Pteridophytes, the seedless vascular plants, had a very flourishing past in dominating the vegetation on the earth about 400 millions years ago (Mehra 1967; Bir 1976a, Khare 1996). Although they are now largely replaced by the seed bearing vascular plants in the extant flora today, yet they constitute a fairly prominent part of the present day vegetation of the World particularly in the tropical countries. Numerous fossil species have also been described. There are about 12838 species and 501 hybrids of ferns under 58 families and 316 genera throughout the World. Pteridophytes are rich in tropical countries. Maximum number of species 3281 has been reported in South America followed by Malesia with 3227 Species. North Central and East Asia are also rich in ferns with the occurrence of 2988 species. Mexico and Central America are also fairly rich in ferns with 2620 species. India is also rich in pteridophytes with around 1000 species.

When compared to flowering plants, the non flowering vascular plants – pteridophytes are comparatively less of importance for daily uses. But there are several reports to show remarkable uses of pteridophytes in various fields. For example the alkaloid Huperzine from *Huperzia* species, is useful to cure Alzheimer’s disease and the drug is commercially available in China. Rhizome of the medicinally important fern *Drynaria quercifolia* is available in local market of Kolli Hills in Tamil Nadu as ‘Attukkal’. Maiden hair fern, *Adiantum* species, (Hindi-Hansraj) is available in Delhi market. In the

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**Review of Literature**
meantime there are numerous reports to show the ethnobotanical uses of pteridophytes throughout the world including India. In general pteridophytes are mostly used as cooling agents. In several species of pteridophytes antioxidant and antimicrobial activities have also been reported (Talukdar et al. 2011). Due to the medicinal importance, several pteridophytes have been pharmacognostically analysed (Irudayaraj, and Senthamarai, 2003) Recent studies have shown the importance of pteridophytes in biotechnology and nanotechnology. By using spores of Ceratopteris richardii, biosensor to detect calcium ion concentration in and around a cell has been developed. By mimicking the dehiscence mechanism of leptosporangium of fern, microactuator, electric power generating device, has been invented by engineers from Michigan University. Thus pteridophytes are the important bioresource on the earth and numerous pteridophytes are yet to be bioprospected for their economic potentiality, particularly from India where the scientific utilization of pteridophytes is comparatively poor when compared to other tropical countries. But the utilization of Indian Sanjeevani, Selaginella species has been mentioned in Ramayana itself. Since this group of plants is not much of importance for daily life of Indians, the efforts to conserve the rare and endangered pteridophytes is also not up to appreciable level. At this situation, based on available literature, a brief account on the bioresource and bioprospecting of Indian pteridophytes along with the conservation activities on rare and endangered pteridophytes has been given in the following review.
RICHNESS OF PTERIDOPHYTES IN INDIA:

The Indian subcontinent and Indo China together house 1598 species of which 1250 species occur in India alone. Himalayas, Western Ghats, Pachmarhi Hills and Eastern Ghats in India are rich in ferns. Western Ghats in India represent unique flora of the World with about 4000 (Nayar, 1986) species of flowering plants. As far as the South India is concerned the earliest comprehensive work on ferns is by Beddome (1864) “The ferns of South India “ in which he has listed and illustrated 271 species with 50 species from Sri Lanka. Beddome (1864) has covered mainly the Nilgiris, Anaimalais and Kerala mountains.

After Beddome’s (1864) work there was a long gap of about 100 years in pteridological studies from South India except the revisionary studies at family level, made by Sledge (1957,1967,1973,1982) along with his studies on ferns from Sri Lanka. Publication of “Problems of cytology and evolution of pteridophytes” by Manton (1954) triggered several Pteridologists throughout the world including India..

Subramanian et al., (1961) have recorded 50 species of pteridophytes from the Cumbum valley and Pachakumatchi hill of the Madurai District, Tamil Nadu. From Kodaikanal alone Bir and Vasudeva (1971) have enumerated 118 species of ferns and fern allies.
A comprehensive work of Manickam and Ninan (1976) shows the presence of 148 taxa of ferns including novelties from the Palni hills. Besides, 3 species are new addition to South India 30 are addition to the Palni hills.

Manickam (1986), in his ‘Fern flora of the Palni hills’, described 36 taxa, of which 4 are new. This book contains general key for all genera together and under each family, key to genera and species are given. The description is detailed and wherever needed critical taxanomic comments are provided. The new taxa pointed out and described in this work are formally treated by Manickam and Irudayaraj (1992).

Manickam and Irudayaraj (1992) published “Pteridophyte Flora of the Western Ghats, South India”. This book deals with 252 species of Pteridophytes. It contains general key for genera and species and detailed descriptions. Every species is illustrated. Eight new taxa are described and chromosome numbers, economic importance of species, wherever available, are also included in that flora.

Nayar and Geevarghese (1993) published Fern flora of Malabar (North kerala). This work includes a general key to all genera nomenclature and detailed morphological description of all the 178 taxa. The anatomical feature of rhizome, stipe and the nature of the spores are used in the keys. Yohanarasimhan et al., (1981) reported 12 species of ferns in the flora of Chikmagalur District, Karnataka. Following this pteridophytes of Karnataka state have been enumerated by Rajagopal & Bhat (1998). They reported 151 ferns and 21 fern allies. A section of Western Ghats also passes from the state of Goa. Vartak (1966) reported 49 species of Pteridophytes from Gomantak region and Rao (1986) described 29 species of Pteridophytes from new localities of Goa. Later Sharmila M. Madaiker (2003) reported and re explored the pteridophytes in Goa and the total list
of 51 Pteridophytes new to Goa is given by Manickam et al., (2004). All the above studies included about 349 species of pteridophytes from the Western Ghats alone. Of which 30 species are listed out to be endangered as they are difficult to relocate (Benniamin 2005). Benjamin and Manickam 2007 reported that 61 species of Pteridophytes of Western Ghats are medicinally important.

**BIOPROSPECTING OF PTERIDOPHYTES:**

The uses of pteridophytes are known from ancient time by utilizing various species to treat various ailments. The historical medicinal ferns are *Asplenium* species and *Dryopteris filix-mas*. The former is used to cure spleen diseases, based on which it the genus has been named as ‘*Asplenium*’ and the later is used as anthelmintic. The heterosporous aquatic weed, *Marsilea minuta* is used to prepare an ayurvedic drug to cure epilepsy. There are several edible ferns like *Diplazium esculentum, Ceratopteris thalictroides* etc. The ethnobotanical uses of several ferns have been confirmed scientifically by phytochemical and pharmacological studies. For example, the weed fern *Cyclosorus interruptus* is being used by local farmers as green manures for banana. The growth promoting effect and antifungal effect have been confirmed by Paulraj (2007) Pauline Vincent (2012). In the same way the spores of the mangrove fern, *Acrostichum aureum* is used as termicidal agent in Kerala and the termicidal effect of spores in that fern has been confirmed by (Mineral Udhayam, 2007).
Phytochemical studies on pteridophytes:

Phytochemistry of ferns and fern-allies is a growing discipline in India and it is at the infant stage. As Wallace (1989) indicated, 50 per cent of the worldwide knowledge relating to fern polyphenolics has been published since 1980 and another 40 per cent between 1970 and 1980. As far as India is concerned, more than 80 per cent of the findings on phytochemical analysis, in general, have been published after 1970.

Alkaloids are a diverse group of compounds and they are known to have a variety of marked effects on animals. Alkaloids often act on the nervous system as stimulators, 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 and Gang 2004). Terpenoids are the main component of many plant essential oils. This group is based on a single unit, isoprene, and thus monoterpenoid, diterpenoids, and triterpenoids, all differ in the number of isoprene units. Terpenoids are also a very diverse group and the 40 pteridophyte species presented here contain: triterpenoids (hopane triterpenoids, epoxytriterpenoid, and serratene triterpenoid), diterpenoids, hemiterpene glycosides, and clerodane diterpene glycosides.
Terpenoids have also been the subject of much study, and many are medicinally significant for a wide range of treatments. For example, triterpenoids isolated from Erica andevalensis are cytotoxic against human cancer cell lines. Also, terpenoids from Calendula officinalis flowers exhibit strong anti-inflammatory activity (Della Loggia 1994). Terpenoids are a very promising class of compounds, and additional studies will only add to the useful knowledge already collected.

Flavonoids are a third class of compound represented in pteridophytes. Most of the pteridophytes contain flavonoids. Flavonoids, like alkaloids and terpenoids, are a diverse group. Only a fraction of flavonoid subdivisions are represented in these pteridophytes: biflavonoids, homoflavonoids, flavone glycosides, and flavonol glycosides. Many flavonoids have medicinal properties. Amentoflavone and ginkgetin are flavonoids found in Selaginella, exhibit neuroprotective activity against cytotoxic stressors. This property suggests their possible use in treatment of neurodegenerative diseases such as stroke and Alzheimer’s (Kang et al 2005). Another flavonoid, mangiferin (found in Trichomanes reniforme), shows antiviral and anti-tumor effects in mice. Mangiferin enhances the immune system’s natural ability to kill cancer cells and also shows inhibitory effect on HIV (Guha et al., 1996).

