Chapter 2

Review of Literature

2.1 Introduction

Philipson (2001) gave history regarding the contribution of plants to medicine. Plants are storehouses of a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids that have demonstrated their antimicrobial properties *in vitro*. Many pharmaceutical products are of plant, bacterial or fungal origin, although synthetic organic compounds are subsequently used and have proved their value in a broad range of human infections. The accessible literature in this regard has been reviewed in the section.

2.2 Medicinal Plants and their antibacterial properties

Medicinal plants have diverse biological activities. A large number of medicinal plants have been identified for antibacterial activities. Antibacterial potential of major plant groups has been explored in this section.

2.2.1 Antibacterial activity of lower group of plants (Bryophytes)

Antibacterial activity of several bryophytes was reported by Gnanaguru et al., (1992). An acetone extract of the moss *Pleurochaete squarrosa* was tested against eleven bacterial strains, some of which were pathogenous for man. The extract was active on some gram-negative strains (Basile et al., 1998). Acetone extract from the moss *Rhynchostegium riparioides* was tested against eleven bacterial strains, some of which were pathogenic. The extract was active on some Gram-negative strains (Basile et al., 1998).

Seven flavonoids were isolated and identified from five moss species. The flavonoids were the flavones apigenin, apigenin-7-O-

triglycoside, lucenin-2, luteolin-7-O-neohesperidoside, saponarine and vitexin; and the biflavonoid bartramiaflavone. Some of these flavonoids were shown to have pronounced antibacterial effects against *Enterobacter cloaceae*, *E. aerogenes* and *Pseudomonas aeruginosa* with a minimal bacteriostatic concentration MIC in the range of 4-20-48 micrograms/ml (Basile et al., 1999).

Most liverworts (Hepaticae) contain oil bodies which are composed of lipophilic terpenoids and aromatic compounds (Asakawa, 2004). The antimicrobial activity of *Bryum argenteum* ethanol extracts has been evaluated by microdilution method against four bacterial (*Escherichia coli*, *Bacillus subtilis*, *Micrococcus luteus* and *Staphilococcus aureus*) and four fungal species (*Aspergillus niger, Penicillium ochrochloron*, *Candida albicans* and *Trichophyton mentagrophyes*). All the investigated ethanol extracts were proved to be active against all bacteria and fungi tested (Sabovljevic et al., 2006). The antimicrobial activity of the methanol extracts of eight moss species was observed at 30 mg/ml concentration against the tested microorganisms (Dulger, et al., 2005). Antimicrobial activities of fifteen Indian mosses have been studied and *Sphagnum junghuhnianum*, *Barbula javanica*, *Barbula arcuata*, *Brachythecium populeum*, *Brachythecium rutabulum*, *Mnium marginatum* and *Entodon rubicundus* were reported to be most active against all the tested organisms (Singh et al., 2007).

2.2.2 Antibacterial activity of Pteridophytes, especially Ferns

Gleichenia linearis, a fern showed antibacterial properties in water extracts (Vasudeva, 1999). Friedelin, epifriedelirol, beta amyrin, beta sitosterol, 3-beta-D-glucopyraroside, and naringin

were isolated from dried rhizome of *Drynaria quercifolia.* The methanol extract of *Drynaria quercifolia* showed broad and concentration dependent antimicrobial activity (Ramesh et al., 2001). Cytotoxic and antioxidant effects of *Drynaria fortunei* were observed (Liu et al., 2001). Flavonoid of *Drynaria fortunei* was found to protect against gentamicin ototoxicity (Long et al., 2004). Flavonoid of *Drynaria fortunei* has use against renal failure (Long et al., 2005). It has been reported the use of *Drynaria quercifolia* against *Neisseria gonorrhoeae* (Shokeen et al., 2005). Antimicrobial compounds have been characterised from a common fern, *Pteris biaurita* (Dalli et al., 2007).

Nine species of *Selaginella* showed bioactivity, and exhibited antiviral, anti-inflammatory, antifungal, antimicrobial, and antioxidant properties (Silva et al., 1995, Sun et al., 1997, Lee et al., 1999, Lin et al., 2000, Woo et al., 2005, Chen et al., 2005). Two *Selaginella* species showed an inhibitory effect on muscle contraction (Perez et al., 1994, Rojas et al., 1999). *Pteris semipinnata* demonstrated anti-tumour activity in two separate investigations (Li et al., 1998, Li et al., 1999). There were other species of *Pteris* possessed antimutagenic, immunomodulatory, and neuronal activity (Goldberg and Cooper, 1975, Lee and Lin, 1988, Wu et al., 2005).

Pteris vittata exhibited carcinogenic activity, (Siman et al., 2000) and provided an example of a secondary compound that was actually harmful to animals. *Christella dentata* also tested for carcinogenic activity and was observed (Somvanshi and Sharma, 2005). *Lycopodium* species showed antiacetylcholinesterase activity in two separate experiments (Zhang et al., 2002, Hirasawa

et al., 2006). *Lycopodiella cernua* was antivirally active and had been patented as a treatment for Hayfever (Cambie and Ash, 1994, Zhang et al., 2002).

Cancer treatments would be an area of much scientific and medical interest. Perhaps it is surprise that the genera, *Pityrogramma* that have been tested the most for bioactivity are those where cytotoxicity has been found in some of the species. The *Pityrogramma calomelanos*, a Moorean, fern, is cytotoxic and contains flavonoids (Star and Mabry, 1971, Sukumaran and Kuttan, 1991). *Pteris semipinnata* and *Pteris multifada* are both cytotoxic, but they contain diterpenes (Li et al., 1998, Li et al., 1999).

2.2.3 Antibacterial activity of higher plants (Angiosperms)

Angiosperm plants are sources of very potent and powerful drugs with antibacterial properties (Iyenger, 1985, Chopra et al., 1992, Behl and Arora, 1993, Youvraj et al., 1995, Ibrahim and Osman, 1995). According to Cristina and Claudia (1994), clinically useful antimicrobials against multi-drug resistant *Salmonella typhi* can be produced from *Heimia salicifolia* aerial parts, *Punica granatum* fruit pericarp and *Rosa borboniana* flowers. *Azadirachta indica* and *Psidium guajava* are potential sources of antibacterial agents (Akpulu et al., 1994).

The results of antibacterial assay of 267 plant extracts from 100 Rwandese medicinal plants, used by traditional healers to treat infections, exhibited prominent activity against various bacterial strains (Vlietinck et al., 1995). Martinez et al., (1996) tested the antimicrobial activities of 23 extracts of 12 traditionally used Cuban medicinal plants by agar diffusion method. Nine

extracts were active against gram positive bacteria, but only two of them showed activity against gram negative bacteria. Of these, the best activity was shown by *Schinus terebenthifolius*.

Ahmad et al., (1998) assayed eighty-two traditionally used Indian medicinal plants for preliminary antibacterial screening against several pathogenic and opportunistic microorganisms. The results indicated that fifty-six exhibited activity against one or more test pathogens. Vincenzo et al., (1998) performed a preliminary screening of the antibacterial activity of *Minthostachys verticillata* oil and showed inhibition of the growth of gram-positive and gram-negative strains.

Arora and Kaur (1999) assayed the antibacterial activity of certain spices and they suggested that spices might have a great potential to be used as antimicrobial agents. On screening eight Nigerian medicinal plants used traditionally in the treatment of infectious and septic diseases in both humans and animals for antibacterial activity, *Angeiossus schimperi* and *Anacardium occidentale* exhibited significant activity against *Escherichia coli* and *Pseudomonas aeruginosa* (Kudi et al., 1999).

Pteleopsis suberosa was used in the traditional medicine of Mali for the treatment of gastric and duodenal ulcers. The decoction and the methanolic extract were active against all the bacterial strains tested. The minimum inhibitory concentrations (MIC) ranged from 62.5 to 500 μgml^{-1} for the decoction and from 31.25 to 250 μgml^{-1} for the methanolic extract (Germano et al., 1998). According to Mansouri (1999) *Myrtus communis* leaves had the greatest activity among the ethanolic extracts of ten plants tested as to the inhibition of the growth of *Staphylococcus aureus*.

