

Phenolic compounds in thalloid liverworts growing in moss house

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ABSTRACT

During the present study Methanolic extracts of 9 thalloid liverworts were analyzed by HPLC which contain Gallic acid, Protocatechuic acid, Chlorogenic acid, Caffeic acid, Rutin, Ferulic acid, Quercetin and Kaempferol known as antioxidants and also have some role in toxicity against cancer cells. Plants were grown in moss House for *ex situ* conservation and multiplication and their use for bio prospection.

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KEYWORDS

Antioxidants;
Cancer cells;
Conservation;
Thalloid liverworts.

INTRODUCTION

Liverworts are rich sources of various unusual compounds and useful for chemosynthetic studies. A large number of new compounds have been isolated and their structures elucidated worldwide. Asakawa^[1] and his group studied time to time about 1000 species of liverworts from different parts of the world with respect to their chemistry, pharmacology and other application as medicinal and agricultural drugs and found that liverworts contain mainly lipophilic mono-, sesqui-, and diterpenoid aromatic compounds (bibenzyls, bis-benzyls, benzoate and cinnamates) and acetogenins. These compounds have various interesting biological activities. These activities are antifungal, antibacterial, insect antifeedant, anticancer and anti HIV^[2-6]. Interestingly there are several bryophytes which are known for their medicinal properties through their use by ethnic people for the cure of several health disorders and in this regard several reports are available^[7-10]. In China, *Conocephalum conicum* and *M. polymorpha* are mixed with vegetable oil and used to treat bites, boils, burns, cuts, eczema and wounds^[11]. However, very few could be scientifically validated. *Plagiochasma*

appendiculatum used by Gaddi tribes of Kangra Valley, Himalaya for the cure of burn, boils and blisters of skin^[12] has been scientifically validated by Laboratory experiments for its antimicrobial and wound healing properties^[13]. According to Shrisat^[14] tribal community of Melghat area, Maharashtra, India is using *Marchantia polymorpha*, *Plagiochasma appendiculatum*, against inflammation and skin disease, *Polytrichum* sp. for hair growth and *Riccia* sp. for curing ring worm disease. Thalloid liverwort *Targionia hypophylla* is used by Mudugar tribes of Western Ghats, Kerala for the treatment of skin diseases^[15]. Hence there is an urgent need for scientific validation of medicinal property of such known plants so that unique natural compound can be isolated from these plants to pave the way for drug development.

The unique and novel chemical compounds which may be helpful to find out the cure of some challenging diseases, could not be isolated from these plants due to very less biomass of these plants available in nature because of their small size and non availability of pure population in field condition. In NBRI, a Moss house has been developed for conservation and multiplication of bryophytes so that pure living germplasm may be

available for such studies. The present study has been done with a point of view to determine the phenolic compounds in thalloid liverworts (Figure 1) growing in moss house and their use for bio-prospection.

EXPERIMENTAL

Investigation of secondary metabolites in liverworts through HPLC

200 mg of air dried plant material was soaked in 80%

methanol over night for HPLC analysis. Filterate is used for analysis.

HPLC system for separation and analysis of polyphenols

Separation followed by qualitative and quantitative analysis of polyphenols was performed by using HPLC-UV (Shimadzu LC-10A, Japan) equipped with dual pump LC-10AT binary system, UV detector SPD-10A at 254 nm, rheodyne injection valve furnished with a 20 μ l loop, on phenomenex Luna RP-C 18 column (4.6



Figure 1A: *Cryptomitrium himalayense* Kash.; B: *Lunularia cruciata* (L.) Dum. ex Lindeb.; C: *Conocephalum conicum* (L.) Dumort.; D: *Reboulia hemispherica* (L.) Raddi; E: *Marchantia papillata* Raddi subsp. *grossibarba* (Steph.) Bischl.; F: *Plagiochasma appendiculatum* Lehm. et Lindenb.; G: *Marchantia paleacea* Bertol.

