2. Review of Literature

In this chapter, the early history of pteridophytes, various phytochemicals present in pteridophytes and their medicinal utilities are described in details. This is followed by a brief description of established reports of the genus Dipteris.

2.1 Early History of Pteridophytes

For thousands of years, nature has been a source of medicinal agent form where an impressive number of modern drugs have been isolated based on their use in traditional medicine. Historically, most of the medicinal preparations were derived from plants, the medicinal value of which lies in some chemical substances that produce a definite physiological action on the human body. The pteridophytes possess an important role in folklore medicine although neglected in modern days. These plants have been successfully used in different systems of medicines like Ayurvedic, Unani, Homeopathic and other systems of medicines. Out of 1250 species of Pteridophytes occurring in India, 173 species have been found to be used as food, flavor, dye, medicine, bio-fertilizers, oil, fiber and bio-gas production (Manickam and Irudayaraj, 1992). The medicinal value of pteridophytes against bacteria, fungi, virus, cancer, rheumatism, diabetes, inflammation, fertility, diuretic, pesticides, hepatoprotective and sedative had been reported. Besides sugar, starch, proteins and amino acids, ferns contain a variety of alkaloids, glycosides, flavonoids, terpenoids, sterols, phenols, sesquiterpens etc. as potential components used in various industries (Kulandairaj and Britto, 2000).

Kirtikar et al. (1935) have described 27 species of ferns having varied medicinal uses. Chopra et al. (1956) have included 44 species and Nadkarni (1954) recorded 11 species of pteridophytes having medicinal importance. Nayar (1959) recorded 29 medicinal
ferns. May (1978) published a detailed review of the various uses of ferns and listed 105 medicinal ferns. In a recent compilation, Singh (1999) reported 160 species of useful Pteridophytes in India on the basis of phytochemical, pharmacological and ethnobotanical studies. The antimicrobial potential of some ferns has been studied (Kumar and Kaushik, 1999; Parihar and Bohra, 2002a & b, 2003). Young leaves of the ferns Diplazium esculentum (Retz.) Sw., Helminthostachys zeylanica (L.) Hook, Nephrolepis cordifolia (L.) Presl and Stenochlaena palustris (Burm.) Bedd. are cooked as vegetables by the tribal in Indian mountains. Azolla pinnata R.Br. is used as rice fertilizer and chicken feed. In the case of water fern Marsilea drummondii the starchy paste of the sporocarps is made in to cakes called “Nardoo” and is eaten by the natives of Australia. Only few Pteridophytic plants are used as medicine e.g. paste of tubers of Nephrolepis auriculata (Linn.) Trimen is used to lower down the brain fever and headache by applying locally. The vegetable of the Croziers of different species of Diplazium Sw. is known to be of laxative nature and often used to treat colitis and constipation. Selaginella bryopteris (Linn.) Bak is considered as highly useful in unconsciousness. Similarly Helminthostachys zeylanica (Linn) Hook. is used to revert the impotency. The paste of Adiantum incisum Forssk. and A. venustum is useful in the healing of wounds (Samant et al., 1998; Kholia and Punetha, 2005). Quite a number of ferns and ferns allies are of great medicinal value. Equisetum arvense Linn. is used in nasal polyps and kidney infections, ashes useful in acidity. E. debile Roxb. is diuretic and given in gonorrhoea. Lycopodium clavatum Linn., in the form of decoction used in rheumatism and diseases of lungs and kidneys. The paste of the leaves of Ophioglossum reticulatum Linn., is used in headache. Botrychium virginianum Sw. is used in dysentery. Helminthostachys zeylanica (Linn.) Hook. is used for vitality and brain tonic. Lygodium flexuosum (Linn.) Sw., is an expectorant and used in ulcers, cut
wounds and sprains. The fronds of the gleicheniaceous fern *Dicranopteris linearis* (Burm.) Underwood is used for asthma and in woman’s sterility. The plant, ‘royal fern’ or *Osmunda regalis* Linn. is used as stypic and tonic. The rhizomes of *Angiopteris evecta* (Forst.) Hoffm. are used for scabies (Vasudev, 1999). The ferns are effective in arresting embryonic development in insects. These substances may be exclusively produced by plants for defense against insect predation. The extracts of pteridophytes have toxic effects on *Spodoptera littura* and *Helicoverpa armigera*. The young fronds of *Phymatosorus scolopendria* (N.L. Burm.) are spread on the bed to keep off bed bugs (Mannan *et al.*, 2008).

