Chapter-1

Introduction

India is one of the 12-mega biodiversity centres having about 10% of the world’s biodiversity wealth, which is distributed across 16 agro-climatic zones. Out of 17,000 species of higher plants reported to occur within India, 7500 are known to have medicinal uses (Shiva, 1996). This proportion of medicinal plants is the highest known in any other country against the existing flora of that country (Kala et al., 2006). Ayurveda, the oldest medical system in Indian subcontinent, has alone reported approximately 2,000 medicinal plant species, followed by the Siddha and Unani medical systems. The Charak Samhita, an age-old written document on herbal therapy, reports on the production of 340 herbal drugs for curing various disease (Prajapati et al., 2003). Approximately 25% of drugs is derived from plants, and many others are synthetic analogues build on prototype compounds isolated from plant species in modern pharmacopoeia (Rao et al., 2004).

The medicinal plants have been continuously getting exploited which lead to the decline in the population of many high value medicinal plant species over the years (Kala, 2003). The primary threats to medicinal plants are those that effect any kind of biodiversity used by humans (Sundriyal and Sharma, 1995). The weakening of customary lose, which have regulated the use of natural resources, is among the causes threatening medicinal plant species (Kala, 2005). These customary lose have often proved to be easily diluted by modern socio-economic forces (KIT, 2003). There are many other potential causes of rarity in medicinal plant species such as habitat specificity, narrow range of distribution,
land-use disturbances, introduction of non-natives, habitat alteration, climatic changes, heavy livestock grazing, explosion of human population, fragmentation and degradation of population, population bottleneck and genetic drift (Kala, 2000; Weekley and Race, 2001; Oostermeijer et al., 2003).

Medicinal plants are a National resource the use of which has continued in an unbroken tradition in India across two millennia. Given their continuing social & growing economic importance as a bio-cultural resource, on one hand, and threats to their survival on the other, it makes imperative for the Government to see these vital floras as a national treasure which must be protected and conserved. Something sort of a national policy on medicinal plants conservation is urgently needed. This must be followed by the immediate implementation of effective programs in line with the national policy.

1.1. Tissue culture

Plant tissue culture is a technique for growing isolated cells, tissues and organs in a defined nutrient medium under aseptic condition (in vitro).

The role of medicinal and aromatic plants in pharmaceuticals, perfumery, flavour and cosmetic industry in strengthening India’s economy needs no elaboration. Because of increased demands, over-exploitation and ruthless collection of these plants from natural resources have resulted in their rapid depletion resulting in endangered status and extinction of various species. Therefore, utmost importance of today is to expedite the pace of propagation of fast depleting species and also promising strategies for their conservation.

Plant tissue culture has stepped in as a promising tool to complete this task because it aims at the production of plants at large scale from small pieces of stock plants in relatively short periods of time in a small area without any
physiological barriers and seasonal interruption which results in biomass increase. Apart from this, plant tissue culture is potentially valuable in producing high yield of bio-medicines and eventually provide efficient means of commercial gains. Plant tissue culture has a good means not only for the propagation and commercialization of existing germplasm but also for the conservation of genetic resources. Moreover, it will also help in the production of genetically upgraded plant population which can fruitfully be employed in plantation crops, aromatic and medicinal plants, spices and condiments most of which are vegetatively propagated (Paramageetham, 2000).

In vitro culture techniques are extensively used for the conservation and large scale propagation of medicinal plants, as there is an increasing need to produce large number of plants of improved quality which holds good promise in this field.

1.2. Phytochemistry

The plant is a biosynthetic laboratory not only for chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by man, but also for a multitude of compounds like glycosides, alkaloids, volatile oils, tannins, etc. that exert a physiological and therapeutic effect. The compounds that are responsible for the therapeutic effect are mostly secondary metabolites. A systematic phytochemical study therefore, involves the thorough screening of primary as well as secondary metabolites derived as a result of plant metabolism.

The botanical industry worldwide is in desperate need of methods for quality control of herbal drugs. Identification of the material and detection of possible adulterants are two of the principal analytical goals. These tasks can usually be completed easily by planar chromatography. Great advantages of HPTLC over HPLC are the speed of method development and the flexibility of
being able to look for several classes of substance with very little effort. As the HPTLC method is highly accurate, selective and precise hence can be used for a routine quality-control analysis and quantitative simultaneous determination of bioactive compounds in pharmaceutical preparations.

