Research Article

High Performance Liquid Chromatography (HPLC) Technique for the Determination of Amino Acids in a Liverwort *Targionia hypophylla* L.

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ABSTRACT

In the present paper high performance liquid chromatography (HPLC) technique has been used for the first time in bryophytes to investigate various amino acids. HPLC method is found to be helpful in qualitative as well as quantitative determination of different amino acids present in liverwort *Targionia hypophylla*. Out of 20 studied amino acids, seven amino acids (methionine, phenylalanine, tryptophan, glutamine, cystine, asparagine and serine) have been detected in this species, of which three (methionine, phenylalanine and tryptophan) are essential, two (glutamine and cystine) are conditionally essential and two (asparagine and serine) are non essential. Among the seven amino acids, glutamine (0.163 mg g^{-1} dw) is found in highest concentration followed by serine (0.134 mg g^{-1} dw), cystine (0.125 mg g^{-1} dw), methionine (0.051 mg g^{-1} dw), phenylalanine (0.045 mg g^{-1} dw), asparagine (0.037 mg g^{-1} dw) and tryptophan (0.003 mg g^{-1} dw) in the lowest concentration.

Keywords: Amino acids, HPLC, qualitative, quantitative, *Targionia hypophylla*, liverwort.

INTRODUCTION

Bryophytes are known to be effective for the treatment of inflammation, arteriosclerosis, rheumatism, hyperlipemia, skin diseases, osteoporosis and bone resorption.[1] In North America, China and Europe, the bryophytes have been used as medicinal plants for a long time.[2]

Liverworts constituting the second largest group of bryophytes exhibit pharmacological and biological activities. They have been used as remedies for burns, bruises, cuts, fractures and pneumonia. *Targionia hypophylla* is also used for curing scabies, itches and other skin diseases by the Irula tribes in India.[3]

Krishnan[1] reported *in vitro* antifungal and antibacterial potentiality of *T. hypophylla*. Lipophilic extracts of *T. indica* shown to have inhibitory effects on the germination and growth of the seeds of *Bidens pilosa* can be used for controlling invasive weeds.[4]

Thakur and Kapila[5] carried out comparative biochemical analysis in vegetative thallus and aechegoniophores of *Marchantia papillata* and reported higher content of proteins, carbohydrates and amino acids along with higher invertase activity in archegoniophores as compared to vegetative thallus.

Analysis of amino acids plays an important role in biomedical, biochemical and pharmaceutical fields.[6]

Amino acids are the basic components of proteins as they are essential for the synthesis of proteins. They have two functional groups-the amino group which gives the basic character and the carboxylic acid group which provides the acidic character.

Amino acids are very essential for regular biological activities in human body. In addition to repairing and building new tissues, amino acids also form antibodies, an important part of our hormonal and enzymatic system.[7]

Not much work has been done so far on amino acids in bryophytes.

Lugg[8] investigated the thalli of *Lunularia cruciata* for the protein composition and determined total N% (3.92) and various amino acids i.e. amide (5.52 % total N), tyrosine (2.43% total N), tryptophan (1.70% total N), methionine (1.37% total N), cystein (1.31% total N) and dilodotyrosine (0.0% total N) as percentage of total N.

Black[9] revealed the presence of total 14 amino acids in *Sphagnum imbricatum.*

Percentage of various amino acids found in 12 species of *Sphagnum* was revealed to range from 0.1% to 1.3% of their dry weight.[10]

In the liverwort *Scapania nemorosa*, the production of the gemmalings was reported to increase with the supply of glutamic acid, histidine and arginine.[11]

Dunham and Bryan[12] studied the effect of exogenous supply of various amino acids i.e. arginine, histidine, hydroxyproline, tryptophan and lysine on the development of gemmae in the liverwort *Marchantia polymorpha* and reported various morphological irregularities and alterations in the normal development pattern.

The variations in the content of different amino acids in the sporophyte of moss *Polytrichum formosum* was studied by Uhel[13] to reveal that both female gametangia and sporophyte of *P. formosum* posses a significant amount of glutamic acid, aspartic acid and arginine.

Recently, Devi[14,15] determined the content of six essential and two non essential amino acids in the species of *Marchantia* and *Plagiochasma* using the technique of HPTLC. A very keen survey of literature available on bryophytes revealed that till now no HPLC method has been reported for the identification and quantification of amino acids in bryophytes.

