

EVALUATION OF ANTIBACTERIAL ACTIVITY OF *BERGENIA CILIATA*

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ABSTRACT:

Bergenia ciliata was subjected to antibacterial analysis. A battery of assays were performed on different extracts of *Bergenia ciliata* for antibacterial activities. Antibacterial effects of ethanol, hexane, ethyl acetate, chloroform, butanol and aqueous extracts of the roots and leaves extract of *Bergenia ciliata* exhibited various degree of inhibition activity. It was observed that root and leaves extract were promising against gram positive and gram negative bacteria viz. *Bacillus subtilis*, *Bacillus megaterium* and *Pseudomonas aeruginosa*. This study showed that *Bergenia ciliata* could inhibit selectively bacteria.

INTRODUCTION

Bergenia ciliata (Haw.) Sternb. belongs to family Saxifragaceae. The genus *Bergenia* comprises of about 6 species, distributed in the temperate Himalayas and central and East Asia, represented in Pakistan by two species namely *Bergenia ciliata* and *Bergenia stracheyi*. It is perennial herbs upto 50 cm tall, succulent, distributed in temperate Himalayan region (from Kashmir to Nepal) from 2000-2700 m. very common rocks in and around the Murree area (Bhakuni D.S. *et al.*, 1974).

The root of *Bergenia* has been considered to enjoy all the useful attributes of Gentiana root and has been regarded as demulcent and deobstruent, relieves pain in ribs and chest due to excessive cold humors, acts as effective diuretic and emmenagogue. Getrid of kidney's and bladder stones and obstructions or toxic waste products, which remain in the alimentary canal, urinary and excretory system. The infusion is considered to be more active than root. In asthma, bronchitis, epilepsy and spasmodic affections and to relieve flatulent colic in children. Root is effective to combat chronic venereal diseases (Akerle O., 1992).

Collection and Identification

The leaves and roots of *Bergenia ciliata* were collected from Thandiani, Abbottabad, North West Frontier Province. The specimen thus obtained was identified and authenticated by Prof. Dr. Muhammad Qaiser and Dr. Suraiya Khatoon, Taxonomy Section of the Department of Botany, University of Karachi. The voucher specimen has been also deposited in Herbarium of Department of Botany, University of Karachi.

Extraction and Isolation

After collection the leaves and roots of *Bergenia ciliata* were dried under shade for 45 days at room temperature. The leaves weighing 1200 g. were soaked in ethanol, while roots weighing 1500 g. also soaked in EtOH for 45 days. The same procedure was repeated thrice (x3). The EtOH

in both the cases was removed under vacuum at reduced pressure to afford a chocolate colored thick viscous syrupy extract weighing 128g of leaves extract, and 270g of root extract as EtOH. This ethanol extract was then diluted in distilled water and organic mass was recovered in hexane. This hexane soluble part was concentrated to 20g in leave and 44 g in root. In continuation, aqueous part was fractionated with ethyl acetate 22g of leaves and 50g of roots. Chloroform 18g of leaves and 48g of roots. Butanol 25g of leaves and 65g of roots and aqueous soluble part 15g of leaves and 30g of roots respectively.

Antibacterial Activity

Sample preparation:

All the roots and leaves extracts (viz. ethanol, hexane, ethyl acetate, chloroform, butanol and aqueous) prepared in distilled water (5 mg/ ml) and aliquots were used to test the anti bacterial activity.

The antibacterial activity had been tested against ten different species of gram positive and gram negative bacteria. Bacterial test cultures were maintained on the stock culture Tryptic soya agar (TSA). From the stock culture a loopful of the fresh culture was inoculated in Tryptic soya broth. The seed broths were incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 hours. Inocula were prepared by diluting 24 hours old cultures in saline. A dilution of 1: 100 was used in all the tests.

MATERIALS AND METHODS

Materials

- Tryptic soya agar media (Merck)
- Tryptic soya broth (Merck)
- Culture plates
- Sterile cork borer
- Streptomycin (Merck)
- 70% ethanol (Merck)
- Autoclave
- Incubators
- Wireloop
- Bacterial Test Cultures

Antibacterial Assay

The tests were run in triplicate. Petri plates (10x10 cm) were prepared with tryptic soya agar (Agarwal R. *et al.*, 1979). 0.1 ml of the diluted culture was poured on each plate and the plates were dried for 30 minutes at 37°C . Wells of 6 mm (approximate) diameter were cut with sterile cork borer in the inoculated agar. The wells were filled with the plant extract. Streptomycin (1 mg/ ml) and 70% ethanol were used as control in the other wells. The plates were incubated for 72 hours at 37°C . At the end of incubation period, the clear zone of inhibition around the wells was measured in millimeter (mm). The results are expressed in Table 1 and Table 2.

