Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2014, Volume 2, Issue 4
ISSN:2321–3647(online)

Evaluation of Antimicrobial Activity of *Bergenia Ligulata*

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ABSTRACT

The rhizome of *Bergenia ligulata*, belonging to the family *Saxifragaceae* was screened for its antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae* and *Salmonella typhii* using agar well diffusion method. Methanol extract exhibited larger inhibition zone 21mm against S*taphylococcus aureus* and *Streptococcus pneumoniae* as compared to S*almonella typhii* and *Escherichia coli*. Ethanol extract showed larger inhibition zone against *Staphylococcus aureus* and *Salmonella typhii.* Acetone extract showed larger inhibition of 24mm zone against *Escherichia coli* as compared to *Staphylococcus aureus, Salmonella typhii* and *Streptococcus pneumoniae*. Water extract showed largest inhibition zone of 25mm against *Staphyloccus aureus* as compared to *Salmonella typhii, Escherichia coli* and *Streptococcus pneumoniae*. The presence of phytochemicals like Saponins, Flavonoids, Phenols, Tannins, Quinones, Carbohydrates, steroids and alkaloids supports its use as medicinal plant for the treatment of various ailments.

Keywords: Berginia ligulata, antimicrobial activity, *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae* and *Salmonella typhii*

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Received 19 March 2014, Accepted 28 March 2014

Please cite this article in press as: Monika *et al.*, Evaluation of Antimicrobial Activity of *Bergenia Ligulata.* American Journal of Pharmacy & Health Research 2014.

INTRODUCTION

Bergenia ligulata belongs to family *saxifragaceae*. It is called Stone breaker because it dissolves slabs. Rhizome is the medicinally used part of this. The plant is mostly found in temperate Himalayas from Kashmir to Bhutan and in Khasia hills at 15, 00 meter altitude where it is popularly known as Paashaanbhed.[1] The roots of *Bergenia ligulata* are used as an antidiabetic drug, diuretic, astringent, cardiotonic, wound healer, expectorant, antipyretic and anti-haemorrhoidal.*Bergenia ligulata* etc have many secondary metabolites such as Bergenin, Tannic acid , Gallic acid , Stigmesterol , β-Sitosterol , catechin , (+)-Afzelechin , 1,8-cineole , Isovalaric acid , (+)- (6S)-parasorbic acid , Arbutin , Phytol , Caryophyllene , Damascenone , β-eudesmol , 3-methyl-2-buten-1-ol ,(Z)-asarone , Terpinen-4-ol and Paashaanolactone which are medicinally important.[2] *Berginia ligulata* is one of the prominent ayurvedic herbs for acute and chronic urinary tract infection.[3] *Bergenia ligulata* is widely used plant in South Asia, mainly India and Pakistan, as a traditional medicine for treatment of urolithiasis.[4] The results of the preliminary investigations revealed the presence of alkaloids, steroids, flavonoids, terpenoids, tannins, glycosides, carbohydrates and saponins.[5] These taxons belong to the widely used medicinal herbs in the traditional Chinese, Nepalese and Indian medicine, for therapy of cough and pulmonary diseases, to stop bleeding, to increase immunity and to dissolve kidney or bladder stone.[6] In the course of screening of plant extracts for anti-influenza viral activity, 50% aqueous methanolic extract exhibited antiviral activity against influenza Virus A.[7]

Figure .1 *Bergenia ligulata*

MATERIAL AND METHODS

Present study was carried out to check the antimicrobial activity of *Bergenia ligulata* and performed phytochemical screening for analysis of chemical constituents.

Procurement and Preparation of the sample

(Bergenia ligulata) `Bergenia ligulata was collected from a garden in Kashmir. It was

authenticated by department of Botany, Mata Gujri College, Fatehgarh Sahib. From whole plant of *Bergenia ligulata* the root part was separated. Then washed and crushed in a kitchen crusher Then shade dried for 3 days. After drying, the powder was again crushed in pestle and motar for fine powder formation and was ready to use.

Soxhlet Extraction-

It is a piece of laboratory apparatus invented in 1879 by Franz von soxhlet. It was originally designed for the extraction of lipids from a solid material [8]. It is required where the desired compound has limited solubility in a solvent and the impurity is insoluble in that solvent.We took 5 grams dried powder of *Bergenia ligulata* and loaded into the main chamber of soxlet extracter. The soxhlet was then equipped with a condenser. The temperature of distillation port was set to boiling point of the solvent used. Repeated cycles were allowed till the colored extraction changes to colorless. Then the liquid extract was evaporated using water bath to get dried extract, the extract was weighed and dissolved in that to get a solution. The plant extract was used for antimicrobial activity and phytochemical analysis.