In the present study, phytochemical studies were carried out to estimate the colchicine content in different parts of *in vivo* plant and *in vitro* tuber.

1.3. Molecular Biology

Molecular biology is the fastest growing of all human endeavors. In ancient days people tried to know the physical and chemical nature of the macromolecules like DNA, RNA and proteins. Once these were understood, tried to know by which these molecules function in the cell. Recently, in the development of the techniques involving gene manipulation and its beneficial use to mankind gave birth to a new discipline, the biotechnology. Molecular biology stresses the study of structure, function, expression and interrelationships of the cells macromolecules including DNA, RNA and proteins (Agarwal, 2001).

Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important (Kalpana Joshi, 2004).

In the present study, protein profiling by SDS-PAGE was done in order to analyse the nature of gene products (proteins) qualitatively in different parts of the *in vivo* and *in vitro* plants.
For polymorphic studies, the plants were collected from eleven different districts of Tamil Nadu, to check the genetic variations between accessions using RAPD markers. The *in vitro* and *in vivo* plants were also used to check the genetic fidelity.

1.4. **Antibacterial assay**

Medicinal plants exhibit antibacterial activity since they contain innumerable biologically active chemical constituents. The use of plant preparations as food stuff, insecticides, CNS active, Cardio active, antitumour and antimicrobial agents are some examples of immense chemical diversity in plants which are as old as man kind (Al-Sereiti *et al.*, 1999). Several studies have revealed the presence of some compounds with antimicrobial properties (Cowan, 1999). The roots, stems, barks and leaves of several plants have been widely evaluated for such bioactive compounds and the results obtained proved that these compounds are the sources of new drugs, antibiotics and agrochemicals. During the last few decades there has been a resurgence of interest in plants as sources of medicines and of novel molecules for the treatment of microbial infections (Kinghorn, 1987; Williamson *et al.*, 1996).

In the present study, the antibacterial studies were done to understand the efficacy of different plant extracts against different human pathogenic bacteria.

1.5. **Pachamalai hills**

Pachamalai hills are a part of Eastern Ghats situated at the central region of the state of Tamil Nadu. These hills are spread across the two districts of Salem and Tiruchirappalli with latitudes 11° 09’ 00”’ to 11° 27’ 00”’ N and longitudes of 78° 28’ 00”’ to 78° 49’ 00”’E. The total geographical area is 527.61 sq. km and altitudes range of 160 to 1072m msl. The vegetative area is distributed into 35 reserved forests. These hills enjoy a sub-tropical climate with
temperature varying from 25° C to 31° C and annual rainfall ranging from 800 to 900 mm. These hills comprises four panchayat unions – Thenpuranadu (16 villages), Vannadu (32 villages), Kombainadu (12 villages) and Attinadu (32 villages). The total tribal population is around 12,000 who live in seventy hamlets scattered all over the plateau area.

Climatically, a sub-tropical climate prevails with a maximum temperature ranging between 23°C to 31°C and a minimum temperature ranging 12°C to 18°C. The annual rainfall varies every year. These hills receive maximum rainfall during the months of September, October and November through the Northeast monsoon. A maximum of 1250 mm has been recorded so far in the past 10 years. The hills receive rainfall in the months of June and August through Southwest monsoon.

The soil of these hills ranges from loam to clay loam and is generally reddish brown to dark yellowish brown in colour. The soil depth is about a meter and soil reaction is near neutral (6.5).

Out of the total 14,122 hectares of Pachamalai, 3420.22 ha (24.5%) are not cultivated and 3806.92 ha (26.96%) are occupied by the forests. The forests are of the evergreen and mixed deciduous type.

The major crop grown here is tapioca apart from paddy, ragi, maize, lablab, beans, gingelly, horse gram, red gram, etc. Vegetables such as tomato, brinjal and beans are extensively grown. Among the fruit trees grown here, guava, cashew, jackfruit, mango, lime oranges are the major ones.
The general constrain in these hills is the transport facility which is not fully developed. Marketing facilities, introduction of high yielding animals, sheep breeding, poultry rearing are to be taken up for large-scale development.

