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ESTIMATION OF QUERCETIN BY HIGH PERFORMANCE CHROMATOGRAPHY AND ANTIFUNGAL ACTIVITY OF MOSS *PHILONOTIS REVOLUTA*

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ABSTRACT: Bryophytes- the small, slow growing group of plants is stockroom of naturally occurring materials. They contain a high number of biologically active compounds, however their use as a natural fungicides is negligible. In the recent years bryophytes has emerged as a potential biopharming tool for production of complex biopharmaceuticals. In order to evaluate potential use of bryophytes as an antifungal compounds their basic chemical contents and the secondary metabolite profile was determined. Among secondary metabolites flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom. Flavonoids like Rutin and quercetin possess many biochemical effects. An HPLC method was developed for the estimation of quercetin from methanol extract of *Philonotis revoluta* bryophyte. The results of the phytochemical screening using different extracts revealed the presence of alkaloids, flavonoids, phenols, etc. in the moss plant and investigated against plant pathogenic fungi using different parameters. Results showed significant reduction in pathogenic growth.

INTRODUCTION: Bryophytes are the second largest group in the plant kingdom with about 25'000 bryophyte species and they can be found in any kind of ecosystems [1-2]. In comparison with higher plants the use of bryophytes for human consumption is negligible due to their low caloric value [3] and poor organoleptic properties. Recent research demonstrates a presence of a large number of biologically active substances in the composition of bryophytes. More specifically bryophytes demonstrate antibacterial, antifungal, antiviral activities, antioxidant, antiplatelet, antithrombin, insecticidal, neuroprotective activities, as well as cytoxicityin respect to cancer cells [4- 5].

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Biological control of plant diseases would help in preventing increase of pathogen population and also health hazards because of the use of various synthetic chemicals. Biological control through the use of plant extracts is a potential, non-chemical means of controlling plant diseases by reducing inoculum level of pathogen. There are reports of studies performed in the last century regarding the chemical composition of bryophytes [6]. However, in the past two decades, biologists, chemists and pharmacologists have been interested in this group of plants. The aim of this work was to analyze phytochemically the extract of the bryophyte *Philonotis rvoluta*- a moss and to investigate its potential on the plant pathogen

MATERIALS AND METHOD:

Collection of plant material and extract preparation: The plant material was collected in rainy season from Mt. Abu district Sirohi (Rajasthan) India from different localities in both vegetative and sporophytic phages.

The collected plant material was washed with tap water followed by double distilled water (DDW) till all the debris and soil particles were removed. The collected plant material was identified with the help of moss flora of Rajasthan, India [7].

Three types of plant extracts were prepared methanolic, acetonic and aqueous.

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. The same method was adopted for acetone and aqueous extract preparation. Except grinding the plant material with acetone and water respectively instead of methanol.

Test organisms: *Helminthosporium turcicum* (Pass). Leonard and Suggs was used for the evaluation of antifungal potential of plant extracts of bryophyte. *Helminthosporium turcicum* was brought from the Department of Pathology, Maharana Pratap Agriculture College, Udaipur.

Bioassay of antifungal activity: The test fungi *Helminthosporium turcicum* was bioassayed by different parameters like colony diameter and fresh weight of test fungi in different concentrations of aqueous, acetonic and methanolic extracts.

Colony diameter and fresh weight assay: The method of poisoned food technique [8] was followed. Autoclaved media and crude extracts of different concentrations were poured in petriplates in 1:1 ratio. After solidification, an agar block containing growing mycelium of test fungi were placed in four corners of petriplates. Different concentrations were used with three replications. Extract free medium served as control. Colony diameter and fresh weight were measured after 72 hrs. Per cent inhibition of mycelial growth was calculated by the formula [9].

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition

C = Colony diameter in control

T = Colony diameter in treatment

Phytochemical Analysis: The extracts of *Philonotis revoluta* were subjected to various phytochemical tests by various methods [10] to detect the presence or absence of certain bioactive compounds such as flavonoids, sterols, saponins, anthroquinones and cardiac glycosides in the plant extracts.