Samy and Ignacimuthu, (2000) screened Indian folklore medicinal plants used by traditional healers using disc diffusion method. Among them, the leaf extracts of *Cassia occidentalis* and *Cassia corniculata* exhibited significant broad-spectrum antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*. Antibacterial activity of Zulu medicinal plants exhibited that methanolic extracts of *Chelianthes viridis, Dioscorea dregeanam, Dioscoria silvatica* and *Molianthus cosmosus* exhibited activity against both gram positive and gram negative bacteria (Kelmanson et al., 2000).

Therapeutic value and wide spectrum antimicrobial action of garlic *(Allium sativum*) and onion *(Allium cepa)* had been studied by Sharma et al., (1977), Dankert et al., (1979), Shashikanth et al., (1981), Elnima et al., (1983), Sreenivasamurthy et al., (1983), Singh and Shukla (1984), Deleha and Gargusi, (1985), Grainge et al., (1985), Adetumbi et al., (1986), Focke et al., (1990), Ghannoum (1990), Choudhary et al., (1991), Forhman et al., (1993), Jain (1993), Rees et al., (1993), Gouz et al., (1994), Wen et al., (1995), Augusti (1996), Cellini et al., (1996), Ankri and Mirelman, (1999), Sasaki and Kita, (2003), Iwalokun et al., (2004), Bakri and Douglas, (2005), Ruddock et al., (2005), Amin and Kapadnis, (2005), Tessema et al., (2006), O'donnell and Gibbons, (2007).

2.3. Phytopharmaceuticals

Plants provide large number of phytopharmaceuticls, these phytopharmaceuticals have different biological activities. Phytochemicals in plants include alkaloids, monophenols, monoterpenes, anthocyanins, carotenes, coumestans, flavan-3-

ols, hydroxycinnamic acids, isoflavones, lignans, organosulfides, phenolic acids, phytosterols, triterpenoids, xanthophylls etc. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. There are more than thousand known phytochemicals. It is well known that plants produce these chemicals to protect it, but recent researches demonstrate that they can protect humans as well against diseases. How do phytochemicals work? There are many phytochemicals and many different mechanisms. These are some possible actions: (a) antioxidant (b) hormonal action (c) Stimulation of enzymes (d) interference with DNA replication (e) anti-bacterial effect; some phytochemicals bind physically to cell walls thereby preventing the adhesion of pathogens to human cell walls. In this section a search for phytopharmaceuticals from various plant-groups is done.

2.3.1 Phytochemicals from lower group of plants (Lichens) and its biological activity

Aphthosin, an example of a tetradepside, has been isolated from *Peltigera aphthosa* (L.) Willd (Lichen). Its structure has been determined from spectroscopic data and hydrolysis to eveminic acid, orsellinic acid and methyl orsellinate (Bachelor and King, 1970). Seven pure flavonoids were isolated and identified from five moss species. The flavonoids were the flavones apigenin, apigenin-7-O-triglycoside, lucenin-2, luteolin-7-O-neohesperidoside, saponarine and vitexin and the biflavonoid bartramia flavone and they showed reduction in spore germination (Basile et al., 2003). Eight components, depsides and orcinol derivatives which exhibit growth-inhibitory activity against lettuce seedlings were isolated

from *Usnea longissima* (Nishitoba et al., 1987). Eleven compounds isolated from the extract of the Central Asian lichens comprised eight new glycosides having murolic, protoconstipatic and allo-murolic acids, as the aglycones and a saccharide moiety linked at C-18 made up of one or two sugars (glucose and apiose or rhamnose or xylose or arabinose) (Rezanka and Guschina, 2000).

Usnic acid [2,6-diacetyl-7, 9-dihydroxy-8, 9b-dimethyl-1, 3(2H,9bH)-dibenzo-furandione] has become the most extensively studied lichen metabolite and one of the few that is commercially available. Usnic acid is uniquely found in lichens, and is especially abundant in genera such as Alectoria, Cladonia, Usnea, Lecanora, Ramalina and Evernia. Many lichens and their extracts containing usnic acid have been utilised for medicinal, perfumery, cosmetic as well as ecological applications. Usnic acid as a pure substance has been formulated in creams, toothpaste, mouthwash, deodorants and sunscreen products, in some cases as an active principle, in others as a preservative. In addition to antimicrobial activity against human and plant pathogens, usnic acid has been shown to exhibit antiviral, antiprotozoal, antiproliferative, anti-inflammatory and analgesic activity (Ingolfsdottir, 2002).

2.3.2 Phytochemicals from lower group of plants, especially from ferns and its biological activities.

Violanthin and isoviolanthin were isolated from the fern *Angiopteris evecta* (Wallace et al., 1979). The molecular structure of the biologically active diterpene alcohol isolated previously from the root-stalks of *Dicranopteris pedata* and *Gleichenia japonica* was confirmed to be (6 S, 1 3 S)-cleroda-3,14-diene-6,13-diol. Further investigation of the root- stalks of *Dicranopteris pedata* resulted in the isolation of two new glycosides, which were characterised as (6S,

13S)-6 -O- [b-D-glucopyranosyl -(1-4)-α-L-rhamnopyranosyl]-l3-O-[α-L-rhamnopyranosyl-(1-4)-β-D-fucopyranosyl]- cleroda-3,14-dieneand (6S,13 S-6-O-[β-glucopyranosyl]- 13-O-[β-fucopyranosyl -(1-2)-α-rhamnopyranosyl] -cleroda-3,14-diene. Of these two glycosides, the former glycoside accelerated the growth of the stems of lettuce and inhibited the growth of the roots (Aoki et al., 1997).

Leaves of the fern *Pityrogramma ebenea* yielded a new compound 2',6'-dlhydroxy -4,3'-dlmethoxy -4',5'-methylenedloxydlhydrochalcone was characterized (2S)-5, 7-dihydroxy-4'-methoxy-6, 8-dimethyl flavanone was identified, another fern, *Blechnum regnelhanum* also yielded the same compound (Miraglia et al 1985). Two new ecdysteroid glycosides reported from *Blechnum minus,* 2deoxyecdysone 3-β-D-glycopyranoside (blechnoside A) and 2deoxyecdysone 25-β-D glucopyranoside (blechnoside B) (Suksamrarn et al., 1986).

2Deoxy-3-epiecdysone and ecdysone were isolated from fronds of the fern *Blechnum vulcanicum* (Russell et al., 1981). Phytochemical called *sulphoquinovosyl diacylglycerol* was isolated exhibited antibacterial activity (Vasange et al., 1997). The flavonoids of the primitive leptosporangiate ferns *Stromatopteris moniliformis, Schizaea bifida*, *Gleichenia cunninghamii*, *Cardiomanes reniforme* and *Hymenophyllum demissum* had been identified as 3-O-glycosides of the flavonols kaempferol and quercetin. None of the examined ferns produced flavonoids, which were also common to the Psilotaceae. The Psilotaceae had previously been shown to produce O-glycosides of amentoflavone

(biflavone) and apigenin (flavone) and traces of C-glycosylflavones (Wallace and Markham, 1978).

Identification of 6 apigeninidin and 6 luteolinidin anthocyanins from the fern *Blechnum procerum* (Forst. f) Schwartz was reported (Crowden and Jarman, 1974). Rosmarinic acid, an ester of caffeic acid and 3, 4-dihydroxyphenyllactic acid was observed in ferns of family Blechnaceae (Hausler et al., 1992). Flavonoids of four species of *Angiopteris* indicated that di-C-glycosylflavones and flavone-o-glycosides might be characteristic of this distinct group of eusporangiate ferns. Derivatives of flavonols, which were typical of leptosporangiate ferns and Ophioglossum, or biflavones, which were characteristic of the Psilotaceae were not detected in *Angiopteris* (Wallace et al., 1981).

New gibberellin-like antheridiogen from gametophytes of the fern *Lygodium circinnutum* has been confirmed as the methyl ester of 9, 11 -didehydro-GA_{20} by synthesis of an authentic sample from gibberellic acid (GA,). Comparative bioassays of the synthetic compound as an antheridium inducing substance in *Lygodium, japonicum* have demonstrated that it is highly potent (Wynne et al., 1998).

