CHAPTER VI

Amino Acid Analysis of Wild Fruits

Amino acids are generally obtained from the most abundant macromolecules found in biological systems called proteins. They serve as precursors of many enzymes, hormones, nucleic acids, neurotransmitters and other molecules essential for life [1]. Non-essential amino acids can be synthesized in the body, while essential amino acids can only be supplied through food. Some amino acids like histidine, cysteine, methionine, lysine, and tryptophan act as antioxidants, and deficiency of essential amino acids causes destruction of cells in adults, causes slowdown of development and growth in children, and generates diseases [2, 3]. Amino acids could help lower blood cholesterol and anti-mutagenicity [4]. Amino acids like aspartic acid, glutamic acid and glycine are known to perform an important role in the process of wound healing [5] and some other amino acids like histidine, methionine, tyrosine, tryptophan and lysine are considered to act as antioxidants [5, 6].

In this study, for the first time we are reporting the amino acid profiles of five wild fruits *viz. G. sapida*, *O. alismoides*, *A. dioica*, *A. bunius*, and *E. operculata* from Assam of North East India.

VI.1 Materials and Methods

VI.1.1 Sample preparation

The sample for determination of amino acid composition was prepared as per the procedure mentioned in the **Section II.2.3 (Page no. 67).**

VI.1.2 Amino acid analysis

The sample (1 mg) was vortexed well with 1 mL of water and the volume was made up to 5 mL with methanol. It was incubated overnight at -20^{o}C. 10 µL of the sample was taken and evaporated completely at 60^{o}C under nitrogen atmosphere. In this sample, 50 µL of PITC reagent was added, vortexed and placed on thermo mixer for 1 h at 45^{o}C and then the sample was vacuum dried. To this pellet, 200 µL of buffer A (10 mM sodium acetate adjusted to pH 6.4 with 6% acetic acid) was added and centrifuged. The supernatant was collected and filtered using syringe filter. 20 µL of the sample was loaded into the reversed-phase HPLC (Agilent 1200 series, Zorbax 300 SB-C18 column with 4.6×250 mm, 254 nm of wavelength). The sample was allowed to run at a flow rate of 1 mL/min for 82 min. The buffer B used was (buffer A + acetonitrile, 40:60). Amino acid identification and quantification were done by comparing the retention times of the peaks with those of standard (acidic and basic amino acid mixture). The amino acid score was determined on the basis of FAO/WHO/UNU (2007) suggested pattern [7].

VI.2 Results and Discussion

Typical HPLC chromatogram of standard amino acid mixture is shown in **Fig. VI.1** and the HPLC chromatograms of five wild fruits are shown in **Fig. VI.2–VI.6**. In this study, identification and quantification of amino acids were done by comparing the retention times of the peaks of individual sample with those of acidic and basic amino acid mixture standard. The amino acid profiles of the standard are presented in **Table VI.1.** Amino acid profiles of five wild edible fruits in % of total amino acids are summarised in **Table VI.2**. It is seen that a total of seventeen amino acids in varying compositions were identified in the fruits. These are eight essential amino acids *viz*. threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, and histidine and nine are non-essential amino acids *viz*. aspartic acid, serine, glutamic acid, proline, glycine, alanine, cysteine, tyrosine, and arginine.

