Nature has been a source of medicines for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Plants are the major component of traditional healing system in developing countries, which have also been an integral part of their history and culture. Plant-based medicinal systems continue to play an important role in primary health care, with about 80% of the world’s inhabitants relies mainly on traditional medicines (Owolabi et al., 2007). In India, higher plants as sources of medicinal compounds have sustained to play a pivotal role in shaping the life of human beings since ancient times. Unfortunately, pteridophytes are the least exploited group of plants in India compared to other countries of the world.

Despite the richness and wide diversity of fern flora in our country, less attention has been devoted to pteridophyte research and the interest has undergone resurgence in the last decade. This renewed special attention of the researchers, plant lovers and horticulturists on ferns from diverse quarters within the scientific community.

**Diversity of Pteridophytes**

Many researchers have carried out significant work on South Indian fern diversity which forms the baseline for the studies even till now. The first illustrated account on the “Ferns of Peninsular India” is that of Rheede (1703) who included few ferns and fern allies from the Malabar coast. However, Beddome (1864) done the only comprehensive work on the ferns of South India. After that, Beddome (1873) in his publication entitled, “The Ferns of Southern India” has listed the various ferns from Southern India. Bir and Vasudeva (1971) recorded 118 species of ferns from the Palni hills. Nayar and Kaur (1974) in their book entitled, “Companion to Beddome’s Handbook to the Ferns of British India” have listed the nomenclatural changes with regard to Beddome’s (1883, 1892) names only. Chandra and
Kaur (1987, 1994) have updated the nomenclature of all the taxa illustrated in Beddome’s Ferns of South India, Ferns of British India and Ferns of South and British India.


**Tree ferns**

Christensen (1905) separated the genus *Cyathea* into three genera: *Cyathea, Hemitelia* and *Alsolpilha* mainly on the basis of presence or absence of indusium. Holttum (1963) worked on the old world species and recognized only one genus *Cyathea*. Holttum (1965) while working on Asian species of tree ferns recognized 11 species of *Cyathea* from the Indian region of which *Cyathea nilgirensis* was described as a new species. Tryon (1970) recognized six genera of paleate Cyatheaceae viz., *Sphaeropteris, Alsopilha, Nephelea, Trichopteris, Cyathea* and *Cnemideria*. Tryon (1976) revised the genus *Cyathea* and recognized 40 species. Pichi-Sermolli (1977) agrees with the view of Tryon regarding the recognition of the six genera in the family Cyatheaceae. Dixit (1984) listed 11 species under the genus *Cyathea* from India. Floristic revision studies on the family Cyatheaceae by Dixit (1998) agrees with the view of Pichi-Sermolli (1977) in recognizing only six paleate genera of Tryon (1970). Out of these six genera, he reported *Alsopilha* and *Sphaeropteris* from
Indian region. Nayar and Geevarghese (1993), Rajagopal and Bhat (1998) and Pullaiah et al. (2003) identified certain species from South India and treated the species under the genus *Cyathea*.

Tree ferns are usually considered under a single family Cyatheaceae except Holttum (1973) who suggested a polyphyletic derivation with four families. The largest of these groups is the family Cyatheaceae which include the world’s tallest tree fern *Cyathea brownii* that reaches height up to 20 m (Large and Braggins, 2004). It comprises about 500 species (Korall et al., 2006) classified into four genera viz., *Cyathea, Alsophila, Cnemidaria* and *Sphaeropteris* (Lellinger, 1987). Among these, 200 species are neotropical, the majority belonging to the genus *Cyathea* (Lehnert, 2009). It is one of the most interesting families among the pteridophytes due to their striking morphology and wide geographical distribution with diversity centers in the tropics, subtropics and southern temperate regions. They are considered as primitive, though they represent different lines of evolution. These ferns display great ecological conservatism as most species are terrestrial plants of moist forests and are intolerant to longer periods of drought or frost. Furthermore, they show a greater provincialism and endemism than most fern groups (Tryon and Gastony, 1975).

In general, all these works are merely a survey of pteridophytic flora which deals with the morphology of pteridophytes. However, a well-resolved taxonomy, which would be the basis for many studies, is still unavailable. With the traditional healing system that is actively searching and expanding its pharmacopoeia in order to treat a huge number of complaints, an environment with great floral diversity is slipping away unlearned by a new generation of healers. The scientific and traditional communities need a resource where data on the phytochemical and molecular aspects of these pteridophytes are collated.
Phytochemistry in Pteridophytes - Preliminary level

Pteridophytes have long been considered as an essential group and a great deal of phytochemical work has recently been aimed at resolving relationships among the disparate groups. At the same time the utility of ferns for analysis of plant function and development has been increasingly appreciated and capitalized upon. Benerjee and Sen (1980) conducted extensive survey on antibiotic activity among ferns and reported about a hundred species having such property. Dixit and Vohra (1984) reported edible and medicinally important pteridophytic species from India. Many ferns, among many other plants, were used for medicinal purposes by the early Greeks and Romans through the middle ages. Dioscorides, a first century botanist, noted the use of spleenworts for curing maladies of the spleen (Irudayaraj and Raja, 1998).

The selection criteria of medicinal plants, which contain potentially new biological agents is based on five principle approaches viz., random, taxonomic, phytochemical, ethnomedicinal and information-managed approach. In the random approach, all the available species are collected, irrespective of prior knowledge and experience. In the taxonomic approach, plants of a specific genus or family are deemed to be of interest, and sought from diverse locations. The phytochemical (chemo-taxonomic) approach is based on a particular compound type, which is of biological interest. Taxonomic and the phytochemical approaches are closely related and cannot be clearly divided from each other. In the ethnomedicinal approach, credence is given to information on the medicinal use of the plant. Based on this information, the plant is collected and evaluated (Cordell et al., 1991). Information-managed plant selection collates taxonomic, biological, ethnomedicinal and phytochemical information to afford a list of plants for specific collection. The information is compiled through computerized databases such as NAPRALERT, a specialized relational database on natural products, based on systematic literature searches (Farnsworth, 1993).
Medicinal ferns from India are gaining importance in recent days due to the fact that they have been subjected to phytochemical screening. Phytochemical analysis on ferns has been done in large scale as compared to other cryptogams and studied to a lesser extent as compared to the phanerogams (angiosperms). Most of the phytochemical works on Indian ferns pertain to the primary metabolites to explain various natural phenomenons in plants. According to Irudayaraj and Raja (1998), though ferns possess less chemotaxonomic value, they are highly important from physiological, ecological and nutritional point of views.

Phytochemical analysis of the edible ferns, *Ampelopteris prolifera* and *Diplazium esculentum* shows the nutritional value (Shankar and Khare, 1985). Phytochemical analysis on rare, endangered and medicinally important spleenworts, *Asplenium* and *Psilotum* was investigated by Lal (1979), Rohtagi *et al.* (1984), Khare and Shankar (1987) and Varma (1992). Quantitative analysis of pigments (chlorophylls, carotenoids), carbohydrates (sugars, starch) and nitrogenous compounds (amino acids, proteins, nitrogen) have been done in a large number of South Indian Thelypteroid ferns (Britto *et al.*, 1991, 1993), *Hypolepis, Pteridium, Histiopteris* and *Cyathea* (Gopalakrishnan *et al.*, 1993), *Pteris* (Jesudass *et al.*, 1993) and Rajasthan ferns and fern allies (Kaur *et al.*, 1986; Vyas and Sharma, 1988; Sharma, 1989; Rathore and Sharma 1990; Harsh and Sharma, 1994; Vyas *et al.*, 1995).

The study of free amino acids in *Ophioglossum* leaves at the time of spike initiation indicates the close relationship between the genetic set-up and incorporation of amino acids into the structural and functional building blocks (Khandelwal and Goswami, 1976). Chark and Dhir (1991) analyzed the soluble proteins in *Pteris vitatta* during rhizoidal differentiation. Yadav (1995) investigated the possible role and behaviour of phytochemical compounds such as sugars, proteins and amino acids during the biorhythmic movements of leaflets in three species of *Marsilea*. 
The behaviour of chlorophylls, carotenoids and phenolics in drought resistance ferns and fern allies (*Selaginella*) from Rajasthan has been studied by Bohra *et al.* (1979), Vyas *et al.* (1989), Rathore and Sharma (1991) and Sharma *et al.* (1992). A reverse trend has been observed in the content of carotenoids by Kumar (1995). Ramachandran *et al.* (1991) and Raja *et al.* (1995) have made ecophysiological studies on the ferns from Kothayar and Palni hills (South India). Ahluwalia *et al.* (2002) investigated the spectrum application of *Azolla* with regard to the effect of various metals.

Britto *et al.* (1994a) studied the phytochemicals present in *Sphaerostephanos* species of Western Ghats. Preliminary phytochemical screening of 19 species of South Indian Thelypteroid ferns showed the occurrence of steroids, alkaloids, phenolics, catechins, saponins and tannins in all the species (Britto *et al.*, 1994b, 1994c). Triterpenoids and anthraquinone are not found in any of the species investigated. Irudayaraj (1996) has reported the presence of triterpenoids in the epidermal glands of *Christella parasitica*. Jesudass *et al.* (2001) screened the phytochemicals in the members of Pteridaceae in Western Ghats, South India. Jadhav *et al.* (2011) analyzed the phytochemicals in eleven species of ferns from the Satara district of Maharashtra.

et al., 2014) have been subjected to qualitative phytochemical screening of different metabolites by various workers.