TABLE 1: PHYTOCHEMICAL SCREENING OF THE BRYOPHYTE EXTRACTS

Phytochemicals	*P.revoluta*		
	Aqueous Extract	Acetonic extract	Methanolic extract
Flavanoids	+	+	+
Saponins	-	-	-
Alkaloids	-	-	-
Terpenoids	+	+	+
Sterols	+	+	+
Anthroquiones	-	-	-
Phenols	+	+	+
Cardiac glycosides	+	+	+

+ = Phytoconstituent present

- = Phytoconstituent absent

HPLC Analysis: HPLC (High performance liquid chromatography) was done for the identification of specific metabolite from methanolic extract of *P. revoluta.* For identification of quercetin present in the moss, standard sample solution of quercetin was run along with the plant extracts, the peak of the analyte was confirmed by comparing it's retention time with that of reference standard [11]. All the HPLC experiments were perfomed at SICART (Sophisticated instrumentation centre for applied research and testing) Anand, Gujarat.

Methanolic extract of *Philonotis revoluta* was analyzed for the presence of secondary metabolites using a perkin elmer series 200 HPLC system Further detail is as follows.

Chromatography

Flow rate : 0.9 ml/min

Detection : 340 nm

Injection quantity : 50 l

Column used : Hichrom C18 (150 mmx4.6 mm i.d., 5)

Column temperature : 35C

Mobile phase ration : 70:30 % v/v

Mobile phase : 0.5% Formic acid: Acetonitrile

RESULTS AND DISCUSSION: HPLC analysis of *Philonotis revoluta* methanolic extract showed

the presence of quercetin as a principle secondary metabolite. Antifungal activity of *Philonotis revoluta* extract against *Helminthosporium turcicum* was assayed and observations revealed that significant reduction in the growth of test fungi was reported in all concentration ranging from 10-100 per cent. Among the three extracts tested, methanolic extract at 100 per cent concentration caused significant inhibition of growth.

Decrease in colony diameter from 25.75 to 9.50mm, fresh weight 1.95 to 0.76gm 35.55 in 10-100 per cent concentration respectively was observed in aqueous extract **(Table 2, Fig. 1).** An increase in per cent mycelial inhibition was found from 12.71 to 67.80 in 10 to 100 per cent concentrations of extract. In acetonic extract **(Table 3, Fig. 2)** 24.73mm colony diameter was reported at 10 per cent which decreased up to 6.50mm at 100 per cent concentration of the extract, fresh weight was 1.89 and 0.52gm at 10 and 100 per cent concentration respectively compared to the control.

TABLE 2: SHOWING THE EFFECT OF DIFFERENT CONCENTRATIONS OF *P. REVOLUTA* AQUEOUS EXTRACT ON *H. TURCICUM*

S.no.	Extract concentrations (%)	Colony diameter (mm)		Fresh weight (gm)		Per cent inhibition	
		Mean	SD	Mean	SD	Mean	SD
1.	Control	29.5000	0.0000	2.1300	0.0000	0	
2.	10	25.7500	0.0000	1.9500	0.0000	12.7100	0.0000
3.	20	20.3700	0.0062	1.7500	0.0000	30.9500	0.0001
4.	40	18.1200	0.0059	1.2100	0.0000	38.5800	0.0000
5.	60	16.8700	0.0057	0.9700	0.0000	42.8100	0.0002
6.	80	12.5200	0.0000	0.8000	0.0000	57.5600	0.0002
7.	100	9.5000	0.0000	0.7600	0.0000	67.8000	0.0000
	GM	18.9471	6.6546	1.3671	0.5396	41.7350	18.3096
	Se	0.0000		0.0000		0.0000	
	CD5%	0.0000		0.0000		0.0000	
	CV	0.00		0.00		0.00	

