Mineral composition and antimicrobial activity of *Swertia cordata* (G. Don) Clarke aerial parts and roots

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The study aim was to evaluate the concentration of mineral elements and antimicrobial activity of *Swertia cordata* (G. Don) Clarke aerial parts and roots. Macro minerals, viz. sodium, potassium, calcium, lithium were estimated by Flame Photometer while micro minerals, viz. iron, copper, manganese, zinc and cobalt were determined by Atomic Absorption Spectrophotometer. Among all the elements, highest concentration of potassium was recorded in both aerial parts (385.6 mg/100 g) and roots (421.3 mg/100 g) of *S. cordata*. Methanol extract of both *S. cordata* aerial parts and roots obtained by Soxhlet extraction were screened for their antimicrobial activity against 6 bacteria and 2 fungi using disc diffusion method. The results showed that both the extracts exhibited moderate antibacterial activity while roots showed good antifungal activity against *Candida albicans* (ZOI = 20 mm).

Keywords: *Swertia cordata*, Medicinal plant, Spectrophotometer, Flame Photometer, Disc diffusion.

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Introduction

*Swertia* Linn. (Gentianaceae) is an annual or perennial herb comprising more than 170 species, found at an altitude of 1200 to 3000 m from Kashmir to Nepal, China and also distributed in Bhutan, Khasi hills and Sikkim1-4. *Swertia* has an erect, about 1 m long stem, leaves are opposite to each other without leaf stalks and have smooth margins. The flowers have four or five united petals, which may be deeply divided. The petals overlap and are twisted in the bud. Nectar-secreting pits are found at the base of the petals and sepalas in some species. Approximately 20 species of this genus are used in Chinese traditional medicine to treat hepatic, choleric and anti-inflammatory diseases. The genus contains different bitter principles, iridoids and secoiridoids, for example amarogentin, swertiamarin, sweroside, amaroswerin, and gentiopicroside, which are mainly responsible for its pharmacological activity1.

Various chemical constituents have been isolated from different species of *Swertia*. Oleanolic acid; gentiopicroside; swertiamarin; daucosterol; swertia decoraxanthone-II; isovitexin; isoorientin have been isolated from *S. delayayi* Franch5. Swertiamarin; mangiferin; oleanolic acid; 1,5,8-trihydroxy-3-methoxyxanthone; 1,8-dihydroxy-3, 7-dimeth-oxyxanthone; 1,8-dihydroxy-3, 5-dimethoxy-xanthone have been reported from *S. mussotti* Franch6. *S. mileensis* T. N. Ho & W. L. Shi was reported to contain swerilactones C and D7. *Swertia cordata* (G. Don) Clarke aerial parts were reported to contain ursolic acid and mangiferin8. Atta-ur-Rehman *et al* (1994)9 isolated 1-hydroxy-3,5,7,8-tetramethoxyxanthone and 1,7-dihydroxy-3, 5, 8-trimethoxy xanthone from this plant. *S. cordata* is used in medicines as an alternative febrifuge, antihelminthic and as a bitter tonic1,8,9.

Previous studies have determined concentrations of mineral elements in a few species of *Swertia*10-12 but the mineral composition in *S. cordata* has not been reported so far. Additionally this is the first antimicrobial activity report of *S. cordata* aerial parts and roots extract.

Materials and Methods

Plant material

Fresh samples of *Swertia cordata* (G. Don) Clarke were collected in the month of October, 2009 from village Vinayak near Pangot (29°25’25”N 79°25’37”E) 15 km away from Nainital, India.
The plant identification was confirmed by the Botanical Survey of India (BSI), Dehra Dun. A voucher specimen (No.112968) was deposited in the Herbarium Section at BSI, Dehra Dun, India.

Mineral analysis

Mineral content in plant was estimated by wet digestion method\textsuperscript{13}. Aerial parts and roots of the plant were air-dried and ground into fine powder. 1.0 g powdered plant material was first digested with conc. HNO\textsubscript{3} (5 mL each), followed by application of 15 mL of tri-acid mixture (HNO\textsubscript{3}, HClO\textsubscript{4} and H\textsubscript{2}SO\textsubscript{4}, 10:4:1, v/v) heated at 200°C and reduce to 1 mL. The residue after digestion was dissolved in double distilled water, filtered and diluted to 100 mL. This solution was used for the estimation of minerals. Macro minerals, viz. Na, K, Ca and Li were estimated by AIMIL, Flame Photometer while micro elements, viz. Fe, Cu, Mn, Zn and Co were estimated by Atomic Absorption Spectrophotometer, model 4129, Electronic Corporation of India Ltd. Estimation of each element was carried out 3 times and the mean values were reported and used. For macro elements, standard blank and sample solutions were passed through flame photometer and concentrations were determined in µg/mL. Total amount of macro elements in mg/kg dry weight of samples were calculated by the formula:

Macro element mg/kg = \frac{Y}{W} \times 50 \quad \text{(or trace element mg/kg =} \frac{Y}{100})

where \(Y\) = flame photometer reading and \(W\) = weight of sample.