**Phytochemistry in Pteridophytes - Analytical methods**

In natural product drug discovery, the conventional approach of extraction, isolation, separation, identification, characterization and test for the desired biological activity suffers from problems like lower yields, de-replication, difficulty in separation and inconsistent biological activity. However, with the introduction of innovative technologies like high throughput screening and combinatorial chemistry with their promise of a seemingly inexhaustible supply of compound libraries has greatly contributed to this declining interest in the screening of natural products by the pharmaceutical industry.

In the recent decades, there has been an increasing interest in the application of chemical evidence to taxonomic problems. Biochemical markers have their own significance and importance in chemical fingerprinting. Allozymes were the best biochemical markers in plants due to various strengths. However, modern and sensitive technologies for identifying markers based on biochemical / gene expression such as UV-Vis, FT-IR, TLC, HPTLC, HPLC, GC-MS and NMR have replaced allozymes. A plant during its life span produces various phytoactive compounds as secondary metabolites for its own growth and survival. Identification and characterization of these active principles can be used in generating a species specific fingerprint (Anandjiwala et al., 2006, 2007). Therefore the marker based on secondary metabolites should be able to discriminate one species from another species and one accession from other accessions.

Micro FT-IR spectra of the cuticles of three Carboniferous medullosan seed-fern leaf species (*Macroneuropteris scheuchzeri*, *Alethopteris lesquereuxii* and *Neuropteris ovata* var. *simonii*) showed oxygenated functional groups (carboxyl and ketone) with strong bands in the aliphatic stretching region revealed the complexity of the molecular structure (Lyons et al., 2014).
FT-IR spectra of seed-fern *Eusphenopteris neuropteroides* and true sphenopterid fern *Oligocarpia brongniartii* were very similar except the true fern does not have aromatic bands in 700-900 cm$^{-1}$ out-of-plane region. Py-GC-MS analysis showed more aromatic compounds for the seed fern than the sphenopterids. Comparison of FT-IR and py-GC-MS characteristics of sphenopterids and other plant groups confirmed that these two techniques have potential to identify chemotaxonomic signals from Carboniferous pteridophylls (Zodrow and Mastalerz, 2002).

Psenicka et al. (2005) studied the functional groups of fossil Marattialeans and chemotaxonomic implications for Pennsylvanian tree ferns and pteridophylls. The species *Pecopteris nyranensis*, *Pecopteris miltonii*, *Pecopteris aspidioides* and *Pecopteris polypodioides* are differentiable from one another by combined FT-IR characteristics and the ratio of CH$_2$/CH$_3$ is hypothesized to be a chemotaxonomic parameter for Pennsylvanian pteridophylls both in seed and true ferns. Baran and Rolleri (2010) characterized the biominerals in ferns (Marattiaceae) using IR spectroscopy. They confirmed the accumulation of biogenic silica in the tissues of *Angiopteris*, *Christensenia*, *Danaea* and *Marattia* and also showed the presence of calcium oxalate probably as weddellite.

Saito et al. (1989) reported the distribution of ptaquiloside and ptaquiloside-like compounds in Pteridaceae by chemical assay (TLC) and observed the widespread occurrence in a variety of ferns, including *Cheilanthes myriophylla*, *Cibotium barometz*, *Dennstaedtia scabra*, *Histiopteris incisa*, *Pityrogramma calomelanos*, *Pteris cretica*, *Pteris nipponica*, *Pteris oshimensis*, *Pteris tremula* and *Pteris wallichiana*. Sharma and Sharma (1992) identified various flavonoids in eight different ferns from Rajasthan by paper chromatography. Krishna and Dawra (1994) reported ptaquiloside in *Pteris quadriaurita* and *Onychium contiguum* for the first time in India, besides bracken fern by TLC method.
Irudayaraj and Johnson (2011a) used TLC separation to study the inter-specific relationship among the three *Asplenium* species. *Asplenium affine* and *Asplenium decrescens* showed 42% of similarity coefficient whereas *Asplenium zenkeranum* was varied from *A. affine* and *A. decrescens* with 36% variance. Pathania et al. (2012) analyzed the flavonoid quercetin in various ferns growing in northern India using TLC. Ferns from Himachal Pradesh viz., *Christella arida*, *Deparia japonica*, *Dryopteris cochleata*, *Dryopteris juxtaposita*, *Hypodematum crenatum*, *Polystichum squarrosum* and *Pteridium revolutum* contain a variable range of quercetin. Similarly, *P. squarrosum* and *P. cretica* from Uttarakhand contained higher concentration of quercetin.

Chikmawati et al. (2012) performed TLC tests in various *Selaginella* extracts to qualitatively analyze the bioactive compounds alkaloids, flavonoids and steroids. Mandal and Mondal (2012) analyzed the free amino acids present in the leaf glands of pteridophytes using TLC. DL-methionine is the common free amino acid of *Pteris vittata*, *Drynaria quercifolia*, *Ampelopteris prolifer* and *Dryopteris filix-mas*. L-tyrosine monohydrochloride are common in *D. filix-mas* and *Selaginella indica*. L-arginine monohydrochloride is also common in *D. quercifolia*, *Ceratopteris thallictroides* and *Marsilea quadrifolia*. Glycine is the only amino acid found in *Helminthostachys zeylanica*.

Srivastava et al. (2008) analyzed the HPTLC profile of *Lycopodium clavatum* stem using the mobile phase toluene: ethyl acetate: formaldehyde (6:3:1) and confirmed the presence of ferulic acid. Paul and Banerjee (2013) determined the HPTLC profile of flavonoids using the mobile phase ethyl acetate - formic acid - glacial acetic acid - water (10 : 0.5 : 0.5 : 1.3) in *Pteris vittata*. Aqueous extract showed six peaks and ethanolic extract showed twelve peaks with varied R<sub>f</sub> values.

Smith et al. (1989) analyzed *Cheilanthes sieberi* from New Zealand and Australia using RP-HPLC and confirmed the presence of ptaquiloside and other potentially
carcinogenic pterosin B precursors. Lai et al. (2005) separated new REE-binding protein from the lamina of *Pronephrium simplex* using HPLC coupled with online UV/ICP-MS. Amino acid composition analysis by RP-HPLC indicated that the protein has relatively high contents of proline and glycine. Chen et al. (2007) identified phenolic antioxidant compounds from the aqueous extract of Sword Brake fern *Pteris ensiformis* by HPLC.

HPLC analysis of *Pteris biaurita* following elution from TLC plate revealed a single peak with the retention time of 8.1 min (Dalli et al., 2007). Ho et al. (2008) identified different ecdysteroids in Polynesian medicinal fern *Microsorum membranifolium* using HPLC. Paulraj et al. (2011) studied the presence of various kinds of terpenoids, alkaloids, tannins, saponins and flavonoids on the epidermal glands extract of the glandular morphotype *Christella parasitica* using HPLC. Zhang et al. (2012a) illustrated the occurrence of flavonoids in different parts of *Dryopteris erythrosora* by means of HPLC.

Mostafa and Ibrahim (2012) analyzed α-tocopherol in *Azolla caroliniana* using HPLC which showed great quantitative variations, whereas ascorbic acid and β-carotene showed marked changes in both number and area of the characterized peaks subjected to UV-B. Chikmawati et al. (2012) determined the highest amentoflavone (6.87 ppm) content in *Selaginella subalpina* using HPLC analysis. Pathania et al. (2012) quantified the carcinogen, ptaquiloside in various ferns growing in certain enzootic areas of Himachal Pradesh and Uttarakhand. *Dryopteris cochleata*, *Hypodematum crenatum*, *Pseudocyclosorus canus* and *Pteris cretica* were identified to contain ptaquiloside for the first time on HPLC and LC-MS analyses.

Flavonoids of four species of *Angiopteris* indicated that di-C-glycosyl flavones and flavone-o-glycosides might be characteristic of distinct group of eusporangiate ferns (Wallace et al., 1981). Yamane et al. (1988) isolated endogenous gibberellins from sporophytes of two tree ferns, *Cibotium glaucum* and *Dicksonia antarctica*. The total gibberellin content in *C.*
*glaucum* (tall) was at least one order of magnitude greater than that of *D. antarctica* (dwarf) based on total ion current response in GC-MS and bioassay data. Oxodihydrophaseic acid was the major component of *C. glaucum* and abscisic acid was the major component present in *D. antarctica*.

