



Received on 09 May, 2014; received in revised form, 21 July, 2014; accepted, 15 August, 2014; published 01 December, 2014

HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC) METHOD FOR THE DETERMINATION OF SOME AMINO ACIDS FROM TWO SPECIES OF *MARCHANTIA*

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Keywords:

Marchantia palmata, *Marchantia nepalensis*, HPTLC, Amino acids.

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ABSTRACT: The objective of the present study is to develop a rapid HPTLC method for the identification, separation and quantification of some amino acids from two species of *Marchantia*. Three different mobile phases were used for the separation and estimation of eight amino acids (alanine, glycine, methionine, threonine, valine, leucine, histidine and isoleucine). The HPTLC analysis of *Marchantia palmata* and *M. nepalensis*, showed the presence of all the eight amino acids. The content of all the amino acids in *Marchantia nepalensis* were higher than *M. palmata*. Of all the amino acids, the glycine content (0.417 mg/g dw in *Marchantia nepalensis* and 0.287 mg/g dw in *M. palmata*) was found to be maximum and valine content (0.053 mg/g dw in *Marchantia nepalensis* and 0.031 mg/g dw in *M. palmata*) was found minimum. The presence and content of amino acids in the liverworts has been for the first time evaluated by a modern technique like HPTLC. This method provides an important tool for the quantitative evaluation of important amino acids and gives more accurate results than the conventional ones reported in the literature.

INTRODUCTION: The genera of family Marchantiaceae are well known in traditional Chinese medicinal herbs and used to treat skin diseases, protect liver and treat hepatitis. In some regions of China these are also used as antipyretic¹⁻³. Many studies on the antimicrobial activities of many species of *Marchantia* such as *M. polymorpha*, *M. convoluta* and *M. paleacea* were reported^{4, 5}. Many *Marchantia* species were studied for the bioactive components, particularly the conjugated polyphenols⁶. In ancient China *Marchantia* species had been used as herbal medicines. *Marchantia polymorpha* was used to cure cuts, fractures, poisonous snake bites, burns, scalds and open wounds and it has antipyretic, antihepatic, antidotal, diuretic activity⁷.

The thick paste of *M. palmata* thalli is directly applied during acute inflammation caused by the touch of fire and hot water⁸. To cure skin borne allergies *Marchantia linearis* is used by Wayanad Kurichya, Kattunaikkans tribes⁹.

Chemical constituents and bioactivities of *M. convoluta* were evaluated in many studies which reported the presence of flavonoids, triterpenoids and steroids¹⁰. From *Marchantia emarginata* bis(bibenzyl and Humulane-type sesquiterpenes¹¹ and from *M. polymorpha* Marchantin A, a macrocyclic bisbibenzyl ether that inhibits protozoal growth *in-vitro* were isolated and characterized¹².

Amino acids are organic substances containing both amino and acid groups. They are building blocks of proteins and required by the human body as they act as precursors of other nitrogen containing compounds, e.g., nucleic acids. Phenylalanine, valine, methionine, leucine, isoleucine, lysine, histidine, threonine and

<p>QUICK RESPONSE CODE</p> 	<p>DOI: 10.13040/IJPSR.0975-8232.5(12).5328-33</p> <hr/> <p>Article can be accessed online on: www.ijpsr.com</p> <hr/> <p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(12).5328-33</p>
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tryptophan are the essential amino acids for humans¹³. Essential amino acids are not synthesized in the body and their exogenous supply is useful in a variety of metabolic activities. Weak immune system, nausea, dizziness, stomach problems and loss of antibody production are sometimes due to the deficiency of amino acids¹⁴. They play vital role in the metabolism of secondary metabolites¹⁵. They are used in industry such as, in the synthesis of cosmetics and drugs and for the production of biodegradable plastics.