2.3.3 Phytochemicals from Gymnosperms and their biological significance

Gymnosperms are naked seed bearing plants and form an evolutionary bridge between pteridophytes and angiosperms (Srivastava, 1995). Six flavonoid constituents (quercetin, isorhamnetin, kaempferol, bilobetin, ginkgetin and sciadopitysin) were isolated from *Ginkgo biloba* leaves (Chi et al., 1997). One compound was isolated for the first time from ethyl acetate extract

of *Ginkgo biloba* leaves and identified as kaempferol-3-O-rhamnoside by spectroscopic methods (Chi and Xu, 1998). A Compound was isolated from the ethyl acetate extract of *Ginkgo biloba* leaves for the first time and was identified as 5,7,4'-trihydroxy-flavone by spectroscopic methods (Chi and Xu, 1998).

Three active constituents were found in the stem barks of *Taxus cuspidata* Sieb. et Zucc. They were baccatin I 1-hydroxy, taxinine J 2-deacetoxy and beta-sitosterol (Mao et al., 1999). The taxoid chinentaxunine has been isolated from the seeds of Chinese yew *Taxus chinensis*, and its structure determined on the basis of spectral and chemical methods. In addition, the known taxol C, paclitaxel, 10-deacetyl taxol A, 10-deacetyl-7-epitaxol, 10-deacetyl-10-oxo-7-epi-taxol, taxinine M, taxchinin A, 10-deacetyl taxinine B and taxuspine X were also isolated and identified from this source (Shen et al., 1999).

A new C-methyl flavonol glycoside, 5,7,8,4'-tetrahydroxy-3-methoxy-6-methylflavone 8-O-beta-D-glucopyranoside was isolated from the needles of *Pinus densiflora*, together with kaempferol 3-O-beta-(6"-acetyl)-galactopyranoside (Jung et al., 2001). A taxine, 5 alpha O-(3'-dimethylamino-3'-phenylpropionyl) taxinine M, together with two known compounds 7-O-acetyltaxine A, and 2 alpha-acetoxy-2' beta-deacetylaustrospicatine, were isolated from the needles of the Himalayan yew, *Taxus wallichiana* Zucc. Their structures were elucidated on the basis of the NMR spectral data, ESI-MS/MS analysis and chemical methods. First and last compounds showed moderate cytotoxic activity against the lung cancer cell line A549 in vitro (Prasain et al., 2001).

Two coumaroyl flavonol glycosides, isorhamnetin 3-O-alpha-L- [6"'-p-coumaroyl-(beta-D) -glucopyranosyl-(1,2)-rhamnopyranoside], and kaempferol 3-O-alpha-L-[6"'-p-coumaroyl-(beta-D)-glucopyranosyl-(1,2)-rhamnopyranoside]-7-O-beta-D-glucopyranoside, were isolated from the n-butanol extract of *Ginkgo biloba* leaves. These two, together with six other flavonol glycosides, kaempferol 3-O-alpha-L- [6"'-p-coumaroyl-(beta-D)-glucopyranosyl-(1,2)-rhamnopyranoside], quercetin 3-O-alpha-L-[6"'-p-coumaroyl-(beta-D)-glucopyranosyl-(1,2)-rhamnopyranoside], quercetin 3-O-alpha-L-[6"'-p-coumaroyl-(beta-D)-glucopyranosyl-(1,2)-rhamnopyranoside]-7-O-beta-D-glucopyranoside, quercetin 3-O-beta-D-glucopyranosyl-(1-2)-alpha-L-rhamnopyranoside, quercetin 3-O-beta-rutinoside and quercetin 3-O-beta-D-glucopyranoside showed profound antioxidant activities in DPPH and cytochrome-c reduction assays using the HL-60 cell culture system (Tang et al., 2001).

A series of new taxoids, named taxus pines A-H and J-Z and taxezopidines A-H and J-L, have been isolated together with 37 known taxoids including paclitaxel from the Japanese yew, *Taxus cuspidata* Sieb. et Zucc. (Taxaceae). Among the new taxoids, some non-taxol-type compounds remarkably reduced $CaCl_2$-induced depolymerization of microtubules, or increased cellular accumulation of vincristine in multidrug-resistant tumour cells as potent as verapamil. On the other hand, chemical derivatization of taxinine, one of major taxoids obtained from this yew, led to the discovery of unusual reactions of taxinine derivatives. Here we describe our recent results on the isolation, structure elucidation, and bioactivity of these new and known taxoids and the formation

of unexpected products of the unusual reactions of taxinine (Kobayashi and Shigemori, 2002).

A new stilbene, gnetifolin M (1), was isolated from the lianas of *Gnetum montanum*, together with seven known compounds, resveratrol (2), gnetol (3), 4', 5, 7-trihydroxy-3'-methoxyflavone, beta-sitosterol, daucosterol, ursolic acid, and tetracosanoic acid. The structure of 1 was determined to be 2-(5'-methoxy-3'-hydroxyphenyl)-4-hydroxybenzofuran (Xiang et al., 2002). Three new lanostane-type triterpenoids were isolated from the bark of *Abies sachalinensis* (Wada et al., 2002). Two new phenol glucosides termed juniperosides I (1) and II (2) were isolated, together with known two biflavones, cupressuflavone and amentoflavone and a diterpene, 3beta-hydroxy sandaracopimaric acid, from leaves of *Juniperus occidentalis* Hook. (Cupressaceae) collected in Oregon, U.S.A., and their structures were established as (1S) - and (1R)-1-(2'-hydroxy-6'-methylphenyl) ethanol 2'-O-beta-D-glucopyranosides (1, 2), respectively, on the basis of spectral, chemical, and synthetic evidence. The glycosides 1 and 2, as well as the corresponding aglycones 1a and 2a, are apparently novel types of naturally occurring compounds; isolation of these types of natural phenol derivatives has only rarely been reported from the vegetable kingdom (Nakanishi et al., 2002).

Bilobetin and 4'''-O-methylamentoflavone were isolated and identified from the needles of *Taxus baccata* for the first time. The antifungal activity of biflavones from *T. baccata and Ginkgo biloba*, namely amentoflavone, 7-O-methylamentoflavone, bilobetin, ginkgetin, sciadopitysin and 2,3-dihydrosciadopitysin towards the fungi *Alternaria alternata*, *Fusarium culmorum*, *Cladosporium*

oxysporum was determined. Bilobetin exhibited a significant antifungal activity with values of ED50 14, 11 and 17 microM respectively. This compound completely inhibited the growth of germinating tubes of *Cladosporium oxysporum* and *Fusarium culmorum* at a concentration 100 μM. Activity of ginkgetin and 7-O-methylamentoflavone towards *Alternaria alternata* was stronger than that of bilobetin. Moreover, slight structural changes in the cell wall of *Alternaria alternata* exposed to ginkgetin at concentration of 200 μM were observed (Krauze-Barnowska and Wiwart, 2003).

Four new cephalotaxus alkaloids, cephalotaxine alpha-N-oxide (1), cephalotaxine beta-N-oxide (2), 11-beta-hydroxycephalotaxine beta-N-oxide (3), and isocephalotaxine (4), were isolated, together with several known alkaloids from an EtOAc extract of the fruits of *Cephalotaxus fortunei*. Compounds 1, 2, 3, and 4 displayed cytotoxicity against nasopharynx KB cells with IC50 values of 30, 14, 31, and 15 μg/ml, respectively (Bocar et al., 2003).

From the autumnal leaves of *Metasequoia glypstostroboides* were isolated: 3'-O-glucoside tricetin and ginkgetin, bilobetin, 2,3-dihydroisoginkgetin--new compounds in this plant (Krauze-Baranowska, 2004). A new homoerythrina alkaloid, C-3-epi-wilsonione, a new tetraflavonoid, taiwanhomoflavone C, and a new stereoisomer of desmethylcephalotaxinone have been isolated from the leaves and heartwood of *Cephalotaxus wilsoniana,* respectively. The structures were elucidated by spectroscopic methods. C-3-epi-wilsonione showed cytotoxic activity against a number of human cancer cell lines in vitro (Wang et al., 2004). Two neolignan glycosides (junipercomnosides

A and B) were isolated from aerial parts of *Juniperus communis* var. *depressa* along with two known neolignan glycosides and seven flavonoid glycosides. The structures of the isolated compounds were determined by spectral analysis, in particular by 2D-NMR analysis (Nakanishi et al., 2004).

