Chemical Investigation of Seeds of Wild Plant 'Citrullus colocynthis' of Chhattisgarh State for Exploring Medicinal and Nutritional Potential

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Citrullus colocynthis (Indrayan) is the plant which seems to grow well in subtropical climates and can stand brief frosts, is probably tolerant of some drought and has many medicinal properties. The proper scientific phytochemical investigation of seeds of Citrullus colocynthis was carried out for estimating and determining various Micronutrients contents, Fatty Acids and Amino Acids present in the seeds along with the proximate and ultimate analysis for determining various other concerned physicochemical properties of the seeds. More than 95% of organic matter contents in the seeds under examination indicated high nutritional quality of the seeds. Proteins were found to be 30.18% in the seeds of Citrullus colocynthis. The seeds under investigation were found to be rich in essential amino acids. All the essential amino acids, Leucin (15.0 g/ 100g protein), Phenyl alanine (5.1 g /100g protein), Lysine (4.0 g /100g protein), Isoleusine (7.7 g /100g protein), Threonine (2.2 g /100g protein), Methionine (1.9 g /100g protein), Valine (8.6 g /100g protein), Histidine (2.5 g /100g protein) were found to be present in the seeds of Citrullus colocynthis. Various non-essential amino acids viz. Aspartic Acid, Serine, Glutamic Acid, Glycine, Arginine, Alanine, Proline and Tyrosine were also found to be present in the seeds in varying amounts.

Key words: Phyto-chemistry, Plant Seeds, Amino-acid Profile.

Since beginning of mankind, wild plant and their derived-products like bark, leaves, roots, seeds etc. have been playing crucial role in traditional medicinal system for human/animal healthcare and/or for fulfilling human/animal nutritional requirement. With the time and progression, these requirements have been magnified manifold, particularly in developing countries, considering the exponential population growth and changing climatic conditions. These factors even continuously widened the scope for traditional medicinal system in health-care and in

fulfilment of nutritious food demands. A great number of natural medicinal products have come to us from the scientific study of remedies traditionally employed by various cultures; most of them being plant derived. Moreover, the Traditional Medicinal System has not only the potential to discover new treatments, but also includes and addresses socioeconomic, conservationist and cultural components¹. Exploring traditional medicinal uses may cut short the discovery of upcoming medicines² and even some such instances are well-marked in the history. Synthetic drugstore medicines, known to sometime have serious, and even lethal repercussions. For this reason too, the popularity of herbal and natural cures has grown, in being a reliable source of naturally nourishing alternative therapy with

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holistic result for the human mind and body. Traditional Medicinal System mostly employs plant-derived products (i.e. make use of biologically active constituents present in that plant product). Most medicinal plants are generally gathered from the wild areas. It is established fact that there is international relevancy of research and development in the field of traditional drug³ considering the rapidly growing demand for alternative and basic therapeutic means. In India, prescribed medicines of almost 95% of the prescriptions are plant based in the traditional system of Unani, Ayurveda, Homeopathy and Siddha⁴.

In general, time to time many phytochemical researchers selected some of local wild plants or their products and investigated scientifically their medicinal and nutritional properties⁵⁻⁷. The scientific phytochemical study of seeds requires the estimation of various Micronutrients, Fatty Acids and Amino Acids present in the seeds under examination along with the determination of various other properties of the seeds. This work presents the phytochemical investigation of the seeds of 'Citrullus colocynthis' (belongs to the family Cucurbitaceae) of Chhattisgarh state.

EXPERIMENTAL

The selected plant seeds were collected from the near-by village around Bilaspur, Chhattisgarh. Then the collected seeds were allowed to get dry gradually in shade. The Hard covers of the dried seeds were stamped out and then seeds were crushed. Then the standard methods⁸ were applied for ultimate and proximate analysis of the crushed seed in order to determine the physic-chemical properties. The various methods/instruments used are given in Table-1.

Proteins from the defatted seeds were extracted by the method reported by Joshi and Nigam¹³. The proteins thus isolated were converted to their respective hydrolysates by the method of Shrivastava *et al.*, ¹⁴. Protein hydrolysates thus obtained were subject to Thin layer and Paper chromatography for qualitative analysis of amino acid composition of the seeds under investigation. Then HPLC was carried out to ascertain amino acid profile of these seeds for quantitative analysis of

amino acid profile with AccQ-Fluor kit (Water Corporation, USA). The digested samples were derivatised with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) following manufacture's instructions. Five pico moles of the samples were loaded on to ACCQ-tag column and eluted with acetonitrile gradient (5-100%). The eluates were monitored with fluorescent detector. The amino acid peaks were compared with standard run under identical conditions. The results of the HPLC analysis were in conformance to qualitative analysis.

