INTRODUCTION

India is a treasure house of biodiversity in its various ecological conditions, rich ethnic diversity and vast traditional knowledge (Pratibha Gupta, Sujata Ganguly and Pratibha Singh, 2012). Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern high throughput drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs (Buenz et al., 2004). Traditional medicinal plants are often cheaper, locally available and easily consumable, raw or as simple medicinal preparations. Nowadays, traditional medicinal practices form an integral part of complementary or alternative medicine. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents (Park and Pezzutto, 2002). Ayurveda is an ancient system of medicine practiced in India since the Vedic period, about 3,500 years ago (Schuppan, Brinkhaus and Hahn, 1999). The Ayurveda system relies strongly on preventive medicine and promotion of positive health (Govindarajan et al., 2005; Sharma, Hanna, Kauffman and Thyagarajan et al., 2002).

The ‘World Health Organization’ estimates in less developed countries that 75-80% of the people rely on plant based medicines for primary health care (WHO 2008). Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc. Recent estimates suggest that several thousands of plants have been known with medicinal applications in various cultures. A large proportion of such medicinal compounds have been discovered with the aid of ethno-botanical knowledge of their traditional uses. Diabetes is a silent killer. If uncontrolled, it can lead to deadly complications. Many drugs commonly used today are herbal origin. Some are made from plant extracts others are synthesized to mimic a natural plant compound. Medicinal plants have been recognized as repository of fungal endophytes with novel metabolites of pharmaceutical importance (Stone et al., 2004). Therefore it is important to explore endophytic mycoflora of the medicinal plants.
Endophytes commonly refer to a group of fungi that reside asymptomatically inside the living plant tissues (Hyde and Soytong, 2008). Elicitation of plant cells is one of the useful biotechnological tools to improve the production of valuable secondary metabolites. Plants respond to endophytic elicitors by activating an array of defense mechanisms including induction of biosynthesis of secondary metabolites. Elicitation by VAM and endophytic fungi is a procedure that has displayed the ability to improve secondary metabolites. The present study is an attempt towards enhancement of gymnemic acids in the Gymnema sylvestre using VAM and endophytic fungi as plant growth enhancers.

**Gymnema sylvestre (G. sylvestre) R. Br.**

*Gymnema sylvestre* R. br. is a wild plant commonly known as gurmar classified in the Asclepiadaceae family and is widely distributed in Southern India, tropical Africa and Australia. *G. sylvestre* is widely used as a health food in Western India (Ye et al., 2000). The leaves of this plant are used for inhibiting the taste of sweetness and is used in the control of diabetes (Agarwal et al., 2000), as a stomachic, diuretic and cough suppressant activity. The leaf extract is also used for the treatment of various physiological effects such as rheumatism, ulcer, jaundice, dyspepsia, constipation, eyes pain, dental caries. It also possesses antimicrobial (Satdive et al., 2003), antiviral (Sinsheimer et al., 1968), antilarvicidal (Khanna et al., 2011), anti hyperlipidemic (Rachh et al., 2010), antihypercholesterolemic, hepatoprotective, anti-allergic (Sawabe et al., 1992), anti-inflammatory (Malik et al., 2008) free radical scavenging activities. (Ohmori et al., 2005). The taxonomic classification of *G. sylvestre* is

<table>
<thead>
<tr>
<th>Division</th>
<th>Angiosperms,</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series</td>
<td>Astrids,</td>
</tr>
<tr>
<td>Order</td>
<td>Gentianales,</td>
</tr>
<tr>
<td>Family</td>
<td>Asclepiadaceae,</td>
</tr>
<tr>
<td>Genus</td>
<td>Gymnema,</td>
</tr>
<tr>
<td>Species</td>
<td>Sylvestre</td>
</tr>
</tbody>
</table>
**Fig1. G. sylvestre plant**

*G. sylvestre* (Asclepiadaceae), a vulnerable species is a slow growing, perennial, medicinal woody climber found in central and peninsular India. Leaves are opposite, usually elliptic or ovate (1.25–2.0 inch × 0.5–1.25 inch). It is a potent antidiabetic plant and used in folk, ayurvedic and homeopathic systems of medicine. *G. sylvestre* leaves contain triterpene saponins belonging to oleanane and dammarene classes. Oleanane saponins are gymnemic acids and gymnema saponins, while dammarene saponins are gymnemasides. Besides this, other plant constituents are flavones, anthraquinones, hentri-acontane, pentatriacontane, α and β-chlorophylls, phytin, resins, dquercitol, tartaric acid, formic acid, butyric acid, lupeol, β-amyrin related glycosides and stigma sterol. The plant extract also tests positive for alkaloids. Leaves of this species yield acidic glucosides and anthroquinones and their derivatives (Dateo and Long 1973). The antidiabetic array of molecules has been identified as a group of closely related gymnemic acids after isolation and purified from the leaves of *G. sylvestre* (Liu, Kiuchi, Tsud, 1992; Sinsheimer and Manni, 1965). Later, the phyto constituents, chemistry and structures were studied (Sinsheimer and Subbarao 1971; Yoshikawa, Nakagawa, Yamamoto, Arihara and Matsuura, 1992).
Structure of Gymnemic Acid

