

## **Chapter 2**



### ***Review of literature***

## **2. Review of Literature**

Nature, ever since its existence has been providing humankind with all the necessary components to sustain life in this mother Earth. It has also been a promising resource of innumerable medicinal plants from where remarkable number of modern drugs have been isolated and successfully employed for treating various diseases. The medicinal values have been known to be imparted by the chemical compounds that produce health promoting action on the human body. Though, Pteridophytes have been used in traditional medicine since ancient times but it has been highly ignored in modern days. Many reports, however, have been produced time to time revealing the efficiency of Pteridophytes as antiviral, antirheumatic, anticancer, antidiabetic, anti-inflammation and antimicrobial agents. In this regard, a brief review has been compiled and presented on the following aspects related to the present work i.e. phytochemical screening of plants, their role as an antioxidative, antimicrobial and antidiabetic agents, followed by a brief discussion on phytochemicals of ferns and their biological efficacies.

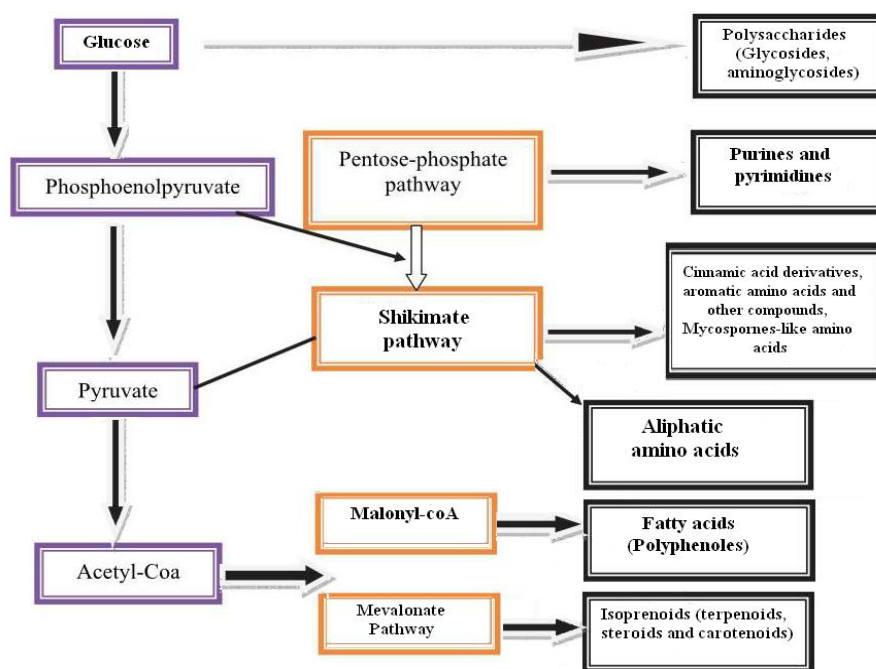
### **2.1. Phytochemical screening of plants**

Phytochemicals (from the Greek word ‘phyto’ meaning plant) are the bioactive, naturally/ universally occurring chemical compounds found in plants which are known to generate specific physiological action on the human body (Hasler & Blumberg, 1999).

They are involved in protecting plants against disease and damage and in contributing the color, aroma and flavor of the plants. Phytochemicals have also been known to play an important role to the health of individuals and communities. Generally, plant chemicals responsible for protecting plant cells from various biotic and abiotic stresses are known as phytochemicals (Gibson *et al.*, 1998; Mathai, 2000). There still remains ambiguity in the classification of phytochemicals as numerous of them are being known or identified. However, they have been classified as primary metabolites which include amino acids, proteins, sugars, purines and pyrimidines of nucleic acids, chlorophyll etc and secondary metabolites such as phenolics, alkaloids, terpenes, flavonoids, lignans, plant steroids, saponins, glucosides etc (Hahn, 1998). A simplified view of secondary metabolites and their interrelationships with primary metabolism is depicted below:

Among the various secondary metabolites, phenolics are the most abundant and structurally diverse plant phytoconstituents.

The importance of medicinal plants lies in the presence of various bioactive compounds and their contribution to the health of individuals and communities are well established which has intrigued researchers all over the world to explore the plants for their biological activities. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952).



Main pathways of some secondary and primary metabolites biosynthesis (modified) Burja *et al.* 2001.

Due to the increasing demand of natural drugs there has been a rapid rise in the search of phytochemical from various plant sources. Thus, Edeoga *et al.* (2005) performed the study to define, compare and quantify the percentage of crude phytochemical present in ten medicinal plants i.e, *Cleome rutidosperma*, *Emilia coccinea*, *Euphrobia heterophylla*, *Physcalis bransilensis*, *Scorparia dulcis*, *Richardia bransilensis*, *Sida acuta*, *Spigelia anthelmia*, *Stachytarpheta cayennensis* and *Tridxa procumbens*, which were used extensively in herbal medicine in South Eastern Nigeria. They reported the presence of alkaloid and flavonoid in all the samples while tannin was absent only in *S. acuta*. Cardiac glycoside was found to be present in all the samples except for *T. procumbens*. Saponin was not detected in *E. heterophylla* and *S. acuta* while it was present in the other samples studied. Steriod was present only in *E. coccinea*, *P. bransilensis*, *R. bransilensis* *S. anthelmia*. Five out of the ten test plants namely *E. heterophylla*, *P. bransilensis*, *S. dulcis*, *S. anthelmia* and *S. cayennensis* revealed the presence of

phlobatannin. Terpenoid was absent in *C. rutidosperma*, *S. dulcis*, *S. anthelmia*, *S. cayennensis* and *T. procumbens* while it was present in the other plants studied. They also quantified the chemical constituents in these medicinal plants and reported the highest percentage crude yield of alkaloids (1.04%) and flavonoid (0.98%) in *S. acuta*. *C. rutidosperma* revealed the lowest yield of alkaloid (0.32%) but the highest yield of tannin (15.25%). Saponin (3.92%) was found to be highest in *P. bransilensis* while phenols in the plants were recorded to be minimal (0.20-0.04%). Presence of chemical constituents in these plants makes them a potential source of useful drugs.

Preliminary phytochemical screening of four common plants of family Caesalpiniaceae was carried out by Awoyin *et al.* (2007). Eight principal bioactive compounds were investigated in the dry leaf of both water and ethanolic extracts of *Cnidocolus aconitifolius*. In all, three active components were positive for both the extracts. These include phenols, saponins and cardiac glycosides. The result revealed that phlobatannin was detected in the water extract while alkaloids in the ethanolic extract. Flavonoids, anthraquinones and combined anthraquinones were absent in both the extracts.

Similarly, phytochemical screening conducted by Musa *et al.* (2008) on the ethylacetate partition portion of ethanolic extract of leaves of *Pseudocedrella kotschyii* revealed the presence of flavonoids, glycosides and tannins as major chemical constituents. Alkaloids saponins, cardiac glycosides and steroids were not detected in the extract. The ethylacetate extract (50 and 100 mg/kg i.p) exhibited significant activity ( $p < 0.05$ ) against acetic acid-induced writhing in a dose dependent manner. In the anti-inflammatory activity the ethylacetate extract (50 and 100 mg/kg i.p.) caused a slight effect against the raw egg albumin-induced oedema. The effect was however observed not to be dose dependent. Chitravadivu *et al.* (2009) had also performed qualitative studies of phytochemical on the leaves and roots of four medicinally important plants such as *Acalypha indica*, *Cassia auriculata*, *Eclipta alba* and *Phyllanthus niruri*. They reported the presence of alkaloids, catechols, flavonoids, phenolic compounds, saponins and steroids and the absence of anthroquinone, tannins and triterpenoids from the root and leaf sample of *Acalypha indica*. Anthroquinone, alkaloids, flavonoids, phenolic compounds, saponins, steroids and tannins were found to be present in the root and leaf sample of *Cassia auriculata*. Phenolic compounds, saponins, steroids, tannins and triterpenoids were present in the leaves and roots of *Eclipta alba*. Phenolic compounds, anthroquinone, flavonoids, saponins, steroids, tannins and triterpenoids were also found to be present in root and leaf sample of *Phyllanthus niruri*.

Phytochemical studies performed by Viji and Murugesan (2010) indicated that the leaf and stem contain a broad spectrum of secondary metabolites. Phenol, tannins and saponins were

predominantly found in all the five tested solvent extracts of leaf and stem followed by steroids, sugars, flavonoids and terpenoids (Benzene and acetone). Acetone and chloroform extracts of leaf had higher inhibitory action against *Salmonella typhi* and *Streptococcus subtilis* respectively. Acetone extracts of stem showed maximum inhibitory action against *S. typhi* and benzene extracts of stem had moderate inhibitory action against *Escherichia coli*. Likewise, various phytochemical constituents including alkaloids, phenols, and ascorbic acids were assessed and compared in the four medicinal plants of different families. In another study, four plants used locally for different medicinal purposes were selected for a comparison of their phytochemical constituents. The plants investigated were *Clerodendrum viscosum*, *Moringa oleifera*, *Cinnamomum tamala* and *Scoparia dulcis*. All these plants were found to contain the active principles including alkaloids, tannin, saponin, terpenoid, flavonoid, cardiac glycoside. Steroid was present in *C. viscosum*, *C. tamala*, and *M. oleifera* but absent in *S. dulcis*. The assay for the quantitative determination of phenols and ascorbic acids revealed that *C. tamala* contained the highest amount of phenol and ascorbic acids whereas *C. viscosum* contained the least amount (Das and Chakraborty, 2011).

Gopinath *et al.* (2012) performed a preliminary screening of the phytochemicals of leaf extracts of *Gymnema sylvestre*, *Phyllanthus amarus*, *Phyllanthus reticulatus* of Siddarabetta, Tumkur district, Karnataka. These plants are used ethnomedicinally as antidiabetic agents in India. The study was carried out using three different extracts namely ethanol, methanol and water. They reported the presence of terpenoids, flavonoids, phenol, quinines and catechin in the water extract of *Phyllanthus amarus*, steroids, tannins, phenol, quinones in ethanolic extract and only tannins, phenol and quinones in methanol extract. Terpenoids, alkaloids, flavonoids, saponins, tannins and quinones were detected in the water extracts of *Gymnema sylvestre* whereas terpenoids alkaloids, saponins and quinones in the methanolic extracts. While, in the ethanolic extracts only alkaloids, tannins and quinones were detected. The water extracts of *Phyllanthus reticulatus* exhibit flavonoids, phenol and quinones. Ethanol extracts showed steroids, tannins, phenol and quinones and methanol extracts showed steroids, tannins, phenol and quinones. The bioactive compounds from different solvent extracts were suspected to exhibit anti-diabetic properties.

Yadav *et al.* (2014) performed a study on six medicinal plants viz. *Ficus religiosa*, *Citrus limonia*, *Phoenix dactylifera*, *S. indicum*, *Swertia chirata* and *Raphanus sativus* of different families to identify and relate their presence with bioactivities of the plants. They reported the presence of tannins and flavonoids in all the samples studied. Moreover, terpenoids were found to be present in all the selected plants except *P. dactylifera*. On the other hand, saponins and steroids were

absent in all the plants except *S. chirata* and phlobatannins were absent in all the plants except *R. sativus*. In addition, carbohydrates, glycosides and coumarins were present in all the selected plants except *P. dactylifera* and *R. sativus*. Alkaloids were present in all the selected plants except *F. religiosa*, *P. dactylifera* and *R. sativus*. Proteins were present only in *F. religiosa* and *S. chirata* whereas emodins, anthraquinones, anthocyanins and leucoanthocyanins were found to be absent in all the selected six plants. From their studies, they concluded *S. chirata* to be of highest therapeutic efficacy possessing majority of phytochemical classes of compounds and *P. dactylifera* is of lowest therapeutic potential due to the absence of majority of phytoconstituents. Likewise, pharmacognostic, total phenolic and antioxidant studies of *Adenantha pavonina* L. (family Mimosaceae) was carried out by Partha and Chowdhury (2015). The leaves and bark of this plant were used by the tribal people for curing various ailments and diseases. Various parameters like micromorphology, anatomy, phytochemical screening and physical constant were considered for pharmacognostic evaluation of different parts of this plant. The methanolic extracts of leaf and bark were found to show positive tests for carbohydrates, proteins, alkaloids, glycosides, saponins, flavonoids, steroids, tannins, etc. According to the authors this study may provide some diagnostic features by which the crude drug of this plant can easily be identified. However, further scientific studies were suggested for proper validation of their preliminary findings.

