Research Article

Determination of Some Amino acids from Three Species of *Plagiochasma* by High Performance Thin Layer Chromatography (HPTLC)

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Accepted on: 16-08-2014; **Finalized on:** 31-10-2014.

ABSTRACT

The objective of the present study is to obtain the information about the presence or absence of eight amino acids and to determine their content in three species of *Plagiochasma* by HPTLC. Out of eight presently studied amino acids, six are essential amino acids (methionine, threonine, valine, leucine, histidine and isoleucine) and two are non- essential amino acids (alanine and glycine). Among the three species of *Plagiochasma*, *P. appendiculatum* shows the highest content of all amino acids except methionine, ranging between 0.138 mg/g dw (of alanine) and 0.644 mg/g dw (of glycine). In *P. articulatum* histidine content (0.177 mg/g dw) is more than the rest of the amino acids. In *P. intermedium* glycine content (0.151 mg/g dw) is highest among the other amino acids. *P. articulatum* and *P. intermedium* show all the eight amino acids, whereas *P. appendiculatum* does not contain methionine.

Keywords: Amino acids, HPTLC, *Plagiochasma*.

INTRODUCTION

Bryophytes are first land plants which grow gregariously during rainy season in wide range of habitats. They are comprised of three groups; mosses, liverworts and hornworts. The liverworts contain cellular oil bodies while the other two classes do not. Oil bodies in the liverworts consist of a number of lipophilic terpenoids with a variety of aromatic compounds several of which show biological activities such as allergenic contact dermatitis, insecticidal, insect anti feedant, cytotoxic, piscicidal, muscle relaxing, plant growth regulatory, anti-HIV, DNA polymerase beta inhibitory, anti-obesity, neurotrophic, antimicrobial and antifungal activities.[1-4] The presence of biological compounds like aromatic and phenolic compounds, sugar alcohols, fatty acids, aliphatic substances, amino acids, oligosaccharides, polysaccharides in bryophytes provide protection against bacteria, fungi and insect larvae, therefore, these plants are not damaged by these microorganisms and have the potential for medicinal use.[5-9] The liverwort *Conocephalum conicum* has antipyretic, antidotal, antifungal activity and used to cure cuts, burns, scalds, fractures, swollen tissue, poisonous snake bites, and gallstones, *Frullania tamarisci* possesses antiseptic activity and *Reboulia hemisphaerica* is used to cure blotches, hemostasis, external wounds, and bruises.[10] The mosses like *Polytrichum*, *Thuidium* and *Atrichum* were also used as antibacterial and anti-inflammatory agents in the Chinese medicines.[11]

In the present study, we have worked on three species of *Plagiochasma* for the quantification of eight amino acids by HPTLC method.

Plagiochasma is a thallose liverwort genus of the family Aytoniaceae. It grows in exposed conditions and on calcium rich substrata. Like other liverworts, it also has medicinal value, but most of the literature pertains to *P. appendiculatum*. In India, *P. appendiculatum* is used by the Gaddi tribes of Himachal Pradesh to cure burns, boils and blisters of skin.[12] It showed the potent antimicrobial and antioxidant activity responsible for wound healing mechanism.[13] Bodade *et al.* (2008) showed that *P. appendiculatum* has more antimicrobial activity than mosses.[14] Singh *et al.* (2011) also reported the medicinal use of *P. appendiculatum* to cure the burn infection.[15]

Amino acids 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. Deficiency of amino acids causes several problems like weak immune system, loss of antibody production, dizziness and stomach problems.[16]

The aim of this work was to provide the information about the presence or absence of amino acids and to determine their content in the three species of *Plagiochasma* which can be useful to the pharmaceutical studies to be done in future in India.

MATERIALS AND METHODS

Collection and preparation of Plant material

Plant materials were collected from Mandi and Shimla, Himachal Pradesh (Western Himalaya). The names of taxa, locality, altitude, nature of substratum and herbarium reference numbers are given in Table 1.

The plants were washed with distilled water, separated, air dried, powdered and stored for further analysis. The methodology of HPTLC as reported by Devi *et al.* 2014[17] is followed.

RESULTS AND DISCUSSION

In our study, we have investigated the presence and content of six essential amino acids (methionine,

threonine, valine, leucine histidine and isoleucine) and two non- essential amino acids (alanine and glycine). The content of amino acids is given in Table 2. Figures 1-3 show the peaks of standards (1a, 2a, 3a) and of samples which were obtained when the plate was scanned at wavelength 484nm, 500nm and 486nm respectively. The content of each amino acid ranged from 0.021-0.644 mg/g dw in three species of *Plagiochasma*. The gametophyte and the sporophyte of *Polytrichum formosum* were also reported to have the same content of amino acids.[18] The content of amino acids was found higher in *P. appendiculatum* than *P. articulatum* and *P. intermedium.* All the studied eight amino acids were present in three species of *Plagiochasma* except methionine which was absent in *P. appendiculatum* and its concentration was found to be very less as compared to other amino acids. Methionine is the precursor of ethylene[19], which is liberated by leaves and fruits of cotton plants under water deficit.[20,21] *Plagiochasma* grows on wet soil in mesic condition. This may be the reason of absence of methionine in *P. appendiculatum* and very less content in other two species of *Plagiochasma*.

