Medicinal plants are a precious natural resource, as they provide raw material for pharmaceutical industry, modern and traditional forms of medicine and generate employment and income.

Medicinal plants are precious part of the world flora. More than 80,000 species out of the 2,50,000 higher plant species on earth are reported to have at least some medicinal value and around 5,000 species have specific therapeutic value. The contemporary phytotherapy and the modern allopathic medicine use raw materials from more than 50,000 plant species (WHO, 2011).

In India, medicinal plants constitute a very important bio resource and has one of the richest plants based tradition in the world being home to around 20,000 medicinal plant species (Hasan, 2009). India is the largest producer of medicinal herbs and is rightly called the “botanical garden of the world” (Umadevi et al, 2013).

India is a home of one of the mega centres of biodiversity of which medicinal plants form an important group. Ayurveda contributes Rs 3,500 crore annually to the international market. Medicinal plant based industries in India are growing at the rate 7-15 % annually, whereas the global herbal drug market is expected to grow to US $ 5 trillion by the year 2050 (Joshi et al., 2004). Plants have been the source of many compounds that are indispensable to food, medicine, and industry. Over 80% of the approximately 30,000 known natural products are of plant origin (Hamideh, 2012, Vimala et al, 2014). The global market for medicinal plants and herbal medicines is estimated to be worth of US $ 80 billion a year. International export trade in medicinal plant for India is 32,600 tones. The demand for medicinal plant has increased globally due to resurgence of interest in herbal medicine standardized plant extract, natural therapeutic essential oil and phytopharmaceuticals (Jabeen et al., 2007).

The consumption of herbal medicines is increasing world over, due to benefits it offer. As per world health organisation (WHO), nearly 80% of the world population in the developing countries relies primarily on herbal medicines for meeting their health care needs (Vines, 2004; Sharma and Arora, 2006; Arora, 2010). Globally, approximately two thirds of the total of 50,000-70,000 plant species used for medicinal purpose are collected from the wild (Schippmann et al., 2006), and in Europe only 10% of the medicinal species used commercially and cultivated (Vines,
Chapter 1

Introduction

Unfortunately, the limited quantity of active metabolites in the plants, slow growth rate and destruction of natural supplies are problems encountered when exploiting plants for medicinal needs. Due to the crude and unscientific extraction methods and absence of any method to cultivate them, the rate of exploitation may exceed the rate of natural regeneration. Thus, there is need to develop propagation as well as conservation strategies for medicinal plants, otherwise, most of the medicinal plants might become extinct while many of them at present fall in the category of endangered species (Kaur et al., 2007). Therefore, alternate sustainable and renewable production systems are urgently needed to protect and preserve plant diversity.

*Podophyllum hexandrum* Royle, commonly known as Himalayan Mayapple, Indian Mayapple or Andiri (a divine drug) is well known for a valuable drug podophyllotoxin which is effective against various diseases being purgative, laxative, cholagogue, emetic and also useful against warts and tumours, growth of skin and anticancer (Chaurasia, 2012). It belongs to the family Berberidaceae and is medicinally important herb.

Classification:

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Ranunculales
Family : Berberidaceae
Genus : *Podophyllum*
Species : *hexandrum*

*Podophyllum hexandrum* Royle is an endangered medicinal herb. In India, *Podophyllum hexandrum* is mostly found in Alpine Himalayas (3000-4000 metre) of Jammu and Kashmir, Himachal Pradesh, Sikkim, Uttarakhand and Arunachal Pradesh (Qazi et al., 2011).

It prefers to grow on dampish shady spots. It has perennial rhizome, while annual aerial parts are annual, emerging in the middle of April. The flower appears in May, the fruit ripens in August or September (Chatterjee, 1952). The plant bears 1 or 2
flowers; consist of 3 sepals, 6 petals and 6 stamens. The two leaves are placed alternate on the flowering stem with petioles of 10-20 cm long. The leaves are circular and 12 to 25 cm across, deeply 3 to 5 lobed, toothed, often spotted and rugose. The fruit is the only part of plant that is not toxic, therefore, edible but insipid (Chatterjee, 1952; Hutchison, 1959; Rix, 1982; Dewik and Shaw, 1988). The chromosome number of *Podophyllum* has been determined to be 2n=12 (Meacham, 1980). *Podophyllum hexandrum* is recognised for its anticancer properties. It has been used by the Himalayan natives and the American Indians (Anon, 1970). The rhizome yields very valuable drug ‘podophyllotoxin’ which has a great demand in pharmaceutical industries throughout the world. The rhizomes and roots of the plant contain anti tumor lignans such as podophyllotoxin, 4’-demethyl podophyllotoxin and podophyllotoxin 4-O-glucoside (Tyler et al., 1988; Broomhead and Dewick, 1990). Among the lignans, American *Podophyllum* contains 4–5% *Podophyllum* resin, whereas Indian species contains 7-16% (Qazi et al., 2011). The variation in percentage of resin is attributed to seasonal differences, different sites of growth and age of the plant (Purohit et al., 1999).

