Chapter1

1.1 Introduction

Nature has always been a hallmark to emplify the remarkable phenomenon of symbiosis[177]. Mankind's existence on this earth was possible only because of the vital role played by nature in sustaining his life. It has bestowed a complete repository of therapies to solve all health issues of humans.[21] From the inception of civilizations, humans have exploited plants for therapeutic requirments. Paleolithic notes, reveal that humans have been utilising herbs as therapeutic purposes since 60,000 years ago.[56] The use of plants, animals, microorganisms and their extracts or pure chemicals derived from them, to treat disease has been a therapeutic modality, which has survived the test of time.[17,444, 53]

The knowledge of drugs compiled over ages provided us many effective means of ensuring the prevention, treatment, and management of illness. Practically, every country developed its own medical system, which includes the civilization of India, China and Egypt. Thus, the Indian medical systems viz. Ayurveda, Unani, Siddha and Homeopathy, came into existence.

The oldest information about plants used as drugs originates from the Sumerians and Akkadians. The Egyptians civilization has given the world- one of its first medical texts, the famous *Ebers Papyrus*, which dates from about 1550 BC. The papyrus is claimed to contain nearly 800 recipes and over 700 references of a large number of crude drugs that are still of great importance, such as aloe, wormwood, peppermint, myrrh, castor seeds, gum arabic etc.[141] Many authors in antiquity described plants and animals that could be used as drugs. Amoung them were Hippocrates - The Father of Medicine

(460-377 BC), Theophrastus (370-287 BC), Pliny the Elder (23-79 AD), Dioscorides (40-80 AD), and Galen (130-210 AD). Around 60-80 AD, Dioscorides compiled of about 600 medicinal plants in "***De Materia Medica***"; and first used the term 'Materia Medica' to define the knowledge of drugs for many hundreds of years.[15, 233, 123]

In India, the experience gained by ages of medical treatment was documented in Ayurveda, the world's oldest medicinal practice. In Rig Veda, sacred text of Hinduism, outlines 67 herbal drugs employed as medicine. Charaka Samhita (900 BC) - the first compiled text on Ayurveda, illustrates use of 341 crude drugs from natural sources for medicinal use.[141, 21]

The ancient civilizations used extracts of plant or animal products, along with some inorganic ores. By the decline of 18th century, crude drugs were yet being used as powders, extracts, fresh juices, fresh pastes or tinctures. However, 1803 Sertürner isolated morphine form opium, and this discovery embarked the rise of a new era of medicinal research, characterized by the isolation and chemical characterization of bioactive isolates from natural sources. Soon many more important compounds such as strychnine, quinine, caffeine, nicotine atropine, cocaine and the mixture of cardioactive glycosides were isolated.[15]

Unaware of the specific significance and explicit chemical profile of natural products, mankind has been using them in their medical system. By the start of twentieth century that natural products encountered a stupendous surge as a robust branch of organic chemistry. Many aspects have led to this change, which include the advancement of new and more potent separation techniques, such as advanced chromatographic

methods, electrophoresis etc; better characterization techniques via modern spectroscopic methods and the change in the perception of the people about chemicals.[18, 32]

An All India Coordinated Project on Ethnobiology has found that, about 8000 different medicinal plants are being used by, about 4638 ethnic community groups in our country. This is a huge number. Several of these plants, which possess valuable medicinal properties, are used in different forms for curative, preventive and promotive purposes including cosmetic and dental care. Many medicinal plants are also used as ingredients in our food. Different medicinal plants are also revered and used during different customs and rituals. Thus, we see a variety of medicinal plants constituting a larger segment of the plants used by humankind.[46]

Herbal medicines are popular as remedies and are critical for providing human health-care solutions to a vast majority of world's population. WHO reports reveal, as much as four billion people, about 80 % of the global population, are dependent on ethanobotanical medicinal practices, principally based on plant material.[338,190] Approximately, 4,000 to 6,000 medicinal plants species are presently traded globally. The world-wide herbal supplements and remedies market has prospered substantially over the decade, without any significant signs of decline during the worldwide recession. The herbal remedies market in the US and Europe soared from $ 1.5 billion in 1992, to over $ 83 billion in 2008. WHO also reported that about 75-80% of the developing countries still very much depend on herbal medicines for primary health care. Currently, the global herbal market is $90 billion, of which, India's contribution is only one billion.[17,440,419,171] However India is witnessing increased deamands in the world market. It has 10 vegetative zones, 16 Agro-climatic zones, 15 biotic provinces and 426 biomes.