Quantitative analysis of pigments (chlorophyll, carotenoids and antocyanins) sugars and starch have been estimated in 12 species of homoporous ferns collected from Kothayar and Palni hills by Patric Raja et al (1992). These authors have also discussed relationship among physical (altitude, light conditions), morphological (lamina texture) and chemical (sugars, starch and pigments) parameters.
Chemical compounds which are more valuable in taxonomy are primary metabolites, secondary metabolities and semantides (information carrying molecules) which consist of DNA (primary semantides), RNA (Secondary semantides), and protein (tertiary semantides). According to Stace (1989) most of the primary metabolites are of universal occurrence or at least occur in a very wide range of plants. The presence of or absence of such compounds is therefore not of much systematic value. Most of the phytochemical works on Indian ferns pertain to the primary metabolites are of less value from the chemo taxonomical point of view, they are very useful from the physiological, ecological and nutritional points of view. The behavior of chlorophylls, carotenoides and phenols in drought resistance in ferns and ferns 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). Patric Raja et al (1995) and Ramchandran et al (1991) have made eco-physiological studies on the ferns from Kothayar and Palni hills (South India). The Phytochemical analysis of the edible ferns, *Ampelopteris prolifera* and *Diplazium esculentum* (Shankar and Khare, 1985) shows the nutritional value of these ferns. The quantative difference of the primary metabolites of closely related species is correlated with the ploidal level as in the case of the diploid *Diplazium polypodioides* ans tetraloid *Diplazium brachylobum* in which all the chemicals investigated (Antonisamy et al, 1992), except phenols are in larger amount in the tetraploid species than the diploid species.
Irudayaraj and Patric Raja (1998) have reviewed phytochemical studies on Indian ferns. According to these authors a large number of fern species have been phytochemically analysed in order to understand ecophysiological adaptations. A good number of medicinal ferns have been analysed phytochemically and pharmacognostically. Analysis of secondary metabolites in several Indian ferns resulted in the discovery or record of many new chemical compounds which are very useful in understanding the taxonomic and phylogenetic relationship among Indian ferns.

Although ferns, among many other plants, were used for medicinal purposes by the early Greeks and Romans and through the Middle Ages, the phytochemical analysis has been done, comparatively on lesser number of non-flowering plants in contrast to flowering plants which have wide range of chemicals in the form of pigments and aromatic substances to favour pollination by insects (Irudayaraj and Patric Raja, 1998). In India, phytochemistry of Pteridophytes has largely been ignored as compared to morphology, anatomy, cytology and other aspects. So far much work has been done on the Pteridophytes of Rajasthan (Kaur et al., 1986; Vyas et al., 1989) and the phytochemical study on south Indian ferns of Western Ghats is very meager (Jesudoss et al., 2003). Only in the recent times certain studies have been done with regard to ethnomedicinal uses of these plants. These data need to be utilized by phytochemists and pharmacologists to determine their true therapeutic compounds that may ultimately lead to the development of new herbal drugs.
Bohm and Tryon (1967) made a survey on the phenolic compounds in ferns. They conducted a survey for hydroxylated cinnamic acid and benzoid acid derivatives in about 46 ferns, representing 28 genera and 8 families. In most plants examined the “basic complement” of cinnamic acids, p-coumaric, caffeic, and ferulic were present.

Bhardwaj et al., (1982) reported the presence of the compound rutin from the fern Asplenium trichomanes. The preliminary phytochemical screening of 19 species of south Indian Thelypteroid ferns showed important secondary metabolites (Britto et al., (1993) and (1994)). Anthocyanins and flavonoids were found to be more in high altitude ferns than the low altitude ferns (Itudayaraj and Patric Raja, 1998). About 22 taxa of western Ghats were evaluated to assess whether they posses renewable energy, oil, hydrocarbon and phytochemical (Augustus et al., 2003). Preliminary phytochemical screening has been done in petroleum ether, benzene, chloroform and ethanol extracts (40-60°C) of the family pteridaceae to assess the presence of secondary metabolites (Jesudoss, 2003). Sixteen flavonol glycosides including new and rare ones, were isolated from Asplenium trichomanes-ramosum on its phytochemical analysis (Iwashina et al., 1995) and two novel flavonol glycosides from the fern Cheilanthes fragrans, have been found (Imperato, 1989) and the edible ferns, Ampelopteris profera and Diplazium esculentum (Retz.)Sw. were subjected to phytochemical analysis by Shankar and Khare (1985) which showed the nutritional value of these ferns.
Phytochemical analysis was done on the dried rhizome of fern *Drynaria quercifolia* which revealed the presence of useful secondary metabolites (Ramesh *et al.*, 2001) and the studies on the chemical constituents of aquatic fern *Azolla nilotica* revealed two new components (Arai *et al.*, 1998).

A phytochemical screening and Thin Layer Chromatography (TLC) analysis on the stem and root of *Microgramma squamulosa* (Kaulf.) showed the presence of flavonoids and tannins which may be related to the antiulcer activity (Ivana *et al.*, 2008) and the analysis on the fern *Cyclosorus interruptus* (Willd.) revealed the presence of three new bioactive coumarin derivatives (Quadri-Spinelli *et al.*, 2000).

Chemotaxonomy and phytochemical studies of bracken, *Pteridium aquilinum* showed the presence of wide range of secondary plant substances including sesquiterpenoids, echydones, cyanogenic glycosides, tannins and phenolic acids (Driver, 2008). A new triterpenoid from the fern *Adiantum lunulatum* has been isolated when it was subjected to phytochemical analysis (Niranjan Reddy *et al.*, 2001). Occurrence of Polygodial and 1-(2, 4, 6-Trimethoxyphenyl)-but-2-en-1-one from some ferns and liverworts are reported (Asakawa *et al.*, 2001).

*Microsorum scolopendria*, (Burn.f.)Copel. a fern of medicinal importance was evaluated for the presence of ecdysteroids, which might be responsible for at least some of their medicinal properties. A combination of normal and reversed phase High Perfomance Liquid Chromatography (HPLC) showed the presence of three minor phytoecdysteroids which were identified by Nuclear Magnetic Resonance (NMR) (Snogan *et al.*, 2007), and through a study on the aerial parts of the fern *Abacopteris*
*penangiana*, five new flavan-4-ol glycosides, abacopteris E-1 (5-9), and seven known flavonoids glycosides (3 and 10-15) have been isolated (Zhao et al., 2007). Thirteen glycosides and methyl (3R, 5R)-5-hydroxy (β-D-glucopyranosyloxy)-hexanoate from the Japanese fern *Hymenophyllum barbatum*, (Toyota et al., 2002) and a New disaccharide, digobios 1 from *Macrothelypteris Digophlebia* (Qiu et al., 2000) have been isolated.

Certain ferns have been subjected to GC-MS analysis for the purpose of separation of volatile components such as essential and fatty oils. Endogenous Gibberellins from sporophytes of two tree ferns, *Cibotium glaucum* and *Dicksonia antarctica* have been identified from Kovats retention indices and full scan mass spectra by capillary GC-MS analysis of purified extracts (Yamane et al., 1988), and from the methanol extract of prothallia of *Lygodium circinnatum*, GA25, GA73, GA73-Me, GA88-Me, and a few unknown GA73 derivatives were detected by GC-MS (Yamauchi et al., 1997) and the fern *Camptonia peregrinia* (L.) (Sweet fern) harvested in the Lake Saint-Jean area (Quebec) has been analysed using GC-MS and Kovats indices techniques in which more than fifty constituents, mainly oxygenated compounds, are reported (Collin et al., 1988).

Most of the primary metabolites are of universal cooccurrence, or at least occur in a very wide range of plants, the presence or absence of such compounds is therefore not of much systematic value. Most of the phytochemical works on Indian ferns pertain to the primary metabolites. Although they are of less value from chemotaxonomical point of view but they are very useful from physiological, ecological and nutritional point of views. The behavior of chlorophylls, carotenoids and phenols in drought resistance in
ferns and fern allies (Selaginella) from Rajasthan has been studied by Bohra et al. (1979), Vyas et al. (1989), Rathore & Sharma (1991) and Sharma et al. (1992). Patric Raja et al. (1991, 1995) and Ramachandran et al. (1991) have made ecophysiological studies on the ferns, from Kothayar and Palni Hills (South India). The Phytochemical analysis of the edible ferns, Ampelopteris prolifer and Diplazium esculentum (Retz.) Sw. (Shankar & Khare 1985, Singh et al. 1989) shows the nutrititional value of these ferns. The quantitative difference of the primary metabolites of closely related species is correlated with the ploidal level as in the case of the diploid Diplazium polypodioides Bl. and tetraploid D. brachylobum (Sledge) Manickam & Irudayaraj which all the chemicals investigated (Antonisamy et al. 1992), except phenol, are in larger amount in the tetraploid species than the Diploid species.

