

**COMPREHENSIVE STUDY OF *EICHHORNIA CRASSIPES* FOR AMINO ACID
PROFILING AND NUTRITIONAL ASSESSMENT**

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ABSTRACT

Eichhornia crassipes (water hyacinth) listed as one of the most productive plants on earth and is considered as the world's worst aquatic weed. It is a severe environmental and economic problem in all of the gulf coast states and in many other areas of the world with a subtropical or tropical climate. In spite of these, as per our present study we can say "the world's worst aquatic plant as a best dietary supplement and therapeutic pool". Understanding the amino acids as basic structural and functional units of proteins, and have immense importance in nutrition, taxonomy and pharmacology; screening of amino acids from different extracts of *Eichhornia crassipes* was done in our research work. Assessment of chromatograms developed in thin layer chromatography (TLC) showed a total of eleven amino acids in water hyacinth (shoot and rhizome). Phytochemical screening revealed the presence of seven essential amino acids and four non-essential amino acids. The detected amino acids from plant were serine, valine, leucine, methionine, aspartic acid, glutamic acid, lysine, tyrosine, tryptophan, histidine and isoleucine respectively. Present work was also designed to evaluate the nutritional potential of *Eichhornia crassipes* collected from the water bodies in Bilaspur. Results showed that plant was characterized by high fibre, high protein content, low lipids and low carbohydrates. In addition the anti-nutritive substance (nitrate) level was also determined and found to be safe as feed. The vital study of amino acid spectrum is of great importance on its strategic food and medicinal significance.

KEYWORDS: Amino acid; *Eichhornia crassipes*; Minerals; Thin Layer Chromatography; Water hyacinth.

INTRODUCTION

Aquatic plants grow profusely in lakes and waterways all over the world and have in recent decades their negative effects magnified by man's intensive use of natural water bodies. Eradication of the weeds has proved almost impossible and even reasonable control is difficult. Turning these weeds to productive use would be desirable if it would partly offset the costs involved in mechanical removal. Among other uses, there has been considerable interest in using aquatic plants as a source of feed. Since aquatic weeds are known to differ widely in their chemical composition depending upon species, season and location,^[1] an insight into their chemical composition is essential if utilization prospects are to be considered.

Eichhornia crassipes (Mart.) Solms-Laubach commonly known as water hyacinth belonging to the family Pontederaceae is listed as one of the most productive plants on the earth and is considered the world's worst aquatic weed.^[2,3,4,5] The water hyacinth which clogs lakes and rivers has attributes which warrant consideration of the plant as a possible food source.

Yount^[6] reported that water hyacinth produce over 534 pounds per acre per day dry weight, which is one of the greatest yields of organic matter ever reported, including even intensive agricultural and sewage algae production.

Water hyacinth is warm water aquatic plant which widespread in many countries, particularly during summer months with its highest growth in July. This plant has been reported to be palatable to fish^[7,8], animal forage^[9,10], to sheep^[11], pigs^[12] and humans.^[13] However, due to its high water content the crude product has a low nutrient concentration. The dry matter of the plant may be of nutritional value as a supplemental source of amino acids since the biological value of leaf protein has been reported to be about equal to that of milk.^[14] Recently considerable attention has been given to harvesting the aquatic plant for practical uses to partially defray the cost of removing plants from waterway and use as economical source of nutrients in many parts of the world.

Amino Acids are the "building Blocks" of the body. There are about 28 amino acids commonly referred to in

human health. They are required by the body as it acts as a precursor. Eight amino acids are essential for humans as phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine and lysine.^[15] Additionally, cysteine, tyrosine, histidine and arginine are required by infants and growing children.^[15,16] Essential amino acids are not synthesized by the body. In addition, the amino acids arginine, cysteine, glycine, glutamine, histidine, proline, serine and tyrosine are considered conditionally essential, meaning they are not normally required in the diet, but must be supplied exogenously as are useful components in a variety of metabolisms. It is important to be aware that amino acids are part of complex pathways and biological systems. Deficiency of amino acid cause weak immune system, loss of antibody production, fatigue, stomach acid/alkaline imbalance, dizziness/nausea, water retention.^[15,16] Considering the fact, investigation of amino acids from *Eichhornia crassipes* has been carried out. In this paper the shoot and rhizome extracts of water hyacinth were screened and assessed for their amino acid spectrum and nutrient values.