## **2.2. Antioxidant activity of plants**

Cellular damage or oxidative injury arising from free radicals or reactive oxygen species (ROS) appears to be the fundamental mechanism underlying a number of human neurodegenerative disorders, diabetes, inflammation, viral infections, autoimmune pathologies and digestive system disorders. Free radicals are generated through normal metabolism of drugs, environmental chemicals and other xenobiotics as well as endogenous chemicals, especially stress hormones (adrenalin and noradrenalin). Accumulated evidence suggests that ROS can be scavenged through chemoprevention utilizing natural /synthetic antioxidant agents. Antioxidants are substances that neutralize free radicals or their actions and have been used as chief protective agents for human health (Sies, 1996). Synthetic antioxidants are associated with various side effects thus the search for natural antioxidants especially from botanicals has been intensified in recent years Ahmad *et al.* (2008). In this regard, a study was performed by Shyur *et al.* (2005) on twenty-six medicinal herbal extracts, popularly used as folk medicine in Taiwan to analyse their anti-oxidative activity. Among, the plants studied, they reported that *Ludwigia octovalvis*, *Vitis*

*thunbergii*, *Rubus parvifolius*, *Lindernia anagallis* and *Zanthoxylum nitidum* exhibited strong DPPH activity while *L. octavalvis*, *V. thunbergii*, *Prunella vulgaris*, *Saurauia oldhamii* and *Rubus parvifolius* showed better superoxide scavenging activity. Significant protection was shown by *Ludwigia octavalvis* and *Bombax malabaricum* on ΨX174 super coiled DNA against strand cleavage induced by UV irradiated H<sub>2</sub>O<sub>2</sub>. Shan *et al.* (2005) investigated the total equivalent antioxidant capacity (TEAC) and phenolic content of 26 common spice extracts from 12 botanical families. The studies demonstrated that many spices containing high levels of phenolics revealed high antioxidant capacity. Different types of phenolic constituents identified in the spice extracts were phenolic acids, phenolic diterpenes, flavonoids, and volatile oils (e.g., aromatic compounds) and rosmarinic acid which were present in varying amount. A highly positive linear relationship were obtained between TEAC values and total phenolic content showing that phenolic compounds in the tested spices may have contributed significantly to their antioxidant capacity.

Aqil *et al.* (2006) studied the antioxidant and free radical scavenging properties of methanolic crude extracts of 12 traditionally used Indian medicinal plants. The study reported that the extracts revealed promising antioxidant and free radical scavenging activity in varying ranges. Phytochemical analysis of the crude extracts revealed the presence of phenolics, alkaloids, glycosides and tannins which may be responsible for the observed antioxidant activity. Similarly, Katalinic *et al.* (2006) analyzed the total phenolic content and related total antioxidant capacity of 70 medicinal plant infusions. Standard assays like FRAP, DPPH radical scavenging activity and ABTS activity were employed for evaluating the *in vitro* antioxidant assay. The study demonstrated that *Melissae folium* contained the highest concentration of phenolics and showed the highest antioxidant activity, concluding that the antioxidant activity must be related to the presence of phenolics in the plant sample.

Khalaf *et al.* (2008) evaluated the antioxidant activity of the methanolic crude extracts of some commonly used medicinal plants. All the methanolic extracts exhibited significant antioxidant activity. Phytochemical analysis detected the presence of alkaloids, glucosides, tannins and flavonoid. Maleki *et al.* (2008) evaluated the *invitro* antioxidant activity, total polyphenolic, flavonoid and flavonol content of petroleum ether, chloroform and methanolic extract of leaves of *Limonia acidissima* Linn. The study demonstrated that the methanolic extract contained good amount of total polyphenolics, total flavonoids and total flavonols which may be responsible its higher antioxidative activity. The methanolic extracts of species *Gmelina arborea* Roxb. (Verbanaceae) were tested for antioxidant potential by Patil *et al.* (2009). It was observed that

the extracts showed potent antioxidant activity which seemed to be correlated with the phenolic content.

Sini *et al.* (2011) studied the antioxidant activity and radical scavenging activity of methanolic extracts of selected plant materials, traditionally used by the tribes of Attapady regions as folk remedies. *Cassia occidentalis*, *Clitoria ternatea*, *Trianthema decandra*, *Capparis zeylanica*, *Anisomeles malabarica* and *Plumbago zeylanica* exhibited strong antioxidant activity as compared to other plants tested with *Trianthema decandra* showing the highest antioxidant activity. Results revealed that these plants are of therapeutic potential due to their high free radical scavenging activity. In another study, the anti-oxidant effects of leaves of *Tinospora cordifolia* were determined by Premanath and Lakshmidevi (2010). Dried and powdered leaves of *T. cordifolia* were extracted with hexane, chloroform, methanol, ethanol and water which was used to determine the total phenolic and flavonoid content along with the antioxidative capacity using different *in vitro* models such as total reducing power, total antioxidant activity, lipid peroxidation inhibitory activity, DPPH radical scavenging activity and superoxide radical scavenging activity. The results have shown that among all the solvents ethanol extract showed highest phenolic and flavonoid content and even the antioxidant activities. Direct correlation between total polyphenols extracted and its antioxidant activity was observed concluding that the active antioxidants may be better extracted in ethanol. Studies conducted by Satheesh *et al.* (2010) revealed the antioxidant activities of *Mucuna pruriens* Linn. Different extracts viz. petroleum ether, ethyl acetate and methanol extract of whole plant of *M. pruriens* were subjected to examine the *in vitro* antioxidant activity by DPPH ( $\alpha, \alpha$ -diphenyl- $\beta$ -picrylhydrazyl) radical scavenging activity, superoxide anion scavenging activity and iron chelating activity. Ethyl acetate and methanol extract exhibited high antioxidant and free radical scavenging activities which may be assumed because of the phenolic compounds as these two extracts were found to contain higher phenolics than the petroleum extract.

Similar investigation was undertaken to appraise the antioxidant properties and the total phenolic contents of 13 important medicinal plants by Narayanaswamy and Balakrishnan (2011). Solvents namely water and ethanol at 5 % concentration levels were used to prepare the herbal extracts of the plants. Radical attenuating abilities of 13 plant extracts were ascertained by 2, 2-diphenyl 1-picryl hydrazyl (DPPH) radical scavenging assay. All the plants tested showed varying degree of DPPH scavenging activity in both the extracts. *Ocimum basillicum* leaf, *Alpina calcarata* leaf, *Jatropha multifida* flower, *Hyptis suaveolens* leaf, *Solanum indicum* leaf and *Clitoria ternate* leaf and flower were observed to possess higher DPPH radical scavenging activity in both the solvent



systems. The total phenolic contents of herbs were found to be maximum in *Ocimum basillicum* and *Clitorria ternate*. The results have shown that the radical scavenging activity of medicinal plants may be due to the hydrogen donating ability of phenolics.

The species *Withania somnifera* L. which is known to possess aphrodisiac property and can cure various diseases is one of the commercially available and most preferred medicinal plants in the Himalayan region. Sharma *et al.* (2013) evaluated the antioxidant properties of *W. somnifera* collected from two different habitats that is, forest and roadside at Kullu, north-west Himalaya and reported that the plants showed antioxidant activities which varied significantly between the habitats.

Keser *et al.* (2012) reported the H<sub>2</sub>O<sub>2</sub> radical scavenging and total antioxidant activity of *Crataegus monogyna* (hawthorn) water and ethanol extracts of leaves, flowers and fruits. Hawthorn leaves, flowers, and berries have been used as medicine traditionally to treat chronic heart failure, high blood pressure, arrhythmia, and various digestive ailments, as well as geriatric and antiarteriosclerosis remedies. The study showed that both the extracts showed appreciable antioxidant activities.

The phytochemical constitution and antioxidant activities of methanolic extract of dried leaves of four medicinally important herbs *Ocimum sanctum*, *Mentha spicata*, *Trigonella foenum-graecum*, *Spinacia oleracea* utilized in the routine diet along with one medicinal important tree *Gmelina arborea* were evaluated by Soni and Sosa (2013). The result of phytochemical analysis revealed the presence of tannins, phlobatannin, saponins, flavonoids, steroids and alkaloids. Among the tested plants, leaf extract of *Mentha spicata* exhibited highest antioxidant and reducing activities which may be attributed to its phenolics content as positive correlation was observed between the antioxidant activity and total phenolic content. Yao *et al.* (2013) studied the antioxidant activity,  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory effects of flavonoids from *Cichorium glandulosum* seeds, and analysed its chemical composition by HPLC-ESI/MS. Traditionally, *C. glandulosum* has been used to treat degenerative or chronic diseases in China. They reported significant *in vitro* antioxidative activity against DPPH, ABTS, hydroxyl radicals, and superoxide anion and  $\alpha$ -amylase and  $\alpha$ -glucosidase activity. *In vivo* treatment of rats with TFs (100, 200, 400 mg/kg) showed a significant decrease in the malondialdehyde level while the superoxide dismutase and glutathione levels were restored to almost normal levels, and the catalase and glutathione peroxidase levels significantly increased compared to the CCl<sub>4</sub>-intoxicated group of rats. The study suggested that *C. glandulosum* seeds may be given special

attention because of their antioxidant and anti-glucosidase, anti-amylase activity and supports the use of *C. glandulosum* seeds as the functional food or a traditional folk medicine.

Though huge numbers of plant have been screened for the antioxidant component, there still remain some lacunae as information regarding many medicinal plants is still obscure. Thus, Coulibaly *et al.* (2014) investigated and reported the antioxidant activity and their phenolic compounds from some of the tropical plants belonging to families Euphorbiaceae, Rubiaceae, Anacardiaceae, Scrophulariaceae and Poaceae. The study on different crude extracts of seven plant species revealed the presence of significant amounts of phenolics with highest amounts in *Feretia apodanthera* and *Ozoroa insignis* along with highest antioxidant activities. In a similar study conducted by Saravanan and Parimelazhagan (2014), they reported the *in vitro* antioxidant, antimicrobial and anti-diabetic properties of polyphenols of *Passiflora ligularis* Juss. fruit pulp. Different solvents like petroleum ether, chloroform, acetone and methanol were used to evaluate its biological efficacies. They reported the highest content of phenolics, tannin and flavonoid in the acetone extract compared to other extracts. The *in vitro* antioxidative activity like metal chelating activity, ferric reducing antioxidant power (FRAP) and free radical scavenging activity like DPPH•, ABTS•+, O<sub>2</sub><sup>-</sup>, 'OH and NO' scavenging activity was exhibited highest by the acetone extract in comparison to the other extracts which was assumed to be because of the higher phenolic content in acetone extract. Likewise, antimicrobial,  $\alpha$ -amylase and  $\alpha$ -glucosidase activity was inhibited highest by the acetone extract in a dose dependent manner among the extracts studied. Analysis of the polyphenolic compounds by HPLC revealed the presence of gallic acid, caffeic acid, rutin, ellagic acid and kaempferol at varying quantity. Overall, it was observed that the extracts containing a higher concentration of phenolic compounds, showed more potent antioxidant, antimicrobial,  $\alpha$ -amylase and  $\alpha$ -glucosidase activity, concluding that the biological activity of *Passiflora ligularis* Juss. fruit pulp may be related to the presence of these substances.

Likewise, studies performed by Medini *et al.* (2014) on the various extracts of *Limonium delicatulum* revealed that the plant was able to exhibit potent antioxidant and antimicrobial activities. Among the different solvents used ethanol extracts had the highest total antioxidant activity, while the acetone extracts had the greatest radical scavenging capacity and the methanol extract the best inhibition of  $\beta$ -carotene bleaching activity. Coumaric acid and chlorogenic acid were identified as the dominant phenol compounds in the extracts which may be assumed to contribute towards the high antioxidant activities of the plant. The search for natural antioxidant from all the possible biological sources has gained so much of attention that, in recent years even

the industrial pomaces, produced by the wine industry have been exploited to evaluate the antioxidant activity. Wine pomace is known to contain high-phenolics which make them one of the potential sources of natural antioxidant. Thus, Gengaihi *et al.* (2014) investigated and reported antioxidant activity of winery pomace (from red and white grapes). Among the different solvent used viz. BuOH, EtOAc, Me<sub>2</sub>Cl<sub>2</sub> and petroleum ether, ethanol extract showed highest antioxidant activity which was correlated with the total phenol content. Various phenolic compounds such as catechin, rutin, rosmarinic, chlorogenic, caffeic, vanillic, coumaric acids were also reported.

The study conducted by Ibraheim *et al.* (2015) demonstrated the antioxidant activity and total flavonoids content of aerial parts of *Ficus pyriformis* Hook. & Arn.(Moraceae) cultivated in Egypt. Different fractions of *Ficus pyriformis* obtained from successive fractionation of the total extracts on vacuum liquid chromatography (VLC) with organic solvents of different polarities like *n*-hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and methanol (MeOH) were subjected to free radical scavenging activity. Among the extracts, MeOH and EtOAc fractions showed the highest activity followed by DCM and *n*-hexane fractions respectively. Phytochemical analysis revealed the presence of steroids, triterpenoids, flavonoids, tannins, carbohydrates and /or glycosides. The antioxidant activity of MeOH fraction and total extract of *Ficus pyriformis* aerial parts may be attributed to the polyphenolic compound such as flavonoids and tannins which are known to possess an antioxidant activity. Similar studies were performed on the aerial parts of *Teucrium barbeyanum* Aschers. and its antioxidant activities were reported along with the various chemical compounds. It was observed that the extracts containing a higher concentration of polyphenols, showed more potent antioxidant activity, which suggested that the antioxidant activity of *Teucrium barbeyanum* must be related to the presence of these class of compounds (Mohamed *et al.*, 2015).

