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Priyanka pokhrelDepartment of pharmacy, Sunsari
Technical College, Dharan, Nepal.**Janmajoy Banerjee**Department of pharmacy, Sunsari
Technical College, Dharan, Nepal.**Prasanna Dahal**Department of pharmacy, Sunsari
Technical College, Dharan, Nepal.**Hemant Khanal**Department of pharmacy, Sunsari
Technical College, Dharan, Nepal.**Amit Kumar Gupta**Department of pharmacy, Sunsari
Technical College, Dharan, Nepal.**Biplab Kumar Dey**Department of pharmacy, Assam
downtown University, Guwahati,
Assam.**Correspondence:****Janmajoy Banerjee**Department of pharmacy,
Sunsari Technical College,
Dharan, Nepal.

Phytochemical screening and biological evaluation of different parts of plant *Bergenia ciliata*

Priyanka pokhrel, Janmajoy Banerjee, Prasanna Dahal, Hemant Khanal, Amit Kumar Gupta, Biplab Kumar Dey

Abstract

Bergenia ciliata of Nepalese origin has been investigated for pharmacognostical, phytochemical and biological activities. The plant was subjected for extraction by ethanol, distilled water, and butanol: hexane (2:1) successfully. The preliminary phytochemical studies of the plant showed the presence of alkaloids, carbohydrates, cardiac glycosides, saponins, phenols, flavonoids, diterpenes and absence of phytosterols. Antimicrobial activity of plant extracts was carried out by cup diffusion method using *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa* bacteria. The root and leaf extract showed activity against tested microorganisms. Brine shrimp bioassay revealed that ethanol extracts of the rhizome of plant had cytotoxic property against brine shrimp nauplii.

Keywords: *Bergenia ciliata*, active constituents, antimicrobial activity, brine shrimp bioassay.

1. Introduction

Bergenia comprises of 6 species distributed in the temperate Himalayas and Central and East Asia. *Bergenia ciliata* is used in the traditional medicine of Asian countries^[1] *Bergenia* species are evergreen herb belonging to the family Saxifragaceae. The leaves, rhizomes, root and other parts of these plants are used in the indigenous system of medicines. Several studies on *Bergenia ciliata* plant have shown is used as demulcent and deobstruent, relieves pain in ribs and chest due to excessive cold humors, acts as effective diuretic and emmenagogue. Get-rid of kidney's and bladder stones and obstructions or toxic waste products, which remain in the alimentary canal, and urinary excretory system^[2, 3, 4]. Furthermore, Juice and powder of the whole plant is taken to treat urinary trouble. Juice of the rhizome is taken in cases of hemorrhoids, asthma and urinary trouble^[5]. All the extracts except chloroform extract of root and leaves of *Bergenia ciliata* (Haw.) Sternb. Were found to possess hypoglycemic activity in Streptozotocin (STZ) treated rats^[6]. However, the studies on *Bergenia ciliata* antibacterial and cytotoxic activity is still lacking. The present study was conducted for the purpose of preliminary analysis of its phytochemical and biological esp. antibacterial and cytotoxic activity of the extracts of various parts of *Bergenia ciliate*.

2. Materials and Method

2.1 Plant collection

The fresh plants were collected from the botanical garden of Manoj Pharmaceuticals Pvt. Ltd located in Vedetar, Dhankuta, Nepal.

2.2 Pharmacognostical studies

The pharmacognostical study of *Bergenia ciliata* was performed to detect the specific features of the plant, which helps in easy identification and avoidance of adulteration and contamination due to misidentification of plant species.

2.3 Macroscopical Analysis

Macroscopical examination was carried out with the naked eye, which gave details concerning the plant aspect, general appearance, colour and odor.

2.4 Microscopical study of Rhizome

The transverse section of the rhizome was observed under the microscope to identify different

anatomical features, characteristic histology in respect to its diagnostic features through the study of tissues, cell contents and their arrangements.

3. Loss on Drying

About 1g of the powdered crude drug was accurately weighed in a tared dish and dried in an oven at 100-105 °C. It was cooled in a dessicator and again weighed. The loss on drying was calculated with reference to the amount of the dried powder taken.

