INTRODUCTION
**Centella asiatica** L. is one of the important medicinal herbs in Asian subcontinents. It is a small herbaceous creeper belonging to the family Apiaceae and comprises of over 50 species (James and Dubery, 2009). This medicinal herb is native to Australia, Pacific Islands, New Guinea, Melanesia, Malaysia, Northern Iran and Asia (Schaneberg et al., 2003). Its common names are Gotu Kola, Asian Pennywort, Antanan, Pegaga, Kula ud and Brahmi. The name ‘Brahmi’ is also shared with *Bacopa monnieri* L as both the medicinal herbs were found to have similar medicinal properties (Daniel, 2005). *C. asiatica* is wildly grown at different elevations and grows extremely well in marshy places under different geographical conditions in India (Schaneberg et al., 2003). It is also predominantly distributed in Sri Lanka and Madagascar. The stems are slender, prostrate or creeping in nature and interconnect one plant to another to form a network of greenish mat of growth on the soil. Wild *C. asiatica* is usually found as a weed in marshy places throughout India. Stems are glabrous with many adventitious roots at the nodal points. The root stock consists of rhizomes, growing vertically down. They are cream- coloured and are covered with root hairs. Leaves are simple usually with smooth texture, palmately netted veins and fleshy, orbicular, reniform, crenate or dentate, base cordate. They are often lobed with a long petiole, smooth on the upper surface and hairy on the surface.

The whole plant of *C. asiatica* contains four important bioactive compounds, collectively known as centellosides, that includes major principle compounds such as asiaticoside, madecassoside, asiatic acid and madecassic acid (Bhattacharya 1956a-c; Rastogi et al., 1960; Singh and Rastogi, 1968, 1969; Tiwari et al., 2000). These bioactive compounds account for medicinal properties for healing a variety of health disorders. *C. asiatica* is one of the key sources of herbal medicines in India system of traditional medicines, namely ayurvedic and siddha and also in traditional Chinese herbal medicines. Considerable amount
of research on pharmacological aspects of this medicinal herb lead to clear understanding of its medicinal properties and presently, *C. asiatica* forms a major components in various herbal formulations. Among centellosides, asiaticoside forms the major compound with regard to its medicinal properties.

It is reported that asiaticoside contributes for stimulation of reticulo-endothelial system, where new blood cells are generated and old ones destroyed. Asiaticoside acts on the various phases of connective tissue development during the process of healing and increases keratinisation, a key process during healing ulcers, skin injuries and connective tissue (Miller, 1978; Sharma et al., 1985; Wyk and Wink, 2004). It is a proven fact that asiaticosides also stimulate the synthesis of lipids and proteins necessary for healthy skin (Miller, 1978). Asiaticoside and other triterpenes extracted from the plant were reported to have modulating properties on the development and metabolism of connective tissues. It improves the synthesis of collagen and other tissue proteins by modulating the action of fibroblasts in the vein wall and stimulates collagen delling in and around the venous wall. Therefore, it improves wound repair and normalization of eri-vascular connective tissue, allowing the improvement of the venous wall tone and elasticity (Wyk and Wink, 2004). It was also reported that in poor connective tissue conditions, triterpenes of *Centella asiatica* are able to renew the collagen in quantity and quality and restore tissue firmness and skin elasticity to improve the appearance of skin. Apart from this, it also has antipsoriatic properties.

*C. asiatica* in India and East countries is traditionally used as a cover crop in tea and rubber plantations and as a leafy green vegetable in Sri Lankan and Indian soils. The leaves are eaten raw in salads or cooked in curry dishes due to its high nutritional value (Bown, 1995; Chadha, 2009). The major nutritional values of *C. asiatica* include calcium, iron, selenium, ascorbic acid, beta carotene, magnesium and or. In addition it contains triterpenes (betulic acid, brahmic acid, isobrahmic acd) saponins (Brahmoside,
thankuniside, asiaticoside A and B brahminoside, madecassoside and flavones (Quercetin). Pharmacological investigations lead to utilization of C. asiatica as one of the potential herbal medicines for its diverse medicinal properties such as anti tumor (Babu et al., 1995), prevention of cognitive (Gupta et al., 2003), anxiolytic (Wijeweera et al., 2006), wound healing (Christopher et al., 2003), immunomodulatory (Punthre et al., 2005), elevation of antioxidant (Shukla et al., 1999) and anti depressant properties (Xin Liang et al., 2008).

Centella asiatica grows in the wild forest as a weed and is exported to rope and other Asian countries for the extraction of herbal medicines. The estimated annual requirement of C. asiatica is 12,700 tonnes of dry mass, primarily met through natural population (Ahmed, 1993; Paramageetham et al., 2004). Because of large scale and unrestricted exploitation, coupled with limited cultivation and insufficient attempts for replacement, wild stock of C. asiatica has been depleted in many parts of India (Paramageetham et al., 2004). The global market of medicinal products is over USD 60 billion with an annual growth rate of seven percent (N and Karki, 2004). This has demanded the medicinal plant sectors to cultivate Centella asiatica as a field crop for the farmers to benefit from its commercialization and to conserve natural population to maintain biodiversity. However, information on appropriate tech for commercial cultivation of C. asiatica is scanty.

