India has a rich culture of medicinal herbs and spices with more than 2000 species and has a broad geographical area catering to Ayurvedic, Unani, Siddha and other conventional medicines. So far only few plants have been systematically studied chemically and pharmacologically for their potential medicinal value (Gupta et al., 2005; Sandhu and Heinrich, 2005). Man has used plants for the treatment of diverse ailments for thousands of years (Sofowara, 1982; Hill, 1989). According to the World Health Organization, most communities still depend upon the traditional medicines for their psychological and physical health requirements (Rabe and Van Stoden, 2000), as they have no access to the products of Western pharmaceutical industries (Salie et al., 1996), and lack healthcare facilities (Griggs et al., 2001). Rural areas of many developing countries still rely on traditional medicine for their primary health care needs.

Herbal medicines are comparatively safer and inexpensive than the synthetic or modern medicines (Iwu et al., 1999; Idu et al., 2007; Mann et al., 2008; Ammara et al., 2009). People living in rural areas use traditional experience to treat ailments although they may not know the scientific basis of these medicines (Maheshwari et al., 1986; Van Wyk et al., 2000).

Herbal medicines are in significant demand in both developed and developing countries as a source of primary health care owing to their properties having wide biological and medicinal activities and lesser costs. Herbal molecules are safe to use and would overcome the resistance produced by pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Lai and Roy, 2004; Tapsell et al., 2006). Similarly with the occurrence of modern or allopathic medicine, Balick and Cox (1996) have noted that a number of important modern drugs have been derived from plants used by indigenous people. Traditional use of herbal medicine is recognized as a way to learn more about potential future medicines.

Researchers have identified number of compounds used in main stream medicine which are derived from "ethno medical" plant sources (Fabricant and Farnsworth, 2001). Plants are used medicinally in different countries and are a source of many vigorous and strong drugs (Srivastava,
et al., 1996; Mahesh and Satish, 2008). Swertia chirata is one of the most important traditional medicinal plant which is used as a source of several drugs and is now regarded as critically endangered/threatened because of its over exploitation from its natural habitats.

Swertia chirata (family: Gentianaceae) is an annual medicinal herb mainly found in north western Himalaya from Kashmir to Bhutan and Khasi hills at an altitude of 1100 - 3300 m. The stems and leaves of the plants are reddish in color when mature. The leaves are lanceolate, and have five nerves varying in the length from 8-9 cm. The Flowers are greenish yellow and tinted with purple. It can attain height up to 1.5 meters. Seed dispersal takes place once the plant has matured in late November or December (depending on the area).

![Swertia chirata plant](image)

**Figure 1.1: Swertia chirata - A Critically Endangered Medicinally Plant**

Swertia chirata (Roxb. ex Fleming) H. Karst. is also mentioned in the literature as Swertia chirayita, Gentiana chirayita Roxburgh 5–8 and Gentiana floribunda Don. In different languages chirata is also named as Anaryatikta, Ardhatikta, Bhunimba, Chiratika, Chiratitka, Haima, Jvarantaka,
Kairata, Kandatikata, Kiranta, Kirataka, Kirata Tikta, Naditikta, Naipala, Nepalanimba, Nidrari, Ramasenka, Sannipatha, Sutikta, Trinanimba and Viktaka in Sanskrit, Cherayata in Patna, Chirrato and Chiraite in Nepal, chiraita and kiraita in Mumbai, chirayatin in Gujrat. The trade name of *Swertia chirayita* is chiretta.

*Swertia chirata* is much prized as a bitter tonic without aroma and astringency. It is an official drug of the Indian Pharmacopoeial list 1946. In Indian medicine, it is prescribed in a variety of forms and combination in disorder of liver, bronchial asthma, fever, chronic fever, anemia, stomach and kidney ailments (Chatterjee and Pakrashi, 1995). Conventional Bhutanese medicine also uses chirata for blood purification and for the treatment of common cold, gout disease and even diabetes and malaria. It is also best known in the ingredient of Maha-Sudarshana churna (a remedy containing more than 50 herbs). The plant has ample metabolites which are medicinally very important having anti-inflammatory, anti-cholinergic, hepato-protective activities which are credited to major secondary metabolites such as swertiamarin, amarogentin, mangiferin (Joshi and Dhawan 2005). Its bitterness, anti-helminthic and hypoglycemic activities are attributed to amarogentin (most bitter compound isolated till date), swerchirin, swertiamarin and other functional principle compound of this important herb.

The medicinal properties of *Swertia Chirata* are reported in Indian pharmaceutical codex, the British and American pharmacopoeias and in the different traditional systems of medicines such as the Ayurveda, Unani and Siddha.