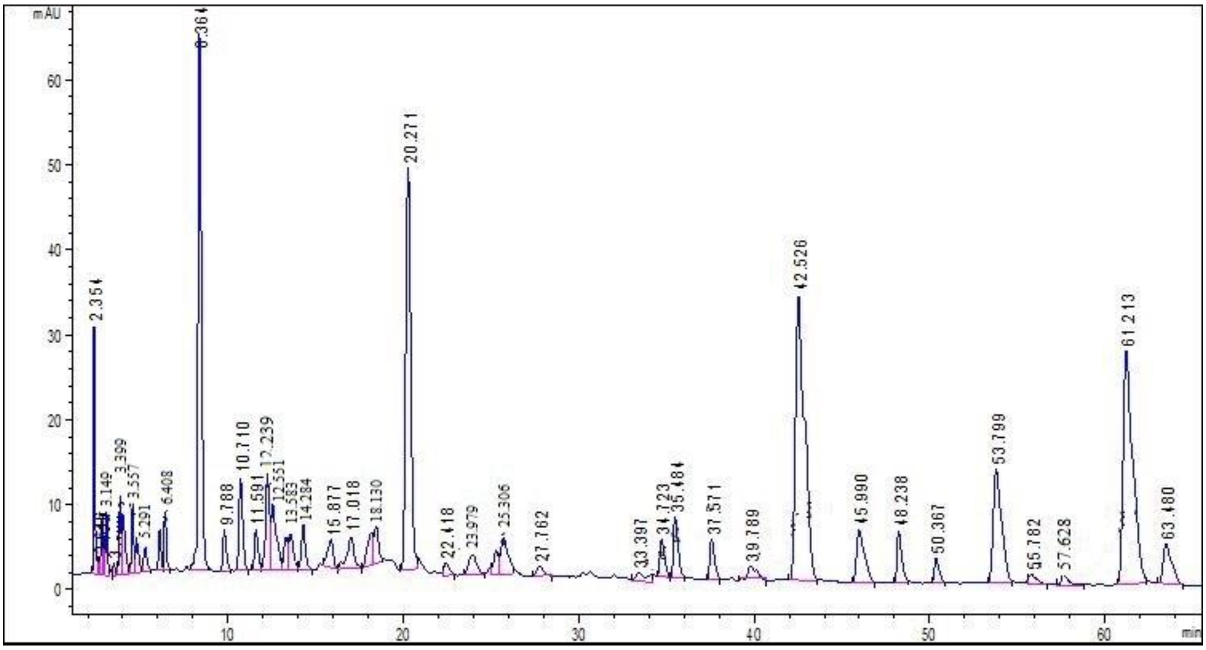

Fig. VI.1: HPLC chromatogram of acidic and basic amino acid mixture standard.

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

Retention times	Amino acids	Retention times	Amino acids
3.48	Phosphoserine	25.706	Proline
4.557	Aspartic acid	27.762	3-Methyl histidine
4.787	Glutamic acid	30.632	1-Methyl histidine
6.408	Amino adipic acid	31.985	Anserine
8.364	OH-Proline	33.393	Tyrosine
9.788	Phosphoenolamine	34.722	Valine
10.71	Serine	35.483	Methionine
11.591	Glycine	37.571	Cystathionine
12.239	Asparagine	39.544	Cysteine
14.284	Taurine	40.124	Isoleucine
15.877	Threonine	42.526	Leucine
17.018	Histidine	45.99	OH Lysine
18.163	Alanine	48.238	Phenylalanine
20.271	Carnosine	50.367	Tryptophan
23.979	β-amino adipic acid	55.782	Ornithine
25.29	Arginine	57.628	Lysine

