

# Evaluation of antioxidant, antibacterial, and antidiabetic potential of two traditional medicinal plants of India: *Swertia cordata* and *Swertia chirayita*

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## ABSTRACT

**Background:** *Swertia cordata* and *Swertia chirayita* are temperate Himalayan medicinal plants used as potent herbal drugs in Indian traditional systems of medicine (Ayurvedic, Unani and Siddha). **Objective:** Assessment of Antioxidant, antibacterial, and antidiabetic potential of *Swertia cordata* and *Swertia chirayita*. **Materials and Methods:** Phytochemicals of methanolic and aqueous extracts of the two *Swertia* species were analyzed. The antioxidant potential of all the extracts was assessed by measuring total phenolic content, total flavonoid content and free radical scavenging potential was assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, antibacterial activity was assessed against various pathogenic and nonpathogenic bacteria *in vitro* by Kirby-Bauer agar well diffusion method and antidiabetic activity was assessed by  $\alpha$ -amylase inhibition. **Results:** Methanolic leaf extracts of both the species of *Swertia* contain significant antibacterial as well as anti-diabetic potential, whereas methanolic root extracts of both species were found to have potential antioxidant activity. However, *Swertia chirayita* showed better activities than *Swertia cordata* although both species have good reputation in traditional Indian medicine. **Conclusion:** Both the species are having high medicinal potential in terms of their antioxidant, antibacterial and antidiabetic activities. Studies are required to further elucidate antioxidant, anti-diabetic and antibacterial potentials using various *in-vitro*, *in-vivo* biochemical and molecular biology techniques.

**Key words:** Antibacterial activity, Anti-diabetic activity, Antioxidant, Chirata, *Swertia chirayita*, *Swertia cordata*

## INTRODUCTION

Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, most medicinal preparations were derived from plants, whether in the simple form of raw plant materials or the refined form of crude extracts, mixtures, etc. In today's world, medicinal plants are gaining attention owing to the fact that the herbal drugs are cost-effective, easily available and with little or no side effects. Plant-based natural constituents can be obtained from any part of the plant like bark, leaves, flowers, fruits, roots, seeds, etc.

Recently, the quest for the isolation of new compounds from medicinal plants has become an intriguing area of research. Plants with ethno pharmaceutical importance were being studied because of their healing properties<sup>[1]</sup> as well as for their efficient antimicrobial, antidiabetic and antioxidant properties.

All the living organisms use oxygen for metabolism and use food nutrients in order to get energy for survival. Thus, oxygen is an essential part of living organisms. Approximately, 5%–7% or more of the inhaled oxygen ( $O_2$ ) is converted to reactive oxygen species (ROS) such as  $O_2^-$ ,  $H_2O_2$  and  $\bullet HO$ .<sup>[2]</sup> Antioxidants remove these free radical intermediates inhibiting oxidation reactions. Antioxidants are those compounds that can delay or inhibit the oxidation of lipids, protein and nucleotides by suppressing the initiation of oxidative chain reactions, thus preventing damage done to the body cells by ROS.<sup>[3]</sup>

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These free radicals are capable of attacking the healthy cells of the body which may lead to diseases, disorders or cell damage. Cell damage caused by free radicals appear to be a major contributor to aging and diseases like cancer, heart disease, decline in brain function such as Alzheimer diseases, Parkinson diseases, and also decline in the immune function.<sup>[4]</sup> *Swertia chirayita* (*S. chirayita*) has shown to have antimicrobial activity against Gram positive and Gram negative bacteria. It is also used as a stringent tonic to the heart, liver, eyes, cough, scanty-urine, melancholia, dropsy, and skin diseases. The plant extracts are also used as a bitter tonic in gastrointestinal disorders, like dyspepsia and/or anorexia, it is used as a digestive, febrifuge, and laxative.<sup>[5]</sup> *Swertia* species contain *xanthones* as secondary metabolites which are used effectively to treat malarial fever and tuberculosis. *Swertia* species also contains amarogentin, a glycoside secondary metabolites that protects liver against carbon tetrachloride poisoning and its believed to be the bitterest component isolated till date.<sup>[6,7]</sup> Almost all species of *Swertia* possess the phytochemical active principles with varying degree of activities, but only *S. chirayita* as the major species has been extensively screened for anti-diabetic, antimicrobial and antioxidant active ingredient.