The thalli of *Lunularia cruciata* were analyzed for total N% (2.04), amide (5.52), tyrosine (2.43), dilodotyrosine (0.0), tryptophan (1.70), cysteine (1.31) and methionine (1.37)¹⁶. Black et al. (1955) listed all the amino acids in *Sphagnum imbricatum*¹⁷. Maass & Craige (1964) reported percentage of amino acids in 12 species of *Sphagnum*¹⁸. Their amount varied from 0.1% to 1.3% of dry weight. In the liverwort *Scapania nemorosa*, enhancement of gemmae production was noticed by the addition of arginine, histidine or glutamic acid¹⁹.

The effect of exogenous amino acids on the development of *Marchantia polymorpha* gemmalings revealed the alteration in normal developmental pattern and morphological irregularities by the addition of arginine, histidine, hydroxyproline, lysine and tryptophan²⁰. The sporophyte of *Polytrichum formosum* was analyzed at different development stages to study the quantitative changes in free amino acids which revealed the presence of glutamic acid, aspartic acid and arginine in significant amount²¹.

Literature survey revealed that no HPTLC method has been reported for quantification of amino acids from liverworts.

In the recent researches, HPTLC plays an important role in quantification of plant products because of its low cost, simplicity, rapidity and need of very small sample. For the quantification of some amino acids, a densitometric HPTLC method has been developed. The present investigation on liverworts provides valuable information about the presence or absence of amino acids that gives an indication of potential medicinal and nutritive value for human welfare.

MATERIALS AND METHODS:

Collection and preparation of Plant material

Plant materials were collected from Mandi district of Himachal Pradesh. Voucher specimens have been deposited in the herbarium of Panjab University, Chandigarh with PAN herbarium reference numbers (*Marchantia nepalensis*; PAN 6102, *Marchantia palmata*; PAN 6103). The plants were washed with distilled water, separated, air dried, powdered and stored for further analysis.

Standard preparation

Standard was prepared by dissolving 1mg/ml amino acid to be studied i.e. alanine, methionine, glycine, leucine, valine, threonine, isoleucine and histidine in distilled water.

Preparation of extracts

The dried and powdered plant material (200mg) was homogenized in methanol: chloroform: water solution (12:5:3). The homogenate was incubated for 24 hours at 4°C and centrifuged at 2500 rpm for 20 min. at 4°C. The obtained supernatant was mixed again with 0.5 ml chloroform and 0.75 ml of distilled water and centrifuged again. Supernatant so obtained was maintained at 38°C for about two hours and then subjected to freeze drying for further analysis.

Mobile phase used

Mobile phases of different composition were tested for HPTLC analysis of amino acids. The desired objective was achieved by use of Butanol: Acetic acid: Water (7: 1.5: 1.5) for the separation of alanine, glycine and methionine, Butanol: Acetic acid: Water: Acetone (3.5: 1: 2: 3.5) for the separation of threonine, valine and leucine and Butanol: Acetic acid: Water (6: 2: 2) for the separation of histidine and isoleucine.

Chromatography

TLC aluminum precoated plate with silica gel 60 F₂₅₄ (10×10 cm) was used with three mobile phases. The extracts of sample and mixture of standards were applied to the plate with an automatic TLC sampler. The plate was dried in the oven. Camag twin trough glass chamber (10×10 cm) with stainless lid was used for the development of TLC plate. The twin trough glass chamber was saturated with mobile phase for 50-55 minutes. The plates were removed from the chamber and oven dried for

one minute at 110°C. Then plates were dipped in 1% ninhydrin in methanol by using camag chromatogram immersion device 111 followed by heating at 110°C for 3-5 minutes. HPTLC finger print profile was snapped by Camag reprostar III before derivatization. The plate was scanned before derivatization using camag TLC scanner III at wavelengths 484nm, 500nm and 486nm. Wincats 1.4.2 software was used for the detection as well as evaluation of the data.

