Chapter 2

REVIEW OF LITERATURE

In this chapter, various fern phytochemicals along with their medicinal potentiality are described exclusively. Followed by this, established reports of works on the genus Cyathea are presented.

2.1 Early History:

For centuries, China, India, Egypt and Greek led the world in the use of natural products in healing and folkloric medicine based on the use of plants and animals have played a significant role in reducing human sufferings and pains. From ancient times, people are well aware of extraordinary medicinal properties of garlic, cinchona etc. in curing many ailments. *Artemisia annual* L. (Qinghao Fan. Compositae) was used in China for curing fever and malaria. In 1972, an antimalarial drug artemisinin was isolated and was found to be superior to other conventional antimalarial drugs. The biological effect of Ginseng (Panax ginseng) was well recognized by the ancient Chinese. Its biological effects are mainly due to synergistic activity between saponins called ginsenosides and flavonoids. *Ginkgo balboa* is another important folk medicine used by the Chinese in improving mental alertness. The main constituents that are associated with *Ginkgo* are due to the activity of ginkgoloids and flavonoids.
2.2 Phytochemical Investigation of Pteridophytes:

The phytochemical potential of pteridophytes is relatively unexplored, although pteridophytes possess great economic potential due to some interesting medicinal and antimicrobial properties (Chen et al., 2005; Dhiman, 1998; Gogoi, 2002; Reddy et al., 2001; Singh et al., 2001; Singh et al., 2008; Vasudeva, 1999). *Pteris vittata* L., a common fern known as 'Brake Fern', is found all over the world, including India, and 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 class of nitrogenous secondary metabolites are known to display a variety of marked effects on animals. Alkaloids often act on the nervous system as stimulants, and sometimes as poisons. Cocaine (which exhibits an anesthetic effect), atropine (which effects motor nerves), and curare (which has been used by South American natives to cause paralysis of prey), are all alkaloids. 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*, a *Lycopodium* alkaloid, isolated from *Huperzia* species among others, 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:

Terpenoids are the main component of many plant essential oils isolated from plants. 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. Terpenoids are of many skeletal types and 40 pteridophytes contains 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. 4α-hydroxyfilican-3-one and fern-9(11)-en-12β-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, 22,29-c-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). Yoko, et al.,(2003) reported ten known triterpenoids, three derivatives of phytol, a stanol, and b-tocopherol from the fresh fronds of *Cyathea podophylla*.

### 2.2.3 Flavonoids in Pteridophytes:

Flavonoids, like alkaloids and terpenoids, constitute another very important group of secondary metabolite of origins in plants. Only a fraction of flavonoid subdivision are 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.

Several studies have reported the presence of leucocyanidin, leucodelphinidin, the flavone ester apigenin 7-O-p-hydroxybenzoate in *P. vittata* L. (Salantino et al.,
The fronds of the fern *Asplenium trichomanes* contain kaempferol 3, 7-dirhamnoside and the new compounds kaempferol 3-O-a-rhamnose-7-O-a-arabinose and kaempferol 3-O-a-arabinose-7-O-a-rhamnose. 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:

The final category of plants is comprised of ferns from which compounds other than terpenoids, flavonoids, or alkaloids have been isolated thus far. 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 of apigenin, luteolin, isocutellarein-8-O-methyl-ether, kaempferol and quercetin (Salantino et al., 1998; Imperato, 2006). In addition it has been shown that *P. vittata* hyperaccumulates arsenic (Ma et al., 2001).
Cooper et al., (1980) reported the importance of flavonoids in understanding of phylogenetic and taxonomic relationship between ferns and fern allies.

Wallace et al., (1983) studied the phytochemical approaches to the Gleicheniaceae and suggested that distribution of flavonol-3-O-glycoside supports the segregation of the Gleicheniaceae, *Sensu lato*, into at least three groupings. Wallace Jr. (1996) while studying the 23 species of Trichomanes s. 1 indicated the presence of C-glycosylflavones, especially mono-C- glycosylflavones, may be a basic characteristic for this genus. Except for the morphologically unique *Cardiomanes reniforme*, this feature approaches to delimit the species of Trichomanes s.l from those of Hymenophyllum s. 1. Several species, primarily those classified within *Didymoglossum*, synthesize flavone-O-glycoside in addition to their C-glycosylflavone profiles. Two species, *T. birmanicum* and *T. radicans*, appear to be unique in synthesizing the di-C- glycosylflavones violanthin and isoviolanthin. Five species (*T. collariatum, T. scandins, T. birmanicum, T. bicorne*, and *T. alatum*) appear to be synthesize di-C- glycosylflavones but lack mono-C-glycosylflavones. Based on this and other studies, it may be appropriate to consider these C-glycosylflavone- producing plants as representing primitive Leptosporangiate stock.

