Chapter - 2: Review of Literature

For thousands of years, nature has been a source of medicinal agent form where an impressive number of modern drugs have been isolated based on their use in traditional medicine. Historically, most of the medicinal preparations were derived from plants, the medicinal value of which lies in some chemical substances that produce a definite physiological action on the human body. The pteridophytes possess an important role in folklore medicine although neglected in modern days. These plants have been successfully used in different systems of medicines like Ayurvedic, Unani, Homeopathic and other systems of medicines. Out of 1250 species of Pteridophytes occurring in India, 173 species have been found to be used as food, flavor, dye, medicine, bio-fertilizers, oil, fiber and bio-gas production (Manickam and Irudayaraj, 1992).

The medicinal value of pteridophytes against bacteria, fungi, virus, cancer, rheumatism, diabetes, inflammation, fertility, diuretic, pesticides, hepatoprotective and sedative had been reported. Besides sugar, starch, proteins and amino acids, ferns contain a variety of alkaloids, glycosides, flavonoids, terpenoids, sterols, phenols, sesquiterpenes etc. as potential components used in various industries (Kulandairaj and Britto, 2000). Kirtikar et al. (1935) have described 27 species of ferns having varied medicinal uses. Chopra et al. (1956) have included 44 species and Nadkarni (1954) recorded 11 species of pteridophytes having medicinal importance. Nayar (1959) recorded 29 medicinal ferns. May (1978) published a detailed review of the various uses of ferns and listed 105 medicinal ferns. In a recent compilation, Singh (1999) reported 160 species of useful Pteridophytes in India on the basis of phytochemical,
pharmacological and ethnobotanical studies. The antimicrobial potential of some ferns has been studied (Kumar and Kaushik, 1999; Parihar and Bohra, 2002a & b, 2003). Young leaves of the ferns *Diplazium esculentum* (Retz.) Sw., *Helminthostachys zeylanica* (L.) Hook, *Nephrolepis cordifolia* (L.) Presl and *Stenochlaena palustris* (Burm.) Bedd. are cooked as vegetables by the tribal in Indian mountains. *Azolla pinnata* R.Br. is used as rice fertilizer and chicken feed. In the case of water fern *Marsilea drummondii* the starchy paste of the sporocarps is made in to cakes called “Nardoo” and is eaten by the natives of Australia. *Nephrolepis auriculata* (Linn.) Trimen tuber paste is used to lower down the brain fever and headache by applying locally. Croziers of different species of *Diplazium* Sw. is known to be of laxative nature and often used to treat colitis and constipation. *Selaginella bryopteris* (Linn.) Bak is considered as highly useful in unconciousness. Similarly *Helminthostachys zeylanica* (Linn.) Hook. is used to revert the impotency. The paste of *Adiantum incisum* Forssk. and *A. venustum* is useful in the healing of wounds (Samant et al., 1998; Kholia and Punetha, 2005). Quite a number of ferns and ferns allies are of great medicinal value. *Equisetum arvense* Linn. is used in nasal polyps and kidney infections, ashes useful in acidity. *E. debile* Roxb. is diuretic and given in gonorrhoea. *Lycopodium clavatum* Linn., in the form of decoction used in rheumatism and diseases of lungs and kidneys. The paste of the leaves of *Ophioglossum reticulatum* Linn., is used in headache. *Botrychium virginianum* Sw. is used in dysentery. *Helminthostachys zeylanica* (Linn.) Hook. is used for vitality and brain tonic. *Lygodium flexuosum* (Linn.) Sw., is an expectorant and used in ulcers, cut wounds and sprains. *Dicranopteris linearis* (Burm.) Underwood fronds are used for asthma and in woman’s sterility. *Osmunda*
regalis Linn. is used as styptic and tonic. *Angiopteris evecta* (Forst.) Hoffm rhizomes are used for scabies (Vasudev, 1999). The ferns are effective in arresting embryonic development in insects. These substances may be exclusively produced by plants for defense against insect predation. The extracts of pteridophytes have toxic effects on *Spodoptera littura* and *Helicoverpa armigera*. The young fronds of *Phymatosorus scolopendria* (N.L. Burm.) are spread on the bed to keep off bed bugs (Mannan *et al.*, 2008). The tribal communities, ethnic groups and folklore throughout the world are utilizing different parts of pteridophytes like rhizome, stem, fronds, pinnae and spore in various ways for the treatment of various ailments since ancient time. The numbers of contributors about the taxonomy, ecology and distribution of Pteridophytes have been published from time to time but enough attention has not been paid towards their useful aspects, specially the phytochemical part of Pteridophytes.

The phytochemical potential of pteridophytes is relatively unexplored, although pteridophytes possess great economic potential due to some interesting medicinal and antimicrobial properties (Dhiman, 1998; Vasudeva, 1999; Reddy *et al.*, 2001; Singh *et al.*, 2001; Gogoi, 2002; Chen *et al.*, 2005; Singh *et al.*, 2008). Maridass and Raju (2009) investigated the phytochemicals of *Huperzia* leaves using methanol by cold extraction method. The result showed the presence of alkaloids, flavonoids, glycosides and terpenoids. Rathore and Sharma (1990) studied the phytochemical composition of three species of *Isoetes* collected from Rajasthan with respect to pigments, aminoacids, proteins, carbohydrates, reducing sugar and glycosides. Sharma and Sharma (1992) investigated the flavonoid content in common ferns from Rajasthan viz., *Actinopteris radiata, Adiantum lunulatum, A. capillus-veneris, Asplenium pumilum, Tectaria macrodonta, Cheilanthes farinosa, Hypodesmatium crenatum* and *Cyclosorus dentatus*. 
Patric Raja et al. (1992) estimated the pigments, sugars and starch content in twelve species of homosporous ferns collected from Kothayar and Palni hills. De Britto and Manickam (1992) carried out preliminary phytochemical analysis on *Sphaerostephanos unitus*, *S. arbuscula* and *S. substruncatus* and confirmed the occurrence of amino acids, sugars and organic compounds. Gopalakrishnan et al. (1993) studied the phytochemical composition of *Cyathea crinita*, *Cyathea nilgirensis* and *Cyathea gigantea* from Western Ghats. Preliminary phytochemical screening on 19 species of South Indian *Thelypteroid* ferns showed the occurrence of steroid, alkaloid, phenol, catechin, saponin and tannin in all the studied species (De Britto et al., 1994). Irudayaraj (1996) reported the presence of triterpenoid in the epidermal glands of *Christella parasitica*.

