Introduction
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Ever since ancient times, in search for rescue for their disease, the people looked for medicine from the nature. The connection between man and his search for drugs in nature dates from the far past, of which there is ample evidence from the original plant medicines. Natural sources especially medicinal plants have been used as traditional treatments for numerous diseases for thousands of years based on experience and folk remedies. Medicinal plants continue to draw wide attention for their role in the treatment of mild and chronic diseases. In recent times, focus on plant research has increased all over the world and a large body of evidence has been accumulated to highlight the immense potential of medicinal plants used in various traditional systems of medicine (Stafford et al., 2008).

Centella asiatica (L.) is one of such imperative medicinal plants used in Ayurvedic medicine. It is a very important medicinal herb used in the orient and is highly popular in India (Chevallier, 1996). *C. asiatica* is a tropical medicinal plant from *Apiaceae* family native to Southeast Asian countries such as India, Sri Lanka, China, Indonesia, and Malaysia as well as South Africa and Madagascar (Jamil et al., 2007). *C. asiatica*, commonly known as “Gotu kola, Asiatic pennywort, Indian pennywort, Indian water navelwort, wild violet, and tiger herb” in English, is a tropical plant, which has been also cultivated successfully due to its medical importance, and it has a long history of utilization in traditional medicines since centuries (Meulenbeld and Wujastyk, 2001).

*Centella asiatica* is a clonal, perennial herbaceous creeper and the leaves are edible, yellowish-green in color, thin, alternate with long petioles, and quite characteristic reniform, orbicular, or oblong-elliptic shapes with seven veins (Ilkay, 2012). The plant grows horizontally through its green to red stolones which combine to each other and roots in underground and thrives in and around water (Jamil et al., 2007). Economically, *C. asiatica* has substantial value and great demand in Indian as well as International market (Dora and Khatri, 2011). A growing body of data supports the folk medicine claims that *C. asiatica* has real therapeutic value. The exact mechanisms and full range of benefits are not fully understood; however,
modern research is revealing new insight into the full range of potential properties and the mode of action in the human body (Blumenthal et al., 2003; Shah et al., 2007). *C. asiatica* is widely used as a blood purifier as well as for treating high blood pressure, for memory enhancement and promoting longevity. In Ayurveda, it is one of the main herbs for revitalizing the nerves and brain cells (Hagemann et al., 1996). It has been reported to own a wide range of biological activities desired for human health such as wound healing, sedative, anti-inflammatory, antidiabetic, antipsoriatic, antibacterial, hepatoprotective, cardioprotective, anticonvulsant antiulcer, antiviral, cytotoxic and antitumor, insecticidal, antifungal, antioxidant, gastroprotective, anti-allergic, anti-osteoporotic, anti-obesity, snake venom antidote, also for lepra and venous deficiency treatments (Ilkay, 2012; Dinda et al., 2010).

The primary active constituents of *C. asiatica* are triterpenoidal saponins, in which a trisaccharide moiety is linked to the aglycone asiaticoside, madecassoside and asiatic acid. These triterpene saponins and their sapogenins are mainly responsible for the therapeutic potential (Kashmira et al., 2010). The triterpene saponins are most well known for their immunemodulating properties (Plohmann et al., 1997). The significant pharmacological potential and furthermore, the availability of well defined chemical profile of triterpene saponins of *C. asiatica* prompted us to take up research on evaluating the untapped stimulating potential on immune system function.

It is certainly axiomatic that *in vivo* treatment of humans with test material, followed by direct measure of immune function or host resistance, represents the most relevant model for human drug development. It is equally true that ethical and practical considerations preclude this approach, at least during the early stages of the drug-development process. This is particularly important in the development of immunomodulatory drugs, which are not routinely intended for life-threatening conditions and may therefore require the demonstration of a higher safety factor. Thus, for the foreseeable future, *in vivo* studies using laboratory animals will continue to represent a key component of the drug discovery and development process. The various models available may yield divergent results with varying degrees of relevance to the human condition. Noteworthy, cyclophosphamide
induced immunosuppression in male Wistar rat serves as a well accepted standardized experimental animal model to evaluate serial immune system functions and to study the efficacy of various natural and synthetic immunomodulatory agents (Hou et al., 2007). The immunosuppression induced by cyclophosphamide has been reported to show many metabolic alterations of immune system and morphologic aberrations in the immune organs (for eg., spleen and thymus) of the experimental animals, similar to those observed in human immunosuppression (Hou et al., 2007; Pratheeshkumar and Girija, 2010a). Thus the present study evaluates immunemodulating effect of triterpene saponins from *Centella asiatica* against the immunosuppressive effects of cyclophosphamide in male Wistar rats.