Loss on drying = (Weight of empty desiccators + Sample weight – Weight after drying)/ Sample weight

4. Thin Layer Chromatography

Thin layer chromatography of different extracts was carried out by preparing glass plates using Silica gel slurry. The plates were then dried in hot air oven and a drop of each extract of leaves, roots and rhizomes were placed in different plates and the plates were kept in beaker containing the appropriate solvent.

$$R.F = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

5. Extraction

The plant materials were cut into pieces and were shade dried at room temperature and they were dried in a hot air oven. Dried sample was crushed into powder by electric blender (electric grinder) and subjected to extraction by using suitable Solvent in the Soxhlet apparatus.

Table 1: Extraction of Plant

S.N	Plant part	Solvent	Amount
1.	Leaves	Distilled water	50 ml
2.	Rhizome	Ethanol	100 ml
3.	Root	Butanol: Hexane	50 ml: 25 ml

6. Procedure ^[7]

36 gram of dried and powdered leaf was extracted with 50 ml distilled water, 23 gram of dried and powdered rhizome was extracted with 100 ml ethanol and 8 gram of dried and powdered root was extracted with 50ml: 25 ml / butanol: hexane respectively. Extracts were then concentrated. The condensed products were weighed and yield percentage was calculated.

6.1 Phytochemical screening

The phytochemical screening was done to identify the main groups of chemical constituents present in different extracts of *Bergenia ciliata* by their colour reactions with different reagents. Each extract was subjected for glycosides (anthraquinone glycoside and C-glycoside), alkaloids, terpenoids, steroids, flavonoids, reducing sugars, tannins and saponins tests using test procedures ^[6, 8].

6.2 Biological studies

The principle process to ascertain the biological activity of plant is the biological screening. In the present work, antimicrobial susceptibility test and brine shrimp bioassay of the plant extracts were investigated.

7. Antimicrobial Screening of the Extracts ^[9]

Preliminary antimicrobial test of *Bergenia ciliata* extracts were carried out by cup diffusion method using Ciprofloxacin as standard. The extracts, which showed preliminary antimicrobial activity, would be subjected for detail antimicrobial screening using Ciprofloxacin as standard.

7.1 Requirement

1. Mueller Hinton agar
2. Sterile distilled water
3. Membrane filter
4. Conical flasks
5. Sterile cork borer (diameter- 9 mm)
6. Micropipette(0.1 ml)
7. Petri plates containing Mueller Hinton agar
8. Sterile cotton swab
9. Inoculating loop
10. Forceps, Ruler

7.2 Microorganisms

i) Gram positive organisms

Staphylococcus aureus, *Streptococcus faecalis*

ii) Gram negative organisms

Escherichia coli, *Pseudomonas aeruginosa*

iii) Anti-microbial agents

Ciprofloxacin 100 µg/ml was used as the standard drug.

Microorganisms and standard Ciprofloxacin were obtained from Microbiology Laboratory of Sunsari Technical College.

iv) Plant extracts

Anti-microbial screening was performed in the leaf and root extract of the plant; distilled water and butanol: hexane-2:1 respectively. 800 mcg/ml, 400 mcg/ml, 200 mcg/ml, 100 mcg/ml, 50 mcg/ml, and 25 mcg/ml concentration of leaves (distilled water) extract and roots (butanol: hexane) extract was prepared by dissolving in suitable solvent i.e. sterile water for injection.

v) Methods

Antimicrobial test of plant extracts was carried out by cup diffusion method.

vi) Procedure ^[9]

Muller Hinton Agar media was prepared by the method mentioned in annex 2. The prepared media was sterilized in an autoclave at 121 °C for 15 minutes. The hot sterilized media was poured into sterile petri plates of size 90 mm diameter such that each plate contained 20-25 ml of medium. The plates were allowed to cool for 15-20 minutes and kept in refrigerator for solidification. Bacterial suspension was prepared by inoculating loop full of bacteria in Brain Heart Infusion (BHI). Cups were made in agar plates with the help of sterile cork borer of diameter 9 mm and labeled properly. The pure form of bacterial suspension was swabbed on the media with a sterile cotton swab in sterile condition and allowed to dry. To the different cups, 0.1 ml of 800 µg/ml, 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml and 25 µg/ml concentration of each extract was placed with the help of micropipette. Also the standard drug was placed as ciprofloxacin. All the plates were incubated at 37 °C for 24 hours. The zone of inhibition was measured and compared with standard.

