Evaluation of antimicrobial and antioxidant activities of *Swertia petiolata*

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Abstract

*Swertia petiolata* is considered as an important medicinal herb and has been used to treat different ailments by various ethnic groups in Kashmir. The present study was designed to evaluate the antioxidant and antimicrobial activity of methanolic extract of *S. petiolata*. The antioxidant activity was evaluated by three different assays namely diphenylpicrylhydrazyl (DPPH), nitroblue tetrazolium (NBT) and ferric reducing power (FRAP). The IC₅₀ values of the methanolic extract were found to be 240 ± 4.14, 258 ± 5.71 and 100 ± 3.53 for DPPH, NBT and FRAP, respectively. The antimicrobial activities were determined by agar diffusion and microdilution methods against Gram-positive (*Bacillus subtilis* ATCC 6051, *Sarcina lutea* ATCC 10054, *Staphylococcus aureus* ATCC 25932), Gram-negative (*Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853) and yeast (*Candida albicans* ATCC 10231). The methanolic extract was found to be more active against Gram-positive bacteria as compared to Gram-negative bacteria and the yeast. The Minimum inhibitory concentration (MIC) values ranged from 67.5-125, 125-250 and 350μg/ml for Gram-positive, Gram-negative and *C. albicans* respectively. Taken together, we propose that the plant is a rich source of antioxidants and antimicrobials.

Keywords: antioxidant, antimicrobial, swertia, minimum inhibitory concentration.

Introduction

*Swertia petiolata* is high altitude perennial medicinal herb found in the alpine pastures of Kumaun Himalaya. It belongs to family *Gentianaceae*, consisting of about 700 species and 80 genera. *Swertia* consists of about 250 species, out of which 25 species are found in India (2). This plant is not grazed by sheep and is widely known for its laxative and antimalarial potential in the folklore of the region (1). The plant is also used for the treatment of skin diseases and mental disorders, liver disorder, ulcers and as bitter tonic, febrifuge, anthelmintic and antidiarrheal (2). Whole of the plant is used in Tibetan medicine for its cooling potency (3). Plants synthesize diverse compounds for their survival in ever changing oxidative environment (4). These molecules act as free radical scavengers and antimicrobials and also help the plant to deter predators. Plants are used in different countries for medical purpose as they are the rich source of many potent and powerful drugs. Hundreds of plants species have been tested for antimicrobial properties, but the vast majority of them have not been adequately evaluated (5). The phytochemical examination of the genus *Swertia* have led to the discovery of about 200 compounds with large diversity in structure belonging mostly to xanthones, terpenoids, irridoid, flavonoids and seco-irridoid, glycosides and alkaloids (6) They are widely used in the treatment of several ailments in various systems of medicine. *S. minor* is used as a substitute for *S. chirata* in the treatment of malarial fever. *S. alata*, is traditionally used as an appetite tonic and febrifuge, *S. davidi* is used as a remedy for acute bacillary dysentery and the plants of *S.
petiolata and S. thomsonii are widely used in the Amchi system of medicine in the Laddakh region (India) (7). Similarly, many studies have reported the benefits of Swertia species as traditional medicines like S. chirayita (8). Despite wide pharmacological importance associated with Swertia species, there is rarely any scientifically validated study on the medicinal properties of S. petiolata. The present study was therefore designed with an aim to investigate the antioxidant and antimicrobial activities of methanolic extract of S. petiolata. Our results indicate that methanolic extract of S. petiolata possess good antioxidant and significant antimicrobial potential and may be a rich source for isolation some important bioactive compounds.

Materials and Methods

Chemicals

DPPH, potassium ferricyanide, trichloroacetic acid, gallic acid (GA), rutin (RU), NBT, and Folin–Ciocalteu’s reagent, were purchased from Sigma–Aldrich (St. Louis, MO, USA). Aluminium chloride, Sodium carbonate, Mueller Hinton media were purchased from Himedia (Mumbai, India). All other chemical reagents used were of analytical grade.

Plant material and extraction

The fresh aerial plant parts were collected from Gulmarg (34° 15' N, 74° 25' E and 2600 m above sea level), Jammu and Kashmir, India. The taxonomic identification of plant was confirmed at the Centre of Plant Taxonomy and Biodiversity, University of Kashmir (India). The collected aerial parts were washed under running tap water to remove the surface infection. The plant material was air dried under shade and then chopped and ground to fine powder using a mechanical blender. Dried root powder was packed in a Soxhlet apparatus and extracted at 60–65 °C for 3–4 h. Extracts were obtained using methanol/water (8:2, v/v) as solvent. The extracts were kept for 24 h at 4°C, filtered through Whatman No. 4 filter paper, evaporated to dryness under vacuum and stored at 4°C for further analysis.

