1. INTRODUCTION

Since his existence on his planet, man has to depend on nature for sustenance and survival; the accumulated knowledge had guided him to discover remedies for common ailments from natural sources even in the remote past. The Indian system of medicine itself is of great antiquity and is believed to be one of the most ancient. Plants are used as medicine since time immemorial. Medicinal herbs constitute an important source for traditional systems of medicine in India.

The traditional system of alternative medicines in India prescribing plant extractives in therapy dates back to the early age of *Rig-veda* (Asima Chatterjee, 1997). Considerable knowledge accumulated the villagers and tribals on herbal medicine remains unknown to the scientists and urban people. Many plant species associated with the rural people are on the verge of disappearing and are on vulnerable list. The impact of deforestation, urbanization and modernization is shifting the rural people from their natural habitats and their well knowledge particularly with respect to herbal drugs is slowly disappearing (Vedavathy, 2003).

Many drugs commonly used today are of herbal origin. Indeed, about 25 per cent of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mime a natural plant compound.
The herbal drug industry is considered to be a high growth industry of the late 90s and seeing the growing demand, it is all set to flourish in the present century. The trend for increasing popularity of medicinal plants in countries like Australia, Germany, China and America are well supported by statistical data.

Ayurveda, the ancient Indian system of medicine, strongly believes in polyherbal formulations and scientists of modern era often ask for scientific validation of herbal remedies. The medicinal herb is a biosynthetic laboratory as it contains number of chemical compounds like alkaloids, glycosides, resins, flavonoids, tannins, saponins etc., These compounds exert therapeutic effect and account for medicinal property of the medicinal herb.

The World Health Organization (WHO) estimates that 4 billion people, 80 per cent of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous traditional medicine and a common element in Ayurvedic, Homeopathic, Naturopathic, traditional oriented, medicine. WHO notes that of 119 plants derived pharmaceutical medicines; almost 74 per cent are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value.

Now-a-days higher plants are playing a prominent role in the treatment of innumerable diseases including cancer, lymphosarcoms,
AIDS, senile dementia and auto-immune diseases. Classically higher plants are occupying a key position in the production of new therapeutic agents. Thus, the plant drugs are able to occupy an important niche in modern medicine.

Even now 75-80% of the world population depends on crude plant drugs to tackle health problems. German and Russian pharmacopeias have shown predominantly the use of plant derived drugs in the treatment of several diseases. Nature has no parallel machinery in constructing at faster rate unimaginable simple or complex molecules used as therapeutic agents. On a global scale the number of plant based drugs which are made by eco-friendly processes are ever increasing for the simple reason that the lesser side effects, efficacy and cultural acceptability.

Recently, nutraceuticals are at great demand in the developed world particularly in the USA and Japan. ‘Nutraceutical’ is a term of recent origin and comprises nutritionally or medicinally enhanced foods with health benefits (Brower, 1998). Many pharmaceutical and biotech companies have extended the term nutraceutical to include pure compounds of natural origin like ‘bacosides’ form Bacopa monnieri, ‘curcumin’ from ‘curuma species, and ‘lovastatin’ from red rice yeast.

Nutraceutical market in the USA alone is about $ 80-250 billion. Such huge markets have arisen because of the Dietary Supplement Health Education Act passed by USA in 1994, which permits
unprecedented claims to be made about food or the dietary supplement’s ability about health benefits including prevention and treatment of diseases (Kamboj, 2000). Thus, medicinal plants are playing a great role in the classical, modern medicines and in nutraceuticals which increased herbal medicine market. As per available records, in 1996 the US herbal medicine market was about $ 4 billion and with current growth rate may be more than double by the turn of this year.

India is one of the 12 mega biodiversity centres having over 45,000 plant species. Twenty plant species including Glycyrrhiza glabra, Comiphora mukul, Plantago ovata, Aloe barbadensis, Azadirachta indica, Centella asiatica, Andrographis paniculata, Phyllanthus species, Withania somnifera, Rauwolfia serpentine, Terminalia chabula, Plumbago zeylanica and Bacopa morrieri were commonly exported as crude drugs and used in modern medicine. Efforts have not been made to preserve their germ-plasm from different localities.

In the folklore of Indian medicine, certain herbs have been used traditionally as brain or nerve tonics. One of the most popular of these used in neurotonics is B. monnieri (L.) Pennell a small, common, amphibious plant growing in marshy areas throughout the Indian subcontinent. Bacopa is also called Brahmi, or Nirbrahmi a name derived from “Brahma”, the creator God of the Hindu pantheon of deities. It is legendary for its diversity of usage. In the Ayurvedic Materia Medica, Bacopa has been recognized for its brain enhancement characteristics.
1.1 Benefits of using Brahmi

- In India this plant is used for all sorts of skin problems—eczema, psoriasis, abscess, ulceration— it is said to stimulate the growth of skin, hair and nails.
- Brahmi extract has been found to be very beneficial in the treatment of anxiety neurosis and mental fatigue. It has been found to significant improve IQ levels, general ability, behavioral patterns and mental concentration in children and also brahmi has antioxidant, cardiotonic and anticancer properties.
- Brahmi is effective against disease like bronchitis, asthma, hoarseness, arthritis, rheumatism, backache, constipation, hair loss, fevers, digestive problems, improving mental clarity, confidence and memory recall. For these uses of Brahmi, it has been widely used by students. etc.,

*B. monnieri* is extensively used in native medicine, modern medicine and nutraceuticals. Much attention has been recently been focused on *B. monnieri* popularly known as ‘brahmi’ due to its wide applciatios in clinical, phytochemical and pharmaceutical studies. In a recent study conducted on Indian medicinal plants. *B. monnieri* was placed second in a priority list of the most important Indian medicinal plants evaluated on the basis of their medicinal importance, commercial value and potential for further research and development (Anonymous, 2, 1997). Due to the exploitation of these resources, in the above fields coupled with destruction of natural habitats and seasonal fluctuations, poor seed setting and viability there has been a concomitant increase in
the demand for raw material. Hence, there is an urgent need to develop approaches for ensuring the availability of raw material of a consistent quality from regular and viable sources.