The plants under investigation are *Swertia cordata* and *S. chirayita* belonging to the family *Gentianaceae*. In this family, many plants are used as medicine. Both plants (*S. cordata* and *S. chirayita*) were selected for phytochemical and biological studies as they both have folkloric reputation.

## MATERIALS AND METHODS

### Chemicals and reagent

Chloroform, dimethyl sulfoxide (DMSO), sulfuric acid, dilute ammonia, ferric chloride, hydrogen chloride, deionized distilled water, Folin-Ciocalteu reagent, sodium carbonate, and gallic acid are used for phytochemical screening and antioxidant assay. Mueller-Hinton broth and agar media are used for antibacterial assay. All the reagent and media were purchased from Hi media. Starch and  $\alpha$ -amylase enzyme are used for anti-diabetic assay.

### Instrumentation

Grinder, water bath, glassware (conical flasks, beakers, test-tubs, petri plates), micropipette, gel puncher, digital balance, incubator, laminar flow hood, hot air oven, spectrophotometer, refrigerator, autoclave, and microwave oven.

### Plant material

The plants *S. cordata* and *S. chirayita* were collected in December, 2013 from Garo hills at 1200 m to 1500 m altitude, Meghalaya. Identity of the plants was confirmed by a taxonomist from ICAR, Umiam, India. The plants

were collected with roots as whole taken out from the soil and then cleaned in running tap water followed by distilled water. Then the plants were dried in the shade for 30 days. Each part (i.e., leaves, stem, and root) was then separated and ground in a clean mechanical grinder separately. The ground powder of root, stem, and leaves was kept in air-tight bag separately in room temperature.

### Microorganisms used

Bacterial strains analyzed were obtained from Institute of Microbial Technology, Chandigarh (*Bacillus megaterium* Microbial Type Culture Collection (MTCC) 8510, *Bacillus subtilis* MTCC 441, *Bacillus flexus* MTCC 7024, *Staphylococcus aureus* MTCC 96, *Clostridium perfringens* MTCC 450, *Lactobacillus rhamnosus* MTCC 1423, *Pseudomonas oleovorans* MTCC 617, *Klebsiella pneumoniae* MTCC 7028, *Salmonella enteric* MTCC 1164 and *Escherichia coli* MTCC 723).

### Extraction of plant materials

The extraction of plant parts was carried out according to the method suggested by Fatope *et al.* 1993,<sup>[8]</sup> with minor modification. In this, 20 g powder of leaves; stem and root of both the species of *Swertia* were percolated at room temperature with 400 ml of methanol for methanolic extraction and with same volume of hot water (98°C initial temperature) for aqueous extraction, respectively. The conical were screw capped and placed in a shaker at room temperature for 24 h at 100 rpm. After 24 h, the extracts were filtered using a muslin cloth and then re-filtered using Whatman filter paper No. 1. The filtrates were concentrated using a water bath at 35–40°C. The extracts were labeled accordingly and preserved in the refrigerator at 4°C, till further work. Extract was dissolved in DMSO to made concentration of the stock solution of 0.5 g/ml.

### Phytochemical screening

Phytochemical screening of root, stem, and leaves of *S. chirayita* and *S. cordata* was carried out according to the methods of Ayoola *et al.* 2008.<sup>[9]</sup> The different plant part extracts prepared were screened for terpenoids, flavonoids, saponins, tannins, and phenols, respectively.

#### Test for terpenoids (Salkowski test)

To 0.5 g of each of the plant part extracts were added with 2 ml of chloroform. Then into it 3 ml of concentrated sulfuric acid was carefully added, to form a layer. A reddish-brown coloration of the interface indicated the presence of terpenoids.

#### Test for flavonoids

In 0.5 ml of filtrate of each of the plant parts extracts, 5 ml of dilute ammonia was added, followed by addition of 1 ml of concentrated sulfuric acid to it. The presence of flavonoids was detected by yellow coloration of the solution that disappears on standing.

**Test for saponins**

To 0.5 g of each plant extracts, 5 ml of distilled water was added in test-tubes. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3–4 drops of olive oil and again shaken vigorously after that it was observed for the formation of emulsions.

