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Biological activities of *Suaeda heterophylla* and *Bergenia stracheyi*

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ABSTRACT

Objective: To evaluate the antioxidant, phytotoxic, antimicrobial, insecticidal, cytotoxic, antiglycative, and xanthine oxidase activities of different extracts of *Suaeda heterophylla* (*S. heterophylla*) and *Bergenia stracheyi* (*B. stracheyi*).

Methods: The extracts of *S. heterophylla* and *B. stracheyi* were evaluated for antioxidant, phytotoxic, antimicrobial, insecticidal, cytotoxic, antiglycative, and xanthine oxidase activities using standard experimental methods.

Results: The overall antioxidant potential of ethyl acetate extract of *S. heterophylla* was the strongest, followed by chloroform extract, methanolic extract and *n*-hexane extract. It is interesting to note that ethyl acetate fraction showed 94.98% inhibition at concentration of 60 μg/mL while standard ascorbic acid showed 98.49% inhibition at same concentration. The crude methanol extracts of *S. heterophylla* and *B. stracheyi* showed significant phytotoxic activity at the highest dose. Moreover, methanol extract of *B. stracheyi* possessed strong activity in xanthine oxidase enzyme inhibition.

Conclusions: Antioxidant, phytotoxic, and xanthine oxidase activities of different fractions of *S. heterophylla* and *B. stracheyi* clearly demonstrate that these fractions possess great potential for the food, cosmetic and pharmaceutical industries.

1. Introduction

Almost 70% medicinal plants are found in Gilgit-Baltistan, Pakistan and 70%-80% people directly or indirectly depend upon the traditional medicinal plants[1]. *Suaeda heterophylla* (*S. heterophylla*) is traditionally used as medicines to cure the scorpion bite, snake bite, and internal ulcer[2,3]. *S. heterophylla* is also used for the stomach and intestinal problems *e.g.* diarrhea, dyspepsia, constipation, bloating and poor appetite. The plant leaves are used as a traditional medicine to cure hepatitis and interestingly it is also used as an ointment for wounds[4]. *S. heterophylla* is a folklore medicinal plant which is used to cure asthma, cancer, skin diseases, ulcer, and paralysis[5].

Bergenia stracheyi (*B. stracheyi*) are present in northern hilly areas of Pakistan and in Pakistan 6 species are present in this genus[6,7]. The height of the plant is 50 cm and it is distributed in temperate Himalayan regions from 2 000 m to 2 700 m and is very common in or around Muree area[8]. *B. stracheyi* is used traditionally for the treatment of various diseases like digestive ulcer and as tonic[9,10]. The

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diverse medicinal uses attributed to *S. heterophylla* and *B. stracheyi* prompt us to carry out biological activities on these plants, and this study, we evaluated the antioxidant, phytotoxic, antimicrobial, insecticidal, cytotoxic, antiglycative, and xanthine oxidase activities of different extracts of these two plants.

2. Material and methods

2.1. Plant material, extraction, and fractionation

The medicinal plant *S. heterophylla* was collected from Naltar and the plant *B. stracheyi* was collected from Nager-Hunza Valley of Gilgit-Baltistan during July-August 2012. The plant species were identified by the plant taxonomist Dr. Sher Wali Khan, Department of Biological Sciences, Karakoram International University, Gilgit-Baltistan. The specimens were deposited in the Herbarium of Department of Biological Sciences, Karakoram International University, Gilgit-Baltistan.

The plants material (*S. heterophylla* and *B. stracheyi*) were dried and made powder and weighed (*S. heterophylla* 803.30 g; *B. stracheyi* 390.54 g). The weighed plant material was soaked in separate flasks with methanol (100%) for 48 h. Then it was filtered and each plant extract was dried by removing the solvent under reduced pressure through rotary evaporator (*S. heterophylla* methanol extract 114.08 g; *B. stracheyi* methanol extract 22.92 g). Each methanol plant extract was dissolved in water and fractionated using *n*-hexane, chloroform and ethyl acetate.

Each dried sample was dissolved in methanol to give concentration of 1 mg/mL and it was further diluted to make the concentrations of 10, 20, 30, 40, 50 and 60 μg/mL. Ascorbic acid was used as standard.