Recent research has focused primarily on Bacopa’s cognitive-enhancing effects specifically memory, learning and concentration, and results support the traditional Ayurvedic claims. Research on asthma, irritable bowel syndrome, and gastric ulcers also supports the Ayurvedic uses of Bacopa. Bacopa’s antioxidant properties may offer protection from free radical damage in cardiovascular disease, certain types of cancer and also reducing oxidation of fats in the bloodstream. Laboratory studies on rats indicate that extracts of the plant improve memory capacity and motor learning ability. Studies in humans show that an extract of the plant has antianxiety effects; it is listed as a nootropic, i.e., a drug that enhances cognitive ability.

In India, this plant has been used traditionally to concentrate newborn babies in the belief that it will open the gateway of intelligence. Recent studies suggest Bacopa may improve intellectual activity.

In recent years, biotechnology has emerged as a frontier branch of science increasingly being used in several areas. Biotechnological approaches are being employed to the production of secondary metabolites for pharmaceutical use. Plant cells are totipotent and possess the capability for regenerating into complete plants.
The uses of this technique for large scale production of secondary metabolites are advantageous, as there are problems in the extraction of metabolites from field grown plants. In many cases, the production of secondary metabolites from cell cultures is higher in comparison to the small amount extracted from in vitro grown plants. Several plant species have been exploited for high quality of secondary plant products for medicinal use.

Plants are the most important source of medicines and play a role in world health (Constable, 1990; Kala, 2005). Collection of medicinal plants on a mass scale from the natural habitats leads to depletion of plant resources. Today’s medicinal plants are important to the global economy, an approximately 80% of traditional medicine preparations involve the use of plants or plant extracts (Viera and Skorupa, 1993; Dhyani and Kala, 2005). The increasing demand for herbal medicines in recent years due to their fewer side effects in comparison to synthetic drugs and antibiotics has highlighted the need for conservation and propagation of medicinal plants. Micropropagation is of special use for the conservation of these valuable genotypes (Abhyankar and Chinchani, 1996), with shoot culture, which is often utilized to maintain clonal fidelity would be of special advantage.

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 provides 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 plants population which can fruitfully be employed in plantation crops, aromatic and medicinal plants, spices and condiments most of which are propagated vegetatively.

After considering the advantages of tissue culture of large scale propagation, conservation, genetic manipulation of commercialization of important medicinal plants, tissue culture studies of *B. monnieri* was carried out in the present study to meet the ever growing requirements.

According to NMPB, annual demand for *B. monnieri*, during the survey conducted in year 2004-205 was 6621.8 tons, with an annual growth rate of 7% annually (Banerjee and Shrivastava, 2008). This requirement is rising rapidly in view of the popularity of the Bacopa-based drugs. In view of the wider market demand, there is a need to conserve the wild stocks of *B. monnieri* and to mass propagate the selected clones. Furthermore, their natural regeneration is hampered by death at two-leaf stage and specific habitat requirement. The sub-merged shoots of *B. monnieri* can hardly ramify to attain the required growth and multiplication. Therefore, it is necessary to develop and standardize the large-scale multiplication through tissue culture and application of bioinoculants to successful acclimatization of this plant in the soil and also the performance of regenerated plants was evaluated in the field.
The species propagates mainly by vegetative means. Though it was found abundantly in wet lands, the drug content of this plant is very low i.e., 0.2% (Tejavathi et al., 1999). Therefore, large amount of plant material is required for drug extraction. Commercial exploitation and elimination of natural habitats consequent to urbanization has led to gradual extinction of this medicinal plant. Micropropagation is one of the effective approaches to conserve such germplasm.

Thus in the present study, an attempt is made to induce callus and multiple shoots from leaf, shoot tip and nodal explants of *B. monnieri*, thereby aimed to report a simple, reproducible and rapid method to the *in vitro* multiplication of this valuable medicinal plant and also to assess the standardization of phytochemicals from callus and *in vitro* plantlets by using GC-MS, HPLC analysis and also to determine the antibacterial activities of *B. monnieri*.

Finally, there are significant challenges associated with ensuring the safety, quality and efficacy of plant based medicinal content, contamination with abiotic and biotic factors, adulteration with misidentified plant species, efficiency of manufacturing processes and product handling. The major constraint in the development of medicinal plants is being experienced by the farmers are non availability of quality planting materials of improved varieties, lack of development and extension support in the cultivation and processing, and unorganized marketing.
Hence, the present study was undertaken with the following objectives:

- To determine the morphometric characteristics and standardize the micropropagation protocol and mass multiplication of \textit{B. monnieri}.
  - Standardization of \textit{in vitro} culture protocol for induction of shoot from shoot tip and nodal explants.
  - Induction of shoot organogenesis from leaf and nodal explants.
  - Selection of suitable explants for the induction of callus from \textit{B. monnieri}.
  - Induction of shoots from \textit{in vitro} cultured callus.
  - Induction of roots from the regenerated shoots.
  - Standardization of hardening in regenerated plantlets of \textit{B. monnieri} using bioinoculants.

- To determine the phytochemical constituents \textit{viz}: qualitative analysis, GC-MS analysis and HPLC analysis from callus and \textit{in vitro} plantlets of \textit{B. monnieri}.

- To determine the antibacterial activities in \textit{in vitro} plantlets of \textit{B. monnieri} against four human pathogenic bacteria.