Chapter - 1: Introduction

India is a mega biodiversity country not only with rich sources of medicinal plants, but also with valuable information on traditional medical practices (Vincent et al., 2012). Modern day food and lifestyle have resulted in an increasing number of diseases and disorders. A major percentage of the population in developing countries depends on herbal medicines for their primary health care needs (Azim et al., 2011). Pteridophytes are vascular cryptograms which possesses medicinal uses since ancient time (Kumar and Kaushik, 1999). They grow luxuriantly in moist tropical and temperate forests. They have been used in different systems of medicines like ayurvedic and homeopathic. In India 160 useful pteridophytic plant species was reported on the basis of phytochemical, pharmacological and ethnobotanical studies (Singh, 1999).

Plants are rich sources of secondary metabolites with interesting biological activities. In general these secondary metabolites are an important source with a variety of structural arrangements and properties (De-Fathima, 2006). The valuable medicinal properties of different plants are due to the presence of several metabolites / chemical constituents i.e. saponins, tannins, alkaloids, alkenyl phenols, glycol alkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters (Cox, 1990). Among them some are act as synergistic and enhance the bioactivity of other compounds.

India is the largest producer of medicinal herbs known as the herbal garden of the world (Seth and Sharma, 2004). In recent years, the use of medicinal products from plant extracts becomes more extensive. However, some products with poor
qualities emerge in the medicinal market along with the popularity of extract products of medicinal materials. Spectroscopic analysis plays a major role in examining the chemical constituents of the tested sample. Hence, there is a need for quick and effective analytical method to entirely monitor and reflect the whole constituents of medicinal plants and their corresponding extract products.

FT-IR is a powerful tool for identification and determination of functional group of the phytochemicals present in the plant sample (Roberts and Xia, 1995; Alagammal et al., 2011; Bharathi et al., 2012; Sumathi and Uthayakumari, 2014; Asha et al., 2014; Nithyadevi and Sivakumar, 2015). Initially, the use of infrared (IR) spectroscopical method is restricted only for structural elucidation of isolated compounds from the herbal matrices. It is also found useful in phytochemical studies as a ‘finger printing’ devices, for comparing a natural with synthetic sample (Harborne, 1998).

Chromatography is an analytical technique used for quality control and standardization of phytotherapeuticals (Andrew, 2007). Gas Chromatography - Mass Spectroscopy (GC-MS) is normally used for direct analysis of components / chemical constituents existing in traditional medicines and medicinal plants. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants. This technique has proved to be a valuable method for the analysis of non-polar components and volatile essential oils, fatty acids, lipids (Jie et al., 1991) and alkaloids (Betz et al., 1997). To reveal the phytochemical profiles of Adiantum latifolium Lam., Angiopteris evecta (Forst.) Hoff. and Marattia fraxinea Sm. the FT-IR, HPTLC and GC-MS analysis were performed.
Antioxidants are substances that can delay or prevent the oxidation process of free radicals. They are classified into two groups, namely, primary and secondary antioxidants, depending on their mechanism of action. The former react with lipid peroxy radicals to convert them to stable products; this group includes chain breakers (or free radical inhibitors) and peroxide decomposers. Secondary antioxidants, such as oxygen scavengers, suppress the formation of radicals, hence reduce the rate of chain initiation and protect against oxidative damage (Duh, 1998; Juntachote and Berghofer, 2005; Lim et al., 2007).

The use of synthetic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole, tert-butylhydroquinone and propyl gallate has been negatively perceived by consumers due to safety and health effects (Sultana et al., 2014). Hence, there is an increasing interest in the search of natural antioxidants from plant sources. Antioxidants play an important role to protect the human body against damage by reactive oxygen species. Plants containing bioactive compounds have been reported to possess strong antioxidant properties (Fabricant and Farnsworth, 2001). Screening of various bioactive compounds from plants has led to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases (Kumar et al., 2004; Sheeja and kuttan, 2007; Mukherjee et al., 2007). In the present study to determine the antioxidant properties of *Adiantum latifolium*, *Angiopteris evecta* and *Marattia fraxinea* the DPPH, ABTS, phosphomolybdenum and FRAP assays were employed.

Cytotoxic studies using brine shrimp lethality bioassay consists of exposing *Artemia salina* to test extracts in saline solution and the lethality is evaluated after 24 hours. It is a useful method due to its in expensiveness and the ease of performing
the assay (McLaughlin et al., 1998). It has been mainly used for the isolation of cytotoxic and antitumor compounds from plant extracts (Oberlies et al., 1998). Numerous studies have illustrated the use of the brine shrimp assay to screen plant extracts (Padmaja et al., 2002; Morshed et al., 2011). To determine the cytotoxic properties of *Adiantum latifolium*, *Angiopteris evecta* and *Marattia fraxinea* brine shrimp lethality bioassay was performed.

Cancer is the cause of more than 6 million deaths worldwide every year (Izevbigie, 2003). For centuries, medicinal plants have been used in the treatment of cancer (Hartwell, 1982). Nearly 50% of breast cancer and 37% of prostate cancer patients use herbal products (Richardson, 2001). More than 60% of currently used anticancer agents are derived from natural sources. Biologically active components from plants are significant and important sources of new drugs that are likely lead to new and better treatments for breast cancer (Cragg, 2003; Balunas and Kinghorn, 2005). To reveal the anticancer properties of the plant extracts various cell line mediated bioassays (MTT and Tryphan Toluene Blue assay) are performed. In the present study the Tryphan toluene blue dye assay was employed to reveal the anticancer properties of *Adiantum latifolium*, *Angiopteris evecta* and *Marattia fraxinea* against DLA and EAC cell line.

