CHAPTER I

INTRODUCTION

1. Introduction

Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products (Pareek, 1998). About 8,000 herbal remedies have been codified in ayurveda. The *Rigveda* (5000 BC) has recorded 67 medicinal plants, *Yajurveda* 81 species, *Atharvaveda* (4500-2500 BC) 290 species, *Charak Samhita* (700 BC) and *Sushrut Samhita* (200 BC) had described properties and uses of 1100 and 1270 species respectively, in compounding of drugs and these are still used in the classical formulations, in the Ayurvedic system of medicine (Suresh Wagh, 2006; Pareek, 1998). Unfortunately, much of the ancient knowledge and many valuable plants are the verge of extinction. *The Red Data Book of India* has 427 entries of endangered species of which 28 are considered extinct, 124 endangered, 81 vulnerable, 100 rare and 34 insufficiently known species (Thomas, 1997).

The World Health Organisation (WHO) estimated that 80% of the populations of developing countries rely on traditional medicines and 85% of the traditional medicines involve the use of the plant extracts (Mukhopadhyay, 1998). Demand for medicinal plant is increasing in both developing and developed countries due to growing recognition of natural products, being non-narcotic, having no side-effects, easily available at affordable prices and sometime the only source of health care available to the poor (Pareek, 1998; Ahmad, 1993). Medicinal plant sector has traditionally occupied an important position in the socio cultural, piritual and medicinal arena of rural and tribal lives of India. Medicinal plants as a group comprise approximately 8000 species and account for around 50% of all the higher flowering plant species of India. Millions of rural households use medicinal plants in a self-help mode (Suresh Wagh, 2006). Over one and a half million practitioners of the Indian System of Medicine in the oral and codified streams use medicinal plants in preventive, promotive and curative applications. There are estimated to be over 7800 manufacturing units in India (Farnsworth and Soejarto, 1991). Allopathic medicine too owes a tremendous debt to medicinal plants: one in four prescriptions filled in a country like the United States is either a synthesized form of or derived from plant materials (Srivastava *et al*., 1997).

In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries. As a result there is a global resurgence in the trade of herbal medicines. International market of medicinal plants is reported to be over 62 billions US dollars per year during 2000 - 2001, which is growing at the rate of 7% annually (Rawat, 2003). India has a rich biodiversity; the growing demand is putting a heavy strain on the existing resources. While the demand for medicinal plants is growing, some of them are increasingly being threatened in their natural habitat. For meeting the future needs cultivation of medicinal plant has to be encouraged. According to an all India ethno biological survey carried out by the Ministry of Environment & Forests, Government of India, there are over 8000 species of plants being used by the people of India. A growing awareness of this new contributor to the foreign exchange reserves of several national treasuries is beginning to emerge. To satisfy the growing market demands, surveys worldwide are being conducted by the pharmaceutical industries and research organization to unearth new plant sources as herbal remedies, medicines and biomolecules (Khanuja, 2003).

Plants continue to be important resource material for therapeutic agents both in the developed and developing countries. These are used in three major modes in health care programme viz., industrial production of pharmaceuticals, utilization as traditional medicines and new drug discovery (Raina *et al*., 1998). It is estimated that 80% population of developing countries rely upon traditional medicinal systems (Ayurvedic, Siddha, Unani and the Tibetan system of medicine) for their primary health care. Also, modern pharmacopoeia still contains at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants. These systems of medicines are based either on their therapeutic uses, active principles, chemical composition or ingredients of the plants (Shiva and Mahatolia, 1998). The tremendous passion for the medicinal plants made the people to use them for a wide range of health related applications from a common cold to memory improvement and treatment of poisonous snakebites to a cure for muscular dystrophy and the enhancement of body's general immunity. Medicinal plant sector has traditionally occupied an important position in the socio cultural, spiritual and medicinal arena of rural and tribal lives of India (Anonymous, 2000).

Factors contributing to the growth in demand for traditional medicine include the increasing human population and the frequently inadequate provision of western medicine in developing countries (Pareek, 1998). Therefore to meet the demand of herbal medicines, the answer lies only when these medicinal plants are cultivated commercially in farm, forestry and plantation sectors to produce the raw material having desired quality standards. To achieve this target, a well defined programme of research and development is needed in a coordinated manner and the recent exciting developments in biotechnology have come as a boon. The powerful techniques in genetic improvement, plant cell and tissue culture, recombinant DNA, bioprocess technologies etc., coupled with most sophisticated analytical tools such as HPLC,HPTLC,NMR,GC-MS, LC-MS etc., have offered mankind the great potency of exploiting the totipotent biosynthetic and biotransformation capabilities of plant cells under in vitro conditions (Stockigt *et al.,* 1985).