The tribal communities, ethnic groups and folklore throughout the world are utilizing different parts of pteridophytes like rhizome, stem, fronds, pinnae and spore in various ways for the treatment of various ailments since ancient time. The numbers of contributors about the taxonomy, ecology and distribution of Pteridophytes have been published from time to time but enough attention has not been paid towards their useful aspects, specially the phytochemical part of Pteridophytes.

### 2.2 Phytochemical Investigation of Pteridophytes

Phytochemicals are chemical compounds or chemical constituents formed in the plant's normal metabolic processes. The chemicals are often referred to as "secondary metabolites" of which there are several classes including alkaloids, steroids, terpenoids, catecholamines, tannins, saponins, anthraquinones, coumarins, fats, flavonoids, glycosides, gums, iridoids, mucilages etc. The naturally occurring phytochemicals offer promise to be used as safe alternatives. The pteridophytes constitute the primitive vascular plant group which are found scattered all over the world. Although, not much consideration has been given towards the utility of pteridophytes yet these possess equal
economic importance including medicinal ones. Caius (1935) is supposed to be the first man who has described medicinal uses of some ferns of India. Filicin, isolated from the rhizome of *Dryopteris filixmas*, is a potential insecticide. Filicin has anti-helminthic properties also. The phytoecdysones present mainly in the ferns, still appear to be somewhat relatively free from insect predation. *Pteris vittata* L., a common fern found all over the world, its young fronds are used traditionally as an astringent. Its decoction is reported to be used in dysentery and the rhizome is eaten as a tonic after boiling in water (Anonymous, 1969). The species has not been studied thoroughly for its pharmacological properties.

2.2.1 Alkaloids in Pteridophytes

Alkaloids, a group of naturally occurring chemical compounds which mostly contain basic nitrogen atoms, produced by bacteria, fungi, plants and animals and are part of group of natural products called secondary metabolites which display a variety of marked effects on animals. Alkaloids often act on the nervous system as stimulators, and sometimes as poisons. Cocaine (which exhibits an anaesthetic effect), atropine (which effects motor nerves), and curare (which has been used by South American natives to cause paralysis of prey), are all alkaloids (Kretovich, 1966). Certain *Lycopodium* alkaloids, which occur naturally in *Lycopodium* and other pteridophytes, have been investigated for their medicinal properties. Alpha-onocerin and lycoperine A, for example, exhibit acetylcholinesterase inhibition activity (Zhang *et al.*, 2002, Hirasawa *et al.*, 2003). Huperzine A, an alkaloid, isolated from *Huperzia* species and other members of Lycopodiaceae, has been shown to enhance memory in animals and is also being investigated for treatment of Alzheimer's disease (Ma *et al.*, 2004).
2.2.2 Terpenoids in Pteridophytes

The terpenoids have been prized for their essential oils isolated from plants and their use as fragrances for over two thousand years. These are mainly classified as sesquiterpenoids, monoterpenoid, diterpenoids, and triterpenoids, depending on whether they contain two, three, four or six isoprene units. Indeed, the triterpenoids are polyisoprenes (Kretovich, 1966). Terpenoids are of many skeletal types and 40 pteridophytes contain triterpenoids (hopane triterpenoids, epoxytriterpenoid, and serratene triterpenoid), diterpenoids, hemiterpene glycosides, and clerodane diterpene glycosides. Many terpenoids are medicinally significant for a wide range of treatments. For example, triterpenoids isolated from Erica andevalensis are cytotoxic against human cancer cell lines (Cordero et al., 2001). Two new migrated hopane triterpenoids, viz. 4alpha-hydroxyfilican-3-one and fern-9(11)-en-12beta-ol, and olean-18-en-3-one and olean-12-en-3-one, the first example of oleanane compounds from Adiantum ferns, were isolated along with many other known triterpenoids from Adiantum capillus-veneris of China and Egypt (Nakane et al., 2002). A new triterpenoid, 22,29ξ-epoxy-30-norhopane-13β-ol was isolated together with six known compounds viz., fern-9(11)-en-6α-ol, fern-9(11)-ene, fern-9(11)-en-25-oic acid, fern-9(11)-en-28-ol, filicenol-B, adiantone and oxidation product of fern-9(11)-en-6α-ol obtained as 6-oxofern-9(11)-ene from the whole plant of Adiantum lunulatum, and their structures were elucidated by means of spectroscopic analysis and antibacterial evaluation of these compounds were conducted (Reddy et al., 2001).

