Chapter I

Introduction
INTRODUCTION

India, with its varying soil and climatic conditions, is a large storehouse of medicinal plants. The classical medical system, Ayurveda, is strictly Indian in origin and development. Plants form an important part of Ayurvedic pharmacopoeia. Charakasamhita, one of the earliest treatises of Ayurveda (600 B.C.) lists a total of 341 plants and plant products for use in health management (Ray and Gupta, 1965). Subsequent authors of later treatises (Nighantu granthas), have extended the list of Ayurvedic single plant drugs to 600 species (Namjoshi, 1979). More than 1500 remedial treatments with Indian medicinal flora have been reported by Sushrutha, Charaka and Vagbhata. Nowadays above 35,000 species of plants are used worldwide for medicinal purposes. It has been confirmed by WHO that herbal medicines serve the health needs of about 80% of the world’s population especially for millions of people in the vast rural areas of developing countries (Kong et al., 2003).

Our nation has made significant progress in bringing to the forefront a large number of herbs used in the indigenous systems for their approved efficiency and administration in modern medicine, by the systematic and scientific study of these plant drugs, from the pharmacognostical, chemical, pharmacological and clinical points of view (Nair, 1984). The results of various studies on Indian medicinal plants are available in so many research publications, a number of books and monographs (Cervellati et al., 2003; Jadhav & Bhutani, 2002; Borek, 2001; Kupeli et al., 2002; Barak et al., 2002; Gedif & Han, 2003; Geetha Thyagarajan et al., 2003; Shenoy et al., 2002; Venukumar & Latha, 2002). Plant derived substances have minimum side effects. Therefore, the latest trend is the preference of natural drugs over synthetic ones. Over the last two decades, the use of herbal medicines in Europe, Australia and United States of America for health care has also been increasing (Roeder, 2000). Work on the chemistry of medicinal plants
will be more useful by close integration with biochemical studies. Thus, the importance of systematic phytochemical and pharmacological screening of medicinal plants is indescribable.

1.1 Investigations on Indian medicinal plants with special reference to flavonoids

The investigations on secondary plant constituents have made phenomenal advance during the past few decades. The development of efficient separation techniques like column, thin layer, paper, high pressure liquid and gas liquid chromatography, paper electrophoresis etc. as well as sensitive methods of instrumental analysis like IR, UV, NMR ($^1$H and $^{13}$C) ESR, ORD, CD and Mass spectroscopy helped a lot in phytochemical investigations. These methods also made feasible the study of micro quantities of substances with considerable precision in determining the chemical structure and distribution patterns in plants. By studying the chemical constituents of different species in one genus, the metabolic pathways leading to the biogenesis of secondary plant constituents were studied. Moreover, a new line of approach of application of chemistry in botanical classification has been developed known as chemotaxonomy or biochemical systematics (Bendz and Santossan, 1974; Swain, 1963; Alston and Turner, 1963; Smith, 1976).

Systematic investigations have been carried out on a very large number of medicinal plants. There are so many books, reviews, and other reports describing the highlights of such investigations (Filippo et al., 1996). Of the different types of plant constituents, polyphenolic compounds are having much importance (Harborne, 1973; Agata et al., 1993). Polyphenols constitute one of the most numerous and widely distributed group of substances in the plant kingdom, with more than 8000 phenolic structures are known (Harborne, 1993). Polyphenols are products of
secondary metabolism of plants and ubiquitous in all plant organs. They arise biogenetically from two main synthetic pathways: the shikimate and the acetate pathways (Harborne, 1993; Bravo, 1998). According to Harborne (1989), polyphenols can be divided into at least 10 different classes depending on their basic chemical structure.

Out of the different types of polyphenolic compounds, flavonoids occupy the top position followed by hydroxy derivatives of anthracene, coumarins, xanthones etc. Flavonoids are a group of naturally occurring polyphenolic compounds ubiquitously found in fruits and vegetables (Aherne & O’Brien, 2002; Hollman & Arts, 2000). Flavonoids constitute more than 4000 compounds described until 1990 (Harborne 1989, 1993). Flavonoids and other polyphenols are partially responsible for sensory and nutritional qualities of plant foods. The astringency and bitterness of foods and beverages depend on the content of these compounds.

The scientists of organic chemistry, botany, physiology, taxonomy, genetics, biochemistry and pharmacology are much more interested in flavonoids than other plant constituents due to many reasons. The important among them are their structural variability, wide spread distribution, comparative stability, ease and speed of identification, little toxicity and their role as potential agents. Flavonols and flavones are flavonoids of particular importance because they have been found to possess antioxidant and free radical scavenging activity in foods (Shahidi & Wanasundara, 1992).

