CHAPTER 1

General introduction
Biodiversity encompasses the variety of all forms of life on earth. India is one of the 17 mega biodiversity nations of the world. With just 2.5% of the land area, India represents 7.8% of the recorded species of the world. Previous studies indicated that, 45,500 species of plants are found in India, out of this around 15,000 - 20,000 plants have great medicinal properties and are being utilized by traditional practitioners whether directly as folk remedies or the medicaments of the distinctive indigenous frameworks of medicine, such as, Ayurveda, Siddha, Unani and so on or indirectly in the pharmaceutical preparations of modern medicine (Pushpangadan et al. 1997; Ballabh and Chaurasia 2007; NBA 2015).

Plants have been employed as an exemplary source of therapeutics for thousands of years (Singh et al. 2011). It is evaluated that up to 80% of the people still depend for the most part on traditional remedies, for example, plants for their medicines. Plants form a source of many modern medicines also (Pezzuto 1997). It is assessed that around one quarter of prescribed drugs contain plant concentrates or active ingredients acquired from plant materials. The most well known pain relieving, aspirin, was originally derived from species of Salix and Spiraea. Probably the most significant anticancer drugs, such as, paclitaxel (Taxus brevifolia) and vinblastine (Catharanthus roseus) are derived exclusively from plant sources (Ekor 2014).

Plant secondary metabolites such as alkaloids, flavanoids, saponins, phenols, steroids and tannins are the key active ingredients of Ayurvedic drugs and restorative preparations. The medicinal plants, besides having natural therapeutic values against various diseases, also provide high quality food and raw materials for livelihood. Right now the pharmacologically active components of numerous Ayurvedic drugs are being distinguished and their usefulness as drugs was determined. Hence the traditional knowledge with its holistic and systematic approach supported by scientific documentation can serve as an innovative and powerful discovery engine for newer, safer and
affordable medicines (Azaizeh et al. 2003). The consumption of herbal medicines is increasing worldwide, primarily due to the benefits it offers. Information provided by conventional medicine has been of extraordinary worth for the disclosure of numerous new drugs or drugs from new sources and hundreds of pharmacologically active compounds for synthetic modifications (WHO 2013).

1.1 The Western Ghats

The Western Ghats form a more or less continuous mountain chain over a distance of about 1600 km along the western coast of India. The western slants and summits of these reliefs experience the full power of the summer monsoon, which, as an after effect of orographic impact, brings abundant precipitation, occasionally more than 7000 mm at few places. Such conditions have enabled the growth of dense, humid forest formations from the coast up to the summit of the Ghats. Then again, the climatic conditions are not uniform all through the Ghats. Since the rainstorm arrives from the south and retreats in the opposite direction, the rainy season is longer in the south than in the north of the Western Ghats (Pascal et al. 2004).

The Western Ghats covers scarcely 5% of India's land area, however its biological richness can be best comprehended when one understands that 27% of the considerable number of species of higher plants recorded in the India are found here (around 4,000 of 15,000 species) (Nayar 1996).

The evergreen forests of the Western Ghats are characterized by a high percentage of species endemic to this region. The aggregate number of endemic plant species is evaluated to be 1800 (MacKinnon and MacKinnon 1986; IISc 2015). Among the evergreen tree species, 56% are endemic. In this way, the Western Ghats are considered as one of the biodiversity hot spots of the world (Myers 1988).
The Western Ghats is also characterised by a series of forest gaps or breaks, that are actually valleys that break the continuity of the mountain ranges and accordingly of the biological components as well. The major ones are the Palghat Gap, the Moyar Gorge or Gap and the Shencottah Gap. These series of gaps have resulted in preventing the spread of certain species and have hence, facilitated local speciation and endemism. The associated mountain ranges such as the Anamalais, the Nilgiris and the Agastyamalais are all separated by clear-cut barriers and besides the interesting floral speciation, a distinct faunal endemism and/or local speciation, are also found. Areas such as this are in urgent need of study and documentation (IISc 2015).