Patitucci *et al.* (1995) used high resolution GC coupled with computerized MS to perform direct analysis of crude extracts and pre-fractions of Brazilian Polypodiaceae members viz., *Pleopeltis angustum*, *Microgramma vacciniifolia*, *Polypodium meniscifolium* and rhizome of *Polypodium aureum*. Wynne *et al.* (1998) confirmed new gibberellin like antheridiogen from gametophytes of the fern *Lygodium circinnatum* as the methyl ester of 9,11-didehydro-GA$_{20}$. Kurumatani *et al.* (2001) isolated the gibberellins A$_{73}$ methyl ester, the most abundant antheridiogen and the methyl esters of GA$_{9}$ and several monohydroxy GA$_{73}$ derivatives from the Schizaeaceous ferns *Lygodium microphyllum* and *Lygodium reticulatum*. Ramesh *et al.* (2001) isolated friedelin, epifriedelinol, β-amyrin, β-sitosterol, β-sitosterol 3-β-D-glucopyranoside and naringin from the dried rhizome of *Drynaria quercifolia*.

Nakane *et al.* (1999) identified six new migrated hopane triterpenoid alcohols from *Adiantum capillus-veneris* viz., pteron-14-en-7a-ol, fern-9(11)-en-3a-ol, fern-7-en-3a-ol, adian-5(10)-en-3a-ol, adian-5-en-3a-ol and fern-9(11)-en-28-ol. Alam *et al.* (2000) isolated normethyl lupine type and lanostane type triterpenes in the aerial parts of *Adiantum venustum*. On the basis of spectral data, the structures of these triterpenes have been established as 30-normethyl lupine-20-one, 30-normethyl olean-3-one-30-betol and lanost-20(22)-ene-30-ol. Bresciani *et al.* (2003) demonstrated the presence of terpenoids as predominant metabolites in *Adiantum cuneatum*. Methanolic crude extracts led to the isolation of four known triterpenoids viz., filicene, filicenal, adiantol and isoadiantone.

Melos *et al.* (2007) isolated a mixture of long-chain carboxylic acid esters as the ethyl esters of palmitic, petroselenic, oleic, (Z)-vacenic, stearic, linoleic, decosahexenoic, α-
linolenic, margaric, arachidic and behenic acids from the ethanolic extracts of *Adiantum tetrphyllum*. Further purifications led to the isolation of β-sitosterol, two triterpenes: 30-normethyl-lupan-20-one and hopan-22-ol, two diterpenes: phytol and phyten-3(20)-1,2-diol, two flavonoids: quercetin and quercetin-3-O-β-D-glucoside, a mixture of ferulic acid, caffeic acid and p-hydroxybenzaldehyde.

GC-MS characterization of *Anemia tomentosa* var. *anthriscifolia* essential oil composition detected the presence of sesquiterpenes (75%) with lower amounts of monoterpenes (15%). The sesquiterpenes were composed mainly of oxygenated components (67%) viz., α-bisabolol (51%), spathulenol (1%), caryophyllene oxide (3%), α-bisaboloxide (1%), 14-hydroxy-9-epi-(E)-caryophyllene (1%) and two unknown components (5%). The monoterpenes were dominated by neral (5%) and geranial (7%) with lower amounts of α-pinene, camphene, 6-methyl-5-hepten-2-one, 1,8-cineole and pinocarveol (Juliani *et al.*, 2004).

Santos *et al.* (2006) reported the presence of isoaficanol, a highly uncommon sesquiterpene in the essential oil of *A. tomentosa* var. *anthriscifolia*. Pinto *et al.* (2007) studied the chemical composition of the essential oil from *A. tomentosa* var. *anthriscifolia* and detected the presence of sesquiterpenes of the silfiperfolane, pre-silfiperfolane, isocumene, caryophyllene, pre-nopsane and nopsane types. They also reported the occurrence of two major components, pre-silfiperfolane-1-ol and silfiperfol-6-ene. In addition to this, Pinto *et al.* (2009a) detected thirty one substances from *A. tomentosa* var. *anthriscifolia* and Pinto *et al.* (2009b) isolated a new triquinane sesquiterpene (−)-epi-presilfiperfolan-1-ol, which was reevaluated and named as 9-epi-presilfiperfolan-1-ol (Nathan *et al.*, 2010).

Methanolic extract of *Selaginella lepidophylla* contains 3-methylenhydroxy-5-methoxy-2,4-dihydroxy tetrahydrofurane, which can act as a slight inhibitory effect on the uterus contraction (Perez *et al.*, 1994). *S. lepidophylla* is also reported to contain volatile oils.
Selaginella uncinata has chromone glycosides, namely uncinoside A and uncinoside B which showed antiviral activities (Ma et al., 2002). Ethanolic extract of S. uncinata contains flavonoids that possess a benzoic acid substituent (Zheng et al., 2008). Selaginella sinensis has a glucoside, namely selaginoside (Dai et al., 2006), a sesquilignan, namely sinensiol A (Wang et al., 2007), secolignans, namely styraxlignolide D and neolloydosin (Feng et al., 2009). Acetone extract of S. sinensis contains selaginellin A, an unusual flavonoid pigment (Zhang et al., 2007). Selaginella labordei contains 4'-methylether robustaflavone, robustaflavone, eriodictyol and amentoflavone (Tan et al., 2009). Selaginella moellendorfii contains several pyrrolidinoindoline alkaloids (Wang et al., 2009). Isocryptomerin isolated from Selaginella tamariscina showed potent antibacterial activity against Gram positive and Gram negative bacterial strains including clinical isolates of antibiotic-resistant species MRSA (Juneyoung et al., 2009). Selaginella species also have large number of bioactive compounds, the most important being biflavonoids which constituted 13 compounds viz., amentoflavone, 2',8'-biapigenin, delicaflavone, ginkgetin, heveaflavone, hinokiflavone, taiwaniaflavone, isocryptomerin, kayaflavone, ochnaflavone, podocarpusflavone A, robustaflavone and sumaflavone (Setyawan, 2011).

GC-MS analysis of antifungal component of Pteris biaurita revealed six major peaks in the retention time range of 7.2-10.9 min which may contain a mixture of eicosenes and heptadecanes (Dalli et al., 2007). Hashemi et al. (2007) studied the volatile components of Artemisia aucheri using GC-MS which includes 1,8-cineol (22.8%), chrysanthene (18.16%), a-pinene (8.33%) and mesitylene (7.41%). Choudhary et al. (2008) isolated two glycosides viz., 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 viz., methyl benzoate, hypogallic acid, caffeic acid, paoniflorin and pikuroside from a fresh water fern Salvinia molesta showing potent antioxidant radical scavenging activity.
Peres et al. (2009) identified the steroid \(\beta\)-sitosterol, the triterpene hopan-22-ol, the flavone glycoside 6-metoxiapinenin-7-O-\(\beta\)-D-allopyranoside and a mixture containing the ethyl esters of hexadecanoic, oleic, 15-methyl-heptadecanoic and linoleic acids from the fronds of *Microgramma vacciniifolia*. Fons et al. (2010) investigated the VOC from five French ferns using GC-MS. The main volatile compound of *Adiantum capillus-veneris* was (E)-2-decenal. The volatile profiles of *Athyrium filix-femina* and *Blechnum spicant* showed similarities with small amounts of isoprenoids. The major volatile compound of *Dryopteris filix-mas* was (E)-nerolidol. Polyketides, as acylfilicinic acids, were mainly identified in this fern. *Oreopteris limbosperma* contained the highest biodiversity of VOC. 80% of the volatiles was obtained from the terpenic pathway. The most important volatiles were (E)-nerolidol, alpha-terpineol, beta-caryophyllene and other minor monoterpenes.

Nilesh et al. (2011) predicted the compounds 3,4-dihydroxy benzoic acid, linoleic acid, chlorogenic acid, 4-hydroxy benzoic acid and ferulic acid from the ethanolic extracts of *Polypodium decumanum*. Narasimhaiah et al. (2012) isolated two new glycosidic compounds viz., 2-(3,4-O-Diglucos cinnamoyl)-4-hydroxyl furan and 1-Heptaloyl, 8-hexyl, 3-(O-diglucos), 10-methyl, 9,10-dihydro napthalene and characterized using TLC, IR, UV spectral analysis, NMR and mass spectra from the ethyl acetate extract of *Actiniopteris radiata*. Mishra and Verma (2013) revealed the presence of a flavonol glycoside, quercetin-3-O-\(\beta\)-glycosyl (1→2) rhamnoside from an antifungal active fraction of n-butanol soluble aqueous ethanolic extract of *Cheilanthes grisea*.

**Phytochemistry - Tree ferns**

The first scrutinization on flavonoid constituents in the genus *Cyathea* was carried out by Harada and Saiki (1955). They examined the leaves of *Cyathea fauriei* and *Cyathea hancockii* during a complete survey on the distribution of flavones, flavonols and flavanones in Japanese ferns. Hiraoka and Hasegawa (1975) detected flavonoid glycosides in five
Cyathea species from Tokyo. Hiraoka and Maeda (1979) isolated new acylated flavonol glycoside from the fronds of Cyathea contaminans and chemically characterized as kaempferol-7-(6''-succinyl)-glucoside. Yamane et al. (1985) identified ten gibberellins from GC-MS analysis of purified extracts of Cyathea australis which include the known GA₁, GA₄, GA₉, GA₁₅, GA₂₄, GA₃₅, GA₅₈ and three new GAs including 12β-hydroxy GA₉ (GA₆₉), 12α-hydroxy GA₉ (GA₇₀) and 12β-hydroxy GA₄ (GA₇₁).

