CHAPTER - 1

INTRODUCTION
1. INTRODUCTION

Medicinal plants have been playing a crucial role in sustaining the health and well-being of mankind. The discovery of the curative properties in plants must have emanated from some human instinct. The primitive man used the plants as therapeutical agents or remedial measures, which he was able to procure most easily. The study that deals with the biological, biochemical and economical features of natural drugs, their constituents and their action is known as Pharmacognosy which has been a part of the medical science since the dawn of civilization. The Babylonians were aware of the medicinal properties of a number of plants.

Hippocrates (460-370 B.C.), the father of medicine have numerous references on the present day drugs. Aristotle (384-322 B.C.) and his disciple Theophrastus (370-287 B.C.) wrote about the medicinal uses of plants, their writings were placed on scientific footing, free from all superstitions. Dioscorides (1st century A.D.) was the writer of the first “Materia Medica” (78 A.D.), where he described 600 medicinal plants, including Belladona, Colchicum, Opium, Hyoscyamus, etc.

In India, the study of drugs started about 5,000 years ago. The first systematic knowledge on the medicinal uses of plants in India was gathered by Von Rheede in a book “Hortus Malabarica” (Kumar 1993). The references to the curative properties of some herbs in the Rig Veda, estimated to be between 3500 and 1800 B.C. seem to be the earliest records of use of plants in medicine. The identity of several plants referred to the Suk of the Rig Veda can be fixed with
reasonable certainty, e.g. of Semal, Pithvan, Palash and Pipal. But references to plants in the *Rig Veda* are very brief. A detailed account is available in the *Atharva Veda*. The most important works on the Indian system of medicine appears in *Charak-Samhita* and *Susruta-Samhita* which deals with about 700 drugs based mainly on plant products (Jain 1968).

Medicinal plants have been the subjects of man's curiosity since time immemorial (Constable 1990). Almost every civilization has a history of medicinal plant use (Ensminger et al., 1983). Approximately 80 per cent of the people in the world's developing countries rely on traditional medicine for their primary health care need, and about 85 per cent of traditional medicine involves the use of plant extracts (Vieira and Skorupa 1993). Interest in phytomedicine has explored in the last few years, and about 500 different plant species are used as key ingredients, and many are still being collected from the wild (Mendelsohn and Balick 1994). Plants play a dominant role in the introduction of new therapeutic agents, and also drugs from the higher plants continue to occupy an important niche in modern medicine (Dev 1997). Many compounds used in todays medicine have a complex structure, and synthesizing these bioactive compounds chemically at a low price is not easy (Shimomura et al., 1997). With deforestation, medicinal wealth is rapidly lost, such that many valuable plants are threatened with extinction. Pharmaceutical companies depend largely upon materials produced from naturally occurring stands that are being rapidly depleted. Plant tissue culture is an alternative method of propagation (George and Sherrington 1984) and is being used widely for the commercial propagation of a large number of plant species, including many medicinal plants (Rout et al., 2000).
The resurgence of public interest in plant-based medicine coupled with rapid expansion of pharmaceutical industries have necessitated an increased demand for medicinal plants, leading to over-exploitation that threatens the survival of many rare species. Also, many medicinal plant species are disappearing at an alarming rate due to rapid agricultural and urban development, uncontrolled deforestation and indiscriminate collection. Combinations of *in vitro* propagation techniques (Fay 1992) and cryopreservation may help in conservation of biodiversity of locally used medicinal plants. Cryopreservation is a reliable method for long-term storage of the germplasm of endangered species. Several medicinal plant species have been successfully cryopreserved (Bajaj 1995, Naik 1998). *In vitro* cell and tissue culture methodology is envisaged as a mean for germplasm conservation to ensure the survival of endangered plant species, rapid mass propagation for large-scale revegetation, and for genetic manipulation studies.

The medicinal plants are in great demand in traditional system of medicine i.e. Ayurveda, Siddha and Unani Tibb as well as folklore prescriptions. The modern pharmaceutical industry also requires a large quality of authentic plants for manufacture of drugs. Extraction of active principles and manufacture of drug formulations is sophisticated technology and capital intensive. However, systematic cultivation of these plants and their drying / preservation at farm level constitute a promising rural industry with attractive remunerations. These semi-processed plants find a ready market with herb dealers / large pharmaceutical industries.

Studies regarding *in situ* and *ex-situ* conservation are limited to very few medicinal plants. Notable among them are *Dioscorea deltoidea* rhizome (Sarin
1970), *Rauvolfia serpentina* root (Sarin 1974), *Costus speciosus* (Sarin et al., 1981). A national Board of Medicinal Plants has been set up, one of its activities is conservation. The Ministry of Environment and Forests is funding an all India coordinated project on conservation of endangered plant species (Raghupathy 2001).

Advances in biotechnology particularly methods for culturing plant cell cultures, should provide new means for the commercial processing of even rare plants and the chemicals they provide. These new technologies will extend and enhance the usefulness of plants as renewable resources of valuable chemicals. There has been considerable interest in plant cell cultures as a potential alternative to traditional agriculture for the industrial production of secondary metabolites (Dicosmo and Misawa 1995). Plant cell culture technologies were introduced at the end of 1960s as a possible tool for both studying and producing plant secondary metabolites. Different strategies using cell cultures systems have been extensively studied with the objective of improving the production of bioactive secondary metabolites. Cell culture systems could be used for the large scale culturing of plant cells from which secondary metabolites can be extracted. The advantage of this method is that it can ultimately provide a continuous, reliable source of natural products.

