ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Preliminary Phytochemical Analysis of Some Bryophytes of District Kangra (H.P) India.

Dr Rajaram Choyal and Sanjay Sharma*

Dept. of Environmental Science M.G.S University, Bikaner, Rajasthan, India
Dept. of Botany, Govt. College, Nurpur, Dist. Kangra, Himachal Pradesh, India

ABSTRACT

Preliminary Phytochemical analysis of metabolites such as Carbohydrates, Lipids, Soluble Sugars and Proteins has carried out for the available species of bryophytes at the selected sites. An overall higher trend of these metabolites has been recorded in the samples collected near the railway track site of Kangra. .Among different species of bryophytes collected, the maximum amount of Average Protein, Soluble Sugars and Ether extract values were found to be in Marchantia palmata Whereas maximum amount of Average Carbohydrate were recorded in Riccia robusta.

Keywords: metabolites, spectrophotometer and centrifuge

Corresponding author
Email: sanjaysharma3269@gmail.com

ISSN: 0975-8585

INTRODUCTION

Plants grow well in two well defined habitats. These are the water and land, the former is best represented by algae and the latter by seed plants or spermatophytes. Between these two extremes of habitats is a transitional zone, represented by the swamps and moist places where water and land meet, called as amphibious zone. Inhabiting the amphibious zone are the mosses (Musci), liver worst (Hepaticae) and hornworts (Anthocerotae) collectively constitute a group of non vascular plants called the bryophytes [1]. Bryophytes rank second after the flowering plants among major group of green land plants with an estimated 15,000- 18,000 species worldwide.(Crum-2001:-25,000 species)

Even with their vascular limitations, bryophytes and mosses is particular can occupy large surface area on rocks, soil, logs and tree trunks, and they can spread negatively to occupy a large are the minute beginnings of a single branch a single spore or a single fragment [2].

Some bryophytes species appear to there in distributed habitat (both naturally distributed and those due to human activities) many bryophytes ,however are quite rare, have extremely local distribution and are at list change in land use and loss of habitat represent the greatly threat to bryophyte diversity [10]. Bryophytes play an important key role in forming communities in environment is very sensitive to pollution and become bio-indicator of environment. They have exhibited antibiotic properties [11] by killing bacterial of vibrio causing cholera and Salmonella causing typhoid. [12] detected chemical constituents like Copper, Nickel and Iron from corticolous mosses from Mahabaleshwar in Western Ghats.

So the present study was undertaken to carry out the Preliminary Phytochemical Analysis of the Bryophytes in Kangra district of H.P state. District Kangra lies in between latitude 30^0 15' to 42^0 and longitude 76^0 and 22^0 46' with altitude varying between 1300-2100 mtrs. Kangra region has hot wet summer and cold winter. The winter extends from December to February while the summer season extends from March to end of June. The rainy season in this region is long (July – September). The maximum temperature at Kangra remains up to 40^0C. There are three principal ranges which run in a South-easterly direction. Kangra district is situated at lower elevation and comparatively warmer but has some hilly ranges covered with pine forests. The proposed study was undertaken in the Kangra district with the following objectives:-1. To identify the Phytochemical substance present in the collected Bryophates.2. Study the presence of Phytochemical in the selected species is helpful in knowing the status of individual plant species in the study area.

METHODOLOGY

Procurement of sample: - Plant species were collected from different localities like. Tanda Medical College Campus, Railway track side etc. of District Kangra..(H.P)
Cleaning and Sorting: - The samples were collected in polythene bags. Damaged plants were discarded. These plants are thoroughly washed in tray with the help of paint brush as plants

were delicate enough, and finally these were washed with distilled water. After removing adhering qualities and cleaning it properly, rated samples were used for further analysis.

Preservation: - The washed and cleaned samples were preserved separately in 10% v/v formalin solution.

