

Evaluation of antioxidant and antimicrobial activities of *Bergenia ciliata* Sternb (Rhizome) crude extract and fractions

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Abstract: Biologically, screening of medicinal plants extracts have been since pre-historic to determine the antioxidants and antimicrobial profile. The present study was aimed to investigate and evaluate crude extract and different fractions of *Bergenia ciliata* Sternb (rhizome) for bioactivity which is most considerable medicinal plants. The chloroform fraction was found to be highly anti-oxidative with the IC₅₀ value (4.15±0.82) as compare to ethyl acetate and n-hexane fraction. In addition, neither crude extract nor any fraction showed inhibition against fungal strains, i.e. *Aspergillus flavus* and *Aspergillus niger*. Furthermore, the crude extract and fractions of *B. ciliata* (rhizome) exhibiting promising activities against *Bacillus atrophoeus*, *Bacillus subtilis*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* strains. In summary, *B. ciliata* is recommended as a source of bioactive compounds which might be used against oxidative stress and drug resistance bacteria.

Keywords: *Bergenia ciliata*, antioxidant, antifungal, antibacterial.

INTRODUCTION

The northern areas of Pakistan are rich in biodiversity and contain more than 400 species of medicinal plant. The evergreen perennial herb *Bergenia ciliata* Sternb is tall from 30 cm to 150 cm (Rajkumar *et al.* 2010), belong to family saxifragaceae (Jan *et al.* 2014), the genus consist of six species found in the Himalayas and Central and East Asia (Siddiqi *et al.* 2012), while with two species namely *Bergenia ciliata* and *Bergenia stracheyi* were found in Pakistan temperate Himalayan region (Awan *et al.* 2011). In the traditional medicine, the genus *Bergenia* is in the curing of several disease Nepal, India, Pakistan, Bhutan and some others developing countries (Roselli *et al.* 2012). Mostly the rhizome of *Bergenia* is used as an ideal tool for kidney and gall bladder stone, cough, cold, wound, cuts, burn and inflammation and wounds (Hamayun 2005; Chowdhary *et al.*, 2009). Moreover, the *Bergenia ciliata* used as folk medicine to cure diabetic mellitus (Mazhar-Ul-Islam *et al.*, 2002), anti-diabetic, expectorant, body tonic, sunburn, pimple, stomachache, muscular pain and running eyes (Bano *et al.*, 2014) and for stomachic, diuretic, cardio active and skin diseases (Ahmad *et al.*, 2009).

Plants have been source of medicinal compound since several decades and it is well established that all parts of plants were used in traditional medicine system for centuries. However, the discovery of new herbal products led popularity as compare to synthetic drugs in the disease therapy (Sajad *et al.* 2010). Similarly, the antioxidant and antimicrobial agents from natural resources provides

protection to human health against infectious and degenerative disease (Rajkumar *et al.* 2010). Furthermore, the crude extract of genus *Bergenia* was more active against microorganisms such as *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* (Kokoska *et al.* 2002). Therefore, the Bergenin compound which isolated from *Bergenia* was found highly anti-oxidative, antimicrobial and as well as for xanthine oxidase inhibitory activities (Nazir *et al.* 2011). The evaluations of plant extracts were usually done through different techniques to ascertain antioxidants and inhibition effects on pathogenic and non-pathogenic microorganism (Sati & Joshi 2010). Thus the aim of the present study was to evaluate antioxidant and antimicrobials properties of crude extract and fractions of *B. ciliata* (rhizome).

MATERIALS AND METHODS

Plant material

The *Bergenia ciliata* rhizome was collected from the Kaghan valley Khyber Pakhtunkhwa, Pakistan (fig. 1). The plant species was deposited in Herbarium of Hazara University Mansehra, Pakistan with the voucher number 3556.