Madhura *et al.* (2015) reported the antioxidant activity of chloroform and ethanol extracts of four Indian medicinal plants, i.e. *Grewia asiatica*, *Caesalpinia bonducella*, *Syzygium samarangense*, *Asteracantha longifolia*. The antioxidant ability of the plants and extracts tested were observed to vary significantly. Amongst, the four plant seeds of *A. longifolia* were found to possess good antioxidant activity. Likewise, the antioxidant activity, antitumor effect, and antiaging property of proanthocyanidins from *Kunlun chrysanthemum* flowers (PKCF) grown in Xinjiang were reported by Siqun *et al.* (2015). The main product of *Kunlun chrysanthemum* in the market of China is dry tea, with almost no refinery processed products and is known to possess various health benefits. The study revealed that the antioxidant activity were remarkable *in vitro* and *in*

*in vivo* with activity stronger than that of vitamin C. The extract was observed to inhibit MDA formation while enhancing the activities of SOD in mice *in vivo* and in *Drosophila*. Antitumor activity was pronounced as it could inhibit the growth of H22, Eca-109, and HeLa cancer cells at low concentrations with average IC<sub>50</sub> values of  $70.96 \pm 0.05409 \mu\text{g/mL}$ ,  $260.4 \pm 0.06887 \mu\text{g/mL}$  and  $113.3 \pm 0.08062 \mu\text{g/mL}$  respectively. It has been suggested that the PKCF could be utilized as a novel natural antioxidant in food and therapeutics as it has been able to reveal potent antioxidant activity. Eddine *et al.* (2015) reported the antioxidant properties of various extracts of *Phoenix dactylifera* L. which has been used to treat a variety of ailments in the various traditional systems of medicine in regions of Southwest Asia and North Africa. Ethanol, methanol, hexane and chloroform extracts were analyzed for the antioxidant properties using the ferric reducing antioxidant power (FRAP), superoxide radical scavenging (O<sub>2</sub>) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assays. The studies demonstrated strong linear correlation of DPPH radical scavenging capacities of the extracts with content of phenolics while FRAP values with significant correlation with total proanthocyanidins content. Among the extracts, ethanol showed higher phenolics and higher antioxidative activity, which was assumed to be because of the phenolics present in the extracts. *Commiphora caudata* Engl. (Syn. *Protium caudatum* Wight & Arn.) commonly known as hill-mango belongs to the family Burseraceae and grows in dry or semi-evergreen forests of South India. The plant has been known to exhibit various biological functions (Sivakumar *et al.*, 2009; Annu *et al.*, 2010). Thus, to further analyze the pharmacological potentials of the *Commiphora caudata* Engl. extracts Reddy *et al.* (2015) investigated the chemical composition, antimicrobial and antioxidant activities of *C. caudata* essential oils isolated from leaves and fruits using *in vitro* assays. The studies demonstrated revealed the presence of fifteen compounds from leaf oil and thirty compounds from fruit oil. Both the leaf oil and fruit oil were observed to exhibit antimicrobial and antioxidant activities at varying degree.

### **2.3. Antimicrobial activity of the plants**

It is well known that plants synthesize antimicrobial compounds as part of their defense mechanism against attacks by pests and pathogens. There is a voluminous record of work by several authors on extraction of these antimicrobial compounds from plants and testing them against microbes *in vitro* with a view to utilize them as source of medicines.

Elizabeth (2005) evaluated the antimicrobial activity of crude and methanol extract of *Terminalia bellerica* dry fruit by disc diffusion method, against 9 human microbial pathogens. The result

showed that both the crude and methanol extract were able to inhibit the growth of the tested organism at varying degree. However, methanolic extract was more effective than crude extract against most of the microbes tested except *E. coli* (enteropathogen) and *P. aeruginosa*. Among the bacterial organisms tested, *S. aureus* was found to be highly susceptible to both the extracts highest suggesting *T. bellerica* to be the strong inhibitor of this organism. The minimal inhibitory concentrations (MICs) of crude extract ranged from 300 to >2400 g/ml and methanolic extracts ranged from 250 g to >2000 g/ml with the lowest MIC value against *S. aureus*, demonstrating *T. bellerica* dry fruit to possess potential broad spectrum antimicrobial activity. Similar antimicrobial studies were performed by El Astal *et al.* (2005) on aqueous, ethanolic, methanolic and phenolic extracts of three Palestinian folkloric medicinal plants along with their commercial oils against ten pathogenic microorganisms. Sage, thyme and parsley has been widely used in folkloric medicine in treating different diseases in Palestine. The studies demonstrated and reported the antibacterial activity of plants against *E.coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Actinobacter haemolyticus*, *Enterococcus* sp. and *Candida albicans* isolated from patients with UTI and the other two bacteria viz. *Salmonella typhi* and *Staphylococcus aureus* from the stool of patients suffering from food poison. The aqueous extracts of sage and thyme were observed to be effective against most of the tested microorganisms. *S. aureus* and *Enterococcus* sp was susceptible to phenolic extract of sage and thyme respectively. On the other hand, the ethanolic extract of parsley was more effective against *E. coli*, however, no pronounced effect was observed against the Gram positive organisms tested. The commercial oils of sage, thyme and parsley was unable to inhibit the growth of *E.coli*, *Proteus mirabilis* and *Salmonella typhi*. Overall, among the 10 tested microorganisms, *S. aureus* was shown to be the most susceptible microbe to most extracts of the three plants studied. The results have thus validated the folkloric medicinal use of thyme, sage and parsley for the treatment of some microbial infections like UTIs and bacterial food poisoning.

The antimicrobial activity of *Bidens pilosa* L., *Bixa orellana* L., *Cecropia peltata* L., *Cinchona officinalis* L., *Gliricidia sepium* H.B. & K, *Jacaranda mimosifolia* D.Don, *Justicia secunda* Vahl., *Piper pulchrum* C.DC, *P. paniculata* L. and *Spilanthes americana* Hieron were tested against five bacteria (*Staphylococcus aureus*, *Streptococcus*  $\beta$  *hemolytic*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Escherichia coli*), and one yeast (*Candida albicans*) by Rojas *et al.* (2006). The results have shown that all the plant studied were effective against three or more of the pathogenic microorganisms, however, they were ineffective against *Streptococcus*  $\beta$  *hemolytic* and *Pseudomonas aeruginosa*. The study also showed that *Bixa orellana* L along with *Justicia secunda* Vahl. and *Piper pulchrum* C.DC may possibly be the probable sources of new

antimicrobial agents, thereby supporting the use of selected plants in folkloric medicine as an antimicrobial agent. It has also been reported by Agyare *et al.* (2006) that both methanolic and petroleum ether extracts of the leaves as well of the bark showed antimicrobial properties when tested against against six microorganisms, namely, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger*, but methanolic extracts were more efficient than the petroleum ether extracts.

Akinpelu and Onakoya (2006) carried out studies on *Psidium guajava* and *Mangifera indica* to analyze their antimicrobial activity. These two plants have been normally used for herbal preparations in the treatment of toothache, gastrointestinal disorders, dysentery, diarrhoea, sore gums and sore throats in South western part of Nigeria. The results have shown that both the plant extracts were able to inhibit the growth of all the fifteen different bacterial isolates used at varying degree, however, *Psidium guajava* was observed to be more efficient.

Duraipandiyan *et al.* (2006) exclusively studied the antimicrobial activity of 18 ethnomedicinal plant extracts against nine bacterial strains (*Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Ervinia sp*, *Proteus vulgaris*) and one fungal strain (*Candida albicans*).The result showed that among the plants tested methanolic extracts of *Acalypha fruticosa*, *Peltophorum pterocarpum*, *Toddalia asiatica*, *Cassia auriculata*, *Punica granatum* and *Syzygium lineare* were most active than the hexane extract. The highest antifungal activity was exhibited by methanol extract of *Peltophorum pterocarpum* and *Punica granatum* against *Candida albicans*.

In a study by Odunayo *et al.* (2007) it was reported that extracts of *Bryophyllum pinnatum* and *Kalanchoe crenata* showed moderate antimicrobial activity against the microorganisms studied viz. gram-negative organisms (*Escherichia coli* ATCC 25922, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Salmonella paratyphi*, *Citrobacter spp*) and gram positive organisms *Staphylococcus aureus* ATCC 25213, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*) and a fungus (*Candida albicans*) used. However, the activity was more pronounced against gram negative organisms. Nonetheless, *Candida albicans* was resistant to the extracts obtained from the traditional method. The methanolic extract of *B. pinnatum* leaves were reported to be more active against gram positive bacteria namely *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. The plants may be assumed to have broad spectrum activities. In a similar study performed by Ushimaru *et al.* (2007) to evaluate the *in vitro* antimicrobial activity of plant extracts (*Allium*

*sativum*, *Zingiberofficinale*, *Caryophyllus aromaticus*, *Cymbopogon citratus*, *Mikania glomerata* and *Psidium guajava*) against Gram-positive and Gram-negative bacterial strains isolated from human infections, it was reported that all the plants exhibited varying degree of antimicrobial activity with methanolic extract of *Caryophyllus aromaticus* being the highest to show the activity. Nair and Chanda (2007) extensively studied the antibacterial activities of ten medicinal plants, namely *Commiphora wightii*, *Hibiscus cannabinus*, *Anethum graveolens*, *Embllica officinalis*, *Ficus religiosa*, *Ficus racemosa*, *Ficus benghalensis*, *Ficus tisel*, *Mentha arvensis* and *Mimusops elengi* against medically important bacterial strains, namely *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Bacillus cereus*, *Alcaligenes faecalis* and *Salmonella typhimurium*. The results have shown that the ethanol extracts of all the plants were more effective than the aqueous extracts. The most resistant strains were *Pseudomonas aeruginosa* and *Salmonella typhimurium* while the most susceptible bacterial strains were *Bacillus cereus* and *Proteus mirabilis*. Among the plants studied, *Embllica officinalis* were able to exhibit strong activity against all the tested bacterial strains making it a potential source of natural drug.

Evaluation of antibacterial and antifungal activity of some of the local flora namely *Acacia nilotica*, *Sida cordifolia*, *Tinospora cordifolia*, *Ziziphus mauritiana* and *Withania somnifera* of Mysore were performed by Mahesh and Satish (2008). The study demonstrated that all the parts of the plants were effective against the bacterial and fungal strains at varying degree. Similarly, Islam *et al.* (2008) extensively studied and reported the antimicrobial activity of some indigenous plants of Bangladesh which have been used for the treatment of infectious diseases by tribal peoples of Chittagong. The study demonstrated that out of the 16 plants screened only 5 plants namely *Mentha arvensis*, *Enhydra fluctuans*, *Blumea lacera*, *Chenopodium album* and *Glinus oppositifolius* were able to show antibacterial activity against *Shigella dysenteriae*, *Salmonella typhi*, *Salmonella paratyphi*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio cholera* and *Bacillus megaterium* at different degrees. Moreover, gram positive bacteria were more susceptible to the crude extracts than gram negative bacterial strains and *Blumea lacera* were able to exhibit more pronounced activity than the other plants.

Joshi *et al.* (2009) exclusively studied the antibacterial properties of some of the locally used medicinal plants viz. *Ocimum sanctum* (Tulsi), *Origanum majorana* (Ram Tulsi), *Cinnamomum zeylanicum* (Dalchini), and *Xanthoxylum armatum* (Timur) against 10 medically important bacterial strains namely *Bacillus subtilis*, *Bacillus cereus*, *Bacillus thuringiensis*, *Staphylococcus*

*aureus*, *Pseudomonas* spp, *Proteus* spp, *Salmonella* Typhi, *Escherichia coli*, *Shigella dysenteriae*, *Klebsiella pneumoniae*. The results have shown that the activity of these plants against the bacterial strains varied noticeably with *Origanum majorana* being the best to exhibit the activity. Gram positive bacteria were reported to be more susceptible than the gram negative bacterial strains. Sengul *et al.* (2009) evaluated the antioxidant and antimicrobial properties of *Inula aucherana*, *Fumaria officinalis*, *Crocus sativus*, *Vicum album*, *Tribulus terrestris*, *Polygonatum multiflorum*, *Alkanna tinctoria* and *Taraxacum officinale* against 32 microorganisms. The study demonstrated that almost all the plants showed different level of antioxidant and antimicrobial activity. The aqueous extracts were observed to be less efficient than the methanolic extracts. Total phenolic contents were not correlated with the antioxidant ability, thus, it may be assumed that the phenolic compounds were not primarily responsible for the antioxidant capacity of the extracts. Rather, several other phytochemicals such as ascorbic acid, tocopherol and pigments may be considered to synergistically contribute to the total antioxidant activity.