Total phenolics and flavonoids content

Total phenolic content was measured using the Folin–Ciocalteu reagent method as described earlier. Total phenolics were expressed as gallic acid equivalent per gram of powder (GAE/g powder) \( (r^2 =0.999) \). Total flavonoid content of both crude extracts was determined using the aluminium chloride colorimetric as described previously. Total flavonoids content were expressed as quercetin equivalent per gram of powder (QE/g powder) \( (r^2 =0.998) \).

Antioxidant activity

DPPH assay

The effect of extracts on DPPH radical was monitored according to the method as described earlier. From the stock solution different concentrations of extract (100 µg–500 µg/ml) were prepared. 200 µl of each concentration was mixed with 3.8 ml DPPH solution and incubated in the dark at room temperature for 60 min. Absorbance of the mixture was then measured at 517nm and Butylated hydroxytoluene (BHT) was used as a positive control. Scavenging ability of the sample to DPPH radical was determined according to the following equation

\[
\%\text{Inhibition} = \frac{Control\ OD - Sample\ OD}{Control\ OD} \times 100
\]

The IC\textsubscript{50} values were calculated as the concentration of extracts causing a 50% inhibition of DPPH radical, a lower IC\textsubscript{50} value corresponds to a higher antioxidant activity of sample. The experiments carried out in three replications.

NBT assay

Superoxide anion scavenging activity was performed as described earlier. From the stock solution (1mg/ml) different concentrations of extract (100 µg–500 µg/ml) were prepared. The reaction was performed in 50 mM phosphate buffer (pH 7.8) containing extracts of various concentrations, 1.5 mM riboflavin, 50 mM NBT, 10 mM DL-methionine, and 0.025% v/v Triton X-100. The reaction was initiated by illuminating the reaction mixture and absorbance of formazan was recorded at 560 nm and percentage scavenging activity was described as inverse of the produced formazan. The experiments were carried out in three replications.

FRAP assay

Ferric reducing/antioxidant power (FRAP) of the extracts was determined by the method described previously (9). Briefly, 1 ml of each concentration of the extracts (100–500 µg/ml) was mixed with 2.5 ml of phosphate buffer (0.2M pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50 °C for 20 min and then 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 10,000 rpm for 10 min. 2.5 ml of the upper layer of the solution was mixed with 2.5ml of distilled water and 0.5ml of 0.1% ferric chloride and the absorbance of the reaction mixtures was measured at 700 nm. Chlorogenic acid was used as a positive control. The extract concentration providing 0.5 of the absorbance (IC\textsubscript{50}) was calculated from the graph of measured absorbance.
Antimicrobial activity

The antimicrobial activity was tested with Gram-positive [Bacillus subtilis (ATCC 6051), Sarcina lutea (ATCC 10054), Staphylococcus aureus (ATCC 25932)], Gram-negative Escherichia coli (ATCC 25922), Enterobacter cloacae (ATCC 13883), Pseudomonas aeruginosa (ATCC 27853) and fungus Candida albicans (ATCC 10231)]. The microbial strains were maintained by subculturing periodically on nutrient agar and preserved at 4°C prior to use. The tested microbes were grown overnight in 10 mL nutrient broth. This broth was used for seeding the microbes onto the agar plates. The agar diffusion method was employed for determination of antimicrobial activities of the solvent extracts, and standards according to the method described by (10, 15). The minimum inhibitory concentration (MIC) values were determined using the broth microdilution method as described by (11) Serial dilutions of the stock solutions of crude extract in broth medium were prepared in a microtiter plate and the microbial suspensions were added in the microwells at the concentration of 5x10^5 organisms/ml. The microtiter plates were then incubated at 37°C (28°C for yeast) for 24 h. The bioactivities were recorded as blue coloration in the wells after the use of resazurin. The MIC values were determined as the lowest concentrations preventing visible growth. Streptomycin for bacteria and Nystatin for yeast were used as a positive controls and each assay was repeated three times.

Results

Total phenolics and flavonoid content

The total phenolic and flavonoid content of the plant was determined by methods described above. The phenolic and flavonoid content of the methanolic extract was found to be 9.20 ± 0.9 and 27.80 ± 1.4 respectively. Our results were in confirmation with studies carried out previously on other species of Swertia (12). Highest phenolic and flavonoid content in the methanolic extracts of Swertia corymbosa were also recorded (13). Studies have reported that the efficiency of the extraction depends on parameters, like the extraction time and temperature, the volume and type of the solvents used.

