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ESTIMATION OF PHENOLIC COMPOUND QUERCETIN BY HPLC ANALYSIS AND ANTIFUNGAL POTENTIAL OF METHANOLIC EXTRACT OF CERTAIN *BRYUM* SPECIES FROM RAJASTHAN (INDIA)

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Abstract

The present study was carried out to estimate the phenolic compound quercetin from moss *Bryum argenteum, Bryum capillare, Bryum cellulare* by high-performance liquid chromatography (HPLC). Further the antifungal potential of these *Bryum* species were evaluated against test fungi Drechslera *maydis,* the causal organism of southern corn leaf blight using pour plate method. Methanolic extract was used against selected test fungi for antimicrobial assay. In HPLC methanol and solvent acetonitrile were used in the ratio of 60:20:20 as mobile phase. Quantification of quercetin was carried out by Athena C18 column and UV absorbance was measured at 262 mm with flow rate of 1.0 ml/min. The results revealed that the retention time of standard was found to be 1.81. The methanolic extract of all the *Bryum sps* also showed a peak with retention time of 1.81 which confirmed the presence of quercetin in *B.argenteum*, *B. capillare*, *B. cellulare* methanolic extracts. Methanolic extracts of all the plants showed antifungal activity potential but in varying degrees. The highest inhibition in colony diameter and fresh weight of colony was observed in methanolic extract of *Bryum argenteum* followed by *B. capillare*, *B. cellulare*.

Key words : Bryophytes, *Bryum,* Antifungal potential, Phytochemical screening, HPLC, Phennolic compound, Quercetin.

Introduction

Bryophytes, including liverworts (Marchantiophyta), hornworts (Anthocerotophyta) and mosses (Bryophyta) are a diverse group of land plants that usually colonize habitats with moist or extremely variable conditions. Traditionally, because of their antimicrobial activity, mosses were used as a natural medicine in the Indian culture (Frahm,2001) and as natural diapers (Ando and Matsuo,1984). Today, mosses and liverworts are interesting for biotechnological use in medicine, agriculture, and pharmacology. Liverworts have been proposed as ideal models for genetic studies and biotechnological applications (Decker *et al.,* 2003). The search for plants with antimicrobial activity has grown in importance in recent years, due to a growing concern about increase in the rate of infection caused by antibiotic-resistant microorganisms Asakawa (2008,2004) has analyzed approximately 1000 bryophyte species from the world total of 27,000. However, few studies have been carried out about the antimicrobial properties of European bryophytes. One of the features that helped bryophytes to survive and maintain their place in today's land plants is their content of biologically active compounds. Although bryophytes are very familiar, their medicinal importance is not exploited completely. However, few studies have been carried out about the antimicrobial properties of bryophytes. In literature, reports have been found about antibacterial activity of 23 bryophytes species (Asakawa, 2007). Donald and Bishop (1953) evaluated that green plants possess antimicrobial substance which inhibit microbial growth. Deora and Guhil (2014) collected bryophytes from various parts of Rajasthan from different ecological conditions and further to divide entire state in different bryoecological zones with phytogrographical aspects.

Mekuria *et al*. (1999) studied the effect of moss extracts against phytopathogenic fungi and showed that alcoholic extract of moss was active against *candida albicans*. Keyhanian *et al*. (2002) studied the effect of

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several fungicidal and insecticidal seed treatment to control rapeseed seedling damaging plants and observed that fungicidal and insecticidal compounds were active against tested pathogen. Subhisha and Subramonian (2005) screened the extracts of *Pallavicinia leyelli* and evaluated that it posses antifungal activity. Iwashina (2003) reported that flavonoid compounds are widely distributed in bryophytes and posses many biological activities against plants, fungi and other microorganisms.

Ilham *et al.* (2006) studied acetonic and methanolic extracts of *Palustriella comutata* against 11 bacteria, 1yeast and 8 moulds. Deora *et al.* (2007) studied three bryophytes *Plagiochasma articulalum, Anthoceros longii, Fissidend bryoides;* liverwort, hornwort and moss respectively for their antibiotic effect on *Agrobacterium tumifacians*. The result showed that mosses are highly antibiotic in nature followed by hornwort and liverworts. The aim of the present study was to find out the antifungal acitivity of *Bryum argenteum, Bryum capillare, Bryum cellulare* against phytopathogenic fungi *Drechslera maydis*. Deora and Guhil (2015) studied the antifungal activity of cold and boiled water crude extract of *Bryum capillare* against a plant pathogen *Drechslera maydis*. The results revealed that *B. capillare* may have some potent active chemicals which showed antifungal activity resulting suppression of fungal growth.