In another study, Arun *et al.* (2010) reported the antibacterial activity of the methanolic extract of *Oldenlandia umbellata* against the pathogenic organisms isolated from the respiratory tract infections. The results have shown that the root and aerial portion (except leaves) were able to effectively inhibit the growth of both gram positive and gram negative bacteria. Seven different anthraquinones, of which 1-2- dihydroxy anthraquinone known as Alizarin was observed predominantly in the methanolic extract of the plant. The plant derived Alizarin and the synthetic Alizarin were found to be equally efficient inhibiting the bacterial growth.

Mathur *et al.* (2011) evaluated antifungal activity of eight medicinal plants *Andrographis paniculata* (Acanthaceae), *Valeriana jatamansi* (Valerianaceae), *Asparagus racemosus* (Liliaceae), *Tinospora cordifolia* (Menispermaceae), *Coleus barbatus* (Lamiaceae), *Berberis aristata* (Berberidaceae), *Achyranthes aspera* (Amaranthaceae) and *Plantago depressa* (Plantaginaceae) against clinical pathogens. The study demonstrated that all the plants exhibited different degree of antifungal activity with hydro-alcoholic extracts to be the potent ones than the hexane extracts. Among the samples, hydroalcoholic extracts of *Andrographis paniculata* and *Achyranthes aspera* were able to effectively inhibit the growth of *Aspergillus niger* and *Candida albicans*. Similarly, Panghal *et al.* (2011) reported antimicrobial activity of ten medicinal plants namely *Asphodelus tenuifolius* Cav., *Asparagus racemosus* Willd. , *Balanites aegyptiaca* L., *Cestrum diurnum* L., *Cordia dichotoma* G. Forst, *Eclipta alba* L., *Murraya koenigii* (L.) Spreng., *Pedaliium murex* L., *Ricinus communis* L. and *Trigonella foenum graecum* L. against clinical isolates of oral cancer cases viz. *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus*



*epidermidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Proteus vulgaris* and the fungal pathogens *Candida albicans* and *Aspergillus fumigatus*. The result has shown that out of the 10 plants tested, eight were able to exhibit significant antimicrobial activity ( $P < 0.05$ ) against most of the isolates with *P. aeruginosa* being the most susceptible bacterium, implying that the medicinal plants could be considered as an effective antimicrobial agent to prevent the secondary infections in treated cancer patients. In another study by Lourens *et al.* (2011), the antimicrobial activity of chloroform:methanol (1:1) extracts (leaf and stem extracts for all 36 *Helichrysum* species and an additional flower extract for *H. rugulosum*) was determined against six microorganisms, including three Gram-positive bacteria (*Bacillus cereus*, ATCC 11778, *Staphylococcus aureus*, ATCC 12600, and *Staphylococcus epidermidis*, ATCC 2223), two Gram-negative bacteria (*Klebsiella pneumoniae*, NCTC 9633 and *Pseudomonas aeruginosa*, ATCC 9027), and a yeast (*Cryptococcus neoformans*, ATCC 90112) and the cytotoxicity was determined against transformed human kidney epithelial (Graham) cells, MCF-7 breast adenocarcinoma and SF-268 glioblastoma cells. Seven out of 36 plants exhibited minimum inhibitory concentration values lower than 0.1 mg/ml against *Bacillus cereus* and/or *Staphylococcus aureus* and less than 25% growth for the Graham and MCF-7 cell lines at the same concentration.

Singariya *et al.* (2012) extensively studied the antibacterial and antifungal activity of crude extracts of fruit coat (calyx) in different solvents (hexane, petroleum ether, toluene, benzene, iso propyl alcohol, chloroform, ethyl acetate, acetone, ethanol, glacial acetic acid and water) of *Withania somnifera* against some human as well as plant pathogen viz. *Proteus merabilis*, *Klebsiella pnemoniae*, *Agrobacterium tumefaciens* (plant pathogen) and one fungus *Aspergillus niger*. The results have shown that 9 out of 11 extracts were effective against the bacterial and fungal growth revealing broad spectrum nature of *W. somnifera*. Among the extracts, ethyl acetate, glacial acetic acid, iso propyl alcohol and chloroform extracts were able to exhibit maximum antimicrobial activities, however, glacial acetic showed pronounced activity with a very low MIC and MBC/MFC values which were similar to the standard drugs (gentamycin and ketoconazole) used. Likewise, the leaves of genus *Murraya koenigii* (L) Spreng (Mitha neem), one of the commonly used flavoring agent in Indian curry was evaluated for its antimicrobial activity by Harish *et al.* (2012). The plants have been known to be traditionally used as medicines and are reported to have activity such as antibacterial, anti-inflammatory, anti-feedant etc (Dahanukar *et al.*, 2000; Bhaskar *et al.*, 2011). The study demonstrated that all the four extracts viz. water, ethanol, chloroform and petroleum ether, were able to exhibit the antimicrobial activity against gram positive organisms (*Staphylococcus aureus*) and gram negative organisms

(*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia* and *Salmonella typhi*) at varying degree. The minimum inhibitory concentration (MIC) of the water, ethanol, chloroform and pet ether extracts were in the range of 12.5 to 50.0 mg/ml while minimum bactericidal concentration (MBC) ranged between 50.0 to 100.0 mg/ml. However, among the extracts, the strongest antibacterial effect was exhibited by the ethanolic extract. The presence of carbazole alkaloids were assumed to be responsible for the antimicrobial activity thereby validating the use of *M. koenigii* as an antimicrobial agent. In similar studies performed by Kagne *et al.* (2012) the antifungal activity of ethanolic, methanolic and distilled water extract of *Blumea lacera* against *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus praraciticus-456*, *A. flavus* and *A. parasiticus* at varying range was reported. Among the extracts, ethanolic extract exhibited better activity than the other extracts which may be attributed to the chemical compounds like oils present in the sample.

Sharma and Patel (2013) evaluated and reported the antibacterial activity of methanolic extract of *Rivea ornata* against four bacterial strains namely *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* in a dose-dependent manner. Thalwal *et al.* (2013) also exclusively studied and reported the antimicrobial activity of *Amaranthus spinosus*, *Orchis muscula* and *Solanum nigrum* against *Staphylococcus aureus* (ATCC96), *Streptococcus mutans* (ATCC890), *Bacillus subtilis* (ATCC6633), *Bacillus amylolique faciens* (ATCC23350), *Aspergillus fumigates* (ATCC1022) and *E. coli* (ATCC483). The methanol, acetone and hexane extracts of *A. spinosus*, *S. nigrum* and *O. muscula* exhibited antimicrobial activity at varying range. However, among all the extracts, methanolic extracts exhibited highest antimicrobial activity while chloroform extract was not effective against the bacterial strains. Phytochemical analysis revealed the presence of tannins and flavonoid in methanolic extracts while it was absent in the chloroform extract which may have contributed towards better antimicrobial activity of the methanolic extracts, thereby, validating the folkloric use of these plants. The antioxidant, antimicrobial activities and phytochemical screening of different crude extracts viz. hexane, ethyl acetate, chloroform, butanol and methanol of *Datura metel* available in Sultanate of Oman were evaluated by Alabri *et al.* (2014). The study demonstrated varying degree of antibacterial and antioxidant activity by all the crude extracts of both dry and fresh leaves extracts. However, the methanolic extracts were moderately better in inhibiting the growth of one gram positive (*Staphylococcus aureus*) and three gram negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) bacteria. Phytochemical analysis revealed the presence of alkaloid, flavonoid, saponin and tannin compounds while steroids and triterpenoid compounds was not detected in all crude extracts of both dry and fresh leaves. The presence of phytochemicals like

tannins and flavonoids may be assumed to have induced the antioxidant and antimicrobial activity.

Extensive studies have been performed by Nanthakumar *et al.* (2014) to evaluate the antimicrobial efficacy of the medicinal plant *Rhinacanthus nasutus* Linn. The study demonstrated that both the aqueous and ethanolic extracts of *R. nasutus* exhibited antimicrobial activity against the human pathogenic bacteria namely *Bacillus subtilis*, *Streptococcus faecalis*, *Salmonella paratyphi*, *Salmonella paratyphi* (A), *Aspergillus flavus*, *Candida albicans*, *Trichoderma viride* and *Pencillium sp* at a varying degree. However, the aqueous extract showed better activity against *Bacillus subtilis*, *Salmonella paratyphi*, *Candida albicans* and *Aspergillus flavus* than the ethanolic extracts making them the potential alternatives as an antimicrobial agent that may be used to prevent resistant microorganisms from causing infectious diseases. In a similar study Haq *et al.* (2014) demonstrated the antioxidant properties and antibacterial activities of different extracts of *Sonneratia alba*. Among the various extracts, ethanol extract of the bark and water extracted fractions demonstrated higher antioxidative and antibacterial properties. According to the authors the high phenolic content in the sample may have contributed towards the biological activities. In another study by Gauniyal and Teotia (2014) the phytochemical and antimicrobial activity of some medicinal plants against five microbial strains causing oral infections was evaluated. The study demonstrated that almost all the medicinal plants studied exhibited varying degree of antimicrobial activity against *Streptococcus mutans*, *Enterococcus faecalis*, *Lactobacillus acidophilus*, *Candida albicans* and *Candida tropicalis*. However, amongst the plants *Acacia nilotica*, *Citrus limon*, *Emblica officinalis*, *Juglans regia*, *Psidium guajava* L. and *Withania somnifera* were observed to effectively inhibit the microbial growth while *Lannea coromandelica* (Houtt) Merr and *Rosa centifolia* could weakly inhibit the growth of the tested microorganism. Phytochemical analysis revealed the presence of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins, reducing sugars and saponins in almost all the plants studied making these plants the potential source for the development of antimicrobial agents against oral microorganisms. Likewise, Tirupatirao *et al.* (2014) evaluated and reported the antimicrobial activity of various extracts namely aqueous, methanolic and petroleum ether extracts of two Indian medicinal plants viz. Neem and Tulsi against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Micrococcus luteus* and *Pseudomonas aeruginosa*. All the extracts were able to inhibit the growth at a varying degree. However, among the extracts, methanolic extract of neem plant and aqueous extract of tulasi showed better antimicrobial activity than the petroleum ether extract.

The *in vitro* antibacterial activity of ethanolic extracts of 16 different traditionally used medicinal plants of Nepal namely *Acorus calamus* L., *Adhatoda vasica* L., *Artemisia vulgaris* L., *Asparagus racemosus* Wild., *Centella asiatica* L., *Cinnamomum camphora* L., *Curculigo orchioides* Gaertn., *Curcuma longa* L., *Cuscuta reflexa* Roxb., *Cynodon dactylon* L., *Drymaria cordata* Wild., *Eupatorium adenophorum* Spreng., *Ginkgo biloba* Spreng., *Psidium guajava* L., *Rauwolfia serpentina* L. and *Swertia chirayita* Roxb. against 13 clinical and 2 references bacterial species was evaluated by Marasini *et al.* (2015). The results indicated that varying degree of antimicrobial activity were exhibited by the plants studied with *Cynodon dactylon* being the efficient to inhibit the growth of even the methicillin-resistant *Staphylococcus aureus*, imipenem-resistant *Pseudomonas aeruginosa*, multidrug-resistant *Salmonella typhi*, and *S. typhimurium*. Furthermore, the ethanolic extracts of *Cinnamomum camphora*, *Curculigo orchioides* and *Curcuma longa* displayed the highest MIC values against *S. pyogenes* while highest antibacterial activity against *S. aureus* was exhibited by chloroform fraction of *Cynodon dactylon*. Some of the extracts i.e. *C. dactylon*, *C. camphora*, *C. orchioides* and *C. longa* with MIC values <100 µg/mL could be considered as the potent source of antibacterial agent against infections by MDR bacteria. Chaitali *et al.* (2015) reported the antimicrobial activity of *Morus indica* leaf extract against *Staphylococcus aureus*, *Aspergillus niger* and *Penicillium*. The ethanolic and methanolic extract exhibited better antibacterial and antifungal activity respectively which may be assumed to be attributed by the various secondary metabolites present in the leaves of *M. indica*. Similarly, Londhe *et al.* (2015) reported the antimicrobial activity of leaf hydrodistillates of *Ocimum tenuiflorum*, *O. gratissimum*, *O. kilimandscharicum* against *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Aspergillus niger*. All the three *Ocimum* species exhibited varying degree of antimicrobial activity. However, *O. kilimandscharicum* displayed effective antibacterial activity against *B. cereus*, *E. coli*, *P. aeruginosa* while leaf distillates of *O. gratissimum* were effective against *A. niger*. Preliminary phytochemical analysis revealed the presence of reducing sugars, monosaccharides, hexose sugar, amino acids, tannins and phenolic compounds, citric, malic and tartaric acids. Presence of the acids along with the other phytochemicals in the sample may be assumed to have attributed towards the antimicrobial activity of the *Ocimum* species.

