1. INTRODUCTION

Next to hunger, diseases are one of the vital problems faced by mankind for its survival. To cure himself of diseases, man has been trying a number of plants, growing around him and rationalization of results of these trials over hundreds of years have resulted in a large number of information which are being used in Ayurveda, Sidha, Unani and other folk medicines. Presently herbal medicines are being commercialized, increasing the demand of medicinal plants for the survival of mankind from diseases.

The plants have been used as healer and health rejuvenator since time immemorial. The concept of use of medicinal plants, that too as processed drug is not a new concept in India. The Rig Veda, considered as the oldest document mentioning herbal medicinal plants is estimated to be formed between 3500-1800 BC. Later written the Charaka-Samhita and the Susruta-Samhita mentioned about 700 drugs based mainly on plant products (Jain 1968).

The first systematic knowledge on the medicinal uses of plants in India was gathered by Von Rheede in a book “Hortus Malabarica” (Kumar 1993). In the beginning of 19th century, John Flemming (1810) wrote “A catalogue of Indian Medicinal Plants And Drugs with their names in Hindustani and in Sanskrit”. “Materia Indica” written by Ainslei (1826) gave a very satisfactory account of drugs in common use in India. Roxburgh (1820, 1824, 1832) referred many medicinal plants in his “Flora Indica” (vol. I, II, III). Henceforth, study of drug plants continued. “The Wealth of India” (CSIR 1948-52) records a number of medicinal plants.
In addition to the systematic studies involving 1500 plants mentioned in Ayurveda, ethnomedicines and other traditional medicinal systems like Siddha and Unani are gaining importance and being listed as sources of drugs. Clinical and pharmacological tests entered in study of medicinal plants towards the beginning of 19th Century. The knowledge which descended through oral folklore, complete records of ancient literature and inscriptions, little supported by the medicinal practitioners started receiving a changed attitude towards the second half of 19th century.

In India, higher plants having medicinal properties have been estimated as 3,000 to 3,500. The “Glossary of Indian Medicinal Plants” has listed 3,000 plants (Chopra et al. 1969, 1956). Two thousand five hundred plants have been reported to be used in ethno-medicine (Jain 1991). This age old process of using plants for medicinal purpose is going on as traditional systems of treatment. Infact, it has increased many fold as the demand of many herbal medicines have increased due to their reduced side effects and toxicity. Approximately 80 per cent of people of developing countries still rely on traditional medicinal systems for their primary healthcare needs (Fransworth and Soejarto 1991). This usually involves the use of plants extracts (Vieira & Skorupa 1993). The development of biomedical practices finds an important place in modern medical therapy and in present time a great number of drugs are based on herbal origin. The less toxicity and high efficacy has increased its use to many fold in the recent years. The pharmaceutical industries are processing many drugs based on plant products.

There is a growing demand for plant based medicines, pharmaceuticals, health and body care products and cosmetics etc. in National and international market. India has great potential in this industry and the country is already a major producer of
herbal medicines, phyto-pharmaceuticals, cosmetics and other products. The World trade figures suggests that India is next to China by exporting 32,000 tonnes of medicinal plants raw material worth US $ 46 million annually (Lange 1997, Dhar et al. 2000, Manjkhola and Dhar 2002). During the last decade Germany alone imported 40,000 tonnes of plant dry material from India (Biswas et al. 2003). Biswas et al. (2000) mentioned that out of about 80,000 tonnes of medicinal plants imported by Western countries, India tops the list of exporters for USA and Europe with the share of over 10,000 tonnes. The rich biodiversity of this country can provide plant based compounds of various therapeutic value. The country exports about a total of 42,000 tonnes of medicinal plant raw material during the year 2000-2001 (Sarin 2003a). The export of material employed in Indian System of Medicine was 9,740 tonnes during 2000-2001 (Anonymous 2001). Indian drug and pharmaceutical and cosmetic industry based on plant products are very fast growing. The annual value of products of this sector is estimated between Rs. 4,000 to 4,500 crores (Handa 1996). For Indian industries ‘Chemical and Pharmaceutical Export Promotion Council’ (CHEMEXIL) has estimated the annual demand of raw materials from 55 species at around 32,000 MT (Prakash 2001).

