CHAPTER 1

General introduction

1.1. INTRODUCTION:

Plants are a major group among the living organisms, which support and help the life line of the human beings as well as other living organisms. They have always sustained human civilization through the biologically active compounds they contain in them.

India is one of the twelve mega diversity countries of the world (Bapat *et al*., 2008), with rich biodiversity. They exhibit 12.51 % of the floral diversity in India. India is a highly biodiversity rich country which has about 48,000 plant species which is 10 % of the total flora of the world (Hajra and Mudgal, 1997). It represents 12.5% of world's phytogeography (Anonymous, 2000).

North East India is a biodiversity hot spot region with highly diversified plant population as well as with ethnic diversity. NE region is the richest reservoir of plant diversity, which supports the 50% of Indian biodiversity (Mao and Hynniewta, 2000). This region is identified as the centre of origin of many crop plant species and has a great diversity of traditional knowledge. It is estimated that the diversity of some major crops in this region includes rice (9650+ varieties), maize (15 varieties and 3 sub varieties), banana (14 species), citrus (17 species + 52 varieties), sugarcane and their relatives (15 species) and bamboo (60 species) (Hore, 1998). The area occupies 7.7 % of the total geographical area of India and it is estimated that about 43%-50% (8000 species) of Indian flora are found in this region (Rao, 1994), while in Assam about 4273 plant species are available in wild condition (Choudhury, 2005). Recent reports support that about 2000 vascular plant species, i.e. 46% of the total flora of Assam, are found in Southern Assam (Hussain, 2013).

1.2. MEDICINAL PLANTS:

Any plant or part of the plants which contain active medicinal chemical constituents with a definite physiological response in the treatments of the diseases in human and other animals are called medicinal plants. In plant kingdom, medicinal plants play an important

role in protecting human health as well as they have varied economic role throughout the world (Govil, 1998). The father of medicine, Hippocrates describes about 400 medicinal plants and advocated," Let food be your medicine and let medicine be your food (Malik *et al*., 2011).

Medicinal plants constitute a group of industrially important crop plants (Rawat, 2003), which play vital role in the socio-cultural, spiritual and medicinal arena of the rural people. It is also one of the most important elements of bio-diversity that usually grows in all kinds of eco-systems and possesses high pharmaceutical, economic and ecological values.

Medicinal plants are a renewable natural resource. WHO estimated that 80% population of the developing countries relies on the traditional medicines, mostly plant based drugs, for their primary health care.

About 90% of the medicinal plants used by the industries are collected from the wild and over 800 species are used in the production of medicine by the industry, while less than 20 species of plants are under commercial cultivation. Over 70% of the plant collections involve destructive harvesting because of the use of parts like roots, bark, wood, stem and the whole plant in case of herbs (Sharma *et al*., 2010). The Task Force Committee on Medicinal Plants of the Planning Commission of India had set the target of exporting the medicinal plants to the tune of Rs 3000 crore by 2005 from rupees 466 crore in 2001 (Anonymous, 2001).

1.2.1. Global Scenario of Medicinal Plants:

According to Schippmann *et al*., (2002) more than 50,000 plant species are used for medicinal purposes worldwide, of which almost 13% are flowering plants (Sakkir, 2012). It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in the developing countries such as China and India, the contribution is as much as 80% (Joy *et al*., 1998). More than 7000-7500 plant species are used for their medicinal values by the traditional communities. India and china are the two largest countries of Asia, which have the richest array of registered and relatively well known medicinal plants (Ravan, 1998).

1.2.2. Scenario of Medicinal Plants in India:

India is a mega-diversity country. There are diverse group of plants, animals as well as microorganisms, which are found among the 34 hot spots of the world, of which two are found in India and in the neighboring countries. India has a long history of the use of medicinal and aromatic plants for various purposes. Our ancient literature right from the Atharvaveda provides rich references on native plants and their properties to alleviate human sufferings (Rao, 1998). A number of native plants have been used in various preparations and the science of medicine based on the herbal preparation has been preserved and carried- forward through the ancient treatises like 'Charak Samhita', 'Shrushut Samhita', 'Bagbatta Samhita' and many similar authoritative reference books. These treatises are still held in high esteem in Ayurvedic system of medicine even today.