Table 1: Nature of substratum and herbarium reference numbers

Name of Taxon	Locality and altitude	Substratum	Herbarium reference No.
Plagiochasma appendiculatum Lehm. et Lindenb.	Mandi; 750m	Wet soil on stony wall	PAN 6105
Plagiochasma articulatum Kash.	Mandi; 911m	On wet soil	PAN 6106
Plagiochasma intermedium Lindenb. et Gottsche	Mandi; 911m	On wet soil	PAN 6107

Glycine content was found maximum in *P. appendiculatum.* In our previous study on the two species of *Marchantia* i.e. *M. palmata* and *M. nepalensis*, glycine was found higher than the other studied amino acids.[17] *P. appendiculatum* grows luxuriantly in exposed conditions on moist soil and rocks in very large patches in plains as well as hilly areas. Due to easy availability of its plants in abundance this taxon can be used as a good source of glycine which is used in the manufacture of glyphosate herbicide.

Alanine helps in the metabolism of sugar and used as a source of energy in the muscle tissue. Perhaps, in these plants, it contributes a role in sugar metabolism.

Leucine, isoleucine and valine are the branched-chain essential amino acids, which cannot be synthesized in the body. The athletes and body builders take supplements and protein powders that contain Leucine to promote muscle recovery without significant changes in the body composition.[22] *P. appendiculatum* can be a better source of leucine (0.594 mg g^{-1} dw), isoleucine (0.367 mg g^{-1} dw and valine (0.171 mg g^{-1} dw) than the other two presently studied species of *Plagiochasma*.

Threonine is synthesized in plants and microorganisms from aspartic acid. The presence of threonine in all the three species of *Plagiochasma* indicates the presence of aspartic acid in these plants. Threonine enhances the immune system by aiding in the production of antibodies and prevents the fat accumulation in the liver. Its presence in species of *Plagiochasma* indicates their role in pharmaceutical industry.

Among three species of *Plagiochasma, P. appendiculatum* showed maximum quantity of histidine (0.424 mg g^{-1} dw), whereas the concentration of histidine *in P. articulatum* (0.177 mg g^{-1} dw) and *P. intermedium* (0.066mg g^{-1} dw) was found even less than that in *Marchantia palmata* (0.254 mg g^{-1} dw) and *M. nepalensis* (0.279 mg g^{-1} dw).[17] Histidine plays a critical role as a metal binding ligand [23] and in plant growth and development.[24,25] Its high concentration in *P. appendiculatum* is responsible for its growth in very large patches on rocks as well as on wet soil in plains as well as hilly areas.

Each amino acid plays a particular role, thus the presence of these amino acids in *Plagiochasma* give the stronger indication of potential medicinal and nutritive value of this genus to human welfare.

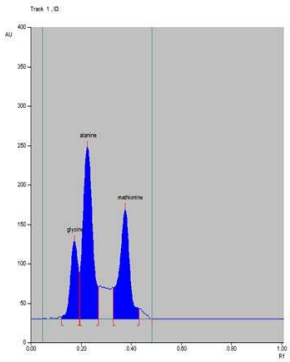

Figure 1a

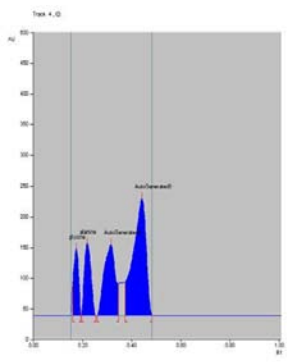

Figure 1b

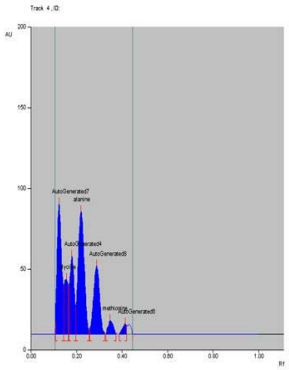

Figure 1c

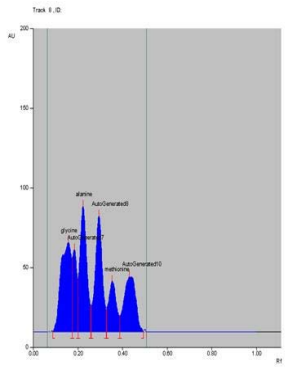

Figure 1d

Figure 1: Chromatograms: 1a-Standards alanine, glycine and methionine; 1b-*P. appendiculatum;* 1c-*P. articulatum;* 1d-*P. intermedium* @ 484nm

