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# An Imperative Study on Phenolic Profiling of some Bryophytes

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**ABSTRACT: The objective of the study is to identify potential active molecules in some bryophytes and their application for plant disease suppression. Four selected bryophytes viz. *Rhodobryum roseum, Isopterygium elegans, Marchantia polymorpha and Plagiochasma appendiculatum* were used for the extraction of the phenolics and were analyzed using Thin layer chromatography (TLC) and High-performance liquid chromatography (HPLC). Data analysis showed that content of vanillic acid, syringic acid, coumaric acid, chlorogenic acid and ferulic acid are higher than that of cinnamic acid, gallic acid, P-hydroxy benzoic acid and protocatechic acid. Presence of high concentration of chlorogenic acid, vanillic acid, syringic acid and coumaric acid represents presence of high anti-oxidant activity in bryophytes and is an important factor for stress resistance of bryophytes.**

**Keywords:** bryophytes, anti oxidant activity, phenolics, stress resistance.

## I. INTRODUCTION

Phytochemicals are the secondary metabolites synthesized in diverse parts of the plants having multiple biological effects, including antioxidant activity. It is well documented that, bryophytes contain a high number of biologically active compounds and are not infected by bacteria and fungi, even they grow in association with the forest floor or decomposing substrates [1-2]. All living plants must protect themselves against microbial infection through cuticle or bark, but bryophytes lack such a shield and utilize chemical weapons that are part of their alternative poikilohydric life strategy. This may be at least one important factor for the evolutionary success of bryophytes and the fact that they survived for more than 350 million years. Since the sixties of the last century, these effects were tested in laboratories. They have remarkable antioxidant activity, the extent of which depends on the extraction conditions and bryophyte species. Several medical uses seems promising, such as anti-leukemic properties and anticancer agents [3].

Traditional medicinal use of bryophytes includes different ailments viz. inflammation, skin disease, wound healing [4], viral diseases [5], treatment of plants and animals etc. In India, people of hilly areas like Uttrakhand, Himachal use bryophytes to cure burns, abscesses and to reduce pus formation, while its paste is applied on the ring worm disease of skin [6-7]. Ethno medicinal use of different bryophytes should be scientifically investigated for active principles in order to bridge between traditional knowledge and pharmacology [7-8].

Bryophytes phenolics are secondary metabolites that encompass several classes structurally diverse of natural products biogenetically arising from the shikimate-phenylpropanoids-flavonoids pathways.

They are known to possess extremely high amounts of terpenoids, phenolics (flavonoids and bibenzyl derivatives), glycosides, fatty acids and also some rare aromatic compounds [9]. Principally, extracts from all bryophyte species showed effects, however, in different degrees with regard to the species and concentration.

Bryophytes posses medicinally important bioactive compounds but with little information, their medicinal importance is not yet exploited completely. These plants produce a great range of biologically active compounds viz. phenolics, terpenoids, aromatic compounds, and acetogenins [2,10]. A lot of these constituents have typical odour, tanginess, and bitterness, and exhibit a fairly curious collection of bioactivities and medicinal properties. The main focus of the study will be to open the door for the use of different bryophyte species for plant biotechnology, suggesting that "Bryotechnology" is a rapidly evolving sector of biotechnology in general [11-12].

The aim of the paper is to carry out profiling of phenolic compounds as a source of natural antioxidants. The study will open and establish new avenues not only in medicine, cosmetics and pharmaceutical applications but also have great potential to be used in a food and agricultural field.

## II. MATERIAL AND METHODS

*A. Study area and collection of samples*

India is one of the 12 mega-biodiversity countries in the world. The large area and the variety of phytoclimatic conditions met within its different bio–geographical zones contribute to the great diversity of the Indian flora. Bryophytes are an important part of any forest ecosystem [13-15]. The northern Himalayan region is known for a luxuriant bryophyte cover, both in frequency and diversity [16]. Kumaon is situated in the state of Uttrakhand and lies between the latitude 28°44' and 30°49' N, and longitude 78°45' and 81°1' E. The topography of the area is irregular due to valleys and plateaus of various dimensions.