Hietz and Briones (2001) investigated the phytochemical constituents present in epiphytic ferns from Mexican cloud forest. Phytochemical investigation of *Cyathea phalerata* showed the presence of an active flavonoid (kaempferol-3-neohesperidoside) with hypoglycaemic activity (Pizzolatti et al., 2007). Kale and Dongare (2007) analyzed total nitrogen, crude proteins and nitrate reductase from *Bolbitis appendiculata*, *Bolbitis virens*, *Osmunda regalis*, *Ceratopteris thalictroides* and *Drynaria quercifolia*. Kale (2007) studied the phytoconstituents present in *Bolbitis appendiculata* from Castle Rock and Anmode, Maharashtra. Johnson et al. (2010) evaluated the phytochemicals of mother plants in-vivo and in-vitro derived callus of *Baliospermum montanum* using ethanol, hexane, chloroform, isopropanol and petroleum ether extract. They reported that calli mediated tissues possesses higher percentage of metabolite constituents. Maridass and Raju (2010) identified the active constituents from different solvent extracts of *Elaphoglossum beddomei*. The results showed the presence of saponins, tannins, polyuronides, alkaloids, sugars, sterol and triterpenes.
Mithraja et al. (2011) evaluated the phytochemical properties of *Azolla pinnata*, *Marsilea minuta* and *Salvinia molesta*. The leaves were extracted successively by petroleum ether, ethylacetate, methanol, chloroform, acetone, benzene and water using soxhlet extractor. They reported that all the extracts contain phenolics followed by tannins, steroids and saponins. Hima et al. (2012) revealed the phytochemical screening of *Hemionitis arifolia* using eight different organic solvents. Yadav and Munin, (2011) investigated the phytochemicals and quantified phenols and flavonoid contents of selected medicinal plants. The plant samples were extracted using hot water extraction and methanol, ethanol and acetone extract. They concluded that crude aqueous and organic solvent extracts contain medicinally important bioactive compounds.

Babu et al. (2012) analysed the phytochemical screening of *Adiantum latifolium* leaves in crude ethanolic extract and reported the presence of flavonoids, triterpenes and saponins. Mithraja et al. (2012) screened the phytochemical properties of some selected medicinally important pteridophytes from Western Ghats, India. They used petroleum ether, aqueous, benzene, acetone, chloroform and ethanol as solvent and reported the presence of phenol, tannin, saponin, steroid, coumarin, carboxylicacid, flavonoids, quinine and alkaloids. Their study concluded that the selected plant extracts may be used as bioactive agents due to the presence of secondary metabolites. Btitto et al. (2012) analysed the phtochemical constituents of five medicinal ferns viz., *Pteris biaurita*, *Lygodium flexuosum*, *Hemionitis arifolia*, *Actiniopteris radiata* and *Adiantum latifolium*. The plants were extracted successively with petroleum ether, benzene, chloroform, methanol and distilled water using soxhlet extractor. Their results showed the presence of more bioactive principles in the studied ferns.
Asim and Amal (2012) performed the qualitative analysis of free aminoacids of some Pteridophytes in West Bengal. The study shows that DLmethionine is the common free amino acid of *Pteris vittata*, *Drynaria quercifolia*, *Ampelopteris prolifera*, *Dryopteris filix-mas*. L-tyrosine monohydrochloride are the common of *D. filix-mas* and *Selaginella indica*. Another free amino acid L-arginine monohydrochloride is also common in *D. quercifolia*, *Ceratopteris thallictroides* and *Marsilea quadrifolia*. Glycine is the only amino acid which is found in the *Helminthostachys zeylanica*. Herin *et al.* (2012) evaluated the qualitative and quantitative analysis of *Pteris confusa*, *Pteris vittata*, *Pteris biaurita* and *Pteris multiaurita* methanolic extract and reported the presence of alkaloids, flavonoids and saponins in the selected fern species. Magda *et al.* (2012) evaluated the phytochemical constituents of some *Salvia* species from Romanian flora. They confirmed that methanolic extracts of *Salvinia officinalis* posseses biologically active principles compared to other studied species. Sukumaran *et al.* (2012) identified the phytococonstituents present in the fronds of *Tectaria zeylanica* using acetone, benzene, chloroform, ethanol, petroleum ether and aqueous extract. Maximum compounds were reported in the aqueous extract followed by benzene and chloroform, acetone and petroleum ether. Magda *et al.* (2012) evaluated the methanolic extracts of nine *Salvia* species present in Romanian flora and reported that *Salvia officinalis* has biologically active principles compared to other species.

Tatik *et al.* (2012) studied the phytochemical composition of *Selaginella* sp. from Java Island Indonesia. The plants were extracted using n – hexane and ethanol. They reported that all the *Selaginella* species contains alkaloids, flavonoids, saponins and steroid compounds. Mithraja *et al.* (2012) examined the phytococonstituents of *Adiantum caudatum*, *Adiantum latifolium*, *Adiantum lumnulatum*, *Christella dentata*
and *Christella parasitica* to provide chemical marker and find inter-specific variation between the medicinally important genuses. They used petroleum ether, ethyl acetate, methanol, chloroform, acetone, benzene and water as solvents. They confirmed that variations of phytochemicals was used to differentiate the two genus *Adiantum* and *Christella*.

Shakoor *et al.* (2013) screened the phytochemical existence in some pteridophytic plant using aqueous, ethanol, methanol and acetone solvent. The samples were extracted by soaking the plant powder in appropriate solvent for 48 hrs and the extract was filtered through Whatman No.1 filter paper. The result showed more of metabolites occurrence in aqueous and ethanolic extract than methanolic and acetone extract. Utkarsh *et al.* (2013) reported the presence of metabolites in *Marsilea minuta* using acetone, benzene, chloroform, aqueous, ethanol and petroleum ether extract.