Purification of the microbial strains

Microbial cultures i.e *Staphylococcus aureus, Salmonella typhii, Escherichia coli* and *Streptococcuss pneumoniae* were maintained in nutrient broth. Purification of cultures was done by streaking method. Colonies were picked from petriplates and inoculated into the sterilized broth present in the flask. Then the cultures were incubated at 37°c for 24 hrs.[9]

Antimicrobial Agents Susceptibility test

Susceptibility to antimicrobial agents was determined by well diffusion method of Kirby Bauer on Mueller Hinton agar as described by clinical laboratory standard institute. Plates of sterilized Mueller Hinton agar were prepared. Cultures were inoculated by spreading inoculums over the entire surface of plates. Extracts used for susceptibility tests were of different concentrations. Then plates were incubated at 37°C for 24 hours and were measured with zone inhibition scale. The antimicrobial activity was then compared with standard antibiotics – Gentamycin, penicillin and ampicillin.[10]

Phytochemical testing

Various tests were performed for analysis of phytochemicals present in *Bergenia ligulata*[11]

Test for Steroids:

To 1 ml of extract, 1 ml of glacial acetic acid and 1 ml of acetic anhydride was added. Then 2 drops of conc. H_2SO_4 was added. If red, then blue and finally bluish green color appears, it shows the presence of steroids.

Test for Saponins:

To 1 ml of extract, 5 ml of water was added and tube was shaken vigorously. If copious lather formation occurs, it shows the presence of saponins.

Test for Phenols:

To extract few drops of 10% aqueous ferric chloride was added. If blue or green color appears it indicates presence of phenols.

Test for Flavonoids:

Shinoda test:

To extract, few magnesium turnings and 1-2 drops of conc. HCl was added. If formation of red color occurs, it shows the presence of flavonoids.

Test for alkaloids:

Hager's test:

To extract 3 ml of Hager's reagent was added. If formation of yellow precipitate occurs, it indicates presence of alkaloids.

Test for tannins:

To extract ferric chloride was added. If formation of dark blue or greenish black color takes place, it shows the presence of tannins.

Test for proteins:

To the extract, 1 ml of 40% sod. Hydroxide solution and 2 drops of 1% copper sulphate solution was added. If Violet color appears, it shows the presence of proteins.

Test for amino acids:

Two drops of ninhydrin solution was added to the plant extract of *Bergenia ligulata* in order to show the presence of amino acid in the plant extract.

Test for carbohydrates: **Fehling's test**:

To the extract, equal quantities of Fehling's solution A and B were added and on heating if brick red color appears, it shows the presence of carbohydrates.

Test for quinones:

To 1 ml of extract, 1 ml of conc. sulfuric acid was added. If red color appears, it shows the presence of quinones.

High performance liquid chromatography (HPLC)

Quantitative analysis of the sample was performed using HPLC. It was carried out in isocratic conditions using a C-18 reverse phase column. Samples were filtered through an ultra membrane filter (pore size 0.45 µm) prior to injection in the sample loop. Quercetin, rutin, gallic acid and

kaempferol were used as standards. Phenolic acids present in each sample were identified by comparing chromatographic peaks with the retention time (R_t) of individual standards and further confirmed by co-injection with isolated standards. The amount of each phenolic acid is expressed as micrograms per gram of fresh weight.[12]

RESULTS AND DISCUSSION

Testing of antimicrobial activity of plant extract different cultures

The liquid extract was evaporated using water bath to get dried extracts. The extracts were weighed and dissolved in different solvents to get a solution. Then the plant extracts were used for antimicrobial activity analysis.

Antimicrobial agent susceptibility test

Susceptibility to antimicrobial agents was determined by well diffusion method of Kirby Bauer on Muller Hinton agar. While using plant extract the results were interpreted as:-

The strength of activity is presented as resistant (>7mm), intermediate (>12mm) and susceptible (>18mm).[13] Effectiveness of different extracts was determined by the size of the control organism growth inhibition zone around the well (diameter of zone in mm).

In the present study, effectiveness of extracts was confirmed by agar well diffusion method and growth inhibition zone diameter were measured in presence and absence of each plant extract in different concentrations. Results are present in table 1 to 4. In table 1 methanol extract showed larger inhibition zone against *Staphylococcus aureus* and *Streptococcus pneumoniae* as compared to *Salmonella typhii* and *Escherichia coli*. In table 2 ethanol extract showed larger inhibition zone against *Staphylococcus aureus* and *Salmonella typhii* as compared to *Escherichia coli* and *Streptococcus pneumoniae*. In table 3 acetone extract showed larger inhibition zone against *Escherichia coli* as compared to *Staphylococcus aureus*, *Salmonella typhii* and *Streptococcus pneumoniae*. In table 4 water extract shows largest inhibition zone against *Staphyloccus aureus* as compare to *Salmonella typhii*, *Escherichia coli* and *Streptococcus pneumoniae*.