Extraction and fractionation

The collected plant material was dried out at room temperature and grinded into fine powder. The powder plant material soaked in methanol (commercial grade) for 12 days three times. The extract was filtered using Whitman filter paper (No.1). The residue plants material was soaked again in methanol and the solvent were

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vaporized at 40°C through rotary evaporator (Sengul *et al.* 2009). The process repeated three times and the methanol crude extract was obtained. The methanolic crude extract was suspended in distilled water with constant stirring and fractionated with n-hexane, chloroform, ethyl acetate as well as n-Butanol respectively (Rahman *et al.* 2008; Nisar *et al.* 2010). The solvents were evaporated at 40°C through Rotary Evaporator and different fractionations were obtained.

Chemicals

Propyl gallate (PG), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 3-Tert-Butyl-4-Hydroxyanisole (TBH), Dimethyl sulfoxide (DMSO), Sabourud dextrose agar (SDA), Nutrient agar and Nutrient broth. All chemical and reagents were purchased from Merck (Germany) and Sigma Aldrich (USA).

Preparation of stock solution

Methanolic crude extract and different fractions (n-Hexane, Chloroform, Ethyl acetate and n-Butanol) of the plant was dissolved in Dimethyl sulfoxide (DMSO) with the concentration of 1 mg/ml evaluated for biological potential (Jacinto *et al.*, 2011).

Antioxidant assay

The plant extract was examined for their antioxidant activity on the basis of scavenging effects using stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method (Miliauskas *et al.* 2004). Freshly DPPH solution were prepared and reserved in dark at 4°C 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (3.2µl) dissolved in 25ml methanol steering regularly up to 30 minutes. Methanolic solution of DPPH (90 µl) was added to 10µl of plant extract solution with different concentration. The mixtures were incubated for 30 minutes at 37°C. After incubation the absorbance were measured at 490 nm using Multiplate reader (Bio-Tek Elx800 TM, Instruments, Inc. USA). Propyl gallate (PG) and 3-tert-butyl-4-hydroxyanisole (TBH) were used as standard while dimethyl sulfoxide (DMSO) as negative control. All determination was performing in triplicate. Percentage inhibition of the radical scavenging activity of test sample as compare with the standard was measure according to the equation.

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

The extracts concentration giving 50% inhibitions (IC₅₀) were calculated from the graph Pad prism soft wear against inhibition percentage of extracts concentration in triplet test for each (Veeru *et al.* 2009).

Antifungal assay

Antifungal potential of plant crude extract and their fractions were determine by pour plate method (Jagessar *et al.* 2008). Media was prepared by dissolving 65g of sabroud dextrose agar (SDA) media in 1000 ml of distilled water. Media was autoclaved for 20 min at 121°C.

C. Petri plates and tips were also autoclaved. Two strains of fungus were used i.e. *Aspergillus niger* and *Aspergillus flavus*. The plant extract was prepared in the dimethyl sulfoxide (DMSO) by dissolving 1mg/ml and 25ml of media was poured in each Petri plate. Media was allowed to solidify. Spores of fungus were applied in the center of the plate. Sterile paper discs were placed in front of the growing end of the mycelium. Sample was applied on the sterile paper discs. Each sample was applied in triplicate. The culture was allowed to grow for four to seven days at 26°C. Fluconazole was used as standard (positive control). Dimethyl sulfoxide (DMSO) was used as negative control. Crescent shape of fungi was appeared in front of paper discs, which shows an inhibition. Zone of inhibition was measured according to (Nisar *et al.* 2011).

$$\text{Inhibition of fungal growth} = 100 - \frac{\text{Linear growth in test (mm)}}{\text{Linear growth in control (mm)}} \times 100$$