In the modern allopathic system of medicine, the plant has been successfully used for the treatment of various disorders, monocytoid leukaemia, hodgkin’s lymphoma, bacterial and viral infections (Gowdey and Carpenter, 1995), venereal warts (Beutner and Krog, 1990) rheumatoid arthalgia associated with the numbers of the limbs and pyogenic infection of skin tissue, AIDS associated Kaposi sarcoma and different cancers of brain, lung and bladder (Blasko and Cordell, 1998).

In the search of novel, effective and non toxic radio protectants, number of plant products have been evaluated for the plant protection against lethal dose of radiation including *Podophyllum hexandrum* (Goel et al., 1998; Rajesh et al., 2005) and it is found that pre-radiation administration of the extracts of *Podophyllum hexandrum* mitigated radiation induced postnatal and physiological alternations and highly effective in control of both planned and unplanned radiation exposure (Goel et al., 2002). Recently, *Podophyllum hexandrum* extracts have been reported to offer radioprotection by modulating free radical flux involving the role of lignans presents (Chawla et al., 2006).

Due to its anticancerous property, podophyllotoxin is in increased demand throughout the world. Total synthesis of podophyllotoxin is an excessive process and availability
of compound from natural resource is an important issue for pharmaceutical companies that manufacture these drugs (Canel et al., 2000). The annual supply is at present estimated as 50-80 tonnes, while the demand is more than 100 tonnes (Alam et al., 2009). To meet this ever increasing demand of crude drug, the rhizomes of *Podophyllum hexandrum* are being indiscriminately collected in large quantities. As, a result *Podophyllum hexandrum* is reported as endangered species in Himalayan region.

Therefore, plant cell and tissue cultures hold great promise for controlled production of a variety of useful secondary metabolites (Vijayasree et al., 2010). In the search for alternatives to production of medicinal compounds from plants, biotechnological approaches, specifically plant tissue cultures are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites.

Cell suspension culture systems are used now a day for large scale culturing of plant cells from which secondary metabolites could be extracted. A suspension culture is developed by transferring the relatively friable portion of the callus into liquid medium and is maintained under suitable conditions of aeration, agitation, light, temperature and other physical parameters (Chattopadhyay et al., 2002). Cell cultures cannot only yield defined standard phytochemicals in large volumes but also eliminate the presence of interfering compounds that occur in the field-grown plants (Lila et al., 2005). The advantage of this method is that it can ultimately provide a continuous, reliable source of natural products (Rao et al., 2002). The major advantage of the cell cultures include synthesis of bioactive secondary metabolites, running in controlled environment, independently from climate and soil conditions (Karuppusamy et al., 2009). A number of different types of bioreactors have been used for mass cultivation of plant cells.

Therefore, the biotechnological production of Podophyllotoxin using plant cell culture derived from *Podophyllum hexandrum* may be an attractive alternative. Podophyllotoxin content are prone to changes due to environment factors of different ecoregions and stage of harvest. These changes could be controlled by *in vitro* culture of *Podophyllum hexandrum* for the synthesis of lignan Podophyllotoxin.
Keeping this background information in view, it was proposed to study the production of *Podophyllum hexandrum* lignan *i.e.* podophyllotoxin by using callus and cell culture for commercial uses and thereby helping in conserving the highly endangered medicinal plant of the Himalayas.

Hence, the present studies have been carried out with the following objectives:

1. Standardization of *in vitro* culture for *Podophyllum hexandrum*.
2. *In vitro* optimization of cell proliferation.
3. Production of podophyllotoxin in callus and cell cultures.
4. Use of enhancers and elicitors for podophyllotoxin production.