Patil *et al.* (2013) qualitatively reported the secondary metabolites present in *Dryopteris filix-mas, Angiopteris evecta, Adiantum lunulatum* and *Adiantum incisum*. The plant was successively dissolved in distilled water and then filtered with Buchanans funnel. The results showed that pteridophytic plants contain valuable secondary metabolites which could be used as therapeutic agent. Britto *et al.* (2013) analysed the presence of phytochemicals in the petroleum ether, benzene, chloroform, methanol and aqueous extracts of *Marsilea minuta*. They reported that methanolic extract contain highest amount of phytochemicals compared with other solvents. Manonmani and Sara (2013) recorded the presence of steroids, triterpenoids, sugars, alkaloids, phenolic compounds, catechins, flavonoids, saponins, tannins, anthraquinones and amino acids in six different solvent extracts of *Actiniopteris radiata*. 
Shakoor et al. (2013) studied the presence of different phytoconstituents in aqueous, methanolic, ethanolic and acetone extracts of 34 species of pteridophytes by qualitative screening. The analyses indicated that 34 species (100%) contained carbohydrates, proteins and free amino acids, 27 species (79.41%) with flavonoids, 26 species (76.47%) with phenolic compounds and tannins, 24 species (70.58%) with glycosides, 23 species (67.64%) with terpenoids, 22 species (64.70%) with saponins, 18 species (52.94%) with volatile oils, 15 species (44.11%) with alkaloids, 12 species (35.29%) with phlobatannins and only 3 species (8.82%) with resins.

Forhad et al. (2014) evaluated the phytochemical screening of Angiopteris evecta root methanolic extract. The preliminary phytochemical screening showed the presence of saponins, tannins, alkaloids and flavonoids in the methanolic extract of A. evecta roots. Manasi et al. (2014) evaluated the phytochemical constituents of Asplenium cicutarium root ethanolic and aqueous extract. The result showed better activity in the ethanolic extract of Asplenium cicutarium. Sivagurunathan and Xavier (2014) qualitatively screened the phytochemicals of Marsilea quadrifolia using benzene, ethanol and aqueous extract and revealed the presence of reducing sugar, amino acids, phenolic compounds, flavonoids, phytosterols, tannins, alkaloids, proteins and saponins. Rupa and Lakshmi (2014) carried out preliminary phytochemical screening in the root and stem methanolic, ethanolic and aqueous extract of Selaginella bryopteris. The result showed high number of phytochemicals in methanol extract followed by ethanol and aqueous extract. Kalpana et al. (2014) evaluated the presence of phytochemicals in the aqueous, ethanol and petroleum ether extracts of Actiniopteris radiata, Drynaria quercifolia, Dryopteris cochleata and Pityrogramma calomelanos. The methanolic extract showed better result than aqueous
and petroleum ether extract. Quantitative analysis showed highest content of tannin and phenol in *Pityrogramma calomelanos* fern extract. Muhammad *et al.* (2014) studied the phytochemical screening of leaf, stem and root extracts of *Adiantum capillus veneris* using aqueous, methanolic, ethanolic, ethylacetate and hexane extracts. Phytochemical analysis showed the presence of flavonoids, alkaloids, tannins, saponins, cardiac glycosides, terpenoids, steroids, and reducing sugars. Awadhesh kumar *et al.* (2014) analysed the phytochemical constituents of *Adiantum* and *Pteris* leaves and stem extract. They reported that the selected plants contain flavonoids, phenols, saponins and tannins. Kanniyan *et al.* (2014) qualitatively determined the metabolites present in *Adiantum lunulatum* and *Hemionitis arifolia* using aqueous, ethanol and petroleum ether extracts.

Ruby and Sara (2014) studied the phytochemical composition of *Pyrrosia lanceolata* frond and rhizome. The fronds and rhizome were extracted with various solvents like water, ethanol, benzene, chloroform, petroleum ether and DMSO. All extracts of *P. lanceolata* frond and rhizome showed the presence of flavonoids, terpenoids, phenolics, anthraquinones, catechins, glycosides, fixed oils and fats. Rajesh *et al.* (2014) studied the presence of phytochemicals in the aqueous, ethanolic and petroleum ether extracts of four ferns viz., *Actinopteris radiata*, *Drynaria quercifolia*, *Dryopteris cochleata* and *Pityrogramma calomelanos* by qualitative and quantitative screening methods. Ethanolic extracts of all the four ferns showed strong positivity for major phytochemicals. Phytochemicals like tannins and phenolics were well expressed in ethanolic extracts of the studied four ferns.

Mismawati *et al.* (2015) studied the phytochemical constituents of *Angiopteris evecta* leaves methanolic extracts. Nishika and Usha (2015) quantified the phytochemicals present in *Dicranopteris linearis* and *Pteris vittata*. Their study result
reported maximum quantity of phenol and flavonoid in *Dicranopteris linearis* and tannin content in *Pteris vittata*. Ahmed *et al.* (2015) reported the phytochemical potential of *Drynaria quercifolia* using petroleum ether, chloroform and methanolic extract. Manisha, (2015) reported the presence of alkaloids, steroids, glycosides, proline, phenol, saponins and tannins in *Bolbitis* species viz., *B. virens*, *B. appendiculata* and *B. presliana*. The methanolic extract showed highest amount of phytochemicals in the *Bolbitis* species. Awe and Amobi (2015) analysed the phytochemical composition of *Pteridium aquilinum* leaves using n- hexane and ethanol as solvent and confirmed the presence of maximum metabolites in ethanolic extracts. Jyothi *et al.* (2015) emphasized the phytochemical screening of *Selaginella bryopteris* using petroleum ether, chloroform, methanol, ethanol and aqueous extracts. The results of the phytochemical screening shows the presence of alkaloids, flavonoids, phenols, tannins in the methanolic extract. Manisha, (2015) analysed the presence of phytochemicals of *Cyathea gigantea* using petroleum ether, benzene, chloroform, methanol and aqueous extracts and reported better result in methanolic extract. Janakiraman (2015) performed preliminary phytochemical analysis in five extracts of *Cyathea* viz., *C. nilgirensis*, *C. gigantea* and *C. crinita* using petroleum ether, chloroform, acetone and ethanolic extract. All the three plant species showed significant indication of various bioactive secondary metabolites.

Suvarnalatha *et al.* (2015) analysed the phytochemicals of *Salvinia auriculata* using acetone, ethanol, methanol, ethyl acetate and benzene extracts and reported maximum metabolites presence in methanolic extracts. Gaya *et al.* (2016) studied the phytochemicals of *Salvinia molesta* from North Paravur, Ernakulam, Kerala. The results showed the presence of total carbohydrates, total soluble protein, tannin, total
carotenoids, alkaloids, flavonoids, terpeois, saponin ad phenol. Gouri et al. (2017) reported the physico-chemical analysis of aerial parts of Diplazium esculentum.

