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AMINOACID PROFILING AND ANTIOXIDANT POTENTIAL OF SOME SELECTED EDIBLE PLANTS

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ABSTRACT
The amino acid is an important aspect and it play pivoted role in human life for healthy growth. Amino acids are easily available in many wild edible plants. Along with several organic compounds, it is now well to cure diseases, but they play important role in many activities in the body. This study was aimed at monitoring the Amino acid profile of some selected ethnic and edible leaves as well as investigating the nutritional status of these species in terms of available amino acids and antioxidant potential. Among all the ten species investigated for their amino acid profile, the plant *Sauropus androgynus* emerged as the most potential one with higher concentration of all the essential amino acids. The highest concentration was shown by cysteine (740.74 mg/g), followed by asparagine (714.28 mg/g) and arginine (380.95 mg/g). The result indicated that non essential amino acids (histidine, alanine, arginine, aspartic acid, glutamic acid, glycine, proline and serine) are higher in concentration compared to essential amino acids (isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine and valine), which constitute 41.1% of the total amino acids analyzed. The chemical score was also analysed.

KEY WORDS: Amino acid, Chemical score, Profiling, Antioxidant potential, Proteins, Edible leaves.

INTRODUCTION

Living organism requires a continuous supply of large number of substances from food to complete their life cycle. This supply is called as nutrition. The amino acid is an important aspect and it play pivoted role in human life for healthy growth. Amino acids are easily available in many wild edible plants. Along with several organic compounds, it is now well to cure diseases, but they play important role in many activities in the body (Malhotra, 1998). Wild plants play an important role in the diet of the humans. These plants tend to be drought-resistant and are gathered both in times of plenty as well as times of need. The evolution towards health and functionality, which is mainly driven by the partial replacement of animal foods with plants, has shown to improve nutritional status (Guillon and Champ, 2002) due to low cholesterol level in plant foods, and increased level of fibre intake which reduces the risk of bowel diseases, including cancer (Adebowale *et al*., 2007). Amino acids are biologically very important organic compounds composed of amine (-NH_2) and carboxylic acid (-COOH) functional groups, along with a side-chain specific to each amino acid. The key elements of an amino acid are carbon, hydrogen, oxygen, and nitrogen though other elements are found in the side-chains of certain amino acids. In the form of proteins, amino acids comprise the second-largest component (water is the largest) of human muscles, cells and other tissues. Outside proteins, amino acids perform critical roles in processes such as neurotransmitter transport and biosynthesis. Amino acids having both the amine and the carboxylic acid groups attached to the first (alpha-) carbon atom have particular importance in biochemistry. Many important proteinogenic and non proteinogenic amino acids also play critical non-protein roles within the body. Nine proteinogenic amino acids are called "essential" for humans because they cannot be created from other compounds by the human body and, so, must be taken in as food. Others may be conditionally essential for certain ages or medical conditions. Because of their biological significance, amino acids are important in nutrition and are commonly used in nutritional supplements, fertilizers, and food technology. Of the 22 standard amino acids, 9 are called essential amino acids because the human body cannot synthesize them from other compounds at the level needed for normal growth, so they must be obtained from food. In addition, cysteine, taurine, tyrosine, and arginine are considered semi essential amino-acids in children (though taurine is not technically an amino acid), because the metabolic pathways that synthesize these amino acids are not fully developed. The nine essential amino acids (EAA) include leucine, isoleucine, valine, lysine, threonine, tryptophan, methionine, phenylalanine, and histidine. Non-essential amino acids include alanine, asparagine, aspartic acid, and glutamic acid. Conditional amino acids include arginine, cysteine, glutamine, glycine, proline, serine, and tyrosine. The foods richest in essential amino acids are those from animal sources such as meats, dairy products, fish and eggs. Although lower in essential amino acids, plant sources also contain protein: whole grains, pulses, legumes, soy, fruits, nuts and seeds. Vegetarians can get enough essential amino acids by eating a variety of plant proteins. Malnutrition affects millions of people all over the world especially in developing nations. Under-nutrition for the poor people and over nutrition for the rich segment of the population are two parallel forms of malnutrition affecting the nations (Weingärtner, 2004). Over-nutrition is associated with nutritional transition which is due to urbanization as such the city dwellers consume mostly refined and junk food ignoring the traditional unrefined food such as fruits and vegetables (Vainio-Maltila, 2000, Weingärtner, 2004). This transition leads to increased risk of non-communicable diseases such as diabetes, hypertension, obesity, cancer and cardiovascular diseases. However, green vegetables have long been recognized as the cheapest and most abundant potential sources of protein because of their ability to synthesize amino acids from a wide range of virtually unlimited and readily available primary materials such as, water, carbon dioxide and atmospheric nitrogen in sunlight (Aletor *et al*.,2002, Byers,1961 and Oke, 1968). Although, leafy vegetables are good sources of protein their use by man and non-ruminants is limited because of their high cellulose content and, in some cases, the presence of inherent toxic factors. However, if the cellulose content was separated mechanically, cellulose free protein from vegetables could supply as much as 10 –20g protein/person/day (Oke, 1973)

