CHAPTER 1

INTRODUCTION

Herbs are playing as a rebound and herbal ‘renaissance’ is occurring everywhere throughout the world. India with its extraordinary biodiversity has an incredible potential and favorable position in the developing field of plant based medicines. Medicinal herbs are a standout amongst the most essential parts of the non-wood forest items sector that supplies more than 80% of India's net forest export profit yearly. The therapeutic properties of medications are because of the existence of complex chemical substances of different composition in at least one section of these plants. These plant metabolites, as per their configuration, were gathered as flavanoids, terpenoids, corticosteroids, carotenoids, alkaloids, glycosides, phenolic compounds and essential oils. These bioactive principles were used in the treatment of various diseases like cholera, diabetes, diarrhoea, fever, skin problems, ageing, wound healing and ophthalmic disorders [1]; scavenging the free radicals produced in the cell thus preventing cancer [2].

In recent decades massive data on drug discovery from terrestrial plants is available from researchers and pharmaceuticals and among them few are in the market and many of those in clinical trials [3]. Around 8,000 plant based medications have been categorized in Ayurveda. As per an ancient report, the Rigveda (5,000 BC) has recorded 67 species, 81 species in Yajurveda, 290 species in Atharvaveda (4,500-2,500 BC), Sushrut Samhita (200 BC) and Charak Samhita (700 BC) had reported uses and properties of more than 1000 plant species, and these are as yet utilized as a part of the traditional preparations in the Ayurvedic medicine system [4]. More than three-fourth of the global populace relies on plants
and plant products for health care. Over 30% of the whole plant species, at one time or other was utilized for restorative purposes.

The global trade in medicinal herbs is roughly around US$ 800 million every year. India exported around 32,600 tonnes of crude medicines that accounts to US$ 46 million which is evident from the Export statistics accessible in the vicinity of 1992 and 1995 [5]. It was assessed that world market for plant determined medicines may represent about Rs.2,00,000 crores. According to a report, the export of medications from India has consistently expanded from Rs.130 crores in 1991-92 to Rs.165 crores in 1994-95 [6]. The international market is dominated by two countries first namely, China with its exports more than 1,20,000 tons for every annum (US$ 264.5 million) and secondly, India with more than 32,000 tons for every annum. The yearly export of medicinal plants from India is esteemed at Rs. 1200 million [7]. All the significant herbal-based pharmaceutical organizations are demonstrating a consistent development of around 15 % or more, following the Information Technology industry.

Plants synthesize and preserve a variety of biochemical products, many of which are recovered for the usage in research purposes. The medicines are developed either from the entire plant or from various organs, similar to stem, root, bark, leaves, seed and flower and so on. A few medications are set up from excretory plant item, for example, resins, latex and gum [6]. Lila and Raskin (2005) defined 2 types of phytochemical interactions that exist in plants namely, endointeractions and exointeractions. Exointeractions usually occurs between constituents inside the plant group and Endointeractions takes place between parts from various plants or amongst plants and engineered drugs. It has been found that Exointeractions and endointeractions between and between and inside the intricate blends of phytochemicals may extremely affect human wellbeing. There are certain interfaces which demonstrated impacts in natural procedures, for example, digestion, body clearance, bioavailability, cell uptake, solvency, and efflux. Current solution has as of late figured out how quickly pathogens and malignancy cells can create
imperviousness to single component medications, requesting the administration of complex medication combinations to circumvent or defer the resistance. Instead of relying on combination therapies, plants possessing a wide stream of phytochemical constituents may be exploited for the treatment of multiple diseases.

1.1 HISTORY OF TRADITIONAL MEDICINE AND DRUG DISCOVERY FROM NATURAL PRODUCTS

Plants have been occurred on globe even before human in entire biological system of our planet. Human beings rely on upon nature for their essential needs like nourishment, clothing, shelter and medication since ages [8].

_Papyrus Ebers_ is a well-known plant which possesses more than 800 formulations and 700 diverse medicines such as fennel, castor oil and acacia. In China, most of the therapeutic plants have been used since 5000 BC. Indians meticulously worked to check and classify herbs which they came across, into groups called *Gunas* (Property of drug). Charaka made fifty groups of ten herbs each. Similarly, they organized 760 medicinal herbs in 7 different groups based on their common properties. A large portion of the Indian population depends on Indian system of medicine called Ayurveda, Siddha, and etc.

The medicinal plants can give crude material to the separation or production of traditional medications. In the current world, discovery of cinchona in seventeenth century, trailed by digitalis, morphine, etc, and after that presentation of aspirin, derived from a plant-based medicine, constrained people to have confidence in the miracles of the differing floristic riches (Ruskin, I. furthermore, Ripoll, C., 2004). An expansive number of plants utilized as a part of the conventional drugs have now turned into a piece of the present day world health care system [9]. Natural product chemistry actually began with the work of Fredrick Surterner (1800 AD), who initially used opium for morphine production. Indian fever bark form *Cinchona officinalis*, was utilized by the natives of the Amazon and Andes as an
infusion to cure fevers; the cocoa tree leaves have been principally masticated to get benefits in Andean cultures, alkaloid pilocarpine isolated from *Pilocarpus jaborandi* is defense against glaucoma, blinding disease. Indians used a kind of enzyme that was isolated from the pineapple juice to break down blood clots to lessen inflammation in skin wounds and other types of wounds.