Calibration curve of amino acids

A stock solution of standard amino acids (100µg/ml) was prepared in distilled water. The different volumes of the stock solution were spotted on the plate in triplicate to obtain concentration 400, 600 and 800 ng/spot. The linear regression line and correlation coefficient are given in the **Table 1**. Peaks of amino acids (standard and samples) were obtained when the plate was scanned at wavelengths 484nm, 500nm and 486nm (**Fig.1-9**).

TABLE 1: AMINO ACIDS SHOW LINEAR REGRESSION EQUATION AND CORRELATION COEFFICIENT.

Amino acid	Linear regression equation	Correlation coefficient
Glycine	y=10.48x+2906	r ² =0.99
Alanine	y=12.70x+4267	r ² =0.98
Methionine	y=8.59x+4241	r ² =0.98
Valine	y=19.86x+3166	r ² =0.98
Leucine	y=11.94x+7234	r ² =0.99
Threonine	y=11.24x+6598	r ² =0.99
Isoleucine	y=8.11x+5290	r ² =0.99
Histidine	y=7.25x+1263	r ² =0.99

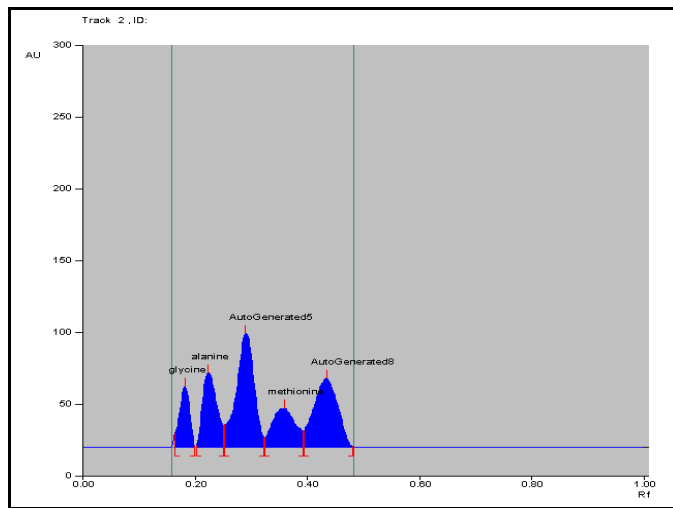


FIG. 2: CHROMATOGRAM OF M. PALMATA 484nm

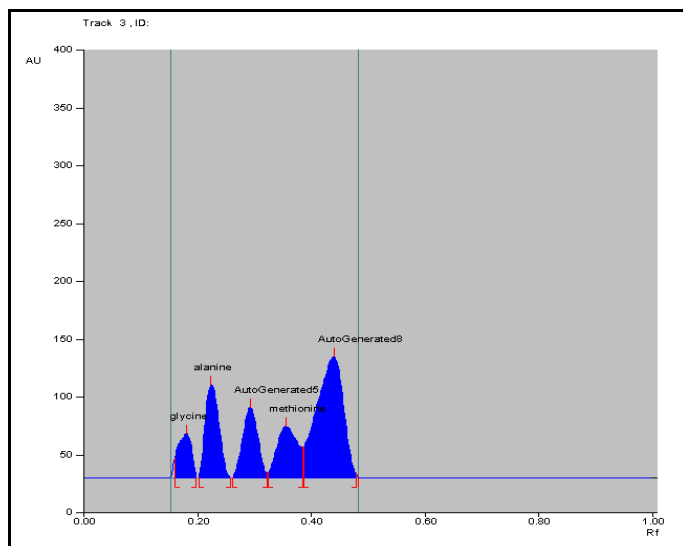


FIG. 3: CHROMATOGRAM OF M. NEPALENSIS AT 484nm

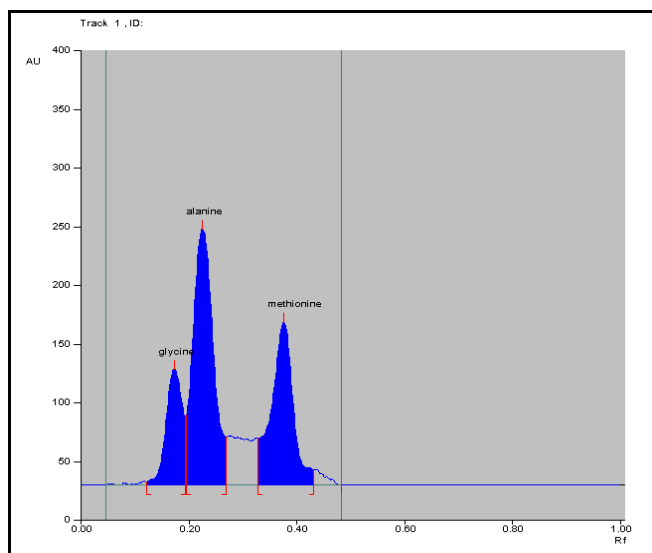


FIG.1: CHROMATOGRAM OF STANDARDS AT ALANINE, GLYCINE, AND METHIONINE AT 484nm

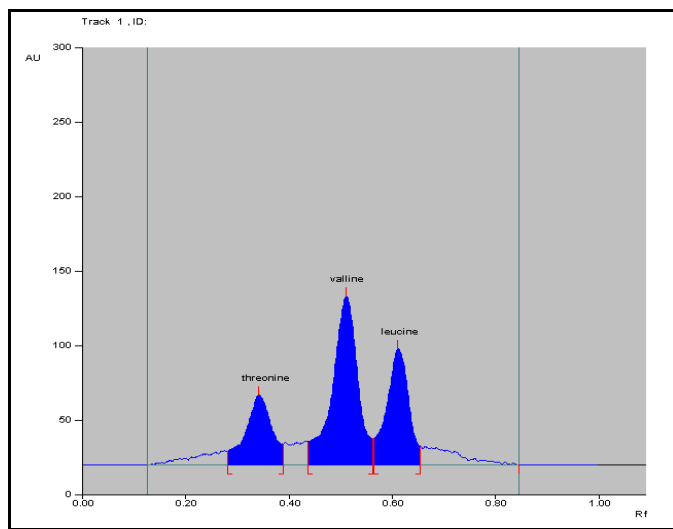


FIG.4: CHROMATOGRAM OF STANDARDS THREONINE, VALINE AND LEUCINE AT 486nm

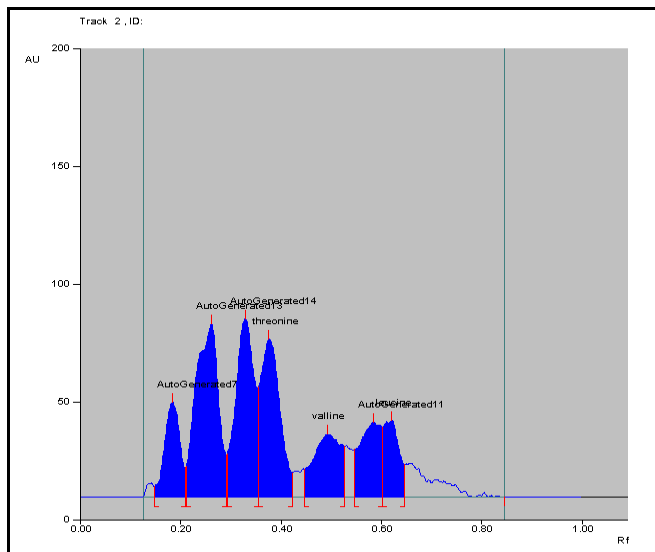


FIG. 5: CHROMATOGRAM OF *M. PALMATA* AT 486nm

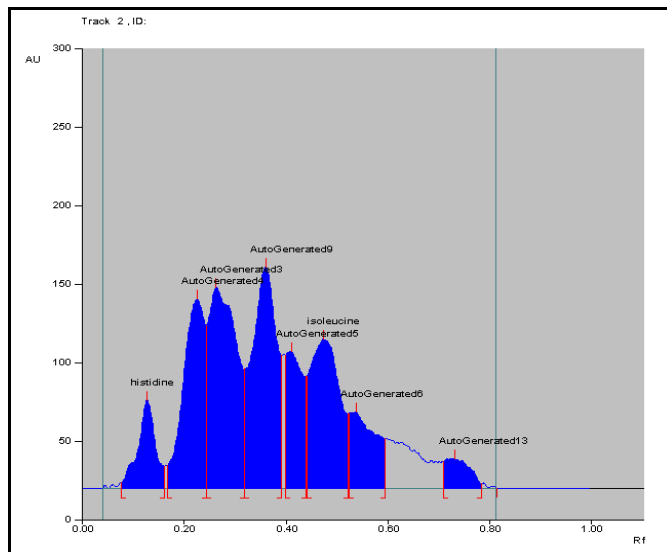


FIG.8: CHROMATOGRAM OF *MARCHANTIA PALMATA* AT 500nm

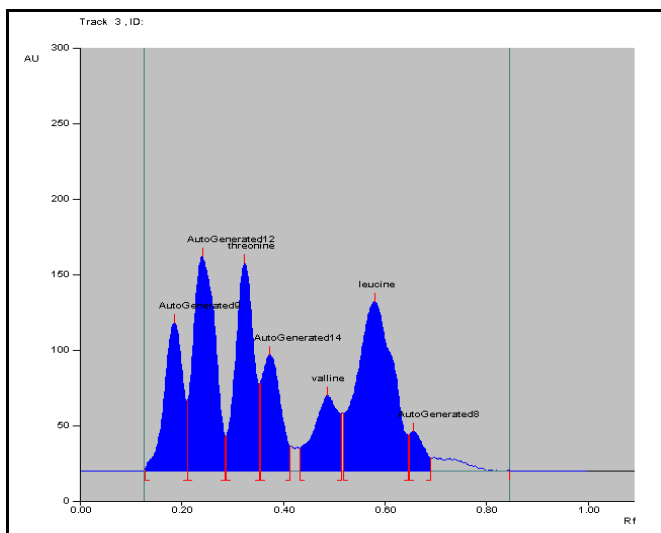


FIG. 6: CHROMATOGRAM OF *M. NEPALENSIS* AT 486nm

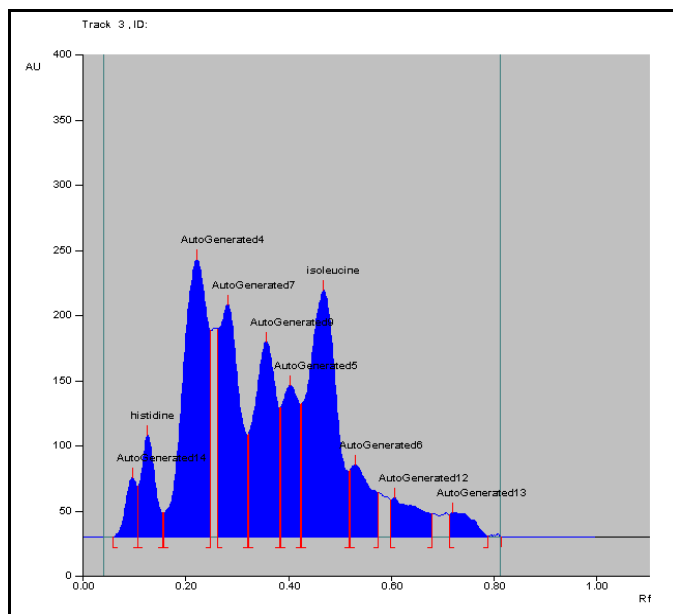