TEST FOR CARBOHYDRATES: - (By Mechre's Method)

Reagents and Chemicals
2.5 N HCl solution
Antherone Reagent

Standard Glucose Solution

Procedure

- To 0.1 g of sample, 5 ml of 2.5 N HCl was added and heated it on the water bath for 1-2 hours.
- It was allowed to cool till room temperature achieved.
- For neutralization of acidic media, Na2CO3 (sodium carbonate) was added till the bubbling stopped. The content was made to 100 ml by adding distilled water.
- The above sample was centrifuged at 4000 rpm for 5 minutes.
- Supernatant was taken in two test-tubes (0.2 ml and 0.4 ml) and the volume was made to 1 ml by adding distilled water. To each of above mentioned test-tube, 4 ml of Antherone Reagent was added.
- The above test-tubes were kept in water bath for 10 minutes and allowed it to cool at room temperature.
- Readings were taken using spectrophotometer by setting the wavelength at 630 nm (for 0.1g of sample).

TEST FOR PROTEINS:- (By Lowrey's Method)

Reagents and Chemicals

- Buffer solution was prepared by adding 0.2 M monobasic solution to 0.2 M dibasic solution till the pH came around 7.6.
- Reagent A was a dilute solution of sodium hydroxide (0.4 g of NaOH in 100 ml of distilled water) to which 2 gm of sodium carbonate was added.
- Reagent B was prepared by dissolving 1 g of sodium potassium in 99 ml of distilled water to which 2 gm of sodium carbonate was added.
- Reagent C was prepared by mixing Reagent A and Reagent B in the ratio of 50:1.
- Reagent D was Folin's Reagent.
- Stock Standard Solution was prepared by dissolving 0.05 g of Bovin Serum in 50 ml of distilled water.

Procedure

- To 0.5 g of sample 5 ml of buffer solution was added and the solution thus obtained was centrifuged at 8000 rpm for 20 minutes.
- The supernatant was collected in two test tubes (0.1 ml and 0.2 ml) and volume of the test-tubes was made to 1 ml by adding distilled water.
- 5 ml of reagent C and o.5 ml of Folin's reagent was added in both the test-tubes. The test-tubes were kept in dark for 30 minutes till the bluish colour appeared.
- Readings were taken on spectrophotometer by setting the wavelength at 660 nm.

DETERMINATION OF SOLUBLE SUGARS: -(By Volumetric Procedure)

Reagents and Chemicals

- Potassium Ferricyanide Solution
- Iodine Solution
- 0.01 N Sodium Thiosulphate Solution
- 5% Glacial Acetic Acid

Procedure

- To 1 g of dried sample, 40 ml of distilled water was added and heated it on water bath for about 10 minutes.
- The above sample was centrifuged at 3000 rpm for 20 minutes and supernatant was collected. Again to same pallet 20 ml distilled water was added and again it was centrifuged at 3000 rpm for 20 minutes. The procedure was repeated 6-8 times. Finally all the supernatants so collected were collected.
- Saturated lead Acetate Solution was added to the collected supernatants and mixed it for 15 minutes.
- The above solution was filtered through Whatman no. 1 filter paper and volume was made to 250 ml with distilled water.
- The excess of lead acetate was precipitated (neutralized) with sodium oxalate.
- 5 ml of concentrated HCl acid was added to 25 ml of above extract and the solution was kept in water bath at 68°C for 8 minutes. After cooling it at room temperature, sodium carbonate (solid) was added to it. The volume was made to 100 ml with distilled water.
- For one reading, 5 ml of potassium ferricyanide was added to the 5 ml aliquot of sample. It was heated on water bath for 15 minutes and cooled at room temperature. To this sample 5 ml of Iodine solution and 3 ml of glacial acetic acid were added. In burette 0.01 N sodium thiosulphate solution was taken and the above sample was titrated till the colour of the solution turned pale yellow. Few drops of starch indicator were added to this solution so that the color of solution would turn into blue from pale yellow. Titration was completed till the disappearance of blue colour.

Standard reading was taken by using 5 ml of distilled water instead of sample and proceeding through the above mentioned (step 7) in same manner.

Calculations

The amount of reducing sugars was collected from the following relationship: -

mg. of reducing sugar in 5 ml of sample extract = μ (x+0.05)

Where: -

μ = 0.338

x = volume of 0.01 N $Na_2S_2O_3$ used for sample, that is, volume of $Na_2S_2O_3$ used in blank -- volume of used in sample

RESULTS AND DISCUSSION

Carbohydrate and Protein analysis were done by using U.V. visible spectrophotometer and centrifuge machine as the main instruments while Soxhlet apparatus was used for the estimation of ether extract. The amount of Soluble Sugars was calculated by volumetric method.