The Western Ghats is extremely rich in its therapeutic wealth. The forests and hills of this region is a treasure house of about 700 medicinal plants. Out of which some are utilized for conventional and folk restorative practices. Many are exploited commercially for their active enzymes and their commercial value. Restorative plant types of Western Ghats represent a variety of life form extending from lichen, algae, herbs, shrubs, climber and trees, which are annuals to perennials. The autecology and synecology of medicinal plant species is complex and their proper understanding requires a sound knowledge on ecology, taxonomy and ethno botany of these species. The limited knowledge on the varied use of the medicinal plants, their availability and extent of distribution are the limiting factors to utilize these resources efficiently. Therefore, it is necessary to bring the information in various sources into one roof (WGBIS IISc 2015).

1.2 Plants and antioxidant properties

The screening for antioxidant properties of therapeutic and food plants have been performed progressively throughout the previous couple of decades with the objective of discovering a productive solution for a few present-day diseases and intends to delay aging side effects (Halliwell 2008).
Oxidation process is one of the most important routes for producing free radicals in food, drugs and even living systems. Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydroperoxide to non-radical structures and capacity as natural antioxidants in human body. Oxidizing agents may damage a number of biological molecules such as nucleic acids, membrane lipids, enzymes, or synovial fluid polysaccharides. Due to depletion of immune system in different illness, consuming antioxidants as free radical scavengers may be necessary (Cook and Samman 1996).

The disorders related to excessive oxidation of cellular substrates (oxidative stress) include type II diabetes, neurodegenerative diseases, or even some types of cancer. There is a tremendous demand for natural antioxidants in food industry, for replacing the synthetic additives used to forestall fat rancidity or colour loss. The oxidative deterioration of the lipid containing food is responsible for the rancid odours and flavours during processing and storage, consequently decreasing the nutritional quality and safety of foods, due to the formation of secondary, potentially toxic compounds. The addition of antioxidant is also form a method for increasing the shelf life of foods (Cook and Samman 1996).

1.3 Antibacterial properties of plants

Herbal medicines are gaining priorities in treating various health ailments of diverse origin in man. Before the inventions of the modern synthetic medicines, man’s dependence was totally on plants. Traditional systems of plant based products have existed with the changes in culture, traditions and mode of life of man; except for a short period when the inventions of the modern synthetic medicines came into existence. Plant based antimicrobials have tremendous therapeutic potential as they can fill up the need without any side effects that are frequently connected with synthetic antimicrobials. Continued exploration of plant derived antimicrobials is needed today (Hussain and Gorsi 2004).
The pathogenic microorganisms can be controlled with the antibiotics presently available; however the need of new antibiotics has increased due to current problems of resistance associated with them (Davies 1994; Prabhat et al. 2010). The drug resistant bacteria and fungal pathogens have complicated the treatment of infectious diseases. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms has necessitated a search for new antimicrobial substances from other sources including plants. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Harbone and Baxter 1995). The substances that can either inhibit the growth of pathogen or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs.

The antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Saxena and Sharma 1999). Plants possessing potential antimicrobial compounds are being discovered as one which is new or a good source for already known phytopharmaceuticals. Therefore, we attempted to screen plants which are using traditionally for various ailments especially against infectious diseases.

1.4 Phytochemical principles from plants

Herbal medicines are used in treating different health ailments of diverse origins in man. Since, time immemorial man has been utilizing plants as medications to cure diseases. Prior to the inventions of the modern synthetic medicines, man's reliance was absolutely on plants. Traditional systems of plant based products have existed with the changes in culture, traditions and mode of life of man except for a short period when the inventions of the modern synthetic medicines came into existence. However, the glory and frame of the traditional plant based medicinal practice still exists, because of their desirable factors such as additional alternative therapy for various ailments (Kochuthressia 2012).
The remarkable component of traditional medicine in India is that it prospers at numerous levels. In present day medicinal practices, secondary metabolities of plants constitute a much more essential source of advanced pharmaceutical drug, and they are turning into an increasingly valuable commodity in expanding market for herbal remedies. Investigations into the chemical and biological activities of plants during the past two centuries have yielded compounds for the development of modern synthetic organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents (Roja and Rao 2000). To date, primary focus of research on medicinal plants has been in the area of phytochemistry and pharmacognosy. In the area of phytochemistry, medicinal plants have been characterized for their possible bioactive compounds, which have been separated and subjected to detailed structural analysis. The identification of biologically active compounds is an essential requirement for quality control and dose determination of plant based drugs. Research in the pharmacognosy of medicinal plants has also involved assays of bioactivity, determination of potential mode of action and target site for active phytomedical compounds (Briskin 2000).