C. gigantea contains several active constituents viz., triterpenes, sterols, saponins, flavonoids, hentriacontane, β-sitostenone, β-sitostanone, diploterol, sitosterol, hopan-29-ol and the whole plant contains oleanolic acid (Juneja et al., 1990). Gopalakrishnan et al. (1993) quantitatively estimated the presence of starch, total sugars, aminoacids, proteins, chlorophyll a, chlorophyll b, total chlorophylls and carotenoids on the lamina of C. crinita, C. gigantea and C. nilgirensis. They also studied the distribution of various aminoacids present in the chloroform and ethanolic extracts using the mobile phase n-butanol: acetic acid: water (12:3:5).

Cyathea has also some records of phytochemical studies related to the production of fernene, lilicene and hopane triterpenes (Arai et al., 1994, 1995), phenolic acids (coumaric and caffeic), protocatechuic acids and flavonoids represented mainly by kaempferol glycosides (Bringmann et al., 1999). Arai et al. (2003) isolated dryocrassyl formate, sitostanyl formate and 12 α-hydroxyfern-9(11)-ene from the fresh fronds of Cyathea podophylla and their structures were elucidated by spectroscopic techniques and synthesis. Ten known triterpenoids, three derivatives of phytol, a stanol and β-tocopherol were also identified from C. podophylla.

Cyathea phalerata determined the presence of an active flavonoid (kaempferol-3-neohesperidopside) with antioxidant and hypoglycaemic activity (Cazarolli et al., 2006). Cyathenosin A, a spiropyranosyl derivative of protocatechuic acid was isolated from the stem.
pith of *C. phalerata*. Its structure was determined by MS, 1D and 2D NMR spectroscopic analyses and confirmed by single crystal X-ray analysis. Cyathenosin A is the first example of a naturally occurring compound containing a spirocyclic orthoester pyranosidic structure (Pizzolatti *et al*., 2007). Brighente *et al*. (2007) reported the isolation of kaempferol-3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, 4-O-β-D-glucopyranosyl caffeic acid and kaempferol from the wood of *C. phalerata*. Further studies by Hort *et al*. (2008) resulted in the isolation and identification of nine substances: kaempferol-3-neohesperidoside, 4-O-β-D-glucopyranosyl caffeic acid, 4-O-β-D-glucopyranosyl p-coumaric acid, β-sitosterol, 3,4-spyglycopyranosyl protocatechuic acid, sitosterol β-D-glucoside, kaempferol, vitexin and ethyl galactoside.

Talukdar *et al*. (2010) performed TLC profiling of *C. gigantea* and *C. brunoniana* in five different solvent systems for the presence of diverse group of phytochemicals. Different Rf values of the compound in caudex and leaves extracts reflects an idea about their polarity and selection of solvents for separation of phytochemicals. Zodrow and Mastalerz (2010) applied FT-IR as an investigative technique in *Cyathea caracasana* for documenting quality spectra suitable for the interpretation of phytochemicals. Based on the absorbance spectra, five sample sets were reduced to three related-chemical groups. The reduction is principally based on aliphatic and oxygen-containing moieties.

Chemical constituents of ethanolic extracts of *Cyathea spinulosa* were isolated by chromatographic techniques and their structures were elucidated by spectral methods. The compounds include (2S,3S,4R)-2-[(2′R)-2′-hydroxytetraicosanoylamino]-stigmast-3,6-dione, β-sitosterol, 1,3,4-octadecanetriol, stigmast-4-ene daucosterol, ergosterol, 3,6-dione, 1-O-beta-D-glucopyranosyl-(2S,3R,4E,8Z)-2-[(2-hydroxyoctadecanoyl)amido]-4,8-octadecadiene-1,3-diol and protocatechuic aldehyde (Jiang *et al*., 2012).
Molecular markers

Information on genetic variation within and among populations is crucial for conservation of rare and endangered species. A species may be considered as a group of individuals organized into populations that share an amalgamation of indicative characters which are not found outside the group. Survival chance of a species is indicated in genetic diversity within the population (Tsuda et al., 2009). Molecular markers have been used for assessing genetic diversity and generating baseline information (Pertoldi et al., 2007; Mirialili et al., 2009). In order to deduct a concrete, reliable data, a marker should meet many criteria; a marker should be inheritable and have the power to discriminate between individuals. It should be easy to generate and interpret. It should be highly polymorphic in nature and frequently distributed throughout the genome. Moreover, a marker should be easy to detect and comparable with similar characters (Hillis and Moritz, 1990).

Though there are no such ideal markers existing, three classes of markers based on morphological, biochemical and molecular traits are routinely explored. Morphological markers are usually visually characterized phenotypic characters such as sorus type, shape and arrangement, indusium attachment, rhizome, type of fertile segments, vein branching, rachis scales and microphyllous leaves (Lasalita-Zapico et al., 2011). Biochemical markers are differences in protein and enzymes that are detected by electrophoresis and specific staining (Pillai et al., 2000). The major disadvantages of these two markers are they may be limited in number and are influenced by environmental factors or the developmental stages of the plant (Winter and Kahl, 1995).

A first map of the human genome based on molecular markers (Botstein et al., 1980) fuelled the development of genomic maps in other organisms. Consequently, polymorphisms have been detected in restricted genomic DNA of plants and paved way to the development of molecular markers for plant breeding. The number of polymorphic morphological markers
is limited, especially in intra-specific crosses, and their expression is influenced by the environment. Therefore, more reliable markers such as proteins or more specifically, allelic variants of several enzymes called isozymes had to be considered (Tanksley and Orton, 1983; Weeden et al., 1988).

DNA sequence-based molecular markers were introduced in 1970s, and they became the preferred method when PCR technology was invented in 1985. They are the most widely used marker predominantly due to their abundance and exhibit a much higher level of allelic variation than morphological and biochemical markers (Weising et al., 2005). DNA markers arise from different classes of DNA mutations such as substitutions, rearrangements or errors in replication of tandemly repeated DNA (Paterson, 1996). They are selectively neutral because they are usually located in non-coding regions of DNA (Winter and Kahl, 1995). DNA markers may be broadly divided into three classes based on the method of their detection viz., DNA-DNA hybridization as in RFLP and AFLP, PCR technique as in RAPD, SNP, ISSR and the method of DNA sequencing (Winter and Kahl, 1995; Gupta et al., 1999; Joshi et al., 1999). These markers reveal genetic differences that can be visualized by using a technique called gel electrophoresis and staining with ethidium bromide, silver nitrate and detection with radioactive probes.

**Proteomic analysis**

Proteomic analysis started to arouse tremendous attention after the completion of sequencing human genome. One of the major goals to sequence the complete human genome is to reveal and understand the linkage between genes and their related proteins. Characterizing and understanding the proteomic composition in any biological sample can provide essential information about complex cellular regulatory networks (Domon and Aebersold, 2006). Although the concept of the "proteome" is currently receiving considerable attention, identification of specific proteins is an established technique whereby isolated
proteins are first separated by their isoelectric point in the one dimensional electrophoresis and then separated by their molecular weight in the second dimension using SDS-PAGE (Mukhlesur and Hirata, 2004).

Rodin and Rask (1990) isolated and characterized 2.2s storage protein (matteuccin) from the ostrich fern *Matteuccia struthiopteris*. Amino acid analysis revealed that the 2.2s protein is rich in arginine. Farrant et al. (2009) isolated total and heat stable proteins from desiccation tolerant and desiccation sensitive fronds of the fern *Mohria caffrorum*. The desiccation mechanism is seasonally regulated and it is due to the *de novo* production of the protein identified as a putative chaperonin. Deeba et al. (2009) carried out proteomic studies in *Selaginella bryopteris* to understand the mechanism of desiccation tolerance. About 250 putatively identified protein spots were reproducibly detected and analyzed. This indicates that the proteins involved in transport, targeting and degradation were expressed more in the desiccated frond *S. bryopteris*.

Wang et al. (2010) identified 138 desiccation-responsive 2-DE spots representing 103 unique proteins in resurrection fern-ally *Selaginella tamariscina*. Hierarchical clustering analyses revealed that 83% proteins were down-regulated upon dehydration. The dynamic expression changes of the desiccation-responsive proteins provide strong evidence that cell structure modification, photosynthesis reduction, antioxidant system activation and protein post-transcriptional/translational modifications are essential to the fern-ally *S. tamariscina* in response to dehydration. Bona et al. (2010) investigated the frond proteome of the arsenic hyperaccumulator fern *Pteris vittata* which differentially modulates expression of 130 leaf proteins with specific responses in *Glomus mosseae* or *Gigaspora margarita* colonized plants.

Revathy et al. (2011) determined the protein expression of *Adiantum raddianum*, *Arachniodes tripinnata*, *Dryopteris sparsa* and *Odontosoria chinensis* collected from
Kothayar, South India using SDS-PAGE. The selected ferns showed unique banding pattern of proteins in different developmental stages viz., gametophyte without sex organ, gametophyte with sex organ, gametophyte with juvenile sporophytes and matured sporophytes which represented the “protein finger print” of that particular species. Sivaraman et al. (2011b) studied the reproductive biology and protein expression studies on the different developmental stages of South Indian ferns viz., Tectaria paradoxa, Araiostegia hymenophylloides and Deparia petersenii. The gametophytes of T. paradoxa and D. petersenii are associated with algae or moss on the surface of the soil. The variation in the total number of bands in different species designates the difference in genetic diversity. The difference is expressed in mature sporophytes with minimum number of bands in T. paradoxa and maximum number of bands in A. hymenophylloides.