Gymnemic acid formulations have also been found useful against obesity, according to recent reports (Yoshikawa, Kondo,Arihara and Matsuura, 1993). This is attributed to the ability of gymnemic acids to delay the glucose absorption in the blood. The atomic arrangement of gymnemic acid molecules is similar to that of glucose molecules. These molecules fill the receptor locations on the taste buds thereby preventing its activation by sugar molecules present in the food, there by curbing the sugar craving. Similarly, Gymnemic acid molecules fill the receptor location in the absorptive external layers of the intestine there by preventing the sugar molecules absorption by the intestine, which results in low blood sugar level (Sahu, Mahato Sarkar and poddar, 1996).

*G. sylvestre* leaves have been found to cause hypoglycemia in laboratory animals and have found a use in herbal medicine to help treat adult onset diabetes mellitus (NIDDM). When Gymnema leaf extract is administered to a diabetic patient, there is stimulation of the pancreas by virtue of which there is an increase in insulin release (Kanetkar,Laddha and Kamat, 2004). These compounds have also been found to increase fecal excretion of cholesterol but further studies to prove clinical significance in treating hypercholesterolemia (high serum cholesterol) are required. Other uses for Gymnema leaf extract are its ability to act as a laxative, diuretic, and cough suppressant. These other actions would be considered adverse reactions when Gymnema is used for its glucose lowering effect in diabetes. Gymnema leaf extract, notably the peptide ‘Gurmarin’, has been found to interfere with the ability of the taste buds on the tongue to taste sweet and bitter, gymnemic acid also has the
similar effect. It is believed that by inhibiting the sweet taste sensation, people taking it will limit their intake of sweet foods and this activity may be partially responsible for its hypoglycemic effect (Nakamura, Tsumura, Tonogai and Shibata, 1999). There are some possible mechanisms by which the leaves and especially Gymnemic acids from _G. sylvestre_ exert its hypoglycemic effects are: 1) it increases secretion of insulin, 2) it promotes regeneration of islet cells, 3) it increases utilization of glucose: it is shown to increase the activities of enzymes responsible for utilization of glucose by insulin dependent pathways, an increase in phosphorylase activity, decrease in gluconeogenic enzymes and sorbitol dehydrogenase, and 4) it causes inhibition of glucose absorption from intestine. The gymnemic acid components are believed to block the absorption of glucose in the small intestine, the exact action being unknown. It could be involve one or more mechanisms (Nakamura, Tsumura, Tonogai and Shibata, 1999). One of the mechanisms responsible for adult onset diabetes mellitus is a form of insulin resistance, which is attributed to the inability of insulin to enter cells via the insulin receptor. _Gymnema_ may overcome this resistance, but require further studies to confirm its validity and also whether the effect is clinically relevant. Should this effect be proven, _Gymnema_ may prove useful in both adult onset (NIDDM) and juvenile onset diabetes mellitus (IDDM) to help insulin enter cells. In the case of IDDM, the insulin is injected by syringe and is not secreted from the pancreas (Agarwal, Singh, Verma, Lakshmi and Sharma 2000). The leaves are also noted for lowering serum cholesterol and triglycerides. The primary chemical constituents of include gymnemic acid, tartaric acid, gurmarin, calcium oxalate, glucose, stigmasterol, betaine, and choline. While the water-soluble acidic fractions reportedly provide the hypoglycemic action, it is not yet clear what specific constituent in the leaves is responsible for the same. Some researchers have suggested gymnemic acid as one possible candidate, although further research is needed (Khare, Tondon and Tewari, 1983). Both gurmarin (another constituent of the leaves) and gymnemic acid have been shown to block sweet taste in humans. The major constituents of the plant material 3B glucuronides of different acetylated gymnemagenins, gymnemic acid a complex mixture of at least 9 closely related acidicglucosides (Maeda, Iwashita and Kurihara 1989; Sinsheimer and Subbarao, 1971).

In general, an endophyte can be defined as microorganisms that include bacteria and fungi living within plant tissues without causing any immediate overt negative effects. They
have been found in every plant species examined to date and recognized as the potential products for exploitation in medicine agriculture and industry (Stone,Bacon and White, 2000). The diversity of endophytes is striking,every plant examined to date harbors at least one endophytic fungal species, and many plants, especially woody plants, may contain hundreds or even thousands of different species (Faeth, 2002). The plants that are endemic, having an unusual longevity or that have occupied a certain land mass are also more likely to lodge endophytes than other plants. Recently various host plants have demonstrated that fungal endophytes are ubiquitous in plant species (Huang et al., 2008; Oses et al., 2008).

There are at least one million species of endophytic fungi which represent an important genetic resource for biotechnology (Ganley et al., 2004). Endophytes have been recognized as potential sources of novel natural products for pharmaceutical, agricultural and industrial uses because of its secondary metabolites produced by fungal endophytes (Strobel and Daisy 2003; Hyde and Soytong, 2008; Mitchell et al., 2008). Now a day’s much attention is paid in endophytic biodiversity, the chemistry and bioactivity of endophytic metabolites, and the relationship between endophytes and their host plants (Tan and Zou, 2001).

