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Original Research Article

QUERCETIN- A FLAVONOID IDENTIFIED IN BRYOPHYTE

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Abstract: Bryophytes the plant amphibians contain numerous potential compounds, including oligosaccharides, sugar alcohols, amino acids, fatty acids, aliphatic compounds, aromatic and phenolic substances etc. These plants show fragrant odours and intensely hot and bitter taste as well as diuretic and anticancer activity. A number of bryophytes have been used as medicinal plants to treat burns, bruises, external wound. Traditionally the bryophytes possess some bioactive compounds and used throughout the world as drugs.

These plants produce a high diversity of natural products or secondary metabolites with a prominent function in the protection against microbial pathogens. Secondary metabolites, including terpenes, phenolics and nitrogen (N) and sulphur (S) containing compounds

These plants also offer a rich source of rare and structurally unique molecules and can serve as a reserve of potentially antifungal compounds for further development as pharmaceuticals. Biological control of plant diseases would help in preventing increase of pathogen. 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 bryophyte. This review presents an overview about presence of some secondary metabolites in bryophyte extract and their antimicrobial activity.

Keywords: Secondary metabolites, rutin, quercetin, antifungal

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Introduction: Extracts of many bryophytes have been shown to possess varying levels of antimicrobial potential and many chemical constituents were isolated from them, which inhibited the growth of pathogenic microorganisms. Bioautographic assay on TLC plates were adopted to guide the fractionation of

the Et_2O extract of *Homalia trichomanoides* (Hedw.) B.S.G., which led to the isolation of the novel p-terphenyl derivative trichomanin (=4,4-dihydroxy-1, and structures were determined on the basis of spectural data. Compounds 3, 5 and 6 exhibited antifungal activity against *Candida albicans*, with minimum inhibitory doses (MID) of 2.0, 2.0, and 0.6g, respectively (Wang *et al.*, 2004[1]).

Frahm (2004[2]) performed the antifungal and antifeedant activity of bryophytes mainly from *in vitro* studies. The first alcoholic extracts of all twenty bryophytes used had an effect on a variety of crops infected with different fungi. Two liverworts showed systemic effects. Based on these results, commercial products from bryophytes have been developed and are sold in Germany.

Crude methanol and flavonoid (free and bound) extracts of *Marchantia polymorpha* L. (Marchantiaceae) were screened against three bacterial strains, viz., *Escherichia coli, Proteus mirabilis* (Gram negative), and *Staphylococcus aureus* (Gram positive) and four fungal strains(Mewari and Kumar, 2008[3]).

In present investigation methanolic extract of *Riccia gangetica* was used for antifungal assays and subjected to various phytochemical tests to detect the presence or absence of certain bioactive compounds.

Materials and Methods

Extract preparation: The collected plant material was washed with distilled water to remove soil particles. For methanolic extract preparation, weighted plant material was grinded in mortar and pestle with equal amount of methanol till the formation of fine paste. The smooth paste of extract was filtered through blotting paper and was centrifuged. It was serially diluted for preparing different concentrations from 10-100 per cent.

Test organisms: Fungal pathogen Helminthosporium *turcicum* causing diseases on maize plant was used as test organisms.

Spore germination assay: Percentage of spore germination was counted using the hanging drop method (Gerald and Lampen, 1962[4]). The germination of the spores was observed under microscope after 12 hr. of incubation

Phytochemical Screening and HPLC analysis: In the continuation of our ongoing study aiming to find novel and biologically active compounds from the moisture loving plants The extracts of bryophyte were subjected to various phytochemical tests. The methods of Trease and Evans (2002[5)] were used to detect the presence or absence of certain bioactive compounds.

HPLC (High performance liquid chromatography) was done for the identification of specific metabolite from methanolic extract of *R. gangetica.*

For identification of quercetin present in the most bryophytes, 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 (Kumar *et al.*, 2009[6]). All the HPLC experiments were perfomed at SICART (Sophisticated instrumentation centre for applied research and testing) Anand, Gujarat.

Tests For Flavonoids

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

Sodium hydroxide test

5 ml of 20% NaOH is added to equal volume of water extract. A yellow solution indicated the presence of flavonoids**.**

Alkaline reagent test

Test solution was taken in test tube and added few drops of lead acetate (10%). Yellow precipitate indicated the presence of flavonoids.

Test for Sterols

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

Liebermann-Burchardt test: Test solution was taken in test tube and few drops of acetic

anhydride were added and mixed well. When concentrated sulphuric acid was added from the sides of test tube, it showed a brown ring at the junction of two layers and the upper layer turned green, indicated the presence of sterols.

Sulphur test: Test solution was taken in test tube and sulphure was added. Sulphur sinked down indicated the presence of sterols.

Test for Terpenoids

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

Liebermann-Burchardt test

Test solution was taken in test tube and added acetic anhydride, mixed well and then added concentrated sulphuric acid from the sides of the test tube. Deep red colour indicated the presence of terpenoids.

Test For Alkaloids

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

Hager's test

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

Test for Phenolic Compounds

Ferric chloride test: In a few ml of filtrate, few drops of 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

Lead acetate test

Few drops of 10% lead acetate solution were added in a few ml of filtrate. A bulky white precipitate indicated the presence of phenolic compounds.