India has 45,000 different species of plant and trees, of which 15,000 are used medicinally in Ayurveda, Siddha, Unani and modern medicine. [189]

Natural products predominated the development of human therapeutics.[288] An estimated 25% of the prescription drugs sold in the world today contain plant-derived active ingredients. Globally, plant-derived drugs sales are estimated at $40 billion per year. In the US alone, about 10% of the prime medicines sold contain plant based contituents. These drugs include the non-steroidal anti-inflammatory agents Naproxen and Diclofenac, the ACE inhibitors Captopril and Enalapril, the immunosuppressant, Cyclosporin, the antibiotics Clavulanic acid / Amoxycillin and the β_2-agonist Salbutamol. Complex plant-derived products account for 25% of the total clinically prescribed drugs and include the classical drug entities such as Digoxin, Codeine, Morphine, Quinine and Atropine.[17,76,191]

Morphine obtained from *Papaver somniferum*, was the first pharmacologically active pure entity isolated about 200 years ago. This paved way for the isolation plant-based purified drug constituents can be isolated and administered in precise dosages.[76,104] Further derivatization of Morphine yielded Diacetylmorphine (Heroin), Codeine (painkiller) and a whole new class of Opioid analgesics.[103] The discovery of Penicillin from *P. notatum*, and its re-isolation, along with progress in derivatization and clinical studies; and marketing of synthetic penicillin derivatives drastically changed the drug development process.[8,90,245,75] With time, medicines from plants and other natural sources (such as animals, microorganisms fungi and marine life) or their analogs gained significance and greatly boosted the commercial drug markets. This led to several discoveries like Aspirin, Reserpine, Quinines; Lovastatin; Digoxin, Pilocarpine,

Artemisinin, Erythromycin etc.[221] Other important examples include Physostigmine, Colchicine, Tubocurarine, Ephedrine, Quinidine, Lovastatin, Podophyllotoxin, Taxol, Camptothecin, Vincristine and Vinblastine.[17,440]

Since immemorial, plant based derivatives have provided new leads for drug discovery process. But due to the advent of modern concept of medical science-robotics, combinatorial chemistry; HTS, genomics, bioinformatics, proteiomics, molecular biology-biotechnology, computer-aided drug modifications and other methodologies.[343] Herbal medicine was left behind, ignored and mandated as superstition. With the scientific age extending its horizon by leaps and bounds, misconceptions on myths persist. One such myth says 'Synthetic is the best'. Medicines that do not contain purified chemicals, and is not manufactured with sophisticated and expensive equipment, highly trained scientists, then the myths say- 'possibly such medicines cannot work'. Pharmaceuticals have turned a blind eye from plants products in their search for newer drug entities. Now-a-days, scientists carry out extensive tests and clinical trials before releasing the drug into the market. But unfortunately there is still room for tragic errors- as the one that occurred with Thalidomine.[141,239,107,25,361, 98]

Unfortunately natural products drug discovery has been neglected, at the time, when several in-roads were made in purification, isolation, characterization and identification of bioactives and newer assay methods are now available with the pharmaceutical industry. Natural products, the sui generis source of chemical diversity, as a result got virtually eliminated from the drug discovery process, contrary to combinatorial chemistry. The ingression of combinatorial biosynthesis led to production vast libraries of new derivatives by combinatorial chemistry complementary to biological

methods. The future prospects of the pharmaceutical industry depends on the systematic bridging of complementary technologies such as combinatorial chemistry, natural products discovery, combinatorial biosynthesis, HTS and integrative and systems biology.[421]

Combinatorial chemistry requires new scaffolds to build newer molecules with minor modifications to that of present-day drugs. Also, the advancements in comparitive genomics revealed newer target sites for drugs. But the screening of such huge number of targets is so enormous task that it requires tremendous investments of time and money to set up the necessary resources to exploit the technology. Such large screening programs, which require libraries of millions of chemical entities can only be managed by HTS methodology. Howerever, High-Throughput Screening (HTS) of these combinatorial libraries failed to provide the number of high-quality leads that were anticipated. Despite the fact that screening natural products on these targets would be excellent, the industry has failed to focus on this front and has opted to save funds by eliminating natural-products departments.[12]