2.1. FT-IR Analysis

The FT-IR has proven to be a valuable tool for characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract (Aysal et al., 2007; Ibrahim, 2008). It is a rapid, non-destructive technique with minimum sample preparation necessary (Singh et al., 2011). Duhita et al. (2013) reported FT-IR analysis of Adiantum philippense frond to identify the biogroups that bound distinctively on the gold and silver surface. The major peak observed in FT-IR of the extract were 3369, 2360, 1585, 1384, 1076 and 514 cm\(^{-1}\). Peak at 3369 cm\(^{-1}\) is attributed to OH stretch in phenols. It allows the qualitative determination of organic compounds as the appearance of the bands in the infrared spectrum. Janakiraman (2015) performed the FT-IR spectra to identify the functional groups of C.nilgirensis, C. gigantea and C. crinita. Prasanna and Anuradha (2016) analysed the Fourier transform infrared spectrum profile of methanolic Drynaria quercifolia rhizome and confirmed the presence of amines, alkanes, denatured amines, alkynes, carboxylic acids, alkenes, alkanes and alkenes. John et al. (2018) analysed the FT-IR profile of methanolic extract of Blechnum orientale and confirmed the presence of functional groups such as aldehydes, monosubst benzenes, 1, 2, 4 – trisubst benzenes, organophosphorous, siloxanes, pyridine n- oxides, aliphatic nitro, aliphatic, aminoacids (or) hydrochlorides, aminoacid esters, aliphatic and aromatic, primary amines.
2.2. HPTLC Analysis

A chromatographic fingerprint of a herbal medicine is a chromatographic pattern of the extract of some common chemical components of pharmacologically active and/or chemical characteristics. By using chromatographic fingerprints, the authentication and identification of herbal medicines can be accurately conducted even if the amount and/or concentration of the chemically characteristic constituents is not exactly the same for different samples of drug.

Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic component of the herbal drug. Fingerprint analysis approach using high-performance thin-layer chromatography (HPTLC) has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. It can serve as a tool for identification, authentication, and quality control of herbal drugs (Prema et al., 2015).

Shweta et al. (2013) studied HPTLC profile of aerial parts of four Adiantum species. It was found that the existence of common bands at $R_f$ value 0.34, 0.52, 0.64 and 0.67 (all red) under UV 366 nm and at $R_f$ 0.63 and 0.80 under UV 254 nm in all the four species. However, A. capillus veneris could be clearly differentiated from other species by the band at 0.57 under UV 366 nm. On the contrary, a red coloured band at the same $R_f$ value 0.57 nm was observed in other three species, viz. A. lunulatum, A. peruvianum and A. venustum.

Teny and George (2014) revealed the phytochemical screening by HPTLC in methanolic leaf extract of Hydnocarpus macrocarpa. The analysis confirmed that the leaf extract of H. macrocarpa is rich in phytochemical compounds like alkaloids, essential oils, flavonoids, glycosides, phenolics, saponins, steroids, tannins and
triterpenoids. Sharad et al. (2008) studied the phytochemical composition of *Lycopodium clavatum* and they reported that HPTLC profile of *L. clavatum* showed the presence of ferulic acid.

### 2.3. GC-MS Analysis

A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies (Milne et al., 1993). Hence a thorough validation of the herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of the phytochemicals have complementary and overlapping mechanism of action.

Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC-MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids, lipids (Jie et al., 1991) and alkaloids (Bertz et al., 1997). Niko et al. (2006) identified 25 compounds in *Equisetum arvense* stem essential oil. Zedan et al. (2011) identified 7 compounds in alcoholic extract of *Adiantum capillus-veneris* dried fronds. Kumar et al. (2011) studied the GC-MS analysis of *Polypodium decumanum* ethanolic extracts and reported more than 13 individual compounds. The main compound identified was long chain fatty acids along with the flavonoids. Rukmini and Dubal et al. (2013) identified 21 constituents in the methanolic extracts of *Tectaria coadunata* rhizome using GC-MS analysis. The most prevailing
compounds are octadec–9 enoic acid (oleic acid), n- hexadecanoic acid (Palmitic acid), Octadecanoic acid (stearic acid), Di -n -octyl phthalate, hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester. Suvarnalatha (2014) revealed the presence of six compounds in acetone extracts of Nephrolepis cardifolia. The phytoconstituents screened were Neophytadiene and 2,6,10–trimethyl, 1,4, ethylene, 2 hexadecen-1-ol and 3,7,11,15 tetramethyl R-R hexadecenoic acid and palmitic acid, 9 octadecenoic acid and oleic acid, stigmast–4–en-3-one and 4- stigmasten –3 one.

Santhosh et al. (2014) identified thirty seven bioactive compounds in the methanolic extracts of Adiantum capillus- veneris. They reported that these identified compounds are having antioxidant, antimicrobial, anti inflammatory, diuretic and analgesic properties. Sivagurunathan and Xavier (2014) reported thirteen bioactive compounds in the ethanolic extract of Marsilea quadrifolia. Among the thirteen compounds eight showed anti microbial activity, six showed anti inflammatory, 4 showed anticancer and 2 other showed antioxidant and hypocholesterolemic activity.

Prasanna and Chitra (2014) studied the phytochemical constituents and chemical composition of Drynaria quercifolia rhizome methanolic and petroleum ether extracts. The methanolic extract showed high number of chemical constituents and about 30 different compounds were identified using GC-MS analysis. Nishanthini et al. (2014) performed GC-MS analysis in the stem and leaves extract of Tiliacora acuminate and six compounds were identified in stem and thirteen compounds were identified in the leaf extract of Tiliacora acuminate. Karikalan and Rajangum (2014) investigated the phytochemical constituents of Marsilea quadrifolia leaf and stem methanolic extracts. The GC-MS analysis showed the presence of 39 phytocompounds in the Marsilea quadrifolia leaf methanolic extract and 29 bioactive compounds in the stem methanolic extract of M.quadrifolia.
Rukmini et al. (2015) reported 10 major chemical constituents in the whole plant acetone extracts of *Hemionitis arifolia*. Manonmani and Cathrin (2015) investigated the chemical composition of *Actiniopteris radiata* ethanolic extract using GC-MS and reported five bioactive compounds. Kanchan et al. (2015) reported 7 compounds in the ethanolic extract and 14 compounds in the chloroform extract. The common compounds in these two extracts are Germacrene D; 1, 3- cyclohexanedione, 2- methyl 2- (3- Oxobutyl); Neoisolongifolene, 8, 9 – dehydro. Pradnya et al. (2015) studied the chemical composition of *Chilanthes farinosa* ethanolic extracts using GC-MS analysis and reported the presence of 7 compounds. Kunnathupura et al. (2016) determined the presence of 21 bioactive compounds in the ethanolic extract of *Azolla microphylla* using GC-MS analysis.

