Chapter 1

Introduction

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Plants have been used as the basic source of energy (oxygen, food, cloth and drugs) for all forms of life. Most importantly, humankind has been exploring the plants as therapeutic agents since early civilization that dates back around 60,000 years (Solecki, 1975). The written evidence of the same has been provided by the great civilisations of the ancient Chinese, Indians, and North Africans. One of the earliest known such document is a 4000 and 4500 year old Sumerian clay tablet and Pun-tsao respectively. In India, the earliest record on the usage of plants as medicine is available in Rig Veda dating between 3500 B.C to 1800 B.C. Thus, traditionally plants played an integral role in primary health care system since ancient times. Theophrastus also known as the "Father of botany" continually provided information, thereby aiding the identification of plants (Phillipson, 1995).

However, it was only during 19^{th} century that man became aware of the active constituents of the plants. Some important breakthrough was the discovery of quinine from *Cinchona* bark by the French scientists Caventou and Pelletier; the isolation of morphine and codeine from the poppy; digoxin from *Digitalis* leaves; reserpine from the *Rauwolfia* species; vinblastine and vincristine from *Catharanthus roseus* etc. Further, pharmacological development led to the first synthetic drug "aspirin" based on natural products "salicylic acid" (Phillipson, 1995). For many years, with the advent of synthetic drug development the importance of natural phytochemicals waned inevitably. However, during past decades search of natural drugs has escalated, as synthetic drugs have been reported to have numerous adverse effects. Some of the natural drugs discovered were taxol, etoposide and artemisinin (Phillipson, 1999) which further stimulated the interest for the use of natural drugs as safe alternatives. Moreover, presence of several chemical compounds may synergistically or additively show pharmacological effect thereby eradicating undesirable side effects caused due to the use of single xenobiotics (Tyler, 1999). The World Health Organization estimates that nearly 80-85% of population both in developed and developing countries rely on traditional medicine for their primary health care needs (WHO, 1985).

India with wide topographical ranges and climatic condition houses emporium of medicinal plants and represents one of the richest countries in tradition, culture and natural biodiversity (resources of medicinal plants) offering everlasting arena for drug discovery (Patrick, 2002). The medicinal use of plants of India has been documented in some ancient literature (Tulsidas 1631 samvat; Charak and Drdhbala, 1996).

Being blessed with four hotspots (Himalaya, Indo-Burma, Sundaland and Western Ghats and SriLanka) of plant biodiversity, India is the seventh mega diversity country with the occurrence of 70% of the world's species. Besides, diversity in higher plants, India has the richest pteridophytic flora encompassing 1,200 pteridophytes out of which 193 are the endemic species (Sanjappa, 2005; Dixit, 1984). About, 13,600 species of fern and fern allies have been known to be distributed worldwide (Moran, 2008).

Pteridophytes (ferns and fern allies) also known as reptile group of plants are the earliest vascular cryptogams originated in the Mid-Paleozoic era during Silurian period i.e. about 250 million years ago and was the dominant vegetation of that era. It still grows luxuriantly in tropical and temperate forests, despite being replaced by the angiospermic plants (Dixit, 2000). The regions richest in the pteridophytic flora of the country are Eastern Himalayas with about 845 taxa belonging to 179 genera followed by Eastern and Western Ghats with more than 300 species in Western Ghats (Manickam and Irudhayaraj, 1992).

As folk medicine, pteridophytes have been known to man since 2000 years and has its application in Ayurvedic, Unani, Homeopathic and other systems of medicines of India. Caius (1935) is probably the first man who illustrated the medicinal importance of ferns of India. Later number of compilations was published regarding the medicinal uses of pteridophytes (Nayar, 1957; Chopra *et al.*, 1958; Singh, 1999; Das, 2007). Traditonally, ferns like *Dicranopteris linearis* have been used as an antibacterial, antihelmintic, laxative,treating asthma, to remove sterility in women and against intestinal worms (Kamini, 2007; Upreti *et al.*, 2009), while the frond of *Drynaria quercifolia* are used as antibacterial, pectoral and expectorant agents and as poultice on swelling (Dixit and Vohra, 1984; Dagar and Dagar, 1987). *Pteris vittata* are ethnomedicinally used as demulcent, hypotensive, tonic antiviral and antibacterial (Singh, 1999; Kumari *et al.*, 2011). The frond juice of *Pteris biaurita* is applied on cuts and bruises while decoction (both of fronds and rhizome) has been given in chronic disorders (Rout *et al.*, 2009; Kumari *et al.*, 2011). Leaf and juice of *Microsorium punctatum* are used as purgative, diuretic, anti-inflammatory and antibacterial (Rout *et al.*, 2009; Sathiyaraj *et al.*, 2015). The frond reduces cough while frond paste of *Nephrolepis cordifolia* controls bleeding when applied to wound. Fresh tubers checks cough, intestinal disorders, stomach ulcer and acidity (Rout *et al.*, 2009; Mannan *et al.* 2008). *Chritella dentata* (*Cyclosorus dentatus*) is used for lowering blood sugar, pain relief while frond paste is applied over body swellings (Tanzin *et al.*, 2013; Sathiyaraj *et al.*, 2015). Medicinal values of plants lies in the presence of various bioactive components which is known to exert various physiological action on human body (Hill, 1952).