Materials and Methods

Plant material and extract preparation

The moss *Bryum argenteum, Bryum capillare, Bryum cellulare* was collected in rainy season (2017-18) from Mt. Abu, District. Sirohi (Raj.) around Nakki Lake, Guru Shikhar and Sunset point in both vegetative and sporophytic phases. All the three plants were washed with distilled water to remove soil particles, attached litter, dead material. For methanolic extract preparation, plant material weighted was grinded in mortar and pestle with equal amount of methanol till the formation of fine paste, then it was centrifuged and filtered. This filtrate was used as (100%) crude extract then it was serially diluted by double distilled water to prepare various concentrations from 10-100 per cent.

Test Organism

The pure culture of test fungi *Drechslera maydis* was obtained from the Department of Pathology RCA, (Udaipur, Rajasthan) India. This test organism was sub-cultured in laboratory at 25ºC temperature to obtain its pure isolates.

Preparation of Medium

PDA (Potato Dextrose Agar) medium was prepared and autoclaved for *Drechslera maydis*.

Screening of antifungal Activity

Antifungal activity of bryophyte fraction was determined by using pour plate method. The plant extracts of 10 ml each were first poured into Petri dishes. Then, 10 ml molten PDA was poured aseptically on the plant extract in the Petri dishes and swirled round for even dispersion of the extract into the agar. The methanolic extract was incorporated at different concentrations of 100, 80, 60, 40, 20 and control. A 5 mm mycelium agar disc of *D. maydis* was released into the poisoned agar/ extracts incorporated into PDA. The treatments were replicated three times, incubation period for antifungal activity was 72 hrs. The average diameter of resultant colony was measured after incubation. The growth of *D. maydis* mycelium on PDA without any amendment was used as control. The percent inhibition of mycelial growth by plant extract was calculated by using the formula given by Vincent (1927).

Phytochemical Analysis

Qualitative phytochemical analysis of a moss *Bryum argenteum*, *Bryum capillare*, *Bryum cellulare* extract was done by the methods of Trease and Evans (2002) to detect the presence or absence of certain bioactive compounds..

Test for Flavonoids

Ferric-chloride test: Plant extract was taken in a test tube and few drops of freshly prepared neutralized ferric chloride solution was added. Intense green colour of the solution indicated the presence of flavonoids.

Lead acetate test

Plant extract was taken in a test tube and added few drops of 10% lead acetate solution. The flavonoids from plant extract get precipitated in the presence of lead acetate giving a bulky white appearance.

Sodium hydroxide test: 5 ml of 20% NaOH is added to equal volume of plant extract. A yellow solution indicated the presence of flavonoids**.**

Test for Terpenoids

Salkowaski test

To find the presence of terpenoids in the extract**,** plant extract was taken in test tube and few drops of concentrated sulphuric acid were added. After shaking well it was allowed to stand. The lower layer turned yellow indicating the presence of terpenoids.

Liebermann-Burchardt test

Plant extract was taken in a test tube and acetic anhydride was added, It was mixed well and then concentrated sulphuric acid was added from the side of

the test tube. Deep red colour indicated the presence of terpenoids.

Test for Sterols

Salkowaski test

To test the presence of sterol plant extract was taken in test tube and few drops of sulphuric acid were added. After shaking well, allowed to stand. The lower layer turned red indicating presence of sterols.

Liebermann-Burchardt test

Plant extract was taken in a test tube and few drops of acetic anhydride were added and mixed well. When concentrated sulphuric acid was added from the sides of the test tube, it showed a brown ring at the junction of two layers and the lower layer turned green, indicated the presence of sterols.

Test for Alkaloids

Mayer's test

In a few ml of plant extract, few drops of Wagner's reagent were added by the side of test tube. A reddish-brown precipitate was not observed; hence presence of alkaloids was not confirmed.

Hager's test

One or two ml of Hager's reagent was added in a few ml of extract. A prominent yellow precipitate was not found, hence alkaloids were found to be absent.

Test for Anthraquinones

Borntrager's test

About 0.5ml of extract was added with 5 ml chloroform and shaken for 5 minutes. The extract was filtered and the filtrate shaken with an equal volume of 100 per cent ammonia. No layer formation indicated the absence of anthraquinones.

Test for Cardiac Glycoside

Keller killeni test

Few ml of extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution then 1ml of concentrate sulphuric acid was added, brown ring obtained at the interface indicates the presence of de-oxysugar characteristic of cardiac glycoside.

Test of Saponins

The extract was diluted with distilled water and made up to 20ml. The suspension was shaken in a graduated cylinder for 15 minutes. No froth formation indicated the absence of saponins.