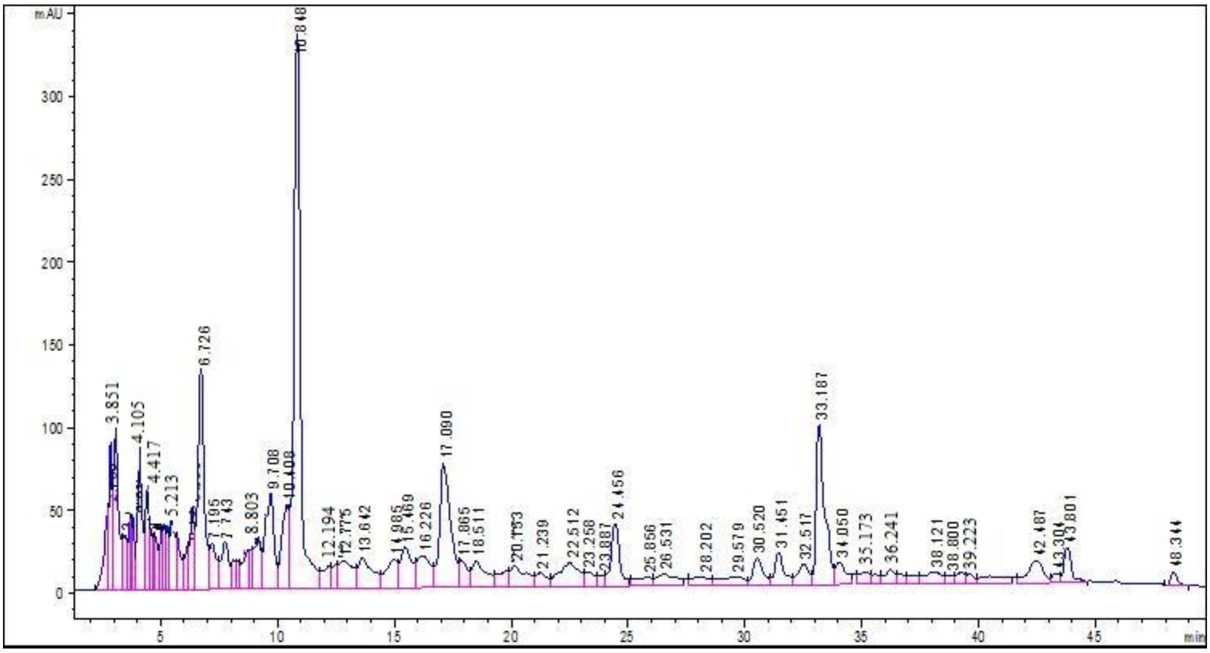

Fig. VI.2: HPLC chromatogram of *G. sapida* fruit.

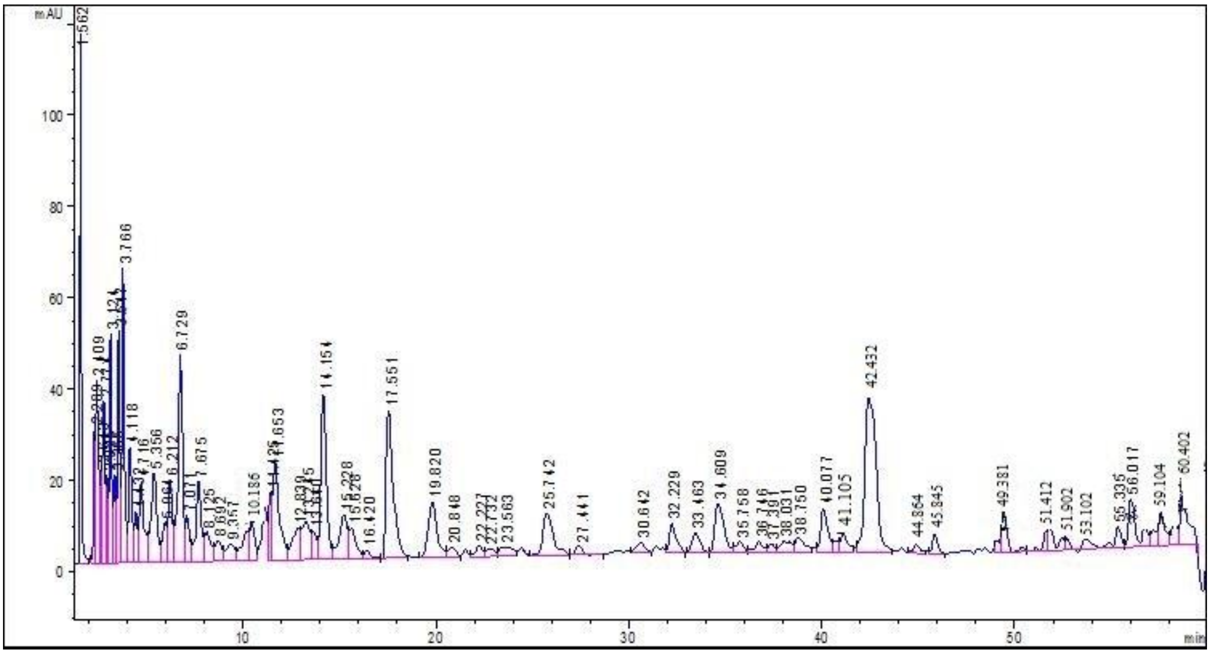

Fig. VI.3: HPLC chromatogram of *O. alismoides* fruit.