Flavonoids represent a very large number of types with different properties. Flavonoids are all structurally derived from the parent substance flavone (2-phenyl chromone or 2-phenyl benzo-γ-pyrene (Figure 1.1).
The term flavonoid was applied by Geissman and Hinreiner (Geissman & Hinreiner, 1976; Swain, 1976). It can be seen that flavone consists of two benzene rings, (A and B) joined together by a three-carbon link which is formed into a $\gamma$-pyrone ring (C). The various classes of flavonoids differ in the level of oxidation of the C-ring of the basic benzo-$\gamma$-pyrone.

### 1.1.1 Different classes of flavonoids

The different classes of flavonoids are recognized and they are (1) Anthocyanins (2) Leucoanthocyanidins (3) Flavones (4) Glycoflavones (5) Biflavonyls (6) Chalcones (7) Dihydrochalcones (8) Flavanones (9) Catechins (10) Isoflavones (11) Neoflavones and (12) Aurones. The basic skeletons of the different classes of flavonoids are given in Figure 1.2.

It should be noted that though the five classes of compounds - chalcones, dihydrochalcones, isoflavones, neoflavones, and aurones - which do not actually possess the basic 2-phenyl chromone skeleton, are so closely related both chemically and biosynthetically to other flavonoid types and hence, they are always included in the flavonoid group.
Figure 1.2: Different classes of flavonoids

<table>
<thead>
<tr>
<th>Class</th>
<th>Structure</th>
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<tbody>
<tr>
<td>Anthocyanins</td>
<td><img src="image" alt="Anthocyanins" /></td>
</tr>
<tr>
<td>Leucoanthocyanidins</td>
<td><img src="image" alt="Leucoanthocyanidins" /></td>
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<tr>
<td>Flavones</td>
<td><img src="image" alt="Flavones" /></td>
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<tr>
<td>Glycoflavones</td>
<td><img src="image" alt="Glycoflavones" /></td>
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<td>Biflavonlys</td>
<td><img src="image" alt="Biflavonlys" /></td>
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<tr>
<td>Chalcones</td>
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<tr>
<td>Dihydrochalcones</td>
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<tr>
<td>Flavanones</td>
<td><img src="image" alt="Flavanones" /></td>
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<tr>
<td>Catechins</td>
<td><img src="image" alt="Catechins" /></td>
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<tr>
<td>Isoflavones</td>
<td><img src="image" alt="Isoflavones" /></td>
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<tr>
<td>Neoflavones</td>
<td><img src="image" alt="Neoflavones" /></td>
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<tr>
<td>Aurones</td>
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</tbody>
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Mainly the number and orientation of hydroxy, methoxy and other groups such as furan rings substituted in the two benzene rings distinguish the individual compounds within each class. The flavonoid compounds exist in the plants mainly as glycosides. The sugar free compounds are referred to as aglycones. Flavonoid glycosides are of different types, tri-, bi- or mono- glycosides. One or more of their hydroxyl groups are joined by a hemiacetal link to C-1 of a sugar. The sugars, which have been found in flavonoid glycosides, include simple hexoses and pentoses (monosides) and bi- and trisachharides (biosides and triosides) always combined through the oxygen at the C-1 usually by a β-link. In many cases more than one phenolic group in the flavonoid molecule may be glycosylated. D-glucose is the most common sugar in glycosides that occur either alone or as part of a disaccharide. Others found are D-galactose and L-rhamnose and to a lesser extent L-arabinose and D-xylose. D-glucuronic acid and D-galacturonic acid are still rare and D-apiose is the rarest. Majority of the sugars are present as pyranosides. Complex anthocyanin glycosides contain acids like p-hydroxy benzoic acid in combination with the sugar residues.

### 1.1.2 Occurrence of flavonoids

Flavonoids occur (Geissman, 1962) in all parts of higher plants - root, stem, leaf, flower, pollen, fruit, seed, wood and bark. Anthocyanins are typically the pigments of fruits, flowers and leaves. Chalcones and aurones are not widely distributed in nature and are usually found in flower petals. But in some species they occur in stems, leaves etc. Flavones, flavonols and flavanones occur in different parts of plants and cannot be said to be the characteristic components of any one kind of tissue. An exception to these are complex polymeric flavonoids, tannins and phlobaphenes. These are largely confined to wood and bark, and are
regarded as the end-products of the condensation of monomers (C$_{15}$-compounds) that arise in the actively metabolizing zones of the stem and are subsequently transformed into immobile polymers and deposited in the woody tissues. Catechins and leucoanthocyanidins have been isolated from woods and barks more often than from any other plant parts. Compounds of these classes occur also in non-woody tissues as tea leaves, cocoa beans, fruit pulps etc. While flavones and flavonols are universal, isoflavones and biflavonols are found in only a few plant families.