**Table 1.1: List of important bioactive constituents isolated from Swertia chirata:**

<table>
<thead>
<tr>
<th>Active constituents</th>
<th>Biological activities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>-------------</td>
<td>---------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Swertiamarin</td>
<td>Analgesic property</td>
<td>Lei et al., 1982.</td>
</tr>
</tbody>
</table>

Some of the Herbaceous medicaments like Ayush-64, Diabecon, Mensturyl syrup and Melicon V ointment restrain *chirata* essence in variable expanse for its anti-pyretic, hypoglycemic, anti-fungal and anti-bacterial effects (Edwin and Chungath, 1988; Valecha et al., 2000; Mitra et al., 1996).

Pharmaceutical value of *Swertia chirata* is increasing day by day. On the contrary its counter claim in pharmaceutical industry is increasing in the same speed. There are different factors like less activity and growing percentage of seeds and gentle field handling of plantlets which distress the advancement of agricultural technologies. Due to these problems, crude material for industrial use is being persistently gathered naturally.

The natural regeneration of plant takes place by seeds, when the seeds become biologically mature having high potentiality of viability during November (Bhattarai, 1996). The viability of seeds is very low if seeds are collected before November. The seeds stored in bad condition have no viability at all. The viability decreases after next October. If seeds are collected after November and washed properly, the percentage of germination is reported up to 90% (Bhattarai, 1991). The seeds should be sown within a year of collection. Before February, the soil is too cold to sow the seeds. It is better to sow between February and April, into moist, fertile nursery beds. The seeds are covered with a thin layer of soil (depth twice the size of the seeds). Mulching is
necessary for superior germination. Periodic water spraying is done to maintain the moisture content of soil. When the seeds start to germinate, the mulching materials should be removed. After the seedlings acquire the height of 6-8 cm, then they are set for transplantation in the field. Generally 15cm spacing between seedlings is needed for optimum production.

1.1 RESOURCE MANAGEMENT

Management System

*Chirata* is mostly collected from government forest, which has no control over collection. Who comes to collect first, will collect more and earns more money. Thus, there is always competition for collection and collectors collect before seed dispersal. Seeds are only the medium for the propagation of this plant, so if the plant is picked up before the maturation of seeds, there will be no future germination. Destructive competition between the collectors has led to the over-harvesting of *Chirata* without the consideration of sustainable regeneration. Not only does early harvesting have a negative effect on regeneration, immature plants decrease the active constituent’s quality of the final product.

Harvesting

November-December is the appropriate time for harvesting. Collection is done manually without using any instruments. Whole plant is pulled out and sun-dried for few days and then wrapped by 'choya'. 'Choya' is the bamboo slip used to tie up bundles of *Chirata*. Then small dried bundles of *Chirata* is collected into big bundles and sold to the local traders.

Sustainability issues

The whole plant is collected for the trade. November - December is the traffic season of this product. Due to its high cost, collectors have high competition for collection and it is collected before maturation. Thus, unmanaged exploitation of *Chirata* has resulted in the decrease in natural production.

Recently an increased demand for *S. chirata* has been noticed. The product has been discovered by the beverage industry as an alternative bitter product (i.e. used in the liquor industries to impart bitter flavor). *Swertia* extract contains Oleanolic acid and Swertiamarin which are used as a hair growth tonic (Suzuki *et al.*, 1989). *Chirata* is also used as one of the ingredients in “Chandra..."
Prabati” which is an Ayurvedic drug for cancer.

**Role of biotechnology in conservation**

Biotechnology can play an indispensable role in conservation of *chirata* species. It can engross different modes of conservation. Farming of *chirata* at low heights is prohibited because of several environmental factors like fertility and textures of soil, pH, humidity etc. Tissue culture technique is useful for conservation of *Swertia chirata* as limited plant material can produce a large number of disease free propagules which can be planted in their natural environment. Systematized cultivation of the plant is important to guarantee continuous supplementation and assertion of drug. Exercise in quality control of raw material of *chirata* is labeling of DNA markers that associate DNA finger printing data with quantity of selected markers. (Joshi *et al.*, 2004). Research is still going on molecular investigation of *chirata* for protection of this wild endangered species, establishing methods for breeding *in-vitro* and making efforts for assuring continuous supply of its raw material.

Collection of *Swertia chirata* has reduced its population to a very low level resulting in its categorization in an endangered species; It will therefore be desirable to apply novel technique of *in vitro* conservation and micropropagation for its conservation and production of a large number of disease-free and true-to-type plants and also save natural populations by *in vitro* production of Swertiamarin, mangiferin and amarogentin, so that plants from natural habitats are not uprooted.

The current study aims to explore the feasibility of cell suspension culture for studying the Swertiamarin, mangiferin and amarogentin accumulation in cell culture by the use of elicitor.

**1.2 OBJECTIVES**

1. Standardization of *in vitro* culture of *Swertia chirata*.
2. Optimization of cell proliferation in liquid culture.
3. Production of Swertiamarin in liquid culture.
4. Antimicrobial activity of *Swertia chirata*. 