Balbuena et al. (2012) characterized the proteome of Equisetum hyemale rhizomes using GeLC-MS spectral counting proteomics strategy. Non-redundant proteins identified in the rhizomes apical tip and elongation zone were 1,911 and 1,860 respectively. Narayani and Johnson (2013) revealed the protein variability among ten Selaginella species using SDS-PAGE. A total of 190 bands with various Rf values were observed. Each region expressed different proteins with varied molecular weight which act as representative of the expression of a particular gene in the studied species of Selaginella.

MALDI-TOF MS analysis

Knowledge of plant development and function can be obtained by determining the distribution of proteins and metabolic processes within plant tissues. MALDI-TOF MS has recently become a popular and versatile method to analyze peptides, proteins and other biomolecules. It is used as a post separation protein identification tool and the mass patterns obtained were used for comparison with known libraries to confirm peptides. Though it is not the most rigorous approach to protein identification, it still represents an economically
convenient alternative to more complex MS systems especially when proteomic analyses are carried out on plant species whose complete genome/protein databases are complete or well annotated.

Lai et al. (2005) separated new REE-binding protein from the lamina of *Pronephrium simplex* and further characterization of the protein showed its molecular mass 5068.4 Da by MALDI-TOF MS and ESI-MS. Moore et al. (2006) purified sporopollenin from *Selaginella pallescens* and *Lycopodium clavatum* using MALDI-TOF MS. The mass spectra obtained from the spores extracted with a base, acid and solvent treatment represents pure sporopollenin. Both indicate ions at approximately m/z 8000 and 4400 to 4800 with the *L. clavatum* analysis also generating ions between m/z 6936 and 7588 (in positive ionisation mode) and at m/z 17654.

Ekman et al. (2008) used 2-DE and MS for comparative protein expression profiling of a cyanobacterium dwelling in leaf cavities of the water-fern *Azolla filiculoides*. Homology-based protein identification using peptide mass fingerprinting, tandem MS analyses and sequence homology searches resulted in an identification success rate of 79% of proteins analyzed in the unsequenced cyanobiont. Martinez-Cortes et al. (2012) purified two cationic peroxidases from *Selaginella martensii* (PRX2 and PRX3) using ammonium sulfate precipitation, adsorption chromatography and cationic exchange chromatography. The molecular mass for PRX2 and PRX3 was 36.3 kDa and 45.6 kDa according to MALDI-TOF/TOF. Both enzymes showed a typical peroxidase UV-Vis spectrum with a Soret peak at 403 nm for PRX2 and 404 nm for PRX3. The specific activities showed against several substrates and the kinetic parameters suggest that PRX2 and PRX3 have specific roles in cell wall formation and especially in lignin biosynthesis. Peptides from tryptic digestion of both peroxidases were also identified through MALDI-TOF MS/MS.
Isozymic analysis

Isozymes have been proven to be reliable genetic markers in plant systematic studies due to consistency in their expression, irrespective of environmental factors. In addition, it allows quantification of genetic homology and distance within and between species (Onus and Pickergill, 2000). These markers can correctly identify several levels of taxa, accessions and individuals since the assumption of homology can be more accurate than some genomic DNA markers (Klaas, 1998). Electrophoretic analyses of isozymes in natural populations allow exploration of levels and patterns of genetic variability. However, a subsequent investigation demonstrated that a homosporous fern with the lowest number for its genus possessed a diploid gene expression profile with Mendelian inheritance (Gastony and Gottlieb, 1982). Furthermore, it was demonstrated that the complex isozyme banding patterns of Chapman *et al.* (1979) were in part attributable to subcellularly compartmentalized isozymes (Gastony and Darrow, 1983; Wolf *et al*., 1987). Continued isozyme investigations supported the observation that homosporous ferns with base chromosome numbers for their genus have diploid expression profiles despite possessing relatively high chromosome numbers (Gastony and Gottlieb, 1985; Haulner and Soltis, 1986; Soltis, 1986; Haufler, 1987).

The use of isozymes to test hypotheses in reticulately evolved complexes has often proved valuable in fern populations, for example in the forest species (Soltis and Soltis, 1987, 1988), epiphytic species (Ranker, 1992; Hooper and Haulner, 1997; Vogel *et al*., 1999; Wubs *et al*., 2010) and colonizing species (Wolf *et al*., 1988). Pryer and Haulner (1993) examined the isozyme, chromosomal and morphological characters for the allotetraploid origin of *Gymnocarpium dryopteris*. Watano and Masuyama (1994) carried out isozymic variation studies on 27 natural populations of *Ceratopteris thalictroides* in Japan. Among the 15 enzyme loci examined, 8 loci were genetically polymorphic. Marked genetic differentiation was observed between populations to the south and north of Okinawa Island at six loci viz.,

Herrero et al. (2001) studied the isozymic variation and genetic relationships among taxa in the Asplenium obovatum group. Electrophoretic analyses of eight enzyme systems encoded by fourteen putative loci were conducted. Alleles of the loci Lap-1, Mdh-2, Mdh-3, Pgm-1, Pgm-19 and 6Pgd-1 emerged as genetic markers for the diploids and were present in an additive pattern in most of the analyzed individuals of the tetraploid. Pajaron et al. (2005) evaluated the different systematic treatments of Asplenium seelosii by means of isozyme electrophoresis. Seventeen populations throughout the range of the complex were studied and fifteen enzymatic systems were assayed. There was no genetic identity within and between populations. Cheng et al. (2008) analyzed the allozyme polymorphisms of Alsophila spinulosa from 9 sites throughout Taiwan using 6 enzyme systems viz., esterase, isocitrate dehydrogenase, menadione reductase, 6-phosphogluconate dehydrogenase, shikimic acid dehydrogenase and malate dehydrogenase. The genetic diversity of A. spinulosa was higher than mean values of other diploid ferns and tree ferns. Highest expected heterozygosity was observed in Nanjenshan population located in South Taiwan.

Johnson et al. (2009) illustrated the isozymic evidence for the common origin of Diplazium species confined to South India and Sri Lanka. Maximum degree of diversity has been observed in Diplazium travancorium with the presence of fourteen bands, followed by Diplazium polypoides with nine bands, in contrast Diplazium cognatum and Diplazium dilatum have only four bands and two bands respectively. Stein et al. (2010) demonstrated the molecular profiles of tetraploids to verify their undiscovered diploid ancestor and
reconstructed *Dryopteris semicristata* using isozymes and restriction site analysis of cpDNA. Johnson *et al.* (2010a) studied the isozymic banding profiles of *Tectaria coadunata*, *Tectaria wightii* and *Tectaria paradoxa* using SDS-PAGE and confirmed that all the three species are morphologically and genetically distinct, but cytologically uniform. Johnson *et al.* (2010b) compared the isoperoxidase banding profile on selected species of *Pteris* from India. A total of thirty eight bands scored in thirty one different positions with eight active zones revealed the biochemical positions of the *Pteris* complex.

Johnson *et al.* (2010c) initiated isozymic analysis on six different species of *Adiantum* from the Western Ghats, South India. Among these, *Adiantum raddianum* and *Adiantum lunulatum* banding profile showed high percentage of similarity index compared to other species. Johnson *et al.* (2010d) studied the genetic distinction of three filmy ferns viz., *Trichomones obscurum*, *Trichomones proliferum* and *Trichomones plicatum* belonging to different morphological forms growing in different ecological niche using isozymic variation. Irudayaraj and Johnson (2011b) explored the identity and phylogenetic relationships of *Sphaerostephanos arbuscula*, *Sphaerostephanos unitus* and *Sphaerostephanos subtruncatus* through isozymic analysis and confirmed the distinctness of three species based on macro-micromorphology, phytochemistry, cytology and isozymic profile.

Johnson *et al.* (2012a) studied the isoperoxidase variation on three South Indian tree ferns viz., *Cyathea nilgirensis*, *Cyathea gigantea* and *Cyathea crinita* to reveal the interspecific variation at molecular level. The banding pattern expressed hundred percentage genetic differentiations among the three species. Johnson *et al.* (2012b) assessed the genetic variation between different populations of *Thelypteris ciliata* collected from different localities of Tirunelveli hills using isoperoxidase. A total of six bands in six different positions with five active regions were observed in the enzyme system.
DNA barcoding

Pteridophytes have a longer evolutionary history than other vascular land plants. Therefore, it has endured greater loss of phylogenetically useful information. This factor has resulted in substantial disagreements in evaluating characters and controversy in establishing a stable classification. The first molecular systematic studies on ferns were published in the mid 1990s (Hasebe et al., 1994, 1995) and set the direction for modern fern systematics. The arrival of phylogenetics and molecular phylogenetics in particular, has rapidly improved our understanding of fern relationships through phylogenetic analyses of DNA sequence data (Hasebe et al. 1994, 1995; Pryer et al. 2004; Schneider et al. 2004a), morphological data alone (Schneider et al. 2009), or combined analyses of molecular and morphological evidence (Lehtonen et al. 2010). Since then, numerous molecular phylogenetic studies have focused on certain classically defined fern groups by sampling members from the group studied, or tested the backbone fern classification by sampling exemplar species of higher taxa. However, both kinds of studies have specific limitations to recover the complete fern tree of life.