Following the anti bacterial assay, all the extracts were tested for their activity against the following bacterial culture.

Table 1
Bio-activity data of roots extracts of *Bergenia ciliata*

Extracts	Zone of inhibition in mm									
	Anti bacterial									
	<i>Bacillus subtilis</i>	<i>Bacillus megaterium</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus viridians</i>	<i>Micrococcus</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Salmonella typhi para B</i>	<i>Shigella sonnei</i>	<i>Pseudomonas aeruginosa</i>
Ethanol	••	••	••	N/A	••	N/A	N/A	N/A	N/A	•••
Hexane	••	•••	••	N/A	••	N/A	N/A	N/A	N/A	••••
Ethyl acetate	••••	••••	N/A	N/A	•••	••	N/A	N/A	N/A	••
Chloroform	••	••	••	N/A	N/A	••	N/A	N/A	N/A	•••
Butanol	••••	••••	••	N/A	••	••	N/A	N/A	N/A	••••
Aqueous	••	•••	••	N/A	N/A	••	N/A	N/A	N/A	•••

Table 2
Bio-activity data of leaves extracts of *Bergenia ciliata*

Extracts	Zone of inhibition in mm									
	Anti bacterial									
	<i>Bacillus subtilis</i>	<i>Bacillus megaterium</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus viridians</i>	<i>Micrococcus</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Salmonella typhi para B</i>	<i>Shigella sonnei</i>	<i>Pseudomonas aeruginosa</i>
Ethanol	N/A	••	••	N/A	N/A	N/A	N/A	N/A	N/A	••••
Hexane	N/A	N/A	••	N/A	N/A	N/A	N/A	N/A	N/A	••••
Ethyl acetate	N/A	N/A	••	N/A	N/A	••	N/A	N/A	N/A	••••
Chloroform	••	••	••	N/A	••	••	N/A	N/A	N/A	•••
Butanol	••••	•••	••••	N/A	••	••	N/A	N/A	N/A	••••
Aqueous	••	••	•••	N/A	••	••	N/A	N/A	N/A	••

N/A : None to very slight activity

• : 01-05 mm

••• : 11-15 mm

•• : 06-10 mm

•••• : 16-more

Gram Positive Bacteria:

Bacillus subtilis (ATCC 6633), *Bacillus megaterium* (ATCC 9885), *Staphylococcus aureus* (ATCC 6538), *Streptococcus viridians*, and *Micrococcus* species (ATCC 9341).

Gram Negative Bacteria:

Escherichia coli (ATCC 10536), *Salmonella typhi* (ATCC 6539), *Salmonella typhi para B* (ATCC 11511), *Shigella sonnei* (ATCC 11060), and *Pseudomonas aeruginosa* (ATCC 9027).

Controls:

Streptomycin (1mg/ml) (Merck)

70% Ethanol (Merck)

RESULTS AND DISCUSSION

Plants have been a source of medicinal compounds since pre-historic time. It is well established that all parts of plants were used in *Unani* systems of medicine for centuries. However, the discovery and use of synthetic drugs led to a dramatic decline in the popularity of herbal products used in the therapy. Nevertheless the realization of harmful toxic effect of a large number of synthetic drug led to for alternative sources which would be safe and effective in various ailments.