According to an all India ethnobiological survey carried out by the Ministry of Environment & Forests, Government of India, there are over 8,000 species of plants being used by the people of India. Among these plants 70% are distributed in Tropical area including Western Ghats and Eastern Ghats and other 30% are found in temperate, alpine and high altitude region, (Report of the Task Force on Conservation & Sustainable use of Medicinal Plants, Government of India Planning Commission March – 2000). From this report it is cleared that among the total medicinal plants 33% are trees, 32% herbs, 20% shrubs, 12% and 3% are other plants. The different parts of the medicinal plants are used for curing different ailments. Among the different parts, roots are used in the highest percentage, i.e. 29%, followed by the whole part 16%, bark 14%, fruits 10%, seeds 7%, stem 6%, leaves 6%, flowers 5%, rhizome 4% and wood 3%.

In the Western Ghats and Indo –Burma region (covering the eastern Himalayas) most of the plants are used in medicinal and aromatic purposes such as Psyllium, Senna, Rauvolfia, Rheum, Aconities ,Opium, Poppy, Cinchonna, Colocynth Jpeeae, Costus, Sweet flag, Periwinkle, Steroidal ,Yams, Azadirachta, Basil, Bhrami, Aswagand, Guggal, Gentians, Agar wood, Jasmine, Spikenard, Rose, Zinger species, Cinamomum species etc. most of them come from agriculture and forestry. The production of medicine and aromatic plants in India is reported to account for about Rs 1000 million annually. There are over 5000 small and medium scale manufacturing units for drug formulations based

on the raw materials of plant origin, which together is estimated to use anywhere between Rs 3000 to Rs 5000 million worth of raw materials.

Across the country, the forests are estimated to harbour 90% of the India's total medicinal plant diversity. Only about 10% of the known medicinal plants of India are restricted to non forest habitats **(**Wakdikar, 2004). According to Schippmann *et al.* (2002), one fifth of all the plants found in India are used for medicinal purposes. The world average stands at 12.5%, while India has 20% plant species of medicinal value, which are in use. But according to Hamilton (2003), India has about 44% of the flora, which are used medicinally. Although it is difficult to estimate the total number of medicinal plants present worldwide, the fact remains that India with rich biodiversity ranks first in the percent of flora, which contain active medicinal ingredients (Mandal, 1999).

1.2.3. Scenario of Medicinal Plants in Northeast India including Assam:

North Eastern Region is the rich source of biodiversity which includes high potential of naturally occurring medicinal plants considered to be the "Cradle of Flowering Plants" (Takhtajan 1969). This geographic region is the mega-diversity hotspot and covers a lot of endemic as well as rare species, since time immemorial. Plants like *Rauvolfia serpentine* Benth., *Andrographis peniculata* (Burm.f.) Wall. ex. Nees., *Angiopteris evecta* (Forst.) Hoffm., *Azadirachta indica* A. Juss., *Homalomena aromatica* (Roxb.) Schott., *Clerodendron* species, *Jasminum* species and many other medicinally important and aromatic plants have been recorded from this Region. Most interestingly out of the nine vegetation types of India, the N.E. India represents six types. The traditional uses of medicinal plants in many parts of N. E. India is still having the ancient medicinal practices with great traditional value and plants with significant medicinal properties (i.e. both in medicinal and aromatic plants) of great importance. It is estimated that NE India is the homeland of about 2000 medicinal plants (Katwal *et al*., 2003).

From the documental study of ethnobotanical plants uses by the indigenous people of North East India, BSI, Eastern Circle, Shillong under the project of Ministry of Environment and Forests (MoEF), Government of India recorded 200 plants from Arunachal Pradesh, which are used for the treatment of 44 different diseases and

ailments, 286 plants from Assam used for 40 different diseases and ailments, 526 from Nagaland for 83 different diseases and ailments, 194 from Tripura for 50 different diseases and ailments (Hynniewta, 1984 and Hynniewta, 1987) and 834 medicinal plants from Meghalaya (FRLHT, 2006) are known for their medicinal uses.