**Test for tannins**

About 0.5 g of each plant extracts was boiled in 10 ml of water in each test-tube and then filtered. Then few drops of 0.1% ferric chloride were added and observed for brownish-green or a blue-black color formation which indicate the presence of tannins.

**Test for phenols (ferric chloride test)**

Small quantities of alcoholic and aqueous extracts were dissolved in 2 ml of distilled water separately, and into it, few drops of 10% aqueous ferric chloride solution were added. A blue or green color was produced which indicating the presences of phenols.

**DETERMINATION OF TOTAL FLAVONOID CONTENT**

The flavonoids content of different plant part extracts of both *Swertia* spp. was measured based on methods described by Ebrahimzadeh *et al.* 2008.<sup>[10]</sup> According to this method, 0.5 ml of each extracts was mixed with 1.5 ml of methanol and then 0.1 ml of 10% aluminum chloride was added, followed by the addition of 0.1 ml of 1M potassium acetate and then 2.8 ml of distilled water was added to the total mixture. Then mixture was incubated for 30 min at room temperature. The absorbance of the reaction mixture was measured at 415 nm in a spectrophotometer. The total flavonoids content was expressed in mg/g of quercetin equivalent, as standard. All the determination was performed in triplicate.

**DETERMINATION OF TOTAL PHENOLIC CONTENT**

The total phenolic content of different plant part extracts of both *Swertia* spp. was evaluated using a method described by Kim *et al.* 2003.<sup>[11]</sup> According to this, 0.5 ml of extract was added to 4.5 ml of distilled water and then 0.5 ml of Folin Ciocalteu reagent was added to it. After mixing, the mixture was maintained at room temperature for 5 min followed by the addition of 5 ml of 7% sodium carbonate and 2 ml of distilled water into the mixture. After mixing, the solution was incubated for 90 min at 23°C. The absorbance was measured by spectrophotometer at 750 nm. The total phenolic content was expressed in mg/g

of Gallic acid equivalent, as standard. Determination of total phenolic content was performed in triplicate.

**DETERMINATION OF 1,1-DIPHENYL-2-PICRILHYDRAZYL RADICAL SCAVENGIN ACTIVITY**

The DPPH radical scavenging activity of all the plant parts extracts for both *Swertia* spp. were determined using the method proposed by Von Gadow *et al.* 1997.<sup>[12]</sup> In this, 50  $\mu$ l of samples of each plant extracts was placed in separate cuvettes, then into it, 2 ml of  $6 \times 10^{-5}$  M methanolic solution of DPPH radical was added immediately and absorbance was measured immediately at 517 nm. Then after 16 min of incubation decrease in absorbance was again measured for the entire samples. Methanolic solution of Ascorbic acid of 1  $\mu$ l/mg concentration was used as a standard. All the determination was performed in triplicates. The DPPH radical scavenging activity was measured in percentage of reduction of the color. The percentage inhibition of the DPPH radical by the sample extracts was calculated by the formula suggested by Yen and Duh 1994.<sup>[13]</sup> Control was taken at time zero that mean before the antioxidant present in plant extracts starts its activities.

$$\% \text{inhibition} = \{(A_{C(0)} - A_{A(t)}) / A_{C(0)}\} \times 100$$

Where,

$A_{C(0)}$  = Absorbance of control at time,  $t = 0$  min

$A_{A(t)}$  = Absorbance of antioxidant at time,  $t = 16$  min.

**METAL CHELATING ACTIVITY**

The metal (ferrous ion) chelating activity by methanolic and aqueous extracts of both the *Swertia* spp. were estimated by a method suggested by Dinis *et al.*<sup>[14]</sup> with minor modification. In this, about 100  $\mu$ l the each plant part extracts samples were added to 50  $\mu$ l solution of 2 mM  $\text{FeCl}_2$ . Then the addition of 200  $\mu$ l of 5 mM ferrozine started the reaction and then the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance for reduction of coloration of the solution by each sample was measured at 562 nm in a spectrophotometer. Ethylenediaminetetraacetic acid solution in place of sample extracts was taken as a positive control and reagent mixture without sample was used as a negative control. The metal chelating activity is expressed in percentage reduction of color. All the determination was performed in triplicate. The metal chelating capacities of the extracts were evaluated using the following equation:

Metal chelating capacity (%) =  $[(A_0 - A_1)/A_0] \times 100$

Where,

$A_0$  = absorbance of the negative control

$A_1$  = absorbance of the sample extracts.