Phytochemical tests performed according to the above described methods showed the presence of flavonoids, terpenoids, sterols and cardiac glycosides in *Bryum argenteum*, *Bryum capillare*, *Bryum cellulare* plant extracts. Alkaloids, anthraquinones and saponins were not detected.

HPLC Analysis

For HPLC analysis methanolic extracts of all the plants along with standard solution were sent to SICART, Anand (Gujarat) which confirmed that methanolic extracts of *Bryum argenteum*, *Bryum capillare*, *Bryum cellulare* contain standard quercetin.

Chromatography Details

Flow rate	:	1.0ml/min
Detection	:	262mm
Injection quantity	:	20µL
Column used	:	C18 (250 mm X4.6 mm X5 µm)
Column temperature	:	Ambient
Mobile phase ratio	:	60:20:20
Mobile phase	:	Methanol
ACN	:	Water

Results and Discussion

Phytochemical analysis

Preliminary phytochemical tests performed according to the authentic methods showed the presence of flavonoids, terpenoids, sterols and cardiac glycosides in *Bryum argenteum*, *Bryum capillare*, *Bryum cellulare* plant extracts. Alkaloids, anthraquinones and saponins were not detected (Table 1).

HPLC analysis

In HPLC analysis the results showed that the retention time of standard Quercetin was found to be 1.81 (Graph 1). The methanolic extract of *B.argenteum*, *B. capillare*, *B.cellulare* also show a peak with retention time of 1.81(Kumar *et al*.2009) (Graph 2,3,4) which confirmed the presence of quercetin in *B.argenteum*, *B.*

Table 1: Preliminary Phytochemical analysis of *Bryum argenteum*, *Bryum capillare*, *Bryum cellulare* methanolic extracts.

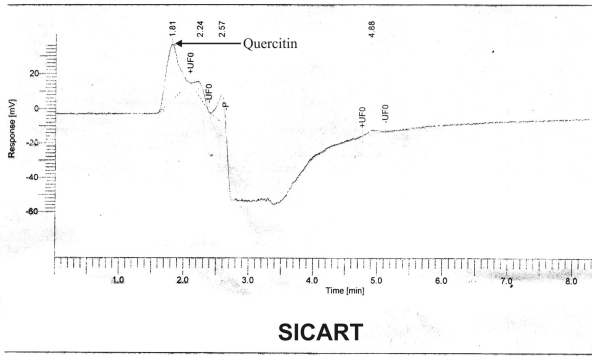
Compound	Methanolic extract
Alkaloids	-
Anthoquinones	-
Cardiac Glycosides	+
Flavonoids	+
Saponins	-
Sterols	+
Terpenoids	+

(+) = phytoconstituents present and (-) = phytoconstituents absent.

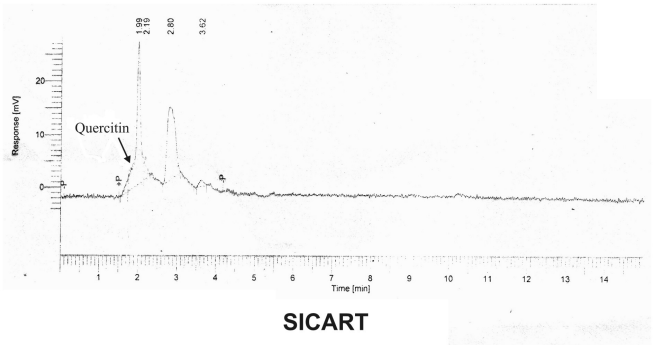
capillare, *B. cellulare* extracts. The outputs of this analysis were received as chromatograms, where the peaks were representing the number of components present and the peak heights and area were representing the concentrations of those components.

Antifungal activity

Antifungal activity of methanolic extract of *Bryum argenteum, Bryum capillare,* Bryum *cellulare* against *Drechslera maydis* was assayed and results are presented in (Table 2) (Fig. 5 and 6). The observations revealed that significant reduction in the growth of test fungi was reported in all concentration ranging from 10-

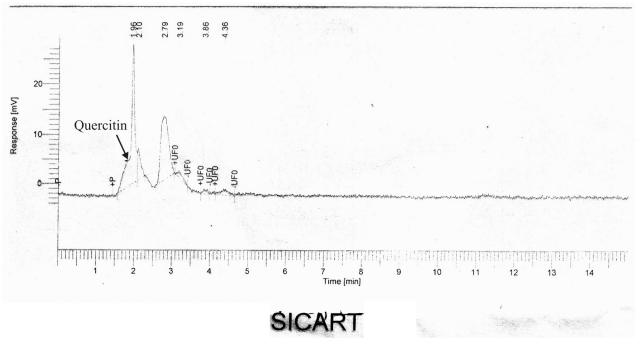


Graph 1: Standard (Quercitin)