#### 2.4. Hypoglycemic activity of the plants

Diabetes mellitus is a serious life threatening disease characterized by chronic hyperglycemia caused due to deficiency or defects in the insulin secretion or insulin resistance and altered metabolism of carbohydrates, lipids and proteins (Bussa and Pinnapareddy, 2010) which in turn may lead to other complications like chronic renal failure, retinal damage, cardio-vascular and neuro-degenerative diseases (Gin and Rigalleau, 2000). Despite the advances made in the management and understanding of diabetes, the rate of disease and disease related problems seem to be developing at an alarming rate. Numerous drugs in use to control diabetes and oxidative stress are associated with various adverse effects which has globally accelerated the effort to search for plants having medicinal properties (Inas *et al.*, 2011) Thus, in this regard Kesari *et al.* (2005) evaluated the hypoglycemic activity of the aqueous extract of *Murraya koenigii* leaves in normal and alloxan induced diabetic rabbits. The result have shown that the aqueous extract was efficient in lowering the blood glucose level in both normal and diabetic rabbits at a single oral administration of variable dose levels (200, 300 and 400mg/kg b.w.). *Murraya koenigii* leaves have been known as a spice for food flavoring and are safe for consumption, thus these leaves may be prescribed as an adjunct along with other treatments like dietary therapy and drug treatment to control diabetes mellitus.

The folkloric use of *Terminalia chebula* (Combretaceae) to treat diabetes has been supported by the study of Rao and Nammi (2006). They evaluated the antidiabetic and renal function protecting activity of the chloroform extract of *T. chebula* in streptozotocin-induced diabetic rats using short term and long term study protocols. Oral administration of the extracts at the doses of 100, 200 and 300 mg/kg in short term study and (300 mg/kg) in long term study ( daily for 8 weeks) to streptozotocin-induced diabetic rats, were shown to significantly reduce the blood glucose level comparable and exhibit significant renoprotective activity. The reduction of blood glucose by *T. chebula* may be assumed to be either through secretion of insulin from the  $\beta$ -cells of Langerhans at an increased rate or through an extra pancreatic mechanism, however, the possible mechanism of renoprotection was unclear. Florence *et al.* (2007) investigated the effect of *Dorstenia picta* methanolic residue extract on blood glucose levels and some biochemical parameters in normal and streptozotocin-induced diabetic rats. The results have shown that the methanolic extract of *D. picta* and insulin were able to prevent body weight loss, polyphagia and polydipsia. The extract at the dose of 75 mg/kg and 150 mg/kg were reported to significantly ( $p < 0.01$ ) reduce the blood glucose levels in the diabetic rats by 53.88 % and 81.96 % respectively. Furthermore, there was significant reduction in serum cholesterol, triglycerides,

plasma alanine and aspartate transaminase levels in both normal and diabetic rats while no change was recorded in creatinine and total protein content. Authors have suggested that *D. picta* may be a potential source of new oral antidiabetic drug as the treatment with *D. picta* extract may improve glucose homeostasis in streptozotocin-induced diabetes by possibly showing insulin-linked activity and ameliorating kidney and liver functions. In a similar study by Sokeng *et al.* (2007) the hypoglycemic effect of *Anacardium occidentale* Linn in streptozotocin-induced diabetic rats was reported. The study demonstrated that the oral administration of the methanolic extract at the doses of 35, 175 and 250 mg/kg could significantly reduce the blood glucose levels in diabetic rats after 3 h of administration with maximum reduction of 37 and 35% at the doses 175 and 250 mg/kg extract respectively. However, repeated administration of the methanol extracts and its fraction, n-hexane, dichloromethane and ethyl acetate fractions significantly reduced the blood glucose ranging between 21-48% besides, urine glucose levels. Among the extracts, hexane and ethyl acetate fractions exhibited the most prominent actions which may be assumed due to the non polar and polar hypoglycemic compounds of the plant.

Likewise, Jie *et al.* (2008) evaluated and reported the *in vitro* antidiabetic and antioxidant activity of *Cirsium japonicum* roots which is known to have various medicinal properties. Traditionally, the plant has been used as an antihemorrhagic, antihypertensive and antihepatitis agent in Korea. The study demonstrated that the methanolic and water extract of roots of *Cirsium japonicum* exhibited antioxidant activity in a dose dependent manner, with methanolic extract being more potent than water extract. However, MeOH extract did not exhibit  $\alpha$ -glucosidase inhibitory activity while moderate but not high inhibitory  $\alpha$ -glucosidase activity was shown by the water extract, though higher phenolic and flavonoid contents was found to be in the MeOH extract. Arumugama *et al.* (2008) reported the antidiabetic activity of both the leaf and calluses obtained from leaf explant of *Aegle marmelos*. Both leaf and the callus were reported to significantly decrease the blood sugar level in streptozotocin diabetic rabbits. However, among the various extracts used, the maximum anti-diabetic effect was exhibited by the methanolic extracts of the leaf and callus, thereby revealing the efficiency of the *in vitro* callus to manage diabetes at par with the original leaf extract.

Sengul *et al.* (2009) exclusively studied the antidiabetic activity of the methanol extract of *Artanema sesamoides* Benth (Scrophulariaceae) (MEAS) in streptozotocin-(STZ) induced diabetic rats. The results have shown that the MEAS extract at doses 200 and 400 mg/kg b.w.p.o significantly reduced the fasting blood glucose level while there was an increase in the liver glycogen level ( $25.84 \pm 1.52$ ) as compared to diabetic control ( $12.31 \pm 0.63$ )

( $P < 0.001$ ). Additionally, significant reduction in the elevated serum glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (SALP) with respect to control was observed. Furthermore, there was a significant reduction in the cholesterol and triglycerides levels with an improvement in the HDL/LDL ratio, thereby validating the cytoprotective activity of the extract. Similarly, Prasad *et al.* (2009) evaluated the antidiabetic activity of aqueous leaf extracts of three herbs namely *Murraya koenigii* (MK), *Psidium guajava* (PG) and *Catharanthus roseus* (CR) in streptozotocin (STZ) induced diabetic albino rats. The study demonstrated that MK, PG and CR at the oral dose of 500 mg/kg body weight once a day for 15 days was able to significantly reduce the blood sugar level ( $p < 0.001$ ) and increase the body weight (MK and PG:  $p < 0.05$ , CR  $p < 0.001$ ) after 15 days with respect to diabetic control. Furthermore, histological parameters altered during diabetic manifestations was significantly restored which was comparable to that of the standard drug glibenclamide used for the treatment of diabetes mellitus. Further, studies performed by Ahmed *et al.* (2010) reported the antidiabetic activity of methanolic extract of *Vinca rosea* (*Catharanthus roseus*) in alloxan induced diabetic rats. Almost all the plant parts of *Vinca rosea* have been used traditionally and validated scientifically to treat various diseases (Cowley and Bennet, 1928; Pillay *et al.*, 1959; Nammi *et al.*, 2003). Additionally, the methanolic whole plant extract of *Vinca rosea* at a concentration of 500mg/kg b.w. exhibited significant antihyperglycemic activity along with the improvement in the parameters like body weight, lipid profile as well as regeneration of  $\beta$ -cells of pancreas in diabetic rats. Possibly, some  $\beta$ -cells may have survived the effect of alloxan, thus these cells were acted upon by *Vinca rosea* extract to produce insulin which may have resulted in antidiabetic activity of the plant. Moreover, histopathological studies further supported the antihyperglycemic activity as pancreas was observed to be healed by *Vinca rosea* extracts. Likewise, Priyadarsini *et al.* (2010) evaluated the *in vitro* and *in vivo* antidiabetic activity of the leaves of *Ravenala madagascariensis* Sonn. The four different extracts namely n-Hexane, ethylacetate, ethanol and aqueous leaf extracts of *Ravenala madagascariensis* at a concentration of 50gram/litre revealed, *in vitro* and *in vivo* antidiabetic at varying degree. Among the extracts, ethanolic and aqueous extracts was observed to significantly inhibit the effect on glucose diffusion *in vitro* and both the extracts exhibited significant *in vivo* antidiabetic activity; however, the ethanolic extract revealed better blood glucose lowering activity than the aqueous extract. The results thereby supported the folkloric use of the plant to treat diabetes.

Studies performed by Omage *et al.* (2011) on the plant *Garcinia kola* regarding its antidiabetic potential reported that the aqueous extract of *Garcinia kola* seeds on oral administration at the dose of 200mg/kg body weight to normal rats, were able to significantly decrease ( $p < 0.05$ ) the

blood sugar level and malondialdehyde, with significant ( $p < 0.05$ ) increase in the superoxide dismutase activity. However, the catalase activity was not observed to be effected significantly ( $p > 0.05$ ). Thus, the study provided an evident that *Garcinia kola* which is traditionally used in Nigeria for improving nervous alertness and stimulation of insomnia, may also acquire hypoglycemic and antioxidative properties. In a similar study, Kumar *et al.* (2011) investigated the *in vitro* alpha-amylase potential and *in vivo* antioxidant activity of *Amaranthus spinosus* in alloxan-induced diabetic rats. *A. spinosus* have been used traditionally to treat various diseases (Vaidyaratanam, 1996; William D'yock, 1976; Kirtikar and Basu, 1987). The results have shown that the methanolic extract of *A. spinosus* (MEAS) could significantly inhibit the alpha-amylase activity, reduce the elevated blood glucose, MDA and restores GSH, CAT and TT levels which may be attributed to the active principle(s) present in the plant extract. Das and Chakraborty (2011) carried out the study to investigate an anti-hyperglycemic effect of *Scoparia dulcis* in streptozotocin induced diabetes. The study demonstrated that the aqueous extract of *Scoparia dulcis* at the dose of 250 mg/kg body wt. /twice a day resulted in a marked decrease in the levels of fasting blood glucose, urine sugar, thiobarbituric acid reactive substances, reduced glutathione and glycogen content with a concomitant increase in body weight.

Besides, *in vivo* antihyperglycemic analysis to understand the mechanism of the plant extracts to treat diabetes, an *in vitro* enzyme activity analysis may also be helpful. Thus, inhibition of amylase and glucosidase enzymes have been studied frequently as it can provide some strategy to control post prandial blood glucose level in type 2 diabetes patients (Ali *et al.*, 2006). In view of this, the genus *Caesalpinia digyna* (root methanolic extract) was evaluated for the *vitro*  $\alpha$ -amylase and  $\alpha$ -glucosidase antidiabetic activity (Narkhade *et al.*, 2011). *C. digyna* have been used traditionally to treat diabetes and anti-inflammatory diseases (Srinivasan *et al.*, 2007). The study demonstrated that the extract was able to inhibit the both  $\alpha$ -amylase and  $\alpha$ -glucosidase activity in a dose dependent manner. The  $\alpha$ -amylase inhibitory activity of the plant may be assumed to be because of the flavanols present in the extract while that of  $\alpha$ -glucosidase be attributed to the intestinal  $\alpha$ -glucosidases inhibitory activity. Since, postprandial hyperglycemia is the major features of diabetes mellitus, thus therapeutic approaches involved in decreasing postprandial hyperglycemia by inhibiting the activity of  $\alpha$ -amylase has been one of the prime focuses in the treatment of diabetes. Hence, Sangeetha and Vedaasree (2012) investigated the *in vitro*  $\alpha$ -amylase inhibitory activity of the leaves of *Thespesia populnea*. The results have shown all the extracts used viz. petroleum ether (PETP), chloroform (CTP), ethyl acetate (EATP) and methanol (MTP) exhibited  $\alpha$ -amylase inhibitory activity at varying degree. However, among the extracts ethyl acetate (EATP) and methanol (MTP) revealed better inhibitory activity. Further,



analysis carried out to understand the nature of enzyme inhibitors suggested the inhibitors to be proteinaceous in nature. Overall, the study validates the traditional use of *T. populnea* to alleviate postprandial hyperglycemia and reduce oxidative stress thereby helping to combat various diabetic complications.

Studies performed by Oboh *et al.* (2012) to assess the inhibitory effect of *Telfaira occidentalis* on  $\alpha$ -amylase and  $\alpha$ -glucosidase revealed that the leaf extracts of both unprocessed and blanched *T. occidentalis* exhibited dose-dependent enzymatic inhibition with better inhibition by unprocessed leaf extract. However, blanched extract revealed significant increase ( $p < 0.05$ ) in the antioxidant properties with decrease in the inhibitory activity. The antioxidant properties along with the enzyme inhibition activity may assume to be the probable mechanism which could be used by *T. occidentalis* leaf in the management of type 2 diabetes. Likewise, Jiju *et al.* (2013) reported that the various leaf extracts of *Carica papaya* L. viz. ethyl acetate, alcohol and aqueous extract revealed increase in the percentage inhibitory activity on  $\alpha$ -amylase in the dose-dependent manner besides delaying the digestion of starch with respect to time. The mechanism by which *C. papaya* may have exerted the inhibitory activity could be related to its action on carbohydrate binding region of  $\alpha$ -amylase which leads to the hydrolysis of the internal  $\alpha$ -1,4glucosidase linkages in starch thereby suppressing the postprandial hyperglycemia.

