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Amino Acid Composition of Five Wild Edible Fruits of Assam, North-East India

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In this study, amino acids profiles of 5 wild edible fruits *viz. Grewia sapida*, *Ottelia alismoides*, *Aporosa dioica*, *Antidesma bunius* and *Eugenia operculata* found in Assam of North-East India were investigated by RP-HPLC equipped with C_{18} column. A total of 17 amino acids in varying compositions were identified and 8 of these are essential amino acids and 9 of these are non-essential amino acids. In all of the 5 wild fruits, 6 different amino acids were identified and these were aspartic acid (1.151-3.837 %), glutamic acid (2.283-9.667 %), arginine (0.904-7.187 %), valine (0.142-1.029 %), leucine (1.849-19.665 %), and histidine (0.467-12.986 %). *A. bunius* fruit showed the highest non-essential amino acid content whereas *O. alismoides* fruit displayed the highest essential amino acid content. Leucine was found to be the most abundant essential amino acid whereas glutamic acid was detected to be the most abundant non-essential amino acid.

Keywords: Wild edible fruits, Proteins, Amino acids, Essential amino acids, Non-essential amino acids.

INTRODUCTION

Indigenous plants have been traditionally an important food sources for rural population. Fruits and vegetables are good sources of minerals, vitamins, carbohydrates, fats, proteins, and amino acids [1,2]. Adequate intake of fruits and vegetables can prevent non-communicable diseases such as diabetes, atherosclerosis and cancers [3]. Amino acids are generally obtained from the most abundant macromolecules found in biological systems called proteins. The 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 [1,4].

Grewia sapida Roxb. ex DC. is a small shrub that grows to about 1-1.5 m in height and it produces edible fruits of small size. The plant belongs to the Malvaceae family and is widespread in Nepal, China, Pakistan and India [5]. *Ottelia alismoides* (L.) Pers. commonly known as Khar in Assam belongs to Hydrocharitaceae family and the fruit is soft, cylindrical, ellipsoid to ovoid, 1-2 cm wide and 1.5-4 cm long. It is widespread throughout the East and South-East Asia and Northern Australia [6]. *Aporosa dioica* (Roxb.) Muell.-Arg. belonging to the family Euphorbiaceae is distributed through the North-East India and eastern Himalayas [4]. The plant yields ellipsoid fruits of 1-1.3 cm long and has a sweet-sour taste. *Antidesma bunius* (L.) Spreng. is a wild plant belonging to Euphorbiaceae family and is distributed in Philippines, Thailand and Southeast Asia. The fruit is very small in size, has a sweet-sour taste and is popular among rural populations [7]. *Eugenia operculata* Roxb., a perennial tree is widely distributed in India, Vietnam, China and other tropical countries [7]. In Assam of North-East India, its fruits are consumed by local communities. All of these five wild fruits selected for this study have good nutritional values, several important phytochemical constituents, and strong antioxidant properties along with high total phenolic and total flavonoid contents [5-7]. 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*, *E. operculata* and *A. bunius*.

EXPERIMENTAL

Sample collection, identification and preparation: Ripe fruits of *Grewia sapida*, *Antidesma bunius* and *Eugenia operculata* were collected in April, 2015 from Chirang district of India whereas the ripe fruits of *Aporosa dioica* and *Ottelia alismoides* were obtained in April and October, 2015, respectively from Kokrajhar district of India. Three herbarium sheets of plants were submitted at Herbarium of Botanical Survey of India (Meghalaya) and authenticated (Ref. No. BSI/ERC/Tech./Plant Iden./2015/211, Dated 25-06-2015) as *Grewia sapida*, *Eugenia operculata* and *Aporosa dioica*. The voucher specimens of *Antidesma bunius* (collection No. 501) and *Ottelia alismoides* (collection No. 502) are deposited in the Herbarium of Botany Department, Bodoland University, Kokrajhar, India. The fresh fruits were washed with clean water and then freeze dried for 72 h. The dry material was powdered using a grinder and kept in air-tight container at 4 ºC.

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 ºC. Around 10 μL of the sample was taken and evaporated completely at 60 ºC under nitrogen atmosphere. In this sample, 50 μL of phenylisothiocyanate (PITC) reagent was added, vortexed and placed on thermo mixer for 1 h at 45 ºC 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.