8. Brine Shrimp Bioassay ^[10, 11]

A method utilizing brine shrimp is proposed as a simple bioassay for natural product research and is considered a useful tool for preliminary assessment of toxicity because they provide a quick and inexpensive alternative to vertebrate testing. The brine shrimp bioassay is based on the ability to kill laboratory cultured brine shrimp (*Artemia salina*) which belongs to phylum arthropoda and class crustacea. The procedure determines LC₅₀ values in µg/ml of test samples against nauplii in the brine medium. If LC₅₀ value of the test sample is less than 1000 µg/ml, the extract is considered biologically active ^[11].

8.1 Requirements

1. Brine shrimp (*Artemia Salina*) eggs
2. Seawater
3. Beaker (500 ml), Volumetric flask (1000 ml)
4. Test tubes
5. Pipettes and micropipettes
6. Magnifying glass
7. Incubator

8.2 Procedures ^[11]

i) Preparation of test samples

50mg of crude extract to be tested was dissolved in 5ml of suitable solvent depending upon its solubility. The ethanol extract was dissolved in distilled water. This solution was called as stock solution.

ii) Preparation of Sea water

Artificial Seawater was prepared by dissolving different chemicals as in stated amount in distilled water.

iii) Hatching of Brine shrimp

Hatching of brine shrimp was done in the beaker with 300ml of seawater by sparkling about 50 mg of brine shrimp eggs. This was then illuminated with table lamp of 100 watt for twenty-four hours to achieve the temperature of about 35 °C to hatch nauplii. After 24 hours, the nauplii were collected by dropper.

iv) Application of the test sample and brine shrimp nauplii to the test tubes

From the stock solution 500 µl (equivalent to 1000 ppm), 50 µl (equivalent to 100 ppm) and 5µl (equivalent to 10 ppm) were transferred to total of fifteen different test tubes, five test tubes for each doses level after evaporating the solvent. Similarly in the other three test tubes, the process was repeated by taking 500 µl, 50 µl & 5 µl of the solvent as control group. The nauplii were counted macroscopically in the stem of the dropper against the lighted background and ten matured and highly motile shrimp larvae were then transferred to each test tube and the volume was made up to 5ml on each test tube by adding the seawater, similarly ten matured brine shrimp larvae were transferred in each test tube of control group and the volume was made 5 ml in each.

v) Counting of nauplii

After 24 hours, the test tubes were observed and the number of survived nauplii in each test tube was counted using magnifying glass. From the data obtained, the LC₅₀ value was calculated.

vi) Data Analysis ^[11]

Cytotoxicity test depends on the calculation of LC₅₀ value. The

LC₅₀ value for the given extract is the lethal concentration that is required to kill the 50% of the brine shrimp nauplii. LC₅₀ value can be calculated from the graph obtained by plotting log concentration verses % mortality.

Where,

% Mortality can be obtained from the following formulae:

$$\% \text{ Mortality} = \frac{\text{Total number of deaths}}{10} \times 100$$

9. Results

9.1 Pharmacognostical Studies

Macroscopical studies

• General appearance

Leaves : Leaves are few, 4-11 x 3-10 cm, glabrous or hirsute, sub orbicular to orbicular or broadly obovate.

Petiole : 1-2(-5) cm long, glabrous or hirsute.

Rhizomes : solid, barrel shaped, cylindrical with ridges and furrows having rootlets.

• Organoleptic properties

Odor: Aromatic, like tea leave

Colour: Leaves are green in colour.

Rhizomes: are dark brown to light black.