Antioxidant activity

DPPH assay

DPPH is a stable, organic free radical. Owing to the ease and convenience of the method and sensitivity of DPPH to detect active ingredients at low concentrations, it is extensively used to evaluate scavenging activity of antioxidants (14). In the DPPH assay, an antioxidant scavenges the free radicals. This test is used to measure the capacity of extracts to scavenge the stable radical DPPH formed in solution by donation of hydrogen atom or an electron. As depicted in Fig.1a, methanolic extracts exhibited strong antioxidant activity and the activity was comparable with that of the BHT used as positive control. The activity of the extract was found to be concentration dependent and increased with increase in the concentration of the extract. The IC_{50} value for methanolic extract as shown in the Table 1 was found to be 240 ± 4.14 as compared to 17.5 ± 1.50 for the positive control, BHT.

NBT assay

Superoxide radical is one the most representative free radicals. In cellular oxidation reactions, superoxide radicals are normally formed first, and their effects can be magnified because they produce other kinds of cell-damaging free radicals and oxidizing agents. Superoxide scavenging activity was evaluated by NBT assay. The IC_{50} value for methanolic extracts was found to be 258 ± 5.71 μg/ml which is comparatively lower than Ascorbic acid used as the positive control (Fig. 1b). The antioxidant activity of extract increased with increase in the concentration of the extract and thus the superoxide scavenging capacity of the extracts showed similar concentration dependent trend as that of DPPH.

FRAP assay

In the present assay reducing activity was measured as the reduction of Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}) in presence of reductants (antioxidants) in the extracts (16). The FRAP assay is a rapid, simple, inexpensive and reproducible procedure used to evaluate the antioxidant capacity of botanicals. The methanolic extract of S. petiolata exhibited electron donating activity with IC_{50} value of 100 ± 3.53 as against 36.22 ± 1.80 for chlorogenic acid used as positive control (Fig. 1c).

Antimicrobial activity

The antibacterial activity of all the extracts was evaluated against Gram-negative and Gram-positive bacteria (Table 2). The methanolic extract of S. petiolata exhibited comparatively stronger activity against Gram-positive bacteria than Gram-negative. For Gram-positive bacteria, the MIC values of methanolic extract ranged from 67.5-125 μg/ml, with lowest against Bacillus subtilis (67.5 μg/ml) and highest against Sarcina lutea (125 μg/ml). However, for Gram-negative bacteria MIC values ranged from 125-250 μg/ml with lowest for Enterobacter cloacae (125 μg/ml) and highest for Escherichia coli and Pseudomonas aeruginosa (250 μg/ml). The antifungal activity of S. petiolata was evaluated against human...
pathogen yeast, *Candida albicans* and the MIC value (350 μg/ml) was found to be comparatively higher than for bacteria. The inhibitory activity of the extract was found to be concentration dependent.

Figure 1: Antioxidant activity of methanolic extract of *S. petiolata* (a) Free radical scavenging activity (b) Superoxide scavenging activity (c) Ferrous reducing capacity. Each value is expressed as the mean ± standard deviation.

Table 1: IC$_{50}$ values for methanolic extract of *Swertia petiolata* observed in three different antioxidant assays.

<table>
<thead>
<tr>
<th>Antioxidant Assay</th>
<th>Methanolic Extract</th>
<th>Butylated hydroxytoluene</th>
<th>Ascorbic acid</th>
<th>Chlorogenic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH</td>
<td>240 ± 4.14</td>
<td>17.5 ± 1.50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NBT</td>
<td>258 ± 5.71</td>
<td>-</td>
<td>11.6 ± 2.11</td>
<td>-</td>
</tr>
<tr>
<td>FRAP</td>
<td>100 ± 3.53</td>
<td>-</td>
<td>-</td>
<td>36.22 ± 1.80</td>
</tr>
</tbody>
</table>
Antimicrobial and antioxidant activities of Swertia petiolata

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Table 2: Minimum Inhibitory Concentration (MIC) of methanolic extract of Swertia petiolata measured as microgram/ml

<table>
<thead>
<tr>
<th>S. No</th>
<th>Microorganism</th>
<th>Methanolic Extract</th>
<th>Streptomycin¹</th>
<th>Nystatin²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Bacillus subtilis</em></td>
<td>67.50</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td><em>Sarcina lutea</em></td>
<td>125.00</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td><em>Staphylococcus aureus</em></td>
<td>67.50</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td><em>Escherichia coli</em></td>
<td>250.00</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td><em>Enterobacter cloacae</em></td>
<td>125.00</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>250.00</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td><em>Candida albicans</em></td>
<td>350.00</td>
<td>5.0</td>
<td></td>
</tr>
</tbody>
</table>