The purpose of this study was to supplement existing knowledge on *Eichhornia crassipes* by evaluating the nutritional value and phytochemical screening of amino acids on active bio-substances of the aquatic plant to examine its suitability as a feed source and therapeutic importance. Examining the amino acid content of water hyacinth is to determine whether its protein could effectively supplement the nutritional deficiencies of grains, because determination of amino acid can only serve as a guide to the nutritional value of water hyacinth protein. The plant was also tested for the presence of any anti-nutritive factors, the presence of which could limit utilization prospects. Many plants contain a wide variety of natural chemical compounds often referred to as anti-nutritive substances or toxic factors which are capable of inducing toxic effects in animals consuming these plants.^[17]

MATERIALS AND METHODS

Water hyacinth (*Eichhornia crassipes*) plants were collected from the water bodies in Bilaspur district of Chhattisgarh (21°37' -25°07' N latitude and 81°12' -83°40' E longitude), India. The general climate of the area is wet and humid, experiences hot summer and moderately cold winters, interspersed with rainy months. The annual potential evapo-transpiration is slightly higher than the annual rainfall. The monsoons are heavy between July and September in this region.

Phytochemical Analysis

Fresh shoots and rhizomes of water hyacinth were washed several times under running tap water followed by surface sterilization by Mercuric Chloride (0.01%). The shoot and rhizome parts were separated and shade dried followed by oven-drying at 50°C for 24 hours. These plant materials were grinded to powder and used for further analysis. Crude fibre, crude fat and ash were

estimated using standard method of AOAC.^[18] Total nitrogen was estimated by Kjeldahl method. Carbohydrate contents were assayed by the phenol-sulphuric acid method.^[19] Crude protein was calculated as percent N times 6.25. Among the anti-nutritive factors, nitrates were estimated using Humphries^[20] method. All the data were subjected to statistical analysis. Observations are presented in tabular form representing the Mean \pm SD.

Qualitative screening of amino acids by TLC study

Thin layer chromatography (TLC) technique of crude extracts was applied for the detection of amino acid using the standard method of Harborne.^[21] Gently washed fresh plant material collected was used for the analysis. The shoot and rhizome was separated. Each fresh sample material grinded in sterile pestle and mortar with ethanol and petroleum ether separately. The contents were centrifuged at 10,000 rpm for 15 min. and then supernatants collected. The extracts of shoot and rhizome were also prepared with boiled ethanol. The final extracts obtained (shoot extract extracted in ethanol (E₁), rhizome extract extracted in ethanol (E₂), shoot extract extracted with boiled ethanol (E₃), rhizome extract extracted with boiled ethanol (E₄), shoot extract in petroleum ether (E₅), rhizome extract in petroleum ether (E₆) were spotted on the TLC plate (plate of silica gel G) and the chromatograms developed using different developing systems-

- BAW₁ (n-Butanol: Acetic acid: Water, taken in 4:1:1 proportion)
- BAW₂ (n-Butanol: Acetic acid: Water, taken in 4:1:5 proportion)
- Phenol (Phenol: water, in the ratio of 3:1)

Later, the spots for amino acids were detected by using standard reagent ninhydrin (triketohydrindene hydrate) and identified by their colour and R_f (x100) values.

RESULTS

Phytochemical composition of water hyacinth was shown in Table 1. The dry matter, ash content, total available nitrogen for dry tissue of water hyacinth and protein contents on dry weight basis were recorded in percentage as 8.6, 25.637, 5021 and 32.56 (N x 6.25) respectively. *Eichhornia crassipes* contained 54.3(%) crude fibre, (1%) fat content and 3.2(%) of total available carbohydrate. The anti-nutritive substance i.e. nitrates studied in this investigation was recorded as 0.5(%)

Table 1: Nutritional Composition of *Eichhornia crassipes*

S. No.	Biochemical parameters	Observed Values (% of dry weight)
1.	Dry matter	8.6 ± 2.621
2.	Ash	25.637 ± 1.586
3.	Total available Nitrogen	5.21 ± 0.594
4.	Crude protein	32.56 ± 1.222
5.	Crude fibre	54.3 ± 1.521
6.	Crude lipid	1.00 ± 0.598
7.	Total available Carbohydrates	3.2 ± 0.28
8.	Nitrate (anti-nutritive factor)	0.5 + 0.08

Mean ± SD (n = 6).