Antibacterial assay

Antibacterial activities of the plant crude extract and different fractions were determined by using paper disc diffusion method (Jagessar *et al.* 2008). Media was prepared by dissolving 28g of nutrient agar and 13g of nutrient broth in 1000ml of distilled water in flask. The nutrient broth was taken approximately 7-8ml per test tube. All the apparatus and media viz Petri plates, tips, Whitman filter paper disc and normal saline etc used in the activity were autoclaved for 20min at 121°C. After sterilization nutrient agar were poured into the Petri plates in the laminar flow hood allowed to solidify and placed in the incubator at 37°C to avoid any contamination. The bacterial stock culture were freshened on the nutrient agar plates by streaking with sterile inoculation loop in laminar flow hood culture were incubated at 37°C for 24 hours. The bacterial culture were inoculated into the sterilized nutrient broth in the flask containing approximately 20 - 25 ml broth media were then incubated in the shaking water bath (Model GLSC-SBR-04-28) for 16 hours at 200 rpm at 37°C. The bacterial cultures from the flask were diluted in test tube containing sterilized nutrient broth for standardization by comparing with 0.5 McFarland (turbidity) standards. 50µl of standardized bacterial culture were spread on each nutrient agar plates with the help of glass spreader. These impregnated plates were refrigerated for 10 mints for absorption after refrigeration impregnated plates brought to laminar flow to place the filter paper disc (6mm in diameter) with the help of sterilized forceps. The samples (6µml) were applied on each paper disc. Azithromycin and tetracycline were applied on separate plates as positive control for both bacteria while dimethyl sulfoxide (DMSO) as negative control. The plates were incubated at 37°C for 24 hours zone of inhibition was recorded around each paper disc in mm. All tests were applied in triplicate.

Microorganisms used

The microorganism used in the assay includes two fungal strains i.e. *Aspergillus flavus*, *Aspergillus niger* two Gram

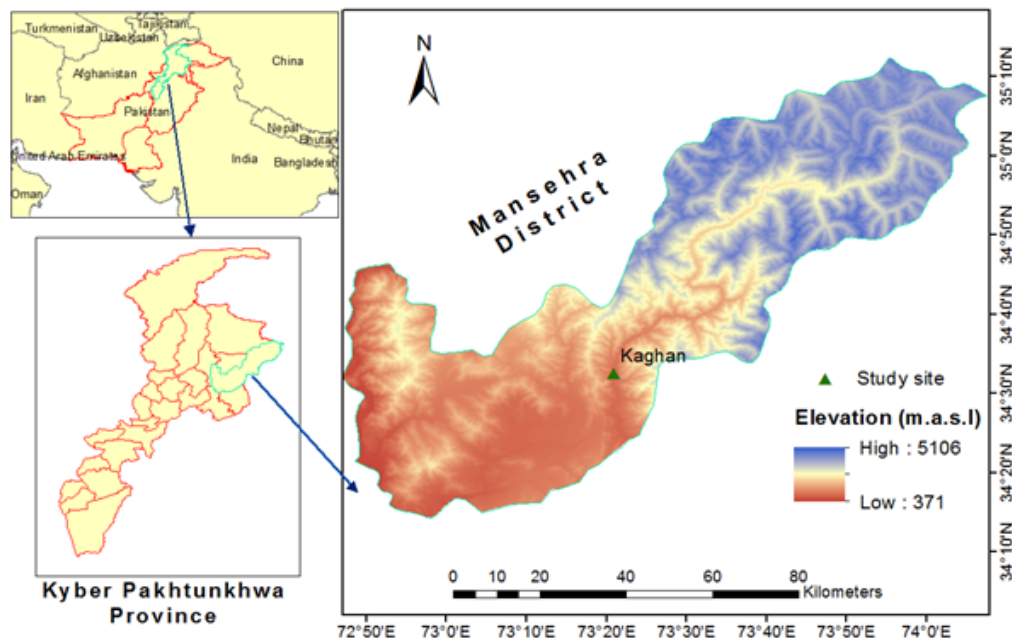


Fig. 1: Study area, the plant was collected from area (Δ) as shown in map.

positive bacterial Strains i.e. *Bacillus atrophoeus*, *Bacillus subtilis* and two Gram negative bacterial strains i.e. *Klebsiella pneumonia*, *Pseudomonas aeruginosa*.