Figure 2 Antimicrobial susceptibility test: showing the presence of microorganism against *Bergenia ligulata* extract

Table 1 Antimicrobial activity of *Bergenia ligulata* extract in methanol solvent

Name of micro organism	Inhibition Zones at concentration(28mg)/100µl	Inhibition zones at concentration(42mg)/100µl
Staphylococcus aureus	18mm	21mm
Salmonella typhii.	15mm	18mm
Escherichia coli.	17mm	20mm
Streptococcus pneumoniae.	18mm	21mm

Table 2 Antimicrobial activity of *Bergenia ligulata* extract in ethanol solvent

Name of micro organism	Inhibition Zones at concentration (28mg)/100µl	Inhibition Zones at concentration (39mg)/100µl
Staphylococcus aureus	20mm	21mm
Salmonella typhii.	17mm	21mm
Escherichia coli.	16mm	18mm
Streptococcus pneumoniae.	19mm	20mm

Table 3 Antimicrobial activity of *Bergenia ligulata* extract in acetone solvent

Name of micro organism	Inhibition Zones at concentration (17mg/100µl)	Inhibition Zones at concentration (26mg/100µl)
Staphylococcus aureus	18mm	20mm
Salmonella typhii.	14mm	18mm
Escherichia coli.	22mm	24mm
Streptococcus pneumoniae.	19mm	20mm

Table 4 Antimicrobial activity of *Bergenia ligulata* extract in water solvent

Name of micro organism	Inhibition Zones at concentration (53mg/100µl)	Inhibition Zones at concentration (71mg/100µl)
Staphylococcus aureus	22mm	25mm
Salmonella typhii.	23mm	24mm
Escherichia coli.	16mm	18mm
Streptococcus pneumoniae.	18mm	20mm

Phytochemical screening of *Bergenia ligulata* showed the presence of constituents like saponins, flavonoids, phenols, tannins, quinones, carbohydrates and absence of constituents like alkaloids, amino acids, steroids, and proteins in methanol extract. In case of ethanol extract it showed the presence of chemical constituents like saponins, flavonoids, phenols, quinones, carbohydrates, steroids and absence of constituents like tannins, alkaloids ,amino acids and proteins. In case of acetone extract it showed the presence of constituents like saponins, flavonoids, phenols ,tannins, quinones, carbohydrates and absence of constituents like alkaloids, amino acids, steroids ,proteins. In case of water extract it showed the presence of constituents like flavonoids, phenols, tannins, quinones, carbohydrates, alkaloids and absence of constituents like amino acids, steroids, proteins.

HPLC Analysis

The HPLC fingerprints of the crude extracts of *Bergenia ligulata* showed four types of the flavonoids i.e. quercetin, rutin, gallic acid, kaempferol that are present in varying amount in the

different solvent extracts (Table 5 and Figure. 3).