Majority of the medicinal plant species, i.e. about 70% are found in the wild habitat and the remaining 30% are found in the non forest land under cultivation. Among them hardly only 5% of these vast natural resources are gathered for marketing (Hore and Hussain, 2004).

1.2.4. Scenario of Medicinal Plants in Southern Assam:

Southern Assam is an important bio-geographical region of Assam which is the meeting ground of different ethnic communities and their culture. Different workers have worked in the field of diversity and conservation of medicinal plants of this region. Barbhuiya *et al*., (2009) encountered a total of 150 medicinal plants belonging to 16 families of Southern Assam and they observed that the tree species have higher potential as medicinal plants compared to other plant species (i.e. 52 trees, 48 shrubs, 37 herbs, and 13 climbers).

Ethnobotany is one of the most important studies for medicinal plants in relation to the ethnic community, i.e. they use the different plant species for curing the different ailments. Southern Assam is one of the homelands of different ethnic communities (i.e. Rongmai, Khasi, H'mar, Reang, Kuki, Mizo, Chiru, Naga, Chakma, Karbi etc.). Many studies have been done by the different workers in the field of Ethnobotany and they have successfully highlighted and documented the important medicinal and aromatic plant species used by the tribal communities and their conservation. The medico-ethnobotanical knowledge of seven tribes settled in Southern Assam has been reported by Dutta and Dutta (2001). Das *et al*., (2002) has reported 72 medicinal plant species used by the tea tribes of Sothern Assam. Dutta Choudhury, (1999) have documented about the ethnobotanical knowledge of the Reang tribe and enumerated 170 medicinal plants comprising fungi, pteridophytes and angiosperms. Dutta and Dutta (2005) have also reported the potential of ethnobotanical studies in North East India with special reference to Southern Assam. Das *et al*., (2008) documented and listed the medicinal plants used by

the different ethnic community of Cachar District of Southern Assam. They enumerated a total of 111 plant species. They also reported one of the endangered medicinal plant species *Angiopteris evecta* (Forst.) Hoffm. from the Cachar district. Das *et al*., (2010) documented the medicinal plants used by the different ethnic communities of Southern Assam. They listed about 245 medicinal plants belonging to 89 families with their habit, habitat, locality, distribution etc. Among the total 245 plants, 232 are angiosperms and 13 are pteridophytes. Nath *et al.,* (2011) reported that 34 plant species belonging to 27 families are used to cure the skin diseases by the Dimasa tribe of Barak Valley (Southern Assam). Das *et al*., (2011) documented the knowledge of H'mar tribe of Southern Assam. They listed about 80 medicinal plant species for the treatment of different diseases. Bhattacharjee *et al*., (2012) encountered 94 medicinal plant species belonging to 51 families used by the Santhal and Munda communities of Cachar district. Nath *et al*., (2013) have reported 42 medicinal plant species belonging to 28 families used in the stomach disorder by the Dimasa tribe of Barak Valley.

1.3. ENDANGERED PLANTS:

Endangered plants may be defined as a taxa in danger of extinction or the taxa where the numbers have been reduced to a critical level or whose habitats have been so drastically reduced that they deemed to be in immediate danger of extinction. The International Union for Conservation of Nature and Natural Resources (IUCN, 1998) has estimated that about 12.5% (33,798) species of vascular plants are under threat.

Wild illegal harvesting has led to biodiversity decline. It is now estimated that out of the 1500 threatened species of plants, 33 have gone extinct, 157 are endangered, 114 are vulnerable and 246 are rare (Anonymous, 2000).