A novel degraded triterpenoid isopseudolaritone A and one new oligosaccharide, 1-O-isopropyl-6-O- [2-O-methyl-alpha-L-rhamnopyranosyl (1 --> 6)]-beta-D-glucopyranose, two new artefacts, 9-O-formacyl cedrusin and 9,9'-O-diformacyl cedrusin, as well as 12 known phenolic Compounds, were isolated from the root bark of *Pseudolarix kaempferi* (Yang et al., 2004). From the needles of *Taxus baccata* the following flavonoids were isolated: 3-O-rutinosides quercetin, myricetin and kaempferol, 7-O-glucosides kaempferol and quercetin, kaempferol, quercetin, myricetin. The composition of flavonols and biflavones in some of the species of the genus Taxus, namely *T. celebica, T. cuspidata, T. media* and cultivar varieties *T. baccata* 'Aurea', *T. baccata* 'Aurea decora', *T. baccata* 'Elegantissima', *T. baccata* 'Fastigiata', *T. baccata* 'Pyramidalis', *T. media* 'Hatfieldii (Krauze-Baranowska, 2004).

A novel dihydroflavonol, C-6, O-7-dimethylaromadendrin, was isolated from a 70% aqueous acetone extract of pine (*Pinus sylvestris* L.) bark (Sinkkonen et al., 2005). Pycnogenol, a standardised extract of *Pinus pinaster* bark, was tested for its antimicrobial activity against 23 different pathogenic prokaryotic (gram-positive and gram-negative) and eukaryotic (yeast and fungi) microorganisms. Pycnogenol inhibited the growth of all the tested microorganisms in minimum concentrations ranging from 20 to

250 μg/ml. These results conformed clinical oral health care studies describing the prevention of plaque formation and the clearance of candidiasis by Pycnogenol (Torras et al., 2005). Chemical investigation on the constituents of the cones of *Cycas beddomei* resulted in the isolation of a new biflavonoid, 2,3-dihydro-4'''-O-methyl amentoflavone, along with 2,3,2'', 3''-tetrahydro hinokiflavone, 2,3,2'',3''-tetrahydro amentoflavone, 2,3-dihydro amentoflavone (Das et al., 2005).

Phytochemical investigation of *Ginkgo biloba* (Ginkgoaceae) has resulted in the isolation of two new biflavone glucosides, ginkgetin 7''-O-beta-D-glucopyranoside and isoginkgetin 7-O-beta-D-glucopyranoside (Hyun et al., 2005). Flavonoids and terpene lactones were isolated from *Ginkgo biloba* by HPLC method (Mesbah et al., 2005). A biflavonoid fraction (BFF) obtained from *Araucaria angustifolia* needles was effective to quench singlet oxygen, to protect plasmid DNA against single strand break (ssb) caused by singlet oxygen or Fenton reaction and to inhibit Fenton or UV radiation-induced lipoperoxidation in phosphatidylcholine liposomes. The activity of the biflavonoid fraction (BFF) was compared with quercetin, rutin (flavonoids), ginkgetin, amentoflavone (biflavonoids), alpha-tocopherol and Trolox. The BFF displayed a higher quenching rate constant compared to flavonoids and biflavonoids and protected against ssb induced by singlet oxygen. Although the BFF was not as efficient as either flavonoid alpha-tocopherol or Trolox in protection against ssb induced by Fenton-reaction or lipoperoxidation, these scavenging properties suggest that BFF is still an excellent candidate for successful employment as an antioxidant and photoprotector (Yamaguchi et al., 2005).

Alkaloids from Ephedra have adverse events in those using whole extracts as "dietary supplements" for weight loss or athletic performance enhancement. Extracts of Ephedra shrubs contain highly active alpha- and beta-adrenergic agonists that have profound effects on the heart and vasculature. Evidence for their effectiveness is limited. Adverse cardiovascular and cerebrovascular effects, including stroke, myocardial infarction, and sudden death, temporally related to their use (Andraws et al., 2005). Two new taxanes with a dimethylamino group on the C-5 side chain were identified for the first time in the needles of the Canadian yew, *Taxus canadensis*. Their structures were characterized as 7beta,10beta,13alpha-triacetoxy-5alpha- (3'-dimethylamino-3'-phenyl propanoyl)oxy-2alpha-hydroxy-2(3-->20) abeotaxa-4(20),11-dien-9-one and 2alpha,10beta-diacetoxy-9alpha-hydroxy-5alpha-(3'-dimethylamino-3'-phenylpropanoyl)oxy-3,11-cyclotax-4(20)-en-13-one (2) (Shi et al., 2006).

2.3.4 Phytochemicals from Higher plants especially from Angiosperms, a brief report

Higher group of plants is flowering plants, angiosperms. A large number of higher plants are identified to have antibacterial compounds, because of the voluminous data a few important ones are cited here. *Guazuma* of Sterculiaceae possessed with lapachol having antiseptic nature (Burnett and Thomson, 1967). The antibacterial activities of isoflavonoid (kievitone and phaseollin), flavonoid (hydroxyflavans), furanoacetylenic (wyerone), and sesquiterpenoid (capsidiol and rishitin), phytoalexins against eight gram-negative and six gram-positive bacteria were examined using

the paper-disc antibiotic assay method (Gnanamanickam and Mansfield, 1981).

A new flavonol glycoslde, gossypetm 8-O-rhamnoside, was isolated from flower petals of *Gossypium arboreum*, Malvaceae along with quercetm 'I-0-glucoade, quercetm 3-0-glucoside and quercetm 7-0-glucoside. These compounds showed antibacterial activity against *Pseudomonas maltophrha* and *Enterobacter cloacae* (Wagge and Hedint, 1984). *Achyrocline* of Asteraceae with Coumarin, Flavone, 5-8-dihydroxy-3-7-dimethoxy, Flavone, 3-5-7-8-tetramethoxy Flavonoids showed antiseptic nature (Mesquita et al., 1986). The alkaloid content, (3-demethylcolchicine, colchifoline, N-deacetyl-N-formylcolchicine, colchicine, cor-nigerine, 2-demethyldemecolcine, 3- demethyldemecolcine, demecolcine) reported from of corms, leaves and seeds of *Colchium turcicum.* In addition to these compounds, corms contained β-lumicolchicine, luteolin, and vanillic acid (Husek et al., 1990).

A new acylated flavonol glycoside quercetin 3-α-arabinopyranoside-2"-gallate, having antibacterial activity, has been isolated from the leaves of *Lasiobema japonica (Bauhinia japonica)* (Leguminosae). Quercetin, hyperin and guaijavarin were also identified (Iwagawa et al., 1990). Flavoniods like Friedelin, Friedelan-3 beta-ol isolated from *Bidens pilosa* exhibited antibacterial activity (Sarg et al., 1991). The aerial parts of *Tanacetum densum* subsp. Sivasicum, compositae, yielded, in addition to known compounds, a new sesquiterpene lactone, sivasinolide which showed antibacterial activity against *Bacillus subtilis* and *Klebsiella pneumoniae* (Goren et al., 1992).

From an acetone extract of the whole plant *Salvia sclarea***,** seven known diterpenes, sclareol, manool, salvipisone, ferruginol, microstegiol, candidissiol and 7-oxoroyleanone, and two new ones, 2,3-dehydrosalvipisone and 7-oxoferruginol-18-al, as well as two sesquiterpenes, caryophyllene oxide and spathulenol, γ-amyrin, & sitosterol and the flavonoids apigenin, luteolin, 4'-methylapigenin, 6-hydroxyluteolin-6,7,3',4'-tetramethyl ether, 6-hydroxy apigenin-7,4'-dimethyl ether were obtained. The diterpenoids and the sesquiterpenoids were tested for antimicrobial activity against standard bacterial strains and yeast. 2, 3-Dehydrosalvipisone, sclareol, manool, 7-oxoroyleanone, spathulenol and caryophyllene oxide were found to be active against *Staphylococcus aureus,* the first and third compound against *Candida albicans* and the last compound against *Proteus mirabilis* (Ulubelen et al., 1994).