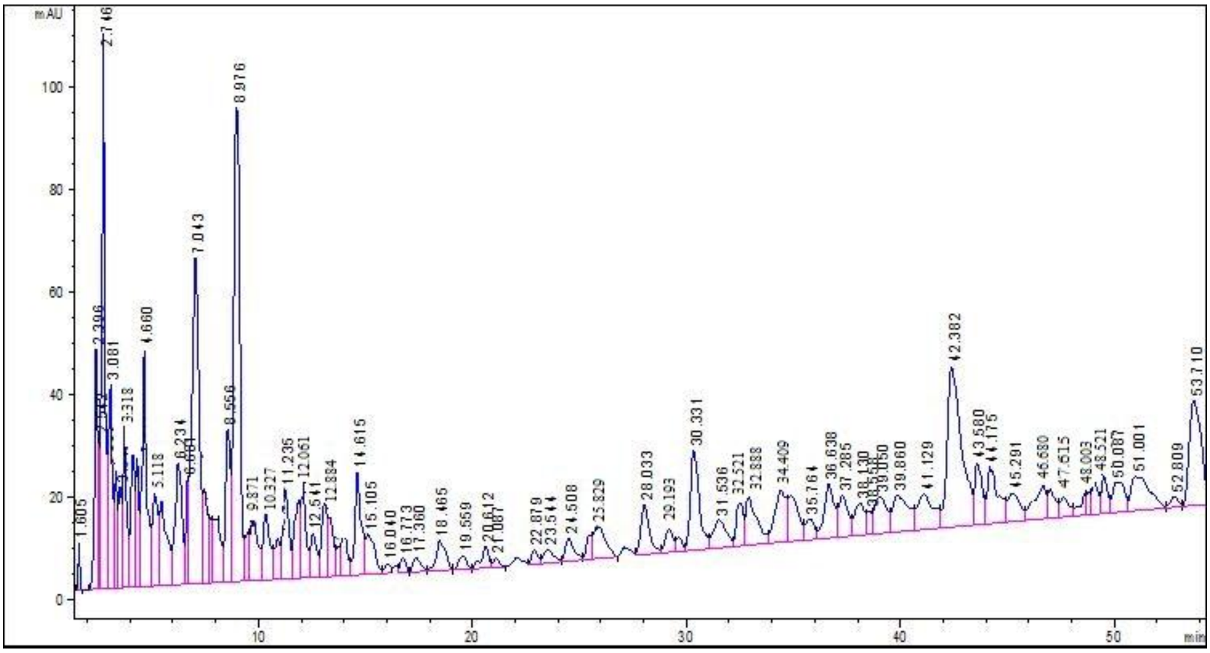

Fig. VI.4: HPLC chromatogram of *A. dioica* fruit.