Carbohydrates have shown a greater accumulation in the Riccia robusta collected from the above mentioned sites. The minimum amount of Carbohydrates was seen to be accumulated in Funeria hygrometrica (sporophyte) (Table-1).

Table-1.Total Carbohydrate values of different species of bryophytes collected in different seasons.

S. No.	Name of Plant species	Total Carbohydrate Average values	
		Sample 1	Sample 2
1	Riccia robusta(gametophyte)	58.15	55.36
2	Marchantia palmata (Gemmae cup)	26.8	—
3	Funeria hygrometrica (sporophyte)	21.4	23.35
4	Funeria hygrometrica (gametophyte)	43.5	46.85
5	Marchantia palmata (sporophyte)	27.43	29.74

The amount of Soluble Sugars was calculated by the volumetric method by titrating the sample against 0.01 N sodium thiosulphate solution using starch solution as an indicator. Its maximum average values were recorded in Marchantia palmata (sporophyte) and minimum values were recorded in Riccia robusta.(Table-2).

ISSN: 0975-8585

Table-2.Amount of Soluble sugar values in different species of bryophytes collected in different seasons.

S.No.	Name of Plant species	Amount of Soluble sugars Average values	
		Sample 1	Sample 2
1	Riccia robusta (gametophyte)	20.28	23.47
2	Marchantia palmata (Gemmae cup)	42.25	43.17
3	Funeria hygrometrica (sporophyte)	52.39	51.87
4	Funeria hygrometrica (gametophyte)	64.22	68.13
5	Marchantia palmata (sporophyte)	76.32	88.63

Among different species of bryophytes collected, the maximum amount of Average Protein values were found to be in Marchantia palmata (sporophyte) and minimum Average Protein Values were recorded in Riccia robusta (gametophyte)(Table-3).

Table-3 .Average Protein values in different species of bryophytes collected in different seasons.

S.No.	Name of Plant species	Amount of Proteins Average values	
		Sample 1	Sample 2
1	Riccia robusta (gametophyte)	4.25	5.10
2	Marchantia palmata (Gemmae cup)	7.45	8.02
3	Funeria hygrometrica (sporophyte)	10.95	9.76
4	Funeria hygrometrica (gametophyte)	9.2	10.01
5	Marchantia palmata (sporophyte)	15.45	17.1

Ether extract values were estimated for the collected species. The values of ether extract were found to be maximum in Marchantia palmata (gemma cup) and the minimum values were found to be in Funeria hygrometrica (gametophyte) (Table-4).

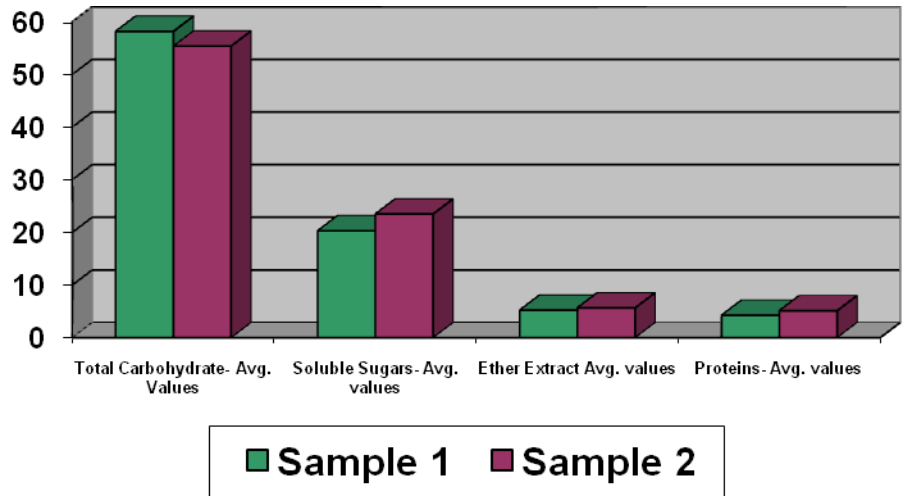
Table-4. Ether Extract values of different species of bryophytes collected in different seasons.