1.1.3 Solubility characteristics of flavonoids

Depending on the nature of the substituents, flavonoids show a large variation in the solubility characteristics. The polyglycosides and diglycosides are soluble in water and sparingly soluble in most organic solvents. Some monoglycosides such as quercimetrin - a 7-glycoside of quercetin - is sparingly soluble in water whereas, some other monoglycosides rutin, isoquercitrin and quercitrin which are 3-glycosides easily soluble in water. Among the aglycones, flavones and flavonols are sparingly soluble in water, whereas dihydroflavonols are more soluble. Catechins and leucoanthocyanidins are soluble in water and ethyl acetate. Anthocyanidins are stable only as salts and processed under acidic condition and preserved as chlorides.

1.1.4 General method of phytochemical analysis

First of all, the plant material is surveyed for flavonoids. The general procedure for surveying a plant tissue for flavonoids has been evolved by Harborne and later modified by Bate-Smith (Harborne, 1973).

The chemical investigation includes extraction, isolation, purification and characterization. The detailed methods of isolation and characterization
of flavonoids are given in different books (Geissman, 1962; Harborne et al., 1975; Mabry et al., 1970; Harborne, 1993).

Flavonoids can be extracted with aqueous ethanol, which remains in the aqueous layer, following sequential solvent extraction with a number of solvents of varying polarity. Distribution between water and an organic phase such as ethyl acetate has been found effective for this purpose.

The above extractives are subjected to different chromatographic techniques. Column chromatography is the single most useful technique for the isolation of large quantities of flavonoids from crude plant extracts. Commonly used adsorbents are silica gel, cellulose and polyamide. Thin layer chromatography (TLC) provides new media for the separation of flavonoids on a small scale, and permits the use of a wider variety of detecting reagents. As in column chromatography, the adsorbents of choice for the separation of flavonoids are silica gel, polyamide and cellulose. The technique of paper chromatography (PC) occupies a dominant position in the field of flavonoid analysis and separation. PC is suitable for the separation of complex mixtures of all types of flavonoids and their glycosides. High pressure liquid chromatography (HPLC) (Kingston, 1979) is also suitable for the analysis. Another method, droplet countercurrent chromatography (DCCC), reduces the separation time and simplifies the isolation of previously unknown or unstable constituents from crude plant extracts. An instrument for rotation locular counter-current chromatography (RLCC) is suitable for flavonoid separation (Hostettmann, 1981). Besides all the above techniques, sublimation and recrystallisation methods are also used for the purification. Nowadays newer techniques like high-speed counter-current chromatography (HSCCC) is being employed for separation and purification of compounds (Ailing Sun et al., 2006).
The purified samples are characterized by determining physical constants, solubility behaviour, characteristic colour reactions with standard reagents, preparation of derivatives, $R_f$ in different solvent systems, colour changes under UV and UV/NH$_3$, and by analyzing percentage composition. IR (Harborne, 1973), UV (Harborne et al., 1975), $^1$HNMR (Ternai and Markham, 1976; Markham, 1976; Chari et al., 1977; Markham et al., 1978) and mass spectroscopic methods (Schulten and A., 1974) must also be made use of. Finally, the structure of the compound can be confirmed by synthesis. Nowadays, electrospray ionization tandem mass spectrometry (ESI-MS/MS) and one- and two-dimensional-NMR spectral studies including $^1$H-$^1$H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond connectivity (HMBC) and nuclear overhauser enhancement spectroscopy (NOESY) are being used for structural elucidation of flavonoids (Kesava Reddy et al., 2006).

1.1.5 Synthesis of flavonoids

Flavonoids can be synthesized in the laboratory. The most important methods are: scheme A- condensation of a $C_6C_2$ unit and (2-hydroxyacetophenone) with a $C_6C_1$ unit (aromatic aldehyde) and scheme B- acylation of phenols ($C_6$ unit) with a cinnamic acid derivative or its equivalent ($C_6C_3$ unit) given in Figure 1.3 which also corresponds to the biosynthetic pathway.