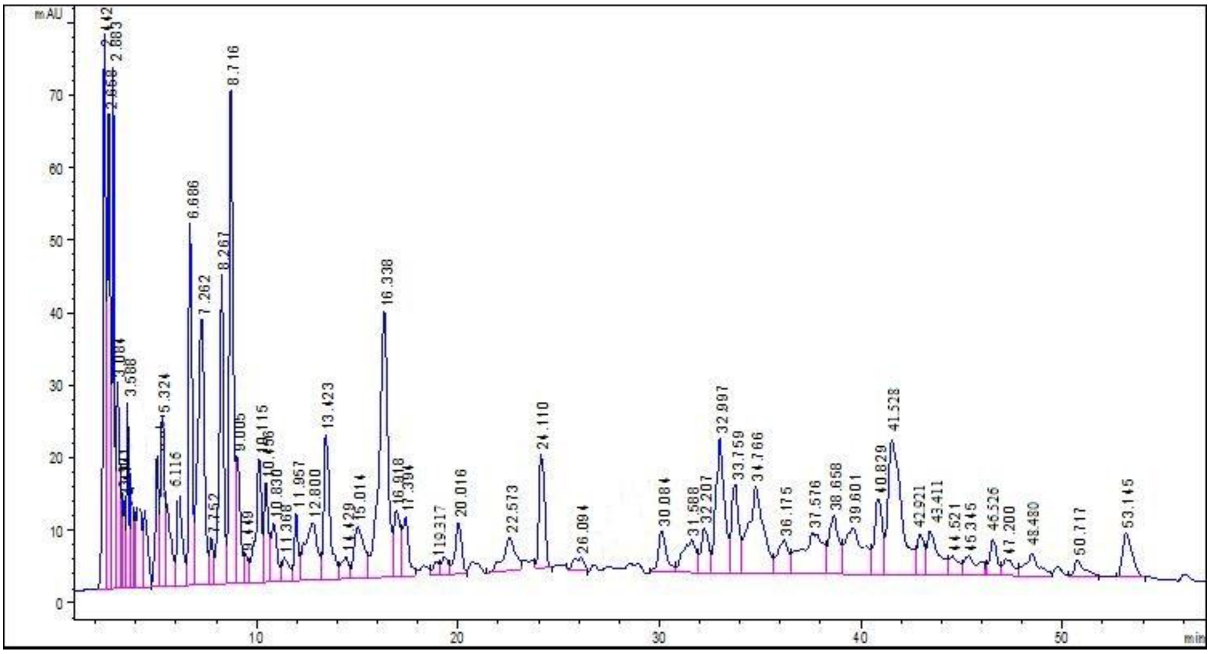

Fig. VI.5: HPLC chromatogram of *A. bunius* fruit.

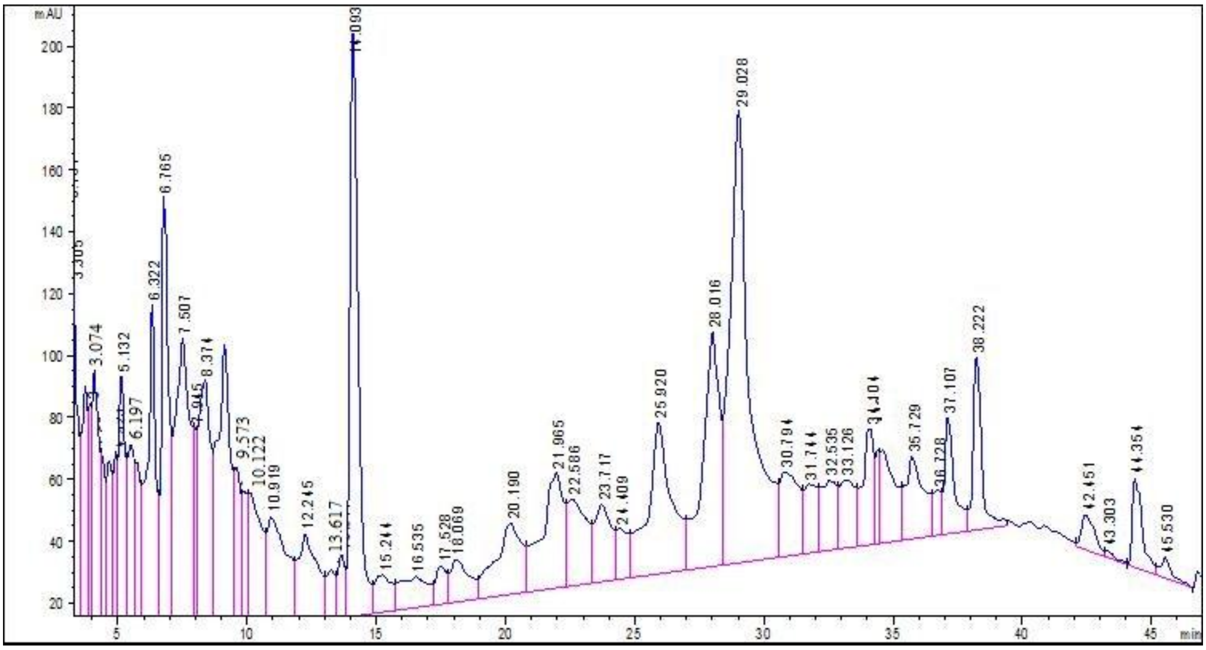

Fig. VI.6: HPLC chromatogram of *E. operculata* fruit.

Table VI.2: Amino acid profiles of five wild edible fruits in % of total amino acids

Amino acids	***G. sapida*** **(% of TAA)**	***O. alismoides*** **(% of TAA)**	***A. dioica*** **(% of TAA)**	***A. bunius*** **(% of TAA)**	***E. operculata*** **(% of TAA)**
NEAA					
Aspartic acid	1.593	1.151	2.276	3.837	3.075
Serine	nd	0.496	0.425	1.105	1.892
Glutamic acid	2.283	4.467	9.667	6.544	3.151
Proline	nd	nd	0.745	nd	nd
Glycine	nd	2.685	1.867	0.978	nd
Alanine	2.564	nd	0.838	nd	1.096
Cysteine	1.814	nd	7.306	17.049	nd
Tyrosine	nd	1.249	3.336	nd	3.094
Arginine	1.423	2.388	1.671	0.904	7.187
Total NEAA	9.677	12.436	28.131	30.417	19.495
EAA					
Threonine	4.602	1.234	nd	nd	1.339
Valine	0.142	0.265	0.214	1.029	0.273
Methionine	0.391	0.249	0.426	nd	nd
Isoleucine	4.434	4.459	nd	nd	nd
Leucine	6.538	19.665	19.431	4.438	1.849
Phenylalanine	nd	nd	3.305	2.701	nd
Lysine	nd	2.592	nd	nd	nd
Histidine	12.986	5.916	0.467	2.702	0.819
Total EAA	29.093	34.38	23.843	10.87	4.28
Total (NEAA + EAA)	38.770	46.816	51.974	41.287	23.775