2.2. Protocol for estimation of DPPH free radical-scavenging capacity

The free radical-scavenging activity of the extracts was evaluated by 1,1,-diphenyl-2-picryl-hydrazil (DPPH) using the reported method[11-14]. In brief, DPPH solution (1.3 mg/mL) was prepared in methanol and plant sample stock and ascorbic acid solution were prepared with the concentration of 1 mg/mL. About 150 μL of DPPH solution was taken in the cell (cuvette) and it was made 5 mL by adding methanol and absorbance was noted immediately at 517 nm for control. A total of 150 μL of various concentrations of standard ascorbic acid and plant samples were taken in separate test tubes and equal volume of DPPH was added to each test tube. Then each solution was made 5 mL by adding methanol.

After incubation in a dark place for 30 min at room temperature, the absorbance of the mixture was measured at 517 nm against methanol as blank using U2020 IRMECO UV-vis spectrophotometer. (L)-ascorbic acid (1 mg/mL) was used as positive control. The activities of the samples were evaluated by comparison with the control. Each sample was measured in triplicate and averaged. The activity was calculated according to the following formula:

$$\% \text{ Scavenging} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test Sample}}{\text{Absorbance of Control}} \times 100$$

2.3. Phytotoxicity bioassay

This phytotoxicity bioassay was performed according to the modified protocol of McLaughlin[15]. The test fractions were incorporated with sterilized E-medium at different concentrations *i.e.* 10, 100, 1 000 μg/mL in methanol. Sterilized conical flasks were inoculated with fractions of desired concentrations prepared from the stock solution and allowed to evaporate overnight. Each flask was inoculated with 20 mL of sterilized E-medium and then 10 *Lemna minor* (*L. minor*) each containing a rosette of three fronds were placed on media. Other flasks were supplemented with methanol serving as negative control and reference inhibitor *i.e.* parquet (0.015 μg/mL) served as positive control. Treatment was replicated three times and the flasks incubated at 30 °C in Fisons Fi-Totron 600 H growth cabinet for 7 d [9 000 lux intensity, (56±10)% relative humidity and 12 h day length]. Growth of *L. minor* in flasks containing fractions was determined by counting the number of fronds per dose and growth inhibition was calculated with reference to negative control[15].

2.4. Antibacterial activity

The antibacterial activity was checked by the agar-well diffusion method[16,17]. Six bacteria *viz.*, *Escherichia coli*, *Bacillus subtilis*, *Shigella flexenari*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi* were used in this assay. In this method one loop full of 24 h old culture containing approximately 10^4-10^6 CFU was spread on the surface of Mueller-Hinton agar plates. Wells were dug in the medium with the help of sterile metallic cork borer. Stock solutions of the test samples at the concentration of 3 mg/mL were prepared in dimethyle sulfoxide (DMSO) and 100 μL dilutions were added in their respective wells. The antibacterial activity of extracts was compared with standard drug imipenem; the standard drug imipenem and DMSO were used as positive and negative control. Zone of inhibition (in mm) was recorded visually. The amount of

growth in each well was determined visually by comparing with the growth in the control wells.

2.5. Antifungal activity

The antifungal activity was determined by agar tube dilution method[18]. Six fungi *viz*., *Trichphyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani*, and *Canadida glabrata* were used. The crude extract was dissolved in DMSO (24 mg/mL). Sterile Sabouraud's dextrose agar medium (5 mL) was placed in a test tube and inoculated with the sample solution (400 μg/mL) kept in slanting position at room temperature overnight. The fungal culture was then inoculated on the slant. The samples were incubated for 7 d at 29 °C and growth inhibition was observed and percentage growth inhibition was calculated with reference to the negative control by applying the formula:

$$\% \text{ Inhibition} = 100 - \frac{\text{Linear growth and test (mm)}}{\text{Linear growth and control (mm)}} \times 100$$

2.6. Insecticidal activity

The insecticidal activity was performed according to reported procedure[19,20].