The use of fungal endophytes and endomycorrhiza as co-culture has opened up a new area of research for improving the production of bioactive molecules in plants. A better understanding of the range of species-specific interactions and their effects on plant growth and metabolism may lead to the development of production schemes utilizing fungal endophytes helps to explore the potential of inoculation of Gymnema sylvestre with of fungal endophyte and VAM.

The plant G. sylvestre is targeted for the isolation of endophytes in this present study. G. sylvestre is one of such important medicinal plants in Ayurvedic medicine. G.sylvestre R.Br. (Asclepiadaceae) is a milk weed commonly known as Gurmar. The plant is known to contain triterpenoid saponins belonging to the oleanane (gymnemic acids) and dammarenene (gymnemasides) classes (Dateo and Long,1973; Khram,Spasov and Samokhina ,2008). The presence of gymnemic acids (GA) the other phytoconstituents include flavones, anthraquinones, hentriacontane, resins, dqruccitol, lupeol, β-amyrin-related glycosides and stigma sterol. The presence of alkaloids has also been confirmed (Dateo and Long,1973). The investigation made by various workers related to its medicinal uses, chemical...
constituents, pharmacological activities and other aspects considering this plant since years till date.

Biofertilizers are the most advanced biotechnological products necessary to support developing organic, sustainable agriculture. It is defined as ready to use live formulations of beneficial microorganisms. The utilisation of mycorrhizae as biofertilisers in the cultivation of medicinal plants is of recent interest. Vesicular Arbuscular Mycorrhiza (VAM) fungi have been used to enhance the plant growth and yield of medicinal crops and to help maintain good soil health and fertility that contributes to a greater extent to a sustainable yield and good quality of the bioactives. The VAM association has been shown to enhance the plant growth (Tinker, 2002) bringing about morphological (Berta et al., 1995), Physiological (Johnson, 1984) and biochemical changes in host plants (Allen et al., 1982).

Mycorrhizal fungi takes carbohydrates from the plant in turn supplies the plant with nutrients, hormones and protects it from root pathogens. These fungi are known to improve the nutritional status of the host, particularly that of phosphorous, and there by enhance their growth, development and yield (Bagyaraj, 2007). The beneficial roles of VAM fungi in nutrient uptake in many plants are well documented in recent years. VAM fungi increase nutrient uptake, physiological parameters and plant growth (Lakshmipathy et al., 2001).

Some endophytes synthesize plant growth hormones such as indole-3-acetic acid, cytokinines and gibberellins that promote plant growth (You et al., 2012; Khan and Lee 2013) and can also increase above ground photosynthesis through the modulation of endogenous sugar and Abscisic acid (ABA) signaling (Zhang et al., 2008). Endophytic fungi may have the metabolic machinery to produce plant growth regulators and there by promote seed germination in crop plants (Bhagobaty and Joshi, 2011). In this study we report endophytic fungi Sordariomycetes sp isolated from R.beddomei and their positive effect on growth of G. sylvestre.

Fungal endophytes play an important role in plant growth and in many complex changes in morphology, biochemistry, HPLC analysis and gene expression takes place leading to altered growth and development patterns that allow the symbiosis to function (Armstrong and Peterson, 2002; Balestrinini and Bonfante, 2005). Enhanced growth, nutrient
use efficiency, stress tolerance and disease resistance have all been demonstrated (Augé, 2001; Redman et al, 2002; Kageyama et al., 2008; Rudgers et al., 2009). These benefits are useful to altered biochemistry in the plant or bioactive compounds produced by the endophyte (Nemec and Lund, 1990; Fester et al., 2002; Bultman et al., 2004). Recently, VAM, PSB and Azotobacter are the most widely used bio fertilizers, significantly contributing N, P and K to plants and also resistance to drought. However, very limited information is available regarding the effect of the biofertilizers on the growth and yield, biochemical parameters and also VAM colonization efficiency of *G. sylvestre* plant. In the present study it was recommended that suitable combination of biofertilizer application for cultivation of *G. sylvestre* at commercial level.

*G. sylvestre* has medicinal, traditional and economic uses in different systems of medicine. The wide varieties of compounds isolated from this plant have extensive range of pharmacological activities which need to be researched in depth to establish their therapeutic potential. This broad range of uses and medicinal values reflects about the idea that, in future, pharmaceutical and drug manufacturing sector mainly relies on plants to obtain life saving therapeutics and drugs. Though it has such medicinal and economic property it is now rarely available and has been categorized as a rare plant could be unawareness about its uses in general public as well as its difficulty in natural reproduction. So different methods like tissue culture techniques play an important for its conservation and propagation (Pratibha Gupta, Sujata Ganguly and Pratibha Singh, 2012). The present study can be explored for large scale production of Gymnemic acid by the cultivars to meet the phytopharmaceutical demand. In view of the above the following objectives were formulated.