International Journal of
Medicinal Chemistry & Analysis
www.ijmca.com
e ISSN 2249 - 7587
Print ISSN 2249 - 7595

ISOLATION OF AMINO ACIDS FROM THE LEAVES OF *CICER ARIETINUM* BY LC-MASS SPECTROSCOPY

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ABSTRACT

The aim of the present study was to isolate and determine the amino acids from different extracts of *Cicer arietinum* leaves belongs to the family Fabaceae by LC-MS technique. It is an annual herb that is spread into Southern Europe, India, Egypt and Southern America. It is extensively cultivated in India mainly in Rajasthan, Hyderabad, Patiala, East Punjab, Haryana and Madhya Pradesh. In India it is very often used as a crash diet and it is one of the most widely made recipes in India kitchen due to its good taste and nutritive values. Traditionally it is used as antibacterial, antifungal, antipyretic, antidiarrhoeal etc. The presence of amino acids has been for the first time evaluated by a modern technique like LC-MS technique. The method presented above provides an important tool for the qualitative evaluation of important amino acids from *Cicer arietinum*.

Keywords: *Cicer arietinum*, Amino acids, LC-MS technique.

INTRODUCTION

Amino acids are molecules containing an amine group, a carboxylic acid group, and a side-chain that is specific to each amino acid [1]. The key elements of an amino acid are carbon, hydrogen, oxygen, and nitrogen. The first few amino acids were discovered in the early 19th century. In 1806, the French chemists Louis-Nicolas Vauquelin and Pierre Jean Robiquet isolated a compound in asparagus that was subsequently named asparagine, the first amino acid to be discovered [2]. Another amino acid that was discovered in the early 19th century was cystine, in 1810, although its monomer, cysteine, was discovered much later, in 1884. Glycine and leucine were also discovered around this time, in 1820. Amino acids are the structural units that make up proteins [3]. They join together to form short polymer chains called peptides or longer chains called either polypeptides or proteins. When taken up into the human body from the diet, the 22 standard amino acids either are used to synthesize proteins and other bio molecules or are oxidized to urea and carbon dioxide as a source of energy. The oxidation pathway starts with the removal of the amino group by a transaminase; the amino group is then fed into the urea cycle [4]. The other product of transamidation is a keto acid that enters the citric acid cycle. Glucogenic amino acids can also be converted into glucose, through gluconeogenesis [5]. In humans, non-protein amino acids also have important roles as metabolic intermediates, such as in the biosynthesis of the neurotransmitter gamma-aminobutyric acid. Many amino acids are used to synthesize other molecules, for example:

- Tryptophan is a precursor of the neurotransmitter serotonin.
- Tyrosine is a precursor of the neurotransmitter dopamine.
- Glycine is a precursor of porphyrins such as heme.
- Arginine is a precursor of nitric oxide.

Cicer arietinum were largely cultivated in most parts of India. Seed is aphrodisiac, anthelmintic, tonic, enriches the blood, cures skin diseases, inflammation; more especially of ear, diuretic [6], halitosis, hepatosis,

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otitis, pharyngosis, pulmonosis and splenosis [7], Ingredient of a Unani anti-hypertensive drug Ajmaloon [8] and ingredient for preparation of Nakhud. Acid exudation is astringent and useful in dyspepsia and constipation. Leaves are sour, astringent; improve taste and appetite, cure bronchitis, causes flatulence. Tart leaves are orexigenic, enterosis. Isoflavonoids isolated from *Cicer arietinum* shows anti-feedant activity. Biochanin-A and formononetin isolated from Cicer arietinum were evaluated for management of diabetes mellitus. Pangamic acid isolated from aqueous extract of *Cicer arietinum* has been evaluated for stamina building, antistress, antihyperlipidimic activity. The aqueous seed coat extract exhibited diuretic activity [9-11].

MATERIALS AND METHODS

Collection and extraction of plant materials:

Cicer arietinum leaves were collected from Kurnool district, India. Air shade dried leaves were used for the detection of amino acids. Extracts were prepared by crushing 0.5 g of the powdered material in a mortar and pestle with different solvents (2 mL each) such as acetone, ethanol and water. The extracts were then filtered and the filtrates were used for LC-MS injection for amino acid detection.

General Experimental Procedure:

For amino acid determination, LC-MS (Shimadzu) was used with UV detector.
The column used: YMC, C18, 50 x 4.6 mm.
Mobile phase used: A- 10mM Ammonium Formate in water + 0.1% Formic acid
B- Acetonitrile + 5% Solvent A + 0.1% Formic acid
Injected Volume: 5.0µL, Flow rate: 1.20 mL/minute
Gradient Programme: 5% B to 100% B in 3.5 minute, Hold till 0.50 min, At 4. 010 min B conc is 5% up to 5.0 min.

