

EVALUATION OF ANTIBACTERIAL ACTIVITY OF *BERGENIA CILIATA* PLANT

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ABSTRACT

Plants used for traditional medicine contain a wide range of chemical constituents which can be used to treat various infectious diseases. *Bergenia ciliata* plant is traditionally used for the treatment of various diseases. In far-flung areas of Kashmir, its leaves are useful in the treatment of kidney stones and wounds. Therefore, antibacterial activities of different extracts of *Bergenia ciliata* were studied against eight bacterial strains four gram positive, four gram negative and six fungal strains. Four **Gram-positive** bacteria were *Staphylococcus aureus* MTCC 122, *Bacillus subtilis* NCIM 5251, *Staphylococcus aureus* NCIM 2492 and *Lactobacillus brevis* MTCC 1750 ; four **Gram-negative** *Serratia marcescens* NCIM 5246, *Pseudomonas aeruginosa* NCIM 2037, *Escherichia coli* NCIM 2109, *Enterobacter aerogenes* NCIM 5139. The activity was performed using four solvents viz., ethanol, methanol, acetone and water. Among all extracts, the ethanol extract of leaf showed significant activity in bacteria except *Staphylococcus aureus*. This activity of ethanol extract might be due to bioactive compounds present in this plant as well as maximum solubility of these compounds in it.

KEY WORDS; *Bergenia*, traditional, infectious, extract, solubility.

INTRODUCTION

For every infectious disease, as far as we can remember up until few years ago we had to take a pill for a few days and would get rid of that infection, but not so any more. The increasing occurrence of antimicrobial resistance represents a worldwide major concern for both human and veterinary medicine (Lorian, 1996). For this reason, there is a growing interest in the antimicrobial screening of extract from plants in order to discover new antimicrobial agents.

Nowadays, about 25% of the drugs prescribed worldwide come from plants and 252 of them are considered as basic and essential by the World Health Organization (WHO). The WHO considers phytotherapy in its health programs and suggests basic procedures for the validation of drugs in developing countries. Infectious diseases are the second leading cause of death worldwide (Fazly-Bazzaz et al., 2005). Herbal medicines that are extracted from different plant parts such as roots, bark, seeds, leaves and flowers contain a variety of naturally-occurring bio-chemicals, which contribute to the plant's medicinal benefits mostly against microorganisms (Folashade et al., 2014). The crude plant extracts of herbal plant in the form of decoction, tincture, infusion or herbal extract are traditionally used for the treatment of many diseases (Wendakoon et al., 2012). Extracts are administered as syrup as well as in the form of essential oils and creams. (Sofowora, 1993;

Wagner et al., 1994; Walters and Storband, 2000; Aarti and Mohile, 2003) World Health Organization defines Traditional medicine as referring to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicine, spiritual therapies, manual techniques and exercise, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being. (WHO, 2003). There is an increase interest to explore the secret of traditional herbal remedies based on information collected from local residents and traditional practitioners in different parts of the world (Mathabe et al., 2006; Abbasi et al., 2010). The search for new antibacterial agents, in particular, has increased in the last decade mainly because of the increasing bacterial infections especially in countries with poor populations and more so because of bacterial resistance to current antibiotics (Ahameethunisa, 2010). Since most of the infectious diseases are commonly treated with antibiotics and some antibiotics like Penicillin is added to items such as chewing gums, mouthwashes and toothpastes. Due to this indiscriminate use of antimicrobial drugs, have lead to the problem of drug resistance (Bidault et al., 2007; Hancock, 2005). Furthermore, the evolution of new strains of disease causing agents is of great concerns to the global health community (Frey and Meyers, 2010). In addition to this problem, antibiotics are sometimes associated with adverse effects on host which include, hypersensitivity, depletion of guts and mucosal microorganism, immune suppression and allergic reaction. Furthermore, antibiotics can also be responsible for killing of useful micro flora within the human body (Ahmad et al., 1998; Walsh, 2003; Koehn and Carter, 2005; Rafii et al., 2008; Rashid et al., 2012). It was also found that production of synthetic drugs results into the pollution of natural resources and ultimately leads to activation of antibiotic resistant genes in bacterial population in the environment (Krintiansson et al., 2011). All these factors associated with the use of synthetic drugs, has always been one of the driving force that encourage the researchers to search for the safe and economic alternatives from bio resource. One approach is to screen the local medicinal plants for possible antimicrobial properties. Thus, the present study is an attempt to investigate and evaluate the bioactivity of *Bergenia ciliata* plant which is of considerable medicinal importance. *B. ciliata* is used in traditional Ayurvedic medicine for the treatment of several diseases in Nepal, India, Pakistan, Bhutan and some other countries. Medicinal activity of plants is due to the presence of secondary metabolites. This plant contains many secondary metabolites like glycosides, alkaloids, terpenoids, steroids, flavonoids, reducing sugars, tannins, fatty acids, and saponins (khan and kumar, 2016). The drug is reported for the treatment of asthmatic disorders in traditional medicine in Jammu and Kashmir. It is a very promising herb with many traditional uses.. Juice of the rhizome of *B. ciliata* has been taken orally by human adult to relieve intermittent fever (Malaria) (Bhattra, 1992). It is reported to be helpful in dissolving kidney stones. The juice of the leaves of *B. ciliata* is used for earache (The Wealth of India, 1998). It has also been used as a poultice, as it is regarded as a locally effective remedy for boils in Kashmir, here it is known as "Zakhhm-e hayat" (Mehra and Raina, 1971).