Antioxidants are defined as a class of compounds which include vitamins, enzymes and phytochemicals. Numerous epidemiological studies suggest that diets rich in antioxidants execute a protective role in health and disease. Antioxidants may serve the task of reducing oxidative damage in humans induced by free radicals and reactive oxygen species (ROS) under oxidative stress conditions. Reactive oxygen species (ROS) causes DNA and protein damage, lipid peroxidation, cancer, ageing and inflammatory activity. Free radicals are naturally produced in the body through normal metabolism of carbohydrates, amino acids and fats. Other factors known to increase free radicals in our body include chronic diseases, smoking, environmental poisons, alcohol and ionizing radiations. Overproduction of free radicals can result in oxidative stress, a deleterious process that damages the cell structure. Antioxidants are chemical compounds that can bind to free oxygen radicals preventing these radicals from damaging healthy cells where as pro-antioxidant act indirectly either by modulation of direct agents or by regulation of the biosynthesis of antioxidant proteins (Halliwell and Guttering, 1998). Plants (fruits, vegetables, medicinal herbs etc.) may contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, and some other endogenous metabolites, which are rich in antioxidant activity. Epidemiological studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti-carcinogenic, anti-bacterial, or anti-viral activities to a greater or lesser extent.

This study was aimed at monitoring the Amino acid profile of some selected ethnic and edible leaves as well as investigating the nutritional status of these species in terms of available amino acids and antioxidant potential.

To complete the study, the following experimental approaches were chosen:

- As an initial part of the work, amino acids from all the selected leaves were isolated and assayed.
- Total essential amino acids, non essential amino acids, aromatic amino acids, sulphur containing amino acids and chemical scores were analyzed.
- Total antioxidant potential of the selected leaves was also analyzed.

The parameters studied are discussed in a framework of amino acid profiling in order to integrate the data into a general sequence of events representing different levels of nutritional aspects of all the selected leaves.

MATERIALS & METHODS

The present study was aimed at monitoring the Amino acid profile of some selected ethnic and edible leaves as well as investigating the nutritional status of these in terms of available amino acids and antioxidant potential.

The following 10 ethnic leaves were used for the present study- *Amaranthus viridis, Sauropus androgynus, Centella asiatica, Vigna unguiculata, Amorphophallus paeoniifolius, Moringa oleifera, Colocasia esculenta, Brassica oleracea, Cassia occidentalis and Amaranthus dubius* .

Quantification of amino acids- Amino acids were determined using the method of Moore and Stein (1948)

Estimation of chemical score- Chemical score (amino acid) was computed for each essential amino acid by the following formula:

$$\frac{Milligram\ of\ amino\ acid\ in\ 1g\ of\ test\ sample}{Milligram\ of\ amino\ acid\ in\ reference\ pattern} x\ 100$$

The lowest amino acid score was used as the chemical score for the amino acid and also predicted the first-limiting amino acid. The reference patterns used were those established by FAO (FAO/WHO.2007)

Total antioxidant potential- Total antioxidant potential of all the selected leaves was done using Reducing Power Assay method (Yen and Duh, 1994)

RESULTS & DISCUSSION

The amino acid profile of the selected edible ethnic plants is given in Tables 1a and 1b. Among all the ten species investigated for their amino acid profile, the plant *Sauropus androgynus* emerged as the most potential one with higher concentration of all the essential amino acids. The highest concentration was shown by cystiene (740.74 mg/g), followed by asparagine (714.28 mg/g), arginine (380.95 mg/g) *etc*. These values are sufficient to match the recommended dietary allowances. *Cassia occidentalis* proved to be the next candidate with potential profile of essential amino acids. The highest concentration was given by cysteine (509.25 mg/g), followed by asparagine (491.07 mg/g), arginine (261.9 mg/g) *etc*. *Amaranthus* and *Brassica* were at the next position with an appreciable amount of essential amino acids. It is surprising to note that *Centella* showed the least concentration of amino acids when compared to the rest of the plants. *Vigna unguiculata*, *Moringa*, *Colocasia* and *Amorphophallus* showed almost similar profile of amino acids.