## ANTIMICROBIAL SCREENING

### Well diffusion method

The antimicrobial activity of the extracts was determined by Kirby–Bauer method in agar well diffusion.<sup>[15]</sup> In this method, initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 h in an incubator. Then agar plates of the Muller Hilton's Agar media were prepared. Each plate was inoculated by swabbing with bacterial suspension or with 18 h fresh culture ( $10^5$ – $10^6$  colony forming unit “CFU”/ml), which was swabbed evenly on the surface of solid agar media by the help of cotton swab. After 20 min, wells of 6 mm diameter were made in solid agar medium with the help of gel puncher and filled with 50 µl of various test samples of *S. chirayita* and *S. cordata* in different solvent extracts. The positive control wells were filled with Amikacin 30 (Standard drug). All the plates were incubated at 37°C for 24 h and then during observation after 24 h, the diameter of the zone of inhibition was measured in millimeter. All the equipment used like media, Petri plates, cotton swab etc., must be sterilized before performing the experiments. As well as proper labeling of the petri plates must be done before performing. All the determination of antibacterial activities of plant extracts were also done in triplicates.

## ANTI-DIABETIC ACTIVITY ASSAY (IN VITRO)

Antidiabetic potential of *S. chirayita* and *S. cordata* was assayed *in vitro* by a method proposed by Prabhakar *et al.* 2013.<sup>[16]</sup> The *in vitro* anti-diabetic activity was determined by assaying the inhibitory activity of the enzyme  $\alpha$ -amylase which involves in the breakdown of starch to produce glucose. In this method, 1 ml of both the methanolic and aqueous extracts of both the *Swertia* spp. was tested separately and thus added to 1 ml of the enzyme  $\alpha$ -amylase in a test-tube and incubated for 10 min at 37°C. Then 1 ml of 1% starch solution was added into it and again incubated for 15 min at 37°C. Then 2 ml 3,5-dinitrosalicylic acid reagent was added into it, in order to terminate the reaction. The reaction mixture was then incubated in boiling water bath for 5 min and then allows it to cool at room temperature. The absorbance of the reaction mixture was then measured at 546 nm in a spectrophotometer. The standard (control) of the reaction

without the extract represents the 100% enzyme activity. The % age inhibition of enzyme activity of  $\alpha$ -amylase was determined by:

%age inhibition of  $\alpha$  - amylase =

Enzyme activity of control -

Enzyme activity of extract  $\times 100$

Enzyme activity of control

### Statistical analysis

The results are represented as mean of three replicates followed by the standard deviation, that is, mean  $\pm$  standard deviation. The data were subjected to student *t*-test analysis, and the significance of the difference was determined by Graphpad. *P* < 0.05 were considered to be significant.

**Table 1: Antioxidant activity in methanolic extracts of different part of *Swertia* spp.**

| Tests        | Plant part extracts | Antioxidant present in plant species |                        |
|--------------|---------------------|--------------------------------------|------------------------|
|              |                     | <i>Swertia chirayita</i>             | <i>Swertia cordata</i> |
| Total        | Root                | 63.25 $\pm$ 2.16                     | 57.60 $\pm$ 4.50       |
| Flavonoid    | Stem                | 125.07 $\pm$ 2.79*                   | 114.9 $\pm$ 3.60       |
| Content      | Leaves              | 207.97 $\pm$ 3.56*                   | 191.7 $\pm$ 3.80       |
| Total        | Root                | 56.60 $\pm$ 4.66                     | 19.50 $\pm$ 2.38       |
| Phenolic     | Stem                | 44.69 $\pm$ 1.69*                    | 14.86 $\pm$ 3.12       |
| Content      | Leaves              | 42.16 $\pm$ 1.48*                    | 14.05 $\pm$ 3.54       |
| DPPH radical | Root                | 38.92 $\pm$ 2.40                     | 38.63 $\pm$ 2.13       |
| Scavenging   | Stem                | 22.75 $\pm$ 3.09                     | 23.53 $\pm$ 3.06       |
| Activity     | Leaves              | 15.73 $\pm$ 2.69                     | 13.67 $\pm$ 2.66       |
| Meta         | Root                | 63.63 $\pm$ 1.86                     | 71.20 $\pm$ 1.73*      |
| Chelating    | Stem                | 65.39 $\pm$ 2.14                     | 62.80 $\pm$ 3.02       |
| Activity     | Leaves              | 56.72 $\pm$ 2.70                     | 47.98 $\pm$ 2.51       |