MATERIALS AND METHODS

Plant Collection

Fresh plants were collected from hilly areas of Kashmir valley from Apharvat nearby Gulmarg and authenticated at the Botany department of Kashmir university. The different parts viz, leaves, roots and stem of the authenticated herbal plant were then collected and dried under sun for six weeks. The dried parts were cut into smaller pieces and ground into fine particles with a grinder. Lowering particle size increases surface contact between sample and extraction solvents. The powdered sample were separately bagged in transparent poly bags and stored in air tight container for further use.

Preparation of extracts

Extraction is an important step in the screening of bio-active compounds from plant materials. The purpose of all extraction is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc (residue) that is not required, with the help of solvents. Different extracts were prepared in different solvents viz, methanol, ethanol, acetone and aqueous.

For aqueous extraction, 8g of dried plant powder was added to 100ml distilled water and boiled on slow heat for 15-20min. It was then filtered through 7 layers of muslin cloth and centrifuged at 5000rpm for 15 min. The supernatant was then collected and concentrated (evaporated) to make the final volume one-tenth of its original volume i.e., 80% extract likewise 60%, 40% and 20% extracts were prepared (Parekh et al., 2005). It was stored at 4°C in air tight bottles for further work.

For solvent extraction, 8g of dried plant powder was taken in 100 ml of organic solvent (methanol, ethanol and acetone) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 hrs. After 24 hours the supernatant was collected and the solvent was evaporated to make the final volume one-tenth of its original volume (Parekh et al., 2005) thereafter, stored at 4°C in airtight bottles for further work.

Microorganisms

Eight bacterial cultures four **Gram-positive** *Paenibacillus polymyxa* MTCC 122, *Bacillus subtilis* NCIM 5251, *Staphylococcus aureus* NCIM 2492 and *Lactobacillus brevis* MTCC 1750; four **Gram-negative** *Serratia marcescens* NCIM 5246, *Pseudomonas aeruginosa* NCIM 2037, *Escherichia coli* NCIM 2109, *Enterobacter aerogenes* NCIM 5139 were used. Cultures were obtained from Codon biotech. All the strains were maintained on agar slants at 4°C for antimicrobial screening tests.

Antimicrobial screening

Screening for antibacterial activity

The agar well diffusion method is the most widely used technique for assaying plant extracts for their antimicrobial activity. In this technique, a well or reservoir containing the test compound at a known concentration is brought into contact with an inoculated medium and the diameter of the clear zone around the reservoir (zone inhibition diameter) is measured at the end of the incubation period. Activity of extracts were tested with agar well diffusion method (Srinivasan et al., 2001; Sen and Batra, 2012). Sterilized nutrient agar media 20ml was poured in petri plates near the flame. After solidification of media, plates were streaked with

bacterial culture by using sterile cotton swabs Wells (8mm) were punched out of the solid agar using μ pipette tips, sealed with molten agar to prevent leaching of the compound then 150 μ l of extract was placed in wells. Control wells containing neat solvents were also run parallel in the same plate. The petri dishes were allowed to diffuse at room temperature for 1hr and then incubated at 37°C for 20 to 24 hrs and average diameter of the inhibition zones surrounding the wells were measured for the result.