1.1. History of the *Centella asiatica*

Gotakola (*Centella asiatica*) has been used as a medicine in India from time immemorial. Originally, the plant was identified botanically as *Hydrocotyle asiatica* Linn., but subsequently, it was named *Centella asiatica*. It was thought to be the plant Manduka parni listed in the Susuita Samita, an ancient Hindu text. In Indian ayurvedic practice as well, its use in promoting longevity was well known. *Centella asiatica* is one of the chief herbs for treating skin problems, to heal wounds, for revitalizing the nerves and brain cells, hence primarily known as a"Brain food" in India. With time it became popular, both internally and externally for treatment of skin diseases which include leprosy, lupus and eczema (Boiteau *et al.,* 1949; Boiteau and Ratsimamanga, 1956; Ratsimamanga *et al.,* 1958). Close relative of *Centella* also grow in the United States and American nineteenth Centure Eclectice were well aware of the medicinal properties of this plant in the treatment of leprosy (Kartnig, 1988). In the 1980 S extracts of

Centella asiatica were accepted as a drug in France. Also in the late 1800S Australian Richmond when applied to wounds and sores in the form of a salve or poultice. In 1852, Dr.Boiteau of India, was cared from leprosy after having being affected for several years, when treated with *Centella* (Boiteau and Ratsimamanga, 1956). In 1888, at the Centennial International Exhibition of Melbourne, Brisbane doctors exhibited the juice of *Centella* as a medicine.

According to the reports of Export and Import Bank of India *Centella asiatica* is one of the important medicinal plants in the International market of medicinal Plant Trade. However, the wild stock of this plant species has been markedly depleted, because of its large scale and unrestricted exploitation coupled with limited cultivation and insufficient attempts for its replacement has been made. Moreover, now it has been listed as Threatened plant species by the International Union for Conservation of Nature and Natural Resources (IUCN), and also as an endangered species (Singh, 1989; Sharma and Kumar, 1998).

1.2. Introduction of the *Centella asiatica*

Centella asiatica (L) Urban. (Mandookaparni) has been used as a medicinal herb for thousands of years in India, China, Srilanka, Nepal and Madagascar. In India, the plant was earlier confused for *Bacopa monnieri* Wettst., as both were sold in the market by the name "Brahmi". However, the controversy has been resolved and it is concluded that Brahmi is *B.monnieri* and Mandookaparni is *C. asiatica* (The Wealth of India, 1992). It is also synonymously known as *Hydrocotyle asiatica* and *Hydrocotyle lunata* Linn. commonly known as *Indian Pennywort*. This genus belongs to the family Apiaceae (Previously known as Umbelliferae) and includes the most ubiquitous species *Centella.* Approximately forty different varieties of *C. asiatica* have been identified throughout the world (Shakir *et al*., 2007). It is an ethanomedicinal plant used in different continents by diverse ancient culture and tribal groups (Peiris and Kays, 1996). *Centella* is a medicinal plant that has probably been used since prehistoric times and has been reported in Ayurveda to have been used for various medicinal and cosmetic purposes (Schaneberg *et al.,* 2003; Nadkarni, 1986). Mass scale collection of *Centella asiatica* from the natural habitat by the pharmaceutical industries as well as local ayurvedic and Unani practioners is leading to the depletion of this plant resource throughout India for medicinal purposes (Schaneberg *et al.,* 2003). *Centella asiatica* is used in the Ayurveda, Siddha, and Unani system of medicine to treat for various diseases. It is one of the spiritual herbs for improved meditation in Ayurvedia (Castiglioni, 1958).