2.4. Antioxidant activity

Reactive oxygen species (ROS) which are generated in living system during metabolism (Aruoma and Cuppette, 1997; Cavas and Yurdakoc, 2005) are produced in different forms such as superoxide anion, hydroxyl radical, hydrogen peroxide and nitric oxide (Chewa et al., 2008). Excessive release of these free radicals become harmful because they induce oxidation of biomolecules which results in cell injury and death, and also generate oxidative stress (Ames, 1983; Stadtman, 1992; Wiseman and Halliwell, 1996). Oxidative stress leads to numerous chronic and degenerative diseases such as atherosclerosis, cardiovascular disorder, aging, diabetes mellitus, cancer, neurodegenerative diseases, etc. (Diaz et al., 1997; Metodiewa and Koska, 2000; Young and Woodside, 2001; Heinecke, 2003). The level of free radicals and their negative effects can be mitigated by either natural or synthetic antioxidants (Pryor, 1991; Larson, 1995; Gazzani et al., 1998; Velioglu et al., 1998). The use of synthetic antioxidants has been negatively perceived by nutritionists and consumers.
due to safety and health concern (Sultana et al., 2007), leading to increased interest in natural sources (Rafat et al., 2011). Hung et al. (2007) evaluated antioxidant activities of six folk medicinal ferns and reported that aqueous extracts had higher antioxidant potencies and polyphenol contents than the ethanolic extracts indicating that the aqueous preparation of Gusuibu is more potent than the ethanolic extract. How et al. (2009) investigated antioxidant activity of leaf extracts of medicinal ferns viz. Acrostichum aureum, Asplenium nidus, Blechnum orientale, Cibotium barometz and Dicranopteris linearis and concluded that the leaf extracts of Blechnum orientale demonstrated the highest antioxidant activity. Consequently, one of the major functional properties of pteridophytes that are relevant to human health is their antioxidative property (Lee and Shin, 2010). Nehete and Bhatia (2010) analysed the correlation of antioxidant activity with phenolic content and reported that methanolic extract was rich in polyphenol and flavonoid content and had significant antioxidant activity which is in positive correlation with phenolic content. How et al. (2010) determined antioxidant activity of Blechnum orientale through successive partitioning with petroleum ether, chloroform, ethyl acetate, butanol and water. The result showed strong radical scavenging activity in ethyl acetate, butanol and water. Zakaria et al. (2011) analysed the antioxidant properties of aqueous, chloroform and methanolic extracts of Dicranopteris linearis leaves and reported that methanolic extract of Dicranopteris linearis produced the highest antioxidant activity. How and Yau (2011) evaluated antioxidant activities of the methanolic extracts of selected ferns in Malaysia. They reported that methanolic extracts of Cyathea latebrosa, Cibotium barometez, Drynaria quercifolia, Blechnum orientale and D. linearis showed very high total phenolic content and are potent antioxidants.
Jutarat and Jindarat (2011) determined the flavonoid content and antioxidant activity of ferns by ultrasonic extraction with acetone, ethanol and ethyl acetate and reported ethanol extraction of all ferns have the flavonoid content and antioxidant activity. How et al. (2011) studied the antioxidant properties of some Malaysian ferns. According to them methanolic extracts of *C. latebrosa, C. barometez, D. quercifolia, B. orientale* and *D. linearis* showed very high total phenolic content and are potent antioxidants. Liliana et al. (2012) carried out antioxidant activity of several pteridophytes of Romania and reported that methanolic leaves extract of *Dryopteris filix-mas, D. affinis* and *Athyrium filix-femina* have shown a good antioxidant activity. A positive correlation was obtained between total phenol and the antioxidant activity. These plants could be a good source of natural antioxidants. Sekendar et al. (2012) reported the antioxidant activity of methanolic extract of *Dryopteris filix-mas*. A dose dependent scavenging activity of DPPH radical scavenging radical was observed with good reducing power of the extract. Tsun and Fai (2012) reported high antioxidant properties of *Selaginella willdenowii* aqueous extracts and reported that antioxidant activity was high in leaf aqueous extracts of *S. willdenowii* which may be due to the presence of high phenolic and flavonoid content.

Jianguo et al. (2013) investigated antioxidant potential of *Dryopteris erythrosora* and reported that flavonoids extracted from *Dryopteris erythrosora* showed similar antioxidant activities with rutin. Tania and Suchitra (2013) evaluated antioxidant potential of *Pteris vittata* aqueous and methanolic leaf extract. According to their result both aqueous and methanolic extract showed the presence of phenolic content. The amount of flavonoids were higher in the ethanolic extract and lower in the aqueous extract. The alpha amylase inhibitory potential of both the aqueous and
ethanolic extract of *Pteris vittata* might be due to its phenolic content. Amit *et al.* (2013) evaluated antioxidant activity of some pteridophytes and reported *Diplazium* has strong antioxidant activity. Sivaraman *et al.* (2013) evaluated antioxidant potential of ethanolic extracts of selected species of *Selaginella* and suggested that *Selaginella* species are a natural source of antioxidants. Milon *et al.* (2013) evaluated antioxidant properties of *Drynaria quercifolia* and reported that ethyl acetate and carbon tetrachloride fractions showed very potent antioxidant activity. Mohammed *et al.* (2013) reported antioxidant activity of *Adiantum philippense* leaves methanolic extract using DPPH assay and reducing power. They reported that antioxidant activity may be due to phenolic and flavonoid content present in the leaves extract. Saleha *et al.* (2014) evaluated antioxidant activity of *Diplazium esculentum* and reported that the leaf extract of *Diplazium esculentum* can be used as a natural antioxidant. Johnson *et al.* (2014) determined antioxidant properties of *Asplenium aethiopicum* using methanol, acetone, chloroform and petroleum ether extract. They confirmed that the best free radical scavenging activity was exerted by methanolic extract of *Asplenium aethiopicum*. Saran *et al.* (2014) evaluated *in vitro* antioxidant activity of methanolic extract of some ferns from Mawsynram of Meghalaya. The result confirmed that the pteridophytic plants are also potential sources of natural antioxidants. A positive correlation was observed between the antioxidant activity and phenolic content.