Table 1: Antioxidant activities of *B. ciliata* rhizome fractions

Extract/Fraction	IC ₅₀ ± SD µg/ml
n-Hexane Fraction	42.39±11.74
Chloroform Fraction	4.15±0.82
Ethyl acetate Fraction	28.15±10.17
Propyl gallate (PG)	1.6±0.2
3-tert-butyl-4-hydroxyanisole (TBH)	1.2±0.1

STATISTICAL ANALYSIS

All experimental results of antioxidants, antifungal and antibacterial are measure statistically as mean ± standard deviation (SD). The measurements are replicated three times. The IC₅₀ values for antioxidants are calculated from linear regression analysis using Graph pad prism5 software.

RESULTS

Antioxidant activities

The antioxidant potential was carried out under the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging methods. There is a strong need for successful antioxidants products from natural sources as alternatives to synthetic food additives in order to prevent degeneration of foods, drugs and cosmetics etc. The IC₅₀ (4.15±0.82µg/ml) value of chloroform fractions of *B. ciliata* consider a strong anti-oxidative as compare to

ethyl acetate fraction (28.15±10.17µg/ml) and n-hexane fraction (42.39±11.74µg/ml). The relationship of these extract/fraction were representing inn such way chloroform> ethyl acetate fraction > n-hexane as shown in (table 1). Thus, the present data indicating that the chloroform fractions of *B. ciliata* might be used as precursor of pharmaceutical drugs that can be used against oxidative stress.

Antifungal activities

The crude methanolic extract and their fractions of *B. ciliata* rhizome were evaluated for their antifungal activities against fungal strains. Interestingly neither crude methanolic extract nor any other fractions showed any activities (table 2) in comparison with standard drugs. In the light of this investigation, we demonstrated that the presence of different chemical constituents in plants responsible for antifungal activities.

Table 2: Antifungal activities of *B. ciliata* rhizome of crude methanolic extract and their fractions

Extract/Fraction	<i>A. flavus</i>	<i>A. niger</i>
Methanolic Extract	-	-
n-Hexane Fraction	-	-
Chloroform Fraction	-	-
Ethyl acetate Fraction	-	-
Fluconazole	24.4±0.5	29±0.0

Antibacterial activities

In order to further investigated the possible antibacterial activity of gram positive and gram negative under disc diffusion method and to determine the parameters associated with it. The strong zone of inhibition 18.3±

1.53, 16 ± 3.61 mm was observed in chloroform fraction against both Gram positive bacterial strains i.e. *B. atrophoeus* and *B. subtilis* respectively. Similarly, ethyl acetate fraction also showed prominent activity against *B. atrophoeus* 16.7 ± 1.53 mm and n-hexane fraction revealed moderate zone of inhibition 15.3 ± 1.53 mm against *K. pneumonia*. Over all zone of inhibition showed by the plant crude extract and fractions were in between 13.3 ± 1.5 , 10 ± 1 mm respectively as shown in (fig. 2). Thus the present result suggested that the *B. ciliata* rhizome might be used in pharmaceutical industry in preparation of bacterial resistance drugs.

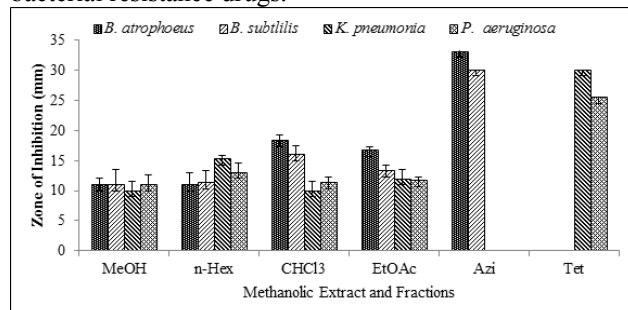


Fig. 2: Graphical representation antibacterial activities of methanolic extract and fraction of *B. ciliata* rhizome in the form of zone of inhibition (mm)

Key: MeOH (Methanol) n-Hex (n-Hexane), CHCl₃ (Chloroform), EtOAc (Ethyl acetate), Azi (Azithromycin), Tet (Tetracycline)