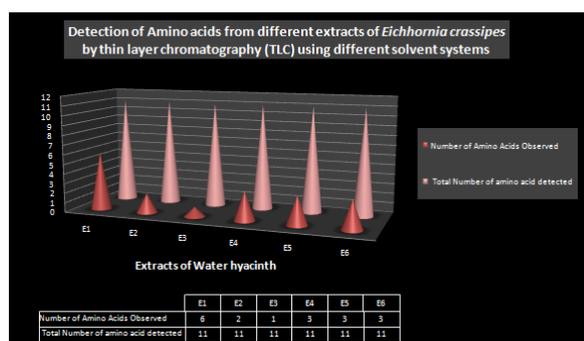
The results shown in Table 2, 3 and 4 revealed the presence of different amino acids in different parts of water hyacinth plant. A total of 11 amino acids were detected namely valine, leucine, methionine, lysine,

tyrosine, tryptophan, isoleucine, serine, aspartic acid, glutamic acid and histidine. The comparative TLC profiles of the test extracts for the number of amino acids present shown in Fig. 1.

Table 2: Detection Of Amino Acids From Different Parts Of *Eichhornia crassipes* By Thin Layer Chromatography (TLC) Using Different Solvent Systems

Extract type	Solvent system: n-Butanol: Acetic acid: Water (BAW, 4:1:1)			
	Rf(X100) Observed values	Rf(X100) Standard values	Colour of the Spot (with or without Ninhydrin)	Amino acid detected
E ₁	33	32	Violet	Valine
	40.8	41	Grey	Tyrosine
	47.8	47	Violet	Tryptophan
E ₂	44.7	44	Violet	Leucine
E ₄	47.4	47	Violet	Tryptophan
Solvent system: n-Butanol : Acetic acid : Water (BAW, 4:1:5)				
E ₁	47.4	47	Grey	Tryptophan
	72.9	73	Violet	Leucine
E ₃	47.3	47	Grey	Tryptophan
E ₄	53	53	Violet	Methionine
E ₅	31.9	32	Violet	Valine
Solvent system: Phenol (phenol-water, 3:1)				
E ₁	9.5	09	Violet	Lysine
E ₂	5.8	06	Violet	Aspartic acid
E ₄	8.8	09	Violet	Lysine
E ₅	20	20	Violet	Serine
	32.3	32	Grey	Histidine
E ₆	10.2	10	Violet	Glutamic acid
	49.2	49	Violet	Methionine
	43.3	43	Violet	Isoleucine

Key: E₁ – Shoot extract in ethanol; E₂ – Rhizome extract in ethanol; E₃ – Shoot extract in boiled ethanol; E₄ – Rhizome extract in boiled ethanol; E₅ – shoot extract in petroleum ether; E₆ – Rhizome extract in petroleum ether.

**Fig. 1: Graph showing the number of amino acids detected from each extract of *Eichhornia crassipes***

Distribution of each amino acid in various test extracts on evaluating with three different solvent systems by TLC is depicted in Fig.2.

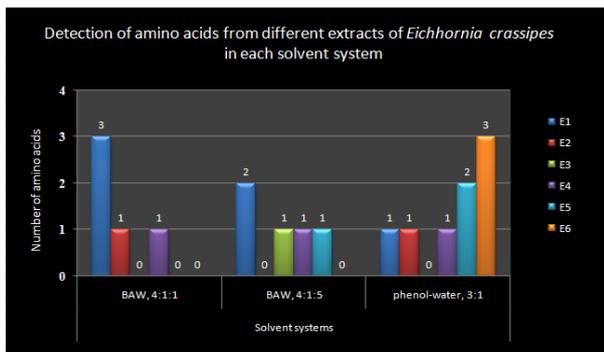


Fig. 2: Graph showing the number of amino acids detected from each solvent system used.

The perusal of Table 3 shows the distribution of amino acids in the shoot and rhizome of *Eichhornia crassipes*. Shoot part consisted of serine, valine, tyrosine and histidine whereas methionine, aspartic acid, glutamic acid and isoleucine were detected from rhizome part. Hence, it's noteworthy that the amino acid profile of water hyacinth is dominated by leucine, lysine and tryptophan since they were found in both shoot and rhizome of the plant as shown in Fig. 3.

Table 3: Distribution of Amino Acids In The Shoot And Rhizome Of *Eichhornia crassipes*

S.No.	Amino acids	Shoot	Rhizome
1.	Serine	+	-
2.	Valine	+	-
3.	Leucine	+	+
4.	Methionine	-	+
5.	Aspartic acid	-	+
6.	Glutamic acid	-	+
7.	Lysine	+	+
8.	Tyrosine	+	-
9.	Tryptophan	+	+
10.	Histidine	+	-
11.	Isoleucine	-	+

(+) is Presence of Amino acid; (-) is Absence of Amino acid.