In addition, flavonoids can be prepared by modifying existing $C_{15}$-structure by oxidation, reduction, isomerisation, partial O- and C-alkylation, dealkylation, selective glycosylation or partial hydrolysis. There are so many reports available on the synthesis of flavonoids and their derivatives (Xud, 1994; Liang et al., 1995; Baltabaeva et al., 1993; Khaitbaev et al., 1994; Khaitbaev et al., 1995; Wagimova et al., 1996).
1.1.6 Flavonoid biosynthesis

It is established that all classes of flavonoids derive their carbon skeleton from compounds of intermediary cell metabolism through the action of phenyl propanoid and flavonoid glycoside pathways (Figure 1.4).
All aspects of the problem of flavonoid biosynthesis have been studied in comparative anatomy (Geissman and, 1952; Birch and, 1953; Robinson, 1955; Whalley 1961), chemical genetic studies (Harborne and Mabry, 1982; Alston, 1961) and radioactive tracer experiments (Grisebach, 1965; Grisebach, 1961; Grisebach, 1968; Grisebach and Barz, 1969). All classes of flavonoids are biosynthetically closely related, with a chalcone being the first common intermediate. A comprehensive report on the biogenesis of flavonoids by Manitto (1981) and a good account of the enzymology of biosynthesis of shikimate derived phenolic compounds by Harborne (1980) were published. Through subsequent hydroxylations and reductions, plants are able to form different classes of flavonoids (Samuelson, 1993) (Figure 1.4).

**Figure 1.4: Flavonoid biosynthesis**

3 Acetic acid  \[ \text{Phenylpropane} \]  \[ \text{A polyketide} \]

Chalcone structure  \[ \text{A Flavonoid} \]
1.1.7 Beneficial effects of flavonoids in plants

The most important function (Harborne, 1965; McClure, 1975) of the sap soluble flavonoids in plants is their ability to impart colour to flowers for attracting the insects, birds and animals. Another important function is their protective role against UV radiation and as feeding deterrents. As feeding deterrents, the more unusual flavonoid structures may offer some protection against overgrazing by the animals. The stimulation of protein degradation as well as inhibition of protein formation by chloramphenicol, tetracyclin and some other preparations resulted in the flavonoid accumulation, implicating the importance of flavonoids in protein synthesis (Margna and Lanest, 1974; Margna, 1975). Other important functions of flavonoids include their properties as antioxidants, enzyme inhibitors, precursors of toxic substances, photosensitizing and energy transferring compounds, phytolexins, in respiration, photosynthesis, morphogenesis and sex determination in plants.

Flavonoids are also useful in biochemical systematics or chemical plant taxonomy (Bendz and Santossan, 1974; Swain, 1963; Smith, 1976; Alston and Turner, 1963; Harborne, 1967; Harborne, 1968; Stace, 1980; Harborne, 1975). Flavonoids have been used in taxonomy as chemotaxonomic markers and phylogenetic indicators. For instance, flavone C-glycosides and biflavonyls are generally found in primitive plants and are either rare or absent in phytogenetically advanced plant families (Krishnaswamy, 1999). As a result of large surveys of distribution of these polyphenols in many families, it is now accepted that flavonoids have considerable potentialities as taxonomic markers in plant classification as they satisfy more than the generally agreed requirements for a chemical character to be of significance in plant taxonomy (Harborne, 1967) viz., chemical complexity and
structural variability, physiological stability, wide spread distribution and easy and rapid identification (Nair, 1984).

1.2 Beneficial effects of phytochemicals with special reference to flavonoids

Phytochemicals have been found to play a protective role in reducing chronic disease risk (Tsao and Drug, 2005). Flavonoids have shown potential health benefits arising from their antioxidant properties, which are attributed to the phenolic hydroxyl groups attached to the flavonoid structure (Rice-Evans & Packer, 1998). Flavonoids have antioxidant capacities that are much stronger than those of vitamins C and E (Prior & Cao, 2000).

Flavonoids possess different biological activities like antiinflammatory (On Wukaeme, 1995; De Whalley, 1990; Kandaswamy et al., 1991), anticarcinogenic (Nagai et al., 1992; Sahu and Gray, 1996; Watanable, 1989), antihepatotoxic (Unnikrishnan and Madistretti et al., 1989; Magistretti et al., 1988; Hikino et al., 1985), antioxidant (Abdul Rahim et al., 1986; Myara et al., 1993), antiviral (Realey et al., 1987), antiallergic (Saija et al., 1995), antibacterial (Baran and Ismailov, 1993), antiproliferative (Wagner et al., 1986), antiulcer (Verma et al., 1988), hypolipidemic (Thompson et al., 1972) and hypoglycemic (Halliwell, 1981) activities. There are also so many other reports on the biological activities of flavonoids like (1) inactivation of cytotoxic substances (Habtemariam, 1997; Wickramasinghe et al., 1996; Tukavkina et al., 1996), (2) antidiabetic (Haraguchi et al., 1997; Nanayakkara et al., 1988), (3) free radical scavenging and protection from lipid peroxidation (Kolhir et al., 1995, Haraguchi et al., 1997), (4) reduction of content of low density lipoproteins in liver and serum (Igarashi et al., 1996; Kolhir, 1995), (5) antitumour effect (Chu et al., 1992; Kandaswami et al., 1992; Devi & Das, 1993), (6) antimitagenic
effect (Huang et al., 1983), (7) radioprotective effect (Iluchenok et al., 1975; Tukavkina et al., 1996), (8) antiviral effect (Biziagos et al., 1987), (9) immunoregulative (antiallergic) and antiinflammatory effect (Kolhir et al., 1995; Bronner & Landry, 1985), (10) normalizing influence on cell enzyme systems (Vladutiu & Middleton, 1986) and (11) lower mutagenic activity and toxicity (Tukavkina et al., 1996; Solimani, 1996).