With increasing number of illustrations regarding the adverse effects of synthetic drugs, past decades has witnessed a remarkable resurgence in the use of herbal medicine. Since ferns and ferns allies having survived from Paleozoic times, adapting to various environmental changes they are expected to contain many more useful metabolites in comparison to other plants (Wallace *et al.,* 1991). Among many useful phytochemicals, ferns were reported to be enriched with flavonoids, steroids, phenols, triterpenoid compounds, varieties of amino acids and fatty acids (Zeng–fu *et al.*, 2008). Focus has been made mainly on the search of natural compounds (phenolics, flavonoids, terpenoids, and steroids etc) rich in antioxidant, anticancer and antimicrobial properties due to its importance in the therapy of various chronic disorders like cancer, diabetes and cardiovascular diseases.

Antioxidants are the substances that can mitigate the harmful effects of free radicals. Free radicals are the extremely reactive unstable molecules produced during the cellular oxidation-reduction reaction in the human body (Ratnam *et al*., 2006). Every individual is endowed with well developed defense system comprising of enzymes such as catalases, superoxide dismutase, glutathione peroxidise, glutathione reductase; or compounds such as glutathione, vitamins E and C etc to neutralize or balance the production of free radicals. However, reduction in the antioxidant levels leads to the generation of excessive free radicals creating oxidative stress which in turn results in numerous diseases and disorders such as cancer, stroke, myocardial infarction, diabetes, septic and hemorrhagic shock, Alzheimer's and Parkinson's diseases (Halliwell *et al.,* 1995; Tsao *et al.,* 2004). Though, synthetic antioxidants such as butylated hydroxyl anisole, butylated hydroxyl toluene etc plays an important role in delaying or preventing oxidative stress related diseases but their major setback is that they impose numerous adverse health effects (Sultana *et al.*, 2007).

Phytochemicals such as phenolics, flavonoids and steroids have received significant consideration in recent years due to their various pharmacological properties such as antioxidant, antimutagenic, antiallergenic, anti-inflammatory, antimicrobial, anticoagulant, antipeptic antiproliferative, antitumor, antibacterial, antiviral, and antiadhesive activities besides their preventive role in human neurodegenerative diseases, cardiovascular disorders and cancer (Kang *et al*., 2006, Katalinic *et al.*, 2006, Cumashi *et al*., 2007; Da Costa *et al*., 2010).

Furthermore, in recent years infectious diseases causing premature deaths have augmented greatly along with the reports of increased number of drug resistant human pathogenic bacteria from across the globe (Pasquale and Tan, 2005; Mahesh and Satish, 2008). Plant-based antimicrobials have been known to be effective in treating infectious diseases as they can

simultaneously alleviate the side effects associated with synthetic antimicrobials. Moreover, multiple effects are being imposed in the body by phytomedicines. For example, besides exhibiting antimicrobial activity, *Hydrastis canadensis* also leads to increase in blood supply to the spleen thereby enhancing the release of mediating compounds (Murray, 1995).

Diabetes mellitus is a non communicable disease caused by abnormal metabolism of carbohydrates, glucose, lipids, and protein. It is mostly associated with abnormality in blood insulin levels either because of improper production from pancreatic β-cells or resistance to insulin by target organs (Jarald *et al*., 2008; Hahm *et al*., 2011). Generally, diabetes mellitus are of two types: insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM). Tremendous increase in the incidence of diabetes has been observed globally with the prediction of International Diabetes Federation (IDF) recently estimating diabetes prevalence to grow from 382 million people worldwide to 592 million in less than 25 years 2030. In India, record reveals that 65.1 million people have diabetes (IDF Diabetes Atlas, 2013).