In view of its versatile medicinal properties, the requirement of *C. asiatica* in pharmaceutical industries has been sharply increasing, thus leading to the over exploitation of this herb (Raghu *et al.,* 2007). It has been used as a traditional vegetable in China, India, Sri Lanka and Indonesia, and is being introduced as a cultivated vegetable in developed countries. It has been used for centuries as a medicinal herb and was referred to in the Indian Ayurvedic medicine some 3,000 years ago. It has also been referred in the French pharmacopoeia in 1884 and the ancient traditional Chinese Shennong Herbal some 2,000 years ago. *Centella* is reported and listed as a drug in the Indian Herbal Pharmacopoeia, the German Homeopathic Pharmacopoeia (GHP), the German Herbal Pharmacopoeia, The European Pharmacopoeia and Pharmacopoeia of the People’s Republic of China (Bonati, 1980; Jacinda and Ian, 2009).

C. asiatica has been preferred as an experimental plant material because of the following reasons. 1. The plant is medicinally very important 2. It reproduces both sexually and vegetatively 3. It produces a new generation of plant within a short span of 3-4 months and it is possible to get four to five generations of new plants within a year.

1.3. Taxonomical Description of the plant

Centella asiatica it is a weekly aromatic, herbaceous, prostrate perennial creeping herb, common all over word growing plentifully in moist area (Sakshi *et al*., 2010; Kavindra *et al*., 2000; Van Wky *et al*., 1997). It is commonly found in India up to an altitude of 600 m above sea level on moist, sandy or clavey soils and marshy banks of rivers, streams, ponds and in irrigated fields (Anonymous, 1992). It has a short vertical rootstock and glabrous axillary stems with long internodes.

The leaves are simple, alternate, stipulate, round to kidney shaped smooth surfaced, and can reach a width of 1-3 cm and a length of 15cm. Leaf margins can be smooth, crenate or slightly lobed. Petioles are 2–6cm long and 1.5-5cm wide, erect, glabrous, furrowed above stipules short, adnate to petiole and farming a sheathing base. The flowers are irregular, bisexual, dark pink to purple, hearly sessile umbels, usually 3 together at ends of short, erect, pubescent, pedundes. The flowering period is from May to October. The fruits are formed throughout the growing season. They are about 0.3-0.4 cm long, dull down, ovoid, hard, consisting of mericarps attached to a slender, central axis with, indehiscent, strongly thickened pericarps. Seeds are solitary in each mericarp, pendulous and albuminous (The Wealth of India, 1992; Sharma, 2003).

1.4. Common Name in Different Language of the *Centella*

Centella is a very popular and economically important medicinal plant found throughout the India and Abroad. *Centella asiatica* (L) Urban., is vernacularly known as Indian Pennywort or Gotukola in English. This plant has been known by an array of vernacular names in different common names of different languages in India and Abroad. The plant is known by following vernacular names.

Hindi: Brahmamanduki, Brahmibhed, Bengsag and Khulakhudi; **Sanskrit:** Mandookaparni; **Marathi:** Brahmi, Mandookaparni, Karinga and Karivana; **Bengali:** Thankuni and Tholkuri; **Gujarati:** Moti Brahmi and Barmi; **Kanarese:** Brahmisoppu, Urage and Vondelaga; **Meghalaya:** Bat-maina; **Telugu:** Brahmi, Mandukbrahmmi, Bokkudu and Saraswataku; **Assam:** Manimuni; **Malayalam:** Kodagam, Kodangal, Kutakam and Muthal; **Tripura:** Thankuni and Thunimankuni; **Bihar:** Chokiora; **Tamil:** Babassa and Vallarai; **USA:** Indian Pennywort and Marsh Pennywort; **Nepal:** Ghodtapre; **Cook Islands:** Kapikapu, (Shakir *et al.,* 2007; Sakshi *et al.,* 2010).

1.5. Medicinal Uses of the *Centella asiatica*

Centella asiatica has also been used in the traditional Indian and Chinese systems of medicine for the treatment of diseases such as leprosy and psoriasis, for wounds and burns, and for insanity (Bose, 1932; Kan, 1986). The plant extracts are used popularly in memory enhancing tonics and used for the treatment of mental retarded patients and stress related disorders (Sharan and Khare, 1991; Moharana and Moharana, 1994; Karting, 1988 ; Appa Rao *et al*., 1973).