Three known phloroglucinols (japonicine A, uliginosin A and isouliginosin B) and a new phloroglucinol (hyperbrasilol A) have been isolated from a petrol extract of the leaves and flowers of *Hypericum brasiliense* All four phloroglucinols were antibacterial against *Bacillus subtilis* in a TLC bioautographic assay. The flavonoids, kaempferol, luteolin, quercetin, quercitrin, isoquercitrin, hyperoside and guaijaverin, were isolated from a methanol extract of the same organs (Rocha et al., 1995). Two novel abietane diterpenoids have been isolated from the aerial material of *Plectranthus elegans,* Lamiaceae11-hydroxy-12-oxo-7,9(11),13-abietatriene and 7α11-dihydroxy-12-methoxy-8,11,13-abietatriene Both inhibited spore germination of the fungus *Cladosporium cucumerinum.* The new diterpenes also inhibited the growth of Gram-positive bacteria (Dellar et al., 1996).

Differential sensitivity of the major pathogens of rice, *Xanthomonas oryzae* pv. *oryzae, Pyricularia oryzae* and *Rhizoctonia solani* to inhibition by certain flavonoids was tested using paper disc/liquid culture and spore germination assays. Naringenin, the first intermediate of the flavonoid pathway, displayed growth inhibition of *Xanthomonas* strains and spore germination of *P. orvzae.* On the other hand, no such inhibition was found with *Rhizoctonia solani*. Crude extracts of leaf and pericarp tissues of a fully purple pigmented rice cultivar, Purpleputtu, also showed growth inhibition of *Xanthomonas* (Padmavti et al., 1997). A new flavonol diglycoside, quercetin-3-O-[3,4 diacetyl-α-L-rhamnopyranosyl -(1-6)β-D-glucopyranoside] and an antifungal dihydrofuranocoumarin, 2'(S),3'(R)-2'-acetoxyisopropyl-3'-acetoxy-2',3'dihydroangelicin together with four other known flavonoids and seven known bioactive coumarins were isolated from the aerial parts of *Tordylium* (Kofinas et al., 1998).

A new favonoid, 3-[1-[[3-di(4-hydroxyphenyl)methyl] 2,4,6-trihydroxyphenyl] 3-di(4-hydroxyphenyl) 1-propanone-2-yl]5,7-dihydroxy -4H-1-benzopyran-4-one, named mohsenone was isolated from the root of *Stellera chamaejasme*, Thymelaeaceae family together with chamaechromone and (-)-epiafzelechin 7-O-b-D-glucopyranoside (Jin et al., 1999). A new compound, 3,39,49,5,6,7,8-heptahydroxyflavan, was isolated from the roots of *Elephantorrhiza goetze*i, Leguminosae. The crude extract and its n-butanol and residual water fractions showed antimicrobial activity. Semi-purified extracts showed higher activity and of the purified compounds only methyl gallate gave activity below 100 μg of sample (Moyo et al., 1999).

From the roots of the plant *Tephrosia aequilata* Baker, five flavonoids were isolated of which, 3,4:8,9-dimethylene dioxypterocarpan is reported for the first time (Tarus et al., 2002). Nineteen flavonoids isolated from licorice (*Glycyrrhiza glabr*a *G. inflata* and *G. uralensi*s were tested for their antimicrobial activities against methicillin sensitive *Staphylococcus aureu*s methicillin resistant *S. aureu*s *Micrococcus luteu*s *Bacillus subtili*s *Escherichia col*i *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Fukai et al., 2002). Five isoflavonoids, (+/-)-7,2',4'-trihydroxy-8,3'-di(gamma,gamma-dimethylallyl)isoflavanone, (3R) -7,4'-dihydroxy-2'-methoxy-6,8-di(gamma,gamma-dimethylallyl) isoflavanone,(3R)-7,2',4'-trihydroxy-6,8-di(gamma,gamma-dimethylallyl) isoflavan,2',4'-dihydroxy-8-gamma,gamma-dimethylallyl-2",2"-dimethylpyrano-[5,6:6,7] isoflavan and (6aS, 11aS)-3,6a- dihydroxy-9-methoxy-4,10-di(gamma, gamma-dimethylallyl) pterocarpan, along with five known compounds, were isolated from the roots of *Erythrina zeyheri*. Certain compounds showed antibacterial activities against methicillin-resistant *Staphylococcus aureus* (Tanaka et al., 2003).

Lycopus europaeus yielded two new isopimarane diterpenes, namely methyl-1alpha-acetoxy-7alpha 14alpha-dihydroxy-8,15-isopimaradien-18-oate and methyl-1alpha, 14alpha-diacetoxy-7alpha-hydroxy-8,15-isopimaradien-18-oate. These compounds and several known diterpenes were tested for in vitro antibacterial and resistance modifying activity against strains of *Staphylococcus aureus* (Gibbons, 2003). Three new isoflavonoids, eryvarins M-O, two new 2-arylbenzofurans, eryvarins P and Q, and a new 3-aryl-2,3-dihydrobenzofuran, eryvarin R, together with three known compounds, were isolated from the roots of *Erythrina variegata*.

Eryvarin Q showed potent antibacterial activity against methicillin-resistant *Staphylococcus aureus* (Tanaka et al., 2004).

The aerial parts of *Carthamus lanatus* (Asteraceae) afforded four new oxygenated bisabolane fucosides, 10-hydroperoxy-bisabola-2,11-diene 7-O-beta-D-fucopyranoside, 11-hydro-peroxy-bisabola-2,9-diene 7-O-beta-D-fucopyranoside, 10-hydroxy-bisabola-2,11-diene 7-O-beta-D-fucopyranoside and 11-hydroxy-bisabola-2,9-diene 7-O-beta-D-fucopyranoside together with the known compounds a-bisabolol beta-D-fucopyranoside, asperuloside, sitosterol 3-O-beta-D-glucoside and stigmasterol 3-O-beta-D-glucoside. The main constituent a-bisabolol fucoside exhibited noticeable antibacterial and cytotoxic activities (Mikhova et al., 2004).

Psoralidin, bakuchicin, psoralin and angelicin, isolated from the seeds of *Psoralea corylifolia*, showed significant antibacterial activities against a number of Gram (+) and Gram (-) bacteria (Khatune et al., 2004). A polyisoprenylated ketone named enervosanone has been isolated from the stem bark of *Calophyllum enervosum* together with three known compounds, cambogin, osajaxanthone and epicatechin. Antimicrobial activity was observed (Taher et al., 2005). Five new iridoids, namely rupesin A-E (1-5, resp.), together with six known iridoids, 6-11, were isolated from the roots of *Patrinia rupestris.* Compounds showed significant antibacterial activities against *Bacillus subtilis, Escherichia coli,* and *Staphylococcus aureus*, respectively (Yang et al., 2006).

Four new dihydroagarofuranoid sesquiterpenes and a new hydroxybenzylsalicylaldehyde, forkienin, together with nine known compounds have been isolated from the roots of Microtropis fokienensis. Four compounds exhibited potent

antitubercular activities against *Mycobacterium tuberculosis* 90-221387 in vitro (Chen et al., 2007). Five new diterpenoids, 18-beta-D-3',4'-diacetoxyxylopyranosyl-ent-kaur-16-ene, 18-beta-L-3',5'-diacetoxyarabinofuranosyl-ent-kaur-16-ene, 18-beta-D-3', 6'-diacetoxyglucopyranosyl-ent-kaur-16-ene, ent-isopimar-8(14), 15-dien-19-oic acid, and 5alpha-hydroxy-ent-rosa-15-en-18-oic acid, isolated from the whole herb of *Sagittaria pygmaea.* The second compound exhibited significant antibacterial activity against the oral pathogens, *Streptococcus mutans* ATCC 25175 and *Actinomyces viscosus* (Liu et al., 2007).

2.4. Phytopaharmaceuticals from Pteridophytes especially Ferns

A general outline of different phytopharmaceuticals from pteridophytes is given in section 2.3.2. Here categorised outline of phytopharmaceuticals from ferns is narrated to point out the importance of this group of plants in their diversity among phytochemicals.