The aim of present study was to use HPLC method for the qualitative and quantitative estimation of different amino acids in bryophytes.

The separation of amino acids and their subsequent identification have become essential because of their increasing role in pharmaceutical industry.

MATERIALS AND METHODS

Collection of Plant Material

The thalli of *Targionia hypophylla* were collected from Kasauli (1927m, Himachal Pradesh).

Voucher specimens of this species have been deposited in the herbarium of Panjab University, Chandigarh with PAN herbarium reference number (PAN 6179).

The collected plants were carefully cleaned to remove other adhering material, washed with distilled water, air dried, powdered and used for analysis.

Preparation of Sample Solution

200 mg sample was taken and diluted with 1 ml of mobile phase which consists of equal concentration of Acetonitrile and HPLC grade water.

The mixture was sonicated for 30 min. and was then filtered through 0.22µm membrane filter to be injected into for HPLC analysis.

Preparation of Standard Solution

Amino acid standard was prepared by dissolving 1mg/ml of each amino acid under study in distilled water.

Chromatographic Conditions and Instrumentation

The Shimadzu HPLC system (LC-2010C HT, Japan) consisting LC-20AT pump and a SIL-20ACHT autosampler, equipped with a SPD-20A UV/VIS detector and SPD-M20A photodiode array detector (PDA) was used.

LC solution version 1.25 was used to process the chromatograms.

The mobile phase consisting of the waters AccQ Tag eluent A Concentrate was diluted to 10% in HPLC grade water and used as eluent A and 60% acetonitrile as eluent B in a linear gradient program with flow rate of 1 ml/min.

The linear gradients used for separation were 0-2 min (100%A), 2.0min (98% A), 15.0 min (93% A), 19.0 min (90.0% A), 32.0 min (67.0% A), 38.0 min (0.0% A) and 55 min (100% A).

The detection for the presence of various amino acids was carried out using PDA detector at 254nm. A volume of 20 µl was injected into amino acid column C_{18} (3.9 mm * 150 mm) for all the standards as well as for the samples.

Principle

The principle is that a solution of the sample is injected into a column of a porous material (stationary phase) and a liquid (mobile phase) is pumped at high pressure through the column.

The separation of sample is based on the differences in the rates of migration through the column arising from different partitions of the sample between the stationary and mobile phase as elution depends upon the partition behavior of different components.

Linearity

The linearity was established using three standard solutions containing 10 µl, 15 µl, 20 µl of each amino acid. The data of peak area *vs*. amino acid concentration were treated by linear least squares regression analysis.

The values of the slope, intercept and the coefficient of determination of the calibration curve for the amino acids present in *T. hypophylla* are given in Table 1.

RESULTS AND DISCUSSION

The present study was aimed to investigate the presence and content of 20 amino acids, of which nine are indispensable (isoleucine, leucine, lysine, methionine, threonine, tryptophan, phenylalanine, histidine and valine), six are conditionally indispensable (arginine, cystine, glutamine. glycine, tyrosine and proline) and five are dispensable (alanine, aspartic acid, asparagine, glutamic acid and serine) for human health.

Indispensable amino acids cannot be synthesized *de novo* by organisms, therefore must be supplied through the diet.

The synthesis of conditionally indispensable amino acids becomes limited under certain health conditions e.g. in infants in catabolic stress.[16]

The dispensable amino acids can be synthesized in the human body, also play a vital role in human nutrition and metabolism, and their deficiency leads to many deficiency diseases.[17]

Figure 1 show the peaks of standards (1a, 1b, 1c, 1d, 1e, 1f and 1g) and figure 2 show the peaks of amino acids present in the sample of *T. hypophylla*.

In *T. hypophylla*, seven (serine, glutamine, cystine, methionine, phenylalanine, asparagine and tryptophan) amino acids are found to be present, out of which three (methionine, phenylalanine and tryptophan) are indispensable, two (glutamine and cystine) are conditionally indispensable and two (asparagine and serine) is dispensable.

The amount of various amino acids present in *T. hypophylla* is shown in the figure 3. It ranged from 0.003-0.163 mg g^{-1} dw.

Out of all the present amino acids, glutamine (0.163 mg g^{-1} dw) showed highest value followed by serine (0.134 mg g^{-1} dw), cystine (0.125 mg g^{-1} dw), methionine (0.051 mg g^{-1} dw), phenylalanine (0.045 mg g^{-1} dw), asparagine (0.037 mg g^{-1} dw) and tryptophan (0.003 mg g^{-1} dw) found in lowest concentration.

