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Study of Phytochemistry, Antibacterial and Anticancer Property of Aqueous and Acetone Extracts of *Bergenia ciliata* (Haw.) Sternb. from Darjeeling Himalayas

Rupam Mandal, Priyankar Sanphui

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ABSTRACT

In modern time researchers are in search of naturally occurring molecule that exhibit medicinal property as they have very less or no side effects. Darjeeling, in the northern most part of West Bengal is located at the foothills of Himalayas and is home to huge number of plants that are used by the locals for medicinal property. It is believed that many more plants are there in this area that are still to be explored and analyzed for their medicinal property. *Bergenia ciliata*, locally known as Paashanbheda belonging to family Saxifragaceae is one such plant used locally. The present study is aimed in studying the phytochemical profile, antibacterial property and anti cancer activity of aqueous and acetone extracts of leaf and rhizome of *Bergenia ciliata* from Darjeeling Himalayas. Our result indicates presence of various phytochemicals in the extracts of *Bergenia ciliata*. The different extracts of *Bergenia ciliata*, exhibited antibacterial property against Gram positive bacteria *Bacillus subtilis* and Gram negative bacteria *Klebsiella pneumoniae*. We found that the acetone extracts of leaf and rhizome showed maximum effect against both gram positive bacteria *Bacillus subtilis* and gram negative bacteria *Klebsiella pneumoniae*. For testing the anticancer property HEK293 cells were used. We found that out of the four tested extracts, the acetone extract of leaf showed significant anticancer activity. Our study indicates that *Bergenia ciliata* contain several phytochemicals, antibacterial and anticancer property. This plant is definitely a rich source of biologically active compounds that contribute to its medicinal importance.

Keywords *Bergenia ciliata*, Antibacterial property, Anticancer property, Phytochemicals, Darjeeling Himalayas.

INTRODUCTION

The relationship between plants and humans dates back to the origin of human civilization. Man depends on plants for clothing, shelter, food and medicine (Sharma *et al*. 2012). Plants have been used from prehistoric times for their medicinal values in traditional medicine. The herbal drugs constitute only those traditional medicines which primarily use preparations

Rupam Mandal[1], Priyankar Sanphui*[2]

[1, 2]Assistant Professor, [1]Department of Botany, [2]Department of Zoology, Sree Chaitanya College, Habra 743268, West Bengal, India

Email: psanphui@gmail.com
*Corresponding author

from medicinal plants. The evidence of use of herbal drugs is found in ancient texts from India, Egypt, China, Rome and Syria. The use of herbal medicine is found in classical Indian texts like Rigveda, Atherveda, Charak Samhita and Sushruta Samhita.

Medicinal plants can synthesize a variety of chemical compound which are capable of performing different biological functions and protect against pathogens. The compound in plants mediates their action on the human body through similar mechanism as that of chemicals in conventional drugs. Thus the herbal medicines are equally effective as conventional medicine but without its side effects.

Ethnobotany is recognized as an effective way to discover future medicine. Research has revealed that many compounds used in modern medicine are derived from the plants. The medicinal properties of the plants are contributed by the presence of different phytochemicals. It is to note that the phytochemicals present in a plant vary with the climate and soil in which it grows.

Darjeeling, "the queen of hills" as it is called is located in the northern most part of West Bengal state of India. It is a mountainous zone in the Himalayas bestowed with rich floral diversity. A huge number of plant are used as food source, however many are still underutilized as their knowledge is confined to only tribal and ethnic communities.

The Darjeeling Himalayas is known to house a huge number of plants that have medicinal benefits (Chakraborty *et al*. 2016). Though many of these plants are used by the locals for their medicinal benefits, proper scientific data about these plants are still lacking.

Bergenia sp. belonging to family Saxifragaceae are hardy perennial wild plants found from Afganisthan to southeast Tibet and the Himalayas. The genus *Bergenia* comprises about 6 species distributed in cold and temperate Himalayas and central and eastern Asia. Hooker in 1888 reported three species from India. Plants of these genus form clumps of large, evergreen leaves with leathery texture and cluster of flowers. They usually grow from 1 to 2 feet in height.