The nutritional value of *Centella asiatica* is promising as it is rich in carotenoids and vitamins B and C (Paramageetham *et al*., 2004; Chew Shio Heong *et al*., 2011). It is commonly used as porridge for feeding pre-school children in Sri-Lanka in order to combat nutritional deficiencies (Cox *et al*.,1993). Modern drugs comprising the pharmacologically active triterpenoid fractions and glycosides such as asiaticoside and madecassoside are being currently used in the treatment of leprosy, lupus, eczema, skin lesions, wound healing, burns, ulcers of the duodenum, skin and cornea tuberculosis and venous diseases (Boiteau *et al*.,1949; King, 1950 a, b; Boiteau and Ratsimamanga, 1956; Ratsimamanga *et al*., 1958; Boiteau and Ratsimamanga, 1959; Tsurumi *et al*., 1973; Allegra *et al*., 1981; Giardina *et al*., 1987).

The whole plant is reported to possess antibacterial (Srivastava *et al*., 1997; Oyedeji and Afolayan, 2005), anti-inflammatory and wound healing (Suguna *et al*., 1996), antileprotic (Sahu *et al*., 1989), antistress (Sharma *et al*., 1996; Appa Rao *et al*., 1977), antifungal (Singh *et al*., 1999 and 2000), anticancer (Babu *et al*., 1995) and antioxidant (Zainol *et al*., 2003) properties and is used as a tonic in ayurvedic formulations. It is also used as an anti-diarrheic, anti-dysentheric, circulatory stimulant and hypotensive (Goh *et al*., 1985).

Centella asiatica has a high level of phenolic compounds and these compounds are believed to be responsible for the antioxidative activity (Hussin *et al*., 2009). The herb was reported to have abundant phenolic compounds that are majorly from flavonoids such as quercetin, catechin, epicatechin, rutin, luteolin, myricetin, kaempferol and naringenin (Hussin *et al*., 2009; Mustafa *et al*., 2010). The phenolic hydroxyl group in flavonoids is found to be a strong antioxidant capable of effectively scavenging reactive oxygen species (Cao *et al*., 1997).

1.6. Chemical Constituents of *Centella asiatica*

The chemical constituents of *Centella asiatica* are mainly terpenes (monoterpenes, sesquiterpenes, diterpenes, triterpenes, tetraterpenes), phenolic compounds (flavonoids, phenylpropanoids, tannins), polyacetylenes group, alkaloids, carbohydrates, vitamin, mineral and amino acid (Chong and Aziz, 2011; Brinkhaus *et al*., 2000; Zheng and Qin, 2007; Jacinda and Ian, 2009). Chong and Aziz, (2011) reported that terpene was the dominant group of chemical constituents in *C. asiatica*. The monoterpenes identified existed in acyclic, monocyclic and bicyclic frameworks while the sesquiterpenes reported in the studies also existed in tricyclic structures in addition to acyclic, monocyclic and bicyclic structures. The triterpenes in *C. asiatica* are pentacyclic triterpenes and the two types of pentacyclic triterpenes reported are ursane and oleanane. Some of these pentacyclic triterpenes carry sugar moieties and are known as pentacyclic triterpenes saponins. Saponines is a major and the most important component of *C.asiatica*, regarded as a marker constituent in terms of quality control. The saponines obtained from *C. asiatica* are mainly pentacyclic triterpenic acids and their respective glycosides, belonging to ursane or oleanane type, including asiatic acid, asiaticoside, madecassic acid, madecassoside, brahmoside, brahmic acid, brahminoside, thankuniside, isothankuniside, centelloside, madasiatic acid, centic acid, cenellic acid, betulinic acid, indocentic acid, etc.

They are most reported chemical compounds were asiaticoside, madecassoside, asiatic acid and madecassic acid (Brinkhaus *et al*., 2000; World Health Organisation, 1999; Jacinda and Ian, 2009). *C. asiatica* is characterised by the presence of essential oil. The essential oil of *C. asiatica* is made up of a wide variety of monoterpenes and sesquiterpenes. According to Yoshida *et al*., (2005), reported that *C. asiatica* was a widespread plant and they produce phenylpropanoids. Flavonoids were another group of phenolic compounds found in *C. asiatica* apart from phenylpropanoids. Being the chief bioactive substances in *C. asiatica*, triterpenoid derivatives play an important role in the aspect of medicinal application.