Many reports are available on the biological studies of flavonoids of which quercetin, kaempferol and gossypol are the well studied. A review (Formica and 1995) of the biological activity of flavonoids shows that these compounds may be used as the starting material for drug development programme. Quercetin has biological properties consistent with its sparing effect on the cardiovascular system. Quercetin and some other flavonoids modify eicosanoid biosynthesis (antiprostanoid and antiinflammatory responses), protect low density lipoprotein from oxidation (prevent atherosclerotic plaque formation), prevent platelet aggregation (antithrombotic effects), and promote relaxation of cardiovascular smooth muscle (antihypertensive, antiarrhythmic effects). Quercetin-4'-O-β-D-glucopyranoside-6'-gallate showed xanthine oxidase inhibitor activity for therapeutic use (Okamura et al., 1994).

Ismailov,
A review (Baran and 1993) of the biological activity of gossypol and its derivatives with 204 references gives an account of the immunosuppressive, antibacterial, antitumour and other pharmacological effects of gossypol derivatives and also the structure-activity relationships and pharmacokinetic data. Antiviral interferon-inducing and immunomodulating activities of gossypol and its derivatives were studied and it was appeared that these effects decreased by modification of hydroxy groups (Baran et al., 1995).
In vitro investigation on mouse brains with hypericum plant extract, hypericin and kaempferol showed antidepressant activities (Mueller et al., 1996). Antioxidant activity of flavonoids like quercetin and luteolin isolated from fresh pepper cultivars was reported by Lee et al. (1995). The antiradical and antioxidant properties of synthetic flavones have been studied by Wallet et al. (1992). Flavonoids like toxofolin and gossypin showed antiinflammatory action (Borissova et al., 1994), and luteolin, apigenin, catechin, naringenin, gossypin etc. showed antiulcer activity. A type of flavonoid, the flavans were generally found to be effective in selective inhibition of HIV-1, HIV-2 or SIV infection (Mahmood et al., 1993). Vachalkova et al. (1995) studied the potential carcinogenicity of homoisoflavonoids and flavonoids from Resino sanguinis draconis.

Moreover, the epidemiological studies (Cock & Samman, 1996) show an inverse correlation between the dietary flavonoid intake and mortality from coronary heart diseases. This may be associated with the ability of the flavonoids to attenuate LDL oxidation, macrophage foam cell formation and atherosclerosis (Fuhrman and Aviram, 2001). It was reported that flavonoids reduce the relative risk of coronary heart diseases to 68% (Schuessher et al., 1995). Thus, the importance of flavonoids in health and diseases is high and our effort concerned is in rediscovering their due role as potential medicinal agents.

1.2.1 Flavonoids as antioxidants

All organisms are steadily exposed to oxygen species like \( \cdot \)OH, \( \cdot \)OR, \( \cdot \)OOR etc. called free radicals (Ernster, 1993). A free radical may be defined as any species that has one or more unpaired electrons. This includes the hydrogen atom, most transition metals and the oxygen molecules itself. i.e., oxygen itself is a radical with two unpaired electrons. If the ground state oxygen molecule accepts a single electron, it must enter
one of the antibonding orbitals and it forms superoxide radical. Superoxide anion (O$_2^-$) is formed in almost all aerobic cells as the byproducts of many biochemical reactions such as the electron transport chain, catabolic steps in mitochondria, microsomal reactions etc. Addition of a second electron to superoxide anion gives the peroxide ion O$_2^{2-}$ that is not a radical. O$_2^{2-}$ formed at physiological pH will immediately promote to give H$_2$O$_2$. In aqueous solution O$_2$ undergoes the so called dismutation reaction to form H$_2$O$_2$ and O$_2$. The fission of O-O bond in H$_2$O$_2$ produces two hydroxyl radicals. A simple mixture of H$_2$O$_2$ and an iron (II) salt form hydroxyl radicals and iron (III). Traces of iron (III) can react further with H$_2$O$_2$ to form O$_2$, H$^+$ and Fe$^{2+}$ and this will follow a series of radical reactions. Thus the free radicals, superoxide anion (O$_2^-$), hydroxyl radical (·OH), hydrogen peroxide (H$_2$O$_2$), singlet oxygen (O$_2^*$) etc. are continuously generated in our body. These free radicals permeate cell membranes and enter in all cellular compartments which results in the peroxidation of lipids, leading to tissue damage \textit{in vivo}. When an imbalance between free radical generation and body defense mechanism occurs, oxidative damage will spread over all the cell targets (DNA, lipids, and proteins).