2.7. Brine shrimp lethality bioassay

Brine shrimp lethality bioassay was performed according to reported procedure[21,22]. Brine shrimp (*Artemia salina* larvae) eggs were hatched in a shallow rectangular plastic dish, filled with artificial seawater, which was prepared by mixing a commercial salt mixture (Instant Ocean, Aquarium System, Inc., Mentor, OH, USA) with double distilled water[21]. An unequal partition was made in the plastic dish with the help of a perforated device. A total of 50 mg of eggs were sprinkled into larger compartment, which was placed under the dark condition while the smaller compartment was opened to ordinary light. After 2 d, naupils were collected. A sample of the test fraction was prepared by dissolving 20 mg of each fraction in 2 mL of methanol. From this stock solution, 1 000, 100 and 10 μg/mL was transferred to 12 vials; three for each dilution, and three vials were kept as control having 2 mL of methanol only. The solvent was allowed to evaporate overnight. When shrimp larvae were ready, 1 mL of sea water was added to each vial along with 10 shrimps and the volume was adjusted with sea water to 5 mL per vial. After 24 h, the number of surviving shrimps was counted. Data were analyzed by a Finney computer program to determine the LD_{50}[22]. Each experiment was replicated thrice.

2.8. Antiglycation activity

The antiglycation activity was performed according to reported procedure[23]. Bovine serum albumin was used (10 mg/mL), dissolved in 67 mmol/L phosphate buffer (pH 7.4). Glucose (50 mg/mL) was dissolved in 67 mmol/L phosphate buffer (pH 7.4). Sodium azide at 3 mmol/L was added in required quantity of phosphate buffer to inhibit bacterial growth. About 1 mg/1 000 μL concentration of each fraction was used to calculate antiglycation activity along with standard inhibitor. The dissolved sample (60 μL in each well of 96-well plate) was incubated for a week at 37 °C. After a week, the samples were taken out and cooled at room temperature. Then 6 μL of 100% trichloroacetic acid was added to each of the well, supernatants containing unbounded glucose; inhibitor and interfering substances were removed after centrifugation at 14 000 r/min for 4 min. Pellets were obtained at the bottom of the wells, supernatant were removed from each well and 60 μL of phosphate buffer saline (pH 10) was added to dissolve the pellets. The comparison of fluorescence intensity at 370 nm excitations and emission at 440 nm was obtained by using spectrofluorimeter[23]. Rutin was used as the standard inhibitor. Percentage inhibition was calculated by the following equation. % inhibition=[100-OD (sample)/OD (blank)]×100.

2.9. Xanthine oxidase activity

The xanthine oxidase inhibitory activity was measured as previously reported[24]. The substrate and the enzyme solutions were prepared immediately before use. The reaction mixture contained 80 mmol/L sodium pyrophosphate buffer (pH=8.5), 0.120 mmol/L xanthine, and 0.1 IU of xanthine oxidase. The absorption at 295 nm, indicating the formation of uric acid at 25 °C, was monitored and the initial rate was calculated. The methanolic dried extract, initially dissolved and diluted in the buffer, was incorporated in the enzyme assay to assess its inhibitory activity at a final concentration of 200 μg/mL. All assays were run in triplicate; thus, inhibition percentages are the mean of three observations. A negative control (blank; 0% xanthine oxidase inhibition activity) was prepared containing the assay mixture without the extract. Allopurinol was used as a positive control in the assay mixture. The xanthine oxidase inhibitory activity was expressed as the percentage inhibition of xanthine oxidase in the above-mentioned assay mixture system, calculated as follows:

$$\% \text{ Inhibition} = (1 - \frac{\text{test inclination}}{\text{blank inclination}}) \times 100$$

3. Results

3.1. Antioxidant activity

The ethyl acetate fraction of *S. heterophylla* exhibited a comparable antioxidant potential with that of ascorbic acid (reference compound) at varying concentration tested (10, 20, 30, 40, 50, 60 μg/mL) (Table 1). The methanol extract, *n*-hexane fraction, chloroform fraction and ethyl acetate fraction at a concentration of 10 μg/mL showed a percentage inhibition of 14.93, 29.81, 41.40 and 43.38 respectively and for 60 μg/mL it was 64.00, 55.86, 88.77 and 94.98. It is interesting to note that ethyl acetate fraction showed 94.98 percentage inhibition at concentration of 60 μg/mL while standard ascorbic acid showed 98.49 percentage inhibition at same concentration (Table 1).

Table 1

Antioxidant activity of different extracts of *S. heterophylla*.