Due to diverse use of C. asiatica, there is an ever increasing demand for raw materials for preparation of herbal products by pharmaceutical industries. In India C. asiatica is one of the chief components of the herbal medicines. The Indian pharmaceutical companies largely depend on natural populations of C. asiatica, leading to rapid decrease in availability of this precious medicinal herb. Thus, the role of cell culture technique to develop alternative production system for Centella metabolites is felt very important. Insufficient availability of raw materials is mainly due to restricted vegetative growth only
during wet season in most of the agricultural lands. Besides, there is a serious concern regarding the purity of raw materials since the raw materials are sourced from agricultural field, often polluted with agrochemicals (fungicides and pesticides) during cycling of various seasonal crops such as cotton, vegetable crops cereals and pulses. It was cautioned that paddy, wheat, mustard, potato, cotton, tea, tomato sugarcane, grape and urban soils were found polluted with persistent organochlorine insecticide residues. DDT and HCH concentrations were found to be higher in paddy fields in many part of India (Kawano et al., 1992). Thus, utilization of C. asiatica by sourcing from agricultural lands is expected to contain residues of various toxic agrochemicals and high risk is involved (Kawano et al., 1992).

Search for alternative methods for the production of phytochemicals of medicinal importance utilizing biotechnological approaches, especially plant tissue culture is reported to have potential as a supplement to traditional agriculture in commercial production of bioactive plant metabolites (Ramachandra Rao and Ravishankar, 2002). Cell suspension culture systems could be used for large scale culturing of plant cells from which secondary metabolites could be extracted. The advantage of this method is that it can ultimately provide a continuous, reliable source of natural products (Nath and Buragohain, 2005). Discovery of cell cultures capable of producing specific medicinal compounds at a rate similar or superior to that of intact plants have accelerated in the last three decades and it was demonstrated that the biosynthetic activity of cultured cells can be enhanced by regulating environmental factors as well as by artificial selection or by improved cell lines or clones (Sivakumar et al., 2006). Some of the medicinal compounds localized in morphologically specialized tissues or organs of native plants have been produced in culture systems not only by inducing specific organized cultures but also from undifferentiated cell cultures.
The potential use of plant cell cultures for the specific biotransformations of natural compounds has been demonstrated (Cheetham, 1995; Scrag 1997; Krings and Berger, 1998; Ravishankar and Ramachandra Rao 2000). Due to the advances, research in the area of tissue culture technology for production of useful phytochemicals has bloomed beyond expectations. The major advantages of cell culture systems over the conventional cultivation of whole plants are:

- Useful compounds can be produced under controlled conditions independent of climatic changes or soil conditions.
- Cultured cells would be free of microbes and insects.
- The cells of any plants, tropical or alpine, could easily be multiplied to yield their specific metabolites.
- Automated control of cell growth and rational regulation of metabolite processes would reduce labour costs and improve productivity and
- Organic substances are extractable from callus cultures.

In order to obtain high yields of phytochemicals for commercial utilization, efforts have been focused on isolation of biosynthetically active cells, optimization of cultural conditions and selection of high yielding strains. In addition, several other viable methods such as precursor feeding, genetic transformation and immobilization have also been employed for enhancing the production (Dicosmo and Misawa, 1995). Transgenic hairy root cultures have revolutionized the role of plant tissue culture in secondary metabolite production. They are unique in their genetic and biosynthetic stability, faster in growth and are maintained more easily. Using this methodology, wider range of phytochemicals has been produced (Shanks and Morgan, 1999; Giri and Narasu, 2000). Advance in tissue culture, combined with improvement in genetic engineering, especially transformation technology, has opened new avenues for volume production of
pharmaceuticals, nutraceuticals and other beneficial substances (Hansen and Wright, 1999). Recent advances in the molecular biology, enzymology and fermentation technology of plant cell cultures suggest that these systems will be viable sources of important secondary metabolites.

The appeal for using natural products for medicinal purposes is increasing and metabolic engineering can alter the production of pharmaceuticals and help to design new therapeutics. At present, researchers aim to produce substances with antitumor, antiviral, hypoglycaemic, anti-inflammatory, antiparasite, antimicrobial, tranquilizer and immune modulating activities through tissue culture technology. Exploration of the biosynthetic capabilities of various cell cultures has been carried out in several countries during the last few decades and advances in the area of cell cultures for the production of medicinal compounds has made possible to produce wide variety of pharmaceuticals such as alkaloids, terpenoids, steroids, saponins, phenolics, flavonoids and acids.

*C. asiatica* is the most popular medicinal herb being used in Indian system of medicine and its diverse medicinal properties were scientifically proven. Of the various medicinally important bioactive compounds, asiaticoside is one of the key phytochemicals used for healing a variety of disorders. Considering its wide range of medicinal properties in addition to over exploitation, wild plants of *C. asiatica* are heavily depleted (Paramageetham et al., 2004). Although, *in vitro* cell culture systems for production of medicinally important compounds from whole plant is quite common, callus culture and transformed root culture of *C. asiatica* was reported in other countries. Effort towards utilization of Indian wild germplasm of *C. asiatica* for production of useful bioactive compounds is not reported using *in vitro* cell culture system. Therefore, the present study is focused on the following objectives:

- Collection and conservation of wild germplasm of *Centella asiatica* in Southern India.
• Characterization of wild germplasm of *C. asiatica*.
• Factors associated with establishment of aseptic leaf tissues and callus induction.
• Optimization of medium composition for regeneration of adventitious roots from leaf derived callus.
• Enhancement of adventitious root proliferation and
• Evaluation of regenerated roots for presence of asiaticoside.