Bryophytes were collected from walls, roofs, or natural rocks where nearly no overhanging vegetation or tree canopy was present. They were collected only from over approx. 1.5 m above ground level, to avoid road-water splashes and areas rich with domestic wastes. Fresh material was collected in March 2010 in the Jageshwar, Almora. They were brought to the laboratory in plastic bags and identified on the basis of morphological examination and with the help of various available literatures [16-21]. The material was cleaned and dried to constant weight at room temperature.

*B. Cleaning and washing of plant material*

Cleaning and sorting of collected samples was done. After removal of foreign material (debris *etc.*), the cleaned moss samples were washed with tap water several times to remove soil and adhering dust particles. After ensuring that no soil or sand particle remained attached, the final washing was done with distilled water.

C. *Extraction of phenolics*

5 g dry mass of each bryophyte sample was used for the extraction of the phenolics by acid hydrolysis procedure of Harborne (1977) and Charpentier and Cowels (1981) [22-23]. The material was previously ground in an electric mill to a rough powder. Extraction was done through Soxhlet extractor, powdered material was taken in to a beaker and treated with 100 ml 2 N HCl and kept in a Soxhlet extractor for one hour, after that it was cooled to ambient temperature and filtered through Whatman No. 42 filter paper. The residue was washed with a fresh batch of 10 ml 2N HCl and filtered. The filtrate was extracted with dichloromethane ($CH_2Cl_2$), (3×20 ml, 20 each) using a separating funnel. The organic layer was washed with 60 ml distilled water and dried by adding about 5g hot anhydrous sodium sulphate ($Na_2SO_4$), concentration to 5ml in a rotary vacuum evaporator. Subsequently, the above method was amended for H.P.L.C. analysis. The concentrated dichloromethane fraction was cooled at 4°C and 100mg active charcoal was added. The contents were instantaneously filtered through Whatman No. 41 filter paper. The residual charcoal was washed with 2ml dichloromethane. The filtered dichloromethane fraction was dried under stream of nitrogen. The material was re-dissolved in 5ml mobile phase (0.2% acetic acid in 80:20(v/v), water and methanol). The sample was filtered through 0.2µm nylon filter disc prior to H.P.L.C.

*D. Thin layer chromatography (TLC) of different bryophytes*

15 µl of each standard as well as test solution was applied on silicagel coated plate. The plate was run in hexane: ethyl acetate solvent system in 70:30 ratios. After running the plate in T.L.C. tank, it was taken out and dried. Yellow spots of phenolics were visualized after putting dry plate in iodine chamber. After T.L.C., it has been revealed that the different bryophytes and standard has different $R_f$ values.

*E. HPLC analysis of the phenolics*

Four different bryophytes viz. *Rhodobryum roseum, Isopterygium elegans, Marchantia polymorpha and Plagiochasma appendiculatum* were selected for High-performance liquid chromatography (HPLC) analysis.

The extracts were analyzed on an analytical HPLC instrument (Waters), consisting of multi solvent delivery pump, 680 automatic gradient controller, 480 lambda max variable UV spectrometer, 7725i rheodyne injector column with 20 µL loop, using a C18 Beckman, ODS column 4.6 mm × 15 cm, ID (made in Ultrasphere, USA) guard column. The mobile phase consisted of 0.2% acetic acid in 80:20(v/v), water and methanol.

The flow rate was 1 mL/min and the injection volume was 0.2 µL. Detection was performed using a programmable photodiode array detector. The phenolic compounds in each sample were identified by comparing their retention times and UV–V is spectra in the 254 nm range with individual standards.

*F. Data analysis*

In the experimental conditions of this study, we established calibration curves for standards and regression coefficients. The linearity of calibration curves for all compounds was very good ($R^2$>0.99). Based on calibration curves and considering the dilutions made, we calculated content of different phenolic compounds. Detection limits were determined in accordance with the standard deviations at minimum concentrations and slopes values of each analyzed compound.