1.5 The need of genetic analysis in plants

A major concern regarding forests health and resilience is the declining in forest genetic diversity as documented as early as 1967 by Food and Agriulture Organization of the United Nations (FAO). Genetic diversity serves several important purposes: (a) as a resource for tree breeding and improvement programs to develop well-adapted tree species varieties and to enhance the genetic gain for a multitude of useful traits; (b) to ensure the vitality of forests as a whole by their capacity to withstand diverse biotic and abiotic stressors under changing and unpredictable environmental conditions; and (c) the livelihoods of indigenous and local communities that use traditional knowledge. Rich genetic diversity within and among forest tree species thus provides an
important basis for maintaining therapeutic and food security and enabling sustainable development (FAO 2013).

Molecular markers have proven to be invaluable tools for assessing plants’ genetic resources by improving our understanding with regards to the distribution and the extent of genetic variation within and among species. Recently developed marker technologies allow the uncovering of the extent of the genetic variation in an unprecedented way through increased coverage of the genome. It provide an accurate assessment of the extent of intra and inter population genetic diversity of naturally distributed plant species on which proper conservation directives for species that are at risk of decline can be issued. This adaptive genetic diversity constitutes important potential for future forest management and conservation purposes (Porth and El-Kassaby 2014).

The assessment of genetic diversity within and between plant populations is routinely performed using various techniques such as (i) morphological, (ii) biochemical characterization/evaluation (allozyme) and (iii) DNA (or molecular) marker analysis. Nowadays molecular markers are widely used for assessing genetic diversity within and among species. Molecular markers may or may not correlate with phenotypic expression of a genomic trait. They offer numerous advantages over conventional, phenotype-based alternatives as they are stable and detectable in all tissues regardless of growth, differentiation, development, or defense status of the cell. Additionally, they are not confounded by environmental, pleiotropic, and epistatic effects (Govindaraj et al. 2015).

Molecular markers may be broadly divided into three classes based on the method of their detection: hybridization based, polymerase chain reaction (PCR) based, and DNA sequence based. At present polymerase chain reaction (PCR) based marker systems are more rapid and require less plant material for DNA extraction. Random amplified polymorphic DNAs (RAPDs) were the first of PCR-based markers and are produced by PCR machines using genomic DNA
and arbitrary (random) primers which act as both forward and backward primers in creation of multiple copies of DNA strands. The advantages of RAPDs include being quick and simple and inexpensive and the facts that multiple loci from a single primer are possible and a small amount of DNA is required (Welsh and McClelland 1990; Collard et al. 2005; Jacobson and Hedrén 2007).

1.6 Micropropagation and its role in conservation

Micropropagation is the true-to-type propagation of selected genotype using in vitro culture techniques. One of the main applications of micropropagation is the mass propagation of superior plants. In many instances, conventional propagation is a slow process during which diseases and pest problem can limit production. Micropropagation offers the possibility to deliver thousands or even billions of plants every year (Debergh and Read 1991).

Micropropagation permits the generation of large number of plants from cells, tissues or organs of stock plant in generally brief period. Contingent upon the species, the first explant is taken from shoot tip, leaf, lateral bud of stem or root tissue. Once the explant is put on an appropriate culture medium, proliferation of buds and adventitious shoots results in tremendous increases in the number of shoots. In vitro strategies have been routinely used for the propagation and rapid duplication of numerous therapeutic plants to take care of the demand of pharmaceutical firms and to protect the natural populations of rare and endangered plant species (Sudha and Seeni 1996; Rani and Grover 1999).