Preliminary phytochemical screening of 19 species of South Indian thelypteroid ferns shows the occurrence of steroid, alkaloid, phenol, catechchin, saponin and tannin in all the species (Britto et al. 1994). Triterpenoids and anthoquinone are not found in any of the species investigated. But Irudayaraj (1996) has reported the presence of triterpenoid, in the epidermal glands of Christella parasitica (L) Lev. Flavonoids have been found to be present in 12 species out of 19 South Indian Thelypteroid ferns screened. (Britto et al. 1993, 1994).

Quantitative analysis of pigments (chlorophylls, carotenoids), carbohydrates (sugars, starch), nitrogenous compounds (amino acids, proteins, nitrogen) have been done in a large number of South Indian Thelypteroid ferns (Britto et al. 1992, 1993, 1994), Pteris, Hypolepis, Pteridium, Histiopteris and Cyathea (Gopalkrishnan et al. 1993 a, b),

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(Jesudass et al. 1993) and Rajasthan ferns and fern allies (Kaur et al. 1986, Vyas & Sharma 1989, Rathore & Sharma 1990, Vyas et al. 1995). These studies explain the physiological adaptation of various species in various habitats. Usually shade loving species have more amount of chlorophylls than the sun plants.

Anthocyanins and flavonoids, which play an important role in protecting the plants against UV irradiation (Gaff 1977) are usually more in high altitude ferns than the low altitude ferns.

**Antimicrobial and anticancer studies on pteridophytes:**

More than hundreds of plants worldwide are used in traditional medicine as treatments for bacterial infection (Martin et al., 2003). Although many have been treated by conventional pharmaceutical approaches, there is a growing interest in the use of natural products by the general public. In addition the pharmaceutical industry continues to examine their potential as sources novel growth factor, immunomodulatory and antimicrobial activity (Ghosh et al., 2003).

Many workers have studied the antibacterial and antifungal activity of plant extract (Gehlot et al 1995; Parihar & Bohra, 2003, 2004; Manickam et al 2005). At present, many scientists are interested to evaluate the importance of ferns from chemical and pharmacological aspects. These non flowering plants produce cynogenic glycosides, nucleotides, flavonoids, terpenoids, alkaloids which are used in deficiency as nutrients as well as in medicine, in the form of astringent, expectorant, diuretic, anti-ulcer, stomachic, stimulant, analgesic, aphrodisiac, appetizer, antihelminthic, carminative etc.
The occurrence of antibiotic activity in the extracts of 114 species of pteridophytes (27 families, 61 genera) has been surveyed. The plants were extracted in water, ethanol, methanol, acetone and ether and assayed against gram positive and gram negative bacteria and fungal plant pathogens. The active substances were in most cases antibacterial and only some possessed antifungal activity (Banerjee and Sen, 1980). The methanol and acetone extracts of the plants *Polystichun pungens* and *Cheilanthes viridis* were subjected to antibacterial screening. Most of them showed significant inhibition against gram positive and gram negative bacteria (Grierson and Afolayan, 1999). Evolution of antimicrobial activity was carried out on the *Adiantum lunulatum* (Niranjan Reddy *et al.*, 2001). Different aqueous, methanolic (70, 80, 90%) extracts of *Pteris vittata*, commonly known as Brake Fern were tested for the antimicrobial properties against the growth of eight intestinal microorganisms, by using disc diffusion and micro dilution methods. The 70 % aqueous methanolic extract showed potent activity against some pathogens (Singh *et al.*, 2008).

The aqueous and ethanol extracts of *Drynaria quercifloia* were also screened for antimicrobial activities against a fungus *Candida albicans* and four bacteria *Klebsiella pneumonaeae, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* and the antimicrobial activity was observed against both gram positive and gram negative bacteria. The presence of antimicrobial activities in the extracts from the rhizome of *D. quercifolia* is in conformity with the traditional medicinal uses of this plant against some pathogenic diseases like typhoid fever (Irudayaraj and Senthamarai 2004). The same fern has been subjected to know the antibacterial effect on urinary tract pathogens by Mithraja *et al.* (2012). Patric Raja *et al* (2012) have screened antimicrobial activity in some ferns.
Plants in general have several pharmacological properties for which usually the macroscopic parts such as entire plant, stems, roots, flowers, fruits or seeds are analysed. But Manickam et al. (2005), Paulraj et al (2011) and Pauline Vincent et al. (2012) interestingly showed the antimicrobial effects on the microscopic epidermal glands of leaves of some ferns. Paulraj et al. (2011) have confirmed the growth promoting effect and pesticidal activities in epidermal glands of Christella parasitica. Due to the presence of various bioactive compounds antitumor and anticancer activities have reported in several ferns as follows: Diplazium esculentum, Vittaria anguste-elongata (Wu et al., 2005), Macrothelypteris torresiana (Lin et al., 2005, 2007, Chiu et al., 2009), Actiniopteris semiflabellata (Mothana et al., 2008), Tectaria singaporeana and Blechnum orientale (Aini et al., 2008), Lygodium flexuosum (Wills & Asha, 2009), Phlebodium decumanum (Gridling et al., 2009), Abacopteris penangiana (Zhao et al., 2007), Cheilanthes farinosa (Krishna et al., 2010), Dryopteris crassirhizoma (Chang et al., 2010), Adiantum venustum (Viral et al., 2011), and Dicranopteris linearis (Desa et al., 2011). Phytochemical, pharmacognostical and antimicrobial studies have been carried out on anticancer spike mosses Selaginella species by Irudayaraj et al. (2010), Haripraya Duraiswamy et al. (2010), Suganya et al. (2011) and Irene Pearl et al. (2011). The extracts from Selaginella ciliaris, Marsilea minuta and Thelypteris prolifer are have been proved to show antitumor properties against Agrobacterium tumefaciens induced tumor (Sarkar et al., 2011). Among the species, highest percentage of tumor inhibition has been found in M. minuta (82.32%) followed by S. ciliaris (80%) and T. prolifer (75.68%) at 1000 ppm. Pharmacognostical, phytochemical and antimicrobial studies on Parahemionitis cordata (Hemionitis arifolia) have been carried out by Irudayaraj and
Senthamarai (2003). Antioxidant activity, polyphenol content and antimicrobial activity have been studied in three native Pteridophytes of Romania by Soare et al. (2012).

Aqueous and alcoholic extracts of *Athyrium pectinatum* were tested against the growth of some human and plant pathogenic bacteria like, *Agrobacterium tumefaciens, Escherichia coli, Salmonella arizonae, Salmonella typhii* and *Staphylococcus aureus*. Nearly all the extracts were found effective against these bacteria (Parihar et al., 2006).

The organic solvent extracts (ethanol, acetone and chloroform) of *Trignospora cordifolia* and *Nephrolepis linearis* were tested against seven important human pathogenic bacterial strains and the result showed variable antibacacterial activity (Davamani et al., 2004).

The water extracts and extracted phenols from gametophytes and different parts of sporophytes of the two ferns *Adiantum capillus veneris* and *Adiantum lunulatum* used as folk medicine in India, China, Tibet, American, Phillipines and Italy were investigated for their antifungal activity against *Aspergillus niger* and *Rhizopus stolonifer*. Both crude extracts phenols of gametophytes and different parts of sporophytes of both fern species were found to be bioactive against the fungal strains (Guha et al., 2005).

About twelve selected *Cyathea* species were tested for their abilities to produce antimicrobial metabolities. Some extracts from the cultivated liquid obviously inhibited human pathogenic fungi *Aspergillus fumigatus, Candida albicans* and *Cryptococcus neoformans*. Activities against six human pathogenic bacteria were also obtained from some of these extracts (Liu and Zhang, 2009) and also aqueous and acetone extracts of
epidermal glands of the ferns, *Christella parasitica* and *Cyclosorus interruptus* were tested both individually and in combination for antibacterial activity against the growth of *Salmonella typhii*. It was found that the acetone extracts was more effective against the bacteria than the aqueous extracts (Vincent and Kanna, 2007).