In the first part of the experiment, standard amino acids were injected to perform an experiment using LC-MS. The spectra were obtained for each amino acid with varying retention times. In second part of the experiment, the plant extracts were injected for LC-MS under similar conditions as above. The spectra were compared with the standard amino acid spectra and the presence of amino acids was evaluated. The amino acids were confirmed by their retention times and [M+1] + peaks.

RESULTS AND DISCUSSION

Amino acids contain a chiral centre, and so they could be used as chiral starting materials and chiral auxiliaries in the synthesis of compounds with high enantiomer excess. For amino acid determination, a number of methods have been reported based on various spectroscopic and chromatographic techniques. The literature reveals that, detection of amino acids could be done by HPLC, paper chromatographic techniques, spectrophotometry etc. In the present study, efforts have been made to establish the presence of amino acids in the leaves of *Cicer arietinum* using LC-MS technique (Table 1). Above work is a qualitative analysis of amino acids present in the leaves of *Cicer arietinum*. The amino acid study shows that *Cicer arietinum* is a source of some important amino acids. The amino acid study showed the presence of d-histidine, Glycine, Lycine, Taurine, Proline.

Fig 1. Structure of aminoacids

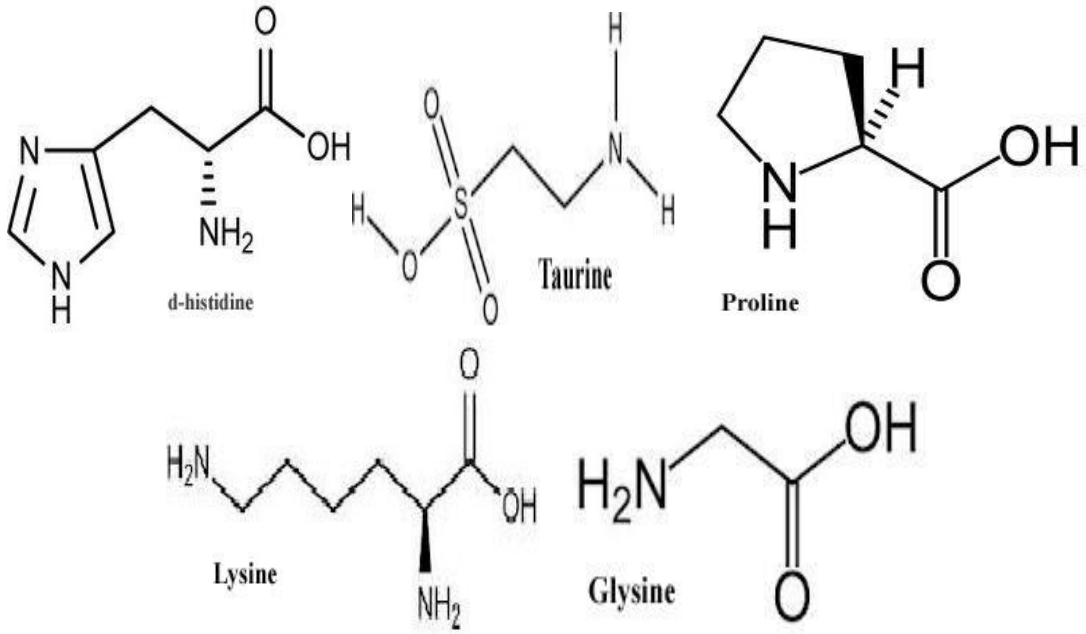

Table 1. LC-MS data for standard amino acids and leaves extracts (@ λ= 220 nm)

Name of amino acid	RT for standard amino acids	RT for plant extract			[M+1]+	[M+Na]+
		E1	**E2**	**E3**	--	--
d-histidine	0.653	--	0.613	0.621	132	128
Glycine	0.432	0.321	--	0.426	125	--
Lycine	0.642	0.591	0.621	0.634	152	--
Taurine	0.813	0.832	--	0.812	--	142
Proline	1.31	--	1.31	1.215	--	--

E1= Alcohol extract, E2= acetone extract, E3= water extract RT= Retention time in minutes

CONCLUSION

The presence of amino acids has been for the first time evaluated by a modern technique like LC-MS. The method presented above provides an important tool for the qualitative evaluation of important amino acids from *Cicer arietinum*. The method gives more accurate results than the conventional ones reported in the literature.

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