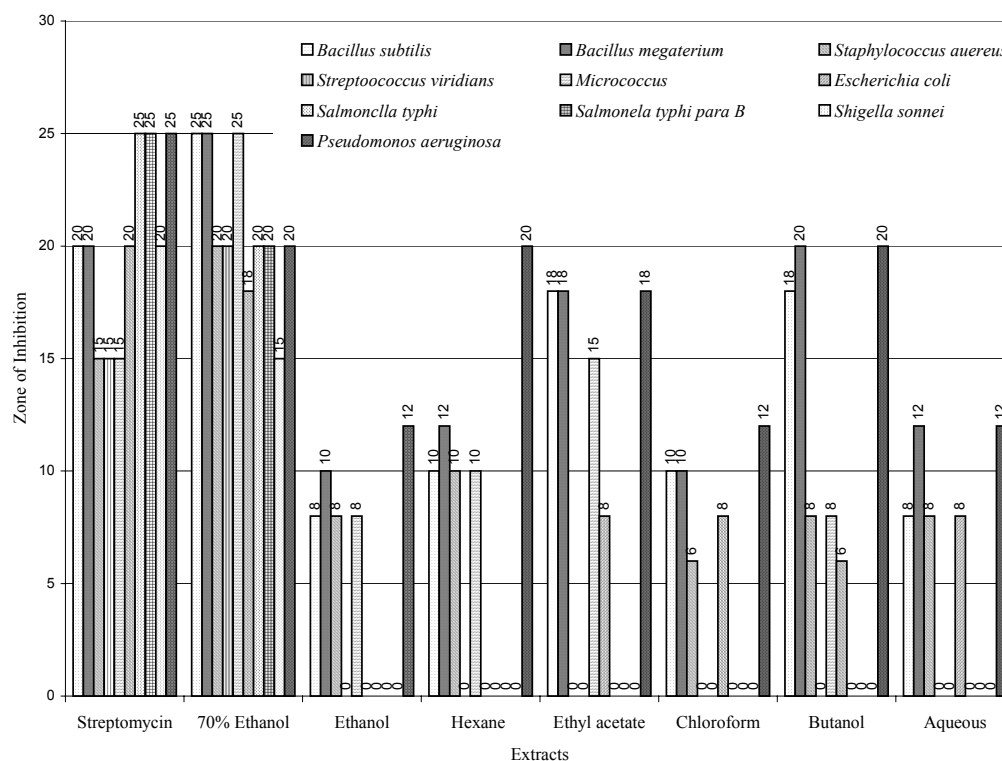


Fig. 1: Graphic representations of antibacterial activity of root extracts of *Bergenia ciliata*

A resurgence of interest in the study and use of medicinal plant taking place during the last three decades. A considerable growth has occurred in official and commercial interest in the use of natural products [4]. In recent years there has been a growing trend to evaluate the bioactivity of the medicinal plants, so that a systematic approach could be adopted for their therapeutic utilization. The present study is an attempt to investigate and evaluate the bioactivity of *Bergenia ciliata*, which is of considerable medicinal importance.

The biological screening of medicinal plant extracts has most frequently been carried out to determine the antibacterial and antifungal profile. These evaluations are usually done through different techniques to ascertain the inhibition effect on pathogenic and non-pathogenic bacteria. In this study antibacterial screening of the different extracts of roots and leaves of *Bergenia ciliata* have been attempted by agar-well diffusion method. In the experimental, ten bacterial strains were selected for the screening activity. The bacterial strains selected are: **gram positive** *Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus*, *Streptococcus viridians*, and *Micrococcus*; **gram negative**: *E. coli*, *Salmonella typhi*, *Salmonella typhi para B*, *Shigella sonnei* and *Pseudomonas aeruginosa*.

Antibacterial Activity of Roots Extract

The results of the antibacterial screening of the roots extract are expressed in table 1 revealed that the ethyl alcohol, hexane, ethyl acetate, chloroform, butanol and aqueous extracts exhibited antibacterial activity against two strains i.e. *Bacillus subtilis* and *Bacillus megaterium* (zone of inhibition range 8mm - 20mm). The ethyl acetate extract did not respond at all against *Staphylococcus aureus* and *Staphylococcus viridians*, whereas the rest of gram positive bacteria were inhibited (range 15 to 18mm) with the ethyl acetate extract. The ethanol, hexane, butanol