The Ministry of Environment and the forest (2012), published about some of the plants of North East India, which gives a clear picture of the threatened plants of the region. They listed the plants state-wise, i.e. Meghalaya has the highest threatened plants (32nos), followed by Assam (15 nos), Manipur (6 nos), Nagaland (5 nos), Mizoram (3 nos), Tripura (1 no) plant species. ENVIS, Centre on Conservation of Medicinal Plants, FRLHT, Bangalore (2010), 16 medicinal plant species were assigned under threatened

status in Assam ,i.e. 1 Critically Endangered, 8 Endangered, 6 Vulnerable and 1 Near Threatened plant species.

Table1.1: Comparative statement of the endangered vascular plants of Southern Assam, Assam, India and Global level.

	Global (IUCN,1998)	India (Rao *et al*., 2003)	Assam (Choudhury,2005)	Southern Assam (Hussain,2013)
Endangered plant	6522	152	149	43

1.4.THREAT TO MEDICINAL PLANTS:

Some anthropogenic activities as well as some other factors are contributing to the threat to the medicinal plants. Some such causes are:

1.Deforestation/degradation of the forest, 2.Conversion of agriculture land, 3.Encroachment, 4.Over Grazing, 5.Forest fire. 6. Illegal extraction of different parts including root, bark and rhizome from natural habitat, 7.Over exploitation, 8.Habitat destruction, 9.Unsustainable harvest, 10.Climate change, 11.Lack of authentic trade information, 12.Lack of appropriate policy etc.

1.5. VEGETATIVE PROPAGATION:

1.5.1. Definition:

Vegetative propagation is a form of asexual reproduction of plants, i.e. production of saplings/ plantlets without using seeds. It involves non sexual reproduction through regeneration of plant organs or parts of a plant body. It can produce cheap, fast, mass genetic copies of the selected individuals with many desirable traits (Lipensky, 2010). It takes place due to the differentiation of the meristematic cells.

It is one of the potential and useful methods of propagation for those species, which are economically important, difficult to raise through seeds and other means (Vashistha *et*

al., 2009). It has advantage of providing saplings at all seasons with the same genetic quality of the parent materials.

1.5.2. Types of Vegetative propagation:

There are mainly two types of vegetative propagation

a) **Natural vegetative propagation**: It is a natural process which involves structural modifications of the stem, any horizontal, underground part of a plant either stem or root or sometimes leaf. It is only found in the herbaceous and woody perennial plants.

b) **Artificial vegetative propagation**: This is a man made process and enhancement of natural process. It is a very commonly used practice by the special methods, i.e. root cutting, grafting, budding etc and *in vitro* artificial propagation by tissue cloning/ culture.

1.5.3. Vegetative structure:

Special vegetative structures are the main component of the vegetative propagation of a plant body. The principal vegetative structures are bulbs, adventitious buds, corms, rhizomes, tubers, suckers and runners.

Bulb: It is a vegetative structure consists of very short stem with closely packed fleshy leaves arranged in concentric circles around the stem e.g. onion, hyacinth, narcissus, tulip etc.

Adventitious bud: It is formed on the root near the ground surface or the damaged stems or on the old roots, develops stem and leaves in the above ground.

Corm: Corm is unique genotypic bulb like structure with short stem enclosed by dry, scale like leaves which swells and stores food e.g. Crocus.

Rhizome: It is a modified underground stem which grow horizontally. The underground stems are swollen with food reserve e.g. Iris, etc.

Tubers: Tubers are special thickened underground modified stem that often develop at the stolen or rhizomes and serve as storage organ e.g. potato.

Suckering: It is a form of budding or regeneration of a plant by shoots that arise from the existing root system.

Runners: This is a prostuate stem from the parent plant above the ground e.g. Strawberry, some fern etc.

1.5.4. Advantages of the vegetative propagation:

(1) The method is inexpensive, rapid and simple and does not require the special techniques necessary in grafting, budding or micropropagation;

(2) Many new plants can be started in a limited space of time from a few stock plants;

(3) Plants can be propagated by vegetative means at any time of the year;
(4) Greater uniformity and superior growth characteristics of plantlets can be obtained compared to the seedlings (Leaky and Coutts, 1998; Legess Negash, 2003);

However, now a days due to the increase in human population and destruction of the natural habitat as well as over exploitation of natural resources have led to the extinction of the valuable species, which are finally identified as endangered. The IUCN (International Union for Conservation of Nature and Natural Resources) published the Red Data Book which includes the list of endangered plants. The book symbolizes the warning signal for those, which are endangered and if not protected are likely to become extinct in the near future.