TAA, Total Amino Acids; NEAA, Non-Essential Amino Acids; EAA, Essential Amino Acids; nd, not detected.

In all of the five wild fruits, a total of six different amino acids were detected (**Table VI. 2**). Three of them are non-essential amino acids (NEAA) *viz*. aspartic acid (1.151–3.837%), glutamic acid (2.283–9.667%) and arginine (0.904–7.187%), and three of them are essential amino acids (EAA) *viz*. valine (0.142–1.029%), leucine (1.849–19.665%), and histidine (0.467–12.986%). The essential amino acid threonine was not detected in *A. dioica* and *A. bunius* fruits. Methionine was found to be absent in the fruits of *A. bunius* and *E. operculata.* Isoleucine was detected only in the two fruits *viz*. *G. sapida* and *O. alismoides*, and phenylalanine was also found in two species only *viz*. *A. dioica* and *A. bunius*. The essential amino acid *viz*. lysine was detected in *O. alismoides* (2.592%) only, and found to be absent in all other species (**Table VI.2**). *A. dioica* fruit showed the highest amino acid content (51.974%) followed by *O. alismoides* fruit (46.816%). The amino acid scores of five wild edible fruits based on FAO/WHO/UNU (2007) consultation pattern [7] are shown in **Table VI.3**.

Table VI.3: Amino acid score of five wild edible fruits based on FAO/WHO/UNU (2007) consultation pattern

Amino acids	**FAO/WHO/ UNU (2007) (mg/g protein)**	**Chemical score (%)**				
		G. sapida	***O. alismoides***	***A. dioica***	***A. bunius***	***E. operculata***
Valine	39	3.642	6.795	5.488	26.385	7.0
Lysine	45	--	57.60	--	--	--
Isoleucine	30	147.8	148.634	--	--	--
Leucine	59	110.814	333.306	329.339	75.221	31.339
Phenylalanine + Tyrosine	38	--	--	174.763	71.079	--
Threonine	23	200.087	53.653	--	--	58.218
Histidine	15	865.734	394.40	31.134	180.134	54.6
Methionine	16	24.438	15.563	26.626	--	--
Total EAA	277	105.029	124.116	86.076	39.242	15.452

EAA, Essential Amino Acids.

Proteins, composed of amino acids, are the major part of our body along with water. Amino acid compositions provide information about the quality of food proteins. NEAA can be synthesized in the body, while EAA can only be supplied through foods [8]. The main purpose of dietary protein is to provide amino acid for biosynthesis of proteins and proper amount of all EAA need to be supplied to the tissues for optimal synthesis of protein [9]. In this study (**Table VI.2**), total NEAA content varied from 9.677% in *G. sapida* (lowest) to