Nishika and Usha (2014) evaluated *in vitro* antioxidant activities of *Pteris biaurita* using methanol, ethanol and hot water extraction. They concluded that hot water, methanol and ethanolic extracts of *Pteris vittata* exhibited appreciable antioxidant activity and good correlation was observed between flavonoids and
antioxidants. Ramakanta et al. (2014) evaluated the antioxidant activity in *Cheilanthes albomarrginata* and reported that ethanolic fraction was found to be the highest polyphenol content which also showed the highest antioxidant activity.

Maneesha et al. (2015) evaluated antioxidant activity of different species of *Pteris vittata* whole plant from Doon valley, Uttarakhand region. The samples were successively extracted with petroleum ether, acetone and ethanol using soxhlet apparatus. They reported that *Pteris vittata* can be used as medicinal plant for the development of health care products for aging and chronic diseases as they are rich in phenolic and flavonoid which are good source of antioxidants. Hassan et al. (2015) analysed the antioxidant activity and phenolic content of eight fern species from North of Iran. They reported that Iranian fern species exhibited strong DPPH and ABTs radical scavenging activity *in vitro* and had the high phenolic compounds content. Kunnathupara et al. (2016) analysed the antioxidant potential of ethanolic extract of *Azolla microphylla* and reported the IC$_{50}$ value of *A. microphylla* as 59.8 µg/ml.

2.5. Cytotoxic activity

*Artemia salina* L. (Artemiidae), the brine shrimp, is an invertebrate of the fauna of saline aquatic and marine ecosystems. BSLB and other *in vivo* lethality tests have been successively employed for bioassay guide fractionation of active cytotoxic and antitumor agents (Krishnaraju and Tsay, 2006; Alim et al., 2007).

Zakaria et al. (2011) evaluated *in vitro* cytotoxic properties of the aqueous, chloroform and methanol extracts of *Dicranopteris linearis* leaves. Rokeya et al. (2013) evaluated the brine shrimp lethality bioassay of the whole plant extract of *Spilanthes paniculata*. They reported that n- hexane, chloroform and ethyl acetate
soluble fractions of methanolic extract possess mild toxicity on shrimp nauplli. Maria et al. (2013) evaluated cytotoxic activity of *Lygodium venustum*. Sreeshma and Bindhu (2014) detected the cytotoxic potential of the ethanolic extract of *Biophytum veldkampii* and *B. reinwardtii* using Brine shrimp lethality assay. They reported that both the plant extracts have potent activity against brine shrimp nauplii.

Saleha et al. (2014) investigated cytotoxic activity of chloroform and methanolic leaf extract of *Diplazium esculentum*. Both extracts produced concentration dependent increment in percent mortality of Brine shrimp nauplii indicating the possible presence of cytotoxic principles in these extracts. Priscilla et al. (2014) reported brine shrimp lethality test in the ethanolic and aqueous extracts of *Selaginella doederleinii*. The 50% lethal concentration (LC$_{50}$) in brine shrimp lethality test using ethanolic and aqueous extract was found to be 1000 ug/ml. Hassan et al. (2015) revealed the cytotoxic activity of eight ferns.

Tsun et al. (2015) studied the cytotoxic potential of six selected edible and medicinal ferns. Meera and Jose kumar (2016) screened the cytotoxicity of epiphytic fern, *Acrostichum heterophyllum* and eporte that *A. heterophyllum* extracts were shown to be non toxic to the brine shrimp nauplii even at a concentration of 1000 µg/ml. The available literature explained the biopotency of the Pteridophytes. But there is no report on the phytochemical profile and antioxidant, wound healing, cytotoxicity, anti-diabetic, hepatoprotective activities of *A. latifolium*, *A. evecta* and *M. fraxinea*.

### 2.6. Anticancer activity

Cancer is one of the most widespread diseases in humans and there is considerable scientific and commercial interest in the continuing discovery of new
anticancer agents from natural product. Currently, over 50% of drugs used in clinical trials for anticancer activity were isolated from natural sources or are related to them (Newman and Crag, 2007). A number of active compounds of plant derived phytochemicals have been shown to possess anticancer activity; these include flavonoids, diterpenoids, triterpenoids and alkaloids (Han et al., 2008).

Somjintana and Nanthita (2005) isolated angiopteroside from the methanolic extract of *Angiopteris evecta*. Angiopteroside showed significant activity for inhibition of HIV – 1 reverse transcriptase and showed anti tumour activity against lung cancer. Nor et al. (2008) evaluated the cytotoxic potential of *Tectaria singaporeana, Blechnum orientale* and *Tacca integrifolia* against breast cancer cells. The result showed that the root methanolic extracts of *Tectaria singaporeana* had the highest cytotoxic potential with an IC$_{50}$ value 28.57 µg/ml.

Hoe et al. (2010) obtained five solvent fractions from the methanolic extracts of *Blechnum orientale* through successive partitioning with petroleum ether, chloroform, ethylacetate, butanol and water. Cytotoxic activity was tested against four cancer cell lines and a non malignant cell using MTT assay. Ethylacetate, butanol and water fractions possesses strong cytotoxic activity towards human colon cancer cell HT – 29. Huanjie and Ping (2010) implicated treatment for breast and prostrate cancer using plant derived terpenoids and reported that terpenoids are able to inhibit tumour cell proliferation and induce tumour cell death by inhibiting multiple cancer specific targets including the proteasome, NF – kB, and antiapoptotic protein Bcl – 2. Zakaria et al. (2010) evaluated in-vitro cytotoxic properties of the aqueous, chloroform and methanolic extracts of *Dicranopteris linearis* leaves. The result showed chloroform
extract was effective only against MCF-7 and HeLa and the methanolic extract was effective against MCF-7, HeLa, HT-29, HL-60, K-562 and MDA-MB-231.

Irene et al. (2011) studied the anticancer activity in Selaginella involvens. Abdul et al. (2011) investigated comparative study on antitumour activity of three pteridophytes viz. Selaginella ciliaris, Marsilea minuta and Thelypteris prolifera. Among the species, highest percentage of tumour inhibition was found in M.minuta extracts. Maria et al. (2013) evaluated cytotoxicity of Lygodium venustum. The ethyl acetate and methanol fractions demonstrated a higher cytotoxic potential. Abdulazeez et al. (2013) evaluated anticancer activity of Peristrophe bicalyculata leaf extracts. The methanolic ethylacetate fraction with an IC<sub>50</sub> value of 15.60 ± 0.5 µg/ml inhibited 50% of the KB cancer cells at concentrations lower than the hexane extract.