RESULTS AND DISCUSSION

Table-2 reports the proximate, ultimate composition and results of other analyses. The amino acid composition of the seeds of Citrullus colocynthis determined through HPCL technique is given in Table-2. An inspection of table-1 indicates that the moisture content in the seeds was 7.8% and the ash content in the same was 4.8%. The low percentage (well below than upper limit of 15%) of moisture content in these seeds indicates that the seeds of Citrullus colocynthis can be stored for longer durations¹⁰. Ash content is a reflector of overall mineral matter in the seed sample. For the seed under examination, total organic matter content was determined as 95.2 %. This higher value indicates that the seeds can be considered to be highly nutritious¹¹.

Total protein contents in the seeds of *Citrullus colocynthis* were recorded to be 30.175. This points out that the seeds under examination contained fairly good amount of proteins. A close examination of Table-3 indicates that 16 major amino acids were present in the seeds. Mainly two essential amino acids namely Leucine and Valine and two non-essential amino acids Arginine and Glutamic acids were present.

The essential amino acid Leucine is essential for growth, lowers blood sugar level and helps in wound healing of skin and bones. Its deficiency causes a biochemical malfunction producing hypoglycemia in infants¹⁵. Valine promotes mental vigor, calm emotions and muscle coordination¹⁶.

The non-essential amino acid, Arginine, is responsible for the improvement of immune responses and causes the release of growth

Table 1. Details of various methods/instruments employed in presented research work

S. No.	Parameters	Methods/Instruments
1	Moisture and ash contents [9, 10,11]	Crucibles, Oven, Muffle furnace,
2	Organic matter contents [9, 10,11]	100- (Ash percentage contents)
3	Carbon, Nitrogen, Hydrogen and Sulphur contents	CHNS analyzer named Elementor Vario EL
4	Calorific Value	Julius Peter Bomb Caloriemeter
5	Refractive Index [12]	Abbe's refractometer
6	Protein estimation	% of Protein = % of Nitrogen x 6.25
7	Fat estimation	Soxhlet apparatus with Whatman filter paper no. 1
8	Micronutrients contents	Atomic Absorption Spectrophotometer (AAS) model: 4141

Table 2. Proximate and Ultimate Composition of the seeds

S.No.	Parameter	Citrullus colocynthis
1.	Carbon (%)	67.53
2.	Hydrogen (%)	10.85
3.	Nitrogen (%)	4.828
4.	Sulphur (%)	0.579
5.	Ash (%)	4.8
6.	Moisture (%)	7.8
7.	Protein (%)	30.175
8.	Calorific Value (cal)	4570.02
9.	Total OrganicMatter (%	95.2
10.	Oil (%)	20.8

Table 3. Amino acid Composition of the seeds of *Citrullus colocynthis*

S. No.	Amino Acid	Amount in gm/100 gm Protein
1.	Lysine	4.0
2.	Phenylalanine	5.1
3.	Leucine	15.0
4.	Iso Leucine	7.7
5.	Methionine	1.9
6.	Valine	8.6
7.	Threonine	2.2
8.	Histidine	2.5
9.	Arginine	13.1
10.	Alanine	8.6
11.	Glycine	2.7
12.	Serine	1.9
13.	Aspartic Acid	3.4
14.	Proline	5.1
15.	Tyrosine	2.7
16.	Glutamic Acid	14.7

hormone. It is crucial for optimal muscle growth and tissue repair¹⁵. Glutamic acid helps in improving mental capacities. It also speeds the healing of ulcers and reduces fatigue. It is also important in controlling schizophrenia and craving for both alcohol and sugar¹⁷.

CONCLUSION

The amino acid profile of the seeds of *Citrullus colocynthis* clearly point out that they possess fairly good amount of proteins and the amino acid composition indicates that these seeds may serve as a possible source to meet out the amino acid requirements of humans as well animals provided that all the toxicants, if present, are first removed from seeds.

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