Fronds of the fern Lophosoria quadripinnata exhibit a thin waxy coating on their lower surface. Five major components of this lipophilic material have been identified by
spectroscopic methods as hopane type triterpenes, one of which is a novel natural product (Tanaka et al., 1992).

### 2.2.3 Flavonoids in Pteridophytes

The flavonoids constitute another very important group of secondary metabolite, contain two benzene rings separated by a propane unit and are derived from flavone. They are generally water-soluble compounds and are found in plants as their glycosides, which can complicate structure determinations. Only a fraction of flavonoid subdivision is represented in pteridophytes. These are biflavonoids, homoflavonoids, flavone glycosides, and flavonol glycosides. Many flavonoids have medicinal properties. Amentoflavone and ginkgetin, flavonoids found in Selaginella, exhibit neuroprotective activity against cytotoxic stress. This property suggests their possible use in treatment of neurodegenerative diseases such as stroke and Alzheimer’s (Kang et al., 2005). Several studies have reported the presence of leucocyanidin, leucodelphinidin, the flavone ester apigenin 7-O-p-hydroxybenzoate in P. vittata L. (Salantino et al., 1998; Imperato, 2006).

The fronds of the fern Asplenium trichomanes contain kaempferol 3, 7-dirhamnoside and the new compounds kaempferol 3-O-a-rhamnoside-7-O-a-arabinoside and kaempferol 3-O-a-arabinoside-7-O-a-rhamnoside. The presence of all of the above mentioned flavonoids has been shown by spectroscopic methods and chemical degradations (Imperato, 2005).

### 2.2.4 Glycosides in Pteridophytes

Glycosides, a group of natural product in which a sugar is bound to a non-carbohydrate moiety, usually a small organic molecule. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. Many
such plant glycosides are used as medications. There are only three species that fit into this category. From these plants benzophenones, ent-pimarene type glycosides, and lactone glycosides are the compounds identified so far. Perhaps the most interesting is the benzophenone that has been isolated in *Davallia solida* (Rancon *et al*., 2001). Benzophenones are involved in the P-glycoprotein removal of harmful substances and may act in the detoxification function of the body (Thews *et al*., 2006). A number of glycosides are apigenin, leutolin, isocutellarein-8-O-methyl-ether, kaempherol and quercetin (Salantino *et al*., 1998; Imperato, 2006). In addition it has been shown that *P. vittata* hyperaccumulates arsenic (Ma *et al*., 2001).

2.3 Biological and Pharmacological activities of phytochemicals of Pteridophytes

Many studies have shown that pteridophytes are responsible for a number of biological and pharmacological activities. Several species have shown to be endowed with antimicrobial, antiulcer, antioxidant, analgesic, antinociceptive, anti-inflammatory, cytotoxic, sedative and even anticonvulsant activities.