The basic principle governing the conservation of any species is the inclusion and maintenance of maximum genetic diversity (Frankel and Soule 1981; Dunham and Minckley 1998; Ananthakrishnan 2001). Incidentally, genetic diversity in plant population is structured in a way that it reflects the
biological characteristics, distribution and ecology of the species examined (Nevo et al. 1988; Nevo and Beiles 1989).

Tissue culture has become crucial in the *ex situ* conservation, especially for species with reduced population, rare and endangered plant species or low seed production. Biodiversity or *ex situ* genetic conservation by using tissue culture technique facilitates the rapid establishment of large number of stock plants, from a minimum of original plant material, thus imposing minimum impact on the endangered wild population (Cuenca and Marco 2000). As medicinal plants are continually under danger due to over exploitation and depletion of natural habitats, the need for their rapid multiplication and conservation have gained importance (Bajaj 1988).

1.7 *Pittosporum*

*Pittosporum* belongs to the family Pittosporaceae and the members are trees, shrubs or climbers, usually with lenticels and often with spines. Several members of this genus are with fragrant flowers and hence are cultivated in gardens (Brickell and Zuk 1997; Wagner et al. 1999).

The family Pittosporaceae is represented by 11 genera and about 250 species, which are distributed in the tropical and subtropical regions of Africa, Asia, Australia, and Pacific Islands. The genus *Pittosporum* alone is found in India with 11 species (Catalogue of Life 2015; Nayar and Giri 1980; 'eFloras 2015). The species such as *P. anamallayense*, *P. ceylanicum*, *P. dasycaulon*, *P. eriocarpum*, *P. ferrugineum*, *P. humile*, *P. neelgherrense*, *P. podocarpum*, *P. tetraspermum*, *P. viridulum* and *P. wightii* are found in India (Nayar and Giri 1980; Mukherjee 1984).

The distribution of *Pittosporum* in India is mainly in the rain forests, occurring in the substages usually at altitudes between 500 to 2800 m and for the most part concentrated in the Himalayan and the Western Ghats regions. The species occurring in peninsular India are *P. anamallayense*, *P. ceylanicum*,
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P. dasycaulon, P. neelgherrense, P. tetraspernum, P. viridulum and P. wightii. The species such as P. anamallayense, P. dasycaulon, P. neelgherrense and P. viridulum are endemic to the Western Ghats (Bhatnagar et al. 1961; Mukherjee 1984).

Different species of Pittosporum are widely used as medicinal plants. Seeds of some species of this genus are commonly used in traditional Chinese medicine for their sedative and cough relieving effects. Phytochemical investigations on Pittosporum species have led to the identification of some physiologically active compounds such as triterpenoid saponins, carotenoids, and essential oils (Feng et al. 2010). A review submitted by Cragg et al. (2006) denotes Pittosporaceae as “hot family” and the genus Pittosporum as “hot genera” due to its antileukemia potential. (The term “hot family” refers to those families having more than 14 individual plants designated as active by the stated selection criteria, and “hot genera” refers to those genera containing three or more active plants).

The flowers, root, bark and leaves of Pittosporum are used as antiinflammatory and antiseptic products and in rheumatic disorders (Weston 2004a; Weston 2004b). In India, the bark of Pittosporum is used in antiinflammatory and antispasmodic roles, as an antidote to snake poison and for narcotic effects. The bark is used for treatment of chronic bronchitis and is also administered for treatment of leprous infections and rheumatic swellings (Binu and Nair 2005; Khare 2007). The stem and bark of P. viridiflorum have been used medicinally (Matshinyalo and Reynolds 2002).

The antimicrobial and antioxidant effects of leaves of various species of this family are reported. The presence of a number of volatile mono and sesquiterpenes in leaves may possibly be responsible for its biological activities (Medeiros et al. 2003; Chou et al. 2008; Momeni et al. 2010). The bark and stem of some species of Pittosporum is found to be effective against intestinal
diseases (Momeni et al. 2010) and *Pittosporum phylliraeoides* var. *microcarpa* has shown antiviral properties (Semple et al. 1998).

As an important wild source of medicine, which is readily available in Indian tropical rain forests, *Pittosporum* plants have yet to receive sufficient attention. Furthermore, little information about the scientific documentation of this genus from the Western Ghats.