In pharmaceutical division, the steps in the drug discovery were target identification, target prioritization, lead advancement and lead recognition. This includes different perspectives like preclinical innovations which covers initial *in vitro* and *in vivo* assays for choice of medicinally active combinations, chemistry manufacturing controls (CMC)/pharmaceutics to detail the medications by thorough investigations of its property and expected for clinical utilize, pharmacology/toxicology to characterize viability and clinical examination on people and at last endorsement as new drug application (NDA) and marketing authorization application (MAA). The medication advancement process is getting more unpredictable and capital intensive with lead disclosure as a more prominent bottleneck. It is evaluated that, to create one effective medication, 12 – 15 years and US $ 900 million is required.

### 1.2 MEDICINAL PLANTS

Most therapeutic plants originated from the tropical territories of Asia, South America, Africa and Caribbean, where they regularly are utilized both as food and pharmaceutical drugs. The material is provided through gathering both from wild populaces and from agriculture. Along these lines the tropical rainforests that spreads 12 percent of the earth are a key resource of plants that can be utilized for pharmaceutical purpose. Nevertheless, just a single percent of the plants have been tried for therapeutic properties in spite of the fact that the truth of the matter is that no less than 25 percent of the present day medications descend from plants. Moreover around 35,000 distinguished plants out of a several hundred thousands are
utilized worldwide for therapeutic purposes and in future the part of plants in pharmaceutical may be of expanded significance for health care industries [10].

### 1.2.1 Drugs from plants

The plant originated drugs for example; quinine, morphine, digitoxin/digoxin, taxol, atropine, vincristine and vinblastine assume a critical part in present day pharmaceuticals. The plant derived drugs are also utilized in malignancy treatment and among those vinblastine (Velbe), paclitaxel (Taxol) and vincristine (Oncovin) are antimitotic and impact G1 and S-phase of the cell cycle thereby providing a difficult cell division. Paclitaxel (Taxol) is the main medication of choice for several types of cancers treatment including breast cancer and ovarian cancer. It is obtained from Taxus brevifolia, a Pacific Yew tree [11]. In the 1950s, two different alkaloids were obtained from Catharanthus roseus namely vincristine and vinblastine. These kinds of drugs have been of incredible pharmacological significance for most of the cancer treatment like malignant melanoma, malignant lymphoma and leukaemia. Subsequently, as the administration of vinblastine increased, the existence percentage of childhood leukemias has expanded by 80 %. The major drawback of this plant is the less alkaloid quantity and it is a significant therapeutic improvement for finding a substitute way of producing the medicines, for instance through in vitro cultures of the Catharanthus roseus cells [11].

The commonly used herbs such as Panax ginseng, Echinacea purpurea, Hypericum perforatum, Ginko biloba and Astragalus membranaceus have been appeared to have a property which modifies the body's defenses in different methods [12]. A few impacts produced are for instance, discerning stimulation of cytokine levels, effects on the cell-mediated immunity and high phagocytic activities [13].

*Echinacea* is an herb utilized for many diseases and in recent times it is known to affect both *in vivo* and *in vitro* immunological functions [14]. A recent study investigated the impacts of *Echinacea purpurea* aqueous extracts on natural
killer (NK) cells in vitro. They have observed that when less concentration of the plant extract was exposed the NK cells are activated naturally. Due to this, the cytotoxicity was upgraded to 100 % and the frequency of CD69 expressing cells was raised. It was also found that the average fluorescence intensity of CD56+ and CD16+ cells were enlarged [15]. Besides there was another study to check changes in White Blood cells (WBC) counts, IL-2 concentrations in vivo and phagocytic activity when Echinacea is supplemented. In animals complemented with the plant extract, the quantity of WBC’s improved considerably amid the initial 2 weeks. As a result, a quick noteworthy rise in monocytes and lymphocytes rates was observed with a huge decline in the percentage of neutrophils.

Lipopolysaccharide (LPS) is a notable outer membrane part of gram-negative bacteria which triggers the inflammatory reaction and generation of pro-inflammatory mediators such as cyclooxygenase-2 (COX-2), TNF-α, cytokines, (Interleukin; IL-6 and IL-1b) and reactive oxygen species (ROS). These inflammatory mediators are carefully related with the pathogenesis of different inflammatory ailments. Additionally, created ROS modify the structure and role of cells and add to cell death [16].

In India, numerous Ayurvedic and Siddha experts are utilizing different ethnic plants to treat various sorts of arthritic problems. The usage of these curative agents has a clear custom and sensible background in the Indian system of Medicine. However, there is a need to explore them for the recent scientific world.