2.3.1 Antimicrobial activity of Pteridophytes

Phytochemicals from pteridophytes show very potent antimicrobial properties. Methanol extract of *Drynaria quercifolia* rhizome showed broad and concentration dependent antibacterial activity against all tested bacteria. Though no activity was observed against fungi, these results may provide scientific support for some uses of the plant in traditional medicine (Ramesha *et al*., 2001). Extracts prepared from fronds and rhizomes of the species *Polypodium brasiliense* Poiret (=Serpocaulon triseriale (Sw.) A.R.Sm.), collected by Barros *et al*. (1989) from Atlantic forest have been tested against seven different microorganisms, four Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus faecalis* and *Mycobacterium smegmatis*),
one Gram-negative bacteria (*Escherichia coli*), one yeast (*Candida albicans*) and one filamentous fungus (*Monilia siliphila*). The result showed that only *M. siliphila* did not suffer growing inhibition in exposure to the extracts of *Serpocaulon triseriale*. Peres et al. (2009) studied the antimicrobial effects of *Microgramma vacciniiifolia* (Langsd. & Fich.) Copel. The result showed that the hexane fraction from ethanolic crude extract was most effective against the *Saccharomyces cerevisae* and *Candida albicans* fungi. Singh et al., (2007) exclusively studied the antibacterial activity of *Adiantum* Linn. of Adiantaceae family. They have selected four important species, i.e. *Adiantum capillus–veneris*, *Adiantum peruvianum*, *Adiantum venustum* and *Adiantum caudatum* and extracted with methanol. These extracts were tested for their antimicrobial agents against five gram positive, six gram negative (including multiresistant bacteria *Staphylococcus aureus*) and eight fungal strains using standard microdilution assay. The maximum activity was exhibited by the methanolic extract of *Adiantum venustum* followed by *Adiantum capillus–veneris*, *Adiantum peruvianum* and *Adiantum caudatum*. The methanolic extract of *Adiantum capillus–veneris* had very low MIC value (0.48 µg/ml) against *Escherichia coli* whereas, *Adiantum venustum* extract against *Aspergillus terreus* with MIC of 0.97 µg/ml. Total phenolic constituents of *Adiantum* species viz. *Adiantum venustum*, *Adiantum capillus–veneris*, *Adiantum peruvianum* and *Adiantum caudatum* were 0.81% (w/w), 0.83% (w/w), 0.71% (w/w) and 0.52% (w/w), implying that the observed activity could be related to the amount of phenolics. Banerjee and Sen (1980) surveyed the occurrence of antibiotic activity in the extracts of 114 species of pteridophytes (27 families, 61 genera). The plants were extracted with water, methanol, 70% ethanol, acetone, ether and assayed against three gram-positive, one acid-fast, five gram-negative bacteria and three fungal plant pathogens. Sixty-seven ferns and six fern allies, representing 64% of the samples.
examined, were shown to possess antibiotic property. The active substances were in
most cases antibacterial and only three possessed antifungal activities. Thirty-three
species were inhibitory to gram-positive bacteria alone, nine exclusively against gram-
negative bacteria and 15 to both, 20 species were inhibitory to penicillin-resistant
Staphylococcus aureus, 16 against Mycobacterium phlei, 24 against Salmonella typhi,
16 against Vibrio cholerae and 3 against Pseudomonas aeruginosa. Only Dryopteris
cochleata was active against both bacteria and fungi. Five other species of Dryopteris
showed remarkable antibacterial activity. Imperato et al., (2000), studied antimicrobial
activity and phytochemical profile of P. vittata, especially against gastrointestinal (GI)
pathogens and reported the presence of flavonoid. Banerjee and Sen (1980) in another
report presented the antimicrobial activity of some polypodiaceous ferns. These include
Microsorum alternifolium, Leptochillus decurrens, Polypodium irioides, Pyrrosia
mannii and Phymatodes ebenipes. Several thelypteroid, davalloid and athyroid ferns
were also found fairly active.

Nine species of Selaginella represented on Moorea to have shown bioactivity. Four of
the nine tested, have cytotoxic activity, while the other exhibit antiviral, anti-
inflammatory, antifungal, antimicrobial, and antioxidant properties (Silva et al., 1995,
Sun et al., 1997, Lin et al., 2000, Lee et al., 1999, Woo et al., 2005, Chen et al., 2005,
Ma et al 2003). Microsorum grossum exhibited antifungal properties in experiments
conducted at the Gump Research Station on Moorea (Baltrushes, 2005). The ethyl
acetate, butanol and water fractions of Blechnum orientale Linn. (Blechnaceae) were
effective against all Gram-positive bacteria tested viz., Bacillus cereus, Micrococcus
luteus, methicillin-susceptible Staphylococcus aureus (MSSA), methicillin-resistant
Staphylococcus aureus (MRSA) and Staphylococcus epidermidis. Phytochemical
analysis revealed the presence of flavonoids, terpenoids and tannins. Ethyl acetate and butanol fractions showed highest total phenolic content (Lai et al., 2010).