DNA barcoding in ferns is potentially of great value when they generally lack the complexity for morphology based identification and underappreciated in ecological studies. A DNA barcode uses genes to identify a species. It can be developed at various sites in a plant such as nuclear, mitochondrial and chloroplast sequences. However, the designed barcode should be in a region of the genome which is variable but conserved enough to design primers that can amplify short regions of 100-150 bp. Since cpDNA evolves faster than mitochondria and shows considerable mutation rate, it is considered a better choice for developing a barcode (Taberlet et al., 1991; Clegg, 1993).

There are many controversies existing over the value of DNA barcoding. Taxonomists consider that the traditional morphology based identification of a plant species would
diminish and result in incorrect species identification as cpDNA relies solely on genetic divergence (Kress et al., 2005). Moreover, taxonomy of science is based on a detailed understanding of morphology, physiology and behavioural attributes (Ebach and Holdrege, 2005; Arvind et al., 2007) and barcoding generates information, not knowledge. Species identification should be based on multigene phenotype rather than a single gene sequence (Moritz and Cicero, 2004). Though DNA barcoding provides rapid species identification, its accuracy relies on PCR technology by using a standardized DNA region as a tag (Hebert and Gregory, 2005).

Molecular phylogenetic hypotheses for extant ferns have utilized data from several chloroplast markers (rbcL, atpA, atpB, accD, rps4, 16S rDNA, ITS), one nuclear gene (18S rDNA) and three mitochondrial genes (atp1, nad2, nad5). A natural outgrowth of these one-gene or few-gene studies on a wide array of ferns has led to broader and increasingly robust multiple gene phylogenetic analyses.

**DNA barcoding - Pteridophytes**

Intergenic spacer sequences such as trnL-F and rps4-trnS were more effective for resolving recent divergence events in the ferns (Small et al., 2005). trnL-F was proved to be useful in studies of Ophioglossaceae (Hauk et al., 1996, 2003), Schizaeaceae (Skog et al., 2002), Polypodiaceae (Haufler et al., 2003; Ranker et al., 2004; Schneider et al., 2004b), Asplenium (Schneider et al., 2004a, 2005) and Cyrtomium (Lu et al., 2005). Another intergenic spacer, rps4-trnS has been applied to infrageneric phylogenetic work in the ferns Hymenophyllum (Hennequin et al., 2003), Elaphoglossum (Rouhan et al., 2004; Skog et al., 2004), Polystichum (Perrie et al., 2003) and in the members of Thelypteridaceae (Smith and Cranfill, 2002).

Kolukisaoglu et al. (1995) indicated that Selaginella and Equisetum emerge earlier than Psilotum based on phytochrome gene. This result corresponds to chloroplast gene
(Raubeson and Jansen, 1992) and supported by \textit{rbcL} gene (Korall \textit{et al.}, 1999). Korall and Kenrick (2002, 2004) proved that the subgenera \textit{Selaginella} and \textit{Tetragonostachys} are monophyletic, \textit{Stachygynandrum} and \textit{Heterostachys} are polyphyletic; while the nature of \textit{Ericetorum} is still unknown yet. Korall and Taylor (2006) indicated that the genus \textit{Selaginella} is monophyletic based on \textit{rbcL} map, however at subgenus level it is monophyletic or paraphyletic.

Dubuisson (1997) used \textit{rbcL} sequences as a promising tool for 18 species of the fern genus \textit{Trichomanes} to test the ability of this gene for resolving relationships within this taxon and to reveal the major phylogenetic tendencies. Gastony and Ungerer (1997) determined nucleotide sequences of the chloroplast-encoded \textit{rbcL} gene for all five species of the onocleoid ferns including both varieties of \textit{Onoclea sensibilis} and for out group member \textit{Blechnum glandulosum}. Yatabe \textit{et al.} (2001) crossed three sympatric \textit{rbcL} sequence types of \textit{Asplenium nidus} and observed that the molecularly distinct types were reproductively isolated because hybrids failed to form between atleast two pairs of \textit{rbcL} types.

Heede \textit{et al.} (2003) investigated the phylogenetic relationships among 20 taxa of the fern genus \textit{Asplenium} subgenus \textit{Ceterach} using DNA sequence data from the nuclear ribosomal internal transcribed spacers and plastid \textit{trnL-F} intergenic spacer. In addition, a single sample per taxon was used in analysis of the plastid \textit{rbcL} gene. Plastid \textit{trnL-F} and \textit{rbcL} analyses resulted in identical tree topologies. The trees produced from the separate plastid and nuclear matrices agree in the recognition of identical groups of accessions corresponding to \textit{A. dalhousiae, A. ceterach, A. aureum, A. cordatum, A. phillipsianum} and \textit{A. haughtonii}. Baracaldo (2004) generated robust phylogeny of the Neotropical fern genera \textit{Jamesonia} and \textit{Eriosorus} based on sequence data of the nuclear ETS of 18S-26S rDNA, the plastid gene \textit{rps4} and the intergenic spacer \textit{rps4-trnS}.
Sequence data from the chloroplast \textit{atpB} gene have proven to be useful for resolving relationships within ferns (Tsutsumi and Kato 2005; Korall \textit{et al.}, 2006), suggesting that the gene may also have phylogenetic utility for \textit{Equisetum}, especially for resolving deep nodes. Zhang \textit{et al.} (2005) carried out phylogenetic analysis of cryptogrammoid ferns and related taxa based on \textit{rbcL} sequences. The resulting cladogram places \textit{Coniogramme}, \textit{Cryptogramma} and \textit{Llavea} into a moderately supported clade, constituting a cryptogrammoid group distantly related to the cheilanthoid ferns. Smith \textit{et al.} (2006) provided the most recent arrangement of Pteridaceae into five monophyletic groups based on morphological and molecular data. Many \textit{rbcL} sequences of Pteridaceae are now available in GenBank which showed that they are variable enough to provide good resolution across the taxonomic diversity of fern taxa.

Schneider and Schuettpelz (2006) tested the principle of DNA barcoding of fern gametophytes using plastid \textit{rbcL} sequence and successfully identified a cultivated gametophyte as \textit{Osmunda regalis}. However, whether \textit{rbcL} shows sufficient variation to allow general identification below genus level remains uncertain. Liu \textit{et al.} (2007) carried out phylogenetic analysis of Dryopteridaceae family using chloroplast \textit{rbcL} and \textit{atpB} genes. The results indicate that Dryopteridaceae form a monophyletic group with the exception of \textit{Didymochlaena}, \textit{Hypodematium} and \textit{Leucostegia}. They are sister to a large clade comprising Lomariopsidaceae, Tectariaceae, Polypodiaceae, Davallia and Oleandraceae. Lu \textit{et al.} (2007) studied the molecular phylogeny of the polystichoid ferns in Asia based on \textit{rbcL} sequences. Guillon (2007) used original (\textit{atpB}) and published (\textit{rbcL}, \textit{trnL-trnF}, \textit{rps4}) sequence data to investigate the phylogeny of the genus \textit{Equisetum}. Analyses of \textit{atpB} sequences give an unusual topology with \textit{Equisetum bogotense} branching within \textit{Hippochaete}.

Madeira \textit{et al.} (2008) conducted the molecular phylogeny of the genus \textit{Lygodium} using \textit{trnL} intron and \textit{trnL-F} inter-genic spacer of cpDNA to determine the relationship of
Lygodium microphyllum and other Lygodium species. Three major clades appeared, one with Lygodium palmatum and Lygodium articulatum, second with Lygodium reticulatum and L. microphyllum, and a third comprising the other species examined. Nitta (2008) explored the utility of three plastid loci (rbcL, trnSGG and trnH-psbA) for biocoding the filmy ferns (Hymenophyllaceae) of Moorea. All the three regions were found to be potentially useful for phylogenetic studies at the appropriate taxonomic level. trnH-psbA has the greatest utility as a potential marker for DNA-based identification because of its high inter-specific variability and high degree of amplification success. rbcL and trnH-psbA were successfully used in combination with morphological characters to identify Polyphlebium borbonicum.

Song et al. (2009) authenticated the members of the family Polygonaceae in Chinese pharmacopoeia using DNA barcoding technique. The amplification efficiency of six DNA barcodes (rbcL, trnH-psbA, ndhJ, rpoB, rpoC1, accD) was 100%. Inter-specific divergence was highest for the trnH-psbA (20.05%) followed by the nrITS (14.01%) across all species pairs. Schneider et al. (2009) used morphological dataset of 136 vegetative and reproductive characters to infer the tracheophyte phylogeny with an emphasis on early divergences of ferns (monilophytes). Independent phylogenetic analyses of morphological evidence recover the same phylogenetic relationships among tracheophytes utilizing DNA sequence data but differ within seed plants and ferns.