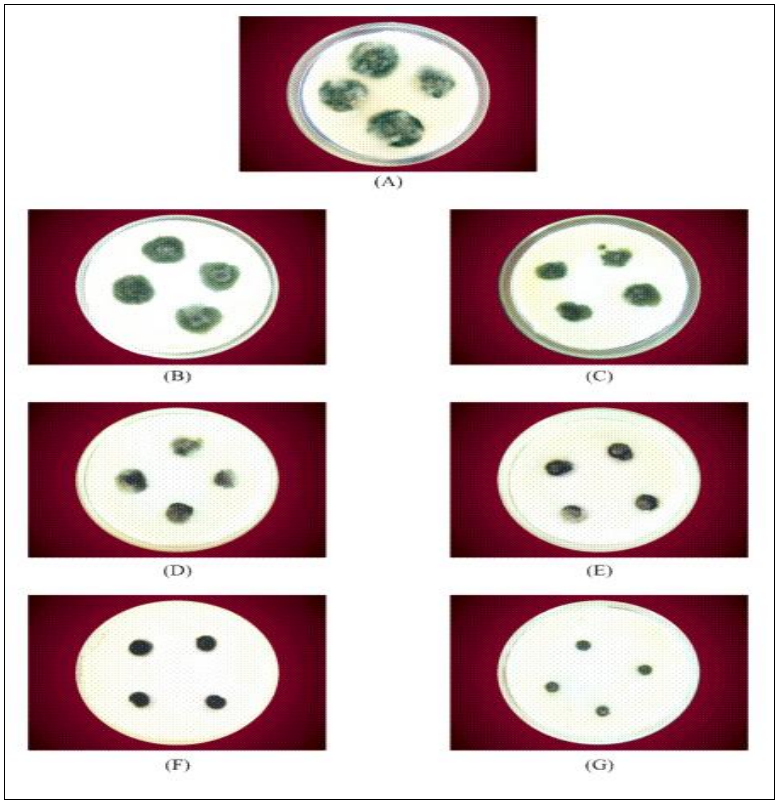


FIG. 1: COLONY DIAMETER, FRESH WEIGHT AND PER CENT INHIBITION OF MYCELIAL GROWTH OF *HELMINTHOSPORIUM TURCICUM* IN THE CONTROL (A); 10(B), 20(C), 40(D), 60(E), 80(F) AND 100 (G) PER CENT CONCENTRATIONS OF *PHILONOTIS REVOLUTA* AQUEOUS EXTRACT

The per cent mycelial inhibition also varied from lower to higher concentrations which was 12.71 at 10 per cent and increased to 67.80 at 100 per cent extract concentration.

TABLE 3: SHOWING THE EFFECT OF DIFFERENT CONCENTRATIONS OF *P. REVOLUTA* ACETONIC EXTRACT ON *H. TURCICUM*

S. no.	Extract concentrations (%)	Colony diameter (mm)		Fresh weight (gm)		Per cent inhibition	
		Mean	SD	Mean	SD	Mean	SD
1.	Control	28.7333	0.2519	2.1000	0.0200	0	
2.	10	24.7333	0.3787	1.8933	0.0153	13.9167	1.3544
3.	20	20.2667	0.2081	1.6933	0.0153	29.4600	1.2179
4.	40	17.5333	0.3215	1.0667	0.0306	38.9767	1.2176
5.	60	14.4000	0.3605	0.8633	0.0306	49.8900	0.8617
6.	80	10.3000	0.3000	0.7500	0.0300	64.1567	0.7313
7.	100	6.5000	0.2646	0.5200	0.0200	77.3767	0.9948
	GM	17.4952	7.4402	1.2695	0.5872	45.6294	21.7692
	Se	0.1750		0.0139		0.6266	
	CD5%	0.5309		0.0420		1.9308	
	CV	1.73		1.89		2.38	