RESULTS AND DISCUSSION

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, and the amino acid profiles of 5 wild edible fruits in % of total amino acids are summarized in Table-1. It is seen that a total of 17 amino acids in varying compositions were identified in the fruits and of these 8 are essential amino acids and 9 are non-essential amino acids. Three non-essential amino acids (aspartic acid, glutamic acid and arginine) and 3 essential amino acids (valine, leucine and histidine) were detected in all the 5 fruits. *A. dioica* fruit showed the highest amino acid content (51.974 %) followed by *O. alismoides* fruit (46.816 %) and *E. operculata* fruit (23.775 %) exhibited the lowest amino acid content. Kivrak *et al*. [8] investigated the amino acid profiles of a wild mushroom (*Calvatia gigantean*) and the essential amino acids reported were tryptophan, leucine, isoleucine, valine, threonine, phenylalanine, lysine, methionine and histidine. The non-essential amino acids reported by Kivrak *et al*. [8] were tyrosine, 4-hyrdroxy proline, proline, arginine, glycine, alanine, serine, glutamine, asparagine, glutamic acid and aspartic acid. These non-essential amino acids were also observed in the current study (Table-1) except 4-hyrdroxy proline, glutamine, and asparagine. The variations in composition of amino acids are due to soil type, cultivar type, hereditary type and environmental conditions [9].

In this study, total non-essential amino acid content varied from 9.677 % in *G. sapida* to 30.417 % in *A. bunius*, whereas total essential amino acids content ranged from 4.28% in

TABLE-1
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)
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.

E. operculata to 34.38 % in *O. alismoides*. The essential amino acids enhances the quality of protein and is used for animal nutrition. Amongst the 17 amino acids detected, leucine was found to be the most abundant essential amino acid followed by histidine, whereas glutamic acid was found to be the most abundant non-essential amino acid followed by arginine. 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 *A. garckeana*. Serine was found in all the fruits except in *G. sapida* fruit (Table-1). Serine is the precursor of cysteine, glycine and tryptophan and used for cell signalling and treatment of Schizophrenia [12]. In this study, glutamic acid (2.283-9.667 %) detected in all the fruits is the most abundant non-essential amino acid. Glutamic acid acts as a fuel for the brain, helps to recover body′s physiological imbalances and is also a good neurotransmitter [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. *E. operculata* fruit (7.187%) showed higher value of arginine than other fruits and it is similar to that of *A. garckeana* fruit (7.01 g/100 g) reported by Nkafamiya *et al*. [11]. Arginine plays important roles in wound healing, cell division, hormone release, maintenance of blood pressure, blood clotting and neurotransmission [12].

Essential amino acids are generally considered as essential for humans. The three essential amino acids *viz*. valine, leucine, and histidine were detected in all the fruits, and leucine was the most abundant essential amino acid followed by histidine. Low levels of essential amino acid such as isoleucine, phenylalanine, threonine, lysine and methionine were detected in some of the species (Table-1). Kivrak [15] reported higher levels of non-essential amino acids *viz*. leucine, valine, isoleucine, phenylalanine, tryptophan, threonine and lysine in Turkish honeys. Threonine detected in *G. sapida* fruit (4.602 %) was the highest than other fruits. Threonine content of bayberry kernel reported by Cheng *et al*. [16] 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 [4]. 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*. [1] 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 *A. daniellii* (4.83 g/100 g) reported by Bouba *et al*. [1]. Deficiency of isoleucine causes physical and mental disorders. Leucine with isoleucine and valine plays very important roles to promote muscle function, bones and skin [1,12]. 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*. [16]. Phenylalanine regulates human mood, promotes alertness, and is used in treatment of Parkinson′s disease, depression, arthritis, obesity, migraine, painful menstruation and schizophrenia [1]. 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 acts as an antioxidant and is used for the treatment of cardiovascular disease [1]. It is also required for removing heavy metals from the body, repair and growth of tissue, and maintenance of myelin sheaths [12]. The amino acid scores of 5 wild edible fruits based on FAO/WHO/UNU (2007) consultation pattern are shown in Table-2, which shows that all of the amino acids with the exception of valine in all the fruits exceeded the amino acid score.

Conclusion

In this study, a total of 17 amino acids in varying compositions were identified and eight of these are essential amino acids and nine are non-essential amino acids. *A. bunius* fruits showed the highest non-essential amino acid content whereas *O. alismoides* fruits displayed the highest essential amino acids content. Leucine was found to be the most abundant essential amino acid whereas glutamic acid was found to be the most abundant non-essential amino acid. This study indicates that the fruits may be useful as good sources of both non-essential and essential amino acids and could be served as good natural supplements for essential amino acid which can contribute to proper maintenance and growth of human health.

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TABLE-2
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 essential amino acids	277	105.029	124.116	86.076	39.242	15.452

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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