FIG. 9: CHROMATOGRAM OF *MARCHANTIA NEPALENSIS* AT 500nm

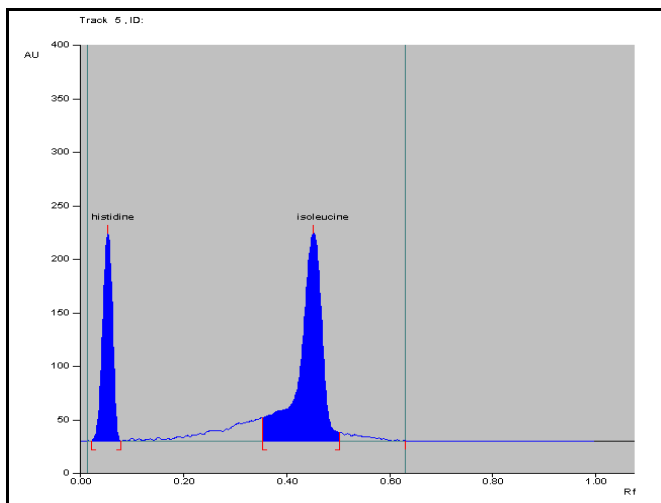


FIG.7: CHROMATOGRAM OF STANDARDS HISTIDINE AND ISOLEUCINE AT 500nm

RESULTS AND DISCUSSION:

Amino acids play central role both as intermediates in metabolism and as building blocks of proteins. They are linked by covalent peptide bonds (-CO-NH-) into the polypeptide chain. The precise amino acid content and the sequence of those amino acids are determined by the sequence of the bases in the gene that encode that protein. The chemical properties of the amino acids of proteins determine the biological activity of the protein. Proteins are essential for normal function and maintenance of body tissues. Essential amino acids cannot be

synthesized by the organism and, therefore, must be supplied in the diet and regarded as essential for humans. The non-essential amino acids also play a vital role in human nutrition and metabolism²².

In our study, we have reported the presence of six essential amino acids (methionine, threonine,

valine, leucine histidine and isoleucine) and two non-essential amino acids (alanine and glycine). All the studied eight amino acids were present in both species of *Marchantia*. The amounts of studied amino acids are given in the **Table 2** and graphically in **Fig. 10**.

TABLE 2: AMOUNT OF EIGHT AMINO ACIDS IN MARCHANTIA PALMATA AND M. NEPALENSIS IN mg/g DRY WEIGHT.

Name of the taxa	Alanine	Glycine	Methionine	Threonine	Valine	Leucine	Histidine	Isoleucine
<i>Marchantia palmata</i>	0.056	0.287	0.058	0.141	0.031	0.047	0.254	0.112
<i>Marchantia nepalensis</i>	0.078	0.417	0.127	0.247	0.053	0.279	0.283	0.207