### 2.3.2 Antiulcer activity of Pteridophytes

In the region of Sao Paulo, Brazil, the population makes use of a tea prepared from rhizomes of *Micogramma squamulosa* (Kaulf.) Sota. for the treatment of ulcers (Suffredinia et al., 1999). They evaluated the action of a crude extract of this species, against acute ulcers caused by ethanol and hydrochloric acid, using misoprostol and cimetidine as reference substances in both tests. The same extract and the control cimetidine were tested also against sub-chronic ulcers induced by acetic acid. The extracts showed significant activity against sub-chronic ulcers, but not against acute ulcers. The mechanism of action must be related to the presence of tannins in the extracts, which would induce a stringent action or to the presence of flavonoids, through a systemic action, perhaps similarly to the cimetidine action. The toxicological sub-chronic action of the crude extract, using a dose of 800 mg/kg for 30 days, was also evaluated. After this time, the animals did not show any significant variation in the consumption of food and water, and neither variation of weight, thus not presenting any evidence of toxicological action.

### 2.3.3 Analgesic activity of Pteridophytes

The species *Adiantum cuneatum* Langsd. & Fish, (=*Adiantum raddianum* C. Presl), which is used as an ornamental plant, is famous for its medicinal properties, mainly in the treatment of pain. Bresciani et al. (2003) studied the crude extracts from the fronds of this species. The hexane extract proved to be responsible for a strong dose dependent analgesic action in rats, demonstrating to be equipotent to acetyl salicylic acid and to acetaminofen. Phytochemical examination of this extract showed it to be
rich in triterpenes. Fractionation of this extract led to the isolation of the triterpenes filicene, filicenal, adiantol and isoadiantone. The analgesic activity of filicene and filicenal was tested; it was shown that these metabolites are responsible, at least in part, for the activity presented by the hexane crude extract. Hence, these results confirm and justify the popular use of this species in the treatment of pain. Stems of the species *Equisetum arvense* L., furnished a hydro-alcoholic extract that has shown analgesic effect against chemical models of pain perception (acetic acid induced writhing syndrome), but not in thermal models (hot plate), with central and peripheral action (Do Monte et al., 2004).

Oral administration of *Drynaria quercifolia* produced significant inhibition of carrageenan-induced paw oedema and granuloma formation in rats, almost comparable to that caused by indomethacin. *Drynaria quercifolia* significantly attenuated acute and delayed phases of formalin-induced pain and acetic acid-induced writhing episodes in mice. The analgesia was comparable to that produced by sodium salicylate and aspirin respectively (Anuja et al., 2010).

### 2.3.4 Anti-inflammatory and antinociceptive activity of Pteridophytes

Nonato et al. (2009) supported the folk medicinal use of fern species, *Blechnum occidentale* L. has been used to treat inflammatory disease. Nonato et al. (2009) evaluated the antinociceptive and anti-inflammatory action of the methanolic crude extract of blades of this species. The study also demonstrated that systematic administration of the methanolic extract of *B. occidentale* did not produce any motor performance alteration. The hydro-alcoholic extract of stems of the species *Equisetum arvense* L. showed anti-inflammatory activity (do Monte et al. 2004). *Cyathea phalerata* Mart. is used to treat various diseases associated with
inflammatory processes. Phytochemical investigations of this plant showed the presence of an active flavonoid kaempferol-3-neohesperidoside, which possess hypoglycaemic activity (Pizzolatti et al. 2007); cyathenosin A, a spiropyranosil derivative of protocatechuic acid also isolated from the stem pith of C. phalerata. Drynaria quercifolia (L.) J. Smith has been widely used by ethnic groups of India to treat inflammation. Phytochemical analysis gave positive tests for catechin, coumarins, flavonoids, phenolics, saponin, steroids, tannins and triterpenes. The results suggest the presence of potent anti-inflammatory principles in Drynaria quercifolia (Anuja et al., 2010).

2.3.5 Antioxidant activity of Pteridophytes

Antioxidants have been used as important protective agents for human health. Santos Jr. et al. (2005a, 2005b) studied the hydro-alcoholic extract of Equisetum arvense L. collected from an undefined locality of the State of Santa Catarina, Brazil, and evaluated its antioxidant activity and the action on the central nervous system (CNS). The study shown that the extract presents sedative and anticonvulsant activities, and improves cognitive deficits in aged rats. Phytochemical analysis detected the presence of tannins, saponins, sterols and flavonoids. The species Dicksonia sellowiana (Presl.) Hook fronds extracts were tested for antioxidant potential and to assess the concentration in phenolic substances. It was observed that the extracts contained a higher concentration of polyphenols which showed more potent antioxidant activity, concluding that the antioxidant activity of D. sellowiana must be related to the presence of this class of substance (Bora et al., 2005).

