19	0.22	0.26			
Alanine	1.53		0.09		0.22			0.05
Proline	0.42		0.11		0.08			3.78
Arginine	0.06				0.01			
Total NEAA	6.40	0.81	0.59	1.44	1.44	0.09	0.07	3.89
Essential amino aci	ids (EAA)						
Histidine		0.06			0.24	0.29	0.06	
Isoleucine	1.57		0.05					0.12
Leucine	1.65		0.01					
Lysine	0.01		0.03					
Methionine		0.11	0.01	0.26				
Phenylalanine	0.60		0.01					
Threonine	0.39		0.01	0.57				
Valine	2.32		0.07		0.06	0.38	0.10	
Tryptophan	0.08			0.12				
Cysteine		1.55	0.01					
Tyrosine	0.15		0.04					
Total EAA	6.77	1.72	0.24	0.95	0.30	0.67	0.16	0.12
Other amino acids	(OAA)							
Phosphoserine	19.79	0.99	23.75	0.47	0.77	0.26	0.39	0.30
Amino adipic acid		7.92		9.79	3.00	0.29		0.6
OH Proline	1.35	0.15	1.19	0.08	0.08	0.03	0.003	0.02
OH lysine			0.01					
Phosphoenolamine		0.005		0.12	0.17	0.12		
Taurine		0.03		0.1	0.16	0.21		
Carnosine					5.79			14.7
3-Methyl histidine					0.01			

Table VI.3: Amino acid profiles of selected wild edible plants in mg/g of dry weight

1-Methyl histidine	1.17		1.21	1.09	0.02			
Anserine					0.19			
Cystathionine		0.08		0.53	0.04			
bABA	0.02					24.25		
aAAA	0.07							
gABA	0.11		0.03					
Ethanolamine	0.08							
Citrulline	0.13		0.01					
Sarcosine	0.09		0.07					
Ornithine	0.01		0.1					
Norvaline	6.88		5.45					
Total OAA	29.70	9.17	31.82	12.18	10.23	25.16	0.39	15.67
NEAA + EAA	13.17	2.53	0.83	2.39	1.74	0.76	0.23	4.01
Total amino acids	42.87	11.70	32.65	14.57	11.97	25.92	0.62	19.68

Sz = S. zeylanica, Ch = C. hirsuta, Sp = S. peguensis, Mp = M. perpusilla, Cs = C.sinensis, Pc = P. chinensis, Lj = L. javanica, Pp = P. perfoliatum, AA = amino acids, $bABA = \beta$ -amino butyric acid, $aAAA = \alpha$ -amino adipic acid, $gABA = \gamma$ -amino butyric acid.

In this study (**Table VI.3**), the total non-essential amino acids (NEAA) content varied from 0.07 mg/g dry weight (DW) in *L. javanica* (lowest) to 6.40 mg/g DW in *S. zeylanica* (highest). A total of eight NEAA such as aspartic acid, glutamic acid, serine, glycine, asparagine, alanine, proline, and arginine were detected in *S. zeylanica* and *C. sinensis*. All these NEAA were also detected in *S. peguensis* except arginine. Among the amino acids, the NEAA viz. aspartic acid and glutamic acid were detected in all the plant species of this study. Glutamic acid detected in all the plant species of this study. Glutamic acid detected in all the plant species of this study. Glutamic acid detected in all the plant samples ranged from 0.02 mg/g DW in *L. javanica* and *S. peguensis* to 0.75 mg/g DW in *M. perpusilla*. These findings are much lower compared with glutamic acid content of edible plant such as *Burnatia enneandra* (35.1 mg/g DW) and similar to *Chenopodium ambrosioides* (0.419 mg/g DW) [7, 8]. Similarly, aspartic acid content varied from 0.01 mg/g in both *P. chinensis* and *P. perfoliatum* to 0.9 mg/g in *S. zeylanica*. Aspartic acid content of current study was found lower compared with the results of edible plant *viz. Brassica oleracea* leaves (3.31 mg/g DW) [9] and comparable with *Parthenium hysterophorus* (0.14 mg/g FW) and *Erigeron bonariensis* (0.03 mg/g FW) [8]. However, the findings