Ma et al. (2010) analyzed five DNA sequence markers (psbA-trnH intergenic region, rbcL, rpoB, rpoC1 and matK) using six chloroplast genomic sequences from GenBank and found psbA-trnH intergenic region was a suitable DNA marker for species identification in medicinally important pteridophytes. Pryer et al. (2010) exposes a case of mistaken identity in the fern horticultural trade using DNA barcoding. The plastid sequences rbcL, atpA and trnG-R were used to demonstrate that a fern marketed as Cheilanthes wrightii in the horticultural trade is in fact Cheilanthes distans. Li et al. (2010) identified diminutive and
featureless stages of ferns using tissue-direct PCR combined with amplifying plant barcodes. It was very helpful for large scale ecological studies surveying distribution and population structure. Ebihara et al. (2010) demonstrated the effectiveness of two plastid DNA barcode regions (\(rbcL\) and \(trnH-psbA\)) for species identification in the Japanese pteridophyte flora.

Groot et al. (2011) evaluated the combination of \(rbcL\) with a non-coding plastid marker \(trnL\)-F to obtain DNA identifications for 86 fern species. All species with non-equal chloroplast genomes formed their own well supported monophyletic clade, indicating a high discriminatory power. Inter-specific distances were larger than intra-specific distances for all the tested taxa. Zhang et al. (2012b) used DNA sequences of four plastid loci (\(rbcL\) gene, \(rps4-trnS\) spacer, \(trnL\) intron, \(trnL\)-F spacer) to reconstruct the phylogeny of \(Dryopteris\). The results confirmed the paraphyly of \(Dryopteris\) and provides the first strong molecular evidence on the monophyly of \(Acrophorus, Diacalpe, Dryopsis, Nothoperanema\) and \(Peranema\). However, all these monophyletic groups together with the paraphyletic \(Acrorumohra\) are suggested to be merged into \(Dryopteris\) based on both molecular and morphological evidence.

Chen et al. (2013) identified field vittarioid gametophytes using DNA barcoding. Combinations of distance-based and tree-based approaches were performed to evaluate the discriminating power of three barcodes (\(matK\), \(rbcL\) and \(trnL\)-F) on 16 vittarioid sporophytes. Sequences of the \(trnL\)-F region were generated from 15 fern gametophyte populations by tissue-direct PCR and were compared against the sporophyte dataset using BLAST. \(trnL\)-F earns highest primer universality and discriminatory ability scores, whereas PCR success rates were very low for \(matK\) and \(rbcL\) regions.

**Molecular variation - Tree ferns**

Tree ferns are a conspicuous component of tropical, subtropical and even south temperate floras, where closely related clades are especially diverse (Smith et al., 2006).
They hold a critical phylogenetic position as the extant sister groups to seed plants. Understanding the organization and evolution of tree ferns can provide useful information for comparative studies across land plants. Outside the extant seed plants, arborescence is today only present in ferns, where it is mostly restricted to the “tree fern” clade (Korall et al., 2006, 2007). Investigations of generation times, along with those of phenology and demography have shown that the members of primarily arborescent clade have longer generation times than closely related non-arborescent lineages (Mehltreter and Palacios-Ríos, 2003; Mehltreter and Garcia-Franco, 2008).

Tree ferns are a well-established clade within leptosporangiate ferns and are strongly monophyletic. Phylogenetic relationships among the tree ferns have been controversial. Bower (1928) classified tree ferns into separate families and placed each of them near different groups, based on morphological differences in the position of sori and epidermal appendages (e.g., marginal sori and hairs in the Dicksoniaceae versus dorsal sori and scales in the Cyatheaceae). Other authors postulated that the tree ferns were closely related (Holttum and Sen, 1961; Kramer, 1990) and even included them in a single family. This was based on similarities in tree habit and oblique annuli of sporangia. Existence of intermediate genera Metaxia and Lophosoria with a combination of dorsal sori and hairs also supports monophyly of the tree ferns.

Cyatheaceae has been the focus of many taxonomic studies in the last 50 years and classifications of Cyatheaceae are still variable at the generic level. The presence of scales and the position of the sorus are the two most conspicuous characters used to separate Cyatheaceae from arborescent species in the Dicksoniaceae. The monophyly of Dicksoniaceae (excluding Cystodium) has been questioned in earlier studies based on DNA sequence data (Hasebe et al., 1994, 1995; Wolf et al., 1999; Pryer et al., 2004) and morphology. Some previous classifications have included it either in Cyatheaceae (Holttum
and Sen, 1961; Holttum, 1963) or divided it into three separate families viz., Dicksoniaceae, Culcitaceae and Thyropteridaceae (Pichi-Sermolli, 1977). The most surprising addition to the tree fern clade is Hymenophyllopsis. All Hymenophyllopsis species have scales and their sporangia resemble those found in the Cyatheaceae (Lellinger, 1984). Other affinities for Hymenophyllopsis have also been proposed (Lellinger, 1984) and it has even been cited as a “genus of uncertain placement” (Kubitzki, 1990).

DNA phylogenies have now greatly clarified the main subgroups of the scaly tree ferns (Holttum and Sen, 1961; Holttum, 1963; Tryon, 1970; Tryon and Tryon, 1982; Conant, 1983; Holttum and Edwards, 1983; Lellinger, 1987; Kubitzki, 1990; Conant et al., 1994, 1995, 1996). In the last decade, phylogenetic studies of restriction site data and morphology led to the recognition of three evolutionary lineages within Cyatheaceae: Sphaeropteris, Alsophila and Cyathea, with Alsophila as sister to the other two (Conant et al., 1994, 1995, 1996). Molecular studies in ferns have generally relied on a subset of the sequences used in angiosperm systematics (Pryer et al., 2004). The gene rbcL has been used extensively in studies for both higher-level and lower-level taxa (Ranker et al., 2004; Schneider et al., 2004a). Phylogenetic studies using nucleotide sequences of the gene encoding the large subunit of rbcL have been successfully revealed the relationships of ferns at both generic and familial levels (Hasebe et al., 1995; Skog et al., 2004). Hasebe et al. (1994) sequenced 1206 nucleotides of the large subunit of the rbcL gene from 58 species representing almost all families of leptosporangiate ferns. Phylogenetic trees proved monophyletic relationship of the tree ferns with 98% of bootstrap probability in the neighbor-joining method and 73% in the parsimony method. Two morphologically distinct heterosporous water ferns viz., Marsilea and Salvinia are sister genera, the tree ferns (Cyatheaceae, Dicksoniaceae and Metaxyaceae) are monophyletic and polypodioids are distantly related to the gleichenioids in spite of the similarity of their exindusiate soral morphology.
Wolf et al. (1999) suggested based on rbcL sequence data from two Hymenophyllopsis species, the family was monophyletic and sister to the single Cyatheaceae species. Lophosoria quadripinnata, the single species in Lophosoiaceae has an affinity to other tree ferns and phylogenetic studies based on plastid DNA sequence data (Tryon, 1970; Tryon and Tryon, 1982; Wolf et al., 1999; Pryer et al., 2004) suggest a close affinity to Dicksonia. Phylogenetic studies based on DNA sequence data concluded that Metaxyaceae belongs to the tree ferns, but there has been no strong support for a specific position within the clade (Hasebe et al., 1994, 1995; Wolf et al., 1999; Pryer et al., 2004). Phylogenetic relationships among the major groups of tree ferns based on protein-coding plastid loci (atpA, atpB, rbcL and rps4) revealed four well-supported clades, with genera of Dicksoniaceae (Kubitzki, 1990) interspersed among them. Dicksoniaceae and Cyatheaceae are not monophyletic and new circumscriptions for these families are needed (Korall et al., 2006).

Ting et al. (2003) sequenced chloroplast trnL intron and trnL-trnF intergenic spacers of Alsophila spinulosa and Cyathea tsangii belongs to Cyatheaceae. The clade showed Sphaeropteris brunoniana, Sphaeropteris hainanensis and Cyathea contaminans diverged from the rest of the members and the latter was further separated into two subclades corresponding to the subgenus Alsophila and Gymnosphaera. Three monophyletic terminal clades were formed separately by Cyathea gigantea - Cyathea pseudogigantea - Cyathea tinganensis - Cyathea pectinata (clade 1), C. contaminans - S. brunoniana - S. hainanensis (clade 2) and C. tsangii - A. spinulosa (clade 3) suggesting that these species could be combined as three separate species: C. gigantea, S. brunoniana and A. spinulosa. The genus Sphaeropteris was placed in the basal position of Cyatheaceae, whereas the genus Alsophila placed as the derived sister group, which supported Tryon's hypothesis accounting for the evolutionary relationships within Cyatheaceae and the derivation of their indusium. Wang et al. (2004) used RAPD analysis and sequences of cpDNA atpB-rbcL intergenic spacers to
characterize the pattern of genetic variation and phylogenetic relationships of *Alsophila spinulosa*. 28 random primers generated 118 bands, out of which 26 (22.03%) were polymorphic loci, distinguishing 17 different RAPD phenotypes. AMOVA showed that 47.44% of the variance was partitioned among regions, 34.01% attributed among populations within regions, whereas only 18.55% occurred within populations. Low level of intra-specific diversity was maintained in *A. spinulosa* with Shannon diversity and gene diversity merely 0.0560 and 0.0590 respectively.