Bergenia ciliata (Haw.) Sternb. is one of the species under the genera *Bergenia* which is very commonly found in Darjeeling Himalayas. This plant is commonly known as Paashanbheda. In hindi Paashan means rock and bheda means piercing. As these plants grow between the rocks and appear to break the rocks, they are called so. *Bergenia ciliata* is found in the Darjeeling Himalayan region between the altitudes ranging from 900 to 3000mt. It is an evergreen perennial herb. It has large rounded and hirsute leaves. They have hairs along the edge of each leaf with the blades being hairless (Ahmad *et al*. 2018).

The aim of the present study was to evaluate the phytochemistry, antibacterial and anticancer property of aqueous and acetone extracts of *Bergenia ciliata* (Haw.) Sternb. from Darjeeling Himalayas.

Bacillus subtilis is a gram positive, free moving, rod shaped endospore forming bacteria, commonly found in soil. They are not considered pathogenic or toxic. *Klebsiella pneumoniae* is a gram negative nonmortile encapsulated rod shaped bacteria. It can cause destructive changes to human and animal lungs if aspirated (inhaled). For the present study *Bacillus subtilis* and *Klebsiella pneumoniae* were taken as representatives of gram positive and gram negative bacteria.

Globally cancer is a disease that affects millions of people. There is a constant demand for new therapies for treating cancer. In modern times the naturally derived compounds are gaining research interest as potential anticancer agents as they have no or less toxic side effects. The present study is also aimed at understanding the anticancer property of the leaf and rhizome extracts of *Bergenia ciliata.*

MATERIALS AND METHODS

Materials

Potassium chloride, Magnesium acetate tetrahydrate, Ammonium solution, Sulfuric acid, Iron (III) chloride, Chloroform, Sodium hydroxide, Sodium chloride, Mercury (II) chloride, LB, Nitric acid were purchased from SRL Acetic acid glacial was purchased from Glaxo India Limited, was purchased from SRL

and plastic wares were purchased from Tarsons.

Plant collection

Leaves and rhizome of *Bergenia ciliata* were collected from the road side areas of Hooker road, Darjeeling. Samples were collected between June to August.

Preparation of plant extracts

Aqueous extract: The aqueous extract of leaves and rhizome were prepared in distilled water as described earlier (Al-Manhel and Niamah 2015). In short, 5 g of dried leaf or rhizome powder was taken and mixed with 50 ml of distilled water in a conical flask. The mixture was kept in shaker for 24 hrs. The mixture was then centrifuged at 5000rpm for 15 minutes and filtered through muslin cloth.

Acetone extract: The acetone extracts were also prepared through the same protocol as mentioned above. Only acetone was used in place of distilled water to dissolve the dried powder.

Test for alkaloids

Ammonium solution was added to the dry powdered sample and mixed. Chloroform was added to the mixture followed by Mayer's reagent. Appearance of a cream colored precipitate indicates the presence of alkaloid as described earlier (Shaikh and Patil 2020).

Test for glycosides

Glycosides were detected as described earlier (Gul *et al*. 2017). Briefly, 4ml of glacial acetic acid, one drop of 2% $FeCl_3$ and 10 ml of test sample were mixed. On adding conc. sulfuric acid, appearance of a brown ring between two layers indicates presence of glycosides.

Test for steroids

Steroid was detected by mixing 1ml of sample with 10 ml of chloroform, followed by addition of 1ml of concentrated sulfuric acid. In presence of steroid the upper layer turns red and the color of sulfuric acid layer turns yellow with green fluorescence (Panchal and Parvez 2019).

Test for saponin

Presence of saponin was detected by formation of foam in the top of the mixture containing extract and distilled water following vigorous shaking for about 15 mins (Gul *et al*. 2017).

Test for tannin

The presence of tannin was detected by mixing 1 ml of sample with 2 ml of distilled water and 2-3 drops of ferric chloride as described earlier (Panchal and Parvez 2019). The change of color from green to blue indicates presence of tannin.