of this study are lower in comparison to the aspartic acid contents of Carissa edulis (12.4 mg/g DW), Xylopia aethiopica (9.65 mg/g DW) and Lannea microcarpa (3.67 mg/g DW) reported by Glew et al. [10]. It was also reported that the aspartic acid and glutamic acid were the most abundant amino acids found in some green vegetables and legumes [11-13] and the results of current study are in accordance with these reported results. Aspartic acid plays important roles in the release and synthesis of luteinizing hormone and testosterone [14]. Glutamic acid plays important roles in central nervous system and recovery of physiological imbalances of the body [15, 16]. In this study (Table VI.3), serine was detected only in five plant species that ranged from 0.03 mg/g in S. peguensis to 0.27 mg/g in M. perpusilla. These findings are comparable to the serine contents found in some tea and tea products (0.03 to 1.85 mg/g DW) reported by Rouba et al. [17] and also to the results of stem, leaves, fruits and roots of an edible plant viz. Rubus amabilis (0.02 mg/g DW to 0.78 mg/g DW) reported by Chaidan et al. [18]. However, higher serine content was reported in Amaranthus viridis (11.1 mg/g DW) by Sena et al. [19] and in Lannea microcarpa (1.74 mg/g DW) reported by Glew et al. [10]. Serine acts as precursor for the synthesis of glycine, cysteine and tryptophan. It participates in cell signalling and also helps in treatment of Schizophrenia [20]. Glycine was detected only in four plant species viz. S. zeylanica (0.06 mg/g), S. peguensis (0.02 mg/g), C. sinensis (0.18 mg/g) and L. javanica (0.02 mg/g). The glycine content was reported higher in Borassus aethiopum (1.87 mg/g) [21] and similar to Millettia auriculata (0.03 mg/g) [22]. Asparagine was found in four plant species and these are S. zevlanica (2.86 mg/g), S. peguensis (0.19 mg/g), M. perpusilla (0.22 mg/g) and C. sinensis (0.26 mg/g). Both alanine (0.05 - 1.53 mg/g) and proline (0.08 - 3.78 mg/g)mg/g) were detected in four plant species viz. S. zeylanica, S. peguensis, C. sinensis and P. perfoliatum (Table VI.3). Glew et al. [7] reported alanine content in Abrus precatorius and Cadaba farinose as 4.32 and 3.62 mg/g DW, respectively; Atanasova [9] reported alanine content in *Brassica oleracea* as 3.73 mg/g DW and Choi et al. [23] also reported alanine content in *Raphanus sativus* as 0.13 mg/g DW. Similarly, Atanasova [9] reported proline content in Brassica oleracea 4.91 mg/g DW, Caidan et al. [18] reported proline content in Rubus amabilis leaves as 3.26 mg/g DW and Glew et al. [10] reported much higher proline content in the fruits of Hibiscus esculentus as 24.5 mg/g DW. In the current study, arginine was detected only in two plant species viz. S. zeylanica (0.06 mg/g) and C. sinensis (0.01 mg/g) which is similar to the free amino acids of Rubus amabilis leaves (0.04 mg/g DW) reported by Caidan et al. [18]. Arginine

is an important amino acid which has a significant role in cell division, wound healing, hormone release, ammonia removal, and immune function [20, 24, 25].