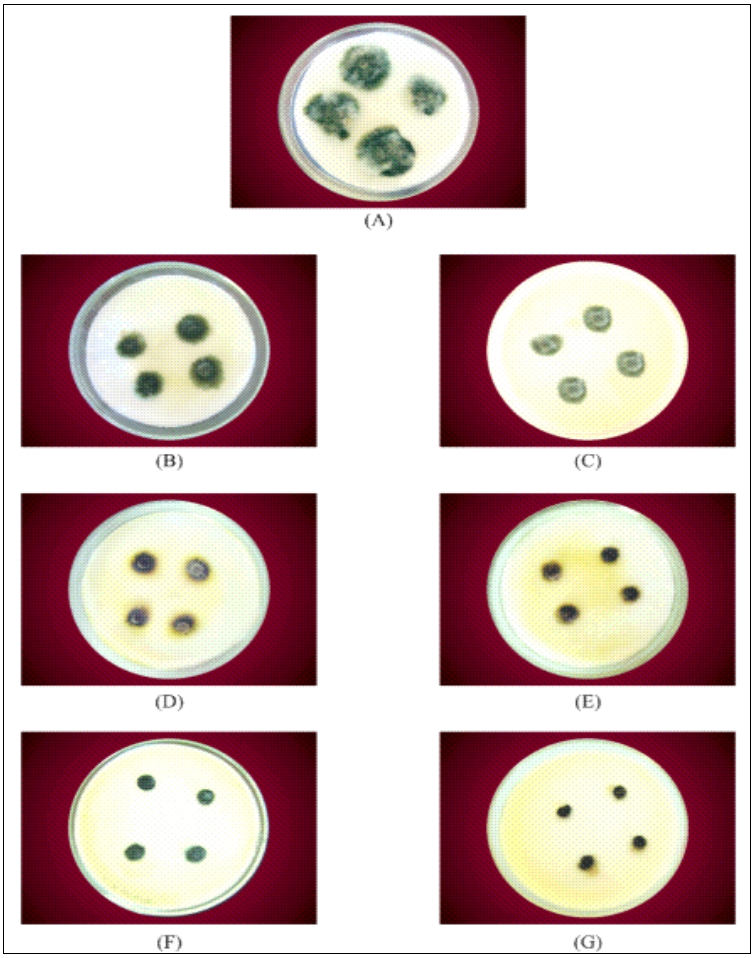


FIG. 2: COLONY DIAMETER, FRESH WEIGHT AND PER CENT INHIBITION OF MYCELIAL GROWTH OF *HELMINTHOSPORIUM TURCICUM* IN THE CONTROL (A); 10(B), 20(C), 40(D), 60(E), 80(F) AND 100 (G) PER CENT CONCENTRATIONS OF *PHILONOTIS REVOLUTA* ACETONIC EXTRACT

The results of methanolic extract **(Table 4, Fig. 3)** are as follows- in the control, colony diameter was maximum (28.16mm) it decreased significantly to 24.16mm at 10 per cent and 4.40mm at 100 per cent concentration of the extract. Similarly fresh weight was maximum (2.06gm) in the control and decreased to 1.85gm at 10 per cent and 0.47gm at 100 per cent extract concentration.

Per cent mycelial inhibition increased along with increasing concentrations and it was reported zero in the control which increased significantly to 13.80 at 10 per cent and reached up to 84.32 at 100 per cent concentration.

TABLE 4: SHOWING THE EFFECT OF DIFFERENT CONCENTRATIONS OF *P. REVOLUTA* METHANOLIC EXTRACT ON *H. TURCICUM*

S.no.	Extract concentrations (%)	Colony diameter (mm)		Fresh weight (gm)		Per cent inhibition	
		Mean	SD	Mean	SD	Mean	SD
1.	Control	28.1667	2.0207	2.0600	0.1389	0	
2.	10	24.1667	1.8930	1.8533	0.0351	13.8000	10.7367
3.	20	19.5000	2.7839	1.6400	0.0889	30.2967	12.7342
4.	40	15.4333	0.5132	0.9533	0.0351	45.0033	4.5344
5.	60	12.8667	0.6110	0.6533	0.0252	54.2667	1.1301
6.	80	8.5000	0.5000	0.4867	0.0603	69.6300	4.0034
7.	100	4.4000	0.3606	0.4733	0.0208	84.3267	1.6348
	GM	16.1476	8.1123	1.1600	0.6444	49.5539	24.9102
	Se	0.8846		0.0405		4.2030	
	CD5%	2.6832		0.1227		12.9508	