1.7. Overview on Plant Bioactive Compounds

Therapeutic value of medicinal plants is due to specific constituents or combination of secondary metabolites present in them. Changes in the proportion of secondary metabolites are often required for the improvement of therapeutic values of medicinal plants. The biosynthetic pathways for the biologically active chemical compounds in medicinal plants are usually complex and high branches. Secondary metabolisms are natural products that often have an ecological role in regulating the interactions between medicinal plants and their environment (Hanson, 2003). The secondary metabolites are characterized by an enormous chemical diversity, every organism has its own characteristic set of secondary metabolites, some of which they may share with other related or totally unrelated organisms (Verpoorte, 1999). Although only 20-30% of higher plants have been investigated so far, tens of thousands of secondary metabolites have already been isolated and identified (Wink, 1999).

Over the past decade, it has become evident that secondary metabolites are not just waste products or otherwise functionless molecules. In fact, the opposite is the case: most secondary metabolites have an important role in the plants producing them. Still our knowledge about the role of the secondary metabolites is limited, but now it is generally accepted that secondary metabolism is involved in the organism's interaction with its environment, e.g. in resistance against pests and diseases, as attractant of pollinators, or as a signal compound (Croteau *et al*., 2000). Secondary metabolites are therefore ultimately important for the fitness of the plant producing them. However, the chemical diversity and the limited knowledge of the role secondary metabolites play in plants, hamper efforts to more sharply define the group.

In this view secondary metabolites are essential for an organism to survive as a species in its ecosystem. Thus, secondary metabolites are closely related to the evolutionary and adaptative ability of a given plant in an endlessly changing environment (Facchini, 1999; Hartmann, 1996). Therefore, most plant species can be characterized by a unique and distinct metabolic fingerprint that explains, in part, the complexity of chemical structures, wide distribution, and variability of secondary metabolites in the plant kingdom (Souret, 2002; Memelink *et al*., 2001; Rhodes, 1994). Individualized metabolite chemistry also implies the existence of both highly regulated basic pathways leading to the biosynthesis of key metabolites that can be suited for biochemical diversification, and specific biosynthetic enzymes that can catalyze the unlimited chemical modifications of these key metabolites, including glycosilation, sulphanation, hydroxylation, o-methylation, esterification, etc., (Souret, 2002; Dixon, 1999).

1.8. Medicinal and Commercial Importance of Bioactive compounds

Natural products once served humankind as the source of all drugs, and higher plants provided most of these therapeutic agents. Today, natural products still represent over 50% of all drugs in clinical use, with higher plant-derived natural products representing 25% of the total (GEN, 1998; Balandrin *et al*., 1993). The World Health Organization estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care, and about 85% of traditional medicine involves the use of plant extracts (Word Health Organisation, 2002). This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs (De Smet, 1997). In the United States plant-derived drugs represent about 25% of the prescription drug market, and in the last decade this equated to a retail value of approximately $15.5 billion (Pezzuto, 1997). From 1983 to 1994 39% of the New Approved Drugs were of natural origin, including original natural products, products derived semi synthetically from natural products, and synthetic products based on natural product models (Cragg *et al*., 1997).

Further evidence of the importance of natural products is provided by the fact that almost half of the world's 25 best selling pharmaceuticals are either natural products or their derivatives (Pezzuto, 1997). Conservative estimates suggest that there are more than 250,000 species of higher plants existing on this planet, and only a very small percentage of plants have been exhaustively studied for their potential value as a source of drugs. Obviously natural products will continue to be extremely important as sources of medicinal agents. The importance of medicinal plant secondary metabolites in medicine, agriculture and industry has led to numerous studies on the biological activity, biochemical analysis, synthesis and biosynthesis. Secondary metabolite lies in the organic molecules possessing pharmacological properties that are produced by them with the advent of new analytical tools and sophisticated instrumental technology. It has become possible to carry out practicable quality assurance profiles for a crude drug, its bioactive constituents and the formulations themselves. Some modern techniques such as UV -Spectroscopy, IR-Spectroscopy, Atomic absorption spectroscopy, Thin Layer Chromatogrphy (TLC), High Performance thin layer chromatography (HPTLC) and High performance liquid chromatography (HPLC) can be very well utilized for the development of chemical markers which, in turn, can be used for identification and quality assurance (Joliffe, 1997). In addition to the natural products which have found direct medicinal application as drug entities, many others can serve as chemical models or templates for the design, synthesis, and semi synthesis of novel substances for treating humankind's diseases. Such clinically useful drugs include the anticancer agents paclitaxel and vincristine the sedative scopolamine, and the muscle relaxant tubocurarine (Faccini, 2001). These substances embrace some of the most exciting new therapeutic agents currently available for use in a clinical setting. Although there are some new approaches to drug discovery, such as combinatorial chemistry and computer-based molecular modeling design, none of them can replace the important role of natural products in drug discovery and development (Pezzuto, 1997).

