INTRODUCTION

Medicinal plants have been the subjects of man’s curiosity since time immemorial (Constable, 1990). India has a centuries old heritage of medicinal plants for alleviating human illness and promotion of health. Medicinal plants are often the only easily accessible health care alternatives for most of the human population and traditional medicine remains an integral part of the health system in India.

Millions of people in the third world countries prefer herbal medicines because they believe in them and regard as “their” medicines in contrast to western allopathic drugs. About 85% of traditional medicines involve the use of plant extracts. Many plant species, possessing medicinally important compounds are disappearing in alarming rate due to destructions of their natural habitats due to rapid agricultural development, indiscriminate deforestation, industrialization, urbanization, and uncontrolled collection of plant materials.

Medicinal plants constitute a very important bioresource of India, because it has one of the richest plant based ethnomedical practices in the world. The global market for medicinal plants and herbal medicines is estimated to be worth US$ 80 billion per year. International export trade in medicinal plants from India is 32600 tons per year (Rawat and Garg, 2005). The demand for medicinal plants has increased globally due to resurgence of interest in standardized plant extracts, culinary herbs, natural therapeutic essential oils and phytopharmaceuticals. As a consequence, the rate of exploitation may exceed those of local natural regeneration. High value medicinal plant species are threatened to critically endangered. Due to their various medicinal properties, they are being over exploited from their habitat. *In vitro* propagation of plants hold tremendous potential for the production of high quality plant based medicines. There is thus an urgent need to develop and implement regeneration conservation strategies for over exploited medicinal plant species.
Many secondary metabolites of plants are commercially important and used in a number of pharmaceutical preparations. Plants, especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity or reduced toxicity. The small fractions of flowering plants that have so far been investigated have yielded therapeutic agents of known structure from different species of plants. In some cases, the crude extracts of medicinal plants may be used as medicaments. In some other cases, the isolation, identification of the active principles from drugs and elucidation of its mechanism of action is paramount important.

Where the active molecule cannot be synthesised economically, the product must be obtained from the cultivation of plant material. The major plant drugs have been identified for which no synthetic one is currently available. The scientific study of traditional medicines, derivation of drugs through bioprospecting and systematic conservation of the concerned medicinal plants are thus of great importance.

*In vitro* cell & 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. Combination of *in vitro* propagation technique (Fay, 1992) and cryopreservation may help in the conservation of biodiversity in general and locally used medicinal plants in particular.

*In vitro* propagation holds tremendous potential for the production of high-quality plant based medicine. *In vitro* production of secondary metabolites in plant cell suspension cultures and callus has been reported from various medicinal plants. Plant micropropagation is the technique of growing plant cells, tissues and organs in an artificially prepared media (static or liquid) under aseptic conditions. It is a vegetative method for multiplication of plants. It has advanced the knowledge of fundamental botany, agriculture, phytopathology, industrial production of plant metabolites and transgenic plants.
Introduction

*In vitro* propagation is a best technique to increase the population of medicinally important plants which do not give seeds or whose seeds have a low germination capacity. Through micropropagation, such plants will be made available throughout the year. Tissue culture helps to produce the uniform clones from highly heterozygous plants and conservation of genetic resources of species and threatened plants.

Micropropagation has many advantages over conventional methods of vegetative propagation, which suffer from several limitations. With micropropagation, the multiplication rate is greatly increased. The micropropagation has the advantage of facilitating the production of uniform and healthy plants, as well as to reduce propagation time. Further, micropropagation techniques do not depend on the climatic conditions and are especially useful in species that have recalcitrant seeds which rapidly loose their viability. It is the case of several tropical perennial species. It is important for long term conservation efforts to preserve the biodiversity of endangered species and ecosystems (Siddique *et al.* 2007). It also permits the production of pathogen free material. Micropropagation of various plant species including many medicinal plants has been using successfully by many workers.

Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries depend, in part, or plants for the production of pharmaceuticals compounds.

In the present study, *Ionidium suffruticosum*, Ging. considered to have highest medicinal values and widely used by traditional healers to treat several diseases like diabetes (Das *et al.*, 2004), malaria (antiplasmodial activities) (Winiger *et al.*, 2004), male sterility in Ivory Coast (Kheraro and Bouquet, 1950), urinary tract infections & water retention and is used as tonic (Pus pangadan *et al.*, 1984). The tender leaf stalks are used as demulcent; the roots are antigenorhhoeic, diuretic, bowel complaints and urinary problems (Deshpande, 2006). Despite its multipotentiality as a medicine there are some limitations in the propagation of this species. Propagation of the species by
seeds or any other conventional methods is not reliable because the plants are perennial but available during rainy season only due to vegetative part of the plant body is removed or grazed by animals. In nature, the seeds are viable for short period and loose their viability within few weeks. To understand the embryological aspects of the plant and phytochemical constituents responsible for various growth events takes place during male and female gametogenesis the histochemical studies were also undertaken. The species is under threat due to their exploitation from their natural habitat by traditional healers, over grazing by animals, seasonal habitat and their short seed dormancy. At the same time, very few reports are available on its micropropagation (Prakash et al., 1999) and no data are available concerning its biological activities (Saxena, 1975; Majumdar et al., 1979). Therefore, methods for rapid in vitro micropropagation and genetic improvement are urgently warranted for this important plant species. Tissue culture method is important tool for large scale multiplication of a number of medicinal herbs and sub shrubs in general. (Bidwell et al., 2001).