Qualitative phytochemical analysis

Test for tannin

About 0.5 g of the dried powdered samples were boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue- black coloration (**Jigna and Sumitra, 2007**).

Test for fatty Acids

0.5 ml of extract was mixed with 5 ml of ether. These extract was allow it for evaporation on filter paper and dried the filter paper. The appearance of transparence on filter paper indicates the presence of fatty acids (**Ayoola et al., 2008**).

Test for anthocyanins

2 ml of aqueous extract is added to 2 ml of 2N Hcl and ammonia. The appearance of pink-red turns blue-violet indicates the presence of anthocyanins (**Paris and Moyse, 1969**).

Test for leucoanthocyanins

5 ml of aqueous extract added to 5 ml of isoamyl alcohol. Upper layer appears red in colour indicates for presence of leucoanthocyanins (**Paris and Moyse, 1969**).

Test for coumarins

3 ml of 10% NaOH was added to 2 ml of Lots of pharmaceutical studies have demonstrated that aqueous extract formation of yellow colour indicates the emodin has many biological effects, such as anticancer, presence of coumarins(**Rizk, 1982**).

Test for emodins

2 ml of NH OH and 3 ml of Benzene was added to the extract. Appearance of red colour indicates the presence of emodins (**Rizk, 1982**).

Test for reducing sugar

To 0.5 ml of extract solution, 1 ml of water and 5 - 8 drops of Fehling's solution was added at hot and observed for brick red precipitate (**Siddiqui and Ali, 1997**).

Test for saponin

0.2 g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing shows the presence of saponin (**Sristisri upadhyaya and Saikia, 2012**).

Test for flavonoid

0.2 g of the extract was dissolved in 10% NaOH solution, yellow coloration indicates the presence of flavonoid. Test for phenol: To 2ml of extract solution, added 2ml of alcohol and few drops of ferric chloride solution and observed for coloration (**Sristisri upadhyaya and Saikia, 2012**).

Test for alkaloid

0.5 g extract was boiled with conc. HCl and filtered. 0.5ml of picric acid and Mayer's reagent was added separately to about 1ml of the filtrate in a different test tube and observed for yellow-whitish or cream colour precipitate (**Evans, 2002**).

Test for anthraquinone

To 0.2g of extract, added 5ml of chloroform and 5ml of 10% ammonia solution. The presence of bright pink colour in the aqueous layer indicated the presence of anthraquinone (**Sristisri upadhyaya and Saikia, 2012**).

Test for trapezoid and steroid

5ml of extract solution was mixed in 2ml of chloroform, and 3ml of conc. sulphuric acid was added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenism. Red colour at the lower surface indicates presence of steroid (**Sristisri upadhyaya and Saikia, 2012**).

Test for reducing sugar

To 0.5 ml of extract solution, 1ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitation (**Sristisri upadhyaya and Saikia, 2012**).

Test for terpenoids

Salkowski test: The extract was mixed with 2ml of chloroform and concentrated H₂SO₄ (3ml) is carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids (**Rimjhim et al., 2014**).

RESULT AND DISCUSSION

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 leaves, roots and stem of *Bergenia ciliata* plant have been attempted by agar-well diffusion method. In the experiment, eight bacterial strains were selected for the screening activity. The bacterial strains selected were: **gram positive** *Bacillus subtilis*, *Paenibacillus polymyxa*, *Staphylococcus aureus*, and *Lactobacillus brevis*; **gram negative**: *E. coli*, *Serratia marcescens*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa*.

The results showed that among the different extracts of *Bergenia ciliata* plant, the ethanol extract of leaves exhibited maximum activity against all bacteria except one, *Staphylococcus aureus*. The activity was less in case of roots and nil in stem. Acetone extract was active against *Paenibacillus polymyxa* and *Lactobacillus brevis* only while methanol and aqueous showed no activity at all. The activity may be due to the secondary metabolites present in this plant and maximum solubility of these in ethanol.