It has been reported that a series of human illness such as cancer, atherosclerosis, cardio- and cerebrovascular diseases, diabetes, immune system impairment, neurodegenerative diseases such as Parkinson’s and Alzheimer’s diseases and arthritis, as well as premature body ageing, can be linked with the damaging action of extremely reactive free radicals (Benavente-Garcia \textit{et al.}, 1997). This causes the pathogenic circumstances including ageing and inflammatory reactions. Normally the cells contain an elaborative network of antioxidant defense mechanisms. Cells are under continuous oxidative stress because of the imbalance between the oxidants and antioxidants. The predominance of oxidants leads to many biochemical changes and contributes to several human chronic diseases such as atherosclerosis and cardiovascular disease, mutagenesis and cancer, several
neurodegenerative disorders, the ageing process etc. Reports of Simbula et al (2007) show that oxidative stress plays a critical role in different degenerative diseases and cancer.

In our body, a wide array of enzymatic and nonenzymatic antioxidant defences exist, including superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, reduced glutathione (GSH) etc. Among the antioxidant defense mechanism in humans, the most important one may be those against oxygen radicals and lipid peroxidation (LPO). LPO can be of major significance to cell injury produced by free radical mechanisms (Guttridge et al., 1986), whether it precedes cell injury or occurs as a consequence of cell injury. LPO is a process in which each step may involve initiation, propagation and termination phases each of which is influenced by a wide range of factors (Aust, 1985). LPO is thought to be an important biological consequence of oxidative cellular damage. The destruction of unsaturated fatty acids that occurs in LPO has been linked with altered membrane structure.

Malondialdehyde (MDA) (Tirmenstein & Read, 1988), a breakdown product of LPO interacts with DNA, thereby leading to the disruption of the cellular organelles. LPO has been demonstrated to occur in isolated mitochondria, lysosomes and microsomes. It seems likely that nuclear membrane peroxidation may disrupt many critical functions.

The sequence of reactions which is now recognized on the basis of LPO was worked out in detail by Barry & Gutteridge, (1989). Fatty acid with 3 double bonds abstracts hydrogen radical and then undergoes molecular rearrangement to form conjugated diene with UV absorbance at 234 nm. This on $O_2$ uptake, peroxyl radical is formed, which abstracts $H\cdot$ from another fatty acid causing an autocatalytic chain reaction. This in turn
fragments into aldehydes including malondialdehyde and polymerization products.

Lipid peroxidation appears to be a major source of endogenous DNA damage in humans. Certain chemicals like NaClO₄, H₂O₂ etc. are found to enhance the lipid peroxidative damage. Perchlorate is an oxidation product of chlorate ion (ClO₃⁻) and releases oxygen radicals. Perchlorate is used as the oxidizer in rocket fuel, in certain fireworks, in the manufacture of matches and also in analytical chemistry. So people working in industries and laboratories are susceptible to perchlorate poisoning which leads to enhanced peroxidative damage resulting in many diseases. Relatively high doses of perchlorate inhibit the normal function of thyroid gland.

Using different tissue homogenate the ascorbic acid induced and ferrous sulphate induced lipid peroxidation are measured by the thiobarbituric acid reaction (Bishayee and 1971). High concentrations of ascorbate have an antioxidant activity and thereby protect the membrane lipids against oxidative damage. The peroxidation of EDTA washed mitochondrial fractions in the presence of 0.1 µM ascorbate show that the presence of iron is obligatory in ascorbic acid induced peroxidation (Ratty and Das,1988). The protective role of brain ascorbate content against LPO at physiological concentration, 1.5 x 10⁻³M, was studied by Seregi et al (1978).