1.9. Triterpenoid Saponins of *Centella asiatica*

Several research workers have reported different biological activities of *Centella asiatica* in various *in vitro* and *in vivo* test models. Therapeutically active compounds of the *Centella asiatica* to produce the secondary metabolites. They are produces as byproducts during metabolism of the plants. *Centella asiatica* contains variety of active compounds; these are usually either genera or species specific. The main active components of the plant are believed to be triterpenoids. Several studies have revealed the triterpenoid derivatives of *Centella asiatica* using different techniques (Diallo *et al*., 1991; Du *et al*., 2001). The first clinical investigation on the medicinal application of this plant and its extracts were completed during the early 1940's. A preliminary approach to the identification of the active compounds of *Centella asiatica* by TLC can be found in European Pharmacopoeia (2005) using drug powder (dried plant parts) as the starting material. A HPLC method was set for quantitative determination of six triterpenes in *Centella asiatica* extracts and commercial products (Schaneberg *et al*., 2003;

Anjana *et al*., 2010). Extract of *C. asiatica* has been formulated into commercial medicinal preparations and are available over-the-counter across the world. The commercial preparations contain TECA (titrated extract of *Centella asiatica*), TTFCA (total triterpenoid fraction of *Centella asiatica*) and TTF (total triterpenic fraction). The European Medicines Agency confirms that while different acronyms are used to describe the content of these preparations, they do in fact describe the same extract.

Centella plant contains variety of therapeutically active compounds, but the active ingredients are asiaticoside (a triterpene glycoside) madecassoside (a glycoside with strong anti-inflammatory properties), brahmoside and brahminoside (both saponin glycosides), madecassic acid, thiamine, riboflavin, pyridoxine, vitamin K, asparate, glutamate, serine, threonine, alanine, lysine, histidine, magnesium, calcium and sodium (Anonymous, 2004). The major therapeutically active compounds of *Centella asiatica* are asiaticoside, madecassoside, asiatica acid and madecassic acid. All the constituents of *C. asiatica*, the most investigated constituent is asiaticoside. *Centella* leaves also contain highly valuable triterpenoid saponins, including oxyasiaticoside, centelloside, brahminoside, thankuniside and isothankuniside and related sapogenins from various chemotypes. They also contain triterpenoid acids like brahmic acid, isobrahmic acid and betulic acid (Sakshi *et al*., 2010; Shakir *et al*.,2007; Inamdar *et al*., 1996; Sing and Rastogi, 1968, 1969; Rasatogi *et al*., 1960; Bhattacharya, 1956 a and b) and triterpenoid glycosides like asiaticoside-C,D,E and F (Zing-Yong Jiang *et al*., 2005).

Various medicinal properties of *Centella asiatica* are ascribed due to active principle compounds saponins and sapogenins like asiaticoside, asiatic acid, madecassoside and madecassic acid (Pointel, 1987).

1.10. Essential of the Present Study on *Centella asiatica*

According to the reports of export and import bank of India *Centella asiatica* is one of the important medicinal plants in the international market of medicinal plant trade. However, at present the increased demand for *Centella* has risen sharply due to the increased popularity of the new mandukparni based drugs.

Because of large scale and unrestricted exploitation to meet its ever increasing demand by the pharmaceutical industry, the population of mandukparni is dwindling in nature. Furthermore, limited cultivation and insufficient attempts for its replenishment have resulted into the depletion of the wild stock. It has now been included in the list of threatened species by the international union of conservation of nature and natural resources and also in the list of endangered species (Pandey *et al.,* 1993; Singh, 1989; Sharma and Kumar, 1998). Unfortunately, low amounts of secondary metabolism contain like, asiaticoside, madecassoside, asiaticoside-B, asiatic acid, madasiatic acid etc. can be isolated form the leaves of the of *Centella asiatica*. Many researchers have attempted to improve the production of these secondary metabolisms through cell or tissue culture (Tanavat *et al*., 2011, Aziz *et al*., 2007; Susana *et al*., 2008; Kim *et al*., 2002; Baek, 1997).