Although there is no doubt that many new drugs emanated from these research trends, but the productivity of pharmaceutical industry continues to be gloomy. However, things seem to be changing due to three prime factors. First, combinatorial chemistry failed in its assurance to provide large number of chemical scaffolds with de novo synthetic. Also, the advancements in isolation techniques and quicker and prescise structural elucidation, eliminated the major difficulties of natural products drug discovery. These were followed by a change in perspective for the intrinsic utility of natural products in drug discovery. Thus, there is growing demand for newer lead

molecules from natural sources, which are known to be safe as compared to synthetic compounds with severe unwanted side effects.[155]

The Renaissance in Natural Products Research

Plants are exquisite chemical workshops producing range structural analogs. However, the concept of chemotaxonomic analysis of plants is more of done with, due to its highly cumbersome nature and it yielded low amounts of compounds.[127] Also it has been an enormous challenge to commercially unviable to produce of these biologically active natural products represents.[141,390]

Regardless of spectacular advances in synthetic drugs in recent years, some of the plant based drugs have still retained their importance. The use of such drugs is increasing; owing to the fact that most plant-based medicines are free from adverse effects. As a result, the past decade has witnessed a revival in natural product research leading to screening of natural materials such as plants, fungi, microorganisms and marine life, as source of potential drug substances.[277]

In the last few decades there has been an exponential growth in the field of medicinal plants.[3] Its has been challenging to design HTS assays capable of hastening bioactives isolation. This would require the collabration of newer technologies. Until now, NMR and MS spear headed the characterization of isolated bioactives. The advancements in these techniques can be employed to further aide medicinal plant drug discovery.[65,353,127] And, high-throughput X-ray crystallography can enhance research in plant-based leads.[364] Natural product isolation produces impracticably minute quantities

of bioactive molecule and such trace quantities are insufficient for lead optimization, lead development, and clinical trials.[123] Hence it is necessary collaborate with synthetic and medicinal chemists to workout possible synthetic or semi-synthetic reactions for largescale productions of the isolated entities.[223,305,173,240] Another method to enhance herbal drug development is the setting up of libraries of natural products and their analogs, which would share data with combinatorial libraries.[389,364,251,43,398,27,240]

Of late, natural products chemistry has witnessed upsurged interest. Elucidation of the biosynthetic pathways for natural products in several cases progressed to isolation and characterization of the enzymes involved and even to the recognition and cloning of the genes which code for these enzymes. This opens up new avenues for discovery of bioactive molecules. This upsurge can be ascribed to the following factors: [261,67,110,197,4,430,216]

- Growing therapeutic demands.
- Wide chemical and pharmacological profile of natural bioactive isolates.
- Utility of these natural bioactive isolates as drug leads.
- Evolution of highly sensitive techniques to detect and characterize natural bioactive isolates.
- Advances in isolation purification and characterisation techniques
- Alternative sources of complex natural bioactives. Such as advancements in the biotechnology have helped in increasing the yield of secondary metabolite produced in the medicinal plant.

Western Ghats

India, with its rich biodiversity, offers a unmatched possibilities for drug discovery from natural resources. It has a very rich flora with nearly 16,000 species of flowering plants. An estimate showed that local healers mostly in rural and tribal areas of India used about 7,500 herbs. The significance of most these herbs still remains unknown to the general public.[317] The detailed investigation, documentation and pharmacological evaluation of taxonomically related medicinal plants used in traditional medicine can pave the way for the discovery of several herbal remedies to numerous diseases.[317,79] It may be noted that in the last two decades there has been a drastic rise usage of natural products by Herbal industry in India and USA.

Western ghat, a veritable emporium of medicinal plants, flora and fauna are of enormous scientific interest due to their major share in earth's biological resources. The Western Ghats are hill ranges lining the the western edge of Indian peninsula, that harbour an incredible diversity of flora and fauna.[373] Their position makes that they are biologically rich and with varied diversity.[125] The rain forests of the Western Ghats are unique vegetation formations as they exist in an environment where there is considerable seasonality in distribution of the rainfall. There are over 6000 vascular plants belonging to over 2500 genera in this hotspot, of which, over 3000 are endemic[277,337,168,373].