The species that have been studied pharmacognastically by various workers are: *Helminthostachys zeylanica* Linn. (Huang *et al*., 2009), *Polypodium vulgare* Linn. (Mannan *et al*. 1989), *Hypodematium crenatum* (Shankar & Khare 1994), *Dryopteris ramosa* (Hope) C. Chr., *D. Chrysoma* (Christ) C. Chr., *D.barbigera*, *D. cochleata* and *D. splendens* (Yadav 1992), *Lygodium flexuosum* Sw. (Wills *et al*., 2006). The chemistry and pharmacology of marsiline, a sedative and anticonvulsant principle isolated from *Marsilea minuta* Linn. and *M. rajasthanensis* Gupta have been studied by Chaterjee *et al*. (1963). *Adiantum lunulatum* Burm. F. has also been studied pharmacognastically (Anand & Srivastava 1994). The rare, endangered and medicinally important spleenworts, *Asplenium sp*. and *Psilotum* have been investigated phytochemically by Lal (1979), Rohtagi *et al*. (1984), Khare & Shankar (1987) and Varma (1992). It has been confirmed that the spores of the mangrove fern *Acrostichum aureum* Linn. seem to be a potential allergen (Yasmeen & Devi 1987). An oil, which can be used as a potential antibiotic and anticancer chemotherapeutic agent (Khandelwal *et al*. 1985, Khandelwal 1986) has been extracted from various species of *Ophioglossum* by Khandelwal *et al*. (1989). Very less work has been done on the antimicrobial activity of pteridophyte, yet ethno botanical importance of these plants have been investigated and studied by various authors. They reported that these plants are of great medicinal importance and are used by the tribal and local people for remedy against various ailments. (Chopra *et al*., 1956,
Vyas, 1987, Manickam & Irudayaraj, 1992, Hansraj, 1996, Kaushik & Dhiman, 1995, Chandra, 2000, Kumar et al., 2003). Dhar et al. (1968) have screened various pteridophytic plants for their biological activity and obtained the similar results. Bhabbie et al. (1972) analyzed the phytochemical composition of *Actiniopteris radiata* and found that the isolated phytochemicals were effective against the microorganisms. Antibiotic activity of pteridophytes were studied by Banerjee and Sen (1980) while the antiviral activity of crude extracts of some pteridophytes and found these effective against conidial germination of *Drachslera oryzae*. Kumar and Kaushik (1999) and Guha et al. (2004) showed the antibacterial activity of *A. capillus-veneris* and found nearly all the extracts effective against the selected micro-organisms. Antifungal effect of pteridophytic plant part extracts have been studied against the dermatophytes. (Davvamani et al., 2005) and that of leaf glands of pteridophytes have also been studied (Manickam et al., 2005). Similar results have been evaluated by Parihar and Bohra (2003, 2004) and Parihar et al. (2005, 2006 a,b,c & 2007 a,b). In the present study, Pharmacognostical and antimicrobial studies are to be carried out on the fronds and rhizomes of medicinally important ferns viz; *Bolbitis virens, Osmunda hugeliana, Acrostichum aureum, Ophioglossum vulgatum, Lygodium flexuosum, Ceratopteris thalictroides* and *Drynaria quercifolia* which are used by various tribal communities of Western Ghats to cure various diseases like hectic fever, dyspepsia and cough (Caius, 1935), typhoid fever jaundice and it is also used as poultice, antifertility agents (Dixit & Vohra 1984)
Pteridophytes as potential phytoremediators:

After knowing the phytoremediation potentiality of Pteris vittata of the fern family Pteridaceae, several species of Pteris and species from the related genera are screened for the phytoremediation potentiality. Thus most of the species of Pteris and related genera have been screened. Zhao et al. (2002) have reported the Arsenic-phytoaccumulating potentiality of Pteris cretica, P. longifolia and P. umbrosa to a similar extent. Meharg (2003) has reported three new species of Arsenic hyperaccumulators in the Pteris genus. He is the first to report members of the Pteris genus, Pteris straminea and P. tremula that do not hyperaccumulate arsenic. Hyperaccumulating ferns identified to date are all located in the order Pteridales, and include a number of Pteris species and Pityrogramma calomelanos. Since pteridophytes are with alternation of generation between independent sporophyte and gametophyte, the phytoremediation potentiality and heavy metal tolerance have been studied not only the sporophyte, but also on gametophyte. Irudayaraj et al. (2011) have studied the effect of heavy metal stress on spore germination of Pteris confusa and Pteris argyraea.

CONSERVATION OF RARE AND ENDANGERED PTERIDOPHYTES:

The pteridophytes being an important part of the flora of a region form the next important part after the angiosperms. There are a large number of indigenous species of which a considerable percentage is rare and threatened. Due to over-exploitation of natural resources and large scale land transformations, the pressure on the threatened and endangered species has increased manifolds and hence, they may face the brunt of extinction in the coming times. The IUCN has done significant job in documenting the

This group of plants faces serious threats to its survival due to climate change, increased demand for natural resources, invasive species, land for agriculture and so on. Ex vitro measures supported appropriately by in vitro tools can become part of the solution for the rescue of these species. Species which are either extinct in the wild (EW) or critically endangered (CR) are high priority for rescue and conservation using in vitro
means (Sarasan et al. 2006). A taxon is EW when it is known only to survive in cultivation, in captivity or as a naturalised population (or populations) well outside the past range and CR when the best available evidence indicates that it is facing an extremely high risk of extinction in the wild (IUCN 1994). It is very important to apply the appropriate tools available for the rescue of plants that produce recalcitrant seeds or are propagated vegetatively. To achieve the targets of recovery, re-introduction and restoration programmes quality propagules need to be developed and also stored in long-term storage repositories. Ferns are among the priority species because of their reliance on a moist atmosphere for spore germination. Because of forest clearance for wood or other human use, the disappearance of shade that is provided by the trees would affect the growth and maintenance of ferns to a great extent.

Two approaches for conservation of plant genetic resources namely in situ and ex situ. As in any conservation strategies, in the cases of ferns also, the best method of conserving the species is by in situ conservation by protecting the natural habitats particularly in ever green forests where they grow commonly. Some cases may require ex situ conservation either by multiplying species by conventional method or by in vitro tissue culture or spore culture method. Such multiplied species may be conserved in the garden. As far as India is concerned the in situ conservation has been made by managing several forest areas and sanctuaries or biosphere reserves eg. KMTR, Nilgiri Biosphere reserves. For ex situ conservation special efforts are not given much to the cases of ferns when compared to the flowering plants. In majority of the gardens there are very few ferns which are grown mostly as ornamental ferns and not as a rare and endangered ferns. In India there are very few fernaries to conserve the rare and endangered ferns eg.
Kodaikanal Botanic Garden, Gurukula Botanic Garden, Nadugani Gene pool forests, and National Botanical Garden. The *ex situ* conservation of rare and endangered ferns may be strengthened by setting up more and more fernaries in different parts of the country particularly near by the sanctuary or biospheres.

**Conservation of rare and endangered ferns through *in vitro* tissue / spore culture:**

The plant tissue culture as an effective tool to conserve plant genes and guarantee the survival of the endemic, endangered and overexploited genotypes is derived from the fact that it makes use of small units (cells and tissues) without losing the mother plant, takes pressure off the waning wild populations and makes available large numbers of plants for reintroduction and commercial delivery.

Tissue culture is a modern innovation for rapid propagation of large number of uniform plants. Conservation of plant germplasm can itself be the goal of tissue culture (Prance, 1997). The process of regenerating an entire plant from a single cell, otherwise the totipotency. But for this regeneration ability of the plant cell, many exciting results in plant tissue culture and genetic engineering could not have been achieved. The emergence of modern biotechnology presets an important approach for establishing a link between conservation and sustainable utilization of genetic diversity. Plant tissue culture and organ culture techniques have emerged as an inseparable tool with possibilities for completing and supplementing the conventional methods of plant breeding. Plant tissue culture has emerged as a powerful tool with the potential not only for rapid multiplication of plant species but also for conservation of rare and endangered ones. Different pathways of *in vitro* techniques have been developed not only to achieve faster
propagation. But also unravel intricacies of morphogenesis involved in these processes. The pteridophytes offer a vast scope of morphogenetic studies as the experimental work can go well beyond the mere callus formation and differentiation. Application of tissue culture methods not only can increase the sporophyte production but also can provide useful insights into fern biology.

Developmental pathways of plants in vitro have been categorized and are well studied in higher plants as either somatic embryogenesis or shoot organogenesis followed by root organogenesis, either directly or via callus formation (Phillips, 2004). However, such developmental pathways in vitro either as organogenesis or somatic embryogenesis remain unexplored in ferns. Among the various biotechnological options, also reported in other agri–horticultural crops, micropropagation through tissue culture are best applied and commercially exploited in fern species (Fay 1994).