Conc. (μg/mL)	% Inhibition				
	SAA[a]	SHME	SHH	SHC	SHE
10	46.30	14.93	29.81	41.40	43.38
20	96.97	17.63	31.65	59.68	78.42
30	97.94	35.00	37.56	66.50	92.80
40	97.98	37.00	39.00	67.15	94.00
50	98.48	43.00	46.75	78.32	94.81
60	98.49	64.00	55.86	88.77	94.98

[a]SAA: Standard ascorbic acid; SHME: *S. heterophylla* methanol extract; SHH: *S. heterophylla n*-hexane fraction; SHC: *S. heterophylla* chlroform fraction; SHE: *S. heterophylla* ethyl acetate fraction.

3.2. Phytotoxic activity

The phytotoxicity of all fractions of plants of *S. heterophylla* and *B. stracheyi* was performed towards *L. minor* (Table 2). This phytotoxic assay was performed at concentrations 1 000, 100 and 10 μg/mL. The crude methanol extracts of plants of *S. heterophylla* and *B. stracheyi* showed significant activity at the highest dose (1 000 μg/mL). However, the tested extracts and negative control did not show any activity at low concentration (10 μg/mL and 100 μg/mL).

Table 2

Results of *in vitro* phytotoxic bioassay of *S. heterophylla* and *B. stracheyi* methanol extracts.

Cocentrtion (μg/mL)	No. of fronds		% Growth regulation
	SHExt	BSExt	
1 000	0	0	100
100	20	20	0
10	20	20	0

SHExt: *S. heterophylla* methanol extract; BSExt: *B. stracheyi* methanol extract.

3.3. Antimicrobial activity

The antibacterial potential of the methanol extracts of *S. heterophylla* and *B. stracheyi* was carried out. Unfortunately, the methanol fractions did not show any promising activity against tested bacteria. The antifungal activity of the crude methanol extracts of plants of *S. heterophylla* and *B. stracheyi* was carried out. Unfortunately, the methanol fractions did not show any promising activity against tested fungi, however, *B. stracheyi* methanol extract showed week activity (10%) against fungus *Fusarium solani*.

3.4. Miscellaneous activities

The insecticidal potential of the crude methanol extracts of *S. heterophylla* and *B. stracheyi* was carried out against five insects *viz.*, *Tribulium castaneum, Sitophilus oryzae, Rhyzopertha dominica, Callosbruchus analis*, and *Trogoderma granarium*. No significant inhibitory effects of these fractions could be observed against these insects. Moreover, crude methanol extracts of *S. heterophylla* and *B. stracheyi* also did not exhibit significant cytotoxic activity against *Artemia salina*. However, crude methanol extract of *B. stracheyi* showed low activity at higher dose level (1 000 μg/mL; LD_{50}: 3314.79 μg/mL) but at lowest doses the tested extract showed no activity. The antiglycation potential of the crude methanol extracts of plants of *S. heterophylla* and *B. stracheyi* was also carried out and these extracts showed week activity. However, antiglycation activity of methanol extract of *S. heterophylla* (36%) was higher as compared to methanol extract of *B. stracheyi* (25.7%). In this study, crude methanol extract of plant of *S. heterophylla* showed weak xanthine oxidase inhibitory activity (8.8%) while crude methanol extract of *B. stracheyi* possessed strong activity (64%) in xanthine oxidase enzyme inhibition.

4. Discussion

Different plants fractions have been investigated for antioxidant potential in the world. Still there is a need of antioxidants from nature because synthetic antioxidants, *viz.*, butylated hydroxyanisole and butylated hydroxytoluene are unsafe[25]. The ethyl acetate fraction of *S. heterophylla* exhibited a comparable antioxidant activity (94.9%) with that of standard ascorbic acid (98.4%). However, the compounds which are responsible for antioxidant potential of *S. heterophylla* are unknown. But it is confirmed from literature that phenolic compounds (tannins and flavonoids *etc.*) may be responsible for antioxidant potential. It has been suggested that antioxidant potential of *S. heterophylla*

might be due to the presence of phenolic compounds in the extract.

Plants and their phytochemicals are potential xanthine oxidase inhibitors; they are used as food or food supplements for many years and found safe for human bodies. Interestingly, crude methanol extract of *B. stracheyi* possesses strong activity in xanthine oxidase enzyme inhibition. Future studies are needed to isolate and identify the bioactive metabolites which are effective in xanthine oxidase inhibitory activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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