Test for flavonoids

For detection of flavonoids, few drops of sodium hydroxide is added to the test sample. Appearance of an intense yellow color which disappears on adding of diluted 50% sulfuric acid indicates the presence of flavonoids (Hossain *et al*. 2013).

Test for quinone

For detecting quinine, a few drops of concentrated sulfuric acid or aqueous sodium hydroxide solution was added to the test sample. Appearance of any color indicates presence of quinine.

Test for phenol

Presence of phenol was detected by adding 5% ferric chloride to the sample. Appearance of dark green color indicates presence of phenol (Shaikh and Patil 2020).

Test for coumarin

A few drops of alcoholic sodium hydroxide solution were added to the test sample. Appearance of yellow color indicates the presence of coumarin (Jain and Joshi 2012).

Test for anthraquinone

For the detection of anthraquinone a few drops of magnesium acetate was added in the extract. Follow-

ing vigorous shaking, appearance of light pink color indicates presence of anthraquinone.

Test for xanthoprotein

For detecting the presence of xanthoprotein ammonia was added to the sample. If the color changes to deep orange yellow, the presence of xanthoprotein is confirmed.

Evaluation of antibacterial property

The antibacterial activity of the extracts was tested by the disc diffusion method. In brief, small round piece of whatman filter paper was made through the punching machine. The paper discs were dipped in plant extract, dried in air and placed on the agar plate containing bacteria. For control, the solvent dipped paper discs were used. The plates were incubated at 37^0 C for 24 hrs. After 24h the zone of inhibition was measured using a Vernier caliper.

Cell culture and MTT assay

Human embryonic kidney 293 cells were cultured as described earlier (Banerji *et al*. 2013). In brief, cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen Life Technologies, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (FBS) and antibiotics (penicillin/streptomycin). Cells were cultured at 37 °C in 95% air and 5% CO_2 humidified incubators. For checking the anticancer property the cells were grown in 96 well cell culture plates. The cells were treated with different doses of the plant extracts for 24 hrs. Following treatment (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) MTT assay was performed as described earlier (Banerji *et al*. 2013). In brief 10 µl of MTT (5mg/ml) was added to medium of each well of the cell culture dish. After 4hrs the MTT solution was removed and the purple crystals were dissolved in DMSO. The absorbance of the solution was measured at a wavelength of 550nm. In this assay MTT, a yellow tetrazole is used to quantify viable cells. MTT is reduced into purple formazan product by mitochondrial dehydrogenase present in metabolically active living cells. Thus the intensity of purple color is a direct representation of the amount of live cells.

RESULTS

Phytochemical analysis of leaf and rhizome extracts of *Bergenia ciliata*

The presence of different phytochemicals in aqueous and acetone extract of leaf and rhizome were tested. A number of phytochemicals were detected in the extracts. Our result indicates the presence of alkaloids, tannin, Phenols and Quinone in all the four extracts (Table 1). Glycosides were only detected in acetone extract of leaf, whereas only aqueous extract of leaf indicates the presence of saponin and flavonoids. The presence of steroids was only detected in the leaf extracts. Xanthoprotein was detected in all the four extracts except aqueous extract of rhizome. None of the extracts showed the presence of coumarin and anthraquinon. In a nutshell our result shows that the leaf and rhizome extracts of the *Bergenia ciliata* are rich source of phytochemicals.

Antibacterial property of leaf and rhizome extracts of *Bergenia ciliata*

The presence of alkaloids and flavonoids in extracts of *Bergenia ciliata* indicated that the plant may have antibacterial property. The antibacterial potential of *Bergenia ciliata* was tested using disc diffusion method. The aqueous and acetone extracts of leaf and rhizome of *Bergenia ciliata* were used and inhibition zones were measured. *Bacillus subtilis* and *Klebsiella pneumoniae* were used as the representatives of gram positive and gram negative bacteria. The result clearly

Table 1. Phytochemical analysis of leaf extracts of *Bergenia ciliata.*

Phytochemicals	Leaf		Rhizome	
	Aqueous extracts	Acetone extract	Aqueous extracts	Acetone extract
Alkaloid	+	+	+	+
Glycosides	-	+	-	-
Saponin	+	-	-	-
Tanin	+	+	+	+
Flavonoids	+	-	-	-
Steroids	+	+	-	-
Phenols	+	+	+	+
Coumarin	-	-	-	-
Quinone	+	+	+	+
Anthraquinone	-	-	-	-
Xanthoprotein	+	+	-	+