30.417% in *A. bunius* (highest), whereas total EAA content ranged from 4.28% in *E. operculata* (lowest) to 34.38% in *O. alismoides* (highest). EAA enhances the quality of protein and is used for animal nutrition. Amongst the seventeen amino acids detected, leucine was found to be the most abundant EAA followed by histidine, whereas glutamic acid was found to be the most abundant NEAA followed by arginine. This study indicates that the fruits may be useful as good sources of both NEAA and EAA. The lowest aspartic acid was detected in *O. alismoides* fruit (1.151%) and the highest was found to be in *A. bunius* fruit (3.837%). The aspartic acid content of *Phoenix dactylifera* fruits reported by Shaba *et al*. [10] was 3.37 g/100 g, while Nkafamiya *et al*. [11] reported higher values of aspartic acid in the fruits (9.67 g/100 g) and leaves (10.97 g/100 g) of *Azanza garckeana*. Serine was found in all the fruits except in *G. sapida* fruit (**Table VI.2**). Serine is the precursor of cysteine, glycine, and tryptophan and plays an important role in cell signalling. It can also be used for the treatment of schizophrenia [12]. In this study, glutamic acid (2.283–9.667%) detected in all the fruits is the most abundant NEAA. Glutamic acid acts as a fuel for the brain, helps to recover body's physiological imbalances, and is also a good neurotransmitter for spinal cord, and central nervous system [13]. The amount of alanine found in this study was lower than that of African pear pulp (4.04 g/100 g) reported by Onuegbu *et al*. [14]. Arginine was detected in all the five fruits studied herein. *E. operculata* fruit (7.187%) showed higher value of arginine than other fruits and it is similar to that of *Azanza garckeana* fruit (7.01 g/100 g) reported by Nkafamiya *et al*. [11]. Arginine plays important roles in wound healing, cell division, hormone release, ammonia removal, and immune function. It also acts as a precursor in biological synthesis of nitric oxide which plays a very important role in maintenance of blood pressure, blood clotting, and neurotransmission. Arginine is supplemented for recovering the number of diseases like pre-eclampsia, sepsis, anxiety, erectile dysfunction and hypertension [12].

EAAs are generally considered as essential for humans. The essential amino acids *viz*. valine, leucine, and histidine were detected in all the fruits (**Table VI.2**), and leucine was the most abundant EAA. Threonine detected in *G. sapida* fruit (4.602%) was the highest than other fruits. Threonine content of bayberry kernel reported by Cheng *et al*. [15] was found in the range of 3.10–3.22 g/100 g. Threonine can be used for various nervous system disorders including multiple sclerosis, spinal spasticity, amyotrophic lateral sclerosis, and familial spastic paraparesis [12]. Methionine content of the fruits (0.249–0.426%) was found lower than African pear pulp (0.81 g/100 g) reported by Onuegbu *et al*. [14]. Methionine acts as an antioxidant [3]. Low level of valine (0.142–1.029%) was found in all the five fruits which is similar to that of some spices reported by Bouba *et al*. [2] and is comparable to that of *Phoenix dactylifera* fruits (1.92 g/100 g) reported by Shaba *et al*. [10]. Isoleucine was detected in the fruits of *G. sapida* (4.434%) and *O. alismoides* (4.459%) only and it was absent in other three fruits. These values are comparable to that of *Aframomum daniellii* (4.83 g/100 g) reported by Bouba *et al*. [2]. Deficiency of isoleucine causes physical and mental disorders. Leucine with isoleucine and valine plays very important roles to promote muscle function, bones and skin [2, 12]. Phenylalanine was detected only in *A. dioica* and *A. bunius* fruits and the results are comparable to that of bayberry kernel reported by Cheng *et al*. [15]. The brain uses phenylalanine to produce a chemical known as norepinephrine which transmits signals between nerve cells. Phenylalanine regulates human mood, promotes alertness, and it is also used in the treatment of Parkinson's disease, depression, arthritis, obesity, migraine, painful menstruation, and schizophrenia [2, 16]. Histidine was detected in all of the five fruits and found to be the highest in *G. sapida* (12.986%) and the lowest being in *A. dioica* (0.467%). Histidine also acts as an antioxidant and is used for the treatment of cardiovascular disease [2, 3, 17]. It is a precursor of histamine. It is also required for removing heavy metals from the body, repair and growth of tissue, and maintenance of myelin sheaths [12]. All of the amino acids with the exception of valine in all the fruits exceeded the amino acid score recommendation given by FAO/WHO/UNU (2007) consultation pattern [7] (**Table VI. 3**).

VI.3 Conclusion

In this study, a total of seventeen amino acids in varying compositions were identified and eight of these are essential amino acids and nine are non-essential amino acids. *A. dioica* fruit showed the highest amino acid content (51.974%) followed by *O. alismoides* fruit (46.816%). *A. bunius* fruits showed the highest NEAA contents (30.417%) whereas *O. alismoides* fruits displayed the highest EAA contents (34.38%). Leucine was found to be the most abundant EAA whereas glutamic acid was found to be the most abundant NEAA. This study indicates that the fruits may be useful as good sources of both EAA and NEAA, and could be served as good natural supplements for EAA which may contribute to proper maintenance and growth of human health.

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