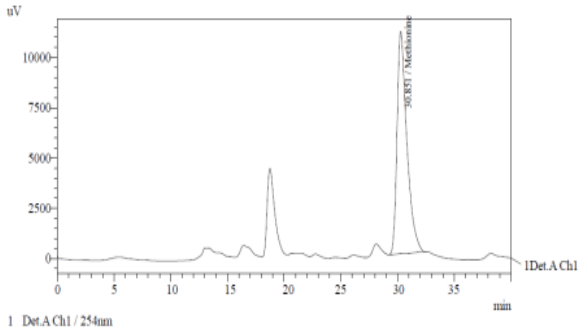


Figure 1a

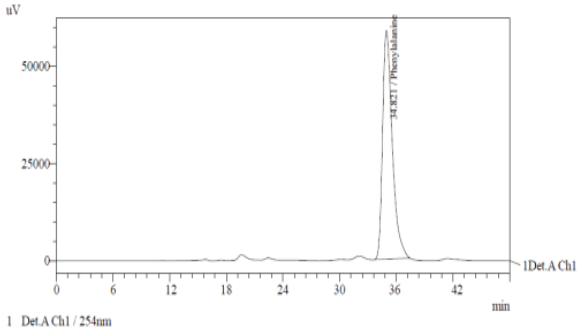
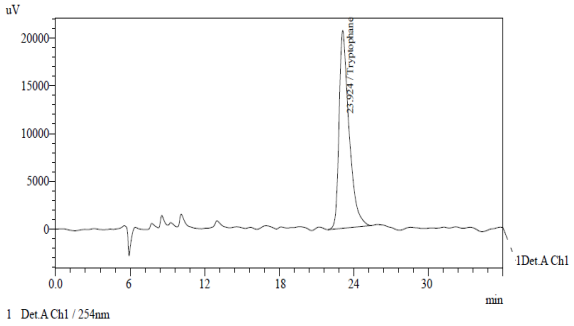


Figure 1b



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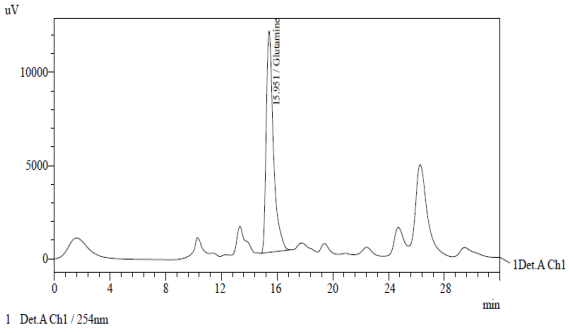
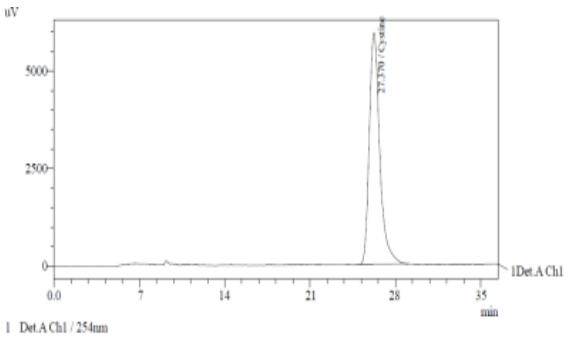


Figure 1d



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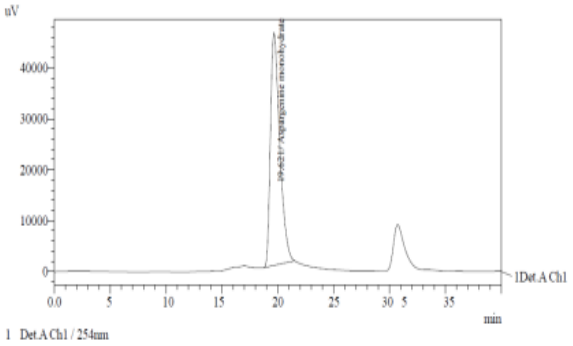