**1.8 *Pittosporum dasycaulon* Miq.**

Kingdom : **Plantae**

Division : **Magnoliophyta**

Class : **Magnoliopsida**

Order : **Apiales Nakai.**

Family : **Pittosporaceae R. Br.**

Genus : **Pittosporum** Banks ex Soland.

Species: **dasycaulon** Miq.

*Pittosporum dasycaulon* Miq. is an evergreen tree of the family Pittosporaceae. The plant is distributed in the moist deciduous and evergreen forests of the southern and central parts of Western Ghats above an elevation of 800 m (Nayar and Giri 1980). *P. dasycaulon* is a small tree with its young branches densely tomentose. Leaves are coriaceous, apex acute, base cuneate, margine entire, petioles 8–18 mm long, purberulous. Inflorescence is terminal or pseudoterminal, umbellate densely brownish
tomentose. Sepals are 2–3 × 1 mm, petals 10–12 × 2.0–2.5 mm, oblong and yellow. Filaments 6–7 mm long and anthers ca 2 mm long. Ovary is ca 3 mm long; style 4–5 mm long, glabrous. Capsules are 8–10 mm in diameter, globose and woody. Seeds may be 4–6, blackish pink (Nayar and Giri 1980).

The stem bark of *P. dasycaulon* has been used in folk medicine as an antibacterial and antifungal agent to treat infection (Khare 2007). Bhatnagar et al. (1961) reported that the stem bark shows antibacterial and antifungal properties.

Essential oil obtained from the stem bark of this plant and various solvent extracts showed antibacterial activity (Sadashiva et al. 2010; Mani and Thomas 2015). Methanol and aqueous extracts of stem bark of *P. dasycaulon* was reported to show antioxidant properties (Mani and Thomas 2014). Several diseases including human colon cancer, can be treated by administration of medicinal plant extracts (Lai and Kim 2010). From the review of literature, it is
confirmed that there are only few reports regarding the scientific documentation of the biological properties of this awesome species and there is no study regarding the genetic analysis of its populations as well as \textit{in vitro} propagation studies.

1.9 Scope of the study

\textit{Pittosporum dasycaulon} is an endemic medicinal plant distributed in the Western Ghats and used as an antibacterial and antifungal agent in traditional therapy. The scientific study of this plant in any area is limited. There are only a few preliminary reports on antibacterial properties of this plant. Therefore, it is important to have detailed studies of biological properties this species in a scientific way and to reveal the phytocconstituents responsible for this activity to certain extent is urgent. \textit{P. dasycaulon} is distributed in the boundary of forest formations. Consequently, the individuals of this species face threat from various natural and anthropogenic agencies such as climate change, habitat destruction etc. To overcome these problems, development of rapid propagation techniques and conservation of germplasm are the most urgent measures to be taken to protect this plant from extinction. Tissue culture provides efficient techniques for rapid and large scale propagation and conservation of germplasm. The study about genetic makeup of individuals in different populations or within a population of a species is important, because it could reveal the degree of genetic diversity existing in a species concerned. This could be valuable information for taking measures in conservation. Therefore, the present study intends to analyse the biological properties such as antioxidant and antibacterial potential to establish its therapeutic usefulness, phytochemical constitution, genetic diversity by RAPD study and to establish an efficient protocol for \textit{in vitro} propagation of \textit{P. dasycaulon}. 
1.10 Objectives of the study

- To evaluate the antioxidant properties of the extracts of stem bark of *P. dasycaulon*.

- To conduct antimicrobial studies against standard strains of bacteria to validate traditional uses of *P. dasycaulon* scientifically.

- To undertake phytochemical analysis of stem bark extracts and structural identification of secondary metabolites by GC/MS analysis.

- Collection of accessions of *P. dasycaulon* growing in different geographical locations of the Western Ghats.

- Isolation of genomic DNA from leaf samples different populations and detection of genetic diversity and phylogenetic relationship among the different populations by RAPD.

- To formulate a reproducible and efficient protocol for micropropagation of *P. dasycaulon* and thereby develop a strategy for its *ex situ* conservation.