2.4.1 *Terpenoids* from ferns

2.4.1.1 Characteristics of terpenoids in general

An enormous range of plant substances are covered by the word 'terpenoid', a term which is used to indicate that all such substances have a common biosynthetic origin. Thus, terpenoids are all based on the isoprene molecule, CH_2=C(CH_3)-CH=CH_2 and their carbon skeletons are built up from the union of two or more of these C_5 units. They are then classified according to whether they contain two (C_{10}), three (C_{15}), four (C_{20}), six (C_{30}), or eight (C_{40}) such units. They range from the essential oil components, the

volatile mono-and sesquiterpenes (C_{10} and C_{15}), through the less volatile diterpenes (C_{20}) to the involatile triterpenoids and sterols (C_{30}) and carotenoid pigments (C_{40}). Terpenoids are derived biosynthetically from the molecule of isoprene (Loudon, 2002), (Harborne, 1973). Terpenoids are the main component of many plant essential oils. This group is based on a single unit, isoprene, and thus monoterpenoid, diterpenoids, and triterpenoids, all differ in the number of isoprene units. Isoprenoids are present in all living organisms, but with an unusual diversity in plants. By 1997 more than 23,000 different plant isoprenoids had been listed and new substances are being constantly identified (Heldt, 2005). Isoprenoids act as antibiotics to protect the plants from pathogenic microorganisms. A number of mainly cyclic compounds containing 10, 15, 20, or correspondingly more C atoms have been isolated from turpentine oil, such substances have been found in many plants and were given the collective name terpenes. Isoprene is the basic constituent of terpenes.

Higher plants have two different synthesis pathways for isoprenoids. Acetyl–CoA is the precursor for the synthesis of isoprenoids in the cytosol. Prenyl transferases catalyse the association of isopprene units (Loudon, 2002). Isoprenoids are also called terpenoids. Terpenoids were common in pteridophyte species with triterpenoids (hopane triterpenoids, epoxytriterpenoid, and serratene triterpenoid), diterpenoids, hemiterpene glycosides, and clerodane diterpene glycosides. Terpenoids had been the subject of many studies, and were medicinally significant for a wide range of treatments. Terpenoids are a very promising class of compounds.

2.4.1.2 Terpenoids isolated from ferns

From aerial parts of the fern *Pteris multifida* Poir. (Polypodiaceae) two diterpenes, entkaurane-2 beta, 16 alpha-diol and ent-kaur-16-ene-2 beta, 15 alpha-diol, were isolated (Woerdenbag et al., 1996). Petroleum ether extract of Rhizome of *Drynariae* with three compounds namely fern-9(11)-ene, hop-22(29)-ene and cyclolaudenol. All the three compounds are triterpenoids (Liu et al., 1999). From a whole plant of a fern, *Diplazium subsinuatum*, three new hopane-triterpene lactone glycosides, diplaziosides V-VII (1-3), were isolated, together with a new monoacetyl derivative (4) of diplazioside VII (3). Compounds 1-3 were defined as the respective 3-O-[beta-D-glucopyranosyl-(1--->2)]-beta-D-glucopyranosides of 3beta,24-dihydroxyhopan-28,22-olide (1), of 3beta, 17, 24-trihydroxyhopan-28,22-olide (2), and of (22R)-3beta,24,30-trihydroxyhopan-28,22-olide (3), and 4 as the 6"-O-acetate of 3, respectively (Inatomi et al., 2000).

Three triterpenoids, 8alpha-hydroxyfernan-25, 7beta-olide, 3alpha-hydroxy-4alpha-methoxyfilicane and 19alpha-hydroxyferna-7,9(11)-diene were isolated from the fresh fronds of *Adiantum caudatum* (Tsuzuki et al., 2001). A new triterpenoid, 22, 29xi-epoxy-30-norhopane-13beta-ol isolated from *Adiantum lunulatum* (Reddy et al., 2001). Diterpenoids isolated from the extract of *Pteris semipinnata* L by HPLC- APCI- MS (Deng et al., 2002). The petrol extract of the whole plant of *Adiantum lunuactum* yielded a new hopane triterpenoid characterized as 6 alpha-acetoxy-16 beta, 22-dihydroxy-3-ketoisohopane, along with the known 3beta, 6 alpha, 16 beta, 22-tetrahydroxyisohopane (mollugogenol A)(Brahmachari and Chatterjee, 2002). Two new

migrated hopane triterpenoids, viz. 4alpha-hydroxyfilican-3-one and fern-9 (11)-en-12beta-ol, and olean-18-en-3-one and olean-12-en-3-one as the first example of oleanane compounds from *Adiantum* ferns were isolated along with many other known triterpenoids from *Adiantum capillus-veneris* (Nakane et al., 2002).

Dryocrassyl formate, sitostanyl formate, and 12 alpha-hydroxyfern-9(11)-ene were isolated from the fresh fronds of *Cyathea podophylla*. Ten known triterpenoids, three derivatives of phytol, a stanol, and beta-tocopherol were also identified from this fern (Arai et al., 2003). Carcinogenic terpene ptaquiloside isolated from bracken fronds, rhizomes of *Pteridium aquilinum* (Rasmussen et al., 2003). Pteroside A2- -a new illudane-type sesquiterpene glucoside isolated from *Pteridium caudatum* L. Maxon (Castillo et al., 2003).

The drimane-type sesquiterpenoids (-)-polygodial, (-)-isopolygodial, drimenin, and isodrimenin were isolated from the fern *Thelypteris hispidula*, along with other terpenoids (Socolsky et al., 2005). Diterpenoids were isolated from *Pteris semipinnata* (Deng and Liang 2005). A new serratane-type triterpene, lycophlegmarin, isolated from *Lycopodium phlegmaria* L. Four known related triterpenoids were also found from the title plant (Shi et al., 2005), the plant is not a fern but a pteridophyte plant. Tetranorclerodanes and clerodane-type diterpene glycosides were isolated from acetone extract of *Dicranopteris dichotoma* (Li et al., 2007).

2.4.2 Flavonoids from Ferns

2.4.2.1 Characteristics of flavonoids in general

Flavonoids, or bioflavonoids, are a ubiquitous group of polyphenolic substances, which are present in most plants, concentrating in seeds, fruit skin or peel, bark, and flowers. A great number of plant medicines contain flavonoids, which have been reported by many authors as having antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic, and vasodilatory actions. The structural components common to these molecules include two benzene rings on either side of a 3-carbon ring (Fig. 2.1). Multiple combinations of hydroxyl groups, sugars, oxygen, and methyl groups attached to these structures create the various classes of flavonoids: flavanols, flavanones, flavones, flavan-3-ols (catechins), anthocyanins, and isoflavones (Alan and Miller, 1996).

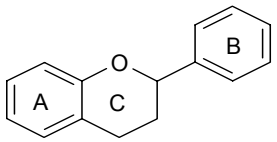
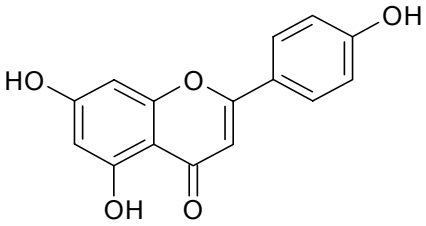
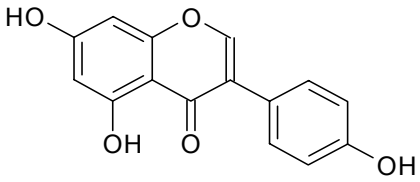


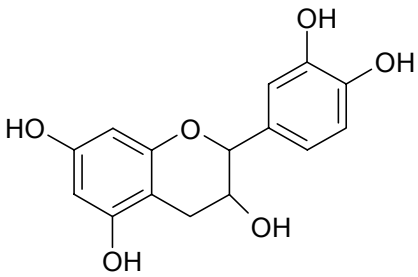
Figure 2.1 Structural component common to flavonoids.