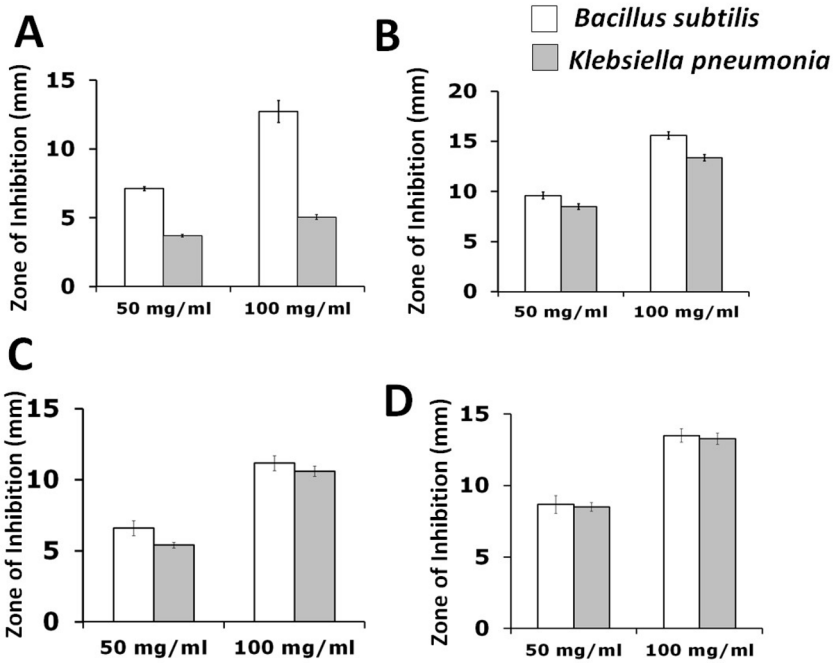

Fig.1. Antibacterial activity of aqueous and acetone extract of leaves and rhizome of *Bergenia ciliata* from Darjeeling Himalayas. The extracts were prepared at a concentration of 50mg/ml and 100 mg/ml. Disc diffusion method was used to study the antibacterial activity of the extracts on gram positive bacteria *Bacillus subtilis* and gram negative bacteria *Klebsiella pneumoniae*. Zone of inhibition (in mm) are represented graphically. Data represented as mean ± SEm of three independent experiments. A: Aqueous extract of leaf, B: Acetone extract of leaf, C: Aqueous extract of rhizome, D: Acetone extract of Rhizome.

indicates that aqueous and acetone extracts of leaf and rhizome of *Bergenia ciliata* show antibacterial property against both gram positive and gram negative bacteria (Fig. 1).

The acetone extract of the leaves and rhizome showed maximum effect against both gram positive bacteria *Bacillus subtilis* and gram negative bacteria *Klebsiella pneumoniae*. Interestingly, all the extracts were more effective against the gram positive bacteria *Bacillus subtilis* compared to gram negative bacteria *Klebsiella pneumoniae*.

Anticancer property of *Bergenia ciliata* extracts

We tested the anticancer potential of the aqueous and acetone extract of leaf and rhizome of *Bergenia ciliata*. Human embryonic kidney 293 (HEK 293) cells were used as model for testing anticancer property of the extracts. We found that out of the four extract tested, acetone extract of Bergenia leaves showed anticancer potential. We used three doses (Dose 1: 0.5mg/ml, Dose 2: 2.5 mg/ml and Dose 3: 5 mg/ml). In the control cells equal volume of respective solvents were added. Out of the three doses, Dose 2 and 3 showed significantly blocked the proliferation of cells. Moreover these doses caused significant cell death. Dose 2 and 3 showed about 40 % and 85% cell death when treated overnight (Fig. 2A). Moreover our observation indicated that the extract destroyed the cell morphology from spread out elongated cells to small round cells (Fig. 2B). In a nutshell our result clearly indicates that the acetone extract of leaf possesses high anticancer activity.