In this study (Table VI.3), the highest concentration of essential amino acids (EAA) was detected in S. zeylanica (6.77 mg/g) followed by C. hirsuta (1.72 mg/g) and the lowest EAA content was found in P. perfoliatum (0.12 mg/g). The histidine was detected only in four plant species and found the highest in P. chinensis (0.29 mg/g) and the lowest was observed both in C. hirsuta and L. javanica (0.06 mg/g). This amino acid is essential for RBC and WBC synthesis in the body and acts as a precursor for histamine protein which is required for sexual stimulation. Isoleucine was detected only in S. zeylanica (1.57 mg/g), S. peguensis (0.05 mg/g) and in P. perfoliatum (0.12 mg/g). These results of isoleucine are lower compared with the reported results of some vegetables and fruits such as Pennisetum americanum (5.54 mg/g DW) and Ziziphus mauritiana (3.0 mg/g DW), [19, 26]. Isoleucine deficiency can cause mental and physical disorders. Leucine along with valine and isoleucine plays significant roles in promoting muscle function, skin and bone formation [27, 20]. Leucine was detected only in S. zeylanica (1.65 mg/g) and S. peguensis (0.01 mg/g). Lower levels of lysine were detected only in two samples viz. S. zeylanica (0.01 mg/g) and S. peguensis (0.03 mg/g). These values are similar with the free amino acids of Rubus amabilis root (0.03 mg/g) and stem (0.08 mg/g DW) reported by Caidan [18]. However, higher levels of lysine were reported in Amaranthus viridis (13.30 mg/g DW) and Lannea microcarpa (1.63 mg/g DW) [19, 10]. A very low amount of methionine was detected only in M. perpusilla (0.26 mg/g), S. peguensis (0.01 mg/g) and C. hirsuta (0.11 mg/g). Similarly, Sena et al. [19] reported methionine contents in Moringa oleifera as 3.3 mg/g DW and in Ceratotheca sesamoides as 1.4 mg/g DW. Glew et al. [7] also reported methionine contents in Pennisetum americanum as 2.05 mg/g DW and Sorghum vulgaris as 1.90 mg/g DW. Methionine is needed in synthesis of choline, inhibits fat deposition in liver and it has antioxidant capacity [28, 12]. Phenylalanine was detected only in two samples viz. in S. zeylanica (0.60 mg/g) and S. peguensis (0.01 mg/g). McCusker et al. [26] reported the phenylalanine content in Beta vulgaris as 2.32 mg/g DW and it was reported as 1.72 mg/g DW in Borassus aethiopum [21]. Phenylalanine is used for the prevention of Parkinson's disease, arthritis, depression, obesity, painful menstruation, migraine, and schizophrenia [27, 29]. In the present study (Table VI.3), threonine was detected only in S. zeylanica (0.39 mg/g), S. peguensis (0.01 mg/g) and M. perpusilla (0.57 mg/g). The result of threenine is also much lower compared with the result of Hibiscus esculentus (4.13 mg/g DW) [10] and Amaranthus viridis (11.0 mg/g) DW [19].

Threonine is essential for the treatment of disorders in nervous system including multiple sclerosis, spinal spasticity, amyotrophic lateral sclerosis and familial spastic paraparesis [20]. Similarly, tyrosine was also detected in *S. zeylanica* (0.15 mg/g DW) and *S. peguensis* (0.04 mg/g DW) which was lower in comparison to the results of *Hibiscus esculentus* (3.98 mg/g DW [10] and with *Amaranthus viridis* (9.9 mg/g DW [19]. Valine was detected in five plant samples which ranged from 0.06 mg/g in *C. sinensis* to 2.32 mg/g in *S. zeylanica*. These results are also lower compared with the reported results of some edible plants like *Amaranthus vdiiris* (15.6 mg/g DW), *Beta vulgaris* (3.61 mg/g), and *Lannea macrocarpa* (2.08 mg/g DW) [10, 19, 26]. Lower levels of tryptophan and tyrosine were detected only in two samples *viz. C. hirsuta* (1.55 mg/g) and *S. peguensis* (0.01 mg/g). Similarly, Glew *et al.* [10] reported cysteine content in *Hibiscus esculentus* (2.65 mg/g DW), *Hibiscus sabdariffa* (0.87 mg/g DW) and *Pennisetum americanum* (3.15 mg/g DW).