The poor propagation coupled with over exploitation of the plant for pharmaceutical use has depleted the species from the natural habitat, thereby widening the gap between demand and supply and thus putting further pressure on the availability of this species. The gradual decline in the population of this species demands concrete conservation efforts so as to ensure continuous and ample supply by establishing a balanced cycle of harvest and renewal. Such conservation efforts would ensure continuous supply of this valuable material which is in great demand by the pharmaceutical industry. Only a small percentage of such medicinal plants used in the industry are cultivated. Most of them are collected from their natural habitat, very often in a destructive and unsustainable manner which led to loss of such precious germplasm from their natural habitat.

Keeping those above facts in mind, the study was undertaken to develop a suitable in vitro regeneration protocol for its rapid multiplication and conservation of genetic diversity. The in vitro propagation is a promising
method to multiply and conserve the critical genotypes of plants because it is independent of geographical and seasonal variations. Various environmental factors offer a defined production system, which ensures the continuous supply of plantlets, uniform in quality and yield.

The beneficial medicinal effects of plant materials typically results from the combinations of secondary products present in the plant. These compounds are mostly secondary metabolites such as steroids, alkaloids, tannins, and phenolic compounds, which are synthesized and deposited in specific part or in all parts of the plant.

Sterols are amphiphilic molecules consisting of hydroxyl groups forming the hydrophilic heads and sterane skeletons with side chains forming the hydrophobic tails (Heldt, 2005). Cholesterol (C\textsubscript{27}H\textsubscript{45}OH) is the main sterol found in mammals where it plays an important role in the structure and function of cell membranes, production of bile, as precursor of hormones and a role in the immune system. Sterols found in plants are known as phytosterols and over 250 phytosterols and their related compounds have been identified (De Brabander et al., 2007) in foods like plant oils, nuts, seeds, cereals, fruits and vegetables (Piironen et al., 2000; Ostlund, 2002). Phytosterols differ from cholesterol in being alkylated at C-24 with C-1 or C-2 substituents (Buchanan et al., 2000). In nature, plants contain sterols with their associated sterolins (glucosides), which are easily destroyed by glycosidic enzymes (Pegel, 1976). Phytosterols cannot be synthesized by humans/animals and are thus consumed from the diet. The most commonly found phytosterols are sitosterol (C\textsubscript{29}), campesterol (C\textsubscript{28}) and stigmasterol (C\textsubscript{29}) (Pegel, 1980; Ostlund, 2002). Phytosterols are incorporated in a variety of food products (functional foods) (Vorster et al., 2003) due to their cholesterol-lowering effect, hence providing protection against cardiovascular disease (Tapiero et al., 2003). Studies with phytosterols, especially β-sitosterol, have shown inhibition of several cancer cell lines including colon (Raicht et al., 1980; Chi et al., 2003 and Awad et al., 1998), prostate (Von Holtz et al., 1998) and breast (Steenkamp and Gouws, 2006; Ju et al., 2004; Awad et al., 2003 and Awad et al., 2001). The role of
phytosterols as immune modulators (Bouic and Lamprecht, 1999; Bouic 2002 and Breytenbach et al., 2001) and anti-inflammatory agents (Quilez et al., 2003; Pegel, 1979) have been also described.

β-sitosterol is the abundant phytosterol found in higher plants specially reference to the experimental plant, along with sitosterol, campesterol and stigmasterol. As the plant claims to produce this valuable drug as secondary metabolite, we had planned to get it from in vitro grown callus and plants, so that, drug can be continuously produced in a commercial scale without disturbing the natural resource. Hence we undertook the HPLC analysis of wild plant and in vitro grown leaf callus of the plant to determine and quantify the β-sitosterol content.

Most of the demand is being met through the collection of large quantities of these plant species and their parts from the wild populations. The method of extraction employed almost crude and unscientific. As a consequence, the rate of exploitation may exceed those of local natural regeneration. Due to its various medicinal properties, it is being over exploited from the world’s habitat. In vitro propagation of plants hold tremendous potential for the production of high quality plant based medicines. The present study reports a high frequency of indirect in vitro regeneration of plantlets and enhanced production of steroid namely β-sitosterol through the callus of leaf explants.

Hence, the present study has been undertaken with the following objectives:

1. Standardization of nutrient media by balancing the growth regulators for direct or indirect organogenesis from the explants of the plant.
3. Standardization of method for continuous culture of callus from any explant of the plant as a source of secondary metabolites.
4. Pharmacognostic analysis of wild plant material to develop data base for identification of crude drug.
5. Preliminary Phytochemical screening of crude extract of wild plants for secondary metabolites.
6. Preliminary Phytochemical evaluation of leaf calli (grown on MS media with different growth regulators) for secondary metabolites.
7. Quantifications of β-sitosterol in wild source and in vitro grown leaf callus.
8. To develop an efficient in vitro regeneration protocol of Ionidium suffrutosum Ging. for commercial propagation and germplasm conservation.