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Detection of amino acids by LC-Mass spectroscopy from the leaves of *Tabernaemontana divaricata*

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ABSTRACT

A small tree, Tabernaemontana divaricata has been mentioned in Ayurvedic medicinal systems mainly for the treatment of diarrhea and cancer. Detection of amino acids was carried out from various extracts of T. divaricata. The study of amino acids was carried out using LC-MS technique. The amino acids from the extracts were compared with the standard amino acids. Total three amino acids were detected in ethanol and acetone extracts. Majority of amino acids were detected in water extract.

Keywords *Tabernaemontana divaricata*, LC-MS, amino acids.

INTRODUCTION

Plants have been used as a major source of medicines.[1],[2] Different plants are being used in the treatment of different ailments.[3] The Indian ancient medicinal therapies like Ayurveda, list majority of the plants found in India. The detailed applications of these plants in herbal medicines have been mentioned as Ayurveda.[4],[5]

T. divaricata, a medicinal plant mentioned in Ayurveda, has been used to cure diseases, diarrhea and neuronal acetylcholinesterase are some of them.[6],[7]

T. divaricata is a small tree commonly found in the western ghats of Maharashtra, India. The literature survey revealed that its stem, bark and leaves are useful in treating various diseases. The plant is known in Ayurveda and has been used for the treatment of ailments such as diarrhea, cancer etc. All parts of *T. divaricata* are used as folk medicines. Whole plant including roots and leaves contains alkaloids which are found to be active against lymphocytic leukemia. Camptothecin has been isolated from the plant which is widely used against various forms of

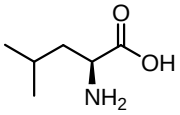
cancer[8]. The leaves of *T. divaricata* are especially used in the treatment of various diseases like cancer, diarrhea, syphilis etc.

Amino acids are building blocks of the proteins.[9] Proteins are an essential part of all the living animals and participate in virtually every process within the cell. Enzymes that catalyze many biological reactions are proteins and these are vital to metabolism. Amino acids are linked with each other through peptide linkage to form a long chain which results in proteins responsible for the growth and well functioning of cells. Identification of amino acid sequence in a protein is of utmost importance in synthesizing new drugs for treatment of diseases such as cancer, diabetes and many more related to genetic disorders. The study of amino acids is important as it reveals the nature of enzymes responsible for the biogenesis of various compounds in the plants. The study of amino acids present in the plant is important from the point of view that it will open up new research avenues.

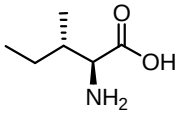
Cysteine has been used in chemothereupatic treatment for leukemia. It is also an important constituent of biologically active molecules like glutathione. Proline and its derivatives are often used as asymmetric catalysts in organic reactions. The CBS reduction and proline catalysed aldol condensation are prominent examples.

L-Proline is an osmoprotectant and therefore is used in many pharmaceutical and biotechnological applications.
Amino acids are major constituents of many drug molecules and also involved in various metabolic activities. It can be said with caution that the presence of cysteine in leaves of *T. divaricata* is responsible for its anticancer activity. Hence it is important to carry out a detailed amino acid study in order to justify the medicinal usage of the plant.

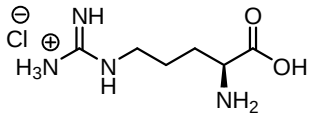
T. divaricata is a source of some biologically important amino acids. The amino acid study will open up new frontiers for research on the plant. The present study has increased the medicinal importance of the plant.



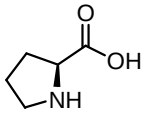
L-Leucine



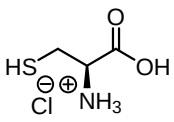
L- Isoleucine



L-Arginine monohydrochloride



L-Proline



Cysteine hydrochloride

Figure 1: Amino acids detected in various extracts.

MATERIALS AND METHODS

Procedure for material collection and extraction:
Plant material was collected from Western Ghats of Maharashtra, India. Air shade dried leaves of *T. divaricata* 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 detecter.

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

The presence of amino acids such as proline, 2-aminobutyric acid etc. in *T. divaricata* therefore opens up a vast scope for new research in this area. Amino acids contain a chiral center, and so they could be used as chiral starting materials and chiral auxiliaries in the synthesis of compounds with high enantiomeric 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,[10] paper chromatographic techniques,[11] spectrophotometry, etc.[12],[13] In the present study, efforts have been made to establish the presence of amino acids in the leaves of *T. divaricata* using LC-MS technique (Table 1).

Above work is a qualitative analysis of amino acids present in the leaves of *T. divaricata*. The amino acid study shows that *T. divaricata* is a source of some important amino acids. The amino

acid study showed the presence of 2-aminobutyric acid in ethanol and water extracts. Proline was detected in acetone and water extracts. As expected, cysteine hydrochloride, being a salt was detected only in water extract. Leucine and iso-leucine were detected in water extract. It was observed that majority of amino acids were detected in water extract.

From the above study, it is now confirmed that *T. divaricata* is a source of important amino acids like proline and cysteine. Amino acids are biologically important components of many drugs.

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			$[M+1]^+$	$[M+Na]^+$
		B_1	B_2	B_3		----
2-Amino butyric acid	0.630	0.630	----	0.660	104	----
Proline	0.62	----	0.65	0.657	116	----
Arginine hydrochloride	0.602	----	0.612	----	174	----
Iso-Leucine	1.183	----	----	1.2	132	----
Cysteine monohydrochloride	0.599	----	----	0.55		145
Leucine	0.975	----	----	1.02	132	

B_1= Alcohol extract, B_2= acetone extract, B_3= 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 *T. divaricata*. The method gives more accurate results than the conventional ones reported in the literature.

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