The culture of tissues in ferns has been utilized as a research instrument for the study of the developing potentialities of the leaf primordia ever since the early 1960’s (Torres, 1988). The first successes in the field of the intensive multiplication of plants through in vitro techniques are cited around 1970, the fern Nephrolepis exaltata being the first plant micropropagated in vitro with a commercial purpose (Cachita-Cosma, 1987). According to Pierik (1991), 157 million plants, i.e. 74% out of the total production of micropropagated plants, have been ornamental species. Out of these, approximately 40 million plants have been pot plants. Top of the list, with 17.8 million plants, is the fern Nephrolepis (Fernández and Revilla, 2003).
Although ferns were some of the earliest plants to be cultured in vitro (Murashige & Skoog, 1962), very few reports are available on the in vitro culture of ferns either directly or via callus formation. Induction of callus in fern culture is considered rare and of spontaneous occurrence rather than a controllable event (Partanen, 1972). Limited callus induction has been reported from shoot apices of *Marsilea vertiata* (Laetsch and Briggs, 1961), leaves of *Pteris cretica* (Bristow, 1962), rhizomes of *Ophioglossum petiolatum* (Peterson, 1967), and roots of *Cyclosorus dentatus* (Mehra and Palta, 1971). Recently, callus induction and plantlet regeneration in ferns have been reported from gametophytic tissue of *Dryopteris affinis* (Fernandez and Revilla, 2003) and from sporophytic tissue of rhizomes in *Platycerium cornarium* and *Platycerium bifurcatum* (Dolinsek and Camloh, 1997).

Ferns have been cultured in vitro to study their growth, development and differentiation as well as for micropropagation purposes, given their ornamental value. Several species of ferns have successfully been propagated by this method (Hartman and Zettler, 1974). Ferns have been traditionally used indoors, but their outdoors use is increasing. An example of this trend is *Polypodium cambricum*, which is becoming popular as a rock plant due to its tolerance to adverse conditions. The effects of auxins and cytokinins on bud formation, yielding a high number of sporophytes, has been reported by several authors (Harper, 1976; Beck and Caponetti, 1983). In certain cases, green globular bodies (GGB) have been described as a response to BA. GGB develop into sporophytes when cultured in a growth regulator-free medium (Higuchi *et al.*, 1987; Higuchi and Amaki, 1989; Amaki and Higuchi, 1991; Fernández *et al.*, 1996).
There are so many reports are available on callus induction from different explants such as young leaves, stolon tips, stipes, rhizome segments etc (Mehra and Sulkyan, 1969; Padhya and Mehra 1981; Cheema and Sharma 1994; Kshirsagar and Mehra, 1978; Mehra and Palta, 1971 and Sara 2001). Callus was induced from rhizome (Kshirsagar and Mehra, 1978) roots (Mehra and Palta, 1971; Sulklyan and Mehra, 1977); runner segments (Sulklyan and Mehra, 1977) and leaves (Sulklyan and Mehra, 1977 and Cheema, 1984) of pteridophytes.

The regeneration of shootlets from callus derived from gametophytes and sporophytes was reported by Cheema and Sharma (1994); Sara (2001); Cheema and Kaur (1985); padhya (1985, 1987); Vallinayagam (2003); Byrene and Caponneti (1992) and Kwa et al., (1995, 1997). Callus induction and regeneration studies were carried out on a medicinal fern, Drynaria quercifolia by culturing the explants on Knop's medium supplemented with various concentration of growth regulators. Yoshihara et al., (2005) reported the induction of callus from a metal hyper tolerant fern, Athyrium yokoscense. Likewise callusing has been induced from gametophyte in certain species.

Caponetti (1990) had propagated the fern Tectaria gemmifera via tissue culture studies adventitious buds as explants. A successful protocol has been evolved for in vitro multiplication of Ceratopteris thalictroides by utilizing adventitious buds as explants on MS medium augmented with various plant growth regulators (Cheema and Sharma 1994). Gametophytic and Sporophytic regeneration from bud scales detached from in vitro grown juvenile shoots of the fern Polypodium bifurcatum has been reported (Dolinsek and Camloh, 1997).
A protocol for rapid propagation of a medicinally and ornamentally useful fern *Adiantum trapeziformae* L through callus culture of its rhizome segments has been standardized employing tissue culture technology. Rhizomes of *Blechnum spicant, Pteris ensiformis* and *Asplenium nidus* initially cultured on MS medium supplemented with only BAP or in combination with NAA and then subcultured in growth regulator free medium produced large number of sporophytes (Fernandez 1996). Bertrand *et al.* (1999) cultured explants from the rhizome, frond, petiole and root tip of juvenile sporophytes of *Polypodium cambricum* on media containing BA or kinetin alone or combined with NAA. Root tip explants exhibited the lowest capacity for organogenesis. Sporophytes are produced from cultured tissues either by axillary shoot proliferation (Murashige, 1974) or as adventitious shoots formed directly or from callus (Amaki and Higuchi, 1991).

Tissue culture which tends to be more sophisticated than spore culture is also advocated and successfully explored for horticulture propagation of selected ferns (Hennen & Sheehan 1978; Padhya & Mehta 1981; Higuchi *et al.* 1987). Successful culture initiation, by it spore culture or tissue culture, depends on a number of physical and chemical factors. A number of workers have studied spore germination under the influence of various physiological and chemical parameters (Mehra & Palta 1971; Sharma & Vangani 1988; Sharma & Sharma 1991).

Ferns are conventionally propagated by both the sexual as well as the vegetative method. The vegetative method of propagation involves the use of rhizomes or other vegetative organs as planting material. This is a reliable method to produce plants that are
genetically identical to the mother plant. New plants are generally produced from pieces of rhizome isolated from the mother plant. Other parts of the plants such as bulbils, e.g. *Asplenium bulbiferum*; proliferating frond tips, e.g. *Adiantum caudatum*; stolons, e.g. *Nephrolepis*; e.g. tree ferns; stipules, e.g. *Angiopteris*; layer ing, e.g. *Lygodium*; tubers, e.g. *Nephrolepis*; root buds, e.g. *Ophioglossum*, are also used as starting material for vegetative propagation.

**The ex situ conservation through in vitro spore culture:**

Pteridophytes are distinct from all the other groups of plants in having alternation of generation with independent autotrophic sporophyte and autotrophic/heterotrophic gametophyte. The haploid spores germinate to form haploid gametophyte which produces the diploid sporophyte after fertilization. Variation of spore germination in different groups of homosporous ferns have been enumerated and described by Nayar and Kaur (1965 & 1971). The effect of temperature over stored spores was studied by Beri and Bir (1995) in *Pteris vittata*. National Botanical Research Institute has raised more than 45 rare and endangered medicinal and ornamental ferns through spore culture (Khare *et al* 1989, Behera 2011). The different germination pattern, gametophyte development and morphology of adult gametophyte of homosporous ferns have been reviewed by Nayar and Kaur (1971).

**General morphology of adult prothalli in ferns:** Fern gametophytes show wide variety of forms, i.e. tuberous, cordate- thalloid, strap-like, ribbon-like, and filamentous shapes. These gametophyte forms have been developed in relation to pteridophyte evolution. The tuberous gametophytes are subterranean with endophytic fungi, and found in *Botrychium*
and *Psilotum*, which are plant groups of early branch of ferns. Furthermore *Osmunda*, the first branch of the leptosporangiate ferns, has been known to have massive gametophytes at early stage of development. The gametophytes of the rest of ferns are mainly cordate, and rarely ribbon-like or irregular in shape. These facts suggest that massive tuberous forms are primitive and cordate forms are derived from tuberous forms, and then ribbon-like or irregular shaped gametophytes are evolved in advanced groups such as Polypodiaceae and Vittariaceae. (Takahashi *et al.*, 2009).


1. **Cordate type:** It is the most common type of prothallus found among Pteridaceae, Adiantaceae, Dryopteridaceae, Dicksoniaceae, Osmundaceae, Marattiaceae and Cytaceae. In most of the cases, the spore germinates to give rise to a green plate of cells with an apical notch. Later the apical cell is replaced by a row of meristematic cells. Under suitable conditions of growth and nourishment this notched plate develops into a heart-shaped or a cordate prothallus. The gametophyte is one cell in thickness except in the region posterior to the notch, where it is many celled thick. The sex organs and rhizoids develop on the ventral side. Under crowded and undernourished conditions the prothallus becomes filamentous. Eg. *Dryopteris* bears only antheridia. It can develop into a more or less cordate type if transferred to suitable environmental conditions. Normally
the prothallus is monoecious. In Cyatheaceae the morphology of adult prothalli is similar as above in shape and structure, but differs in the earlier mode of development. In this case the short filament of cells divides longitudinally to form a plate of green cells. The apical cell later appears in this thin plate of cells. Later it is replaced by a group of apical cells. Further development is similar to the previous cases.