Diabetes mellitus (DM) is a disease commonly associated with elevated glucose level in blood (hyperglycemia) with the ability to alter metabolism of fats and protein and to a large extent carbohydrates metabolism (Onyesom et al., 2002). In this condition, insulin secretion by the beta-cells of the islet of Langerhans in the pancreas is partially deficient (insulinoplethoric) or absolutely deficient (insulinopenic) or either cells cannot utilize the insulin generated (Azu et al., 2014; Shoback, 2011).
Nowadays, Tolbutamide, Metformin, Gliclazide etc that are highly preferred as anti-diabetic drugs. Sulfonylureas and biguanides which are known to cause various adverse effects (Jain et al., 2005). Hence in the recent years medicinal plants have become a subject of interest for drug development owing to their phytochemical constituents and their therapeutic potential.

Hepatic damage is a global metabolic and epidemic disease affecting essential biochemical activities in almost every age group (Anju et al., 2012). Excess consumption of certain drugs like antibiotics, chemotherapeutic agents, acetaminophen, and exposure to some chemicals such as peroxidised oils, aflatoxin, CCl₄, alcohol etc make liver vulnerable to variety of disorders viz., jaundice, hepatitis etc which are the two major hepatic disorders that account for high death rate (Nirmala, 2012).

Normal wound healing response begins the moment when the tissue is injured. An injury is defined as an interruption in the continuity of tissues. Thus, the objective of repairing the injury is mainly to re-establish the continuity by proliferation, migration, and differentiation of involved cells (Goss, 1992). The first body response to injury is inflammation which allows control of blood loss and fends off bacterial invasion. It signals the cells necessary for repair and regeneration to come to the site of injury. The second body response is proliferation. In this phase, new tissues are built to fill the gap left by damaged and debrided tissues.

There are four crucial events occur; angiogenesis (formation of new blood vessels), granulation tissue formation (proliferation of fibroblasts and scar tissue formation), wound contraction (generation of forces within the wound pull surrounding tissue over the wound that lead to its size reduction) and epithelization
(migration and multiplication of keratinocytes across the wound bed) (Myers, 2004). Lastly, in maturation and remodeling phase, there will be reorganization of scar tissue to reach maximum strength and function. This remodeling phase also involves both the resorption and synthesis of components to form the healed skin. But there is no report on anti-diabetic, hepatoprotective, wound healing properties of *A. latifolium, A. evecta* and *M. fraxinea*.

Forhad *et al.* (2014) evaluated the phytochemical screening of *Angiopteris evecta* root methanolic extract. The preliminary phytochemical screening showed the presence of saponins, tannins, alkaloids and flavonoids in the methanolic extract of *A. evecta* roots. Babu *et al.* (2012) analysed the phytochemical screening of *Adiantum latifolium* leaves in crude ethanolic extract and reported the presence of flavonoids, triterpenes and saponins. Patil *et al.* (2013) qualitatively reported the secondary metabolites present in *Dryopteris filix-mas* and *Angiopteris evecta*. The results showed that pteridophytic plants contain valuable secondary metabolites which could be used as therapeutic agent. Britto *et al.* (2012) analysed the phytochemical constituents of five medicinal ferns viz., *Pteris biaurita, Lygodium flexuosum, Hemionitis arifolia, Actinopteris radiata* and *Adiantum latifolium*. The plants were extracted successively with petroleum ether, benzene, chloroform, methanol and distilled water using soxhlet extractor. Their results showed the presence of more bioactive principles in the studied ferns. The available literature clearly explained the preliminary phytochemical studies on *A. latifolium, A. evecta*, and *M. fraxinea*. But there is no report on the spectroscopic and chromatographic analysis on *A. latifolium, A. evecta*, and *M. fraxinea*. To reveal the phytochemical (spectroscopic and chromatographic) profile, the present study was performed using FT-IR, HPTLC and
GC-MS analysis. In addition, the biological properties (antioxidant, hepatoprotective, antidiabetic, anticancer, cytotoxic) of *A. latifolium*, *A. evecta*, and *M. fraxinea* was also determined.

The present study was carried out with the following objectives:

- To know the secondary metabolites presence in *Adiantum latifolium*, *Angiopteris evecta* and *Marattia fraxinea*
- To study the functional groups and chemical composition of *Adiantum latifolium*, *Angiopteris evecta* and *Marattia fraxinea* using FT-IR, GC-MS and HPTLC analysis
- To find out the toxicity level of *Adiantum latifolium*, *Angiopteris evecta* and *Marattia fraxinea* crude extracts using brine shrimp lethality bioassay
- To determine the antioxidant activities of *Adiantum latifolium*, *Angiopteris evecta* and *Marattia fraxinea* crude extracts using various assays viz., DPPH, ABTS, phosphomolybdenum and FRAP
- To evaluate the anticancer potential of *Adiantum latifolium*, *Angiopteris evecta* and *Marattia fraxinea* using DLA and EAC cell line
- To determine the antidiabetic, hepatoprotective and wound healing activity of *Adiantum latifolium*, *Angiopteris evecta* and *Marattia fraxinea*