In 1990, the estimated annual requirement of *Centella asiatica* was around 12,700 tonnes of dry biomass valued at Rs.1.5billion (Ahmad, 1993). According to Suresh Wagh (2006) the demand of Brahmi during the year 2001-2002 was 3,822.5 metric tonnes. In the year 2004-05 it was 6,621.8 metric tonnes. He has predicted an increase in demand of *Centella asiatica* by 20.1 % per annum. In recent years, the growing demand for herbal products has led to a quantum jump in volumes of plant material treaded within and across countries. Considering the word wide demand of the *Centella asiatica* plants, there is an urgent need to develop high yielding varieties having superior and desirable quality. Many pharmaceutical companies are now focusing their attention on genetic improvement of *Centella asiatica* so that the plants could yield higher quantities of medicinal compounds.

1.11. Strategies to Increase Secondary Metabolite Production in *Centella*

Artificial polyploidy is an alternative technique for increasing productivity of secondary metabolites in a variety of plant species (Dhawan and Lavania, 1996). Often the polyploid plants are bigger due to increased complement of the chromosomes (Gao *et al*., 1996; Lavania, 1988), the phenomenon which may enhance the accumulation of commercially important bioactive compounds. This was the case when artificial autotetraploids were produced by colchicine treatment in the essential-oil-bearing vetiver grass (*Vetiveria zizanioides* L. Nash). Tetraploids were vigorous with thicker and longer roots, and the percent of essential oil increased from 0.98% to 1.4% in freshly harvested roots of tetraploid plants compared to the control (Lavania, 1988). Similarly, roots of a diploid medicinal plant, *Salvia miltiorrhiza*, contained higher amounts of tanshinones as a result of colchicine treatment due to the autotetraploid chromosome complement (Gao *et al*., 1996).

Experimentally induced tetraploid vegetables may have increased vigour and produced larger fruits and higher yields. Polyploidy is often induced in plants of economical interest in order to produce variability that can improve the yields (Allard, 1960). This approach has been applied to Coffee (Cruz *et al*., 1993), orange (Romero - Aranda *et al*.,1997), *Nicandra physaloides* (Gupta and Roy, 1986) and *Clitoria ternate* (Gandhi and Patil, 1997). Induced polyploids of certain medicinal plants are known to produce higher amounts of therapeutically active compounds. Several authors have reported 50% higher contents of solasodine in the induced autotetraploids of *Solanum viarum* as compared to their diploid progenitors (Bhatt and Heble, 1978; Maheshwar, 1983; Krishnan, 1995). Krishnan, (1998) has also emphasized that chromosome doubling through induction of autotetraploidy is the only successful avenue for genetic up gradation of solasodine content in the berries of this plant. A similar 154 % increase in the alkaloid content in autotetraploid plants of *Atropa belladonna* as compared to diploid plants has been reported by Jackson and Rawson, (1953). Gao *et al*., (2002), have reported higher productivity of baicalin in autotetraploid plants of *Scutellaria baicalensis* as compared to their diploid counterparts. Colchicine induced polyploids in *Salvia miltiorrhiza* showed much higher contents of tanshinone than their diploids (Gao *et al*., 1996). Similar increase in alkaloid content has also been reported in autotetraploid plants of *Datura* by Rawson, (1944). These reports clearly indicate that secondary metabolite production can be increased by induction of autotetraploidy using colchicine.

In the present investigation an attempt is made to induce autotetraploidy in *C. asiatica* employing colchicine with an objective of improving the yield of therapeutically active compounds. Colchicine has been used for chromosome doubling of many crops including *Solanum incanum* (Anaso and Uzo, 1991), blueberry (Perry and Lyrene, 1984), and coffee (Nsumbu, 1979). Even though most crop plants have been exposed to this chemical in attempts to improve their production, little or no research has been applied to medicinal plants especially to brahmi (*Centella asiatica* (L) Urban). Selection is one of the age old practices followed for breeding crop plants. In its simplest form, selection is, choosing plants of one's choice.