In *Centella* and *Vigna unguiculata* the amino acid profile was noticed to be very poor when compared with other selected plants. *Moringa oleifera* and *Colocasia esculenta* at almost the same level with regard to the levels of essential, none essential, aromatic and sulphur contatining amino acids. Leaf samples of *Brassica oleracea* and *Amorphophallus paeonifolius* showed to be moderate candidates with respect to the amino acid profiling. The result indicated that non essential amino acids (histidine, alanine, arginine, aspartic acid, glutamic acid, glycine proline and serine) are higher in concentration compared to essential amino acids (isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine and valine), which constitute 41.1% of the total amino acids analyzed. Similar observations have been reported (Aremu, and Akintayo, 2006, Hassan and Umar, 2006). It is observed that lysine and methionine were the most abundant nonessential amino acids while cysteine and asparagine were the most abundant essential amino acids in all samples. Similar observations had been made in other plants (Adeyeye and Afolabi, 2004)

TABLE 1a: Amino acid profile of the leaves

Amino Acids (mg/g)	*Amaranthus viridis*	*Amaranthus dubius*	*Sauropus androgynus*	*Centella asiatica*	*Vigna unguiculata*
Arginine	190.47	235.71	380.95	4.761	78.571
Histidine	53.908	66.711	107.81	1.347	22.237
Isoleucine	71.428	88.392	142.85	1.785	29.464
Leucine	11.11	137.49	222.22	2.777	45.833
Lysine	118.34	146.44	236.68	2.958	48.816
Methionine	96.385	119.27	192.77	2.409	39.759
Phenylalanine	44.692	55.307	89.38	1.117	18.435
Threonine	95.011	117.57	190.02	2.375	39.192
Tryptophan	48.780	60.365	97.56	1.219	20.121
Valine	116.95	144.73	233.91	2.923	48.245
Proline	90.090	111.48	180.18	2.25	37.162
Serine	76.775	95.009	153.55	1.919	31.669
Tyrosine	102.82	127.24	205.65	2.57	42.416
Alanine	125	154.68	250	3.125	51.562
Aspartate	51.216	63.380	102.43	1.28	21.126
Asparagine	357.142	441.96	714.28	8.92	147.32
Cysteine	370.37	458.33	740.74	9.25	152.77
Glutamine	45.045	55.74	90.09	1.12	18.581
Glycine	186.04	230.23	372.09	4.651	76.744

TABLE 1b: Amino acid profile of the leaves

Amino Acids (mg/g)	*Moringa oleifera*	*Colocasia esculenta*	*Brassica oleracea*	*Cassia occidentalis*	*Amorphophallus paeonifolius*
Arginine	121.42	57.14	238.09	261.9	110.71
Histidine	34.36	16.172	67.38	74.12	31.33
Isoleucine	45.53	21.42	89.285	98.21	41.517
Leucine	70.83	33.33	138.88	152.77	64.583
Lysine	75.44	35.50	147.92	162.72	68.78
Methionine	61.44	28.91	120.48	132.53	56.02
Phenylalanine	28.49	13.40	55.865	61.45	25.97
Threonine	60.57	28.50	118.76	130.64	55.22
Tryptophan	31.09	14.63	60.975	67.07	28.35
Valine	74.56	35.08	146.19	160.81	67.98
Proline	0.057	27.02	112.61	123.87	52.36
Serine	48.94	23.03	95.969	105.56	44.62
Tyrosine	65.55	30.84	128.53	141.38	59.76
Alanine	79.68	37.5	156.25	171.87	72.65
Aspartate	32.65	15.36	64.02	70.42	29.76
Asparagine	227.67	107.14	446.42	491.07	207.5
Cysteine	236.11	111.11	462.96	509.25	215.2
Glutamine	28.71	13.51	56.306	61.93	26.18
Glycine	118.604	55.81	232.5	255.81	108.13

The amino acid, cysteine is one of the best free radical destroyers, and works best when taken with selenium and vitamin E. Cysteine is also precursor to glutathione, a substance that detoxifies the liver by binding with potentially harmful substances there. It helps to protect the liver and brain from damage due to alcohol, drugs, and toxic compounds in cigarette smoke. Asparagine is needed to maintain balance in the central nervous system; it prevents us from being either overly nervous or overly calm. It promotes the process by which one amino acid is transformed into another in the liver. This amino acid is found mostly in our selected samples. All the samples were also analysed for their chemical score to find out the limiting amino acid (Tables 2a and b). From *Sauropus androgynus,* highest chemical score was demonstrated in cysteine and the lowest score was recorded in phenyl alanine. From *A.viridis,* highest chemical score was demonstrated in cysteine and the lowest score was recorded in phenyl alanine. In almost all samples, the result was same.