Tissue culture techniques are now providing a powerful tool for manipulating plants. It is one of the most exhilarating achievements of plant sciences, which may lead to revolutionary progress in agriculture, horticulture and even forestry in the future. The main objective of plant cell culture in the
India with a geographical area of 329 million hectares is a store house and treasure trove of rich biological diversity – both flora and fauna. There are more than 45,000 plant species with about 7,000 endemics and 75,000 animal species. India is recognised as one of the 25 ‘mega-biodiversity’ centres and one of the earth’s biologically wealthiest nations in the world. There are two biodiversity rich areas within our territory, termed as hotspots – the Western Ghats and the Indo-Burmese region which include all the north-eastern states of India. According to IUCN, nearly 10 per cent of the flora in the world are endangered and threatened for extinction. An assessment of the endangered status of various plant species in N.E. region by the Botanical Survey of India reveals that about 300 species are endangered needing immediate conservation measures; while equally the same number of species are vulnerable if remedial measures are not taken (Hegde 2000).

N E India one of the hot spots of biodiversity of the world holds a great number of medicinal plants. Of late, N E India witnessed wanton destruction of forests. In fact N E region is loosing many important medicinal plants and many of them are on the verge of extinction. Some woody plants die after their medicinal components are harvested. For example, the medicinal part of *Eucamomia ulnoides* Oliv. is the phloem and the plant rarely survives which it is harvested. Two other important plants *Terminalia arjuna* and *Holarrhena antidysenterica* are ruthlessly exploited by removing their barks. Such plants also die easily as their food transport system is disturbed. Many important medicinal plants have been exploited out by the MNCs without the knowledge of the local people. Such a
situation calls for mass awareness campaign and rapid propagation of some of the threatened medicinal plants of the N E India by exploiting the tissue culture technique. Keeping in view the above circumstances the following plants have been selected for mass propagation through tissue culture:

1) *Boerhaavia diffusa* L.

2) *Dioscorea deltoidea* Wall

3) *Houttuynia cordata* Thunb.

1. **Boerhaavia diffusa** L

Commonly known as ‘punarnava’. The genus *Boerhaavia*, one member of Nyctaginaceae, is represented by about 20 species in almost all tropical and subtropical areas of the world (Fosberg 1978; Bittrich and Kühn 1993). It is a perennial herb growing to 0.1m by 0.5m. The flowers are hermaphrodite. The plant prefers light (sandy) and medium (loamy) soils and requires well-drained soil. The whole plant is a source of Ayurvedic drug “Punarnavine”. Roots have been found to be more potent than the stem, having 0.05 per cent alkaloid (Majunath 1948); which possesses diuretic, anti-inflammatory, antibrinolytic, antiviral, antibacterial activities (Kurup et al., 1958). The plant is bitter, astringent, cooling, anthelmintic, diuretic, aphrodisiac, cardiac stimulant, diaphoretic, emetic, expectorant, anti-inflammatory, febrifuge, laxative and tonic. It is useful in all types of inflammatious; stranguary, leucorrhoea, ophthalmia, lumbago, myalgia, scabies, jaundice, anaemia, dyspepsia, constipation, cough, bronchitis and general debility.
The species is becoming rare in the natural habitat, due to over exploitation of the plant for root and it is also consumed as a leafy vegetables (Kanjilal et al., 1982).

2. *Dioscorea deltoidea* Wall.

Commonly known as ‘Yams’. Plants of genus *Dioscorea* (Family Dioscoreaceae) are perennial climbers with tuberous roots. Approximately 600 species of *Dioscorea* occur rather abundantly in tropical area, sub tropical regions with a few species in the temperate regions. Only 15 species are reported to contain steroidal saponogenin chiefly diosgenin. In India, *D. deltoidea* and *D. prazeri* occurring wild in North West and North East Himalayas respectively are the natural sources of diosgenin. *Dioscorea deltoidea* is found in India, Pakistan, Nepal and Bhutan extending to South Western China while *D. prazeri* occurs in Eastern India, Burma and northern parts of Malaysia (Hussain 1988).

The tubers of some species of *Dioscorea* are important sources of diosgenin, a chemical used for the commercial synthesis of sex hormones and corticosteroids (Coursey 1967). *Dioscorea* species is frequently used as tonic in Chinese traditional medicine. The most active ingredient discovered in the tuber is diosgenin, which can be used as a precursor for many important medicinal steroids such as prednisolone, dexamethasone, norethisterone and metenolone etc. (Tsukamoto et al., 1936).


*Houttuynia cordata* Thunb (family – Saururaceae) is a perennial herb native to mountainous regions of eastern Asia, while it exists also in China, mainly in its
middle, southeastern and southwestern provinces and regions, such as Sichuan and Chongqing. The plant grows to about 15 inches high with an indefinite spread as a creeping rhizome in moist locations. This is a rampant growing herbaceous plant, though it dies right down in the winter. It succeeds in moist and wet soils as well as in shallow water. The whole plant has a beneficial effect in the treatment of haemorrhoids, acute conjunctivitis and other ocular infections due to *Bacillus phyocyaneus*. It is also used in treating measles. The juice of the crushed fresh plants is also administered orally. In case of contusions and ophthalmia, crushed fresh leaves are applied externally in a poultice (Prajapati and Kumar 2005).

Considering the above facts and its pharmaceutical characters, tissue culture work was designed on the three medicinal plants of high potential value from North Eastern part of India with the following objectives:

1. Collection of plant materials and maintenance of the germ plasm in the Botanical Garden of the Department of Botany, Gauhati University.

2. To identify the best culture medium for each species.

3. Initiation of callus and organogenesis by modifying the nutrient media from time to time by supplementing with PGRs and other growth adjuncts.

4. Acclimatization of plantlets under controlled condition.

5. Transplantation of hardened plantlets to natural habitat.