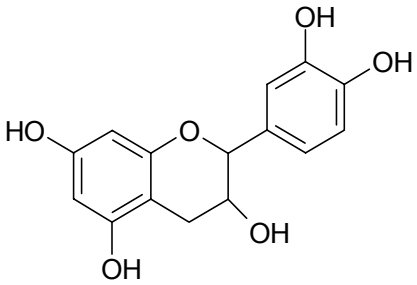
(a) Flavone



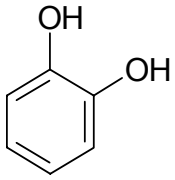
(b) Isoflavone



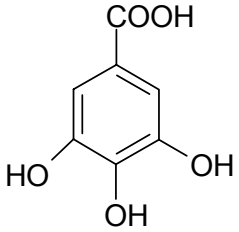
(c) Anthocyanin



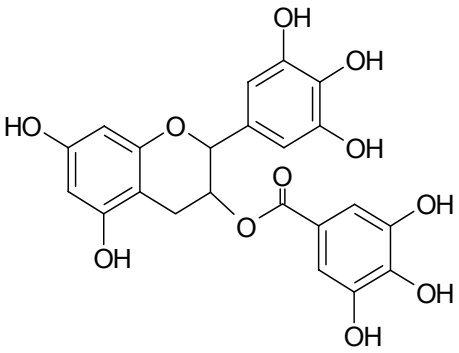
(d) Catechin



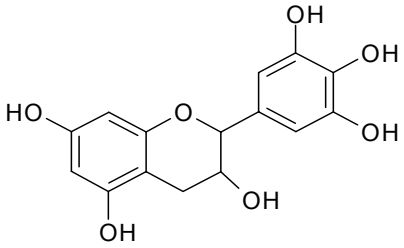
(e) Catechol



(f) Gallic acid



(g) Epigallocatechin gallate



(h) Epigallocatechin

Figure 2.2 Structure of flavonoids

Flavonoids have been shown in a number of studies to be potent antioxidants, capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals. Flavonoids are a class of water-soluble plant pigments. The basic flavonoid structure allows a multitude of variations in chemical structure, giving rise to flavonols (quercetin, kaempherol, myricetin), flavones (apigenin, luteolin), flavanones (catechin, epicatechin), anthocyanins and isoflavonoids (Fig. 2.2).

Some of the best-known flavonoids, such as genistein in soy, and quercetin in onions, can be considered subcategories of categories. Although they are all structurally related, their functions are different. Flavonoids also include hesperidin, rutin, citrus flavonoids, and a variety of other supplements. While they are not considered essential nutrients, some flavonoids support health by strengthening capillaries and other connective tissue, and some function as anti-inflammatory, antihistaminic, and antiviral agents. Quercetin has been reported to block the "sorbitol pathway" that is linked to many problems associated with diabetes. Rutin and several other flavonoids may also protect blood vessels. As antioxidants, some flavonoids, such as quercetin, protect LDL ("bad") cholesterol from oxidative damage. Others, such as the anthocyanidins from bilberry, purple cabbage, and grapes, may help protect the lens of the eye from cataracts. Animal research suggests that naringenin, found in grapefruit, may have anticancer activity (So et al., 1996).

Quercetin chalcone (QC), a novel flavonoid, is quercetin with an opened C ring and the oxygen found in the C-ring of quercetin converted into a hydroxyl group (Figure 2.3). QC also retains the

C-ring double bond from quercetin, and should retain the antioxidant properties of quercetin as well. In fact, with the addition of the extra hydroxyl group in the C ring, quercetin chalcone could be a more potent antioxidant than quercetin (Alan and Miller, 1996).

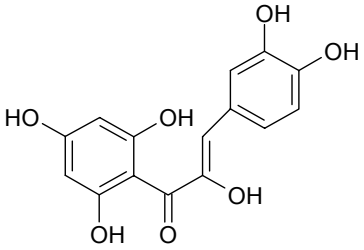


Figure 2.3 Structure of quercetin chalcone

In a small, preliminary trial, rutoside (500 mg twice daily), a derivative of the flavonoid, rutin, combined with vitamin C. (500 mg twice daily) produced marked improvement in three women with progressive pigmented purpura (PPP), a mild skin condition (Reinhold et al., 1999). Although not a serious medical condition, cosmetic concerns lead persons with PPP to seek treatment with a variety of drugs. Flavonoids are found in a wide range of foods. For example, flavanones are in citrus, isoflavones in soyproducts anthocyanidins in wine and bilberry, and flavans in apples and tea. In 1980, quercetin was reported to induce cancer in animals (Pamukcu et al., 1980). Most of the further research did not find this to be true (Hirono et al., 1981), while quercetin is mutagenic in test tube studies, it does not appear to be mutagenic in animal studies (Aeschbacher et al., 1982). In fact, quercetin has been found to inhibit both tumour promoters (Nishino et al., 1984) and human cancer cells (Kuo, 1996). People who eat high levels of

flavonoids have been found to have an overall lower risk of getting a wide variety of cancers, (Knekt et al., 1997). Though preliminary human research, studying foods with high in quercetin has found no relation to cancer risk one way or the other (Hertog et al., 1994). Despite the confusion, in recent years experts have shifted their view of quercetin from concerns that it might cause cancer in test tube studies to guarded hope that quercetin has anticancer effects in humans (Stavric, 1994).

2.4.2.2 Flavonoids isolated from ferns

Four species of *Agiopteris* indicated di-C-glycosylflavones and flavone-O-glycosides (Wallace et al., 1981). Leaves of the fern *Pityrogramma ebenea* yielded a new compound 2',6'-dlhydroxy-4,3'-dlmethoxy-4',5'- methylenedloxydlhydrochalcone was characterized (2S)-5,7-dihydroxy-4'- methoxy-6,8- dimethyl flavanone was identified; another fern *Blechnum regnelhanum* aslo yielded the same compound (Miraglia et al 1985). A new gamma-lactone derivative named davallialactone and the 7-O-beta-D-glucuronide of (+/-)-eriodictyol have been isolated from *Davallia mariessi* Moore along with caffeic acid, 4-beta-D-glucopyranosylcaffeic acid and 4-O-beta-D-glucopyranosyl-p-coumaric acid (Cui et al., 1990). A new flavonoid, 2,3-trans-5,2'-dihydroxy-7, 8-dimethoxy-dihydroflavonol-3-O-acetate, was isolated from the farinose coating on the lower leave surface of the fern, *Notholaena sulphurea* (Wollenweber et al., 2001). Four compounds were obtained from *Dryopteris sublaeta* and identified as 2(S)-5,7,3'-trihydroxy-6,8-dimethyl-5'- methoxyflavanone, matteucinol, desmethoxymatteucinol and 5,7,2'-trihydroxy-6,8-dimethylflavanone (Feng et al., 2005).

Eight compounds were isolated from *Lygodium japonicum* and identified as tilianin, kaempferol-7-O-alpha-L-rhamnopyranoside, kaempferol, p-coumaric acid, hexadecanoic acid 2, 3-dihydroxy-propyl ester, daucosterol, beta-sitosterol, and 1-hentriacontanol respectively (Zhang et al., 2005). Three new flavonoids, protoapigenone, 5',6'-dihydro-6'-methoxyprotoapigenone, and protoapigenin, along with four known compounds, protoapigenin 4'-O-beta- D-glucoside, apigenin 4'- O-beta- D-glucoside, kaempferol 3-O-alpha- L-rhamnopyranoside, kaempferol 3,7-di- O-alpha- L-rhamnopyranoside, were isolated from *Thelypteris torresiana* using bioactivity-guided fractionation methods (Lin et al., 2005).