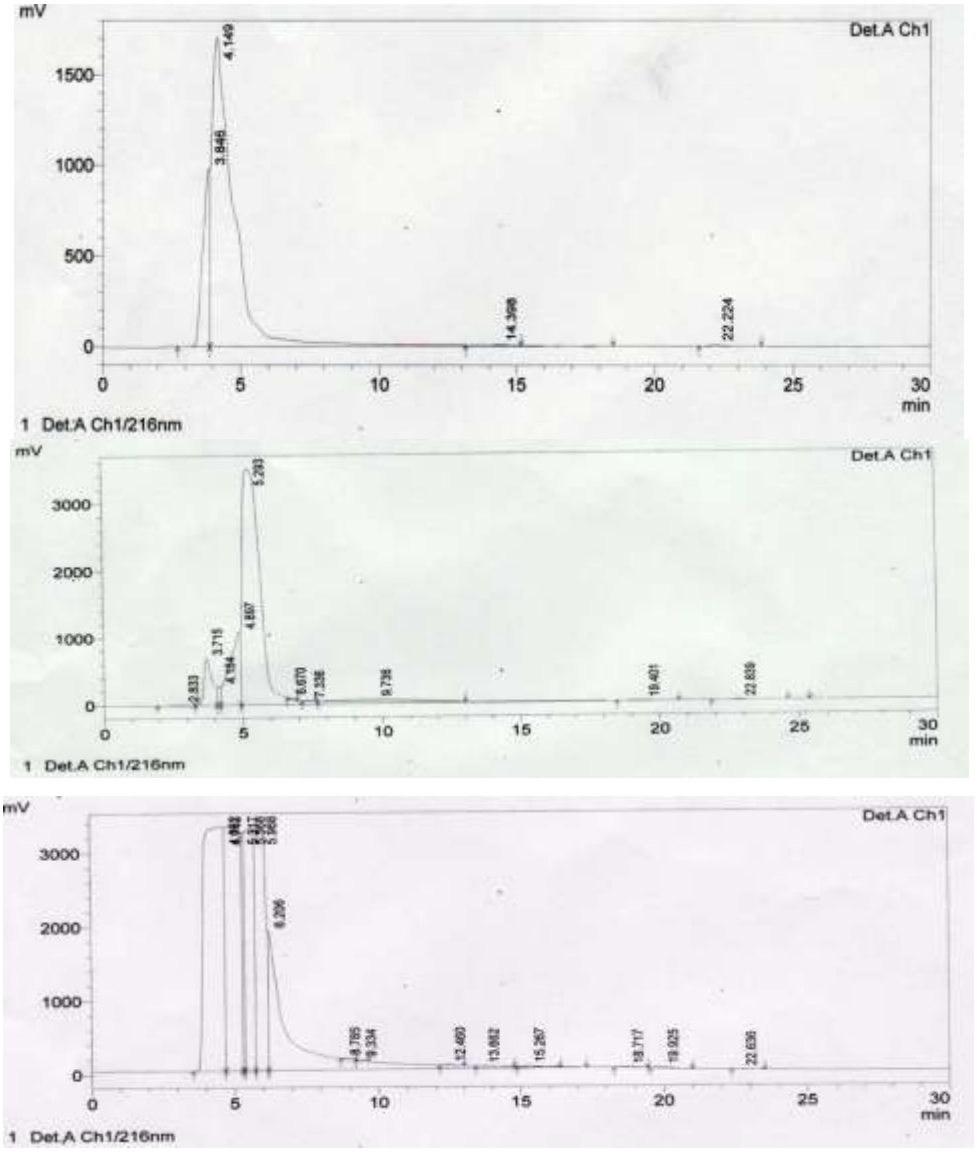

Figure 3 HPLC chromatogram of components present in *Bergenia ligulata* in ethanol, methanol and acetone solvents respectively

Table 5: Flavonoids in the crude extracts of *Bergenia ligulata*

Crude extract in solvents	Flavonoids (mg / gm of dry weight)			
	Quercetin	Rutin	Gallic Acid	Kaempferol
Methanol	1µg/g	-	50mg/g	20µg/g
Ethanol	-	0.7µg/g	88mg/g	12µg/g
Acetone	4 µg/g	580	44mg/g	60 µg/g

Present study reveals the antimicrobial susceptibility of herbal extracts against four cultures. Among all acetone extract has shown highest inhibitory effect against *Escherichia coli*. *Bergenia ligulata* water extract shows better antimicrobial results as compared to penicillin and ampicillin against *Salmonella typhii*. Where as its acetone extract showed better results as compared to

penicillin and gentamycin against *Streptococcus pneumoniae*. As we increase the concentration of *Bergenia ligulata* extract the antimicrobial activity will also get increased. Phytochemical screening of *Bergenia ligulata* showed presence of flavonoids and phenolic compounds which may be responsible for pharmacological activities. The anti-inflammatory and the antimicrobial effects of *Bergenia ligulata* can be attributed to the presence of sterols, glycosides and other chemical constituents present in *Bergenia spp*. In the roots of *Bergenia ligulata* bergenin and β-sitosterol and recently paashaanolactone have been isolated.[14,15] HPLC analysis of *Bergenia ligulata* extract showed presence of quercetin, rutin, gallic acid and kaempferol, but concentration of gallic acid is very high as compared to quercetin, rutin and kaempferol. This result is similar according to which the rhizome of *Bergenia ligulata* were found to contain higher concentration of gallic acid than other part of plant. *Bergenia* is a source of Bergenin, Quercetin-3-0-α-L-rhamnoside, Kaempferol-3-0-α-L-rhamnoside constituents[16].

CONCLUSION

The in-vitro studies of different extracts of *Bergenia ligulata* rhizome prove its use as antimicrobial agents against *Salmonella typhii, Escherichia coli* (isolated from urine sample of patient), *Streptococcus pneumoniae* and *Staphylococcus aureus.* Hence, *Bergenia ligulata* extract and their components can be recommended for therapeutic purposes and be used as an alternative medicine.

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