DISCUSSION

Medicinal plants have been used for last few decades in daily life to treat human health disorders as well as animals disease all over the world. Whereas, in the ongoing study we have focused on the chemical constituents present in the plants. For this purpose we prepare different crude extracts with chemical on basis of their polarity and evaluated for antioxidants and antimicrobial activities. Antioxidants due to their scavenging potential are valuable for the controlling of human and animal's diseases. DPPH stable free radical method is a sensitive approach to determine the antioxidant activity of plant extracts (Koleva *et al.* 2002; Kanatt *et al.* 2007). The methanol and water extract of the *B. ciliata* rhizome possess strong antioxidant activities while n-hexane fraction with the least one such as, methanol > water > n-hexane (Uddin *et al.*, 2012). Then it was confirmed that the medicinal plants with the presences of highly chemicals constituent such as steroids, terpenoids, tannis and flavonoids responsible for the antioxidant activity, while the n-hexane less active due to low chemical components. In contrast, the chloroform fraction showed (4.15 ± 0.82 μ g/ml) with IC₅₀ value (table 1). Thus another previous investigation also confirmed that both methanol and aqueous were found to be more active radical scavenger (Rajkumar *et al.* 2010). The present data indicating that the chloroform fractions

of *B. ciliata* might be used as precursor of pharmaceutical drugs that can be used against oxidative stress. As Table 2 showed antifungal activities of crude extracts in which all extracts were found to be inactive against tested fungal strains *A. niger* and *A. flavus*. This results is in line with previous study which reported that the ethanolic, chloroform, hexane, butanol and water extract of were evaluated for antifungal activities against fungal strains i.e. *A. niger*, *Alternaria solani*, *Penicillium funiculosium*, *Fusarium solani*, *Microsporum canis*, *Nigrospora oryzae*, *Curvularia lunta*, *Pleurotus oustreatus* and *Candida albicans*. All extract were effective against *Microsporum canis*, *Pleurotus oustreatus* and *Candida albicans* while non-active against *Aspergillus niger* and other remaining fungal strains (Mazhar-UI-Islam *et al.* 2002). This is may be due to absence of some chemical constituent in the *B. ciliate* which showed resistance against these fungal strains. Whereas the ethyl acetate, chloroform and other extracts of *B. stracheyi* were investigated against *Alternaria alternate*, *Aspergillus niger*, *Colletotrichum gloeosporioides*, *Fusarium oxysporium*, *Ganoderma lucidum* and *Rhizoctonia solani* fungal strains and revealed different extant of antifungal activities against all the tested fungal strains (Kumar & Tyagi 2015). On the other hand, the metholic extract *B. ciliate* rhizome showed 11.1 ± 2.1 mm zone of inhibition against *B. subtilis* and 11.3 ± 0.58 mm against *P. aeruginosa* (Fig. 2), bergenin is compound which isolated from *B. stracheyi* showed weak zone of inhibition against *Saureus*, *B. subtilis* and *K. pneumonia* (Nazir *et al.*, 2011). In addition, the methanol extract of *B. ciliata* at 1000 μ /disc 13.5 mm zone of inhibition was investigated against while 8 mm zone of inhibition against *P. aeruginosa* (Sinha *et al.* 2001). In contrast, our present results showed strong zone of inhibition of against *B. subtilis* and *K. pneumonia*. It has been proved that antibacterial effects of genus *Bergenia* can be attributed to the presence of sterols, glycosides and all other chemical constituents to showed exhibition against the different infectious microorganisms (Rajbhandari *et al.*, 2003).

CONCLUSION

The chloroform fraction of *B. ciliata* rhizome is important source of compounds with health protective potential and other extracts/ fraction exhibited a broad spectrum of activities at all tested bacterial strains. However, it is interested to be note that the *B. ciliata* rhizome might be used in pharmaceutical industry and can be implemented as anti-oxidative and antibacterial resistance agent. Further studies prospected for isolation and identification of active compounds.

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