Figure 1f

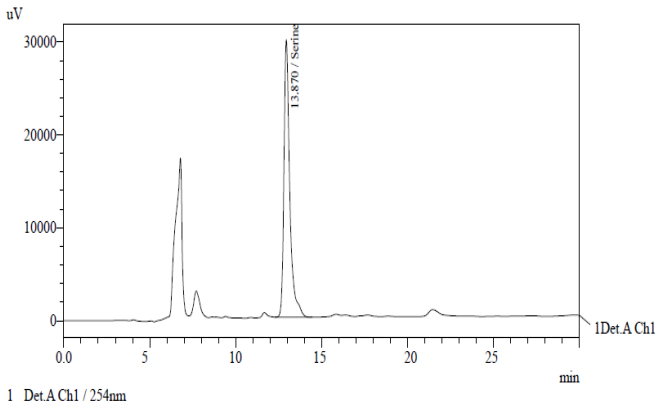

Figure 1g

Figure 1: Chromatograms of the standard amino acids present in *T. hypophylla* i.e. methionine (1a), phenylalanine (1b), tryptophan (1c), glutamine (1d), cystine (1e), asparagine (1f) and serine (1g).

Table 1: Linear regression equations and correlation coefficient of amino acids present in *T. hypophylla*

Amino acids	Linear Regression Equation	Correlation Coefficient
Methionine	y= 40265x + 10656	R^2=0.993
Phenylalanine	y= 21440x + 53107	R^2=0.999
Tryptophan	y= 14543x + 40289	R^2=0.996
Glutamine	y= 27054x + 25904	R^2=0.998
Cystine	y= 37608x + 11441	R^2=0.988
Asparagine	y= 75597x + 35236	R^2=0.996
Serine	y= 11293x + 15288	R^2=0.998

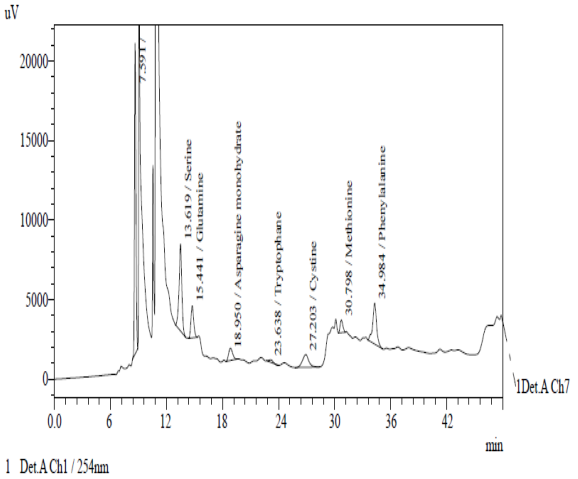

Figure 2: Chromatogram of *Targionia hypophylla* showing the presence of serine, glutamine, asparagine, tryptophan, cystine, methionine and phenylalanine.

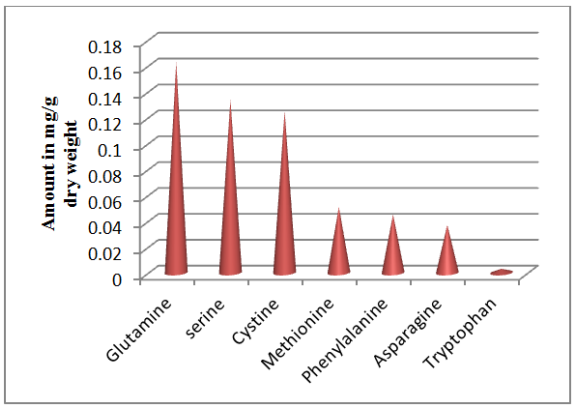

Figure 3: Amount of various amino acids present in *T. hypophylla*

Glutamine plays a vital role in nitrogen metabolism during the normal growth and development of plants. Presence of high content of glutamine in the presently studied species might be helpful in fulfilling the needs of energy required for respiration and other essential metabolic processes by catabolizing its reserve food material to help this species to thrive and colonize successfully in harsh conditions of exposed sites inhabited by it. Earlier, Yemm[18] also attributed the role of glutamine in cell respiration and breakdown of carbohydrates in barley. Serine plays an important role in the catalytic function of many enzymes.

Nerve agents and other substances used in insecticides, act by combining with a residue of serine. They block the activity of enzyme acetylcholine esterase, which is required for the breakdown of a neurotransmitter i.e. acetycholine and disrupt the transmission of neurons.[19]

Presence of this amino acid in *T. hypophylla* suggests that these plants can also be used as alternative source for the manufacture of insecticides.