Gokcora et al (1992) showed a decreased level of lipid peroxide and an increased level of glutathione by the ip. injection of epidermal growth factor (EGF), a growth promoting polypeptide. A closed inhibition of LPO by aqueous turmeric in the liposomal system was reported (Shalini & Srinivas, 1987). The peroxide induced DNA damage is found to be protected by aqueous turmeric extracts. Subramanian (1989) found that the dialyzed turmeric extract significantly inhibited the LPO of the brain
homogenate up to 85% at the highest concentration of turmeric extract studied.

Lipid peroxidation could be prevented (a) by reducing the formation of free radicals (b) by destroying the free radicals already formed (c) by supplying a competitive substrate for unsaturated lipids in the membrane and (d) accelerating the repair mechanism of damaged cell membrane. Many natural and synthetic antioxidants are in use to prevent the lipid peroxidation.

Now natural plant products like flavonoids are noteworthy as safe antioxidants (Frankel et al., 1996) and suppressing the peroxidation of membrane phospholipids induced by the attack of aqueous oxygen radicals (Junji et al., 1994). The reports on the antioxidant action of flavonoids are supported by numerous data on the inhibition of low density lipoprotein (LDL) oxidation and the reduction of platelet aggregation. This inhibition in LPO can be brought about either by chelating of transition metals (Clemetson et al., 1966; Afnas’ev et al., 1989) or by scavenging of free radicals with the formation of less reactive flavonoid aroxyl radicals. At present radical scavenging is clearly the favoured mechanism as evidenced by the lopsided ratio of reports on scavenging versus chelating properties of flavonoids. The inhibition of LDL oxidation by pure isolated flavonoids has been shown in numerous in vitro studies (De Whalley et al., 1990).

1.3 Brief description on the plants selected

1.3.1 Cochlospermum religiosum (Linn.) Alston

*Cochlospermum religiosum* (Linn.) Alston (synonym: *Cochlospermum gossypium* DC.) of the family, Cochlospermaceae, is a small or medium sized deciduous tree distributed in Garhwal,
Bundelkhand, Bihar, Orissa, Bengal, Madhya Bharat, Deccan, West Peninsula, and South India in dry forests, especially on stony hills, but less common on the West coast. (Chopra et al., 1999).

The vernacular names of the plant are as follows (Nadkarni, 1976):

<table>
<thead>
<tr>
<th>Language</th>
<th>Name</th>
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</tbody>
</table>

The leaves, flowers, and the gum obtained from the trunk is used in medicine. The dried leaves and flowers of the tree are used as stimulant (Chopra et al., 1999). The tree yields a gum called Indian “tragacanth” obtained from the trunk, is sweetish, cooling, sedative and used in coughs and gonorrhea (Wealth of India, 1950). The gum (Nadkarni, 1976) is demulscent and astringent and is used in hoarse throat and scalding in the urine. It is largely used, being mixed with curds or whey to cure diarrhea and dysentery. Tender leaves are used to make a cooling wash for the hair. The seeds are used for the preparation of pan cakes among indigenous tribes of South India.

*C. religiosum* is a small or medium sized deciduous, soft-wooden tree, 2.4-5.4 m in height and up to 1 foot in diameter. Bark is smooth and ash coloured. Leaves are scattered about the ends of the branchlets, 7.5-18
cm in diameter, palmately 3-5 lobed, glabrous above, white tomentose beneath, lobes are entire and acute, petioles 10-18 cm in length, pubescent when young. Flowers are 10-12.5 cm in diameter, in terminal subcorymbose panicles, bright yellow, appearing before the leaves, pedicels are stout, 5-12.5 cm in length, grooved, twisted and pubescent. Sepals are unequal, oblong, concave and silky outside. Petals are obovate and deeply emarginated. Capsules are 5-7.5 cm in length, obovoid and striate outside. Seeds are 6 mm in length, cochleate and covered with an abundance of white silky wood (Kirtikar & Basu, 1993). The flowers appear after leaf fall from December to April and are succeeded by large, pear-shaped capsular fruits, 2-3 inches in diameter and ripening in June-July.

The gum contains over 50% pentosans and galactans, and on hydrolysis with mineral acids yields 14% acetic acid, gondic acid, acochlospermic acid, xylose and galactose (The Wealth of India, 1950). This gum and the gum derived from Sterculia urens have assumed great importance in recent years, and several million pounds are exported annually from India for use in cigar paste and ice-cream industry. The gum is used as a substitute for tragacanth in calico printing, marbling paper and leather dressing. It is also used for giving polish to tusser silk. The gum has been much studied. It is characterized by giving off acetic acid recognized by smell on opening a bottle containing the gum. This gum also absorbs large quantities of water with consequent swelling. No enzyme could be found in it.