The genus *Cassia fistula* Linn. have been used traditionally in the treatment of hematemesis, pruritis, intestinal disorders, leucoderma, diabetes, and as antipyretic, analgesic & laxative (Bhakta *et al.* 2001). The folkloric claim of *C. fistula* to be useful in diabetes management has been supported by Shrikant and Manjunath (2013). They studied and reported the *in vitro*  $\alpha$ -amylase inhibitory activity of the total alcoholic bark extract of *C. fistula* along with its ethyl acetate and butanol fractions. The most significant inhibitory activity was exhibited by the ethyl acetate fraction. Presence of flavonoidal moiety in the ethyl acetate fraction may be considered responsible for  $\alpha$ -amylase inhibitory activity.

Mary and Gayathri (2014) evaluated the *in vitro*  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activities of bark of *Terminalia bellirica*. The petroleum ether, chloroform and ethanol extract of *T. bellirica* revealed varying percentage of alpha amylase and glucosidase inhibition. However, comparatively, ethanolic extract exhibited appreciable inhibitory activities which may be attributed to the secondary metabolites present in the sample. Likewise, Shareef *et al.* (2014) reported the *in vitro*  $\alpha$ -amylase activity of the petroleum ether, chloroform, ethyl acetate and methanol extracts of leaves of *Tinospora cordifolia*. Among the extracts potent inhibitory activity was exhibited by ethyl acetate and methanol fraction. Additionally, *in vitro* and *in vivo*

antidiabetic activity of extracts from *Actinidia kolomikta* was reported by Yuan *et al.* (2014). The study demonstrated that alcohol and water extracts of both roots and leaves of *A. kolomikta* were able to exhibit dose dependent  $\alpha$ -glucosidase inhibitory activity. However, aqueous leaf extract revealed comparatively better  $\alpha$ -glucosidase inhibition than the other extracts. Furthermore, aqueous extract was observed to reduce blood glucose level without causing hypoglycemia besides controlling various diabetes complication related blood indicators such as protein metabolism, lipid profile and liver functions suggesting *A. kolomikta* to be one of the probable and sustainable bioresources for diabetes management. In another study by Aditya *et al.* (2014) methanolic bark extract of *Pseudovaria macrophylla* at concentration of 200 and 400 mg/kg bw were capable of decreasing the blood glucose levels in STZ-nicotinamide induced diabetic rats besides significantly increasing the antioxidant enzyme, reduced glutathione, improving serum insulin, C-peptide levels with decrease in the level of lipid peroxidation (LPO). Furthermore, *P. macrophylla* strikingly reduced the levels of serum pro-inflammatory cytokines while effectively preserving the islet and  $\beta$ -cells structure. Additionally, the enhanced expression of insulin protein, GLUT -1, GLUT -2 and GLUT -4 in the pancreatic cells could be one of the mechanisms responsible for antidiabetic activity exhibited by the plant extract.

Till date, the use of traditional medicines to manage diabetes has been extensively used across the globe. Despite developments achieved in the diabetes treatment, rural as well as urban communities in Africa widely rely on traditional remedies. Many plants available in Sudan have been reported to possess hypoglycemic activity. In this view, some plants namely *Mitragyna inremis* (Wild) O. Kundze, *Nauclea latifolia* Smith., *Randia nilotica* Stapf., *Tinospora bakis* (A.Rich.) Miers and *Striga hermonthica* (Del.) Benth. have been evaluated for its hypoglycemic activity. The study demonstrated that the aqueous extracts of *T. bakis*, *N. latifolia* and *R. nilotica* at the doses of 400mg/kg bw could significantly reduce the blood glucose level ( $p < 0.05$ ) in case of acute treatment, while chronic treatment of diabetic rats exhibited the ability to rectify the metabolic disturbances besides significantly reducing the blood glucose level ( $p < 0.05$ ). Furthermore, the aqueous extracts of the plants revealed differential ability to significantly lower the total cholesterol, total triglycerides, LDL-C, plasma AST, ALT, LDH, urea and blood urea nitrogen and increase HDL-C. However, *S. hermonthica* did not exhibit any hypoglycemic activity against the diabetic rats. The study thereby supports the use of these plants in the Sudanese traditional medicine (Maha *et al.*, 2015). Significant reduction ( $p < 0.05$ ) in the blood sugar level of alloxan induced diabetic mice by the ethanolic leaf extract of *Phyllanthus amarus* has been reported (Shetti and Kaliwel, 2015). Similarly, Gupta *et al.* (2015) reported that oral administration of 1.5g/kg bw of ethanolic bulb extract of *Urginea indica* significantly reduced the

blood sugar, total cholesterol and triglyceride level with improvement in HDL of STZ-treated diabetic rats.

## 2.5. Phytochemicals of ferns

It is not surprising that pteridophytes played an important role in folkloric medicine and have been successfully used from ages in Ayurvedic, Unani, Homeopathic and other systems of medicines; but its importance in modern era has been highly neglected. However, the search for novel and safer alternatives for drugs has gained so much of interest in recent times that even the lower groups of plant like Pteridophytes have been occasionally investigated for its phytochemicals and medicinal values. In this context, certain available literature has been reviewed below:

Irudayaraj and Senthamarai (2004) reported the presence of steroid, phenolic groups like catechin and tannin with very good positive result in the rhizome of *Drynaria quercifolia* (L.) J. Smith. Further, study on the phytochemical constituents of *D. quercifolia* rhizome in the different extracts namely acetone, benzene, chloroform, water, ethanol and petroleum ether revealed the presence of phenols and tannins, and an absence of alkaloids, proteins, xanthoproteins, carboxylic acid and coumarins in the extracts tested. However, saponin was reported only in the benzene and petroleum ether extract (Muraleedharannair *et al.*, 2012). Similarly, presence of tannins, saponins and flavonoids in the hydro-alcoholic extract of *Equisetum arvense* L. collected from some unknown region of the State of Santa Catarina, Brazil has been reported by Santos Jr. *et al.* (2005a, 2005b).

Likewise, presence of various types of flavonoids, particularly, types of flavonol 3-O- glycosides, and triterpenes, saponins along with high content of steroids in the leaves of *Dicranopteris linearis* has been reported by Raja *et al.* (1995) and Zakaria (2007). Furthermore, presence of flavonoids, saponins, triterpenes, tannins and steroids but absence of alkaloids was reported from the leaves of *D. linearis* (Zakaria *et al.*, 2011). Additionally, many species of *Microsorium* were reported to contain ecdysteroids (from *Microsorium scolopendria*) /phytoecdysteroids which have been considered responsible for the medicinal property of these plants (Eva *et al.*, 2007; Raimano *et al.*, 2007).

The presence of phytochemicals like leucocyanidin, leucodelphinidin, the flavones ester apigenin 7-O-p-hydroxybenzoate and a number of glycosides of apigenin, leutolin, isocutellarein-8-O-methyl-ether, kaempferol and quercetin were reported by Salantino and Prado (1998) and

Imperato (2006) in *Pteris vittata*. Further, flavonoid (mainly rutin), tannins, resins, glycosides and terpenoids have also been reported (Singh *et al.*, 2008). In a study performed by Rani *et al.* (2010), phytochemicals like flavonoids, tannins, alkaloids, reducing sugars, triterpenoids and steroids were reported in *Psilotum nudum*, *Nephrolepis biserrata* and *Nephrolepis cordifolia*. Similarly, eleven species of ferns namely *Microsorium membranacum* (D. Don.), *Nepobolus lanceolatus* Trim., *Pleopeltis wightiana* (Beddome), *Lygodium flexuosum* (L.) Sw., *Marsilea minuta* L., *Athyrium hohenackeranum* Kunze, *Pityrogramma calomelanos* (L.) Link, *Christella parasitica* (L.) H. Lev., *Diplazium esculentum* (Retz.) Sw., *Pteris vittata* (L.), *Tectaria coadunata* (Wall. Ex Hook. & Grev.) C. Chr. were investigated for the presence of phytochemicals. Phytochemicals such as chlorophylls, carotenoids and polyphenols at varying range were quantified (Bharat *et al.*, 2011).

Likewise, phytochemical screening of acetone, benzene, chloroform, aqueous, ethanol and petroleum ether extracts of some important medicinal Pteridophytes of Western Ghats namely *Drymoglossum heterophyllum* (L.) Trimen, *Dicranopteris linearis* Burm.f., *Blechnum orientale* L., *Ceratopteris thalictroides* (L.) Brong, *Hemionitis arifolia* (Burm), *Lindsaea ensifolia* SW, *Nephrolepis multiflora* (Roxb.) Jarrett, *Pityrogramma calomelanos* (L.), *Pteris confuse* T.G. Walker and leaves and rhizomes of *Drynaria quercifolia* (J.) Smith was performed by Mithraja *et al.* (2012). The results have shown that out of the 66 extracts tested only 47 showed the presence of tannin. Abraham and Aeri (2012) analysed the phytochemicals present in the methanolic extracts of *Azolla microphylla* and reported the presence of tannins, steroids, anthraquinone glycosides, phenols and sugar, and an absence of alkaloid, triterpenoid and amino acids.

Britto *et al.* (2012) investigated the presence of phytochemicals in five medicinal ferns namely *Pteris biaurita* L., *Lygodium flexuosum* (L.) Sw., *Hemionitis arifolia* (Burm.f.) T. Moore, *Actinopteris radiata* (J. Koenig ex Sw.) Link and *Adiantum latifolium* Lam. Twenty five extracts of the samples extracted in five different solvents viz. petroleum ether, benzene, chloroform, methanol and distilled water were used for the study. The results have shown the difference in the presence of the phytochemicals with respect to the solvents used. Presence of flavonoids and steroid in all the samples were detected, however, phenolics were present only in the methanolic extracts. Triterpenoids and catechins were detected in five extracts, tannins and saponins in eight extracts, alkaloids in fifteen extracts and anthraquinones and amino acids in only three of the extracts studied. However, among the extracts, methanolic extracts of all the plants showed maximum number of the compounds. In another study, Muraleedharannair *et al.* (2012) examined the phytoconstituents present in five different ferns namely *Adiantum caudatum*, *Adiantum*

*latifolium*, *Adiantum lunulatum*, *Christella dentata* and *Christella parasitica* growing on the Kanayakumari District of Western Ghats, India. Total of thirty extracts prepared in acetone, benzene, chloroform, aqueous, ethanol and petroleum ether were employed in the study. The study has demonstrated varied degree of presence and absence of phytochemicals with reference to the solvents used. Phenols were present in almost all the plants extracts while flavonoids, alkaloids, proteins, quinones and steroid (with exception in petroleum ether extract of *A. latifolium* and *C. parasitica*, and in acetone extract of *A. caudatum*) were absent in the extracts. Saponins were detected in fifteen extracts, tannin in sixteen extracts, xanthoprotein in seven extracts, carboxylic acid in nine extracts, coumarins in seven extracts and carbohydrates in twenty one extracts.

Gracelin *et al.* (2013) investigated the phytochemicals present in the methanolic extracts of five ferns belonging to Pteridaceae family such as *Pteris argyreae* T. Moore, *Pteris confusa* T.G. Walker, *Pteris vittata* L., *Pteris biaurita* L., and *Pteris multiaurita* Ag. The results have revealed the presence of alkaloids, phenolic compounds, flavonoids, saponins and tannins in all ferns studied while tannin was absent in *P. multiaurita*. Likewise, steroid was present in all the ferns while triterpenoids was not detected in *P. confusa* and *P. multiaurita*. Reducing sugars were present only in *P. confusa* and *P. vittata* while sugar was present only in *P. biaurita*. Amino acids were present only in *P. vittata* and *P. biaurita* while *anthraquinones* were absent in all the ferns except *P. biaurita*. Besides, phytochemicals like tannins, reducing sugar, cardiac glycosides, carbohydrate and saponin, the proximate analysis had revealed the presence of protein, fibre, ash, moisture and carbohydrates with appreciable amount of vitamin C in the fresh leaflets of *Nephrolepis cordifolia* (Oloyede *et al.*, 2013).

Kalpna *et al.* (2014) investigated the presence of various phytochemicals in the aqueous, ethanol and petroleum ether extracts of four ferns namely, *Actinopteris radiata*, *Drynaria quercifolia*, *Dryopteris cochleata* and *Pityrogramma calomelanos*. Presence of tannins, saponins, flavonoids, quinines, cardoglycosides, terpenoids, phenol, alkaloid, betacyanin and coumarins were revealed by almost all the ethanolic and aqueous extracts of the four ferns. On the other hand, petroleum ether extract showed negative responses to almost all the phytochemicals examined. Further quantification revealed highest and lowest content of the total phenol and tannin in *P. calomelanos* and *D. quercifolia* respectively.