Though, insulin therapy is the effective and widely employed therapy for IDDM patients, yet it has been observed to have many shortcomings like short shelf life, excess doses leading to fatal hypoglycemia, ineffectiveness while orally administered etc. Similarly, treatment with sulfonylureas and biguanides causes diverse side effects in NIDDM patients (Rang and Dale, 1991). Diabetes management with minimal side effects and at a lesser cost is still one of the enduring challenges to the medical system (Sun *et al.,* 2008). In this context, phytochemicals being cost effective and with lesser side effects provides an open and new avenue for the treatment of various chronic diseases like diabetes (Li *et al.,* 2011).

Consequently, it is clear that plants are enriched with combination of bioactive compounds that may have synergistically attributed to the biological activities of plants. Thus, screening the active compounds and understanding the chemical nature is important for the development of pharmaceutically important lead compounds discovering safer drugs (Mariswamy *et al.,* 2011). Separation of the bioactive compounds for identification, quantification and characterization has been employed using various chromatography techniques (column chromatography, thin layer chromatography, high performance layer chromatograpy, flash chromatography and Sephadex chromatography) (Sasidharan *et al.,* 2011). Though, difference in the polarites of the phytochemicals still remains the continuing challenge for isolation of pure compounds. However, invention of many new medicinal drugs having protective role against diseases like cancer and Alzheimer has been reported (Sheeja and Kuttan, 2007; Mukherjee *et al.*, 2007). With the advent of many purification techniques and isolation of pure compounds or phytochemicals, drug

discovery could be made less cumbersome as these compounds might as reported be the potential key player in regulating many mechanisms and could be used as ligand for *in silico* drug designing.

Of-late a humungous number of bioinformatic tools have been developed that offer novel approaches for drug discovery which provide a preliminary idea about the probable behaviour or activities of the compounds/ligands in the active site of the target/protein/enzymes. Such interaction between protein and ligand is similar to lock and key hypothesis in which lock encodes the protein and the ligands resembles the key. Non-covalent interactions mainly hydrogen bonding, vander waals etc are considered as the major driving force for enabling the binding between protein and the ligand (Kubinyi, 1998). Generally, docking study facilitates in determining computational aided binding affinity between molecules (target protein and ligand) which in turn is important for the identification of effective and reliable drug against target enzymes and to study the protein-inhibitor interaction activity. *In silico* screening thus helps in decreasing the number of compounds that are used in evaluating various biological activity assays, thereby reducing time and costs as compared to time consuming assay-based screening or to biophysical screening methods of drug discovery (Osguthorpe *et al*., 2012; Kalyani *et al.,* 2013).

Many *in silico* molecular docking studies with view to identify potent compounds against target proteins of diabetes mellitus are in rise in recent years (Angadi *et al.,* 2013; Ahmed *et al.,* 2014). Various antidiabetic targets are 11β-hydroxysteroid dehydrogenase type 1, DGAT1, dipeptidyl peptidase-IV, glucokinase, peroxisome proliferators activated receptor gamma, glycogen phosphorylase, protein tyrosine phosphatase 1-beta, fructose 1,6- bisphosphatase, aldose reductase, insulin receptor, and so forth (Carpino and Goodwin, 2010; Guttulu *et al.,* 2011). Phytoconstituents have been documented to act through multiple mechanisms, thus docking studies would greatly aid in understanding the underlying mechanism of action of the compounds against multiple target enzymes and select the better and safe candidate to treat diabetes.

Thus, considering the beneficial role of phytochemicals in human health, research leading to the isolation and characterization of individual compounds has gained remarkable impetus across the globe for safer drug discovery. Moreover, ferns and ferns allies known to contain myriad of phytochemicals have now been regarded as the potential group of plants for drug isolation (Shil and Choudhury, 2009).

Though, numerous studies focusing the medicinal properties of angiospersms are available very less consideration has been made towards the useful aspects of pteridophytes (Bir and Vasudeva,

1971; Benniamin *et al*., 2008). Moreover, despite Eastern Himalaya being floristically rich in pteridophytic flora and with a very long history of medicinal uses, ferns have been highly neglected in terms of economical and medicinal values with lesser application in pharmaceuticals. Although, in past years plentiful number of works in line to its medicinal application has been produced globally, however very less attention has been paid towards the ferns available in Darjeeling district of North Bengal region, West Bengal. Thus, considering the importance of ferns in this region and their potential in pharmaceuticals, the present study has been undertaken. The objectives of the present study are:

- Collection and identification of the some locally available ferns
- Phytochemical screening of the active principles of plants
- Estimation of the total phenol, protein and flavonoid of the test plants
- Determination of the antioxidative activities of plants
- Study of the antimicrobial activities of plant samples
- Study of the antidiabetic properties of plants
- Separation, isolation and characterization of the compounds showing potent anti-microbial, anti-oxidative and antidiabetic activities.