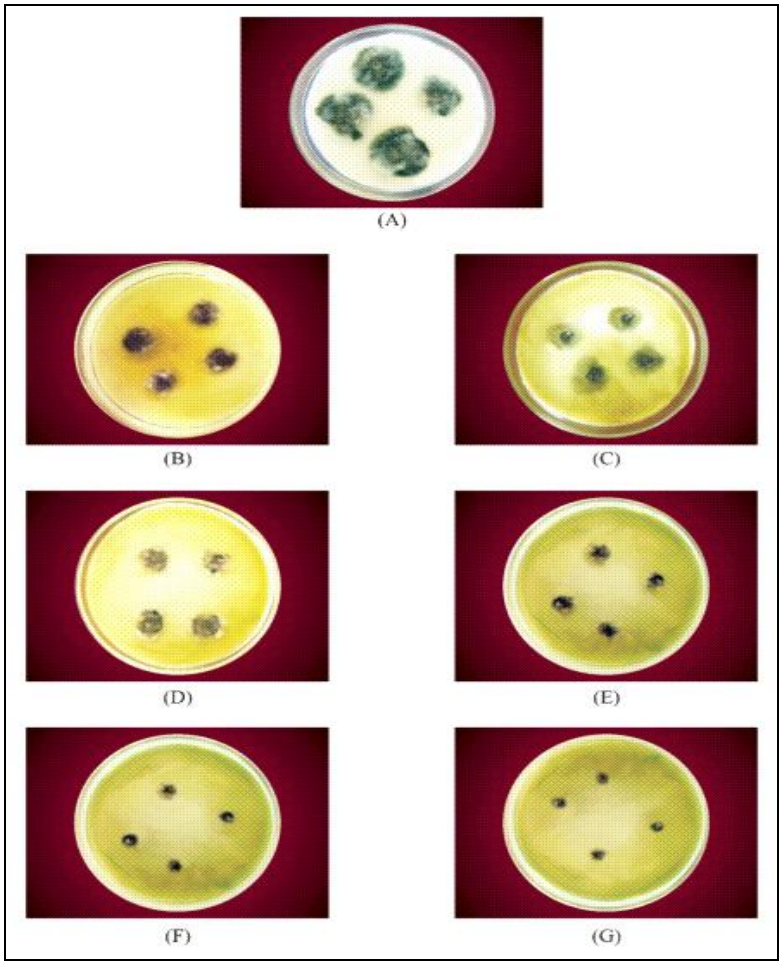
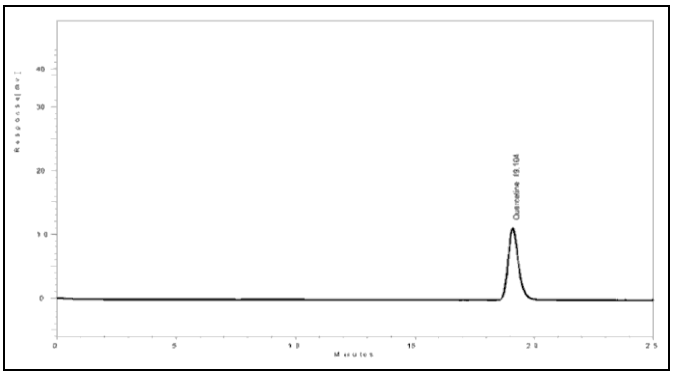


FIG.3: COLONY DIAMETER, FRESH WEIGHT AND PER CENT INHIBITION OF MYCELIAL GROWTH OF *HELMINTHOSPORIUM TURCICUM* IN THE CONTROL (A); 10(B), 20(C), 40(D), 60(E), 80(F) AND 100 (G) PER CENT CONCENTRATIONS OF *PHILONOTIS REVOLUTA* METHANOLIC EXTRACT