The systematic investigations to check the real efficiency and the restrictions to use these medicines will certainly broaden its space for later use in case if they are truly viable. This is especially vital, initially because of the depth of the rheumatism disease and furthermore because of the nonexistence of the correct kind of medication which is chemical based for its cure. The currently accessible medicines give just symptomatic relief and side effects are common. The aim should be to find more up to date medicines based on plant sources that might give healing
treatment and will be devoid of adverse impacts and in addition cost-effective, which would be acknowledged by the developing countries like India.

In traditional medicines including Ayurveda, Siddha, etc. several herbal drugs are used to treat diseases. However, their efficacy and safety are not clear. Herbal drugs have a major role in the traditional medicine. In a study in India, 43% of arthritic patients had used complimentary alternative medicine therapies, which are mainly herbs. Herbal drugs are promising for the development of effective and safe drugs against arthritis in light of modern medicine. In the recent past, a few reviews have appeared on this topic [17].

A vast number of plant species consisting numerous bioactive compounds exhibiting beneficial activities; like anti-oxidative, anti-inflammatory, anti-cancer, hepatoprotective, anti-febrifuge, anti-hypertensive, anti-allergic and antimicrobial effects etc. Numerous natural compounds have been isolated and tested on various in vitro and in vivo models for the development of new anti-inflammatory therapeutics and other health problems [18].

1.3 APPLICATIONS OF THE MEDICINAL PLANTS

1.3.1 Anti-inflammatory study

Based on survey of literature from AYUSH, Government of India, in this present investigation we are intend to scientifically explore the traditionally used anti-inflammatory Indian medicinal plants like *Acalypha alnifolia*, *Blepharis maderaspatensis*, *Dregea volubilis* and *Ipomoea staphylina* which were collected from agricultural field in Chengalpattu, Kancheepuram district, Tamil Nadu, India. They are used for skin diseases, sprain, wound healing and bone fracture. These four medicinal plants were evaluated for phytochemical estimations and antioxidant activity. Among four medicinal plants, *Blepharis maderaspatensis* shows promising results.
Blepharis maderaspatensis belongs to the family Acanthaceae, called as sathai otti, elumbu otti in Tamil and it was collected from agricultural field in Chengalpattu district, Tamil Nadu. B. maderaspatensis is utilized in Indian system of medicine for the cure of uneven heart beats. Root extract is used for circulatory system disorders, arrhythmia, Infections/Infestations, syphilis, and leaf juice is used for bronchial complaints and especially leaf paste is used for external wound healing and plant-ash used for dropsy, swellings, oedema, gout.

1.3.2 Anticancer Activity

Cancer is described by cells in the human body persistently multiplying with the failure to be stopped or controlled subsequently, forming malignant tumorous cells with the possibility to be metastatic [19]. Present-day treatments consist of radiotherapy, chemotherapy and usage of synthetically derived drugs. However, there is an interest on alternative treatment using medicinal plants. Many plant species are as of now being utilized to cure or avoid improvement of malignancy. Compounds which are extracted from the plants which are necessary for plant survival and “housekeeping” of the organism are being investigated for their capacity to inhibit growth and initiate apoptosis of cancerous cells. Paclitaxel was the first drug originally extracted from the Pacific Yew tree, Taxus brevifolia. These types of drugs promote microtubule stability, preventing their disassembly. Recently, taxanes have drawback as medicines in formulation because they are poorly soluble in water [20].

1.4 SCOPE OF THE THESIS

According to Indian System of medicine, the experimental plant Blepharis maderaspatensis is used as a plant ash for swellings, dropsy, gout and edema. The dry alcoholic extract of B. maderaspatensis is used as an effective diuretic and its parts are used to treat venereal diseases. Juice extracted from the plant leaves can treat asthma and throat troubles. The leaves are a better
Cure for bone fracture and its paste is used to heal wounds. Literature survey clearly indicated that medicinal properties of *Blepharis maderaspatensis* (L.) Heyne ex Roth extensively studied whereas leaves of this plant scientifically were poorly studied for its medicinal potential against various human diseases. Reports on potential of *B. maderaspatensis* in treating the inflammation are inadequate. The leaves of the plant will be evaluated for various phytochemical and their anti-inflammatory properties on human cell lines. Inflammatory markers and molecular markers will be employed in order to evaluate the *B. maderaspatensis* efficiency against the LPS induced anti-inflammatory activity. Based on this, the thesis has been divided into following four objectives where we aimed to investigate potential anti-inflammatory and anticancer activities of protocatechuic acid from the *B. maderaspatensis* leaves on LPS induced production of inflammatory mediators in activated macrophages.

1. To isolate and estimate the phytochemical constituents of selected medicinal plants
2. To quantify and screen *in vitro* antioxidant and cytotoxic activities of selected medicinal plants
3. To purify, check and characterize the anti-inflammatory compound from *Blepharis maderaspatensis*
4. To study the anticancer activity of protocatechuic acid isolated from *Blepharis maderaspatensis*