## III. RESULT AND DISCUSSION

The chemistry of bryophytes is not well known. The available data indicate interesting chemical constitutions of some bryophyte species, i.e., active and new compounds are to be found within bryophytes, especially mosses and liverworts. In this study, two liverwort and two moss species were studied: *Rhodobryum roseum, Isopterygium elegans, Plagiochasma appendiculatum and Marchantia polymorpha*.

In TLC the $R_f$ values were obtained in 6 different standards 0.35 for Gallic acid, 0.39 for Protocatechuic acid, 0.44 for Chlorogenic acid, 0.46 for Caffic acid, 0.47 for Vanillic acid and 0.61 for Syringic acid. However, in case of *Rhodobryum giganteum* 4 different compounds were separated showing 0.18, 0.33, 0.45 and 0.73 as $R_f$ values. In the same way, *Isopterygium elegans,* also depict differentiation of 4 compounds with 0.18, 0.32, 0.60 and 0.72 as $R_f$ values. In addition, 3 compounds were separated in *Marchantia polymorpha* and *Plagiochasma appendiculatum* showing $R_f$ values as 0.32, 0.45, 0.52 and 0.19, 0.32, 0.45 respectively.

Data analysis of HPLC showed that content of vanillic acid, syringic acid, Coumaric acid, chlorogenic acid and ferulic acid are higher than that of cinnamic acid, gallic acid, P-hydroxy benzoic acid and protocatechic acid (Fig. 1). Content of vanillic acid is found to be on an average of 8 mg/gm on three bryophyte species *Plagiochasma appendiculatum, Rhodobryum roseum, Marchantia polymorpha*, while in the case of *Isopterygium elegans,* it is found to be only 1.9851 mg/gm (Fig. 2-5). Coumaric acid content found to be on an average of 4.7 mg/gm on all four species of bryophytes. Syringic acid content of *Isopterygium elegans* and *Marchantia polymorpha* found to be on average of 5.6 mg/gm while for *Plagiochasma appendiculatum* and *Rhodobryum roseum,* it is found to be 7.2954 and 7.2612 mg/gm respectively (Table 1).

Content of P-hydroxybenzoic acid and protocatechic acid was found to be on an average of 1 mg/gm for all the four bryophyte species. Similarly ferulic acid content was found to be on average of 2.2 mg/gm for all four species. Chlorogenic acid was found to be in different concentration for all the four different species as mentioned in the table.