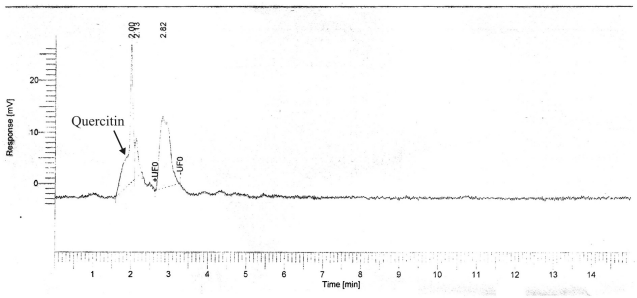


Graph 2: Methonolic extract of *B. argenteum*

100 per cent concentration. In *Bryum argenteum, Bryum capillare, Bryum cellulare* colony diameter was 13.46, 18.43 and 26.43 mm whereas colony fresh weight was 62.80,62.03 and 71.13 gm respectively in 10 per cent concentration. In 100 per cent concentration colony diameter was 2.90, 4.40 and 6.43 mm and colony fresh weight was 22.50, 28.00 and 39.46 gm. The bryophyte extracts prepared in different solvents were found effective in reducing fungal growth as they posses various secondary metabolites which acts as antifungal agent. The activity of different bryophytes was in order of *B. argenteum>B. capillare>B. cellulare* as the bioactive



Graph 3: Methonolic extract of *B. capillare*



Graph 4: Methonolic extract of *B. cellulare*

compounds are more soluble in organic solvents. The present results showed similarity with the results of Deora *et al.* (2010) who determined the antifungal activity of a moss against certain phytopathogenic fungi. Deora and Suhalka (2010) studied the effect of liverwort *R. gangetica* against *F. moniliforme* and found cold water extract was more effective than boiled water extract. Bodade *et al*. (2008) evaluated the antimicrobial effect *of Plagiochasma appendiculatum*, *Thuidium cymbifolium, Bryum cellulare, Bryum argenteum* and *Racomitrium crispulum* on 12 microorganisms. Solubility data and antibiotic spectra of the active plants indicated the occurrence of the variety of antibiotic substances among bryophytes.

Conclusion

Result of the present study concludes that Quercetin is present in all the three species of *Bryum* and the methanolic extract of all the selected mosses (*Bryum argenteum, Bryum capillare, Bryum cellulare*) exhibited varying degrees of antifungal activities against *D. maydis* but methanolic extract of *Bryum argenteum* was found to be most effective against *D. maydis* followed by *Bryum capillare and Bryum cellulare*. The result of this work corresponded to the findings of Deora and Guhil [21] who find out that the *Bryum argenteum* has a broad

Table 2: Comparative effect of methanolic extracts of *Bryum argenteum*, *Bryum capillare* and *Bryum cellulare* against *Drechslera maydis*.

S.No.	Extract Concentration	*Bryum argenteum*		*Bryum capillare*		*Bryum cellulare*	
		Colony Diameter (Mean)	Fresh weight (Mean)	Colony Diameter (Mean)	Fresh weight (Mean)	Colony Diameter (Mean)	Fresh weight (Mean)
1	Control	26.1333	75.8667	30.6667	81.1333	35.3333	90.2667
2	10	18.1733	65.6667	22.5000	62.9333	27.3333	73.9000
3	20	14.5000	58.8667	15.0000	56.2667	17.3667	71.4333
4	40	10.2667	43.4667	12.2000	53.9333	14.9333	67.1333
5	60	8.4333	40.0333	9.5000	44.4000	11.6667	47.8000
6	80	6.3667	33.4000	7.2667	38.8667	9.1000	45.0333
7	100	4.6633	29.9000	6.1333	32.8000	7.0667	41.9000
	GM	12.5724	49.6000	14.5667	52.9048	19.8286	62.4952
	Se	0.2401	0.2296	0.1826	0.2917	0.2350	0.2074
	CD5%	0.7283	0.6963	0.5538	0.8847	0.7129	0.6291
	CD1%	1.0115	0.9671	0.7691	1.2288	0.9901	0.8737
	CV	3.31	0.80	2.17	0.95	2.05	0.57

*** Significant at 5% and 1% respectively.

spectrum antifungal activity against the phytopathogenic fungus *Curvularia lunata*.

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