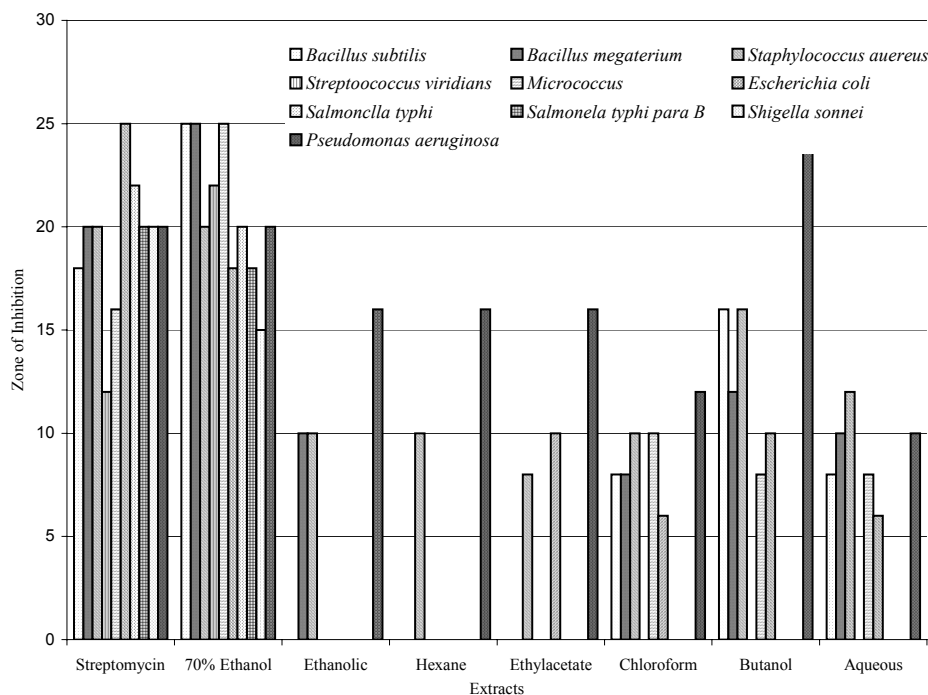


Fig. 2: Graphic representations of antibacterial activity of roots extracts of *Bergenia ciliata*

and chloroform extracts displayed no activity against *Streptococcus viridians* only. Furthermore chloroform and aqueous extract were also found devoid of any activity against *Micrococcus* spp. Thus it can be inferred that all the extracts of *Bergenia ciliata* roots have been shown to possess varying degree of different antibacterial activity against gram positive bacteria. Whereas the evaluation of antibacterial activity against the gram negative bacteria revealed that all the root extracts did not exhibit the activity against gram negative such as *Salmonella typhi*, *Salmonella typhi para B* and *Shigella sonnei*. Whereas the entire root extracts exhibited activity against *Pseudomonas aeruginosa* (zone of inhibition 12mm-20mm) and all the extracts except ethanol and butanol exhibited activity against *Escherichia coli* (zone of inhibition 6mm-8mm).

Thus it can be concluded that *Bergenia ciliata* root extract was found to inhibit the growth of two of the gram negative bacteria i.e. *Pseudomonas aeruginosa* and *Escherichia coli*. The graphic presentation of antibacterial activity of root extracts of *Bergenia ciliata* given in Fig. 1 revealed that gram positive bacteria were inhibited to the large extent as compared to gram negative strain which responded poorly. Therefore in a way it can be inferred that *Bergenia ciliata* extracts exhibit rather a narrow spectrum antibacterial activity.

Antibacterial Activity of Leaves Extract

The screening results of various leaves extract of *Bergenia ciliata* (Table 2) exhibited activity against the gram positive *Staphylococcus aureus* (zone of inhibition range [8mm-12mm]), whereas chloroform, butanol and aqueous extracts were found active against *Bacillus subtilis*, *Bacillus megaterium* and *micrococcus* (zone of inhibition range 10mm-20mm). No leave extract showed activity against *Streptococcus viridians*. All the leave extracts found active against gram negative *Pseudomonas aeruginosa* (zone of inhibition 10mm-20mm), whereas ethyl acetate, chloroform, butanol and aqueous extracts have shown activity against *Escherichia coli* (zone inhibition 6mm-10mm). All the leave extracts found inactive against *Salmonella typhi*, *salmonella typhi para B* and *Shigella sonnei*. The graphic presentation is given in Fig. 2. Consequently it can be suggested that the activity of root extract is much higher as compared to the leave extract of *Bergenia ciliata*.

In vitro antibacterial activity against pathogenic gram positive and gram negative bacteria has been reported in the essential oils of several plants viz. *Nigella sativa* (Sinha A.K. *et al.*, 1977), *Oenanthe garanicia* (Zutshi S.K. *et al.*, 1976), *Anaphalis controta*, *Hedychium spicatum*, *Salvia lanata* and zingiber officinale (Krishanaswamy M. And Purushotaman K.K., 1980), *curcuma langa*, *Laggea aurita*, *blumea membrancea* and *Psoralea corylifolia* (Sharma V.D. *et al.*, 1977). Similarly Plumvagin, *Plumbago zeylinica* (Gaitonde B.B. *et al.*, 1975), *alium sativum* (Sharma V.D. *et al.*, 1977) and *Berberis* spp (Gaitonde B.B. *et al.*, 1975) also exhibited antibacterial effect against gram positive and gram negative bacteria.

As such the different chemical constituents elaborated by different spp. of *Bergenia* displayed are coumarin benzenoids, steroids and tannins and the antibacterial activity of *Bergenia ciliata* may emancipate due to either of these constituents.

In conclusion, the summary of the biological activity evaluation of the leaves and root extracts of *Bergenia ciliata* can be delineated as follows:

The antibacterial activity of roots and leaves extract were promising against *Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. It is inhibiting selective both gram positive and gram negative bacteria. The antifungal activity of roots and leaves extract were effective against *Microsporium canis*, *Pleuroetus oustreatus*, and *Candida albicans*.

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