Secondary metabolites are important for humans because of many pharmacological activities. Most pharmaceuticals are based on plant chemical structures and secondary metabolites. Secondary metabolites have been isolated from plants which give pharmacological effects in humans so that is used as medicine (William, 1996; Bidlack and Wayne, 2000; Karban et al., 1997; Rosenthal et al., 1991). Various secondary metabolites present in this plant are Bergenin, Tannic acid, Gallic acid, Stigmesterol, β -Sitosterol, Catechin, Hydroquinone, (+) Afzelechin, 1,8-cineole, Isovaleric acid, (+)-(6S)-parasorbic acid, Arbutin, Phytol, β -Caryophyllene, Damasconone, β -eudesmol, 3-methyl-2-buten-1-ol, (Z)-asarone, Terpinen-4-ol, Paashaanolactone (Chauhan et al., 2015). Among these metabolites parasorbic acid, afzelechin, Gallic acid, asarone and β -caryophyllene possess antimicrobial activity as reported by (Chung et al., 1998; Pokhrel et al., 2014; Ahmad et al., 2017; Khan et al., 2015). So, it might be possible that the antimicrobial activity of this plant is due to the synergistic effect of these metabolites.

Table(1): Antibacterial activity of different extracts of *Bergenia ciliata* leaves:

Bacterial strains	Zone of inhibition in mm								
	Ethanol		Methanol		Acetone		Aqueous		
	80%	40%	80%	40%	80%	40%	80%	40%	
Lactobacillus brevis	46	07	NA	NA	07	NA	NA	NA	NA
Pseudomonas aeruginosa	35	08	NA	NA	NA	NA	NA	NA	NA
Bacillus subtilis	50	20	NA	NA	NA	NA	NA	NA	NA

Escherichia coli	50	11	NA	NA	NA	NA	NA	NA
Serratia marcescens	55	25	NA	NA	NA	NA	NA	NA
Paenibacillus polymyxa	62	11	NA	NA	10	NA	NA	NA
Enterobacter aerogenes	52	23	NA	NA	NA	NA	NA	NA
Staphylococcus aureus	NA							

Table(2):Antibacterial activity of different extracts of *Bergenia ciliate* roots

Bacterial strains	Zone of inhibition in mm							
	Ethanol		Methanol		Acetone		Aqueous	
	80%	40%	80%	40%	80%	40%	80%	40%
Lactobacillus brevis	09	NA	NA	NA	NA	NA	NA	NA
Pseudomonas aeruginosa	14	NA	NA	NA	NA	NA	NA	NA
Bacillus subtilis	14	NA	NA	NA	NA	NA	NA	NA
Escherichia coli	14	NA	NA	NA	NA	NA	NA	NA
Serratia marcescens	11	NA	NA	NA	NA	NA	NA	NA
Paenibacillus polymyxa	17	15	NA	NA	NA	NA	NA	NA
Enterobacter aerogenes	10	NA	NA	NA	NA	NA	NA	NA
Staphylococcus aureus	17	NA	NA	NA	NA	NA	NA	NA

Table(3):Phytochemical analysis of different extracts of *Bergenia ciliata* plant:

Chemical compounds	Ethanol			Methanol			Acetone			Aqueous		
	Leaf	root	Stem	Leaf	root	stem	Leaf	root	Stem	leaf	root	stem
Alkaloids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Flavonoids	+ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve
Tannins	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Saponins	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Reducing sugars	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Phenols	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Terpenoids	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
Trapezoid	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve
Steroid	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve
Fatty acids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Emodins	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Leucoanthocyanins										-ve	+ve	-ve
Coumarins										+ve	-ve	+ve
Anthocyanins										-ve	-ve	-ve

+ = present, - = absent

From the results above it is evident that *Paenibacillus polymyxa* exhibited the highest zone of inhibition with 62mm in ethanol leaf extract and 17mm in ethanol root extract while extract was the least active against *Enterobacter aerogenes*, this shows that the leaf extract of *Bergenia ciliata* has higher antibacterial activity as compared to root extract. The extracts were more active against Gram-positive bacteria than the Gram-negative bacteria. This is in agreement with previous reports that plant extracts are more active against Gram-positive bacteria than against Gram-negative bacteria (Parekh and chanda, 2006). So, it can be stated that ethanol leaf extract exhibits maximum activity perhaps due to the intense solubility of secondary metabolites in it.

CONCLUSION

It can be concluded from the above results that ethanol extract of *Bergenia ciliata* has significant activity against all test organisms perhaps due to the secondary metabolites present in this plant. The results of the present study support the folkloric usage of the studied plant and suggest that most of the extracts of the plant possess compounds with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. In future, further work should be done to isolate and purify the bioactive compounds associated with the antimicrobial activity of *Bergenia ciliata* plant.

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