TABLE 2a: Chemical Score of the amino acids

Amino Acids (mg/g)	*Amaranthus viridis*	*Amaranthus dubius*	*Sauropus androgynus*	*Centella asiatica*	*Vigna unguiculata*
Arginine	90700	112242.8	181404.7	2267.1	37414
Histidine	7264.1	8990.5	14529.6	181.53	3969.6
Isoleucine	12753.5	15783.9	25508.9	318.7	5260.7
Leucine	30863.8	38191.6	61727.7	771.38	12730.5
Lysine	35000	43325.4	70023.6	875.14	14440.8
Methionine	23224.09	28739.7	46450.6	580.48	9578.3
Phenylalanine	4993.2	6178.7	9986.5	124.80	2059.2
Threonine	22567.6	27926.3	45135.3	564.13	9308.7
Tryptophan	5948.7	7360.9	11897.56	148.65	2453.6
Valine	34195.9	42318.7	68394.7	854.67	14105.2
Proline	20290.5	25108.1	40581.08	506.75	8369.3
Serine	14735.1	18235.8	29472.1	368.33	6076.77
Tyrosine	26426.7	32709.5	52866.3	660.6	10902.3
Alanine	39062.5	48337.5	78125	976.5	16112.5
Aspartate	6556.9	8115.23	13115.2	163.89	2704.2
Asparagine	318875	394607.1	637750	7964.2	131517.8
Cysteine	342935.1	424379.6	685870.3	8564.8	141388.88
Glutamine	5072.07	6277.02	10145.2	126.12	20923
Glycine	86530.2	107083.7	173065.1	2163.2	35693

TABLE 2b: Chemical Score of the amino acids

Amino Acids (mg/g)	*Moringa oleifera*	*Colocasia esculenta*	*Brassica oleracea*	*Cassia occidentalis*	*Amorphophallus paeonifolius*
Arginine	57819.04	27209.5	113376.1	124714.2	52719.04
Histidine	4630.7	2179.2	134.77	9989.2	4222.37
Isoleucine	8130.3	3825	15942.8	17537.5	7413.37
Leucine	19675	9258.3	38577.7	42416.6	17938.8
Lysine	22319.5	10502.9	43763.3	48136.09	20349.1
Methionine	14804.8	6966.2	29031.3	31927.7	13498.7
Phenylalanine	3183.2	1497.2	6241.3	6865.9	2901.6
Threonine	14387.1	6769.5	28209.02	31021.3	13116.3
Tryptophan	3791.4	1784.1	552846.6	8179.2	3457.3
Valine	21801.1	10257.3	42745.6	47020.4	19877.1
Proline	1283	6085.5	25362.6	27882.8	11792.7
Serine	9393.4	4420.3	18418.4	2024905	8564.2
Tyrosine	16850	7928.02	33041.1	36344.4	15362.4
Alanine	24900	11718.7	48828.1	53709.3	22703.1
Aspartate	4180.5	1966.7	8197.1	9016.6	3810.4
Asparagine	203276.7	95625	398589.2	438455.3	185267.8
Cysteine	218620.3	102879.6	428666.6	471527.7	199259.2
Glutamine	3233.10	1521.3	6340.09	6974.09	2948.1
Glycine	55162.7	25958.1	108139.5	118981.3	50293.2

From the amino acid profiling of *Sauropus androgynus* leaf, it was observed that its nutritive value is superior to other commonly consumed leafy vegetables in India. *Sauropus androgynus* leaf was previously reported to contain considerable amounts of the alkaloid papavarine. Excessive consumption of the leaf reportedly caused dizziness, drowsiness, constipation etc. Before it could be recommended for wide and frequent use, further work on the subject is necessary to set safe levels for its consumption. In the present study, we have evaluated the free radical scavenger activity of ten selected wild plants (Table 3). Among the ten extracts tested for the in vitro antioxidant activity using reducing power assay, *Cassia occidentalis* and *Amaranthus viridis* showed the highest activity i.e. 5% followed by *Moringa oleifera (*4.666%) *Colocasia* (2.333 %) and *Sauropus androgynus* (1.666%).