The ever expanding herb based pharmaceutical industry has put a great pressure on the raw materials, majority of which are obtained from plants growing in the forests or other natural habitat. While the developed technologies remain unused and cultivation of the plants given up, the Indian drug and pharmaceutical industry continues to get almost 90 per cent of its supplies from the collections made from wild (Sarin 2003b) and over 70 per cent of the plant collection involves destructive harvesting mainly because of the use of plant parts like roots, bark, wood and whole
plants (Tiwari 1999). The high demands of pharmaceutical industries are being met from the nature and the plants are being over exploited as very little importance is being given to their conservation. This has led to a gross depletion of the number of medicinal plants.

In addition to indiscriminate collection, rapid agricultural and urban development, deforestation along with lack of scientific approach towards conservation has contributed to the disappearance of medicinal plants at an alarming rate. Hence, many of the species that are in danger list or near-extinct in wild demand immediate strategy for conservation and large scale production, so that they can be saved from extinction, their germ-plasm can be conserved and at the same time high demand from pharmaceutical industry can be met which is essential for the health of human population.

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 Forest is funding an all India coordinated project on conservation of endangered plant species (Raghupathy 2001).

Of the different conservation techniques presently being employed tissue culture has proved to be a very useful tool. Tissue culture provides a quick method of large scale multiplication, a method for germ-plasm conservation, clonal propagation, production of autoploids and production of virus free plant material (Dodds and Roberts 1985). This technology has been successfully used for commercial production of pathogen free plants (Debergh and Maene 1981) and to conserve the
germ plasm of rare and endangered species (Fay 1992). For conservation of valuable genotype of medicinal plants, micropropagation is of specific use (Sen and Sarma 1990).

Micropropagation represents the optimum efficiency in terms of vegetative plant propagation and allows large-scale production in a relatively shorter period of time under controlled conditions throughout the year in a relatively small space.

Hence, tissue culture of medicinal plants can give following outcome of significance –

1. Quick large-scale production of endangered plants for conservation.
2. Rapid multiplication, speedy release of varieties, improved phenotypes, maintaining clonal and phenotypic uniformity among offsprings, yield in large number that too in small space, round the year production for pharmaceutical industries.
3. Disease free plant material for proper evaluation of drug yielding capacity.
4. Screening of improved genotype having high yielding capacity and more adoptability, for saving them from extinction.

These significant possible outcomes make it necessary for development of protocol of micropropagation for different medicinal plants. This is most important in North East India. North East India is one of the hot spots of biodiversity of the World and holds a great number of medicinal plants. But due to unplanned developmental activities the habitats of a large number of medicinal plants are fast depleting. In addition to this there is another danger to the survival of these valuable genetic resource of medicinal plants and that is of bio-pyrating.

To protect these plants from unreliable hands, to protect the germ plasm of this area for the future generation, to preserve rich natural floral resource of bio-
diversity a strategic conservation technique is necessary. Developing protocol of in vitro propagation of medicinal plants of this area will not only provide a conservation technique but also give plants that can fulfill the need of pharmaceutical industries. Considering the possible significant outcome following three plants were selected to determine the micropropagational protocol. The plants are of common use in North East India specially in Assam in traditional healing systems.

The plants are:

1. *Bacopa monnieri* (L) Penn.  
3. *Costus speciosus* (Koen. ex Retz.) Sm.

1. *Bacopa monnieri* (L) Pennall. Commonly known as Brahmi in Sanskrit and in most of the other common languages of India, Zarazab in Arabian, Lunuwalia in Sinhalese, Otomeazent in Japanese, Pa-chi-Thien in China etc. This small creeper of Scrophulariaceae family has lathery opposite leaves of 1-1.5 cm length and light green in colour. Flower is white and the plant extracts has a bitter taste.

Brahmi is often called memory vitalizer. Brahmi with a number of traditional medicinal uses, has been a revered household name in India over 3,000 years. In Charak Samhita it is mentioned that if juice of Brahmi is taken with one fourth powder of *Glycyrrhiza* with milk, it cures all kinds of diseases and bestows longevity (Verma 1955). Traditionally it is being used in bronchitis, chronic coughs, asthma, hoarseness, arthritis, rheumatism, back-ache, inflammatory condition and fluid retention. It is also used as blood cleanser, in chronic skin clearness, constipation, hair loss, fever, digestive problems, epileptic fits, depression
including post natal depression, and for improving circulation and strengthening capillaries, impotency etc. It is an well known memory vitalizer (Anonymous 1950). The entire plant is used as a nerve tonic and as a cure of epilepsy and insanity (Anonymous 1948). Koman (1919,17,48) found Brahmi herexta a medicated ghee prepared from the plant, beneficial in case of epilepsy and insanity.