Ilkay et al. (2013) evaluated cytotoxicity of Lycopodium clavatum and Lycopodium complanatum sub sp. Chaeajparis. Sw extracts and reported no toxicity was exerted by L. clavatum and L. complanatum chloroform extracts on rat skeletal muscle myoblasts. Sayema and Dewan (2016) studied the cytotoxic activity of Adiantum incisum using N-hexane, chloroform and carbon tetrachloride extract showed better cytotoxic activity. Janakiraman and Johnson (2016) studied anticancer activity of ethanolic extracts of selected Cyathea species. The results showed that ethanolic extracts of Cyathea species had a moderate anticancer activity against MCF 7 cells. Anitta et al. (2016) reported the antitumour potential of phytosterol compositions present in the leaves of mangrove fern Acrostichum aureum. They identified five phytosterols in the leaves of mangrove fern.

2.7. Antidiabetic activity

Diabetes is a multifactorial disease which has become pandemic nowadays in India and worldwide with the highest population engulfed by this disease causing
enormous health problem (Sampath et al., 2012). The free radicals generated due to oxidative stress pose life-threatening state which is common in hyperglycemic condition (Kirana et al., 2009). Alloxan monohydrate (2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetetron) being a toxic glucose analogue, selectively destroys insulin-producing pancreatic β-cells and causes Diabetes Mellitus in experimental animal (Sharma and Kumar, 2011).

Diabetes mellitus Type I is characterized by chronic hyperglycemia, through pathophysiology that involves destruction of pancreatic cells. Type I diabetes affects an estimated 5~10% of all diabetes patients (Daneman and Lancet, 2006). Streptozotocin (STZ) is widely used to induce type 1 diabetes mellitus in experimental animal models (Hayashi and Kojima, 2006; Tian et al., 2010). The STZ-induced diabetic rodent model is usually characterized by hyperglycemia in both the fasting and non-fasting state, reduced serum insulin levels and hyperlipidemia (Mikio et al., 2001). STZ is a nitric oxide donor, nitric oxide was found to bring about the destruction of pancreatic islet cells and STZ by itself was found to generate reactive oxygen species, which contributed to DNA fragmentation and evoked other deleterious changes within the pancreatic tissue (Elsner, 2004; Szkudelski, 2001). STZ-induction cause a production of reactive oxygen species within the pancreatic tissue, and thus it has been used to study natural compounds whether exhibit the antioxidant and protective activity of STZ-induced oxidative stress (Coskun, 2005).

Type II diabetes (Diabetes Mellitus) being non-genetic can be worked out in laboratory by effectively inducing diabetes in Sprague dawley rats by a single dose of alloxan, which is a toxic glucose analogue that selectively destroys insulin producing cells in the pancreas leading to non insulin dependent diabetes mellitus (Sharma and
Kumar, 2011). Nowadays the antidiabetic drugs that are highly preferred are mainly sulfonylureas and biguanides which are known to cause various adverse effects (Jain et al., 2006). In the past few decades there is an upsurge of interest in the therapeutic potential of the medicinal plants. Many natural extracts are known for their hypoglycemic and antioxidant property in laboratory animals (Venkataratnam et al., 2006). However, it was found that they are less effective in lowering glucose levels in severe diabetes (Semwal et al., 2008).

Tania et al. (2012) evaluated the antidiabetic potential of Adiaantum philippense in hyperglycaemic induced alloxan monohydrate ethanolic and aqueous extracts. They reported that Adaintm philippense possess antihyperglycemic activity. Tania et al. (2012) investigated antihyperglycemic activity of aqueous and ethanolic extracts of Pteris vittata in alloxan induced Sprague Dawley rats. The study result employed that aqueous and ethanolic extract of Pteris vittata showed almost 77% and 72% decrease in the plasma glucose levels respectively in the experimental animal at an interval of 21 days.

Chand et al. (2013) studied the in-vitro antidiabetic activity of whole plant extract of Actiniopteris radiata. The result showed that chloroform extracts have more significant antidiabetic activity compared to n-hexane, ethanolic extracts. Rabiea et al. (2013) evaluated antihyperglycemic activity of Christella dentata whole plant methanolic extract through oral glucose tolrance tests in glucose loaded Swiss albino mice. The methanolic extract showed dose dependant significant lowering of blood sugar levels when orally administered to glucose challenged mice at doses of 100, 200 and 400 mg/kg bodyweight.
Samira et al. (2014) reported the preliminary antihyperglycemic activity of *Angiopteris evecta* leaves in Swiss Albino mice. Administration of whole plant methanolic extract of led to dose dependent reductions in blood. They concluded that leaves extract of *Angiopteris evecta* can be used for lowering blood sugar level. Cheng et al. (2015) isolated three novel compounds, 13-chloro-spelosin 3-O-β-D-glucopyranoside, (3R)-Pterosin D 3-O-β-D-(3'-p-coumaroyl) - glucopyranoside and (2R,3R) - Pterosin L 3-O-β-D- (3'-p-coumaroyl) -glucopyranoside, from four fern species viz. *Ceratopteris thalictroides*, *Hypolepis punctata*, *Nephrolepis multiflora* and *Pteridium revolutum* and reported these compounds have potential antidiabetic activity.

Mordi et al. (2016) examined the glucose lowering effect possibly associated hypolipidaemic property of *Dryopteris dilatata* leaf extract in STZ induced diabetic rats. They reported that treatment of diabetic rats with the aqueous extract of *Dryopteris dilatata* caused a significant decrease in liver blood glucose level (400, 800 and 1000 mg/kg). Cheng et al. (2017) evaluated the effect of (-) epicatechin3-O-β -d allopyparanoside isolated from *Davallia formosana* rhizome on type I diabetes mellitus and dislipidemia in streptozotocin (STZ) induced diabetic mice and reported (-)- epicatechin 3-O- β -D allopyparanoside (BB) in streptozotoan induced diabetic mice may have the ability to lower blood glucose and triglyceride levels there by ameliorating hyperglycemia and hypertriglyceridemia.