S.No.	Name of Plant species	Amount of Proteins Average values	
		Sample 1	Sample 2
1	Riccia robusta (gametophyte)	5.2	5.64
2	Marchantia palmata (Gemmae cup)	6.72	6.89
3	Funeria hygrometrica (sporophyte)	4.76	5.27
4	Funeria hygrometrica (gametophyte)	3.97	4.33
5	Marchantia palmata (sporophyte)	5.10	6.78

The present studies shows comparative analysis of above mentioned species in Fig.(1,to 5).

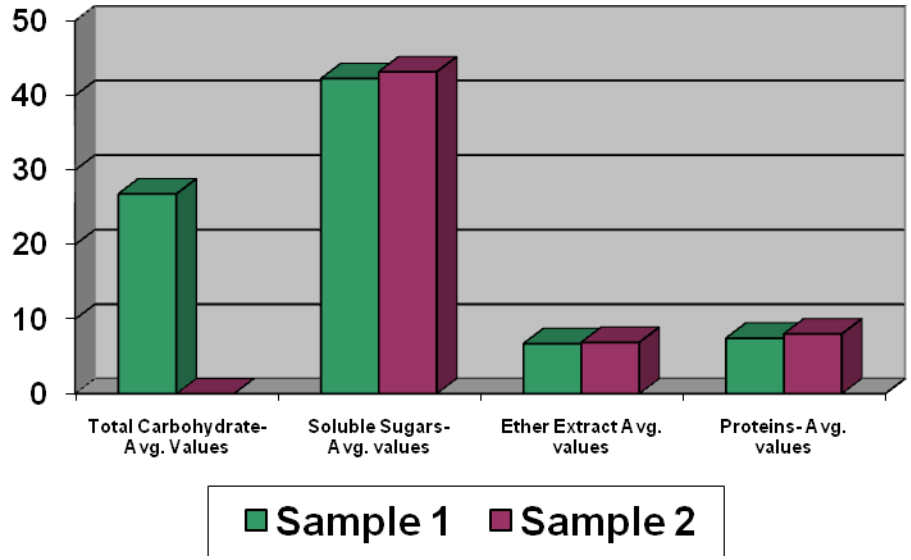
ISSN: 0975-8585

TEXT FIG.1



GRAPH SHOWING AVERAGE VALUES OF TOTAL CARBOHYDRATES, SOLUBLE SUGARS, ETHER EXTRACT AND PROTEINSIN Riccia robusta (gametophyte)

TEXT FIG. 2



GRAPH SHOWING AVERAGE VALUES OF TOTAL CARBOHYDRATES, SOLUBLE SUGARS, ETHER EXTRACT AND PROTEINS IN Marchantia palmata (gemmae cup)