In this study, besides EAA and NEAA, some other amino acids (non-protein amino acids) were also detected which are presented in the Table VI.3. In this study, phosphoserine and OH-proline were detected in all the selected plant species. Phosphoserine was found the highest in S. peguensis (23.75 mg/g) followed by S. zeylanica (19.79 mg/g) and the lowest phosphoserine was detected in P. chinensis (0.26 mg/g). However, lower levels of phosphoserine (0.14 to 0. 23 mg/g) were reported in different varieties of potato by Choi et al. [30]. The OH-proline ranged from 0.003 mg/g in L. javanica to 1.35 mg/g in S. zevlanica. The OH-lysine (0.01 mg/g) was detected only in one plant species viz. S. peguensis. 3-Methyl histidine (0.01 mg/g) and anserine (0.19 mg/g) were detected only in C. sinensis and ethanolamine (0.08 mg/g) was detected only in S. zeylanica. The results of OH-lysine and ethanolamine of current study are in accordance with the results of different varieties of potato reported by Choi et al. [30]. The levels of amino adipic acids detected in five plant species ranged from 0.29 mg/g in P. chinensis to 9.79 mg/g in M. perpusilla and α -amino adipic acid was detected only in S. zeylanica (0.07 mg/g). Choi et al. [30] reported the lower values of these amino acids (0.02 to 0.04 mg/g DW) in different varieties of potato peel in comparison to current study. In present study, the β-amino butyric acid was detected in S. zeylanica (0.02 mg/g) and P. chinensis (24.25 mg/g) and γ -amino butyric acid was detected only in S. zeylanica (0.11 mg/g) and S. peguensis (0.03 mg/g). The concentration of γ -amino butyric acid is found similar to the result of *Rubus amabilis* leaves (0.03 mg/g DW) reported by Caidan *et al.* [18]. β -amino butyric acid can help in

prevention of obesity by increasing gut hormone (leptin) production [31]. Ornithine was detected only in two samples *viz. S. zeylanica* (0.01 mg/g) and *in S. peguensis* (0.1 mg/g). Similar results (0.01 and 0.07 mg/g DW) of ornithine was reported in *Rubus amabilis* fruits and stem by Caidan *et al.* [18]. Ali *et al.* [32] also reported comparable ornithine content (0.01 to 0.07 mg/g DW) in tobaccos of Philippines. Ornithine can help in relieving stress and improving the quality of sleep related to fatigue [33]. Higher levels of carnosine were found in *C. sinensis* (5.79 mg/g) and *P. perfoliatum* (14.75 mg/g). Norvaline was also detected only in *S. zeylanica* (6.88 mg/g) and in *S. peguensis* (5.45 mg/g). Lower levels of other amino acids such as phosphoenolamine, taurine, 1-methyl histidine, cystathionine, citrulline and sarcosine were also detected in some of the plant species (**Table VI.3**). The dietary roles of most of these uncommon amino acids are still not known but they are reported as bioactive compounds [31].

VI.3 Conclusion

In this study, a total of sixteen essential and non-essential amino acids were detected in *S. zeylanica* and *S. peguensis* plant species. The total NEAA contents varied from 0.07 mg/g dry weight in *L. javanica* to 6.40 mg/g dry weight in *S. zeylanica*. A total of eight NEAA such as aspartic acid, glutamic acid, serine, glycine, asparagine, alanine, proline, and arginine were detected in *S. zeylanica* and *C. sinensis*. All these eight NEAA were also detected in *S. peguensis* except arginine. Among the amino acids, aspartic acid and glutamic acid were detected in all the selected plant species of this study. In this study, the highest concentration of EAA was detected in *S. zeylanica* (6.77 mg/g) followed by *C. hirsuta* (1.72 mg/g) and the lowest EAA content was found in *P. perfoliatum* (0.12 mg/g). Besides EAA and NEAA, some other amino acids (non-protein amino acids) were also detected in this study. The total amino acid content was found to be the highest in *S. zeylanica* (42.87 mg/g) followed by *S. peguensis* (32.65 mg/g) and the lowest amino acid content was detected in *L. javanica* (0.62 mg/g). Therefore, the consumption of these wild plants may fulfil the amino acid requirements in the diet and can prevent diseases caused by amino acid deficiencies.

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