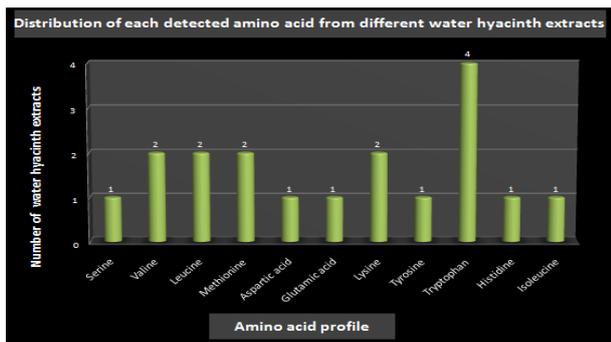


Fig. 3: Graph showing the presence of each amino acid in number of *Eichhornia crassipes* extracts

Essential and non-essential amino acids detected from water hyacinth were categorized in Table 4 and Fig. 4. Among the 11 amino acids detected in TLC analysis, 7 are essential amino acids and 4 are non-essential amino acids (Table 4).

Table 4: List of Essential and Non-essential Amino Acids Detected From *Eichhornia crassipes*

Essential amino acids	Non-essential amino acids
Valine	Serine
Leucine	Aspartic acid
Methionine	Glutamic acid
Lysine	Histidine
Tyrosine	
Tryptophan	
Isoleucine	

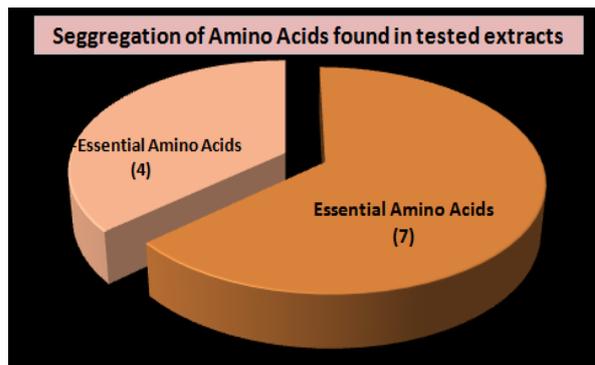


Fig. 4: Graph showing distribution of essential and non-essential amino acid in test material (extracts of *Eichhornia crassipes*)

DISCUSSION

In the present study dry matter, ash, total available nitrogen, crude protein, crude fibre, crude fat and total available carbohydrates and nitrates (as anti-nutrient factor) were considered when analyzing the selected plant *Eichhornia crassipes* as potential feed. The value of dry matter obtained in this investigation was found to be in consistent with the study by Anjana and Matai.^[9] The recorded ash value of the plant is little higher than the values reported in literature.^[8,9] Excessive concentration of ash decreases the amount of organic constituents per unit weight and lowers food value.^[22] The level of total available nitrogen and crude fibre studied is also higher than the values reported earlier.^[8,9]

Most of the floating and emergent types had more fibre content than the submersed plants. This was probably because they require more strength to support aerial vegetation.^[9] Due to the presence of high value of fibre content, water hyacinth plants could be used as potential substitutes for hay and other roughages eaten by ruminants. At the same time the studied macrophyte was characterized by high protein content in the present study which is again a higher value than the values reported by Anjana and Matai^[9,23,24], whereas the fat content recorded is lower than the earlier reports.^[25] Our findings are in agreement with Banerjee^[26] who reported that comparing the composition of aquatic plants with those of conventionally used livestock feed, aquatics on an average contained larger amounts of crude protein.

Coming in agreement with our study the finding of EL-Serafy *et al.*^[27] who reported that the extraction of water hyacinth showed satisfactory results for both extracted protein and fibrous residues these processes may eliminate some anti-nutrients, such as tannins, nitrates and oxalates. Nitrates are known to accumulate in forage plants and in some weeds at level high enough to be toxic to animals.^[28] A nitrate content of less than 1.5% has been considered to be safe for use as animal feed^[17] and the value obtained in this study lies absolutely within safe range.

Amino acids play central roles both as building block of proteins and as intermediates in metabolism and their presence was detected by using Thin Layer Chromatographic technique. Amino acids are the basic structural and functional units of proteins, thus amino acids have immense importance in nutrition, taxonomy, pharmacology. The study revealed the presence of maximum number of amino acids essential in the diet of humans.