### 2.3 Medicinal potentiality of Pteridophyte phytochemicals:

There are many reports in the literature indicating the potentiality of pteridophyte phytochemicals. These phytochemicals are widely used as potential antibacterial, antifungal and antiviral agents. Some of these are acts as active antioxidants. There are also some reports establish the Cytotoxic potentiality of some fern phytochemicals. Fronds of the fern *Microsorum scolopendria* are widely used in traditional medicine in the Society Islands. They were investigated for the presence of ecdysteroids, which might be responsible for at least some of their medicinal properties. *M. scolopendria* represents an excellent source of ecdysone (0.16% of dry weight) and 20-hydroxyecdysone (0.20%), and also contains significant amounts (0.01-0.02%) of makisterones A and C, inokosterone and amarasterone A, together with lower amounts of postosterone and of
2.3.2: Antimicrobial Potentiality of Pteridophyte phytochemicals:

A compound tentatively identified as 24,28-diepi-cyasterone (Eva Snogan et al., 2007).

2.3.1 Antioxidant Potentiality of Pteridophyte phytochemicals:

Appel et al., (2008) in their paper “Antioxidant and Hepatoprotective effects of Cyathea phalerata Mart. (Cyatheaceae)” reported the antioxidant potentiality of Cyathea phalerata. The in vitro antioxidant potential of the crude extract (CE), precipitate (PPT), aqueous fraction (AQF), n-butanolic 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. In these assays they 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 defenses (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).
2.3.2 Antimicrobial Potentiality of Pteridophyte phytochemicals:

Phytochemicals from some pteridophyte species specially *Adiantum, Selaginella, Pteris vittata* etc. shows very potent antimicrobial properties. Singh et al., (2007) exclusively studied the antibacterial activity of *Adiantum* Linn. of Adiantaceae family. Ethnomedicinally, the genus is important and popularly known as "Hansraj" in Ayurvedic System of Medicine. It has been used in cold, tumors of spleen, liver and other viscera, skin diseases, bronchitis and inflammatory diseases. It is also considered as tonic and diuretic. In their experiment 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 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), respectively (as gallic acid equivalent); implying that the observed activity could be related to the amount of phenolics.

Banerjee (2008) surveyed the occurrence of antibiotic activity in the extracts of 114 species of pteridophytes (27 families, 61 genera). The plants were extracted in 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. Twenty species were inhibitory to penicillin-resistant *Staphylococcus aureus*, 16 against
**2.3.2: Antimicrobial Potentiality of Pteridophyte phytochemicals:**

*Mycobacterium phlei,* 24 against *Salmonella typhi,* 16 against *Vibrio cholerae* and 3 against *Pseudomonas aeruginosa.* Only *Dryopteris coch-leata* 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 screening of *P. vittata,* especially against gastrointestinal (GI) pathogens and reported the presence of flavonoid.

Banerjee (2008) in another report presented the antimicrobial activity of some polypodiaceous ferns. These include *Microsorium alternifolium,* *Leptochillus decurrens,* *Polypodium irioides,* *Pyrrosia mannii* and *Phymatodes ebenipes.* Several thelypteroid, davallloid and athyroid ferns were also found fairly active.

Species in seventeen of the genera present on Moorea have exhibited medicinal bioactivity in experiments. Nine species of *Selaginella* represented here have shown bioactivity. However, no studies have been performed on the *Selaginella sp., Selaginella banskoti.* Which can be found on Moorea. 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). Two *Selaginella* species show an inhibitory effect on muscle contraction (Rojas et al., 1999, Perez et al., 1994). Six species of *Pteris* have been tested for bioactivity thus far, but the two *Pteris* species that live on Moorea have not been tested. Two of the six tested have shown bioactivity. *Pteris semipinnata* has demonstrated anti-tumor activity in two separate investigations (Li et al., 1998, Li et al., 1999). The other *Pteris* species have anti-mutagenic, immunomodulatory, and neuronal activity (Wu et al., 2005, Lee et al., 1988, Goldberg et al., 1975). *Pteris vittata* has carcinogenic activity, (Siman et al., 2000) and provides an example of a secondary compound that is actually harmful to animals.