Hort et al. (2008) studied the antioxidant and hepatoprotective potential of the crude hydro-alcoholic extracts of Cyathea phalerata Mart. and of fractions obtained
by treatment with organic solvents of increasing polarity. The results have shown that the ethyl acetate fraction of the crude extract displayed the best antioxidant activity and is assumed that the flavonoids contained in this fraction could be responsible for this activity. Peres et al. (2009) evaluated the antioxidant activity of an ethanolic crude extract and fractions of Microgramma vaccinifolia (Langsd. & Fich.) Copel. fronds using 2,2-di-phenyl-1-picrylhydrazyl (DPPH). The ethyl acetate fraction showed good activity in DPPH assay.

Appel et al. (2008) reported the antioxidant potentiality of Cyathea phalerata. The in vitro antioxidant potential of the crude extract (CE), precipitate (PPT), aqueous fraction (AQF), n-butanol fraction (BUF) and ethyl acetate fraction (EAF) from C. phalerata was evaluated through the scavenging of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), superoxide anion (O$_2^{-}$) (nitroblue tetrazolium assay), hydroxyl radicals (OH$^-$) (deoxyribose assay), and lipid peroxidation in rat liver homogenate. The assays reported that, EAF had marked antioxidant potential, especially as a scavenger of the OH$^-$ radical and in inhibiting lipid peroxidation. The in vivo evaluation of oxidative stress (DNA fragmentation, membrane lipoperoxidation and carbonyl protein formation) and the antioxidant defences (concentration of reduced glutathione, as well as catalase and glutathione S-transferase activities) were measured in mice pre-treated with EAF (10, 30 or 100 mg/kg, orally) and later exposed to carbon tetrachloride (CCl$_4$). The EAF decreased thiobarbituric acid reactive substances levels, DNA damage carbonyl protein contents, and increased catalase and glutathione S-transferase activities. Based on these results, it was concluded that the EAF from C. phalerata protects liver from oxidative stress induced by CCl$_4$ in mice and these effects are probably related to the antioxidant activity associated with the free radical scavenging property of this fractions. Two
glycosides, 6′-O-(3,4-dihydroxy benzoyl)-β-d-glucopyranosyl ester, and 4-O-β-d-glucopyranoside-3-hydroxy methyl benzoate along with five known compounds methyl benzoate, hypogallic acid, caffeic acid, paeoniflorin and pikuroside were isolated for the first time from a fresh water fern *Salvinia molesta* D.S. Mitch. These compounds showed a potent antioxidant radical scavenging activity in a non-physiological assay (Choudhary *et al.*, 2008). The ethyl acetate, butanol and water fractions of *Blechnum orientale* Linn. (*Blechnaceae*) possessed strong radical scavenging activity. Phytochemical analysis revealed the presence of flavonoids, terpenoids and tannins. Ethyl acetate and butanol fractions showed highest total phenolic content (Lai *et al.*, 2010).

### 2.3.6 Cytotoxicity of Pteridophytes

A total of six pteridophytes *viz.* *Adiantum australasicum, Adiantum plantagineum, Lycopodium. reticulatum, B. lonchophora, Microsorum commutatum,* and *Lycopodium ernua* exhibited cytotoxic activity in a brine shrimp lethality assay conducted at the Gump Research Station on Moorea. Differences between cytotoxicity of root, leaf extracts, ethanol and water extracts were found in the cytotoxicity experiments suggesting a diversity of compounds within the pteridophytes (Baltrushes, 2005). *Selaginella* has many cytotoxic species and each species with such activity contains bioflavonoids. Four of the nine species of *Selaginella* tested, have cytotoxic activity (Silva *et al.*, 1995, Chen *et al.*, 2005, Woo *et al.*, 2005). *Pityrogramma calomelanos*, a moorean fern, is cytotoxic and contains flavonoids (Star *et al.*, 1971, Sukumaran *et al.*, 1991). *Peris semipinnata* and *Pteris multifida*, both have cytotoxic property and contain diterpenes (Li *et al.*, 1998, Li *et al.*, 1999). These plants have different active compounds but show similar effects. Analysis of the chemical constituents of plants is
thus an important tool for identifying useful plants, but without assays it is not sufficient to indicate medicinal usefulness. The ethyl acetate, butanol and water fractions of *Blechnum orientale* Linn. (*Blechnaceae*) possessed cytotoxic activity towards human colon cancer cell HT-29. Phytochemical analysis revealed the presence of flavonoids, terpenoids and tannins. Ethyl acetate and butanol fractions showed highest total phenolic content (Lai *et al.*, 2010).