The genetic basis of selection is the genetic diversity arising out of spontaneous mutations and its subsequent incorporation into populations through natural hybridization and recombination over generations. Thus choosing the best ones out of the large population, continued over generations, results in the development of superior genotypes (Chaudhary, 1997). Clonal selection is selection of elite clones exhibiting desired phenotypic traits among the total population. So in this plant efforts are also made to exploit this breeding method to obtain superior phenotypes which are potentially capable of yielding higher amounts of therapeutically active compounds. The applications of *in vitro* somatic cell genetic methods which include a combination of approaches seem very promising, though the clonal uniformity of elite genotypes was originally believed to be one of the principal applications of plant tissue culture. In recent years, there has been an increased interest in *in vitro* culture techniques which offer a viable tool for mass multiplication and germplasm conservation of rare, endangered and threatened medicinal plants (Ajithkumar and Seeni, 1998; Sahoo and Chand, 1998; Prakash *et al*;, 1999). Tissue culture techniques can play an important role in the clonal propagation of elite clones and germplasm conservation of *C. asiatica*.

However, during last few years *in vitro* technology has proven its potential and feasibility for the induction of genetic variability in various crops and plant species of medicinal and aromatic values. Induction of somaclonal variations in medicinal plants seems to be a promising area, aimed at genetic improvement of plants. Pharmaceutical companies largely depend upon material procured from naturally occurring stands which are being depleted rapidly raising concern about possible extinction of the species. This provides justification for the development of *in vitro* propagation of this plant. With this objective in mind we have attempted to generate genetic variability through *in vitro* cultures and produce somaclonal variants in *C. asiatica* so that promising variants can be isolated.

1.13. Objectives of the Present Research

In many plant species, chromosome doubling is accompanied by conspicuous changes in the secondary metabolism of plants as well as the primary metabolism (Levin, 1983). Some of these changes include an overall increase in enzymatic activity, isozyme diversity, and alteration in plant phytochemicals etc., which may lead to enhanced production and certain qualitative changes in the biosynthesis of secondary metabolites (Dhawan and Lavania, 1996; Levin, 1983). Such inherent potential could be exploited by resorting to experimental manipulations to achieve commercial gains with respect to the production of useful secondary metabolites.

There has been tremendous emphasis on breeding of medicinal plants, especially those, which are used in traditional system of medicine and folk medicine during the last 15 years. Constant efforts are made to explore alternate ways for improving the medicinal plants for their therapeutically active compounds. Genetic improvement of *C. asiatica* for higher yields of major saponins viz. asiaticoside, madecassoside, asiaticoside-B, madecassic acid and asiatic acid has been the major objective of the present investigation. For achieving this objective we wish to explore all experimental possibilities to improve the quantity of medicinal compounds well known for their therapeutic activity in *C. asiatica*, employing popular methods of plant breeding like, induction of autotetraploidy and induction of somaclonal variations, which have been widely accepted as a supplementary approaches for genetic improvement of crop plants. The present work has been undertaken with the following objectives:

1. To assess adequate colchicine concentration and exposure times for induction of autotetraploidy of *Centella asiatica* plants.

2. To verify that autotetraploidy in *Centella asiatica* plants have been obtained.

3. Induction of somaclonal variations in *Centella* through *in vitro*

techniques and if successful, isolate the somaclones showing desired traits,

4. Propagation of control, autotetraploid as well as somaclonal elite clones for several generations in the field and analyzing them for morphometric traits like plant height, leaf area, internodal length, petiole length, fresh and dry weight of 100 leaves and number of stomata per unit area,

5. Quantitative estimation of major saponins viz., asiaticoside, madecassoside, asiaticoside B, madasiatic acid and asiatic acid in the control and induced autotetraploid plants as well as elite clones obtained through somaclonal variation.

1.14. Significance of the Present Research

Genetically improved, high yielding *Centella asiatica* plants will immensely help us in increasing the yield of therapeutically active compounds like Asiatic acid, Medecassic acid, Terminolic acid, and Asiaticoside. These superior, novel and improved varieties of *Centella* can be made available to the local farmers whose main occupation is cultivation of medicinal plants and supplying them to the Ayurvedic and Homeopathic manufacturing concerns. This will therefore be, immensely helpful to both farmers and to the Ayurvedic and homeopathic manufacturing concerns.

The present work will also help us in establishing the fact that the technique of ploidy breeding holds great promise for increasing the yield of therapeutically active compounds of large number of important medicinal plants.