Three new flavonol glycosides, kaempferol-3-O-(6-trans-caffeoyl)-beta-D-glucopyranosyl-(1-->2)-beta-D-glucopyranoside, kaempferol-3-O-(6-trans-caffeoyl)-beta-D-glucopyranosyl-(1--> 2)-beta-D-glucopyranoside-7-O-beta-D-glucopyranoside, and kaempferol-3-O-(6-trans-p-coumaroyl)-beta-D-glucopyranosyl-(1-->2)-beta-D-glucopyranoside-7-O-beta-D-glucopyranoside were isolated from the aerial part of *Camptosorus sibiricus* (Li, et al., 2006). A new kaempferol glycoside, kaempferol-3-O-beta-D-glucopyranoside-7-O-alpha-L-arabinofuranoside, was isolated from the ethanol extract of *Pyrrosia petiolosa* together with six known flavonoids already reported from the same plant (Wang et al., 2006). Six new flavanone glycosides were isolated from the methanol extract of the rhizomes of *Cyclosorus acuminatus* (Fang et al., 2006). Two new compounds, a novel flavonoid, flavotorresin and a flavonoid diglycoside, multiflorin C, along with five known compounds, were isolated from *Thelypteris torresiana* (Lin et al., 2007).

2.4.3 Glycosides isolated from ferns

Two new ecdysteroid glycosides reported from *Blechnum minus,* 2deoxyecdysone 3-β-D-glycopyranoside (blechnoside A) and 2deoxyecdysone 25-β-D glucopyranoside (blechnoside B) (Suksamrarn et al., 1986). A glycoside showing a strong growth inhibition of lettuce was isolated from root-stalks of Gleichenia japonica and its structure was established to be the 3-O-x-rhamnopyranosyl-(1→2)-β-glucopyranoside of 13-O-α-rhamnopyranosyl-(+)-3β-hydroxymanool. In addition, two related glycosides were also isolated and they were characterized as the 3-O-β-fucopyranosyl- (l→3)-α- rhamnopyranosyl-(1→2)-β-glucopyranoside of 13-O-α-rhamnopyranosyl -(+)-3β-hydroxymanool and the 13-O-rhamnopyranoside of the same diterpene alcohol (Munesada et al., 1992). A new flavone glycoside isolated from the fern *Pteris cretica* has been shown to be luteolin 7-O-robinobioside (Imperato, 1994). Clerodane glycosides and flavonoids in *Dicranopteris pedata* and three varieties of *D. linearis* were investigated. All the ferns contained a new glycoside (Raja et al., 1995). Hymenosides G--J were newly isolated from the Japanese fern *Hymenophyllum barbatum* in addition to hymenosides A—F (Toyota et al., 2001). Thirteen glycosides and methyl (3R,5R)-5-hydroxy-(beta-D-glucopyranosyloxy)-hexanoate were newly isolated from the Japanese fern *Hymenophyllum barbatum* (Toyota et al., 2002).

A trinorsesterterpene glycoside was isolated from the North American fern *Woodwardia virginica* (L.) Smith (Hanus et al., 2003). A new phenolic glycoside, dryopteroside (1-butanoyl-3-C-beta-D-glucopyranosyl-5-methyl-phloroglucinyl -6-O-beta-D-glucopyranoside), was isolated from the rhizomes *of Dryopteris*

crassirhizoma (Chang et al., 2006). Acetylated flavanone glycosides were isolated from the rhizomes of *Cyclosorus acuminatus* (Fang et al., 2006). Acetone extract of *Dicranopteris dichotoma* yielded Tetranorclerodanes and clerodane-type diterpene glycosides (Li et al., 2007).

2.4.4. Alkaloids isolated from ferns

Alkaloids are a diverse group of compounds and they are known to have a variety of marked effects on animals. Alkaloids often act on the nervous system as stimulators, and sometimes as poisons. Cocaine (which exhibits an anaesthetic effect), atropine (which effects motor nerves), and curare (which has been used by South American natives to cause paralysis of prey), are all alkaloids (Harbone, 1973).

There is a single report of alkaloid from ferns, the reason is that alkaloid is an evolved compound and is usually observed in evolved plants like angiosperms. A new beta-carboline alkaloid, 1-acetyl-8-hydroxy-beta-carboline, was isolated from the fern, *Hypodematium squamuloso-pilosum*, along with a known compound, 1-acetyl-beta-carboline. Their structures were elucidated from spectral evidence. This is the first report of this type of alkaloid being found in ferns (Zhou et al., 1998).

2.4.4.1 Alkaloids of Lycopdium a pteridophyte plant but not a fern.

Certain lycopodium alkaloids, which occur naturally in *Lycopodium* and other pteridophytes, have been investigated for their medicinal properties. *Lycopodium* alkaloid Sauroxine was isolated (Ayer et al., 1965). In addition to huperzinine, a new alkaloid, N-demethylhuperzinine, was isolated from the aerial

parts of *Lycopodium casuarinoides* by bioassay-directed fractionation (Shen and Chen, 1994). Four alkaloids, lucidine B, oxolucidine A, lucidine A, and lucidulinone from Lycopodium lucidulum (Tori et al., 2000). Three lycopodium alkaloid N-oxides, huperzine J, huperzine K and huperzine L were obtained from Huperzia serrata (Thunb.) (Gao et al., 2000). Serratezomines A--C, new alkaloids from Lycopodium serratum var. serratum (Morita et al., 2000). A novel alkaloid, lycoposerramine-A, which has a 1,2,4-oxadiazolidin-5-one residue in the molecule, was isolated from the club moss *Lycopodium serratum* Thunb (Takayama et al., 2001). Lyconadin A, a novel alkaloid from *Lycopodium complanatum* (Kobayashi et al., 2001).

Huperzine R, a novel 15-carbon Lycopodium alkaloid, was isolated from the whole plant of *Huperzia serrata*, (Tan et al., 2002). Phlegmariurine B, a known alkaloid, along with three new analogous compounds, 2alpha-hydroxyphlegmariurine B, 2-oxoyphlegmariurine B and 11-oxophlegmariurine B, were isolated from the CHCl3 fraction of total alkaloids of whole plant of the Chinese medicinal herb *Huperzia serrata* (Tan et al., 2002). Three new lycopodium alkaloids, 11alpha-hydroxyphlegmariurine B, 7alpha-hydroxyphlegmariurine B and 7alpha,11alpha-dihydroxyphlegmariurine B along with a known compound, phlegmariurine B, were isolated from the herb *Huperzia serrata* (Tan et al., 2002). Alpha-onocerin and lycoperine A, for example, exhibit acetylcholinesterase inhibition activity (Zhang et al., 2002, Hirasawa et al., 2003). Huperzine A, a lycopodium alkaloid, isolated from *Huperzia* species among others, has been shown to enhance memory in animals and is also being investigated for treatment of Alzheimer's disease (Zangara, 2003). Ten new

alkaloids, lycoposerramines-F, -G, -H, -I, -J, -K, -L, -M, -N and -O, having lycopodine-related structures, were isolated from the club moss *Lycopodium serratum* THUNB. (Takayama et al., 2003) Two new Lycopodium alkaloids, miyoshianines A and B, together with five known alkaloids, lycopodine, lycodoline, 12-epilycodoline, clavolonine, and flabelliformine, were isolated from *Huperzia miyoshiana (*Makino) Ching (Huperziaceae)(Tong et al., 2003). A novel C(27)N(3)-type Lycopodium alkaloid consisting of a fastigiatine-type skeleton (C(16)N(2)) and a quinolizidine moiety (C(11)N), himeradine A, has been isolated from the club moss *Lycopodium chinense* (Morita et al., 2003).

Lycopodium alkaloids are quinolizine, or pyridine and alpha-pyridone type alkaloids. Some Lycopodium alkaloids are potent inhibitors of acetylcholinesterase (AChE). Huperzine A (HupA) is reported to increase efficiency for learning and memory in animals, and it shows promise in the treatment of Alzheimer's disease (Ma and Gang, 2004). A novel, fused-tetracyclic Lycopodium alkaloid, nankakurine A, consisting of a cyclohexane ring and a 3-aza-bicyclo[3.3.1]nonane ring connected to a piperidine ring through a spiro carbon, was isolated from the club moss *Lycopodium hamiltonii* (Hirasawa et al., 2004). Three new alkaloids, lycopodatines A, B, and C, have been isolated from the club moss *Lycopodium inundatum* (Morita et al., 2005).

Four new alkaloids, lycopladines B-D and lyconadin B have been isolated from the club moss *Lycopodium complanatum* (Ishiuchi et al., 2006). Two new dimeric Lycopodium alkaloids, complanadines C and D have been isolated from the club moss *Lycopodium complanatum* (Ishiuchi et al., 2007).