2.3.7. Hepatoprotective activity of Pteridophytes

The hepatoprotective potential of *Lygodium flexuosum* (L.) Sw. was evaluated in male wistar rats against carbon tetrachloride-induced liver damage in preventive and curative models. Pre-treated rats were given *n*-hexane extracts at 200 and 100 mg/kg dose 48, 24 and 2 h prior to CCl4 administration. In post-treatment groups, rats were treated with *n*-hexane extract at a dose of 200 and 100 mg/kg, 2, 24 and 48 h after CCl4 intoxication. Rats pre-treated with *Lygodium flexuosum* remarkably prevented the elevation of serum AST, ALT, LDH and liver lipid peroxides in CCl4-treated rats. Rats treated with the extract after the establishment of CCl4 induced liver injury showed significant protection of liver as evidenced from normal AST, ALT, LDH and MDA levels. Hepatic glutathione levels were significantly increased by the treatment with the extracts in both the experimental groups. Histopathological changes induced by CCl4 were also significantly reduced by the extract treatment in preventive and curative groups. Phytochemical studies revealed the presence of saponins, triterpenes, sterols and bitter principles in *Lygodium flexuosum* *n*-hexane extract which could be responsible for the possible hepatoprotective action (Wills and Asha, 2006).

The rhizomes of *Helminthostachys zeylanica* (L.) are used by the Kattunaikan tribe of Kerala, for the treatment of various hepatic disorders. The effect of the methanolic
extract of *Helminthostachys zeylanica* rhizomes on carbon tetrachloride (CCl4)-induced liver damage in Wistar rats was studied. The results showed significant hepatoprotective effect against CCl4-induced liver damage, by oral administration of *Helminthostachys zeylanica* methanolic extract. The extract was effective in increasing the choleretic activity of anaesthetised normal rats. It also shortened hexobarbitone-induced sleeping time in mice, which was increased by CCl4 treatment, besides showing significant antilipid peroxidant effect in vitro. This provides a scientific rationale for the traditional use of this plant in the management of liver diseases (Suja *et al.*, 2004).

### 2.4 Ethnopharmacological investigations of genus *Dipteris*

A very few records are available regarding ethnomedicinal and phytochemical investigation of this genus *Dipteris*. During the course of a comparative study of the vascular organization in the homosporous ferns, some interesting morphological peculiarities in the vasculature of the rhizome in three species of *Dipteris* viz., *D. conjugata* Reinw., *D. lobbiana* (Hook.) Moore and *D. wallichii* (R.Br.) Moore was noticed. The remaining two being *D. papilioniformis* Kjellberg from Celebes and *D. quinquefurcata* (Bak.) Christ from Borneo could not be studied due to a lack of material. On the basis of the vasculature of the rhizome, it is suggested that the complexity of the leaf trace in these species may be held as forming a series progressive from the simple condition (*D. chinensis*, *D. conjugata*, *D. nieuwenhuisii* and *D. novoguineensis*) through *D. lobbiana*, leading to the more complex state in *D. wallichii* which probably exhibits a derived condition. It is reported that *D. wallichii* is a tetraploid which further strengthens the evolutionarily advanced condition of the species (Chandra S. and Kaur S., 1993).
Reports have been found that many important species of plants of Garo Hills, Khasi Hills, and Jaintia Hills, representing the Western, Central, and Eastern parts of the state of Meghalaya, are under threat and many species have become extinct because of various developments, including intensification of agriculture and infrastructure development. *Dipteris wallichii* (R.Br.) T. Moore represents one of those species which has been extinct in Meghalaya (Kataki, 1983). *Dipteris conjugata* is currently the only member in the family Dipteridaceae extant in Singapore. It is considered critically endangered in Singapore (Tan et al., 2008). Regarding ethnomedicinal utility, records showed that the rhizome of *D. wallichii* is used as hepatoprotective agent (Dutta Choudhury *et al*., 2002). Since the plant is endemic to North East region, it has become essential to study the chemical entities as well as pharmacological evaluation of this plant which might possess high potentiality to become drug.