9.2 Microscopical studies

T.S of rhizome shows cork divided into two zones, outer zone which is denser made up of compressed cells and inner multilayered zone composed of thin wall, tangentially elongated cells. Cortical zone is parenchymatous having few rosette crystals, large number of starch grains and tanniferous cells. Vascular bundle are arranged in a ring and are conjoint, ollateral and open. Xylem consists of vessel elements, tracheids, xy lem parenchyma and xylem fibers.



Fig 1: Dried rhizome of *Bergenia ciliata*

10. Extractive Values

The yield percentage for each extract was calculated and it was found to be the highest in case of ethanol extract. The second highest was that of Butanol: Hexane extract followed by Distilled water extract.

Table 2: shows the respective percentage yield for each extract.

Extracts	% Yield
Distilled water	0.982 gm
Ethanol	3.083 gm
Butanol:Hexane(2:1)	0.764 gm

11. Phytochemical Screening

Phytochemical screening of the plant showed the presence of

different constituents in different solvent extracts. The phytochemical analysis showed that the various groups that were found to be present in the different extracts are listed in table 3.

Table 3: Phytochemical Screening of Plant Extract

Plant(extracts)	Alkaloids	Glycosides	Saponins	Carbohydrates	Phenols	Flavonoids	Diterpines	Phytosterols	Proteins
Leaves	+	+	+	+	+	+	+	-	-
Rhizome	+	+	+	+	+	+	+	-	-
Roots	+	+	+	+	+	+	+	-	-

12. Loss on Drying

Initial Weight (Weight of Petri plate + Leaf powder before drying) = 90.0199

Final Weight (Weight of Petri plate + powder after drying in oven) = 89.968

Therefore,

Loss on drying

= (Weight of empty desiccator + Sample weight – Weight after drying) / Sample weight

= (89.046 + 0.936 – 89.968) / 0.936

= 0.0149

13. Melting Point

The melting point was tested by open capillary tube method. The leaf extract was placed in capillary tube and it was tied with a thread to the paraffin holder tube. The tube was heated from the bottom by burner and the melting point of extract was noted. The melting point test was carried for leaves extract and it was found to be 250 °C.

14. Thin Layer Chromatography

Chromatography is an important technique to identify the formulation of new compound and also to determine the purity of new compounds. The Rf value is characteristic of each compound. Thin layer chromatographs of different compound were carried out by preparing glass plates using Silica gel slurry. The plates were then dried in hot air oven and a drop of each extract, were placed in different plates and the plates were kept in beaker containing the appropriate solvent.

R.F = Distance travelled by solute / Distance travelled by solvent.

Table 4: TLC of extract

S.N	Extract	Solvent system	Rf value
1.	Leaves	Distilled Water	0.5616
2.	Roots	Butanol:hexane	0.8
3.	Rhizome	Ethanol	0.58

14.1 Comparison of RF of roots, leaves and rhizomes (Thin Layer Chromatography)**i) Antibacterial Activities of Different Extract****A. Leaves****Table 5:** Antibacterial Activity of Leaves in different concentration.

S.N	Bacteria	Standard (100 µg/ml)	800 µg/ml	400 µg/ml	200 µg/ml	100 µg/ml	50 µg/ml	25 µg/ml
1.	<i>S. faecalis</i>	2.6cm	2.6 cm	2.5 cm	1.5 cm	1.3 cm	1.2 cm	1.1 cm
2.	<i>S. Aureus</i>	2.5cm	2 cm	2 cm	1.8 cm	1.3 cm	1.2 cm	1.1 cm
3.	<i>Pseudomonas aeruginosa</i>	2.2cm	2.2 cm	2 cm	2 cm	1.1 cm	-	-
4.	<i>E. coli</i>	2.2cm	1.1 cm	-	-	-	-	-

B. Root**Table 6:** Antibacterial activities of Roots in different concentration

S.N	Bacteria	Standard (100 µg/ml)	800 µg/ml	400 µg/ml	200 µg/ml	100 µg/ml	50 µg/ml	25 µg/ml
1.	<i>Pseudomonas aureginosa</i>	2.2	2.1	2	2	1.5	-	-
2.	<i>S.aureus</i>	2.3	1.8	1.4	1.3	1.2	-	-
3.	<i>S. faecalis</i>	2.2	2.0	1.3	1.1	1	-	-
4.	<i>E.coli</i>	2.1	1.8	1.4	1	0.7	0.5	-

ii) Brine Shrimp Bioassay of Rhizome (Ethanollic Extract)**Table 7:** Effects of Rhizome (Ethanollic extract) on Brine Shrimp.