Studies carried out by Bahadori *et al.* (2015) on the methanolic extract (leaves and rhizome) of eight Iranian ferns namely *Polypodium interjectum*, *Polystichum woronowii*, *Polystichum aculeatum*, *Dryopteris affinis*, *Athyrium filix-femina*, *Asplenium scolopendrium*, *Asplenium*

*adiantum* and *Pteris cretica* revealed the presence of terpenoids and polyphenols in all the tested plants while anthocyanins were absent in all the plants studied. Quinones and anthraquinones were detected only in *P.woronowii*, *P. aculeatum* and *D. affinis*. Similarly, Chai *et al.* (2015) carried an analysis to determine the phytochemicals present in six medicinal and edible ferns namely, *Christella arida*, *Christella dentata*, *Cyclosorus interruptus*, *Microsorium punctatum*, *Nephrolepis acutifolia* and *Pleocnemia irregularis*. Appreciable amount of total phenol, hydroxycinnamic acids, flavonoids and proanthocyanidins were reported in these ferns

## **2.6. Biological efficacy of Pteridophytes**

Many studies have been conducted to evaluate biological activities like antioxidant, antimicrobial, hypoglycemic, antiulcer, cytotoxic, anti-inflammatory etc of pteridophytes in recent days. However, focus in this review will be primarily on antioxidant, antimicrobial and antidiabetic activities.

### **2.6.1. Antioxidant activity of Pteridophytes**

Bora *et al.* (2005) evaluated and reported the antioxidant activity of *Dicksonia sellowiana* (Presl.) Hook fronds extracts. The results have shown that the extracts with higher concentration of polyphenols revealed potent antioxidant ability of *D. sellowiana*, suggesting that the polyphenols present in the sample could be responsible for the activity. Likewise, Jair *et al.* (2005) reported the ability of the hydroalcoholic extract of stems from *Equisetum arvense* (HAE) to reverse the cognitive impairment in aged rats and to exhibit pronounced antioxidative activity. The result of *in vitro* antioxidant assay had shown that the HAE was able to reduce the thiobarbituric acid reactive substances as well as nitrite formation without altering the catalase activity. The antioxidant activity of HAE may be assumed to have contributed in cognitive enhancement in aged rats.

Zhongxiang *et al.* (2007) reported the antioxidant activity of glycosides isolated from the aerial parts of the fern *Abacopteris penangiana*. The study revealed the presence of five new flavan-4-ol glycosides, abacopterins E-I (5-9) and seven known flavonoid glycosides. In a study performed by Mariana *et al.* (2008), marked *in vitro* and *in vivo* antioxidant activity of ethyl acetate fraction (EAF) of *Cyathea phalerata* in comparison to the other extracts were reported. Similarly, studies carried out on the crude hydroalcoholic extract and of fractions of *Cyathea phalerata* reported the antioxidant and hepatoprotective activity of the plants. Among the fractions, the ethyl acetate fraction of the crude extract exhibited the best antioxidant and

hepatoprotective activities, and assumed that the presence of the flavonoid in this fraction may probably have contributed towards the biological activities studied (Hort *et al.*, 2008). In similar studies conducted by Peres *et al.* (2009) to evaluate the antioxidant activity of ethanolic crude extracts and fractions of *Microgramma vacciniifolia* (Langsd. & Fich.) Copel., the ethyl acetate fraction was revealed to exhibit highest antioxidant activity.

Similarly, Zakaria *et al.* (2011) performed a study to evaluate the *in vitro* anticancer and antioxidant activities of the aqueous (AEDL), chloroform (CEDL) and methanolic (MEDL) extracts of *D. linearis* leaves. The study revealed that MEDL exhibited highest antioxidant activity in comparison to AEDL and CEDL and was effective against all the cancer cells tested. The high antioxidant and anticancer activity exhibited by MEDL was correlated with its phenolic content as highest phenolic content was estimated in methanol extract of *D. linearis* leaves. Further, the use of fern as traditional medicines in Malaysia was validated by the study of Lai and Lim (2011). They evaluated the antioxidant property of methanolic extracts of *Cyathea latebrosa*, *Dicranopteris linearis*, *Pteris vittata*, *Cibotium barometz*, *Drynaria quercefolia*, *Blechnum orientele*, *Adiantum raddianum*, *Diplazium esculentum*, *Pityrogramma calomelanos*, *Lygodium circinnatum*, *Microsorium punctatum*, *Nephrolepis biserrata*, *Pteris venulosa*, *Pyrossia numularifolia* and *Acrostichum aureum*. The result has shown that among the extracts, *C. latebrosa*, *C. barometz*, *D. quercifolia*, *B. orientele*, *D. linearis* showed very high radical scavenging, reducing and BCB antioxidant activity, thereby considering them as a potent primary antioxidants. On the contrary *P. vittata* and *P. venulosa* were considered effective as secondary antioxidants since they revealed strong chelating power. The presence of phenolics was assumed to be responsible for the antioxidant activity of the plant extracts.

The frond extracts (both sterile and fertile) of *Stenochlaena palustris* (Burm. F.), an edible fern were evaluated to assess the concentration of phenolic substances (total polyphenols, flavonoids, hydroxycinnamic acids and anthocyanins) and for its antioxidant potential. The study demonstrated that both the fronds were able to exhibit better antioxidant activity. It was concluded that the presence of these class of substance may have contributed towards the antioxidant activity of *S. palustris* (Chai *et al.*, 2012).

Chai *et al.* (2013) investigated and reported the antioxidant potential of aqueous extracts of the leaves and rhizomes of *Cyathea latebrosa*, *Dicranopteris curranii*, *Gleichenia truncata* and *Phymatopteris triloba*. Among the ferns *P. triloba* exhibited better antioxidant activity and showed high correlation to its flavonoid and hydroxycinnamic acid derivatives which may in turn have contributed towards the antioxidant activity.

Similarly, antioxidant potential of *Adiantum* and *Pteris* ferns have been reported, which may be contributed by the presence of various phytochemical constituents like flavonoid, phenols, saponins, tannins and saponins (Shrivastavaa *et al.*, 2014). Valizadeh *et al.* (2015) evaluated the antioxidant activity of methanolic extracts of rhizomes and fronds of eight ferns namely *Polypodium interjectum* Shivas, *Polystichum woronowii* Fomin, *Polystichum aculeatum* (L.) Schott. *Athyrium filix-femina* (L.) Roth, *Asplenium scolopendrium* L., *Asplenium adiantum-nigrum* L., *Dryopteris affinis* (Lowe) Fraser-Jenk and *Pteris cretica* L. widely growing ferns in North Iran. The study demonstrated that almost all the ferns exhibited better to moderate antioxidant activity. Between the plant parts, rhizome extracts were more efficient antioxidants than the frond extracts. Very weak correlation between the total phenol content and DPPH was reported suggesting that phenolic derivatives may not be the sole components contributing to the antioxidant activity of the plant extracts. In another study performed by Ahmed *et al.* (2015) the antioxidant activity of methanol, hexane and aqueous extracts of *Adiantum caudatum* leaves were reported. Except for the DPPH activity, all the other antioxidant activities were exhibited highest by the methanolic extracts followed by aqueous and hexane extract while the aqueous extract was observed to reveal the highest DPPH activity. Like in most cases, presence of phenols was assumed to have played an important role in the antioxidant activities exhibited by the extracts.

### **2.6.2. Antimicrobial activity**

Kshirsagar and Mehta (1972) evaluated and reported the antibacterial activity of the methanolic extract of *Adiantum trapiziforme* against *Bacillus megaterium* and *Staphylococcus aureus* B-43-5. It was also observed that the older plants were able to exhibit more prominent activity than the young ones and fertile fronds showed better activity than the vegetative ones. Likewise, Barros *et al* (1989) evaluated the antimicrobial activity of the fronds and rhizomes extract of *Polypodium brasiliense* Poiret (= *Serpocaulon triseriale* (Sw.) A.R.Sm.) collected from the Atlantic Forest (Mata de Dois Irmaos, Recife, PE, Brazil) against seven different microorganisms viz. four Gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus faecalis* and *Mycobacterium smegmatis*), one Gram-negative bacteria (*Escherichia coli*), one yeast (*Candida albicans*) and one filamentous fungus (*Monilia silophila*). The study demonstrated that all the microorganisms except *M. silophila* were susceptible against the extracts of *Serpocaulon triseriale*.

The traditional use of the rhizome of *Drynaria quercifolia* was supported by the work performed by Ramesh *et al.* (2001), where the methanol extract revealed broad and concentration dependent



antibacterial activity against all the tested bacteria. However, fungi tested were resistant to the extract. The ethno-medicinal use of *D. quercifolia* rhizome was also validated by the study of Muraleedharannair *et al.* (2012) wherein, the antibacterial potential of six different extracts namely acetone, benzene, chloroform, water, ethanol and petroleum ether of this fern against eight clinically isolated urinary tract infecting bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis* and *Streptococcus pyogenes* (gram positive) and *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter sp* and *Proteus mirabilis* (gram negative) were studied. Among the different extracts, acetone extract was reported to exhibit better inhibitory activity against UTI related bacteria i.e, *S.pyogenes* and *E.faecalis*. However, all the extracts were ineffective against *E. coli*, *K. pneumoniae*, *S. aureus*, *Acinetobacter sp* and *P. mirabilis*.

Similar study was conducted by Dalli *et al.* (2007) to investigate the antimicrobial activity of methanolic fraction of *Pteris biaurita* L. against fungal pathogens namely *Curvularia lunata*, *Fomes lamaoensis*, *Poria hypobrunnea*, *Fusarium oxysporum* and *Aspergillus niger* and a bacterial culture of *Bacillus sp*. The study demonstrated that the fraction III (which was the hydrolysates of aqueous fraction further extracted with ethyl acetate) revealed better antifungal and antibacterial activity than the other two fractions.

Singh *et al.* (2008) extensively studied the antimicrobial activity of the different extracts (70, 80 and 90% aqueous methanol) of *Pteris vittata* against the pathogenic gastrointestinal microflora namely *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Streptococcus pyogenes* and *Shigella flexneri*. The results have shown that among the extracts, 70% aqueous methanolic extract was more efficient in inhibiting the growth of all the bacteria tested than the other two extracts. However, *B.cereus* was reported to be resistant against 80 and 90% aqueous methanolic extract. Further, the flavonoid rutin isolated were tested and reported to exhibit potent antimicrobial activity against *B.cereus*, *P.aeruginosa* and *K.pneumoniae*. The presence of rutin was assumed to be partly responsible for the antimicrobial activity thereby validating the ethnobotanical claims of *Pteris* species as an antimicrobial agent. Further, Singh *et al.* (2008) tested the antimicrobial activity of methanol extract of *A. capillus-veneris*, *A. peruvianum*, *A. venustum* and *A.caudatum* against five Gram positive, six Gram negative (including multi-resistant *Staphylococcus aureus*) bacterial and eight fungal strains. The study determined that almost all the plant extract had varying degree of inhibitory activity. The extract of *A. capillus-veneris* had very low MIC value (0.48µg/mL) against *Escherichia coli*, whereas *A. venustum* extract activity against *Aspergillus terreus* showed an MIC of 0.97µg/mL. that showed good correlation with the phenolic contents of the sample,

implying that the observed activity may be related to the content of phenolics. Likewise, Peres *et al.* (2009) evaluated the antimicrobial potential of *Microgramma vacciniifolia* (Langsd. & Fich.) against *Saccharomyces cerevisiae* and *Candida albicans* fungi. The most effective inhibitory activity was exhibited by the hexane fraction from ethanolic crude extract.

Ethno-medicinal uses of a fern *Blechnum orientale* Linn. (*Blechnaceae*) was scientifically supported by the work of Lai *et al.* (2010). They reported potent antibacterial activity of ethyl acetate, butanol and water fractions of *Blechnum orientale* Linn. (*Blechnaceae*) against all Gram-positive bacteria tested viz., *Bacillus cereus*, *Micrococcus luteus*, methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis*. Among the three extracts, ethyl acetate and butanol fraction revealed better biological activities such as antimicrobial and antioxidant activity which was highly correlated to its poly-phenolic contents. In a similar study, Parihar *et al.* (2010) reported the varying levels of antibacterial activity of *Adiantum capillus-veneris* L., *Adiantum incisum* Forsk., *Adiantum lunulatum* Burm. F., *Actiniopteris radiata* (Swartz.) Link. , *Araiostegia pseudocystopteris* Copel., *Athyrium pectinatum* (Wall ex Mett.) T. Moore, *Chelienthes albomarginata* Clarke, *Cyclosorus dentatus* (Forsk.) Ching. , *Dryopteris cochleata* (Don.) C. Chr., *Hypodematum crenatum* (Forsk.) Kuhn., *Marsilea minuta* L. and *Tectaria coadunata* (J. Smith) C. Chr. (*T. macrodonta*) against *Agrobacterium tumefaciens*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. Further, studies on the antimicrobial activities of *Psilotum nudum*, *Nephrolepis biserrata* and *Nephrolepis cordifolia* was performed by Rani *et al.* (2010). The extracts viz. hexane, chloroform, ethanol and water were tested against bacterial strains (*Proteus mirabilis*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Bacillus cereus* and *Streptococcus faecalis*) and fungal strains such as *Microsporium gypseum*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*. Among the extracts, water extracts of all the three plant samples were effective in inhibiting the growth of both bacteria and fungi at varying degree. However, among the plant species *P.nudum* was observed to exhibit better antimicrobial properties than *Nephrolepis biserrata* and *Nephrolepis cordifolia*.