Values represent mean $\pm$ SD of triplicate, \*Significant at *P*<0.05. Methanolic extracts of *Swertia chirayita* when compared with *Swertia cordata*. DPPH=1,1-diphenyl-2-picrylhydrazyl; SD=Standard deviation

**Table 2: Antioxidant activity in an aqueous extract of different part of *Swertia* spp.**

| Tests        | Plant part extracts | Antioxidant present in plant species |                        |
|--------------|---------------------|--------------------------------------|------------------------|
|              |                     | <i>Swertia chirayita</i>             | <i>Swertia cordata</i> |
| Total        | Root                | 28.64 $\pm$ 1.92*                    | 23.50 $\pm$ 2.21       |
| Flavonoid    | Stem                | 42.70 $\pm$ 5.65                     | 43.60 $\pm$ 3.23       |
| Content      | Leaves              | 70.22 $\pm$ 3.91                     | 63.88 $\pm$ 2.31       |
| Total        | Root                | 44.39 $\pm$ 4.27*                    | 14.86 $\pm$ 4.61       |
| Phenolic     | Stem                | 62.32 $\pm$ 4.66*                    | 21.14 $\pm$ 3.41       |
| Content      | Leaves              | 54.40 $\pm$ 3.74*                    | 18.41 $\pm$ 3.42       |
| DPPH radical | Root                | 33.26 $\pm$ 3.06                     | 30.82 $\pm$ 4.06       |
| Scavenging   | Stem                | 27.76 $\pm$ 1.98                     | 28.39 $\pm$ 2.80       |
| Activity     | Leaves              | 19.45 $\pm$ 1.46                     | 20.48 $\pm$ 3.60       |
| Meta         | Root                | 59.54 $\pm$ 3.59*                    | 51.21 $\pm$ 1.96       |
| Chelating    | Stem                | 47.05 $\pm$ 3.41                     | 45.56 $\pm$ 3.05       |
| Activity     | Leaves              | 55.73 $\pm$ 4.32*                    | 60.69 $\pm$ 2.31       |

Values represent mean $\pm$ SD of triplicate, \*Significant at *P*<0.05. Aqueous extracts of *Swertia chirayita* when compared with *Swertia cordata*. DPPH=1,1-diphenyl-2-picrylhydrazyl; SD=Standard deviation

**Table 3: Antibacterial activity of *Swertia chirayita* and *Swertia cordata* with diameter of the zone of inhibition in millimeter**