2.8. Hepatoprotective activity

The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges like xenobiotics, drugs, viral infections and chronic alcoholism (Ramachandra et al., 2007). An exposure to the above mentioned
challenges shows that the natural protective mechanism of the liver are over powered and leads to hepatic injury (Wolf, 1999). Hepatic damage is always associated with the cellular necrosis, the increase in tissue lipid peroxidation and the depletion in the tissue glutathione (GSH) levels (Sandy and Ben, 1998). Moreover, serum levels of many biochemical markers like aspartate transaminase (AST), alanine transaminase (ALT), serum alkaline phosphatase (ALP), triglycerides, cholesterol and bilirubin are also elevated. In short, liver disorders still remain common and unconquered.

Modern medicines have little to offer for alleviation of hepatic diseases and only limited numbers of drugs are available for the treatment of liver disorders. The herbal based preparations were effective for the treatment of liver disorders (Karan et al., 1999; Chaterrjee, 2000). Therefore, several herbal medicines are experimented for their possible antioxidant and hepatoprotective effects against various chemical induced liver damages in animals. Carbon tetrachloride (CCl₄) is a widely used hepatotoxic agent in rodents and its trichloromethyl radical (•CCl₃) induced toxicity in rat's liver which closely resembles human cirrhosis (Al-Shabanah et al., 2000). Hence, it is an acceptable animal model for analyzing hepatoprotective activity.

Royal et al. (2012) evaluated hepatoprotective activity of Adiantum incisum methanolic leaf extract against CCl₄ induced hepatotoxicity in rats. They reported flavonoid constituents of Adiantum incisum possess hepatoprotective properties. Administration of methanolic extraction of A.incisum leaf at two different doses level attenuated the increased levels of the serum enzymes, produced by CCl₄ and caused a subsequent recovery towards normalization almost like that of silymarin treatment. Madhu et al. (2012) investigated hepatoprotective activity of Cyathea gigantea methanolic leaves extract against paracetamol induced hepatotoxicity in rats. They concluded that phytochemical screening of methanolic leaf extract of
C. gigantea showed the presence of triterpenes, sterols, flavonoids, phenols and saponins and these antioxidant phytochemicals of C. gigantea might contribute to its hepatoprotective activity.

Divya et al. (2013) studied the hepatoprotective effect of methanolic whole plant extract fractions of Marsilea minuta. The results of the study revealed that the steroidal triterpenoidal and flavonoidal glycosides and phenolic compounds are present in butanol fraction Marsilea minuta methanolic extract. The literature reveals that the plants containing steroid, triterpenoids and flavonoids can control liver diseases Pradeep and Ajudhia (2013) evaluated the hepatoprotective effect of hydroalcoholic extract of Drynaria quercifolia fronds. They concluded that the plant Drynaria quercifolia exhibited hepatoprotective potential due to the presence of compounds of Dq-4 like flavonoids substances.

Devika and Prasanna (2016) evaluated the in vitro hepatoprotective activity of methanolic extract of Drynaria quercifolia rhizome. They reported that potential hepatoprotective activity of Drynaria quercifolia may be due to the presence of various phytochemical such as flavonoids and alkaloids compounds.

2.9. Wound healing activity

Wound healing is currently a clinical challenge due to inconsistencies encountered in the healing processes. Medical treatment includes administration of drugs either locally (topical) or systemically (oral or parenteral) with the aim to either shorten the time required for healing or to minimize the undesired consequences during wound repair (Myers et al., 1980). Medicinal plants have generated much interest for treatment of skin ailments as they are affordable and purportedly safe from
hypersensitive reactions (Raina et al., 2008). Wound is defined as a breaking of cellular and anatomical or functional continuity of living tissue (Prabu et al., 2008).

There are three phases in the process of wound healing. Phase 1 is the coagulation and inflammatory phase that involves migration of neutrophils at the margin of incision. Phase 2 is the proliferative phase which is characterized by angiogenesis, collagen deposition, epithelization and wound contraction. Angiogenesis involves new blood vessel growth from endothelial cells. Granulation tissue progressively invades the incision space. Collagen fibrils become more abundant and begin to bridge the incision. At this phase, the epithelization depends on the migratory, proliferative and differential abilities of keratinocytes and these are regulated by growth factors such as epidermal growth factor family and fibroblast growth factor family (Fu et al., 2007; Nayak et al., 2009). Phase 3 is a remodeling phase involving continuous accumulation of collagen and proliferation of fibroblasts. This phase involves synthesis of collagen fibers, leading to increase in tensile strength of the skin (Jorge et al., 2008). Alterations in any of these steps can lead to healing delay or even the inability to heal completely (Akkol et al., 2009). How et al. (2011) examined wound healing activity in the aqueous extract of Blechum orientale on Sprague Dawley rats. The result confirmed that 25 % (w/w) concentration was capable of producing significant wound healing activity.

Manjunatha et al. (2007) reported the wound healing activity of aqueous and ethanol leaf extracts of Lycopodium serratum. The result confirmed that ethanolic extract treated animals showed faster epithelisation of wound. Hendy et al. (2013) studied the wound healing properties of Acrostichum aureum and A.speciosum ethanolic extract. The result confirmed that topical application of both extracts shown significant effect in wound healing process. Marco et al. (2016) isolated flavonoid
oligoglycosides from *Ophioglossum vulgatum* which possesses wound healing properties. Two new glycosylated and acylated flavonols, viz. quercetin-3-O-[(6-caffeoyl)-β-glucopyranosyl (1→3) α-rhamnopyranoside]-7-O-α-rhamnopyranoside (2), and kaempferol-3-O-[(6-caffeoyl)-β-glucopyranosyl (1→3) α-rhamnopyranoside]-7-O-α-rhamnopyranoside (3), together with the known quercetin-3-O-methyl ether were isolated from the aerial parts of the fern *Ophioglossum vulgatum*. These 3 compounds were all found to be active in scratch wound healing assays on keratinocytes.

Ranjan *et al.* (2014) studied wound healing activity of *Drynaria quercifolia* rhizome. The rhizome was subjected to successive extraction using n- hexane, petroleum ether, chloroform and methanol and finally with distilled water using soxhlet extractor. They reported that the rate of wound contraction was significantly higher in the animals treated with methanol herbal extracts compared to the reference drugs (ie) Neosporin. Furthermore the methanol herbal (DQ) extract exhibited significantly decreased period of epithelisation compared to controls. Wound dressed with both methanolic extract (105) and chloroform (105) found to be epithelised fast. Azam *et al.* (2015) studied the effect of *Equisetum arvense* ointment on wound healing activity. According to their results *Equisetum arvense* ointment promoted wound healing and relieved pain during the 10 day period after episitomy.