Conc. (µg/ml)	No of Survivors					No of Deaths					Average
	A	B	C	D	E	A	B	C	D	E	
500	1	1	-	-	-	9	9	10	10	10	9.6
50	4	5	4	5	5	6	5	6	5	5	5.4
5	7	7	5	7	5	3	3	5	3	5	3.8

iii) Mortality of Brine Shrimp

Table 8: Mortality of Brine Shrimp

S.N	Concentration	Log concentration	% Mortality
1.	500	2.69	96
2.	50	1.69	54
3.	05	0.69	38

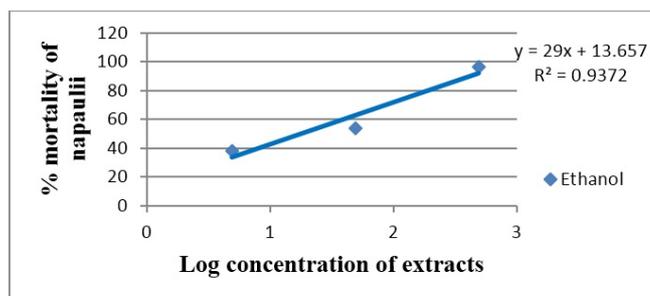


Fig 2: % Mortality Vs Log Concn

iv) Cytotoxic Effects of Extracts on Brine Shrimp (LC50 Concentration Inµg)

Table 9: LC 50 Concentration

S.N	Extract	LC 50	
		Log Cocn	Cocn
1.	Rhizome	1.2535	17.92

15. Discussion

The plant, *Bergenia ciliata*, was collected and pharmacognostical study was performed. The leaves, root and rhizome part was dried, grinded and extracted by distilled water, ethanol, butanol: hexane (2:1) respectively. These extracts were subjected to phytochemical and biological screenings. However, due to the lack of sufficient quantity of extracts, the pharmacological screening was conducted for ethanol extract for cytotoxic activity and roots and leaf extract for microbiological assay. Pharmacognostical analysis of the leaves showed the presence of cilia. Leaves are few, spreading, and 4-11 x 3-10 cm, glabrous or hirsute, sub orbicular to orbicular or broadly obovate. Petiole is 1-2(-5) cm long, glabrous or hirsute. Inflorescence a one sided raceme or corymbose. These distinct characteristics of the plant help in identifying the authentic plant and to find out possible adulteration and substitutes. The preliminary phytochemical screening of the plant was found to exhibit the positive tests for terpenoids, alkaloid, saponins, tannins, deoxy sugar and flavonoids; and negative tests for anthraquinone glycoside, and phytosterols. Brine shrimp bioassay revealed that extract showed LC50 value of 1.25 mcg/ml. The LC50 values, thus, indicate the cytotoxic property of the extracts since these values are less than 1000 mcg/ml. Both extracts showed concentration-dependent cytotoxicity in each of the three cell lines. According to the American national cancer institute, the LC50 value to consider a crude extract promising for development of anticancer drugs is lower than a limit threshold (30 µg/ml) here the ethanolic extract of rhizome showed

profound anticancer properties with LC 50 value of 1.25 µg/ml. *Bergenia ciliata* extracts exhibit rather a narrow spectrum antibacterial activity. Consequently it was suggested that the activity of root extract is much higher as compared to the leaves extract of *Bergenia ciliata*. Similarly, while performing antibacterial activity, the root extract was found to be more active against Gram Positive bacteria than Gram Negative. Also the activity of leaves extract was found to be lesser than that of root extract.

16. Conclusion

The study concluded that the extracts are active against both Gram Positive and Gram negative Bacteria tested. The Ethanolic extract (rhizome) shows good cytotoxicity activity with significant LC50 values. The study encourage futher future research to explore additional information on these therapeutic activities of this plant.

17. Acknowledgements

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