Medicinal compounds often have the potential to be harmful, thus it is not surprising that a genus would have both the dangerous and medicinal plants. *Christella,* by this reasoning deserves some attention as well, because of the tested carcinogenic activity of one species, *Christella dentata* (Somvanshi et al., 2005). Perhaps, other species in this genus have medicinal properties, instead of harmful effects. *Lycopodium*
species have shown antiacetylcholinesterase activity in two separate experiments (Hirasawa et al., 2006, Zhang et al., 2002). *Lycopodiella cernua*, the single *lycophyte* present on Moorea, is antivirally active and has been patented as a treatment for Hayfever (Zhang et al., 2002, Cambie and Ash 1994). Out of all the species present on Moorea, only eight have been tested for bioactivity.

### 2.3.3 Cytotoxicity of Pteridophyte phytochemicals:

A total of six pteridophyte e.g, *Adiantum australasicum, Adiantum plantagineum, Lycopodium reticulatum, B. lonchophora, Microsorum commutatum,* and *Lycopodium ernua* exhibited cytotoxic activity in a brine shrimp lethality assay, and one (*Microsorum grossum*) exhibited antifungal properties in experiments 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). It is interesting to note that, seven of the species, in three genera, exhibited cytotoxicity. *Selaginella,* as mentioned before has many cytotoxic species and each species with such activity contains biflavonoids (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). *Pteris semipinnata* and *Pteris multifada* have both 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.

### 2.4 Phytochemicals in *Cyathea* Species:

The first investigation on flavonoid constituents in the genus *Cyathea* was carried out by Harada et al., (1955). They analyzed the leaves of *C. fauriei* and *C. hancockii* during a comprehensive survey on the distribution of flavones, flavonols and flavanones in Japanese ferns. According to them, the latter species had kaempferol, cyrtominetin and farrerol, on the other hand, the former had cyrtominetin, farrerol and an uniden-
2.4: Phytochemicals in Cyathea Species:

tified flavone which was identified later as vitexin by Ueno et al., (1962). Concerning the distribution of flavonoid glycosides in this genus. Soeder et al., (1972) showed the occurrence of flavone C-glycosides (vitexin and isovitexin), in leaves of 3 species of Latin America. Very little is known at the present time, however, about the patterns of flavonoid glycosides. They examined the pattern of flavonoid glycosides which might have taxonomic value for distinguishing groups of species within the genus. In this report, the differences between the glycosidic patterns of kaempferol in leaves of the 3 species and two other species. All the five species analysed, contain Vitexin, Orientin, Kaempferol arabinoside, Astragalin. Afzelin. C. fauriei Contain Kaempferol 3-sophoroside, C. mertensiana and C. leichhardtiana have Kaempferol 7-rhamnogluco- side. C. podophylla and C. hancockii have Kaempferol 3-galaetoside (trifolin) and Kaempferol 3- rhamnogluicoside (Hiroaka et al.,1975).

Presence of hentriacontane, β-sitostenone, β-sitostanone, diploterol, sitosterol, hopan-29-ol, and oleanolic acid from the hexane soluble fraction of Cyathea gigantea .(Juneja et al., 1990).

Yōko, et al., (2003) reported the presence of Dryocrassyl formate, sitostanyl formate, and 12α-hydroxyfern-9(11)-ene along with ten known triterpenoids, three derivatives of phytol, α stanol, and β-tocopherol from the fresh fronds of Cyathea podophylla . Further, they analysed the each fraction by subjecting them to rechromatography and preparative HPLC to obtain a total of nineteen compounds: three new compounds Dryocrassyl formate, sitostanyl formate, and 12α-hydroxyfern-9(11)-ene and known compounds: trisnorhopane neohop-13(18)-ene, fern-9(11)-ene ,ferna-7,9(11)-diene , neohopa-11,13(18)-diene, squalene , 9α ,11α -epoxyfernane ,9β ,11β -epoxyferene, friedelin , hydroxyhpane , and esters of phytol8 , sitostanol , β-tocopherol and phytoic acid (3,7,11,15-tetramethyl-2-haxadecen-1-oic acid.

Cyathenosin A, a spiropyranosyl derivative of protocatechuic acid was isolated from the stem pith of Cyathea phalerata .Cyathenosin A is the first example of a naturally occurring compound containing a spirocyclic orthoester pyranosidic structure.(Pizzolatti et al., 2007)

Recently in 2008, Appel et al., in their paper "Antioxidant and Hepatoprotective effects of Cyathea phalerata Mart. (Cyatheaceae)" reported the antioxidative poten-
tiality of *Cyathea phalerata*.

Very little works has been done so far in genus *Cyathea*. So, It was the need of time to explore some of the species of this genus specially *Cyathea gigantea* and *Cyathea brunoniana* which are available in Southern part of Assam, India, for better upgradation of knowledge regarding the phytochemicals of this genus.