INTRODUCTION

Medicinal plants are a rich source of bioactive compounds and have been used for various purposes across the world. With technological advancements, these compounds have found usage in pharmaceuticals products, and also as food additives, dyes, fragrances and pesticides (Zhi-lin et al. 2007). A study by the World Health Organization, gave an estimate that about 80 % of the world’s population relies on traditional medicine for their primary healthcare needs (Sieniawska et al. 2013). It is also estimated that about 60 % of antitumor drugs and 25 % of other modern drugs are derived from natural products (Newman and Cragg 2012). These drugs have gained wide acceptance among consumers as they are effective, safe to use and have lesser side effects. It becomes essential to undertake large scale production of medicinal plants to cater the demand of plant bioactive compounds. The cultivation of medicinal plants and also the quantity as well as quality of bioactive compounds are largely affected by the plant genotypes, environmental factors and nutrient uptake leading to their unstable production (Ann Lila 2006).

Several biotechnological strategies have thus been utilized to achieve stable yield and quality of medicinal plants (Hussain et al. 2012). Plant tissue culture techniques have been deployed for selection of high yielding cell lines and strain improvement for production and accumulation of valuable chemical compounds. Another approach involves use of an elicitor to induce or enhance the biosynthesis of a specific compound. Genetic manipulation offers an alternative for secondary metabolite production by plant cells; various approaches foe genetic modification such as, Agrobacterium tumifaciens and A. rhizogenes can be used to transfer the gene(s) of interest. Alteration of the metabolic pathway and manipulation of cellular activities have also been undertaken to enhance the yield of compounds of interest (Zhao et al. 2001a; Karuppusamy 2009). These methods of plant secondary
metabolite production are however high on cost and require scaling up. Besides these methods, studies on beneficial interaction between host plant and symbionts have laid emphasis on exploring their role in biomass production and accumulation of plant bioactive compounds. The interactive effect of *Arbuscular mycorrhiza* (AM) with medicinal plants is well documented. Several reports have shown that AM symbiosis has beneficial effects on yield and quality of medicinal plants (Zeng et al. 2013b). However, the commercial production and large scale use of AM fungi faces a challenge as the mass production of pure inoculum and axenic cultivation of AM fungi has not been achieved to this date.

A plethora of other microorganisms inhabit the rhizospheric ecosystem and form symbiotic association with the plants. Fungal root endophytes involving a diverse group of fungi inhabit the plant roots having beneficial effect on plant growth and enhance their resistance to biotic and abiotic stresses (Hermosa et al. 2012). One of the well-studied root endophytic fungus is *Piriformospora indica*, belonging to the group *Basidiomycota*. (Oelmüller et al. 2009). The fungus can be seen growing inter- and intra-cellularly in diverse plant species, leading to formation of pear shaped chlamydospores. It is however not seen to enter plant endodermis and aerial parts (Oelmüller et al. 2009; Weiss et al. 2004). *P. indica* colonization has been shown to enhance growth of host plant, increase resistance to biotic and abiotic stresses (Sherameti et al. 2008; Vadassery et al. 2009) and heighten assimilation of essential nutrients (Varma et al. 2013). Both, fungal spores and culture filtrate of *P. indica* have shown beneficial effects on plant growth suggesting better nutrient uptake and/or hormonal signaling by the fungus. Morphological changes in the root and physiology of *P. indica* colonized plants have also been observed suggesting the induction of regulatory pathways (Grunewald et al. 2009). *P. indica* has the potential to grow on a number of complex and semi-synthetic media, thus it can be cultured axenically for mass production (Prasad et al. 2005). It has also been established as a potent bio fertilizer, bio protectant and biological hardening agent (Johnson et al. 2014). Association of *P. indica* with medicinal plants has
shown to enhance plant secondary metabolite production. Use of this plant-fungus interaction can thus pave an alternative way for enhanced accumulation of commercially important bioactive compounds.

Rhizospheric bacteria have also been known to enhance plant growth through nitrogen fixation, phosphate solubilisation and quorum sensing (Bhattacharyya and Jha 2012). They have been shown to improve the secondary metabolite content in plants by improving the phosphorus uptake and/or by altering the hormonal balance of the plants (Köberl et al. 2015). Therefore, engineering of the plant rhizosphere through inoculation of specific microorganisms can provide cumulative benefit to the plant. The study of antagonistic or synergistic effect of microbial inoculants on medicinal plants is critical for the development of an effective host-microbe interaction system. The symbiotic association of *P. indica* and *A. chroococcum* in a co-culture system was established by Bhuyan et al. (2015) making them a promising bio-inoculant.

*Stevia rebaudiana* is a medicinally and industrially important herb and is known to accumulate sweet tasting steviol glycosides (Brandle et al. 1998). The sweetening compounds have a great demand worldwide as they are 300 times sweeter than cane sugar and are non-caloric in nature (Chatsudthipong and Muanprasat 2009). The cultivation of *S. rebaudiana* with high quantity and quality of steviol glycosides is thus essential to meet their emerging need.

In view of the above, the present study was designed with the aim to study the synergistic interplay of dual inoculation of the root endophytic fungus *P. indica* and plant growth promoting rhizobacteria *A. chroococcum* on plant growth characteristics and steviosides yield in *S. rebaudiana* through following objectives;

**Objectives**

1. Interaction of *P. indica* with *S. rebaudiana*; singly or in combination with PGPR(s)
2. Analysis of growth and yield attributing traits after interaction with *P. indica* and PGPR(s)
3. Biochemical analysis of steviol glycosides

4. Expression profiling of genes of steviol glycosides biosynthetic pathway
5. Phytochemical analysis of crude extract of *S. rebaudiana* for antioxidant activity and phenolic content