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X 250 mm, i.d., 5µm pore size) preceded with guard column of same chemistry. Data was integrated by Shimadzu class VP series software and results were obtained by comparison with standards. Results are the mean values of three replicates of the same sample. Elution was carried out at a flow rate of 0.6 ml/min with water: acetic acid (99.0:1.0 v/v) as solvent A and acetonitrile as solvent B using a gradient elution in 0-14 min with 20-35% of solvent B, 14-40 min with 35-50% of solvent B.

RESULTS AND DISCUSSION

After HPLC analysis of the thalloid liverworts viz., *Asterella multiflora* (Steph.) Kachroo, *Cryptomitrium himalayense* Kash., *Lunularia cruciata* (L.) Dum. ex Lindenb., *Targionia hypophylla* L., *Conocephalum conicum* (L.) Dumort., *Reboulia hemispherica* (L.) Raddi, *Plagiochasma appendiculatum* Lehm. et Lindenb., *Marchantia papillata* subsp. *grossibarba* (Steph.) Bischl. and

Marchantia paleacea Bertol., it was found that they contain Gallic acid, Protocatechuic acid, Chlorogenic acid, Caffeic acid, Rutin, Ferulic acid, Quercetin and Kaempferol known as antioxidants and also have some role in toxicity against cancer cells.

The present results have shown that Gallic acid is present in maximum amount as compared to other phenolic compounds in the thalloid liverworts investigated. *Lunularia cruciata* has maximum amount of Gallic acid (2355.41 µg/g), Chlorogenic acid (29.06 µg/g), Quercetin (29.79 µg/g) and Kaempferol (144.86 µg/g) as compared to other liverworts. Jockovic *et al.* [16] studied methanolic extract of *L. cruciata* and found that flavonoid heteroside Luteolin-7-*O*-glucoside and flavonoid aglycone quercetin is present in this species. Protocatechuic acid (195.58 µg/g), Caffeic acid (122.80 µg/g), Rutin (378.10 µg/g) and Ferulic acid (288.11 µg/g) is present in maximum amount in *Cryptomitrium himalayense*, *Marchantia papillata* subsp. *grossibarba*, *Asterella multiflora* and *Conocephalum conicum* respectively (TABLE 1).

TABLE 1 : Comparative account of presence of chemical contents in Thalloid liverworts

Sample code	Gallic acid (µg/g)	Protocatechuic acid (µg/g)	Chlorogenic acid (µg/g)	Caffeic acid (µg/g)	Rutin (µg/g)	Ferulic acid (µg/g)	Quercetin (µg/g)	Kaempferol (µg/g)
1	775.57	4.53	12.64	17.88	378.10	80.60	8.58	15.50
2	1406.74	195.58	20.97	35.56	60.71	56.41	4.23	1.35
3	2355.41	14.25	29.06	35.25	17.7	122.18	29.79	144.86
4	1230.00	30.07	12.27	38.11	11.95	193.75	10.13	1.79
5	965.56	16.13	19.99	72.82	171.56	288.11	4.72	2.43
6	1702.05	14.90	19.44	36.14	38.04	132.25	5.51	2.44
7	2269.03	16.02	11.58	66.75	193.20	220.71	21.64	8.83
8	1769.00	23.62	11.91	122.8	175.5	86.35	4.94	12.09
9	1069.94	3.10	25.10	15.94	23.95	156.12	9.67	13.37

Sample code 1: *Asterella multiflora*; 2: *Cryptomitrium himalayense*; 3: *Lunularia cruciata*; 4: *Targionia hypophylla*; 5: *Conocephalum conicum*; 6: *Reboulia hemispherica*; 7: *Plagiochasma appendiculatum*; 8: *Marchantia papillata* subsp. *grossibarba*; 9: *Marchantia paleacea*.

Caffeic acid is already known from some mosses^[17].

plants in nature.

CONCLUSIONS

By growing bryophytes in moss house we can easily obtain plants in desired amounts for biological tests and other experiments within a reasonable time, without overharvesting them from their natural sites. It is very important for conservation of potential medicinal

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