2. Filamentous type: The filamentous type of prothallus is characteristic of *Schizaea* and some members of the family Hymenophyllaceae. Filamentous prothalli have no doubt, been seen to develop in several ferns but they are the result of abnormal environmental conditions. In *Schizaea* and *Trichomanes* filamentous condition persists under all circumstances. The prothalli in both these genera resemble some branched algal filaments or moss protonemata. Some of the branches penetrate the soil and act as rhizoidal branches, whereas others are green and transversely septate. In *Schizaea pusilla* the rhizoids are unicellular and develop from specialized short branches whose cells are infected with a mycorrhizic fungus. The sex organs are seated on unicellular lateral branches. In *Trichomanes*, the antheridia occur on any branch of the filamentous prothallus but archegonia appear in clusters on specialized multicellular structures called the archegoniophores.

3. Strap-shaped prothallus: This is found in the family Grammitidaceae and some Lomariopsidaceae and Polypodiaceae. In *Elaphoglossum stenophyllum* the thalli are long and strap-like with fimbriated and hairy margins. The breadth of the thallus is intermediate between cordate type and ribbon-shaped prothalli. They are much longer than broad and are slow growing and unbranched with cordate apex. The apical meristem
is pluricellular, the midrib is thin and interrupted. Sex organs and rhizoids are borne on the midrib. The rhizoids may occur along the margins too.

4. **Ribbon-shaped prothalli:** It is found in Loxogrammaceae, Vittariaceae and in some members of Hymenophyllaceae and Polypodiaceae. The thalli are thin, one cell in thickness, flat, dorsiventral, perennial and slow growing. It is devoid of a mid-rib and has rounded apex that has no well defined meristem. It is usually profusely branched. The branching is lateral. The sex organs are born on irregularly scattered 2-4 celled thick cushions. In Hymenophyllaceae these cushions are marginal and in others they are superficial. The rhizoids arise in marginal clusters.

5. **Tuberous prothalli:** This type of prothalli is prevalent in the Ophioglossaceae. *Helminthostachys* affords a very good example of such a type of prothallus. In this case the prothallus is saprophytic in nutrition and is mostly underground or subterranean. The underground portion of the prothallus is lobed and is attached to the soil by means of rhizoids. It is also called the vegetative region of the prothallus. From the vegetative region arises a cylindrical branch that grows vertically upwards and bears the sex organs. This cylindrical and generative region may remain underground or it may slightly project above ground. The cylindrical region grows by means of 4-sided apical cell. The endophytic fungus is largely confined to the lower vegetative region but it may extend up to the cylindrical region. The thalli are perennial and very slow growing.

The mature gametophytes of *Ophioglossum* may be cylindrical, conical or irregular in shape. They may be branched or unbranched. They are usually wholly subterranean or in some cases partly above ground and partly underground. In *O.*
pedunculosum the exposed portion of the porthallus assumes green colour and may become flattened and irregularly lobed. The gametophytes of Botrychium are also mycorrizic and may be cylindrical or have a tendency towards flattened and dorsiventral form (B. virgianum). There is no well defined meristem in Helminthostachys and Botrychium. Such prothalli are also found in Actinostachys and Lophidium of Schizaeaceae, and Stromatopteris of Gleicheniaceae.

**Vegetative propagation of the prothallus:** Vegetative propagation of mature prothalli of homosporous ferns is quite common. It is effected by following methods:

1. **Regeneration by branches:** It is very common among the ribbon-like and filamentous-branched prothalli. The older portions of the prothalli degenerate setting free the younger branches, which under certain conditions develop into new prothalli. In some cases branches arising from superficial cells develop into new prothalli. This type is common among Hymenophyllaceae, Schizaeaceae, Vittariaceae and some Polypodiaceae.

2. **By the formation of germ filaments:** In Ceratopteris mollissima the young prothallus produces monoliform germ filaments that easily break into fragments each of which develops into a new prothallus.

3. **By the formation of gemmae:** Thease are specialized, unicellular or multicellular structures borne on sterigmata and are capable of germinating into new prothalli. In some Polypodiaceae (Kaulinia, Colysis, Letochilus and Paraleptochilus) the gemmae are unicellular or dumbbell-shaped and densely chlorophyllous. In Vittariaceae and some Hymenophyllaceae the gemmae are multiseriate and spindle shaped are attached by an
end cell to the sterigmata. In Trichomanes and Polyphlebium venosum the multicellular and filamentous gemmae are borne perpendicular to the sterigmata. Such filamentous gemmae may become 10-12 cells long. Such gemmae produce germ filaments that develop into prothalli. On detachment the gemmae attach themselves to the substratum by producing rhizoids.

4. **By the formation of tubers:** The cordate prothalli of Anogramma bear storage tubers that grow into the soil and serve the dual function of perennation and vegetative propagation.

**Independent fern gametophytes in the wild:** The existence of perennial, independent populations of fern gametophytes is a well known phenomenon in eastern North America. They also occur to some extent in western North America, Japan, India, Hawaii, and Central America. It is likely that this phenomenon occurs in mesic temperate and tropical regions throughout the world. Gemmae play an important role in the perennial existence of independent gametophytes. Gemmae occur in three families: Vittariaceae, Hymenophyllaceae and Grammitidaceae. Thus the phenomenon is of considerable importance in the biology of pteridophytes (Farrar, 1985).

The *in vitro* spore culture is an important and effective technique used to multiply the rare and endangered ferns particularly the economic ferns that are being lost and threatened due to deforestation and increasing population (Amoroso and Amoroso, 1998; Lusby *et al.*, 2002; Soare, 2008). The *in vitro* culture technique has been applied in several ferns mainly as botanical interest rather than conservation point of view (Nester and Coolbaugh (1986); Von Aderkas and Raghvan (1985); Whittier (1981); Camloh
The age and the viability of the spores vary from a few days to few years. In *Osmunda* they remain viable for a few days, whereas in *Onychium* they remain viable for one year. Ferns spores have been successfully germinated in axenic cultures for experimental as well as for horticultural purposes. (Ford and Fay, 1990, 1999).

Fern gametophytes have been described as ideal experimental organisms for use in scientific studies and as model multicellular systems (e.g., Miller, 1968). One of the main attractions of these plants is their minimal nutritional requirements, and most researchers have exploited them in cultures employing artificial media. Agar-solidified medium has been the substrate used most routinely for the artificial culture of fern gametophytes, but disadvantages have been reported. Agar can be toxic to plants (e.g., Debergh, 1983), it can perturb sexual expression of ferns (Rubin & Paolillo, 1983), and can slow fern development (Dyer, 1979). Douglas & Sheffield (1992) showed dry weight yield of two species of fern gametophytes, *Pteridium aquilinum* and *Anemia phyllitidis*, to be significantly higher in liquid than in the same medium solidified with agar. Simabukuro *et al.*, (1998) in *Cyathea delgadii* and camloh (1993) in *Platycerium bifurcatum*. The deterioration of spores during storage by reduction in germination and gametophyte development is also reported by Towill and Lkum (1975) and Bir and Goyal (1983). The use of various techniques of raising ferns from spores has been discussed (Jones, 1987; Goudey, 1985 and kaur *et al.*, 1989). The gametophyte morphologies are known for several Indian species viz., *Bolbitis semicordata* (Moore) Ching, *B. quoyana* (Gaud.) Ching, *B. subcrenata* (Hook. et Grev.) Ching in C. Chr. (Nayar, 1960; Nayar and
Kaur, 1964a, 1964b, 1965a, 1965b, 1965c, 1971); and for Old World species such as *B. angustipinna* (Hayata) Ito, *B. heteroclita* (Presl) Ching and *B. repanda* (Bl.) Schott (Hennip-man 1970, 1977). Bower (1923-28) and Holttum (1947) pointed out that the comparative morphology of fern gametophytes could be of significance in understanding evolutionary relationships. According to Stokey (1951, 1960), Atkinson and Stokey (1964), and Atkinson (1973) comparison of gametophyte structure and their development strengthens our understanding of the relationships among various genera and higher groups.

Prothallial morphology in the family Lomariopsidaceae is known only for *Bolbitis* (Nayar, 1960), *Egenolfia* (Nayar and Kaur, 1965), and *Elaphoglossum* and *Rhipidopteris* (Stokey and Atkinson, 1957). Few details are known about the gametophyte of *Lomagramma sinuata* (Atkinson, 1973).

Endangered ferns of the Western Ghats such as *Diplazium cognatum, Histiopteris incisa, Hypodematum crenatum, Thelypteris confluentes, Athyrium nigripes, Pteris vittata, Metathelypteris flaccida, Pteris gongalensis, Pteris confusa, Cyathea crinita, Cheilanthes viridis, Pronephrium articulatum, and Nephrolepis multiflora* have been multiplied through *in vitro* spore culture as a part of *ex situ* conservation by Manickam and co-workers (Sara 2001; Johnson 2003; Manickam et al. 2003; Vallinayagam 2003; Johnson et al. 2005; Sara & Manickam 2005; Johnson & Manickam 2006; Sara & Manickam 2007; Johnson & Manickam 2007; Johnson et al. 2008).

