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Phytochemical analysis of the rhizomes of *Bergenia ciliata* (How) Sternb

Ahatsham Hussain*[1], Mudassir Kanth[1], Pradeep K Shrivastva[1], Manik Sharma[2], Jagrati Tripath[3], Mohammed Azaz Khan[4]

[1] Department of Limnology, Barkatullah University Bhopal, India

[2] Department of Zoology Carrier College Bhopal, India

[3] Dept of Botany LBS College Sironj, India

[4] Pinnacle Biomedical Research Institute (PBRI), Bhopal, (M.P), 462003

ABSTRACT

Bergenia ciliata of family Saxifragaceae is known as Kodiya or Pashanbheda in Dhanolti (Uttranchal). It is a second good source of bergenin after *Bergenia ligulata*. It has many medicinal properties such as antibacterial, anti-inflammatory, anticancer, antidiabetic. *B. ciliata* is used mainly for kidney disorder. Its phytochemical constituents are Gallic acid, Tannic acid, (-)-3-0- Galloylepicatechin, (-)-3-0-Galloylcatechin, (+)-Catechin, Gallicin. The bioactive compounds that are produced by plants are collectively called as Phytochemicals. The phytochemical ingredients are plant derived compounds which protect the plants from environmental stresses, including insects, bacteria, fungus and weather changes. Though phytochemicals are not considered essential nutrients, it has become apparent that they offer many health benefits to the plants. It is well-known that plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect humans against diseases. There are more than thousand known phytochemicals and they offer protection to many chronic diseases such as diabetes, cancer, heart disease and alzheimer's. The aim of the present study is to examine *B. ciliata* rhizomes for phytochemical profile. Qualitative analysis of various phytochemical constituents and quantitative analysis of total saponins and alkaloids were determined by the well-known test protocol available in the literature. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids ects. It is expected that the important phytochemical properties recognized by our study in the indigenous medicinal plants will be very useful in the curing of various diseases when taken along with our food.

Keywords: *Bergenia ciliata*, Phytochemical screening, Total saponins, Total alkaloids.

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***Address for Correspondence:**

Ahatsham Hussain, Department of Limnology, Barkatullah University Bhopal, India

INTRODUCTION

India is the largest producer of medicinal herbs and appropriately called the Botanical garden of the world [1]. Since ancient times plants have been traditionally used in therapeutic practices for the treatment of different types of ailments [2-5]. There are a number of crude drugs where the plant source has not yet been scientifically identified. A phytochemical is a natural bioactive compound found in plants foods that works with nutrients and dietary fibre to protect against diseases. Many researchers suggest that, phytochemical working together with nutrients found in fruits, vegetables and nuts. They can have complementary and overlapping mechanism of action in the body including antioxidant effect. *Bergenia ciliata* is the member of family Saxifragaceae. It consists of about 30 genera and 580 species worldwide. The plant is commonly known as Pashanbheda because it is the main source of Pashanbheda which is highly used in indigenous system [6]. It itself shows that the plant originate between rocks and appears to break them or that it possesses lithotriptic property. It is found in Afghanistan, South Tibet and Bhutan. In India it is found in Himalayas (Kumaon), Meghalaya, Lushai hills West Bengal (Darjeeling, Labha, Takdah, Rimbick(Kalimpong), Arunachal Pradesh (Nyam Jang Chu), Kyongnosla, Changu, Karponanag, Lachen to Thongu, Nathang, Prekchu-Tsokha, Pangolakha-Subaney Dara, Gangtok (domesticated) in Sikkim [7]. It is considered as a miracle herb because it is used to cure several diseases viz; gastrointestinal problems, kidney stone, malaria etc. This plant showed the prescence of various phytochemicals viz; tannins, terpenoids, flavonoids, steroids, saponins, coumarins and glucosides. The rhizome is the rich source of alkaloids, tannins and coumarins. There are approximate 58 phytochemicals present in *Bergenia ciliata* species. Out of these 48 volatile organic compounds are classified into 11 categories such as phenols, terpenoids, fatty acids,