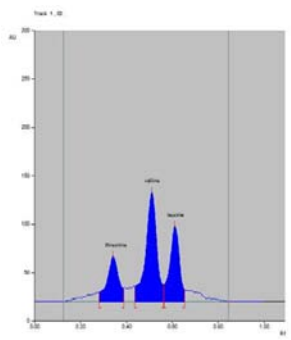

Figure 2a

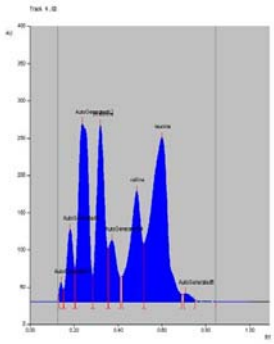

Figure 2b

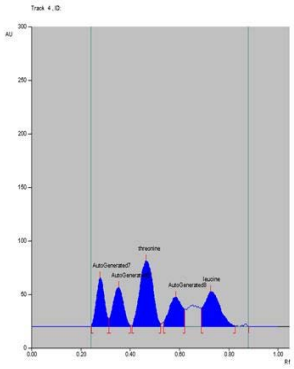

Figure 2c

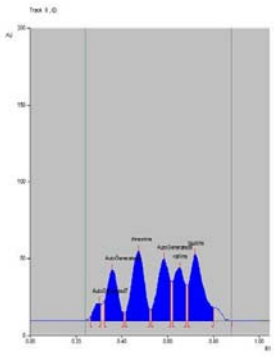

Figure 2d

Figure 2: Chromatograms: 2a-Standards threonine, valine and leucine; 2b-*P. appendiculatum;* 2c-*P. articulatum;* 2d-*P. intermedium* @ 486nm

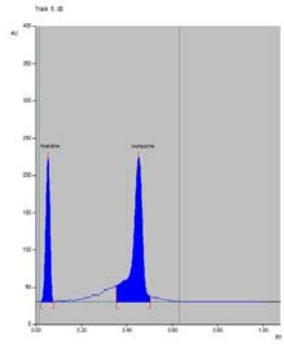

Figure 3a

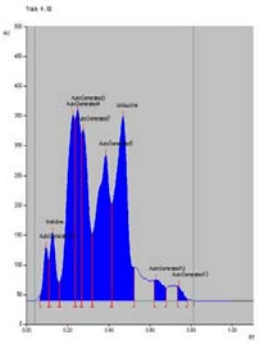

Figure 3b

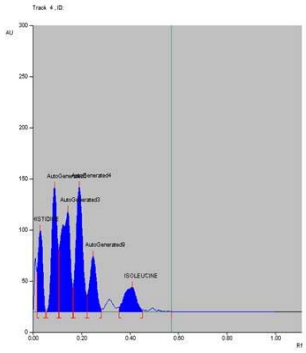

Figure 3c

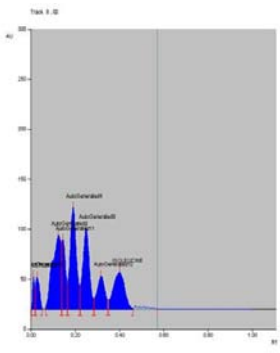

Figure 3d

Figure 3: Chromatograms: 3a-Standards threonine, valine and leucine; 3b-*P. appendiculatum;* 3c-*P. articulatum;* 3d-*P. intermedium* @ 500nm

Table 2: Content of eight amino acids in three species of *Plagiochasma* in mg/g dry weight

Name of the taxa	Ala	Gly	Met	Thr	Val	Leu	His	Iso
P. appendiculatum	0.138	0.644	_	0.437	0.171	0.594	0.424	0.367
P. articulatum	0.058	0.094	0.020	0.131	0.031	0.101	0.177	0.046
P. intermedium	0.045	0.151	0.031	0.097	0.034	0.119	0.066	0.074

Literature survey revealed more ethnotherapeutic value of *P. appendiculatum* than the other two species of *Plagiochasma*. The high content of amino acids in *P. appendiculatum* than that in the other two presently studied species of *Plagiochasma* and two species of *Marchantia* studied by Devi *et al.,* 2014[17] may be one of the factors for their medicinal properties.

CONCLUSION

The present study reported the content of eight amino acids in the three species of *Plagiochasma* by HPTLC method. This method proved to be a linear, precise and powerful method for the amino acids quantification and can be used further in other bryophytes. All the eight amino acids were present in the three species of *Plagiochasma* except methionine which was absent in *P. appendiculatum*. Among the three species, *P. appendiculatum* showed more amount of amino acids than the other two species.

Acknowledgment: The first author is grateful to the University Grant Commission, New Delhi for financial assistance.

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Source of Support: Nil, **Conflict of Interest:** None.