Due to over exploitation and habitat destruction many of these medicinal/ aromatic and endemic plants are becoming rare and endangered. The need for conservation of medicinal plants is being increasingly recognized at local, national and international level. As medicinal herbs not only provide raw materials for manufacturing of drugs but have also served the inhabitants for long time in traditional health care system and various other domestic consumptions (Purohit, 1997; Akerele *et al*., 1991).

1.6. Medicinal and aromatic plants of Southern Assam having export potential (A survey report):

According to the present survey and investigation Southern Assam is a highly demanded homeland for medicinal and aromatic plants. Mainly three medicinal and three aromatic plants parts are exported from Southern Assam every year. Chuksini (*Smilax glabra* Roxb.), Salmugra (*Hydnocarpus kurzii* (King.) Warb.) and Gumchilota (local name) are the three highly demanded medicinal plants of this geographic region. Chuksini and gumchilota are climbers and the Salmugra is a tree species. On the other hand Gondhikochu (*Homalomena aromatica* (Roxb.) Schott.), Agar (*Aquilaria malaccensis* Lamk.) and the flower of Nagashwar (*Meesua ferrea* L.) are the three highly demanded export item of aromatic plants. Gondhikochu is a shrub and shade loving plant and other two are tree species. The above mentioned both aromatic and medicinal plants are found in the wild habitat and they are collected illegally from the wild. The economic utility of the parts of these species differ from species to species. Rhizome of chuksini, seeds of Salmugra and whole plant parts of Gumchilota are mainly exported from Barak Valley. On the other hand rhizome of Gondhikochu, whole stem of agar and flower of Nagashwar are highly demanded plant parts from the economic utility point of view. From the present survey work it is clear that the wild stocks of these important plant species are gradually decreasing. The availability (habitat) of these medicinal and aromatic plants is mostly from Cachar district followed by Hailakandi and Karimgang districts. The different parts of these plant species are exported to Uttar Pradesh, Kanuj, Kolkata, and Delhi every year. Actually, the export of these medicinal and aromatic plants has started from Barak Valley since 1970 onwards, while 1972 has been the main peak time. However there are no official primary extraction unit and garden of medicinal and aromatic plants in the Valley. Only 3-4 private extraction units are available in the valley.

1.6.1. *Smilax glabra* Roxb.

Smilax glabra Roxb. is locally known as chuksini. It is a highly demanded medicinal plant of export from Barak Valley. This species is found in the wild habitat in the mateetilla (slopy hillocks). The associated plant species is *Lantana camera* L. Once upon

a time this plant was also available in Assam University campus also (Ecoforest), but during the present investigation only a single plant was detected. Some years back a merchant had found a *Smilax* plant in the Ecoforest which contained 200 kg of rhizome in the year 2000 (Ref. personal communication).

The main demand of the part of this plant species is rhizome. After the extraction of rhizome of chuksini from the wild habitat, it is exported in two ways, one as Chuksini and other as lokondi. The main difference of the two trades is that chuksini is exported as pink coloured rhizome without bark and dried under sun while lokondi is the black coloured dried rhizome processed by smoke heat and vaporization treatment. One of the important points is that lokondi is prepared within 2 days from the fresh rhizome, while chuksini takes long time for preparation. The exported amount is also different in these two products. Annually 50,000kg of chuksini is exported to Calcutta from Bark valley and in case of Lokondi it is 30,000kg/year exported to Delhi. The life span of these products is also different. Chukchini is only one month and the Lokondi is for 2 to 3 years.

The export of the rhizome of *Smilax glabra* Roxb. has started from the year 1970 onwards. At that time the price of the rhizome was Rs.15/kg. The market price has increased with time, i.e. Rs. 120-130/kg in 2010-2011, Rs. 185/kg in 2012-2013and now it is Rs.200-500/kg (2013-2014/2015). However, selling of Lokondi has started probably from 1990 as Rs.100-150/kg. But presently its market price has gone upto Rs.200-500/kg.