The botanical immunomodulators being nontoxic and having no side effects are attracting the global attention. The plant derived saponins based immunomodulators are of importance in improving the immunity levels by optimizing the immune functions (Pragathi et al., 2011). The chemical synthesis of immunomodulators is difficult and economically infeasible due to their complex nature. Many such important bioactive molecules are harvested from the natural habitants. The excessive harvesting is resulting in lack of adequate and continuous supply of raw materials besides the threat to the important medicinal plant species becoming endangered. To overcome this situation there is a need to adopt biological techniques which improves the *in planta* production and accumulation of botanical immunomodulators. Growing demand for natural medicines from medicinal plants is spurring interest in novel and more efficient production methods (Rai et al., 2001).

The targeted 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. The newly emerged efficient biotechnique have the potential for broad usefulness in organic and alternative production in conventional systems. 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 (Richard, 2010). If explored and maximized, the benefits could lead to less dependency on synthetic inputs, and improved crop yields and stress resistance in all systems. Due to the high demand for plant based
immunomodulators, and the potential for increasing the content of desirable natural products in plant tissues, the natural products industry could greatly benefit as well (Richard, 2010). Thus, this study seeks to explore the potential of inoculation of *Centella asiatica* with of fungal endophyte and endomycorrhiza as part of a commercially viable production scheme. The primary focus of this investigation is the efficacy and effect of endophyte and endomycorrhizal inoculation on growth and production of immunomodulators (triterpene saponins) in *Centella asiatica*.

It is established that plants harbour microorganisms, collectively known as endophytes (Alvin et al., 2014); endo” meaning “in” and “phyte” meaning “plant”. These organisms cause no damage or disease symptoms and can confer various degrees of benefit to the host (Gunatilaka, 2006; Ownley et al., 2008). 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. Furthermore, the plants growing in areas of great biodiversity also have the prospect of housing endophytes with great biodiversity (Yu et al., 2010).

Considering the above selection strategy, the plant *Pterocarpus santalinus* is targeted for the isolation of endophytes in the present study. *Pterocarpus santalinus* is endemic, endangered, globally threatened medicinal tree taxa, known as “Red Sanders” in India. Tirumala hill forest, the biosphere reserve of Eastern Ghats in India has a great diversity of endemic medicinal plants. *Pterocarpus santalinus* Linn (Family: *Fabaceae*) is found throughout the Tirumala hills (at elevations of 900 m or higher), Seshachalam biosphere reserve of Eastern Ghats, India. The fungal endophytes associated with *P.santalinus* which can grow on organic substrates in the harsher and or extreme conditions, might include specialists that have evolved specific adaptations, some of which could also be of technical interest.

For this reason, the present study was undertaken 1) to isolate endophytic fungi from *Pterocarpus santalinus*, (2) to identify them by morphological and molecular techniques based on internal transcribed spacer (ITS) sequence analysis and (3) to investigate the efficacy and effect of endophyte and endomycorrhizal inoculation production of *in planta* natural immunomodulators. The fungal
endophytes play an important role in many facets of plant growth and development. The foundations of the mechanisms that allow these relationships have evolutionary origins and exist in the genomes of both endophyte and plant. Complex changes in gene expression, morphology and biochemistry take place in both partners, leading to altered growth and development patterns that allow the symbiosis to function (Armstrong and Peterson, 2002; Balestrinini and Bonfante, 2005; Strack et al., 2003). The plant host can benefit in various ways. 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). Some of these benefits are a direct result of altered biochemistry in the plant or bioactive compounds produced by the endophyte (Nemec and Lund, 1990; Fester et al., 2002; Bultman et al., 2004).

The utilisation of mycorrhizae as biofertilisers in the cultivation of medicinal plants is of recent interest. Arbuscular mycorrhizal (AM) 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. AM fungi can also alter plant water relation and response to drought. Due to their ability to increase nutrient uptake and water transport, AM fungi are being frequently used in sustainable agriculture (Richard, 2010). To mention, recent studies have clearly brought out host preference in AM fungi, thus emphasizing the need for selecting efficient AM fungi for inoculating a particular host. Arbuscular mycorrhizae has been shown to alter phytochemistry in *Echinacea purpurea*, and other endophytes are well known to have the same effect in a diverse range of other plant species (Strack et al., 2003; Toussaint et al., 2007; Yuan et al., 2007; Araim et al., 2009).

The establishment of symbiosis with potential strains of fungal endophyte and endomycorrhiza enhanced the metabolite production (Sherameti et al., 2005; Strack and Fester, 2006; Richard, 2010). Lin et al., (2007) reported the promotion of enzyme activities and terpenoid production in *Euphorbia pekinensis* in presence of endophytic and endomycorrhizal association. However, there have been no efforts on effect of endophyte and AM inoculation on *Centella asiatica* plant. This research
could address useful insights and the potential role played by fungal endophyte and endomycorrhizae as co-culture systems in the regulation of growth and metabolism of immunemodulating saponins in *Centella asiatica* and other plant species.