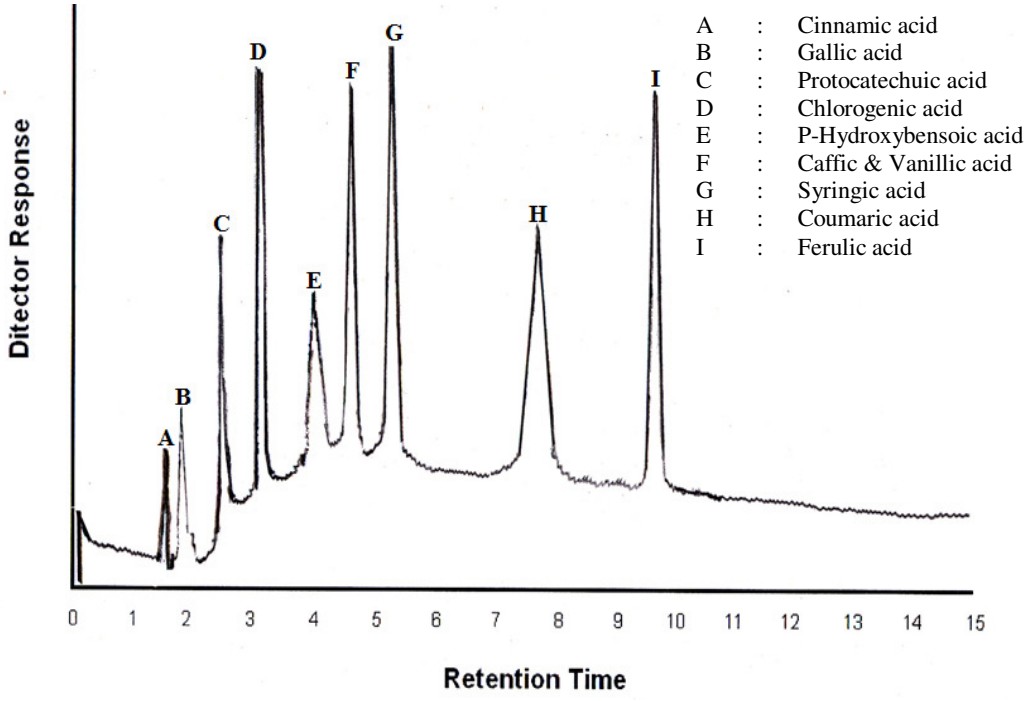


**Fig. 1.** Chromatograms of the phenolics of different standards.

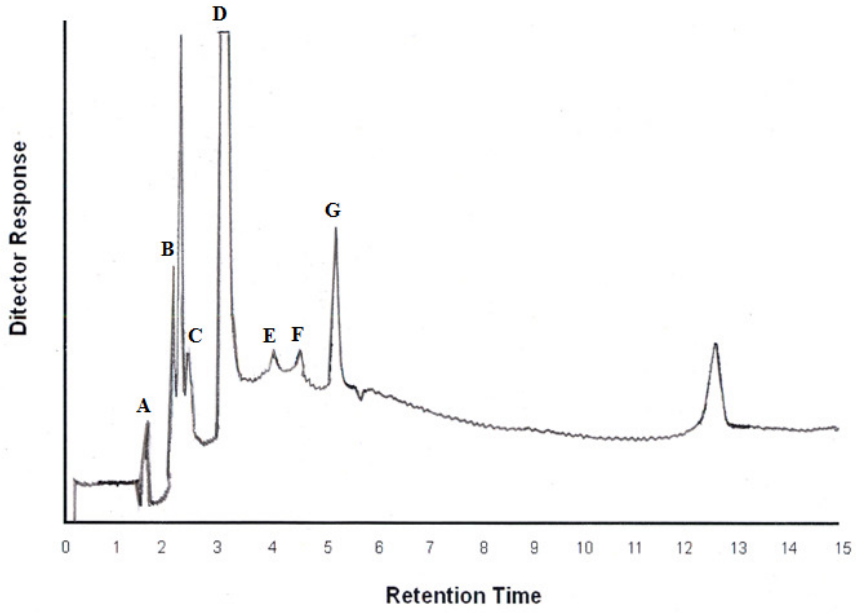


**Fig. 2.** Chromatograms of the moss methanol extract of *R. roseum* [(A). Cinnamic acid, (B). Gallic acid, (C). Protocatechuic acid, (D). Chlorogenic acid, (E). P-Hydroxybensoic acid, (F). Caffic & Vanillic acid, (G). Syringic acid] of different phenolics.