Due to the high demand, now a days the rhizome of *Smilax glabra* is imported from Tripura and Mizoram to Barak Valley and after processing these products are exported to Calcutta, Delhi etc.

1.6.2. ***Homalomena aromatica*** **(Roxb.) Schott.**

It is locally known as Gondhkochu or Gondhikochu. This is distributed in the whole of Barak Valley. The rhizome is the main important part of this species. This is an important NTFP of this region. Young leaf and buds are edible which is consumed by the tribal people of Southern Assam. The dry rhizome and extracted oil is exported from this region. Due to the presence of aromatic chemical compounds, the oil of the rhizome is

highly in demand in the International market. About 400 MT of dry rhizome is exported annually from Southern Assam (Ahmed, 2005).

Sugandhimantri and Montria oil are the two trade names of the rhizome and the oil extracted from this species. In Southern Assam export of rhizome and oil have began from 1987 onward. The market price is also increasing day by day. In 1987 it was Rs.6/kg, in 1991-1995 Rs10-20/kg, in 1997-2004 Rs. 20-50/kg, in 2006-2010 Rs. 50-70/kg, in 2011-2012 Rs.70-100/kg, in 2012-2014 Rs. 100-150/kg.

One of the important points is that the dry rhizomes are collected by the different gutkha companies from this region. Therefore the merchants of this region believe that the dry rhizome of the plant is also used as a raw material in gutkha industry.

Keeping the above in view the present research work was carried out on the vegetative propagation of the selected medicinal and the endangered plants of Southern Assam *in vivo*. Subsequently and attempt for *in vitro* propagation through micropropagation technique was also taken up for the plant species (i.e. *Smilax glabra* Roxb., *Homalomena aromatica* (Roxb.) Schott., *Bulbophyllum careyanum* (Hook.) Spreng. and *Paphiopedilum spicerianum* (Rchb.f.) Pfitz. respectively.

Therefore, in the present work, attempt was made for the vegetative/ micropropagation of four endangered and medicinal as well as aromatic plants, so that they can be sustainability utilized without any possible threat of their extinction in the near future.

The specific objectives of the research work are as follows:

1. To study the different mode of vegetative propagation of a few endangered medicinal /aromatic plants i.e. *Smilax glabra* Roxb., *Homalomena aromatica* (Roxb.) Schott., *Bulbophyllum careyanum* (Hook.) Spreng.
2. To study the effect of different seasons/times (i.e., spring, summer, autumn and winter) on the vegetative propagation of *Smilax glabra* Roxb., *Homalomena aromatica* (Roxb.) Schott. and *Bulbophyllum careyanum* (Hook.) Spreng.

3. To study the effect of varied substrate (i.e. soil/ sand/ leaf mould) on the vegetative propagation of *Smilax glabra* Roxb., *Homalomena aromatica* (Roxb.) Schott. and *Bulbophyllum careyanum* (Hook.) Spreng.
4. To study the effect of cuttings on the vegetative propagation of *Smilax glabra* Roxb., *Homalomena aromatica* (Roxb.) Schott. and *Bulbophyllum careyanum* (Hook.) Spreng.
5. To study the effect of different inorganic (i.e. NPK) and organic soil amendments (i.e. Cow dung, vermicompost) on the vegetative propagation of the test plants.
6. To study the effect of growth substances and nutritive media on the vegetative propagation of different parts (i.e. root, shoot, plantlets etc) of the plants under study.
7. To study the potential of *in vitro* propagation through tissue culture technique of three endemic/endangered plant species (i.e. *Smilax glabra* Roxb., *Bulbophyllum careyanum* (Hook.) Spreng., *Paphiopedilum spicerianum* (Rchb,f.) Pfitz.) to produce large numbers of the plantlets. So that they can be disseminated for growing/conservation in the different habitats (forest), regions/districts at large.