carboxylic acid, flavonoids, nitro compounds, cinnamic acid, glycosides, alcohols, volatile organic compounds and sterol [8]. The present study was designed to investigate the presence of various phytochemicals constituents in *B. ciliata* rhizomes. Extensive effort have now been channelled towards screening of plants for more active and effective new drugs to eliminate diseases which have strains of pathogenic organism that resist the effect of drug in use today [9]. Based on the many ethnomedicinal values of this plant, it is becomes imperative to determine the active ingredients present in different parts of the plant as well as their composition.

MATERIALS AND METHODS

Plant materials

The rhizomes of *B. ciliata were* collected from Himalayan region of Jammu and Kashmir, District Udhampur. The sample was identified by senior Botanist Dr. Zia-Ul-Hassan, Professor and head of department of Botany, Safia College of Arts and Science, peer gate Bhopal. A herbarium of plants was submitted to the specimen library of Safia College of Arts and Science, peer gate Bhopal and The specimen voucher no. of *B. ciliata* is 119/Bot/Saf/18.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction of plant material

Cold maceration

The rhizomes of *B. ciliata* were collected, washed and rinsed properly. They were dried in shade and powdered mechanically. About 1kg of the Powder rhizomes was successive extracted with different organic solvents viz; Pet. ether, ethyl acetate and Methanol and allow to stored for 72 hours in ice cold condition for the extraction of phytochemicals. At the end of the third day extract was filtered using whatmann No. 1 filter paper to remove all un-extractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts [10].

Qualitative phytochemical analysis of plant extract

The *B. ciliata* rhizomes extract obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate [11, 12]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein, amino acid and tannins.

Tests for Carbohydrates

Molish Test

2 ml of aqueous extract was treated with 2 drops of alcoholic α-naphthol solution in a test tube and then 1 ml of concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.

Fehling's Test

To 1 ml of aqueous extract, 1 ml of Fehling's A and 1 ml of Fehling's B solutions were added in a test tube and heated in the water bath for 10 minutes. Formation of red precipitate indicates the presence of reducing sugar.

Benedict's test

Equal volume of Benedict's reagent and extract were mixed in a test tube and heated in the water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presence of reducing sugar.

Tests for Protein and Amino acids

Biuret's Test

The extract was treated with 1 ml of 10% sodium hydroxide solution in a test tube and heated. A drop of 0.7% copper sulphate solution was added to the above mixture. The formation of violet or pink colour indicates the presence of proteins.

Ninhydrin Test

3 ml of the test solution was heated with 3 drops of 5% Ninhydrin solution in a water bath for 10 minutes. Formation of blue colour indicates the presence of amino acids.

Tests for Glycosides

Borntrager's Test

To 3 ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and shakes it welled. The rganic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammonical layer indicates presence of anthraquinone glycosides.

Legal's Test

1 ml of test solution was dissolved in pyridine. 1 ml of sodium nitropruside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red color indicates the presence of Cardiac glycosides.

Keller-Killiani Test

To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Formation of blue color in the acetic acid layer indicates the presence of Cardiac glycosides.

Tests for Alkaloids

To the extract, dilute hydrochloric acid was added, shake it well and filtered. With the filtrate, the following tests were performed.

Mayer's Test

To 2-3 ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of white or creamy precipitate indicates the presence of alkaloids.

Hager's Test

To 1-2 ml of filtrate, few drops of Hager's reagent were added in a test tube. Formation of yellow color precipitate indicates the presence of alkaloids.

Wagner's Test

To 1-2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish brown precipitate indicates the presence of alkaloids.

Tests for Saponins

Froth Test

The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

Tests for Flavonoids

Lead Acetate Test

The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate may indicate the presence of flavonoids.

Alkaline Reagent Test

The extract was treated with few drops of sodium hydroxide separately in a test tube. Formation of intense yellow color, which becomes color less on addition of few drops of dilute acid, indicate presence of flavonoids.