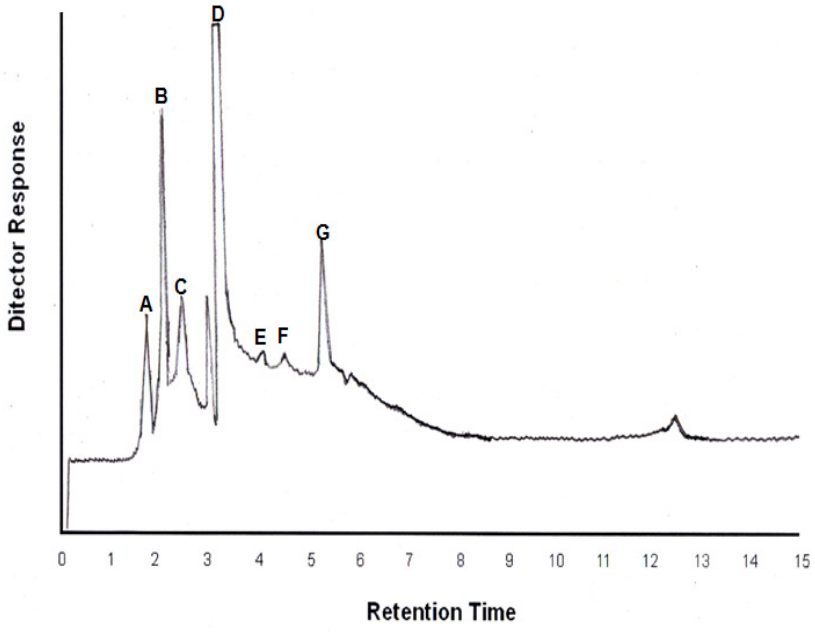


**Fig. 3.** Chromatograms of the moss methanol extract of *I. elegans* [(A). Cinnamic acid, (B).Gallic acid, (C). Protocatechuic acid, (D). Chlorogenic acid, (E). P-Hydroxybensoic acid, (F). Caffic & Vanillic acid, (G). Syringic acid] of different phenolics.

*Plagiochasma appendiculatum* contains 9.0872 mg/gm amount of chlorogenic acid, while *Isopterygium elegans* contains 5.0387 mg/gm, where as *Marchantia polymorpha* contain 5.51 mg/gm of chlorogenic acid (Table 1). Presence of high concentration of chlorogenic acid, vanillic acid, syringic acid and coumaric acid represents presence high anti-oxidant activity in bryophytes. More specifically, chlorogenic acid production is usually initiated in plant by several factors including changes in environmental conditions, plant stress and pest infestation. It has been reported in higher plants that chlorogenic acid concentration gets increased more than twice while grown in harsher condition.

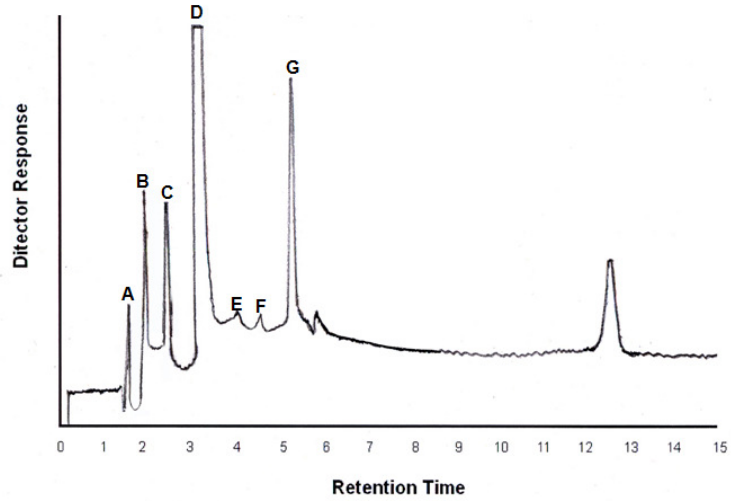


**Fig. 4.** Chromatograms of the liverwort methanol extract of *P. appendiculatum* [(A). Cinnamic acid, (B). Gallic acid, (C). Protocatechuic acid, (D). Chlorogenic acid, (E). P-Hydroxybensoic acid, (F). Caffic & Vanillic acid, (G). Syringic acid] of different phenolics.