| Bacterial strain               | Zone of inhibition (mm) by Amikacin 30 (positive control) | Plant part | Diameter of zone of inhibition (in mm) |                        |
|--------------------------------|---|------------|--|------------------------|
|                                |   |            | <i>Swertia chirayita</i>               | <i>Swertia cordata</i> |
| <i>Bacillus flexus</i>         | 33.50±3.54  | Root       | -                                      | -                      |
|                                |   | Stem       | 12.5±0.35                              | 12.0±0.8               |
|                                |   | Leaves     | 12.5±0.71                              | 15.0±1.3               |
| <i>Bacillus subtilis</i>       | 26.00±2.83  | Root       | -                                      | -                      |
|                                |   | Stem       | 7.5±0.35                               | 8.0±1.0                |
|                                |   | Leaves     | 11.0±1.41                              | 12.0±1.8               |
| <i>Bacillus megaterium</i>     | 22.50±2.12  | Root       | -                                      | -                      |
|                                |   | Stem       | 12.5±0.35                              | 17.0±0.5               |
|                                |   | Leaves     | 13.0±1.41                              | 20.0±1.0               |
| <i>Lactobacillus rhamnosus</i> | 22.0±1.41   | Root       | -                                      | -                      |
|                                |   | Stem       | -                                      | 17.0±1.2               |
|                                |   | Leaves     | 11.5±0.71                              | 20.0±0.8               |
| <i>Pseudomonas oleovorans</i>  | 21.5±0.71   | Root       | -                                      | -                      |
|                                |   | Stem       | 8.5±0.71                               | 9.0±1.3                |
|                                |   | Leaves     | 12.0±1.41                              | 12.0±1.0               |
| <i>Klebsiella pneumoniae</i>   | 29.5±0.71   | Root       | -                                      | -                      |
|                                |   | Stem       | 8.0±1.41                               | 7.0±0.5                |
|                                |   | Leaves     | 9.5±0.71                               | 9.0±1.0                |
| <i>Salmonella enterica</i>     | 27.0±1.41   | Root       | -                                      | -                      |
|                                |   | Stem       | 13.5±1.12                              | 13.0±1.1               |
|                                |   | Leaves     | 17.5±4.24                              | 15.0±0.5               |
| <i>Clostridium perfringens</i> | 33.0±1.41   | Root       | 13.0±4.24                              | 7.0±1.0                |
|                                |   | Stem       | 12.5±0.35                              | 9.0±0.2                |
|                                |   | Leaves     | 21.5±0.71                              | 19.0±2.0               |
| <i>Staphylococcus aureus</i>   | 27.00±4.24  | Root       | -                                      | -                      |
|                                |   | Stem       | 9.0±0.71                               | 10.0±1.0               |
|                                |   | Leaves     | 13.05±0.71                             | 14.0±0.7               |
| <i>Escherichia coli</i>        | 23.0±1.41   | Root       | -                                      | -                      |
|                                |   | Stem       | -                                      | -                      |
|                                |   | Leaves     | -                                      | -                      |

## RESULTS AND DISCUSSION

The different solvent extracts from various plant parts of both the species of *S. chirayita* and *S. cordata* exhibited varying degree of antioxidant activity *in vitro*. Only methanolic extract of stem and leaves of both the *Swertia* spp. showed antimicrobial activity.

Both the *Swertia* spp. plants showed significant [Tables 1-4].

## CONCLUSION

Antioxidant, antimicrobial and antidiabetic activity of *Swertia cordata* and *Swertia chirayata* plants were analyzed in this experiment. Among the different plant extracts analyzed methanolic leaf extracts, aqueous root extracts *S. chirayata* and methanolic stem extract of *S. cordata* showed most significant antioxidants potential. Very significant antidiabetic activity was observed in both methanolic and aqueous leaf extract followed by methanolic and aqueous stem extracts of both the *Swertia* species. Root extracts also showed minor antidiabetic activity.

**Table 4: Anti-diabetic activity of *Swertia chirayita* and *Swertia cordata* assay *in vitro* with percentage of enzyme  $\alpha$ -amylase inhibition activity**

| Plant extracts      | Plant parts | Percentage inhibition of $\alpha$ -amylase |                        |
|---------------------|-------------|--|------------------------|
|                     |             | <i>Swertia chirayita</i>                   | <i>Swertia cordata</i> |
| Methanolic extracts | Root        | 30.12±0.67                                 | 31.02±0.74             |
|                     | Stem        | 52.43±0.43                                 | 48.93±0.49             |
|                     | Leaves      | 74.21±0.73*                                | 62.31±0.68             |
| Aqueous extracts    | Root        | 21.56±0.84                                 | 23.91±1.24             |
|                     | Stem        | 45.67±0.21                                 | 43.87±0.94             |
|                     | Leaves      | 75.25±0.84*                                | 70.83±0.42             |

Values represent mean±SD of triplicate, \*Significant at  $P<0.05$ . Both the methanolic and aqueous leaves extracts of *Swertia chirayita* when compared with *Swertia cordata*. SD=Standard deviation

Methanolic leaf extracts of both *S. chirayita* and *S. cordata* showed good antibacterial activity.

These results reveal significant antibacterial antioxidants and antidiabetic activities present in different plant parts of *S. cordata* and *S. chirayata*. Local populations have already realized the medicinal importance of these rare plants and have been using them for treatment of medical ailments for past many centuries.

Further studies are required to elucidate and understand the antibacterial, antidiabetic and antioxidants potential of these both *Swertia Spp.* plants using various biochemical and molecular biology tools.

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