¹Positive control for bacterial strains, ²Positive control for yeast

Discussion

In the present study we determined the total phenolics and flavonoid content of the methanolic extract of *S. petiolata* which were found to be 9.20 ± 0.9 and 27.80 ± 1.4 respectively. We also evaluated the antioxidant activity of the extract which exhibited the capacity to scavenge different types of free radicals. The Antioxidant activity of the extract could be attributed to the presence of high phenolic and flavonoids content. Phenolic compounds are important plant constituents because of their free radical scavenging ability facilitated by their hydroxyl groups and the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Phenolic compounds are also involved in conferring plants with oxidative stress tolerance (17). Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various other free radicals implicated in several diseases (18). Flavonoids, on the other hand, suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species, and up-regulate the antioxidant defenses. Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics and flavonoids, are increasingly being used in the food industry for their antioxidant properties and health benefits. Studies carried on other related *Swertia* species have shown similar results. Methanolic extract of *S. longifolia* showed similar results for antioxidant activity (19). The ethanolic extract of *S. chirayita* possesses both in vitro and in vivo antioxidant activity (12). Similar results have been observed in *S. bimaculata*.

Further, we also evaluated the antimicrobial activity of methanolic extract of *S. petiolata*. Gram-positive bacteria were found to be more susceptible which is depicted by their low MIC values (Table 2). The reason for higher sensitivity of the Gram-positive bacteria than Gram negative bacteria could be attributed to their differences in cell membrane constituents. Gram-positive bacteria contain an outer peptidoglycan layer, which is an ineffective permeability barrier. Similar results were also been obtained previously. It was reported that the Strawberry tree (*Arbutus unedo* L.) leaf extracts inhibited only Gram-positive bacteria but showed no activity against Gram-negative bacteria (20). The antimicrobial activity of the extracts could be attributed to the presence of flavonoids which have been reported to be involved in the inhibition of nucleic acid biosynthesis and other metabolic processes (21). Flavonoid rich plant extracts from species of Capsella and Chromolaena (22) have been reported to possess antibacterial activity. Flavonoids have also been reported to inhibit spore germination of plant pathogens (23). Some flavonoids are formed as antimicrobial barriers in plant’s response to microbial infection. Therefore, it should not be surprising that they show antibacterial activity in vitro against a wide array of microorganisms. The antibacterial activity of *Swertia* species has also been reported previously. Sultana et al. observed the antibacterial activity of *S. chirata* against 12 pathogenic bacteria (24). Antimicrobial activity of rhizome of *S. petiolata* has also been recently reported against 4 human pathogenic bacteria by agar well diffusion method (1). Besides flavonoids, the antimicrobial activity of methanolic extract may be due to the presence of
xanthanones such as swerchirin which is considered to medicinally important xanthone with known antimicrobial activities (25). Further, the amphipathicity of phenolic compounds of the extract may explain its antifungal activity. The interactions of these compounds with biomembranes are known to cause the inhibitory effect (26). The extracts rich in phenolics interact with enzymes and proteins of the membrane, thus producing a flux of protons towards the cell exterior which induces changes in the cells and, ultimately their death (27). Also, owing to the widespread ability of flavonoids to inhibit spore germination of plant pathogens, they have widespread antifungal activities. The antifungal activity of many plants against various fungal species has been attributed to their flavonoid contents. The flavonoid 7-hydroxy-3′, 4′-(methylenedioxy) flavan, isolated from *Terminalia bellerica* fruit rind, has also been shown to possess activity against *C. albicans* (28). Another important reason for the antimicrobial activity of the methanolic extract of *S. petiolata* may be the presence of multiple bioactive components which may exhibit synergism. Synergistic effects can be produced if the constituents of an extract affect different targets or interact with one another in order to improve the solubility and thereby enhance the bioavailability of one or several substances of an extract.

While as the development of resistance against one single compound is comparatively easier, the development of resistance against combined action of multiple compounds is difficult (29).

**Conclusion**

These findings demonstrated that methanolic extract of *S. petiolata* possessed antimicrobial activity against a range of microorganisms as well as antioxidant property with the capacity to neutralize different types of free radicals. This suggests that it may serve as rich sources of antioxidants and antimicrobials. Although a number of phenolic and flavonoid compounds were putatively identified, further phytochemical analysis for isolation of these bioactive molecules is urgently required.

**Acknowledgement**

None declared

**Conflict of Interest**

Authors declare that there is no conflict of Interest to reveal.

**References**