Vigna unguiculata showed the least antioxidant activity (0.1%) when compared to other plant extracts. The leaves of *Centella*, *Amaranthus dubius* and *Colocasia* also showed an appreciable level of antioxidants.

TABLE 3: Total antioxidant potential of leaves

Plants	Total antioxidant potential (%)
Amaranthus viridis	5
Amaranthus dubius	1.2
Sauropus androgynus	1.666
Centella asiatica	1
Vigna unguiculata	0.1
Moringa oleifera	4.666
Colocasia esculenta	2.333
Brassica oleracea	0.3666
Cassia occidentalis	5
Amorphophallus paeonifolius	1.466

Every 100g of the leaf sample of *Sauropus androgynus* contains 1513g of essential amino acids, 3087.53 g of non essential amino acids 1277.0 g of sulphur-containing amino acids (Cysteine and Methionine) and 392.59 g of total aromatic amino acids (Phenylalanine, Tryptophan, Histidine and Tyrosine) (Table 4).Similarly, in *Amaranthus viridis,* 756.604mg of essential amino acid concentration was observed whereas the amount of non essential amino acids was 1543.752 mg. An appreciable amount of Cysteine and Methionine and total aromatic amino acids were also noticed. In case of *Amaranthus dubius,* the data was quite surprising. Even though it is proved to be a widely accepted vegetable with high protein value, it contained only 936.275 mg of essential amino acids, 1910.379 mg of non essential amino acids, 790.179 mg of sulphur-containing amino acids and 242.912 mg of total aromatic amino acids.

TABLE 4: Total amino acid profile of the leaves

Plants	Essential Aminoacids (mg/g)	Non essential Aminoacids (mg/g)	Hydroxyl or sulfur/ selenium-containing (mg/g)	Aromatic aminoacids (mg/g)
Amaranthus viridis	756.604	1543.752	638.541	196.292
Amaranthus dubius	936.275	1513.2	790.179	242.912
Sauropus androgynus	1513.2	3087.533	1277.08	392.59
Centella asiatica	18.91	312.102	15.953	4.906
Vigna unguiculata	482.31	226.942	263.39	80.972
Moringa oleifera	945.735	926.741	407.06	125.13
Colocasia esculenta	226.942	463.1	191.55	58.87
Brassica oleracea	945.735	1929.635	798.169	245.37
Cassia occidentalis	1040.32	2122.64	877.98	269.9
Amorphophallus paeonifolius	439.75	897.11	371.06	114.08

Understanding the importance of antioxidants and amino acids is critical, because a failure to eat foods that contain these essential amino acids and antioxidants can lead to deficiency and adverse health effects. These effects can include but are not limited to fatigue, allergies, loss of memory, and even heart disease. When one considers the pain and suffering caused by any of these four ill health effects, and the myriad of subsequent ailments that they can provoke, it becomes readily apparent that a knowledge of amino acids, and especially "essential" amino acids, must be a part of our daily diet.

Plants under the current investigation can be considered as good sources of natural antioxidants as their extracts were found to possess high antioxidant activity. Further studies needed to identify the active constituents present in these plant extracts. Their antioxidant potential and amino acid profile further lend credence to the biological value of these plants. Thus, it can be concluded that these leaves can contribute significantly to the nutrient requirements of man and should be used as a source of nutrients to supplement other major sources.

CONCLUSION & FUTURE OUTLOOK

Proteins are particularly important substances of human cells and play an important role in human nutrition. The amino acid content, their proportions and digestibility by humans characterize protein's biological value. Proteins consist of 20 amino acids, but the most important are essential amino acids which the human body needs to take from food. The amino acid composition of food proteins provides important information and perspective about their nutritive value. Alternative sources of feeds offering high-quality nutrients, especially proteins, limiting amino acids but also energy, could be found in unconventional plants. The relatively high content of essential amino acids in *Sauropus androgynus* predetermines its use as a substitution of conventional cereals. The most of all essential amino acid profile of all samples of the species compared favorably with FAO/WHO requirements.

Results showed that, these plants possess high antioxidant properties in general and hence it can be recommended to be included in our daily diet. Further studies are essential in order to use these plants as a source of production of natural antioxidants. This work will also be a prelude to replenish our ancestral knowledge about these underutilized vegetables that will be helpful for the betterment of upcoming generations.

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