The spores of *Diphasiastrum sitchense* germinate in the dark on a nutrient medium containing inorganic nutrients and glucose. Dark-grown prothalli develop into
white, carrot-shaped gametophytes with a tapering base, constricted neck, and gametangial cap. The antheridia are large and sunken, and the archegonia have long necks with numerous neck canal cells. The tapering base has a zone of radially elongated cells that is comparable to the inner mycorrhizal zone of *Diphasiastrum* gametophytes from nature (Dean, 2003).

Gametophyte Development, Sex Expression and Antheridiogen System in *Pteris incompleta* were established in order to analyze developmental features of its gametophytes by Carmen Prada *et al.*, (2008)

In the Marattiaceae, gametophytes have been documented for a number of species, including *Angiopteris evecta* (Farmer, 1892; Haupt, 1940), *Danaea simplicifolia* (Brebner, 1896), *Marattia sambucina* (Stokey, 1942), *Marattia douglasii* (Campbell, 1894), and *Macroglossum smithii* (Stokey, 1942). The gametophytes of these species are large, conspicuous, and perennial. They are dark green, relatively thick, and look more like liverworts than the gametophytes of leptosporangiate ferns. The antheridia and archegonia are sunken (Nayar and Kaur, 1971).

**Factors influencing/affecting spore germination:** The factors controlling the gametophyte/sporophyte/gametophyte cycle in the Pteridophyta are re-examined in the light of current knowledge of gametogenesis, sporogenesis, apospory and apogamy. The dependence of spore germination on light in most species of ferns was established by nineteenth century biologists including Borodin, Schmidt, Kny, and Beck (Sussman, 1965). However, there are conflicting reports on the ability of spores of certain species to germinate in the dark. For example, Borodin (1865), Schulz (1902), and Life (1907) were
unable to demonstrate the germination of spores of *Anemia phyllitidis* in the dark, although Schelting (1875) claimed that it occurs. The light requirement for germination in various species has been found to be complex. Ability of spores to undergo dark germination may vary, for example, with age (Laage, 1907), with pretreatment at various temperatures (Schulz, 1902), or with exposure to various plant hormones. Reliable action spectra for light-induced promotion and inhibition of germination in species of *Dryopteris* and *Osmunda* (Bunning and Mohr, 1955; Mohr, 1956; Mohr et al., 1964) show maxima at the absorption maxima of phytochrome.

Although there have been reports of *Asplenium phyllitidis* spores which are able to germinate in the dark at 30°C (Schelting, 1875), such dark germination was absent in Life's (1907) studies and was very rare in our experiments. Spores were inoculated into MES-Moore's medium at pH 4.5 and left in the dark at 10°, 20°, 30° and 37° C. Final counts made three weeks after inoculation showed less than 0.5 % germination at any of these temperatures. Under parallel conditions using the same spore inoculum, GA3-treated spores gave extensive germination at 20° and 30° C, but no germination at 10° and 37° C.

According to Lloyd and Klekowski (1970), chlorophyll bearing spores occur in only a few taxa in the Pteridophyta. These spores have a germination time of less than 3 d and have viability lengths of 1 yr or less. Non-chlorophyll bearing spores occur in the majority of ferns, requiring longer periods for germination, the average being 10 d, and have longer viabilities of up to 3 yr. Species with green spores occur consistently in wet
mesophytic habitats, and most of the small island and biodiversity hotspots come under this category.

The pH optima for germination have been reported for a limited number of ferns and fern allies (Sussman, 1965). In general they lie on the acidic side of neutrality, from pH 5.0 to 7.0. Mohr (1956) reported failure of germination at either pH 3.0 or 10.0, and maximum germination between 5.0 and 5.5 for Dryopteris filix-mas. We have shown a striking pH dependence of gibberellic acid stimulated dark germination in Anemia phyllitidis (Weinberg and Voeller, 1969). Maximum dark germination in this species occurs at pH values of 4.5 and lower.

Numerous methods of surface sterilization and germination of spores have been described. The commonly employed surface sterilization and germination of spores have been described. The commonly employed surface sterilizing agents are Sodium hypochloride and Mercuric chloride reported for ferns (Basile, 1973; Dyer, 1979; Warne et al., 1986; Raine and Sheffield, 1997; Shimabukuro et al., 1998 & Camloh, 1993). Spores of Schizaea dichotoma were subjected to sterilization using Sodium hypochloride (NaOCl₂), Streptomycin, or a combination of both Streptomycin and Sodium hypochloride and their effect on the spore germination was investigated (Cox et al., 2003).

The effect of season on the in vitro germination of spores and development of gametophytes has been studied in various ferns such as Drynaria quercifolia (Hedge et al., 2006), Athyrium nigripes, Thelypteris confluens, Cyathea crinita and Nephrolepis multiflora (Sara, 2001), Diplazium cognatum, Histiopteris incisa, Hypodematum
crenatum and Pteris vittata (Vallinayagam, 2003). These studies affirmed that the seasonal changes affect the spore germination and the development.

Reduced nitrogen source is necessary for spore germination and early growth of gametophytes in Arthromeris tenucauda and Botrychium dissectum (Melan and Whittier, 1990).

The commonly used sugar has been sucrose (Dyer, 1979). In comparative studies with several sugars, sucrose and glucose have given the best growth (Hurel-Py, 1955; Whittier, 1964). Other sugars which have supported the growth of some gametophytes are fructose, maltose, ribose, and xylose (Hurel-Py, 1955; Courbet, 1957; Whittier, 1964). The result of Camloh (1993) suggest that sucrose has no promotive effect on either spore germination or early gametophyte growth and this is probably due to spores having enough endogenous carbohydrates for germination and early gametophyte development, but old gametophytes at presexual stage attain optimal growth only in the presence of sucrose. But Kata (1964) reported that the sugar had great influence on development and growth of gametophytes in Pteris vittata. Biological and other nutritional aspects were also studied in Asplenium nidus, Dryopteris affinis sp. affinis, Osmunda regalis, Pteris ensiformis and Woodwardia virginica (Fernandez et al., 1999). On the other hand, Kato (1967) reported that the sugar had great influence on development and growth of gametophytes in Pteris vitatta. He observed that growth and development increased with increased sugar concentration until an optimum is reached and then decreased at very low concentration. Also by manipulating the sucrose concentration in the culture medium.
production of haploid, diploid and tetraploid sporophytes as well as gametophytes were demonstrated in some ferns (Johnson, 2003).

Sucrose is the most widely used carbon source in *in vitro* culture (Dyer, 1979) and when added to the nutrient media promotes gametophyte growth of leptosporangiate ferns. The optimum sucrose concentration is afforded by its promoter effect as a nutrient and its inhibitory effect as an osmotic agent. In general terms, gametophyte dry weight increases when the sucrose concentration of the culture medium increases. However, differences among species are observed and the gametophyte of *Osmunda regalis* is able to grow independently of the presence of sucrose in the culture medium, indicating autotrophy of this organism when cultured *in vitro* (Fernandez et al., 1997).

Light and Cytokinins were required to developmental process in plants (Chory et al., 1994). There are various reports available on the requirements of light for spore germination, gametophyte development and sporophytes formation (Haupt, 1985; Nester and Coolbaugh, 1986). However Cox et al., (2003) reported that the spore germination was not affected by the presence or absence of light. Growth in darkness was apparently sustained by mobilization of reserves present in the spore (Racusen 2002).

The importance and effect of various auxins IAA, NAA and Cytokinins and BAP on spore germinaton and gametophytes development have been studied in *Adiantum lunulatum, Adiantum capillus veneris* and *Actinopteris radiata, Cheilanthes farinosa, Equisetum arvense, Blechnum spicant* (Kuriyama and Maeda, 1999).
Antheridiogenesis in the fern gametophytes could be induced by the addition of gibberelline (or) Antheridiogen was also studied in *Blechnum spicant* (Fernandez *et al.*, 1997), *Lygodium flexuosum* (Trivedi and Usha 1977), *Anemia phyllitidis* (Grill, 1988), *Cheilanthes farinosa* (Sharma and Vangani 1988) and *Phanerophelebia* spp. (Yatskievych, 1993) was also studied.

Effect of plant growth hormones/regulators on spore germination, gametophyte and sporophyte and regenerative capacity of gametophyte were studied in *Pteris longifolia* (Albaum, 1938), *Marsilea drummondii* (Allsop and Alicja, 1960), *Ceratopteris thalictroides* (Hickok and Kiriluk, 1984), *Adiantum capillus veneris* (Gupta and Bhambie, 1991) and *Nephrlepis multiflora* (Sara *et al.*, 1998) and reported that the Kin and BAP are effective in the sporophytic regeration from the gametophytes.