The tribals living in Pachamalai hills are Malayalis. They are generally forest dwellers are engaged in the collection of tamarind, walnut and tapioca, etc. Several of them also work as landless farm labourers. The villages are scattered all over the hills. They are generally very backward and illiterate. Their income is very low which is earned mainly from the proceeds of tamarind and walnut. Due to poverty and illiteracy, they suffer from malnutrition and other ailments like cold, coughs, etc. Children are the worst sufferers due to the malnutrition and under nutrition. Personal hygiene and environmental sanitation are far from satisfactory level. The general condition if the livestock is also poor. The housing condition in this area is deplorable and particularly during the monsoon, life becomes miserable. The tribals usually carry their products by head loads to the nearest market centre.

The Pachamalai hills are well known for their wealth of medicinal plants which have been tremendous medicinal properties.

1.6.  **Gloriosa superba L.**

*Gloriosa superba* L. belonging to family Liliaceae is among some of the modern medicines most important plants actually facing local extinction. The plant derives its name *Gloriosa* from the word ‘gloriosus’ which means handsome and *superba* from the word ‘superb’ means splendid or majestic kind. This plant has been a source of medicine right from the ancient time.
1.6.1. Habitat

The plant grows in sandy-loam soil in the mixed deciduous forests in sunny positions. It is very tolerant of nutrient poor soils. It occurs in thickets, forest edges and boundaries of cultivated areas in warm countries up to height of 2530 m. it is also widely grown as an ornamental plant in cool temperate countries under glass or in conservatories. (Neu Winger, 1994: Inchem, 2004).

1.6.2. Morphology

It is a glabrous climbing herb with tuberous root stock grows over hedges and small trees. Stem is 6m long which grows to a height of 1.2-1.5m before the stem branches. Leaves are simple, alternate or whorled, sessile, ovate-lanceolate, 17x4.5cm, tip elongating into a spirally coiled tendril, base cordate and margin entire. Flowers are large in terminal racemes; perianth segments 6, linear, flexuosus and deflexed, basal half bright yellow, upper half red; stamens 6; ovary glabrous, 3-celled. Fruits are capsules, linear-oblong, upto 6.8cm long, 3 equal lobes, one or two lobes shorter in malformed fruits; green dried to pale and then black colour, dehisced into three sections. Seeds are oval in shape, testa spongy, embryo cylindric, 30-150 seeds per capsule, pale orange attached to the sutures. Tubers are cylindric, large, simple, ‘V’ shaped with the two limps equal or unequal in length pointed towards end brownish externally and yellowish internally. (Narain, 1977)

1.6.3. Distribution

A native to tropical jungles of Africa, is now found growing naturally in many parts of tropical Asia including India, Myanmar, Malaysia, Sri Lanka (Jayaweera, 1982). In temperate countries, Gloriosa superba. L is propagated as an ornamental in conservatories, best suited to green houses (Neuwinger, 1994). In India it is mainly found in Nasik, Ratnagiri, Savanthwadi (Maharashtra), Uttara Kannada, Hassan, Chikmangalur, Coorg, Mysore (Karnataka),
Cannanore, Palakkad, Trivandrum,(Kerala), Tamil Nadu and Goa. (CES 2004). Today it is under cultivation in fairly large areas of India, but seen less in Pachamalai hills, Tamil Nadu.

1.6.4. Medicinal importance

- **Leaf**

  The sap from the leaf tip is used for pimples and skin eruptions; leaf juice is instilled into the nose in case of fainting. In Congo crushed leaves are applied to the chest to treat asthma. In Burundi a leaf decoction is recommended for treating dropsy of the scrotum, while the leaf pulp serves against rheumatism. The Ulanga people of Tanzania burn the herb and apply ash on wounds to promote healing. They also drink the plant juice as anti malarial

- **Tubers**

  The tuberous roots are useful in curing inflammations, ulcers, scrofula, bleeding piles, white discharge, skin diseases, leprosy, indigestion, helminthiasis, snake bites, baldness, intermittent fever debility (Prajapati et al., 2003)

  Tubers are given internally as an antidote for snake poison. Considered useful in promoting labour and expulsion of placenta. It is also used for the treatment of bruises, sprains (Rastogi and Mehrotra, 1993), colic, hemorrhoids, cancer, impotence (Nadkarni, 1978), nocturnal seminal emissions and leprosy. Many cultures believe the species to have various magical properties (Watt & Breyer-Brandwijk, 1962; Neuwinger, 1994; Burkill, 1995). The plump roots of the plant have been used in the treatment of parasitic skin infections, leprosy, and internal worms (Mutshinyalo, 2001; Dhushara, 2004).
• **Seeds**

The seeds are used for relieving rheumatic pain and as a muscle relaxant (Prajapati *et al.*, 2003).