The concentrations of micro elements were calculated by the same formula, where \(Y\) = reading of atomic absorption spectrophotometer and \(W\) = weight of sample.

Extraction procedure

The aerial parts and roots of the plant \textit{S. cordata} were shade dried, powdered and extracted separately with 70% methanol using Soxhlet apparatus. After extraction each filtrate was concentrated on a rotary evaporator under vacuum at 20°C till a residual mass was obtained. Each extract was then stored separately in screw capped vials under refrigeration, till needed.

Phytochemical screening

Various tests for the presence of alkaloids, flavanoids, glycosides, terpenoids, xanthones and steroids were performed on the methanol extract of \textit{S. cordata} aerial parts and roots.

Microorganisms

Three Gram positive, three Gram negative and two fungi were used for antimicrobial activity studies. Gram positive bacteria were \textit{Staphylococcus aureus} (MTCC 3160), \textit{Bacillus subtilis} (MTCC 441) and \textit{Bacillus mycoides} (MTCC 645). Gram negative bacteria were \textit{Escherichia coli} (MTCC 406), \textit{Pseudomonas aeruginosa} (MTCC 424) and \textit{Proteus vulgaris} (MTCC 426). Yeast like fungi used were \textit{Candida albicans} (MTCC 227) and \textit{Aspergillus niger} (MTCC 404). Required microorganisms were procured from Institute of Microbial Technology, Chandigarh, India.

Determination of zone of inhibition

The disc diffusion method\textsuperscript{14} was used to evaluate the antimicrobial activity. The test solutions of aerial parts and roots methanol extract at the concentrations of 4000, 2000, 1000, 500 and 250 µg/mL were prepared by dissolving the extracts in dimethylsulphoxide (DMSO). 0.1mL of test solutions were injected into sterilized discs of 5 mm in diameter. Chloramphenicol (25 µg/disc), ampicillin (25 µg/disc) and fluconazole (25 µg/mL) were used as positive controls. As a negative control, a blank disc impregnated with DMSO was used. The test discs, standard discs and blank discs were placed in petri dish with a particular microorganism. The petri dishes were then incubated at 37°C for 24 h for bacterial growth and at 27°C for 48 h for the growth of yeast. Nutrient agar and potato dextrose agar medium were used for the growth of bacteria and yeast, respectively. Each extract was analyzed in triplicate.

Results and Discussion

Mineral content of samples

The mineral profiles of \textit{S. cordata} aerial parts and roots are shown in Table 1. Among all the elements, highest concentration of K was recorded in both aerial parts and roots of \textit{S. cordata}. The concentration of other elements in aerial parts decreased in the order Ca>Na>Fe>Zn>Mn>Cu>Li>Co while in roots the decreasing order of elements was Ca>Fe>Na>Zn>Mn>Cu>Li>Co (Fig. 1).

The analysis showed that both aerial parts and roots have low amount of sodium with relatively high concentration of potassium. K/Na ratio in diet is an important factor in prevention of hypertension and arterosclerosis since K depresses and Na enhances
Table 1—Mineral composition of Swertia cordata aerial parts and roots (dry wt mg/100g)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Minerals</th>
<th>Aerial parts</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sodium (Na)</td>
<td>18.29</td>
<td>32.92</td>
</tr>
<tr>
<td>2</td>
<td>Potassium (K)</td>
<td>385.6</td>
<td>421.3</td>
</tr>
<tr>
<td>3</td>
<td>Calcium (Ca)</td>
<td>277.00</td>
<td>166.20</td>
</tr>
<tr>
<td>4</td>
<td>Lithium (Li)</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>5</td>
<td>Iron (Fe)</td>
<td>21.6</td>
<td>140.2</td>
</tr>
<tr>
<td>6</td>
<td>Cobalt (Co)</td>
<td>0.1</td>
<td>0.7</td>
</tr>
<tr>
<td>7</td>
<td>Zinc (Zn)</td>
<td>5.1</td>
<td>14.5</td>
</tr>
<tr>
<td>8</td>
<td>Manganese (Mn)</td>
<td>5.1</td>
<td>6.9</td>
</tr>
<tr>
<td>9</td>
<td>Copper (Cu)</td>
<td>4.3</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Fig. 1—Mineral composition of Swertia cordata aerial parts and roots