Tree ferns support the regeneration of a number of flowering plants in reunion tropical forests. According to *Riviere et al. (2008)*, in order to preserve biodiversity, it is recommended to include tree ferns in future tropical forest restoration programmes. A shortage of rainfall during summer decreases fertility in the following yr in the case of *Botrychium multifidum*, grazing reduces the number of spores for dispersal later on, and this could lead to low recruitment and extinction (Mesipuu *et al.*, 2009). *In vitro* propagation of ferns has contributed to the development of germplasm for conservation of many ferns (Goller and Rybczynski 2007). The protocols published by Pence (2008) on cryopreservation of ferns describe various methods available to store valuable genetic diversity in fern species. Fern spores can be successfully stored at subzero temperatures as effectively as seeds (Ballesteros and Walters 2007). However, at different storage
temperatures above 0°C, it has been noticed that viability has decreased and, in some cases, development of sporophytes as well (Cha-Cha et al. 2005).

Douglas and Sheffield (1992) have reported extensive studies on favourable conditions for in vitro spore germination and gametophyte development. The germination time for fern spores has been found to vary from a few days to a year. *Nephrolepis* spores, as reported by Smith and Yee (1975), germinate in 3–4 days in culture, while in members of *Ophioglossaceae* germination, this is known to occur at the end of 6 months of incubation in the dark (Whittier, 1981).

**Induced apospory, apogamy and polyembryony under in vitro condition:** Fertilization is a prominent feature in the sexual lifecycle of ferns. However, a number of fern species exhibit another way to produce sporophytes: they are born out of gametophyte cells without fertilization, in a process known as apogamy. Many characteristics have been used to infer the occurrence of apogamy in ferns, e.g., ploidy levels, spore number per sporangium, spore size, the development of gametangia, and the formation and morphology of young sporophytes. Apogamous reproduction is strongly associated with ploidy level. More than 75% of apogamous ferns are polyploid (Walker, 1962; Kanamori, 1972; Park and Kato, 2003). Heilbronn (1932) found that opportunities for apogamy increase with the addition of chromosomes. Furthermore, Raghavan (1989) suggested that an important provision for apogamy is an increase in gene dosage. That implies that polyploidy might induce apogamous occurrence. More, it may increase the probability that occasional apogamous sporophytes will be selected because they are the
only viable sporophytes that can be produced when unbalanced chromosome combination prevents typical sexual reproduction.

Apogamy and apospory are interesting deviations from the normal sexual life-cycle of the ferns. In apogamy a morphological sporophyte arises directly from growth of gametophytic cells without a prior fusion of gametes or formation of an embryo. Sometimes only isolated sporophytic structures such as leaves are differentiated, and a few instances are known in which sporangia are borne directly on the gametophyte (Pace 1910, Lang 1929). Apospory results in the growth of a gametophyte from part of the sporophyte without reduction division and spore formation. The phenomenon of apogamy provides an excellent opportunity to study the direct origin of a vascular plant from a non-vascular fern gametophyte. In a large number of ferns, apogamy is known to be a constant phenomenon, but apospory of this nature has been found in only a single fern, *Scolopendrium vulgare*. The discovery of its constant occurrence was made as a result of the studies of the inheritance of apospory in certain ferns by Andersson-Kotto (1931, 1932) and Andersson-Kotto and Gairdner (1936). Both apogamy and apospory are sometimes described as abnormalities in the life cycle of a plant. It is commonly assumed that if one of these phenomena occurs in a life cycle, it must be followed by the other one. However, it is known that only apogamy occurs in some ferns, and that apogamy is the only way in which the sporophyte is reproduced in those cases. In some apogamous ferns apospory may occur, but it may also be found in non-apogamous ones. It is suggested by the Whittier that both apospory and apogamy may be considered as normal phenomena in the life cycle, and are not necessarily related. For a brief discussion of the independence of these phases in a life cycle, the reader is referred to an earlier paper by
the writer (Steil, 1944). Studies of apogamy and apospory in the Pteridophytes during the
decade just past (Steil, 1944a, 1944b, 1949) have not been numerous. Hence this group of
plants, so favorable for cytological study of these phenomena and others of a
physiological nature, has interested comparatively few botanists. During the past ten
years, however, a number of papers have appeared on various phases of apogamy and
apospory as follows:

Approximately 10% of ferns and an unknown proportion of other pteridophyta
have life cycles of this kind (Sheffield and Bell, 1987). A good example of this is the
apogamous fern *Dryopteris affinis* subsp. *affinis* (Fernandez *et al*., 1996a). It must be
emphasized that this process was strictly apogamic, since sexual reproduction was not
possible due to the absence of archegonia. Apogamy can be induced by changes in *in
vitro* culture conditions (Whittier and Steeves, 1960; Fernandez *et al*., 1996a). Apogamy
may be considered the organogenesis of sporophyte from gametophytic tissue, and as is
well known, promotion or inhibition of organogenesis in plant tissues is affected by
growth substances (Skoog and Miller, 1957).

Apogamy was discovered by the wighttier in three ferns: *Tectaria trifoliata* (Steil
1944a), a *Dryopteris* species (Steil 1944b). In *T. trifoliata* the gametophytes are usually
variegated and are similar to those described in several ferns by Andersson (1923) and
Andersson-Kotto (1930). The gametophytes produce antheridia in abundance, but no
archegonia. Embryos are formed apogamously, usually on a projection produced from
the sinus of the prothallium. In an unidentified species of *Dryopteris* the writer (Steil
,1944b) found that the embryos are formed apogamously, although the gameto-phytes
produced in abundance both sex-organs. The gametophytes are more distinctly variegated than those of *T. trifoliata*. *Pellaea ovata* was found to be constantly apogamous. The gametophytes of this fern are somewhat smaller than those of *T. trifoliata* and the *Dryopteris* species. In some of the numerous cultures of prothallia of the fern made by the writer over a period of 14 years, antheridia were always produced in abundance, but archegonia have never been observed on any of the gametophytes. No detailed study of the cytology of apogamy of all these ferns was made by the writer. It has been found, however, that there is no change in the chromosome number in the life cycle of *Pellaea ovata*. Apogamy was also discovered in *Angiopteris australis* by Stokey (1948). The discovery of apospory in *Tectaria trifoliata* by Steil (1944a). A study of the aposporous growths by the writer (Steil 1949) in *Pteridium aquilinum*, in which Farlow (1889) described apospory. Induced apogamy in *Doodia caudata* by Duncan (1941). Apogamy of the obligate type was also reported by Stokey (1942, 1948) in *Marattia sambucina* and in *Trichomanes auricu-latum*. The origin and development of the apogamous embryo by Duncan (1941, 1943). The cytology of apogamy by Drpp (1939) and others. Neither apogamy nor apospory has recently been discovered in any of the fern allies, and parthenogenesis has not been reported in any pteridophyte since 1939.

The emergence of multiple seedlings from a single seed was first observed by Leeuwenhoek as early as 1719 (Maheswari, 1950; Tissera *et al.*, 1979). This condition, referred to as polyembryony is widely Prevalent among angiosperms (*Tisserat et al.*, 1979).
Polyembryony has been documented in sexual ferns and is usually attributed to multiple fertilizations (Mottier, 1925; Klekowski, 1970, 1972). The occurrence of polyembryos may be not caused by multi-gametophyte growth. Even in cultures of density experiments, polyembryos occurred in the lowest density condition (Cousens, 1979). Therefore, polyembryos may arise from intergametophytic or intragametophytic mating in sexual ferns. It is thought that polyembryony would increase the probability of intergametophytic mating in sexual ferns through the neighboring sporophytes and subsequently adjacent gametophytes (Klekowski, 1970, 1972; Lloyd, 1974). Our discovery of polyembryony in *P. cadieri* and *P. grevilleana* is the first report of this phenomenon in apogamous ferns. No mating occurs in these apogamous ferns, and the meaning of their polyembryony is not clear yet.

As reviewed above, numerous ferns and fern allies have been raised through *in vitro* spore or tissue culture. Practically, spore culture is more successful technique when compared to tissue culture technique in ferns. But spore culture is impossible in sterile hybrids and it is very difficult in chlorophyllous spores as in the cases of hymenophyllaceous and grammitidaceous ferns. Moreover, usually the spores of common ferns germinate easily under *in vitro* condition, but the spores of rare and endangered ferns will not germinate easily. Thus, in conclusion, selection and application of particular technique is important for particular ferns depend upon the requirement. For example multiplication of common ornamental or medicinal fern can be done easily by spore culture. But multiplication of rare and endangered ferns with chlorophyllous spores needs effective and risky tissue culture technology.