Methionine is an important component of S-adenosylmethionine which serves as a building block for the synthesis of polyamines, vitamin B_1, osmoprotectants (dimethylsulphoniopropionate) and dimethylsulphide which supply sulfur.[20] Methionine is the most important amino acid in all the eukaryotes as it initiates protein synthesis. *In vitro* studies on *Taxiphyllum barbieri* showed that the treatment with methionine resulted in inhibition of growth in length of shoots.[21] Methionine can be used for the production of cystine in the body. Cystine acts as an antioxidant and has a vital role in the endogenous detoxification.[22] Plants use methionine to synthesize ethylene which is used on a large scale in the chemical industry.[23] Production of ethylene-a stress hormone might help this species to cope with the adverse conditions. Methionine also helps to initiate the synthesis of proteins in *T. hypophylla*. The presence of methionine alongwith cystine in the presently studied species indicates the use of this plant in chemical and pharmaceutical industry.

Phenylalanine found in many proteins such as hemoglobin, is essential in the human diet and can be readily converted into the amino acid tyrosine in the human body. It also helps in the synthesis of proteins and secondary metabolites i.e. phenylpropanoids which enable the plant to survive in biotic as well as abiotic stresses.[24] Presence of phenylalanine helps this species to flourish luxuriantly in the stressful conditions.

Asparagine, one of the conditionally essential amino acid, improves the functioning of the liver and also increases the resistance to fatigue in human body. It gets accumulated in the absence of protein synthesis due to adverse conditions as well as in water deficient conditions and helps in the survival of the plant by maintaining optimum osmotic pressures.[25] Asparagine also plays a significant role in the transport of nitrogen in the

developing as well as germinating seeds and also in the vegetative tissues in the stress conditions.[26] The presence of asparagine might be responsible for the growth of *T. hypophylla* in exposed habitats and also for its potential to be used as an antioxidant.

In infants tryptophan is needed for normal growth and in adults for nitrogen balance.[27] Amino acid tryptophan is the precursor for the synthesis of plant hormone auxin[28], which plays a significant role in the growth and development of plants by regulating various processes such as elongation of cells, initiation of roots, growth of fruits and flowers. Some synthetic auxins in higher concentrations are toxic to the plants and can be used as herbicides to control the proliferation of weeds. Presence of tryptophan in *T. hypophylla* might be responsible for its growth as a single patch without intermixing with other species. It is also suggestive of its role as herbicide and also in the *in vitro* studied for initiating roots and shaping the final development.

Each amino acid found in the present species plays a significant role in the growth and development of *Targionia.* Methionine, phenyalanine, tryptophan are essential amino acids for human so their presence in the presently studied species is indicative of its use in pharmaceutical industry. The presence of these amino acids in *Targionia* is indicative of its potential to be used as food supplement in nutrition industry as well as in pharmaceutical industry for human welfare.

The qualitative as well as quantitative study of various chemical constituents also plays a vital role in solving existing taxonomic problems in liverworts and mosses and even within families. The composition of fatty acids[29] and their evolutionary significance[30] has also been observed in bryophytes. Lewis[31,32] reported the presence of sugar alcohols for generic and species segregation in *Plagiochila* and *Jamesoniella*. Suleiman[33] used the presence and absence of carbohydrates as a taxonomic tool in bryophytes.

Similarly, the amino acids presently found in *T. hypophylla* are observed to be entirely different from those recorded in *T. indica* by Devi[34] except methionine which is common in both the species but differs quantitatively, more in *T. hypophylla* (0.051 mg g^{-1} dw) than in *T. indica* (0.019 mg g^{-1} dw). So the qualitative as well as quantitative study of amino acids might also be helpful in chemotaxonomy.

CONCLUSION

Today HPLC is one of the most important tools of analytical chemistry and is most advanced and accurate technique in the modern era for the quantification of amino acids as compared to other conventional methods found in literature. The rapid HPLC method presently used for the simultaneous characterization and quantification of amino acids from a liverwort *Targionia hypophylla* and also to detect, separate and quantify amino acids from other bryophytes also. This method is an advanced, specific, accurate and precise analytical tool to determine extremely low to extremely high quantity of various amino acids present in the studied sample.

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