Kumar and Kaushik (2011) reported the antibacterial activity of *Christella dentata* Frosk. against *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. Likewise, various extract viz. petroleum ether, benzene, chloroform, methanol and water of five medicinal ferns namely *Pteris biaurita* L., *Lygodium flexuosam* (L.) Sw., *Hemionitis arifolia* (Burm.F.) T. Moore, *Actiniopteris radiata* (J. Koenig ex Sw.) Link and *Adiantum latifolium* Lam. were tested

against the gram negative plant pathogenic bacteria *Xanthomonas campestris*. The results have shown that among the five samples, methanolic extract of *P. biaurita* was highly effective against the tested pathogen which may be attributed to the phytochemicals present in the samples (Herin *et al.*, 2012). Methanol, ethyl acetate, hexane and butanol fraction of *Trichomanes chinense* L. and the compounds isolated viz. 3,4- dihydroxybenzoic acid and 3,4- dihydroxybenzaldehyde were reported to exhibit significant antibacterial and antioxidant activities (Syafni *et al.* 2012).

Similarly, Patric *et al.* (2012) examined the antimicrobial activity of ethanolic extracts of *Cyathea nilgiriensis* Holttum, *Cyathea crinita* (Hook.) Copel., *Leptochilus lanceolatus* Fee and *Osmunda hugeliana* Presl against *Proteus aureus*, *Klebsiella pneumoniae*, *Streptococcus* sps, *Aspergillus niger* and *Fusarium* sps. The study demonstrated varying degree of inhibitory activity, with highest activity exhibited by *C. crinita* against *Pseudomonas aureus* and *Klebsiella pneumonia* followed by *L. lanceolatus* against *K. pneumonia* and *Aspergillus niger*. However, none of the plant extracts inhibited the growth of *Streptococcus* sp. In a similar study conducted by Kumarpal (2013), antimicrobial activity against *Escherichia coli* and *Bacillus megaterium* of some commonly available ferns of Darjeeling district, West Bengal, India has been reported. The three plant parts like rhizome, rachis and frond extracts of *Athyrium filix-femina*(L.)Roth., *Dicranopteris linearis*(Burm.f.)Underw., *Pleopeltis macromarpa* (Bory ex Willd.) Kaulf. was observed to show varying degree of antimicrobial activity. Among the plant parts, frond and rhizome extracts revealed good antimicrobial activity than rachis, which may be attributed to the presence of good amount of phytochemicals like phenolic compounds, glycosides, flavonoids and alkaloids.

Chai *et al.* (2013) evaluated antibacterial activity of aqueous extracts of the leaves and rhizomes of some Malaysian highland ferns namely *Cyathea latebrosa*, *Dicranopteris curranii*, *Gleichenia truncata* and *Phymatopteris triloba* against two gram-positive bacteria (*Staphylococcus aureus* and *Micrococcus luteus*) and two gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The results have shown that the leaf and rhizome extracts of *P. triloba* and *G. truncata* were efficient in inhibiting the growth of both Gram-positive and Gram-negative bacteria while the tested bacteria were resistant to *C latebrosa* extract suggesting that *P. triloba* and *G. truncata* may be the probable source of broad-spectrum antibacterial agents. Likewise, Pal (2014) studied the antimicrobial activity of all the parts of three ethnomedicinal ferns of Darjeeling district namely *Adiantum capillus-veneris*, *Neohrolepis cordifolia* and *Pteris vitata* against *Escherichia coli* and *Bacillus megaterium*. Varying degree of antibacterial activity was

exhibited by the plants extracts. Among the plant parts, frond extracts revealed better activity which may be because of the presence of good amount of antibacterial compounds in the frond. Ahmed *et al.* (2015) evaluated the antibacterial activity of methanolic, hexane and aqueous extracts of *Adiantum caudatum* against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The results have shown that the aqueous extract exhibited potent inhibitory activity followed by methanolic and hexanic extract against all the bacteria tested. Among the three bacteria tested, the most susceptible strain observed was *P. aeruginosa*. Moreover, appreciable amount of phenols were quantified in the samples which may have attributed towards the antibacterial potential of the plant extracts. Bahadori *et al* (2015) evaluated the antibacterial activity of eight ferns of Iran viz. *Polypodium interjectum*, *Polystichum woronowii*, *Polystichum aculeatum*, *Dryopteris affinis*, *Athyrium filix-femina*, *Asplenium scolopendrium*, *Asplenium adiantum* and *Pteris cretica* against *Escherichia coli* and *Staphylococcus aureus*. The rhizome and leaves of both the plants extracted with methanol were employed in the study. The result have shown that among the extracts, rhizome and leaves extracts of *Dryopteris affinis* revealed better antimicrobial activity which was evident with its low MIC values (2 µg/mL) followed by *Asplenium adiantum* (4 µg/mL) and *Asplenium scolopendrium* (8 µg/mL against *E.coli* and 4 µg/mL *Staphylococcus aureus* ) than the other ferns studied. Likewise, the varying range of minimum bactericidal concentrations (MBCs) was exhibited by the plant extracts making them the potential source for the antibiotic drug discovery.

### **2.6.3. Hypoglycemic activity**

Study performed by Rastogi & Mehrotra (1991) to investigate the blood sugar lowering activity of the *Adiantum capillus-veneris* reported an initial rise in the sugar level at  $t_0$  ( $p < 0.001$ ) with a decrease in the blood glucose level of 20 and 16 percent at  $t+60$  and 90 min respectively, when water extract of *A.capillus-veneris* was administered 60 min prior to dextrose load. The hydrosoluble chemical components present in the water extract may be assumed to have contributed to its hypoglycaemic activity. Similarly, 50 percent ethanolic extract of *A.incisum* was also able to exhibit hypoglycaemic activity. Further, Neef *et al.* (1995) investigated the hypoglycaemic activity of water and alcoholic extracts of *A.capillus-veneris* using Oral Glucose Tolerance Test (OGTT). The study demonstrated that only the water extract was able to exhibit the hypoglycaemic activity, but with an initial rise in the glucose levels, while, the alcoholic extract treated mice was observed to show increased level of blood glucose throughout the experimental set up.

Similarly, the aqueous and ethanolic extracts of *Hemionitis arifolia* (Burm.) Moore, a folkloric anti-diabetes fern was evaluated for its anti-diabetic and hypoglycaemic properties in the alloxan diabetic rats. The study demonstrated that the ethanol extract was more efficient in lowering the blood glucose level in the glucose fed rats, showing highest activity at the concentration of 200mg/kg. Likewise, the ethyl acetate fraction of ethanolic extract exhibited anti-diabetic activity even at a low dose of 50mg/kg which may be attributable to the presence of steroids and coumarins in the fraction (Nair *et al.* 2006).

Zheng *et al.* (2011) evaluated the anti-diabetic activity of total flavonoids of *Selaginella tamariscina* (Beauv.) Spring (TFST) on high fat diet and STZ (35mg/kg) induced diabetic rats. The results have shown that the oral administration of TFST at varying doses was able to decrease serum levels of fast blood glucose, glycosylated hemoglobin, triglyceride, total cholesterol, free fatty acid, low density lipoprotein- cholesterol, as well as increased high density lipoprotein- cholesterol. Further, studies on the ethanolic and water extract of *Selaginella tamariscina* (Beauv.) Spring revealed the antihyperglycemic activity of the extracts at varying degrees. Between the extracts, ethanolic extract showed better antidiabetic activity.

Paul *et al.* (2012) exclusively studied the anti-hyperglycemic potential of aqueous and ethanolic extract of *Adiantum philippense* L. on alloxan induced diabetic rats. The result showed significant ( $p < 0.05$ ) reduction in the blood sugar level, triglycerides, cholesterol, serum urea and creatinine, SGPT and SGOT level with significant ( $p < 0.05$ ) increase in HDL-cholesterol level of the induced diabetic rats with the administration of both the extracts at 250 and 500 mg/kg bw. Similar evaluation was performed by Paul *et al.* (2012) on the lithophytic pteridophyte *Pteris vittata*. Besides, significantly ( $p < 0.01$ ) increasing the body weight and the HDL- cholesterol level of the diabetic rat, significant ( $p < 0.01$ ) reduction in the blood sugar level, triglycerides, cholesterol, serum urea and creatinine, SGPT and SGOT level was reported in the induced diabetic rats with the oral administration of aqueous and ethanolic extracts at 250 and 500 mg/kg bw. With the report of *P.vittata* being hypoglycemic, further study on supporting its role in lowering blood glucose level was carried out by Paul and Banerjee (2013). The study demonstrated that both the extracts viz. ethanolic and aqueous were able to appreciably inhibit the activity of alpha amylase and the phenolics constituents present in the sample may be considered responsible to its inhibitory activity.

In a study conducted by Basha *et al.* (2013), anti-diabetic activity assayed through  $\alpha$ - amylase inhibitory activity of the ethanolic extract and fraction was reported in *Actiniopteris radiata* Linn. Likewise, aqueous extracts of the leaves and rhizomes of some highland ferns of Malaysia

namely *Cyathea latebrosa*, *Dicranopteris curranii*, *Gleichenia truncata* and *Phymatopteris triloba* were evaluated to determine its anti-glucosidase potential. Among all the extracts, *P. triloba* leaf extract had the highest anti-glucosidase activity while *C. latebrosa* revealed the lowest activity. The high positive correlation was reported between anti-glucosidase activity and the total proanthocyanidin content of both the leaf and rhizome extracts, which may have contributed to its anti-glucosidase activity (Chai *et al.* 2013). Similarly, the common fern species of Bangladesh viz. *Christella dentata* was analysed for its anti-hyperglycemic activity on the diabetic induced Swiss albino mice. The study revealed significant reduction of blood glucose level of the diabetic rats after the oral administration of the methanolic extract of *C. dentata* (Tanzin *et al.* 2013).

The traditional uses of *Drynaria quercifolia* Linn. rhizome as an antidiabetic and hypolipidemic agent was supported by the work of Rajimol *et al.* (2014). The study demonstrated that both the rhizome extracts viz. chloroform and ethanolic administered at the dose of 400mg/kg were able to significantly reduce the fasting blood glucose level and all lipid profile parameters. Likewise, the methanolic and aqueous extracts of *Adiantum capillus veneris* Linn. were studied to determine its anti-hyperglycemic activity. The results have shown that the aqueous extract could efficiently lower the blood sugar level at the dose (100mg/kg bw) lesser than the dose (400mg/kg bw) of methanolic extract. Likewise, the extracts were efficient in increasing the body weight of the diabetic induced rats. The phytochemicals such as flavonoids, steroids and tannins were the major constituents in the extracts which may have contributed towards the antidiabetic activity of the extracts (Vadi *et al.* 2014).

Furthermore, anti-glucosidase activity of some medicinal and edible ferns, namely *Christella arida*, *Christella dentata*, *Cyclosorus interruptus*, *Microsorium punctatum*, *Nephrolepis acutifolia* and *Pleocnemia irregularis* were investigated by Chai *et al.* (2015). The study revealed the anti-glucosidase activity of all the ferns in the concentration dependent manner with highest activity exhibited by *C. dentata* followed by *P. irregularis*, *N. acutifolia*, *C. arida* and *M. punctatum* while *C. interruptus* showed  $\alpha$ -glucosidase stimulatory activity rather than inhibitory activity. The positive correlation between anti-glucosidase activity and the phenolic content of *C. dentata*, suggested that the phenolic compounds may have contributed significantly to their anti-glucosidase activity. Likewise, some other ferns like *Blechnum orientale* L. (Blechnaceae), *Davallia denticulata* (Burm.) Mett. (Davalliaceae), *Diplazium esculentum* (Retz.) Sw. (Athyriaceae), *Nephrolepis biserrata* (Sw.) and *Pteris vittata* L. were also evaluated for the anti-glucosidase activity by Chai *et al.* (2015). The  $\alpha$ -glucosidase inhibitory activity was reported to

be concentration dependent for all the extracts, except in *D. denticulata*. Among the extracts, *D. esculentum* showed the highest  $\alpha$ -glucosidase inhibitory activity followed by *B. orientale* and *P. vittata*. The bioactivity did not reveal positive correlation with the phenolic constituents thereby suggesting that phytochemicals other than phenolics may have contributed to the bioactivity.

Telagari and Hullatti (2015) evaluated the *in-vitro*  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity of the hydro-alcoholic extract and four fraction of *Adiantum caudatum* Linn. and *Celosia argentea* Linn. Varying degree of inhibitory activity was reported by the different fractions of *A. caudatum* and *C. argentea* which may have been attributed by the phenolic and triterpenoid content in the samples.

Increasing demand of herbal medicine and the richness of pteridophytic flora in the Eastern Himalayas and its neighboring areas has provided an arena for pteridological research. Pteridophytes being the second largest vascular plant after angiosperms have been over-exploited for various land transformations. Thus there is an urgent need in understanding the various biological utilities of these uncared plants before it becomes extinct. Moreover, information regarding the biochemical compounds along with the pharmacological evaluation of these ferns may make them the potential source of modern drug therapy.