CONCLUSION

The traditional medicine system is a prehistoric sys-

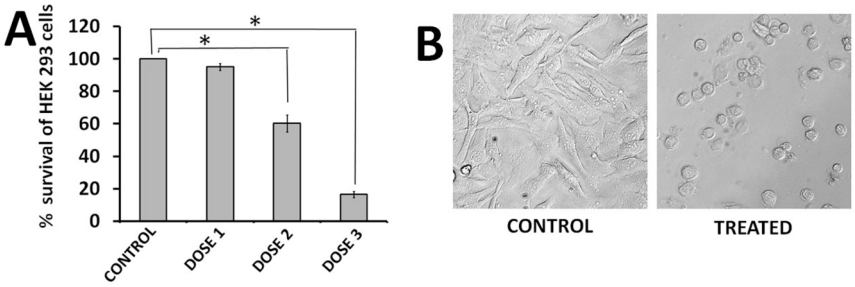

Fig. 2. Anticancer activity of acetone extract of *Bergenia ciliata* leaf from Darjeeling Himalayas. A: Graphical representation of cell survival in presence or absent of acetone leaf extract. Dose 1: 0.5mg/ml, Dose 2: 2.5 mg/ml and Dose 3: 5 mg/ml. Data represented as mean ± SEm of three independent experiments. Asterisks denote statistical significance differences between control cells and treated cells: *$p < 0.001$. B: Microscopic image of HEK 293 cell, in presence and absence of the leaf extract. The images were taken at 40X objective.

tem of medicine practiced all over the world from ancient times and now recognized as a trustworthy health care resource. Plants are definitely the most important component of traditional medicine. More than 2500 plant species have been recognized for their medicinal values and about 6000 more plants are estimated to be explored in traditional or folk medicine.

Our study was confined to *Bergenia ciliata* from the Darjeeling Himalayas. This plant is used by the local peoples of Darjeeling and Nepal in traditional medicine. Our investigation revealed the presence of various phytochemicals in the aqueous and acetone extract of leaf and rhizome of *Bergenia ciliata*. Alkaloids, Tannin, Phenols and Quinone were present in all the extract tested whereas coumarin and anthraquinon were absent in all the extract tested. As the plant extracts indicate presence of alkaloids and flavonoids, the plant is most likely to possess antibacterial property. This was also evident from our investigations. Presence of phenolic compound indicate that *Bergenia ciliata* may possess ant-carcinogenic or anti-inflammatory effect (Abotaleb *et al*. 2020). Quinone present in the extract may contribute to some anticancer and anti-aging potential of *Bergenia ciliata* (Madeo *et al*. 2013). It is known that spatial and climatic condition affects the phytochemical diversity and accordingly the medicinal property of a plant (Kumar *et al*. 2017, Lei *et al*. 2016). Therefore it would be interesting to look into the anti-oxidant and anti-inflammatory potential of *Bergenia ciliata* from Darjeeling Himalayas.

We have checked the antibacterial property of *Bergenia ciliata* from Darjeeling Himalayas on one gram positive bacteria *Bacillus subtilis* and one gram negative bacteria *Klebsiella pneumoniae*. All the extracts showed antibacterial potential against the bacteria tested. However the extracts were more effective against gram positive bacteria *Bacillus subtilis* than gram negative *Klebsiella pneumoniae*.

Cancer being a global disease of concern, researchers all over the world are in search of naturally derived compounds that can combat the disease as the present available treatment have a lot of side effects. We have checked the extracts of *Bergenia ciliata* for its anticancer property. Out of all the extracts, the acetone extracts of leaf exhibited significant anticancer property.

In conclusion, it can be said that our study indicates presence of various phytochemicals in *Bergenia ciliata* and the plant have antibacterial and anti-carcinogenic property. It would be interesting to investigate the active compounds present in the extracts and the mechanism of action of these extracts. Further investigation in this direction may help in formulation of effective herbal antibacterial preparations from *Bergenia ciliata* from Darjeeling Himalayas.

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