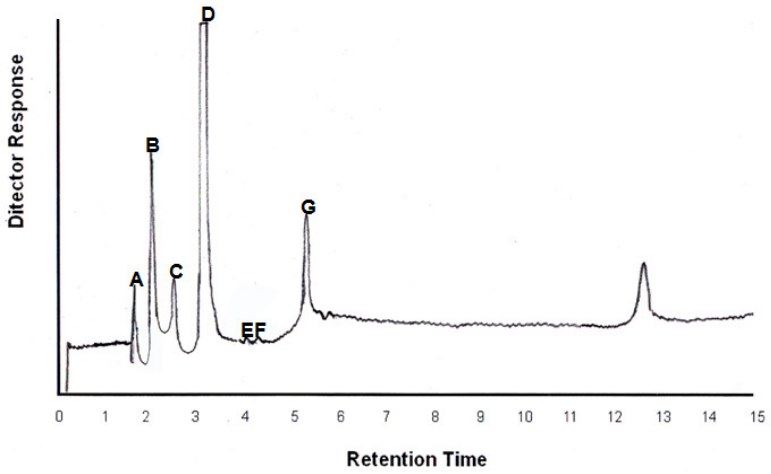


**Fig. 5.** Chromatograms of the liverwort methanol extract of *M. polymorpha* [(A). Cinnamic acid, (B). Gallic acid, (C). Protocatechuic acid, (D). Chlorogenic acid, (E). P-Hydroxybensoic acid, (F). Caffic & Vanillic acid, (G). Syringic acid] of different phenolics.

So presence of constitutive high level of chlorogenic acid is an important factor for stress resistance of bryophytes. Bryophytes are regular producers of polyphenols including flavonoids, but it is in the vascular plants that the full range of polyphenols is found [24]. Moreover, the existence of a characteristic phenolic pattern, which taxonomists use to separate species, can also have enough adaptive value for survival through natural selection. Phenolic compounds have been synthesized during the course of evolution by different plant species when the presence of a particular secondary metabolite has conferred a selectionary advantage on the plant containing it. Caffeic acid is already known from some mosses [25]. Apigenine is a pale yellow pigment present in many plants from the families Apiaceae and Asteraceae with an antitumor effect. Apigenin and its derivates are known to be present in mosses and to have biological effects [26]. In mosses, p-coumaric and ferulic acids are known to be present in moss spores. They are precursors of lignin, which is not common in moss gametophytes, but both p-coumaric and ferulic are present in moss gametophytes where lignin was not detected [27]. Although phenolic compounds are known to be present in bryophytes, this knowledge is mainly based on liverworts not mosses and their presence; diversity and distribution within different species remain for further studies [28-29].

**Table 1: Quantification of phenolic compounds (mg/g DW) from different bryophytes.**

| Genus | Phenolic compounds (mg/g DW) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cinnamic acid | P-hydroxy benzoic acid | Protocatechic acid | Chlorogenic acid | Caffeic acid | Coumaric acid | Syringic acid | Ferulic acid | Vanillic acid | Gallic acid |
| *I. elegans* | 0.029 | 1.0387 | 1.2292 | 5.0364 | 1.6629 | 4.7921 | 5.931 | 2.2958 | 1.9851 | 0.0965 |
| *P. appendiculatum* | 0.0363 | 1.0654 | 3.5326 | 9.0872 | 2.8185 | 4.7163 | 7.2954 | 2.2595 | 8.3027 | 0.2187 |
| *R. roseum* | 0.0285 | 1.0943 | 1.3407 | 4.0879 | 5.7453 | 4.7329 | 7.2612 | 2.288 | 8.1442 | 0.058 |
| *M. polymorpha* | 0.0367 | 1.0899 | 1.1998 | 5.5138 | 9.3227 | 4.7145 | 5.6009 | 2.2842 | 8.1695 | 0.0761 |

Thus, present study is one first approach to the identification of phenolics in the bryophytes *Rhodobryum roseum, Isopterygium elegans, Plagiochasma appendiculatum and Marchantia polymorpha* in India.

An overview of the bioactivity data obtained from the current investigation, it can be highlighted that the tested possesses potent antioxidant activity. There is a need for further investigation to explore the promising antibacterial and antifungal properties of the plants screened in the current study.

## IV. CONCLUSION

This study indicates that the extracts obtained from bryophytes have remarkable antioxidant activity, the extent of which depends on the extraction conditions. The principal factors that contribute to the efficiency of extraction are the type of solvent, temperature, ratio solvent: bryophyte mass, etc. Bryophytes are potentially valuable source of bioactive materials but very scanty of them have been studied chemically. They are small plant groups, with number of terpenoids and phenolic compounds, may show interesting biological activity.

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