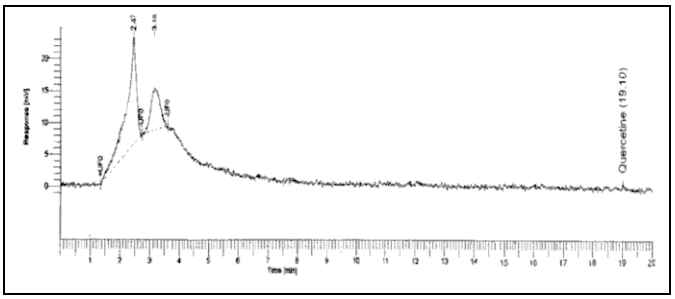
Various Phytochemical constituents were reported in plant extracts **(Table 1).** The presence of secondary metabolites was tried to detect in methanolic extract of the plant through HPLC.

Along with this standard quercetin was also analyzed which showed the retention time 19.104. The methanolic extract of *P.revoluta* also showed a peak with retention time 19.101 which confirmed

the presence of quercetin in methanolic extract of *P.revoluta* **(Graph 1, 2)** The first evidence of flavonols in bryophytes were kaempferol and quercetin in *Corsinia coriandrina* [12].



GRAPH 1: STANDARD (QUERCETIN) THE RETENTION TIME OF STANDARD QUERCETIN WAS FOUND TO BE 19.104 (GRAPH 1)



GRAPH 2: METHANOLIC EXTRACT OF *P. REVOLUTE* THE RETENTION TIME OF QUERCETIN IN *P. REVOLUTE* FOUND TO BE 19.10 (GRAPH 2), WHICH IS MATCHING WITH STANDARD Rt VALUE

Steroids and terpenoids occur in both mosses and liverworts, of all the diterpenoids only one is present in mosses. Terpenoids or aromatic compounds are very significant chemosystematic markers in bryophytes [13].

Flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom. Flavonoids like Rutin and quercetin possess many biochemical effects. An HPLC method was developed for the estimation of rutin and quercetin from methanol herbal extract of *Amaranthus viridis*. Seven pure flavonoides were isolated and identified from moss species. Extract of certain Bryophytes such as *Plagiochasma articulatum, Anthoceros longii, Fissidens bryoides* showed Antibiotic property against *Agrobacterium Tumifacian* [14-15]. Antifungal activity of a moss was determined against certain Phytopathogenic fungi [16]. Effect of liverwort *R.gangetica* against *F.moniliforme* and found cold water Extract more effective than boiled water Extract.

Antibiotic activity of 52 species of the Bryophytes tested against 12 microorganisms. Solubility data and antibiotic spectra of the active plants indicated the occurrence of the variety of antibiotic substances among Bryophytes [17- 18]. Phytotochemical analysis and antimicrobial Activity of moss *Bryum celluiare* (Hook.) (Bryales: Bryaceae) was tested against test fungi *Drechslersa maydis* (Drech.) and *Curvularia lunata* (Wakker) Boedijn the causal organisms of leaf Blight of *Zea mays* L. (Poales: Poaceae) and Leaf spot of wheat respectively and reported that *B. Cellulare* is a store house of various bioactive compounds. Methanolic crude extract of *Bryum argentum* had significant antibacterial potential.

The results were confirmed with the different concentrations of commercially available antibiotic drugs amoxillian and streptomycin.

Further HPLC analysis of *B.argentum* confirmed the presence of α-terpenol which exhibited antimicrobial activity [19-20]. Antifungal potential of *Bryum cellulare* aqueous and methanolic crude extracts against fungi *Curvularia lunata* spore germination and reported that the various concentrations of this plant inhibit spore germination and malformation resulted in the hyphae also [21].

CONCLUSION: As the methanolic extract showed efficacy better than other extracts, this may be due to the possibility of solubility of active chemicals which are responsible for antifungal activity *in vitro*. A flavonoid, quercetine was confirmed in methanolic extract of the plant. This study suggests that bryophytes can be used as an alternative natural antifungal agent as green fungicides instead of synthetic fungicides.

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