Natural products as Anti-cancer agents

Cancer kills over sixty lakh people every year worldwide, and is the second largest factor for mortility. Depending on the stage of cancer, surgery, radio therapy and chemotherapy are the most commonly used treatment modalities. Since time immorable, nature has provided an array of anticancer molecules with diverse chemical structures and biological potentials. Several chemotherapy drugs are isolated from medicinal plants, including vinblastine and vincristine, from Catharanthus roseus, etoposide and teniposide, semisyntheticaly derivative from epipodophyllotoxin isomer of podophyllotoxin (isolated from kind of Podophyllum species), taxanes isolated from Taxus species, the semisynthetic derivatives of camptothecin: topotecan and irinotecan, isolated from the plant *Camptotheca acuminata*.[358,335,333] In the present scenario the search for newer molecules is changing towards the natural bioactive substances owing to their fewer side effects and low cost. About 60% of currently existing anticancer drugs are rooted to natural sources.[214, 125]

Diospyros species are known to produce a range of naphthoquinones and pentacyclic triterpenoid saponins.[140] *Diospyros* is an important member genus of the Ebanaceae family. Ebenaceae members should be pharmacologically active, going by strong evidence for the presence of well bioactive metabolites. There is enough evidence that detailed profiling of these lattices would results in the isolation of several antibacterial, antiviral, cytotoxic, mano amino oxidase inhibitors, antioxidant monomers, dimers or oligomers of naphthoquinones.[140] In Asia, many Ebenaceae members are employed as anthelmintic and antiviral.[140,108,60,323,256,174,355,153,30] Many *Diospyros* species

are known to exhibit unique biological and pharmacological activities viz. termicidal activity**,** piscicidal and and fungicidal activities molluscicidal activities**.**[244,303,144]

Taking cue from the brief overview presented and based on the rich ethnobotanical profiles and thorough literature review, the author has selected three species of *Diospyros* viz. *D. oocarpa*, *D. nigrescens* and *D. candolleana* to study their chemical profiles and biological potentials.

1.2 AIMS AND OBJECTIVES

Cancer involves an abnormal growth of cells in our bodies, which invade and destroy normal cells, thus leading to death. These cells arise from errors in the cell cycle owing to body imbalances and by rectifying these may help in cancer treatment. Cancer is major health hazard causing death spread across all classes of nations and only second to heart disorders. Modern cancer therapies such as chemotherapy and radiation therapy are proven to possess serious adverse effects, thus patients are seeking alternative complementary therapies involving the use of plants and other natural products.[84,233]

Survey of literature and documented tribal knowledge reveals that various parts like roots, leaves, bark and flowers of *Diospyros* species are known to be employed in tribal medicine for is different pharmacological activities, especially in treatment of tumours, warts, wounds which may be considered to be signs of cancer.[249] The present study involves exploration of three unexplored *Diospyros* species from Western Ghats Karnataka for their dynamic biological profile i.e. Anticancer; and isolation and characterization of their active constituents.

Specifically this study aims at:

1. **Phytochemical evaluation**

   - Authentication of plants by taxonomist
   - Collection of plant material
   - Identification of phytoconstituents by preliminary investigation
   - Isolation of bioactive molecules will be tried

- To conduct systemic chemical investigation of

  -Roots of *Diospyros oocarpa* Dalz.

  -Roots of *Diospyros nigrescens* (Dalz.)

  -Roots of *Diospyros condolleana* Thw.

2. **Biological Evaluation**

The root extracts of *Diospyros oocarpa* Thw *Diospyros nigrescens* (Dalz.) Sald and *Diospyros candolleana* Dalz. for its cytotoxic activity.

Acute Toxicity Study: Determination of the LD_{50} value for the the *Diospyros* extracts, in accordance to the OECD guidelines specified. The LD_{50} value would aid in Dose selection for Cytotoxity study in

Cytotoxicity Screening

- ***In-vitro cytotoxic evaluation of extract and their isolates by Brine Shrimp Lethality (BSL) Assay***
- ***In-vitro cytotoxic evaluation of extracts and their isolates using DLA Cell lines.***
- ***DMBA induced Skin carcinoma in mice***