In 1945 Central Drug Research Institute (CDRI) began extensive study of this treasure to show active ingredients for modern therapeutic use. In 1963 two active molecules Bacoside A and Bacoside B were identified. The alkaloid contents are brahmine, herpestaine ($C_{34}H_{46}N_{2}O_{6}$) and mixture of 3 bases.

2. **Clerodendrum colebrookianum** (L.) Walp. syn. *C. glandulosum* colebrook ex. Wall., the second species selected, is a small shrub with tender whole leaves with glands at the base of petiole on the lower side. Flowers white with pink colouration. Fruits red when ripe. The leaves have a pungent smell. Planning Commission and National Medicinal Plant Board listed 19 prioritized medicinal plants from Arunachal Pradesh and *Clerodendrum colebrookianum* is one of them (Haridasan et al. 2003).

Chemically the plant contains triaconatane, clerodin (2,4,5)-ethyl-cholesta 5, 22,25 tri-Ca-3β-ol; α-amyrin,β-sistosterol and clerodolone with two new compounds 2-methyleicosa2, 1-diene and 10,11,32-trimethyl tetetrecontanol (Singh et al. 1995). Another compound glycoside clerosterol 3β-O-[β-D-glucoside] from the plant (Goswami et al. 1996). Recently a new diglycoside of fatty acid ester of glycerin, Clerobroside (A) and nine other known compounds have been reported (Yang et al. 2000a) and five steroids, colebrin A, B, C, D,E were isolated from aerial parts (Yang et al. 2000 b).
The tender leaves of this plant are used to reduce high blood pressure and colic pain by different communities of North East India (Borthakur 1976, Nath 1988, Nath and Bardoloi 1988, 1991, Majumdar 1980, Baruah et al. 1992, Gupta et al. 1994). In Assam it is also used in insomnia (Borthakur 1976). In Meghalaya some tribes use it to kill intestinal worms (Borthakur 1976). People also use decoction of its roots, leaves and bark for curing malaria fever (Rao and Jamir 1982 a, b).

Due to high exploitation from natural population and destruction of habitat caused by rapid urbanization this species once abundant, is now considered to be a threatened one. Hence micropropagation protocol for this species is necessary.

3. The third species taken for study is Costus speciosus (Koen. ex Retz.) Sm. This is an erect herb of Zingiberaceae family. It develops rhizomatous root stock. Leaves 15-30cm, almost without petiole, silky pubescent beneath. Flowers white, tubular with red bracts, in dense long spike. Fruit red, seed black.

Drug of Costus is derived from rhizome. It is used as tonic, anthelmintic and aphrodisiac. Traditionally it is used in fever, cough, dyspepsia, worms, skin diseases and snake-bites. Steroids from plant has the prospective in antifertility, anti-inflammatory and anti-arthritis drugs. There are reports of possible use of Costus speciosus as a source of orahypoglycemic agent (Musihuzzaman et al. 1994).

Costus is rich source of steroids. CDRI has published it as a source of diosgenin (Asolkar and Chandha 1979). Six compounds diosgenin, prosapogenin B of dioscin, diosgenone, cycloartanol, 25-en-cycloartenol and actacosanoic acid were recently derived from Costus rhizome and later four for the first time (Qiao et al. 1988).
2002). Diosgenin is an important precursor for corticosteroids and anti-fertility drugs. Hence, Costus a source of diosgenin is gaining importance.

With the premise as stated the present investigation was designed for _in vitro_ micropropagation of three medicinal plants of high potential value from North Eastern part of India. For the purpose, following line of works were designed:

1. Collection of plant materials and maintenance of the germ plasm in the Botanical Garden of the Department of Botany, Gauhati University.
2. Standardization of basic culture steps for regeneration of multiple shoots.
3. Initiation of callus and organogenesis by modifying the media from time to time.
4. Acclimatization of plantlets under controlled condition.
5. Transplantation of hardened plantlets in natural habitat.

And with this plane of work it was tried to reach the objective of finding a standard protocol for _in vitro_ regeneration of each of the three medicinal plant species.