1.6.5. **Other uses**

*Gloriosa superba* L. is the state flower of Tamil Nadu. Except miscellaneous pharmaceutical products and other therapeutic preparations, it is also a popular plant for providing colour in green houses and conservatories even immature flowers are beautiful to be hold. All parts of the plant, especially the tubers, are extremely poisonous and can be fatal. (Senanayake and Karalliedde, 1986). The tubers may be mistakenly eaten in place of sweet potatoes since the tubers resemble those of sweet potatos. The juice of the leaves is used as an ingredient in arrow poisons. The flowers are used in religious ceremonies.

1.6.6. **Chemical constituent**

Studies reveal that all the parts of the plant especially the tubers are extremely toxic due to the presence of a highly active alkaloid, Colchicine. The species also contains another toxic alkaloid, Gloriosine (Gooneratne, 1996; Angunawela and Fernando, 1971). Other compounds such as 3-desmethyl colchicine, beta-lumicolchicine, N-formyldesacetyl-colchicine, 2-desmethylcolchicine, Chelidonic acid and Salicylic acid (Duke, 1985).

1.7. **Reasons for choosing *G. superba* for the studies**

This plant has been a source of medicine right from the ancient time. So, many books and articles have been written so far on the medicinal and other values of this plant. It is one of the most popular herb in all discussions, symposia and seminars, etc. related to herbal conservation. This glorious herb was found in abundance once upon a time in Pachamalai hills. Nowadays, over-
exploitation of this plant by local healers, this plant is becoming rare and endangered in these hills.

The plants growing in the wild are dependent on soil, seasons and weather conditions. Hence, they may not be available throughout the year. *G. superba* which is having tremendous medicinal properties found only during the monsoon season. When the season is over, not only the plant gets dried up, but also the subterraneous rhizomes cannot be collected. Pharmacies and drug-manufactures often fulfill upto 75% of their raw material demand of this species from the wild. Of this 75%, about 60% comes from destructive collections which include the entire plant or underground parts like corms and roots or reproductive parts like fruits and seeds. Although it is conventionally propagated through corms, the proliferation rate is very low. Daughter corms flower only 2-3 years once they have achieved a critical size.

The germination of its seed is very difficult or impossible because of susceptibility to many pests, and over-exploitation in habitats for medicinal purpose have pushed this taxon to endangered (Sivakumar, et al., 2003). The seeds of this glorious herb are highly priced in the world market as they are the main source of Colchicine and Colchicoside (Sivakumar et al., 2002). In order to provide enough plant material for commercial exploitation, the cultivation of the plant using corm is not sufficient (Sivakumar et al., 2003). Thus, mass propagation through *in vitro* techniques of this plant is urgently needed not only to conserve but also to meet the demands for this medicinal plant as a source of colchicines and to make available throughout the year.

Due to these reasons, the plant was selected for the study and makes it an important issue so that conservationists, botanists, entrepreneurs and NGOs come forward to rescue and save this plant in these hills.
Objectives

Because of multifold medicinal uses, *Gloriosa superba* L. has been selected for the present study with the following objectives:

- Survey and understanding the status of medicinal plants in Pachamalai hills, a part of Eastern Ghats, Tamil Nadu, India.

- *In vitro* standardization of *Gloriosa superba* L, an endangered medicinal plant of Pachamalai hills.

- Phytochemical analysis of *G. superba* to understand the novel bioactive compounds.

- Chromatographic studies to elucidate the bioactive compounds.

- Protein profiling of *G. superba* to understand the proteins, a pioneer for gene action and functions.

- Polymorphic studies using RAPD markers in different accessions of *G. superba* collected from different locations of Tamil Nadu.

- Checking the genetic fidelity between *in vivo* and *in vitro* plant, through RAPD markers.

- To screen the anti-bacterial activity of various parts of the plant against growth of some pathogenic bacteria under *in vitro* conditions.

- To compare the efficacy of different parts of the plant extracts on the test organisms with standard antibiotic.