Tests for Triterpenoids and Steroids:

Salkowski's Test

The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layers turns red, sterol are present. Presence of golden yellow layer at bottom indicates the presence of triterpenes.

Libermann-Burchard's Test

The extract was treated with chloroform. To this solution few drops of acetic anhydride were added, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. Formation of brown ring at the junction of two layers, if upper layer turned green, indicate presence of steroids and formation of deep red color indicate presence of triterpenoids.

Tests for Tannin and Phenolic compounds

Ferric Chloride Test

Some amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.

Lead Acetate Test

Some amount of extract was dissolved in distilled water. To this solution few drops of lead acetate solution was added. Formation of white precipitate indicates presence of phenolic compounds.

Gelatin Test

Some quantity of extract dissolved in distilled water. To this solution 2 ml of 1% gelatin solution containing 10% sodium chloride was added. Development of white precipitate indicates presence of phenolic compounds.

Tests for Fats and Oils

Solubility test

To 2-3 ml of the alcoholic solution of extract, add few ml of chloroform and solubility was observed.To 2-3 ml of the alcoholic solution of extract, add few ml of 90% ethanol and solubility was observed.

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this total saponins and alkaloids are determined. Extracts obtained from rhizomes of *B. ciliata* plant material of subjected to estimate the presence of total saponins and alkaloids by standard procedure

Determination of total alkaloids content

The plant extract (1 mg) was dissolved in dimethyl sulphoxide (DMSO), 1 ml of 2 N HCl was added and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) were prepared. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Vis spectrophotometer. The total alkaloid content was expressed as mg of AE/gm of extract [13].

Estimation of total saponins content

Estimation of total saponins content was determined by the method described by Makkar et al. based on vanillin-sulphuric acid colorimetric reaction with some modifications [14]. About 50 µl of plant extract was added with 250 µl of distilled water. To this, about 250 µl of vanillin reagent (800 mg of vanillin in 10 ml of 99.5% ethanol) was added. Then 2.5 ml of 72% sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60°C for 10 min. After 10 min, it was cooled in ice cold water and the absorbance was read at 544 nm. The values were expressed as diosgenin equivalents (mg DE/g extract) derived from a standard curve.

RESULTS AND DISCUSSIONS

The crude extracts so obtained after cold maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The percentage yield of extraction is very important in phytochemical extraction in order to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from the rhizomes of the plants using petroleum ether, ethyl acetate and methanol as solvents are depicted in the Table 1.

Table 1 Results of percentage yield of rhizomes extracts

Plant Name	Percentage yield (%)		
	Pet. ether	Ethyl acetate	Methanol
Bergenia Ciliata	2.5	3.6	5.6

The results of qualitative phytochemical analysis of the crude powder of rhizomes of *B. ciliata* are shown in Table 2. Methanolic extracts of sample of *B. ciliata* showed the presence of alkaloids, terpenoids, flavonoids, phenols, tannins, carbohydrate, glycosides and saponins, Ethyl acetate extracts show the presence of carbohydrates, alkaloids, saponins, flavonoids treterpenoids, steroids, tannin and phenolics but in petroleum ether extracts all phytoconstituents was absents.

Table 2 Phytochemical evaluation of *Bergenia Ciliata* rhizomes

Tests	Pet ether	Ethyl acetate	Methanol
Carbohydrates			
Molish	- ve	+ ve	+ ve
Fehlings	+ ve	+ ve	+ ve
Benedit's	+ ve	+ ve	+ ve
Protien & amino acids			
Biurets	- ve	- ve	- ve
Ninhydrin	- ve	- ve	- ve
Glycosides			
Borntrager	- ve	- ve	+ ve
Killer killani	- ve	- ve	+ ve
Alkaloids			
Mayers	+ ve	+ ve	+ ve
Hagers	- ve	+ ve	+ ve
Wagners	- ve	+ ve	+ ve
Saponins			
Froth	- ve	+ ve	+ ve
Flavonoids			
Lead acetate	- ve	+ ve	+ ve
Alkaline reagent test	- ve	+ ve	+ ve
Treterpenoids & Steroids			
Salwoski	+ ve	+ ve	+ ve
Liebermann	- ve	+ ve	+ ve
Tannin & Phenolics			
Ferric chloride	- ve	+ ve	+ ve
Lead acetate	- ve	+ ve	+ ve
Gelatine	- ve	+ ve	+ve