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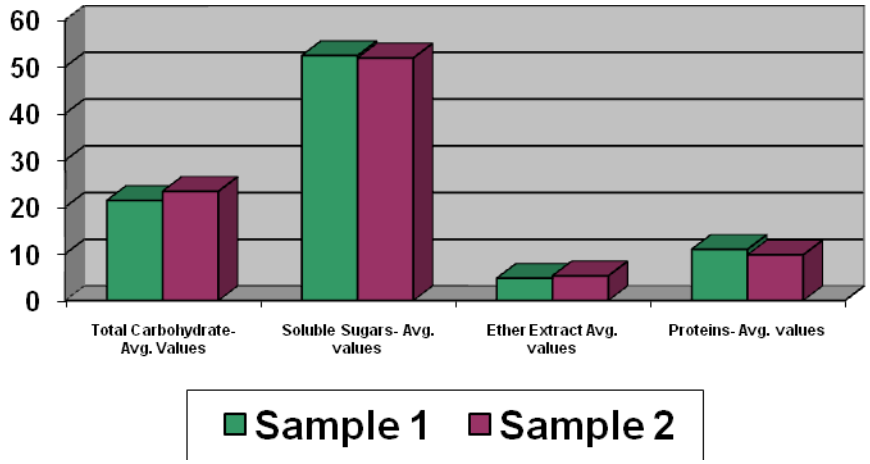
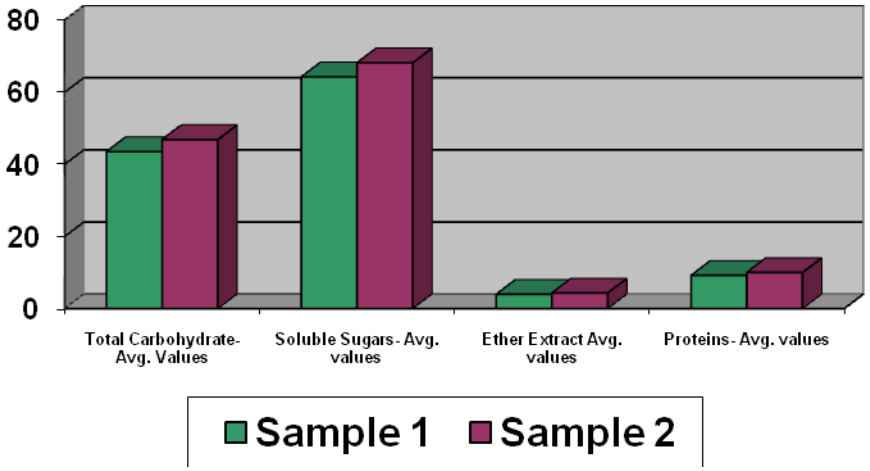
TEXT FIG.3



GRAPH SHOWING AVERAGE VALUES OF TOTAL CARBOHYDRATES, SOLUBLE SUGARS, ETHER EXTRACT AND PROTEINS IN Funeria hygrometrica
(sporophyte)

TEXT FIG. 4



GRAPH SHOWING AVERAGE VALUES OF TOTAL CARBOHYDRATES, SOLUBLE SUGARS, ETHER EXTRACT AND PROTEINS IN Funeria hygrometrica
(gametophyte)

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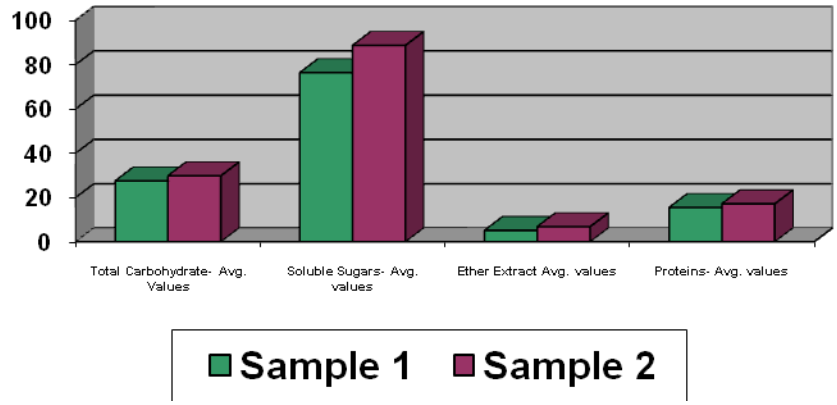
TEXT FIG.5



GRAPH SHOWING AVERAGE VALUES OF TOTAL CARBOHYDRATES, SOLUBLE SUGARS, ETHER EXTRACT AND PROTEINS IN Marchantia palmate (sporophyte)

In Fig.1Carbohydrates have shown a greater accumulation in the Riccia robusta. The maximum amount of Average Protein values and souble sugar were found to be in Marchantia palmata (sporophyte)(Fig5). The Maximum values of ether extract were found in Marchantia palmata (gemma cup)(Fig2) .An overall higher trend of these metabolites has been recorded in the samples collected near the railway track site of Kangra. .Among different species of bryophytes collected, the maximum amount of Average Protein, Soluble Sugars and Ether extract values were found to be in Marchantia palmata Whereas maximum amount of Average Carbohydrate were recorded in Riccia robusta.

This Preliminary phytochemical analysis of Bryophytes is helped in knowing the status of individual Bryophyta species in the study area. The investigations carried out towards a future line of research on these aspects.

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