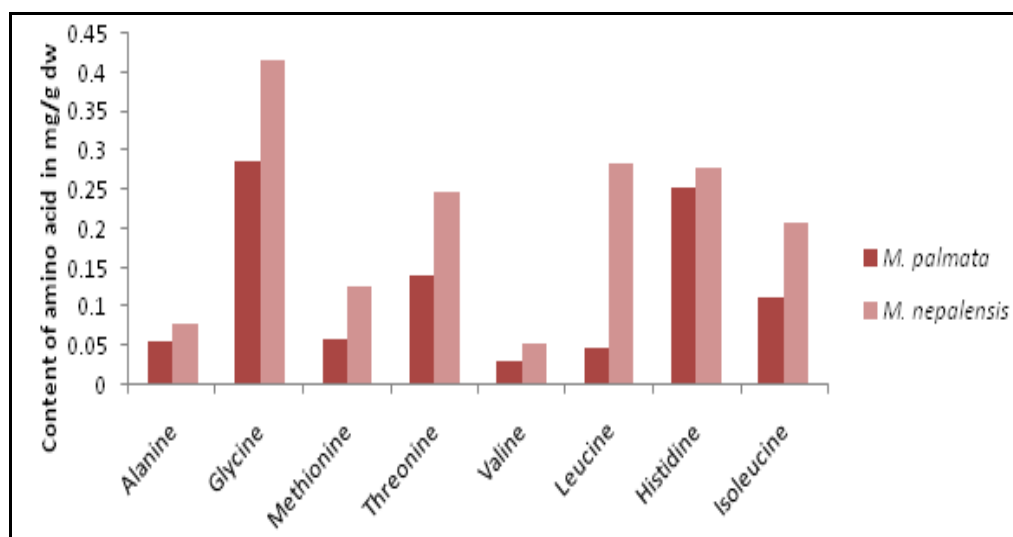


FIG. 10: CONTENT OF AMINO ACIDS IN M. PALMAT AND M. NEPALENSIS

The content of studied amino acids ranged from 0.031-0.417 mg/g dw. The similar content of amino acids has been also reported from the gametophyte and sporophyte of *Polytrichum formosum*²¹. The content of all the amino acids in *Marchantia nepalensis* were higher than *M. palmata*. Of all the amino acids, the glycine content (0.417 mg/g dw in *Marchantia nepalensis* and 0.287 mg/g dw in *M. palmata*) was found to be maximum and valine content (0.053 mg/g dw in *Marchantia nepalensis* and 0.031 mg/g dw in *M. palmata*) was found minimum. Glycine is used in the manufacture of herbicide glyphosate. The higher content of glycine in both the species suggests that these plants can also be used as alternative source for the manufacture of this herbicide.

Alanine helps in the metabolism of sugars, strengthens the immune system by producing antibodies and used as source of energy for muscle

tissue. The content of alanine in these plants may be one of the factors in sugar metabolism. Methionine is used by the plants for synthesis of ethylene which is widely used in the chemical industry. The presence of methionine in both the species of *Marchantia* indicates the use of these plants in chemical industry. Leucine and isoleucine provide important ingredients for manufacturing biochemical components in the body¹⁴.

Valine plays role in the repairing of tissue, regulating blood sugar and responsible for the normal human growth. Lack of it causes mental and neurological disorder. Histidine is precursor of histamine which performs a number of functions. It plays an important role in central nervous system as a neurotransmitter and neuromodulator. Leucine, isoleucine, valine and histidine are essential amino acids for humans so their presence in *Marchantia* species indicates their use in pharmaceutical

industries. Hence the presence of these amino acids in this genus is definitely related to the use of these plants since ancient days.

CONCLUSIONS: A TLC densitometric method for the quantification of amino acids from two species of *Marchantia* using HPTLC was established. This method is simple, precise, rapid, accurate and economic and can also be used for the estimation of amino acids content from the other bryophytes.

ACKNOWLEDGMENTS: The authors are thankful to University Grant Commission, New Delhi for financial assistance.

REFERENCES:

- Xiao JB, Jiang XY and Chen XQ: Antibacterial, anti-inflammatory and diuretic effect of flavonoids from *Marchantia convoluta*. African Journal of Traditional Complementary and Alternative Medicines 2005; 2:244-252.
- Zou DF, Zhu H, Xiao JB, Cao H and Zhou CS: Identification of UASLG for *M. polymorpha*, *M. convoluta* and *M. paleacea*. Natural Product Research and Development 2005; 17:463-464.
- Chen XQ and Xiao JB: In vitro cytotoxic activity of extracts of *Marchantia convoluta* on human liver and lung cancer cell lines. African Journal of Traditional Complementary and Alternative Medicines 2006; 3:32-36.
- Mewari N and Kumar P: Antimicrobial activity of extracts of *Marchantia polymorpha*. Pharmaceutical Biology 2008; 46:819-822.
- Khanam R, Chaudhary BL, Khanam S and Kumar P: Antibacterial activity of *Marchantia palmata*. Nees. Asian. Journal of Biochemical and Pharmaceutical Research 2011; 1:27-36.
- Asakawa Y, Tori M, Takikawa K, Krishnamurthy HG and Kar SK: Cyclic Bis (Bibenzyls) and related compounds from the liverworts *Marchantia polymorpha* and *Marchantia palmata*. Phytochemistry 1987; 26:1811-1816.
- Parihar NS, Lal B and Katiyar N: Hepatics and Anthocerotes of India. A new annotated checklist. Central Book Depot, Allahabad 1994.
- Tag H, Das AK and Loyi H: Anti-inflammatory plants used by the Khamti tribe of Lohit district in eastern Arunachal Pradesh, India. Natural Product Radiance 2007; 6:334-340.
- Bhagobaty RK and Joshi SR: Antimicrobial and antioxidant activity of endophytic fungi isolated from ethnomedicinal plants of the "Sacred forests" of Meghalaya, India. Mikolgia Lekarska 2012; 19:5-11.
- Zhu H, Xiao JB and Zhou CS: Isolation, Purification and Identification of apigenin-7-O-D- glucuronide in *Marchantia convoluta* with silica column chromatography, RP-HPLC and LC-ESI-MS. Natural Product Research and Development 2005; 17:38-41.
- Alam A: Some Indian bryophytes known for their biologically active compounds. International Journal of Applied Biology and Pharmaceutical Technology 2012; 3:239-246.
- Jensen S, Omarsdottir S, Bwalya AG, Nielsen MA, Tesdemir D and Olafsdottir ES: Marchantin A, a macrocyclic bisbibenzyl ether, isolated from the liverwort *Marchantia polymorpha*, inhibits protozoal growth *in vitro*. *Phytomedicine* 2012; 19:1191-1195.
- Young VR: Adult amino acid requirements: the case for a major revision in current recommendations. Journal of Nutrition 1994; 124:5117S-1523S.
- Gaikwad SA, Kale A, Mundhe K, Deshpande NR and Salvekar JP: Detection of amino acids present in the leaves of *Cassia auriculata* L. International Journal of PharmTech Research 2010; 2:1092-1094.
- Swati MD, Gayatri SK, Rasika CT, Asha AK, Nirmala RD and Jyoti PS: Preliminary screening of amino acids from a medicinal plant: *Morinda pubescence*. Journal of Chemical and Pharmaceutical Research 2011; 3:192-195.
- Lugg JWH: Further studies of cryptogam tissue proteins: protein composition and tissue function in plants. Biochemical Journal 1943; 37:132-137.
- Black WAP, Cornhill WJ and Woodward FN: A preliminary investigation on the chemical composition of *Sphagnum* moss and peat. Journal of Applied Chemistry 1955; 5:484-492.
- Maass WSG and Craigie JS: Examination of some soluble constituents of *Sphagnum* gametophytes. Canadian Journal of Botany 1964; 42:805-813.
- Basile DV: Growth, development and gemma formation in the liverwort *Scapania nemorosa* as influenced by L-arginine, L-histidine and L- glutamic acid. American Journal of Botany 1965; 52:443-454.
- Dunham VL and Bryan JK: Effects of exogenous amino acids on the development of *Marchantia polymorpha* gemmalings. American Journal of Botany 1968; 55:745-752.
- Uhel C: Quantitative changes in free amino acids in *Polytrichum formosum* during development. Phytochemistry 1975; 14:2337-2340.
- Hassan LG, Umar KG, Dangogo SM and Ladan MJ: Protein and amino acid composition of African locust bean (*Parkia biglobosa* L.). Journal of Tropical and Subtropical Agroecosystems 2005; 5:522-529.

How to cite this article:

Devi K, Kapila S and Rao A: High-Performance Thin Layer Chromatography (HPTLC) Method for the Determination of Some Amino Acids from Two Species of *Marchantia*. Int J Pharm Sci Res 2014; 5(12): 5328-33.doi: 10.13040/IJPSR.0975-8232.5 (12).5328-33.

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