Except cystine, phenylalanine and threonine, other essential amino acids were detected. Presence of methionine, valine, glutamic acid, threonine, tryptophan, tyrosine, leucine and lysine in water hyacinth has been reported in literature.^[23] A point of interest is whether the amino acid of water hyacinth could serve as a supplemental source to improve the nutritional quality of grain proteins. Lysine and tryptophan are the first two limiting amino acids in grains; therefore interest centers on the two amino acids in water hyacinth since both were detected from the test plants in this study. On comparing to the FAO reference pattern^[29] it has been reported that the protein content of *Eichhornia crassipes* shows deficient levels in only two of the essential amino acids i.e. valine and methionine. The protein of water hyacinth may be developed into useful dietary supplements for grain diets, especially in the underdeveloped countries. If a number of uses are found for the other constituents of water hyacinth so as to make processing economically feasible, the protein could be extracted. Such a protein concentrate could be used as an effective supplement to grains for animal and human consumption.

Amino acids are precursors for the synthesis of secondary metabolites such as alkaloids, which provide chemical defense for plants that confer beneficial physiological effects in consumers. Alkaloids provide protection to plants from a variety of herbivores and some of them possess significant pharmacological activity such as analgesic, antibacterial and antibiotic.^[30]

Valine maintains the balance of branched chain amino acids.^[31] The results in this study show its presence in shoot part of the test plant.

Leucine provides ingredients for manufacturing of other essential biochemical components in the body, some of which are utilized for the production of energy; stimulants to the upper brain and helping the body to be more alert.^[32] Leucine regulates the protein turnover (mTOR signaling) and gene expression.^[31,33,34] The present study reveals the presence of Leucine an essential amino acid in E₁ extracted with BAW (4:1:1) and E₂ extracted with BAW (4:1:5).

Methionine is a sulfur-containing essential amino acid that plays a crucial role in metabolism. As an essential amino acid, methionine cannot be synthesized in the body and must be obtained from foods. It is an antioxidant, it helps in breakdown of fats and aids in reducing muscle degeneration.^[16,35] It is also good for healthy skin and nail. Methionine also plays an important role in proper functioning of the immune system. Presence of methionine in water hyacinth makes them more efficient in having therapeutic value.

Lysine helps to maintain intestinal integrity and health.^[36,37]

Although it has the lowest concentration in the human body relative to the other 19 primary amino acids, tryptophan is a critical component of numerous metabolic functions. As the sole precursor of serotonin, tryptophan's role in brain serotonin synthesis is an important factor involved in mood, behavior and cognition.^[38]

Isoleucine is beneficial for skin, bone and tissue wound healing. This essential amino acid which has been detected in our research from E₆ extracted with solvent system phenol-water (3:1), promotes growth hormone synthesis that is essential for healthy nervous system function.

Aspartic acid function is essential for purine, pyrimidine, asparagine and inositol synthesis.^[31] This amino acid is related to detoxification and excretion of ammonia.^[31,36] Recent studies have shown that it may increase resistance to fatigue and increase endurance.^[35] In our study Aspartic acid was detected in E₂ extract of test plant.

Glutamic acid has a wide range of applications in food industry, in pharmacology and cosmetics.^[39] Glutamic acid participates in the synthesis of glutathione increasing the antioxidant capacity of the plant.^[31] The result in our study reveals the presence of glutamic acid in E₆ extract. Hence *Eichhornia crassipes* can be a promising substrate in nutraceutical industries, being good source of glutamic acid.

As mentioned before *Eichhornia crassipes*, could easily be available and used in pharmaceuticals and food products. Therefore, the potency of these extracts could provide a chemical basis for some of the health benefits claimed for water hyacinth in medicine and an effective supplement to grains for animal and human consumption. Specific metabolic processes in which these amino acids participate may be related to the therapeutic properties of plants as per their use in medicine and therefore may facilitate the understanding of their beneficial properties. However, further studies are needed to determine and isolate compounds responsible for the specific therapeutic properties of the plants.

CONCLUSION

On the basis of an overall assessment of biochemical profiling, nutrient composition and phytochemical analysis, *Eichhornia crassipes* was found to contain sufficient quantities of nutrients and number of bioactive substance amino acid and were also safe enough to be considered as potential livestock feed. The study further reveals that the consumption of water hyacinth would exert several beneficial effects by virtue of their antioxidant activity. Therefore, it is suggested that further studies aimed on other aspects of feed quality such as palatability, digestibility experiments; presence of any other anti-nutritive substances like phenolic contents and therapeutic agents to be performed. Owing to the acute shortage of fodder in the world, utilization of aquatic plants as feed is particularly attractive for the better productivity of livestock which comprise an integral part of the agriculture system. These results showed that *Eichhornia crassipes* is a rich source of nutrient contents which can serve as suitable economic feed and also possessing pharmaceutical ingredients. Because of the rich diversity of this aquatic plant it is expected that biochemical analysis and scientific evaluation of plant may prove beneficial for the mankind along with the management of the weed.

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