Test for Anthroquinons

Borntrager's test: About 0.5ml of extract was added with 5ml 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 anthroquinons.

Test for Cardiac Glycoside

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

Test for Saponins: The extract was diluted with distilled water and made upto 20 ml. The suspension was shaken in a graduated cylinder for 15 minutes. No froath formation indicated the absence of saponins.

Results and Discussion:

In the present investigation it was reported that methanolic extract of *R. gangetica* extract showed significant inhibitory effect against spore germination of *H.turcicum*

Spore germination was `affected by this extract and caused malformation or shrinkage of the test fungi at 100 per cent concentration.

40 per cent spore germination was recorded at 10 per cent concentration, beyond 60 per cent extract concentration spore germination was completely inhibited.

The extracts of *Riccia gangetica* were subjected to various phytochemical tests. Extracts showed the presence of flavonoids, terpenoids, sterols, phenols and cardiac glycosides.

HPLC Analysis: The presence of secondary metabolites were tried to confirm in methanolic extract of both the plant through HPLC. Along with this standard quercetin was also analyzed which showed the retention time 19.104. The methanolic extract of *R. gangetica* also showed a peak with retention time 19.101 which confirmed the presence of quercetin in methanolic extract of *R. gangetica.*

The first evidence of flavonols in bryophytes was given by Reznik and Wiermann 1966[7] who reported kaempferol and quercetin in *Corsinia coriandrina.*

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 Asakawa, 1981[8]

The first isoflavones from a bryophyte was investigated by Anhut *et al.*, 1984[9].

Iwasshina 2003[10] found that flavonoid compounds are distributed widely in vascular plants and bryophytes, and 5,000 kinds have been reported as naturally occurring substances. Flavonoids are biological active compounds. They include pollinator attractants, oviposition stimulants, feeding attractants and deterrents, allelopathy and phytoalexins. They reviewed function and activity of flavonoids against plants and other organisms. Deora *et al.*,2010[11] determined the antifungal activity of a moss against certain phytopathogenic fungi. Deora and Suhalka, 2010[12] studied the effect of liverwort *R.gangetica* against *F.moniliforme* and found cold water extract more effective than boiled water extract. Deora and Suhalka 2012[13] studied the effect of bryophyte against fungal pathogen. Suhalka 2013[14] evaluated the effect of bryophytes as antimicrobial compounds.

Table 1. Effect of *R. gangetica* methanolic extract on *H. turcicum*

S.No.	Extract concentrations (%)	Spore germination (%)	
		Mean	SD
1.	Control	90.0000	3.3300
2.	10	40.0000	3.3300
3.	20	30.0000	3.3300
4.	40	15.5567	1.9284
5.	60	6.6667	3.3350
6.	80	0.0000	0.0000
7.	100	0.0000	0.0000
8.	GM	26.0319	30.4341
9.	Se	1.5136	
10.	CD5%	4.5909	
11.	CD1%	6.3761	
12.	CV	10.07	

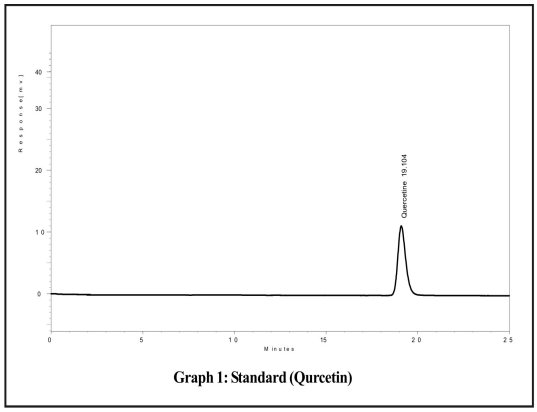
Result based on mean of three replicates

Table 2. Phytochemical screening of the methanolic extract of *R.gangetica*

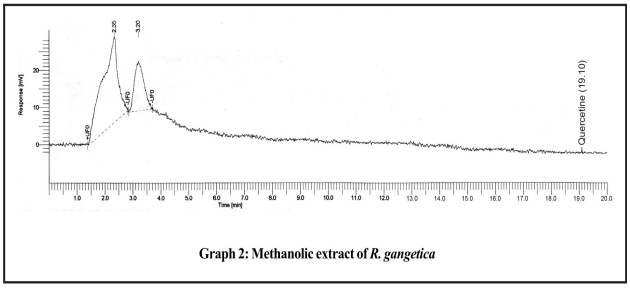
Phytochemicals	Present/Absent
1.Flavanoids	+
2.Saponins	-
3.Alkaloids	-
4.Terpenoids	+
5.Sterols	+
6.Anthroquiones	-
7.Phenols	+
8.Cardiac glycosides	+

+ = Phytoconstituent present

- = Phytoconstituent absent



Graph 1: Standard (Qurcetin)



Graph 2: Methanolic extract of *R. gangetica*

The retention time of standard quercetin was found to be 19.104 (Graph 1). The retention time of quercetin in *Riccia gangetica* found to be 19.10 (Graphs 2), which is matching with standard Rt value

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