Su *et al.* (2004) sequenced cpDNA *atpB-rbcL* intergenic spacers of individuals of a relict tree fern *Alsophila spinulosa* collected from ten populations in Southern China. Sequence length varied from 724 to 731 bp showing length polymorphism and base composition was with high A+T content between 63.17% and 63.95%. A total of 19 haplotypes were identified based on nucleotide variation. High levels of haplotype diversity and nucleotide diversity were detected in *A. spinulosa* which allowed the accumulation of genetic variation within lineages. Su *et al.* (2005) inferred genetic differentiation and phylogeographical pattern of *A. spinulosa* in southern China using sequence variations of *trnL-F* noncoding regions of cpDNA. AMOVA analysis indicated that most of the genetic variation was partitioned among regions. Korall *et al.* (2007) investigated the phylogenetic relationships of scaly tree ferns based on DNA sequence data from five plastid regions (*rbcL, rbcL-accD* IGS, *rbcL-atpB* IGS, *trnG-trnR* and *trnL-trnF*). A basal dichotomy resolves *Sphaeropteris* with conform scales is sister to all other taxa having marginate scales. The marginate-scaled clade consists of a basal trichotomy with the three groups viz., *Cyathea, Alsophila* and *Gymnosphaera*. In recent phylogenetic analyses, tree ferns were shown to be the sister group of polypods, the most diverse group of living ferns. Gao *et al.* (2009) sequenced the complete chloroplast genome of a scaly tree fern *Alsophila spinulosa*. It shares some unusual characteristics with the previously sequenced genome of the polypod fern.
Adiantum capillus-veneris, including the absence of 5 tRNA genes that exist in most other chloroplast genomes. The genome shows a high degree of synteny with that of Adiantum, but differs considerably from two basal ferns (Angiopteris evecta and Psilotum nudum).

All these studies have given rise to growing confidence in relationships and correspondingly to the composition of taxa at familial and ordinal ranks. Nowadays it is widely accepted that any valid plant barcode will be multi-locus, preferably existing of a conservative coding region like rbcL, in combination with a more rapidly evolving region, which is most likely non-coding (Kress et al., 2009). The non-coding trnL intron and trnL-F intergenic spacer have been repeatedly suggested for this purpose (Taberlet et al., 2006; Hollingsworth et al., 2009) and were successfully used by Li et al. (2009) for identification of a mysterious aquatic gametophyte. Besides the technical issues of primer universality, sequence quality and complexity, Schneider and Schuettpelz (2006) mentioned three potential difficulties for any tested marker to overcome: incomplete sampling of the online records to be used as a reference for identification (GenBank), the occurrence of mis-identified and erroneous sequences in these online databases and the potential inability of the marker to discriminate among species.

A genotypic marker in comparison to a phenotypic marker does not reveal the active principle or the chemical constituent. Therefore, the current trend on chemotype-based fingerprint to support the genotype based molecular markers helps in the proper structuring of a species beyond any level of ambiguity. Considerable work has been carried out to correlate DNA markers with phytochemical compositions among closely related species (Baum et al., 2001; Fico et al., 2003; Joshi et al., 2004). Merging of these profiles will certainly help in developing a comprehensive understanding of a species.

Thus the present study area is growing rapidly on both basic (chemotaxonomy) and applied (discovery of new natural medicines) points of view with the availability of modern
techniques. The chemical taxonomy has grown in a rapid way and up to the level that today it is applied to distinguish not only species, but also individuals within a population as in the case of forensic science by applying DNA fingerprinting techniques (Irudayaraj and Raja, 1998). There is also a challenge for studying variation among plants despite having many advanced molecular techniques available to assess genetic variation. The level of polymorphism that different methodologies reveal is also important. If it reveals too little variation, then it may not be possible to discriminate taxa. If the variation found is too high, then the relationship between the taxa is concealed. This results in inaccurate prediction of relationships.

**Biological activities**

A large number of bacteria are involved in various skin infections and plants contain phytochemicals to kill bacteria involved in skin diseases (Kar and Kumar, 2010). Ethyl acetate, butanol and aqueous extracts of *Blechnum orientale* were effective against all the tested Gram positive bacteria (Lai et al., 2010). Soare et al. (2012) demonstrated the most evident antibacterial effect in *Cystopteris fragilis* gametophytic extract which inhibited the growth of all the tested bacteria. Raja et al. (2012) studied the antimicrobial activity of *Cyathea nilgiriensis*, *Cyathea crinita*, *Leptochilus lanceolatus* and *Osmunda hugeliana* using paper disc diffusion assay. All the selected ferns showed inhibitory effect against *Proteus aureus*, *Klebsiella pneumoniae*, *Aspergillus niger* and *Fusarium* sp. with the maximum inhibition in the highest concentration (100 µg/ml).

Ethyl acetate fraction of *Cyathea phalerata* had marked antioxidant potential, especially as a scavenger of the hydroxyl radical and in inhibiting lipid peroxidation (Hort et al., 2008). Methanolic extract of *Cyathea gigantea* produced a dose dependent inhibition of *in vitro* free radical generation of superoxide anion, hydroxyl radical and DPPH radical (Madhukiran and Rao, 2011). Revathi and Sara (2014) screened *Marsilea minuta* for free
radical scavenging properties using DPPH assay and found maximum activity in frond followed by petiole and rhizome. Johnson et al. (2014) determined the antioxidant potential of *Asplenium aethiopicum* in different extracts using DPPH radical scavenging, phosphomolybdenum assay and scavenging of hydrogen peroxide. The best free radical scavenging activity was exerted in methanolic extract of *A. aethiopicum*.

*Gleichenia linearis* showed antibacterial properties in aqueous extracts (Vasudeva, 1999). Methanolic extracts of *Drynaria quercifolia* showed broad concentration dependent antimicrobial activity (Ramesh et al., 2001). There are reports of *D. quercifolia* against *Neisseria gonorrhoeae* (Shokeen et al., 2005). Friedelin, epifriedelienol, beta amyrin, beta sitosterol, 3-beta-D-glucopyranoside and naringin were isolated from dried rhizome of *D. quercifolia*. Flavonoids of *Drynaria fortunei* were found to protect against gentamicin ototoxicity and renal failure (Long et al., 2004, 2005). Species of *Lycopodium* demonstrated anti-acetylcholinesterase activity in two separate experiments (Zhang et al., 2002; Hirasawa et al., 2006). *Lycopodiella cernua* was antivirally active and had been patented as a treatment for Hayfever (Cambie and Ash, 1994; Zhang et al., 2002).

*Selaginella* species showed an inhibitory effect on muscle contraction (Perez et al., 1994; Rojas et al., 1999). Different species of *Selaginella* showed bioactivity and exhibited anti-inflammatory, antimicrobial and antioxidant properties (Silva et al., 1995; Sun et al., 1997; Lee et al., 1999; Lin et al., 2000; Chen et al., 2005). Aqueous extract of *Selaginella delicatula* has antioxidant activity and degrades blood cholesterol (Gayathri et al., 2005). Isocryptomerin from *Selaginella tamariscina* showed antibacterial potential against Gram positive and Gram negative bacteria (Lee et al., 2009). Amentoflavone from *S. tamariscina* inhibits several pathogenic fungi (Woo et al., 2005; Jung et al., 2007).

*Pteris semipinnata* demonstrated anti-tumour activity in two separate investigations. *P. semipinnata* and *Pteris multifida* are both cytotoxic, but they contain diterpenes (Li et al.,
1998, 1999). Pteris vittata exhibited carcinogenic activity (Siman et al., 2000). Other species of Pteris possessed antimutagenic, immunomodulatory and neuronal activity (Goldberg and Cooper, 1975; Lee and Lin, 1988; Wu et al., 2005). Antimicrobial compounds have also been characterised from a common fern, Pteris bifurcata (Dalli et al., 2007). Fronds of Pteris quadriaurita showed antibacterial potential towards pathogenic multi-drug resistant strains involved in skin diseases in human beings (Thomas, 2011). Pityrogramma calomelanos is cytotoxic and contains flavonoids (Star and Mabry, 1971; Sukumaran and Kuttan, 1991). An oil substance which can be used as a potential antibiotic and anticancer chemotherapeutic agent has been extracted from various species of Ophioglossum (Khandelwal et al., 1985). Aqueous extracts of Drynaria fortunei showed cytotoxic and antioxidant properties (Liu et al., 2001). Christella dentata showed carcinogenic activity (Somvanshi and Sharma, 2005). Cytotoxic activity in terms of brine shrimp lethality bioassay was found to be most effective in A. aethiopicum. Larvicidal activity of A. aethiopicum against Culex quinquefasciatus showed highest mortality in crude acetone extracts (Johnson et al., 2014).

From the above review, it is clear that phytochemical analysis and molecular variation studies has been done on large number of Indian ferns and fern allies but less work has been done on the tree ferns of South India. Hence in the present investigation, an attempt has been made to study the phytochemical and molecular characterization of selected Cyathea species from Western Ghats, South India.