blood pressure. Calcium is associated with growth and maintenance of bones, teeth and muscles. Comparatively, S. cordata aerial parts have higher Ca content than roots, however, this Ca content was found lesser than those in S. chirayita (Roxb. ex Fleming) Karsten, S. speciosa D. Don and S. paniculata Wall. Iron is an essential trace element for haemoglobin formation, normal functioning of central nervous system and in the oxidation of carbohydrates, proteins and fats. From the results, aerial parts of S. cordata have lower Fe content than roots. In human body copper exists as an integral part of Cu proteins ceruloplasmin, which is concerned with the release of iron from the cells into the plasma and is involved in energy metabolism. The Cu content in S. cordata roots and aerial parts were present in equal amounts. Manganese is another micro element essential for human nutrition and it acts as activator of many enzymes. The Mn content in S. cordata aerial parts and roots was found comparable with the values reported in S. speciosa while lesser than S. chirayita and S. paniculata. As shown in Table 1, roots have higher Zn content than aerial parts while Li was found in equal proportion. Cobalt was found in traces in both aerial parts and roots.

Antimicrobial activity

Antibacterial and antifungal activity of S. cordata aerial parts and roots methanol extract are shown in Table 2. Antimicrobial potential of the extracts were evaluated by measuring the diameter of zones of inhibition (mm), including the diameter of disc and the results (mm of zone of inhibition) were expressed as average values. The zone of inhibition markedly decreased on decreasing the concentration of the extracts for all the strains used for study. The results showed that S. cordata aerial parts exhibited good antibacterial activity (Fig. 2) while roots were found to possess good antifungal activity (Fig. 3). Aerial parts showed good antibacterial activity against B. mycoides while E. coli was found insensitive for roots. Roots exhibited very good antifungal activity against C. albicans while aerial parts showed moderate activity against both the fungal strains. Antifungal activity of roots was found even comparable with Fluconazole while antibacterial activity for both aerial parts and roots was found low as compared to the controls (Ampicillin, Chloramphenicol).

The inhibitory effects of S. cordata methanol extract against tested microorganisms might be related to the role of mineral contents, active and effective phytochemical constituents like flavonoids, xanthones, alkaloids, etc. The presence of micronutrients and biologically active constituents in plant extract usually interfere with growth and metabolism of microorganisms to destroy them. It has been reported in many studies that the antimicrobial property of plant extracts is partly contributed by minerals. The antibacterial effect of zinc on Streptococci and Staphylococcus was described as early as 1949. It has been documented that the basic mechanism that lies behind the antibacterial activity of zinc ion depends on its ability to bind to the membranes of microorganism, thereby prolonging the lag phase of growth cycle and increasing the generation time of the organisms which takes for each organism more time to complete cell division. Relying upon the results of the present investigation it could be suggested that these findings may be exploited for the remedies of a wide range of microbial diseases of plants as well as animals.
Table 2—Antimicrobial activity of *Swertia cordata* aerial parts and roots

<table>
<thead>
<tr>
<th>Zone of Inhibition (mm)</th>
<th>Aerial Parts (µg/mL)</th>
<th>Roots (µg/mL)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4000 2000 1000 500 250</td>
<td>4000 2000 1000 500 250</td>
<td>AM CP FZ</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>12 9 7 6 - 9 8 7 6 -</td>
<td>9 8 7 6 -</td>
<td>32 12 -</td>
</tr>
<tr>
<td><em>B. mycoides</em></td>
<td>14 13 10 8 6 9 7 6 -</td>
<td>9 8 7 6 -</td>
<td>- - 25 -</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>12 10 8 7 - 9 8 7 6 -</td>
<td>9 8 7 6 -</td>
<td>20 30 -</td>
</tr>
<tr>
<td><em>P. vulgaris</em></td>
<td>11 10 8 7 - 9 8 7 6 -</td>
<td>9 8 7 6 -</td>
<td>16 14 -</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>13 11 9 7 6 - - - -</td>
<td>11 9 7 6 -</td>
<td>21 23 -</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>12 7 6 - - 7 6 - -</td>
<td>7 6 - -</td>
<td>23 10 -</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>13 10 8 7 6 7 6 -</td>
<td>10 8 - -</td>
<td>- - 15</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>12 10 9 8 7 7 6 -</td>
<td>9 7 6 -</td>
<td>- - 13</td>
</tr>
</tbody>
</table>

AM = Ampicillin (25 µg/disc), CP = Chloramphenicol (25 µg/disc), FZ= Fluconazole (25 µg/mL)

Fig. 2—Antimicrobial activity of *S. cordata* aerial parts

Fig. 3—Antimicrobial activity of *S. cordata* roots
The study has also provided some biochemical basis for the ethnomedical use of the extracts from the whole plant of *S. cordata*.

**Conclusion**

From the results it can be concluded that potassium is present in highest concentration in the whole plant of *S. cordata*, followed by Ca>Na>Fe in case of aerial parts while in roots, the minerals are found in the order K>Ca>Fe>Na. The plant was found to exhibit good antifungal activity as well, the most susceptible fungi was *Candida albicans*. Thus the plant could be used in the treatment of fungal infections caused by these microbes.

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**References**