The determination of the total saponin content, expressed as mg dioscenin equivalents and per 100 mg dry weight of sample. Total saponin of methanolic and ethyl acetate extract of *B. ciliata* rhizomes showed the content values of 97.33 and 32.33mg/gm respectively. But petroleum ether extracts of *B. ciliata* rhizomes have no saponin content. The total alkaloids content of the extracts was expressed as percentage of Atropine equivalent per 100 mg dry weight of sample. The total alkaloids estimation of methanolic and ethyl acetate extract of *B. ciliata* rhizomes showed the content values of 285 and 112mg/gm respectively. The above results showed that ethyl acetate extract contain less saponin and alkaloids content than the alcoholic extract. It may due to the solubility of principle contents presence be higher in case of alcoholic solvent, thus it has been accepted that it is a universal solvent for the extraction of plant constituents. Results are provided in (Table 3 and Fig. 1, 2).

Table 3 Estimation of total saponins and alkaloids content of *Bergenia Ciliata* rhizomes

S. No	*Bergenia Ciliata* Extracts	Total saponin content (mg/100mg of dried extract)	Total alkaloids content (mg/ 100 mg of dried extract)
1.	Petroleum ether	-	-
2.	Methanol	97.33	285.00
3.	Ethyl acetate	32.33	112.00

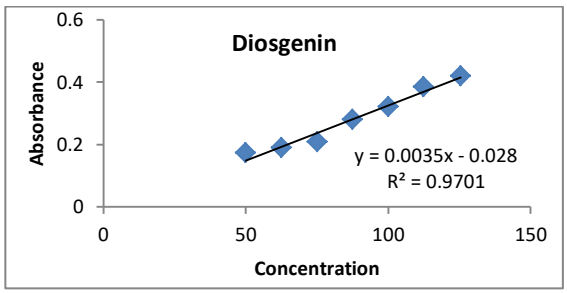

Fig. 1 Graph of estimation of total saponins content

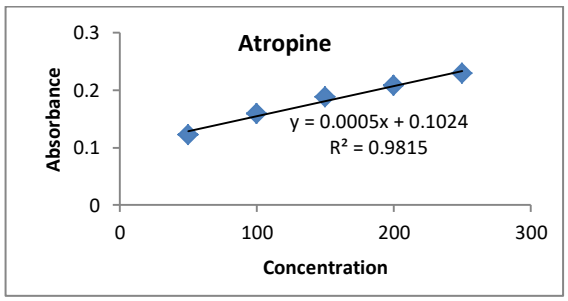

Fig. 2 Graph of estimation of total alkaloids content

CONCLUSION

It is found that this miracle herb had been traditionally used among the various communities across the Himalayan region of world for urinary, gastrointestinal, skin, respiratory, skeletal, gynecological, inflammatory, infectious diseases. In total of 104 different disease disorders were reported to be treated by this species while its highest potential was observed to cure gastrointestinal disorders primarily. In addition to this, the species is also well known to treat kidney stones and kidney disorders by the traditional healers. Almost all parts of the plant are used for curing different ailments; the most frequent part used is rhizome followed by root, leaf, flower, latex and whole plant. *B. ciliata* rhizomes have potential to act as a functional food and a source of useful drugs because of the presence of various phytochemical components. Methanolic extracts shows good results regarding presence of phytoconstituents hence these plants may directly use in medicine preparation or for the development of novel agents for various pathological disorders. Further research on the health benefits of phytochemicals in this plant may be warranted.

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