It is particularly common in hot, dry and strong regions. It is one of the few species capable of withstanding forest fires and is considered useful in afforestation of bare rocky and denuded hills. It is often cultivated in gardens and near temples for its beautiful yellow flowers. The seed is small, reniform in shape with a hard shell. The kernel has a sweetish somewhat almond-like flavour and a slight bitter taste.
Figure 1.5: Cochlospermum religiosum (Linn.) Alston

Whole Plant

Leaves
The analysis of seeds gave the following values. Moisture content 9.25, ether extractives 14.25, albuminoids 20.94, carbohydrates 34.78, crude fibre 14.63 and ash 5.15 %. The expressed oil is brown when freshly prepared, turning pale yellow on exposure to diffused light for several days. It has a peculiar taste and smell. It possesses the following characteristics.

- Specific gravity : 0.922
- Saponification value : 186.29
- Iodine value : 95.97
- Acid value : 14.24
- R.M. value : 0.19
- Solidifying point : 1°C

It is a non drying oil which can be used for making soaps. The residual cake after expressing the oil contains albuminoids - 21.5; oil - 8.1; carbohydrates - 39.8; fibre - 15-17 and ash - 5.45 %. It can be used as cattle feed or manure. The leaves and the gum obtained from the trunk are used as medicines. Powdered bark is applied to broken limbs of cattle. The plant is used in sores and tuberculosis (Asolkar et al., 1981).

Earlier phytochemical reports show that only a dihydroflavone, naringenin was isolated from the flowers of Cochlospermum religiosum (Linn.) Alston. (Ram. P. Rastogi and Mehrrotra, 1995).

1.3.2 *Adenanthera pavonina* Linn.

*Adenanthera pavonina* Linn. (Family – Leguminosae) is a moderate-sized deciduous tree, distributed in the eastern sub-Himalayan tract and in the Western Ghats. The tree grows well in moist areas and is easily propagated by cuttings. It is often planted along roadside, especially in southern India (Chopra et al., 1999a).
The vernacular names of the plant are as follows (Nadkarni, 1976a):

<table>
<thead>
<tr>
<th>Language</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assam</td>
<td>Chandat</td>
</tr>
<tr>
<td>Bengali</td>
<td>Raktha-kambal, Ranjan</td>
</tr>
<tr>
<td>Gujarati</td>
<td>Badi gumchi, Hati-gumchi</td>
</tr>
<tr>
<td>Hindi</td>
<td>Barigumchi</td>
</tr>
<tr>
<td>Kannada</td>
<td>Manjutti</td>
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<tr>
<td>Malayalam</td>
<td>Manjadi</td>
</tr>
<tr>
<td>Sanskrit</td>
<td>Kuchandana</td>
</tr>
<tr>
<td>Tamil</td>
<td>Anai-kundumani, Manjadi</td>
</tr>
<tr>
<td>Telugu</td>
<td>Bandi gurvina, Mansenikottae</td>
</tr>
<tr>
<td>English</td>
<td>Coral wood</td>
</tr>
</tbody>
</table>

*Adenanthera pavonina* is a deciduous fast-growing tree, 18-24 m in height and 2.0-2.4 m in girth. Bark is rough on old trees, dark brown or greyish outside and brownish-white or white inside. Leaves are pinnate, 20-30 cm in length; leaflets are alternate, glabrous, obtuse, elliptic-oblong, papery and in 4-8 pairs. Flowers are borne in short-peduncled axillary racemoses or panicked at the end of branches, fragrant and yellowish. The five petals are united at the base. The calyx is small and bell-shaped, with short teeth. Pods twisted, linear, narrow, 15-20 cm in length, containing small hard lustrous scarlet seeds. There are 10 to 12 seeds in a pod, which are usually shining, lenticular and compressed. The plant flowers during summer and fruits during winter (Asima Chatterjee and Satyesh Chandra Prakashi, 1995).

The heart-wood is red and is reported to be used as a substitute for true red sandalwood. The wood is used in South India for building purposes and cabinet-making.
Traditionally, the different parts of the plant - seeds, leaves, root and bark - are used as medicines (Chopra et al., 1999a). The decoction of leaves or bark is used in chronic rheumatism, gout, piles, haemateria and haematemesis. Seeds are used for boils and inflammation. Powdered seeds externally applied, hasten suppuration of boils, inflammation etc. When used for a long time it acts as aphrodisiac. Root is used as an emetic.

Earlier reports show the presence of triterpenoids (Yadav et al., 1976; Chandra et al., 1982), alkaloids (Chopra et al., 1999a), steroidal glycosides (Misra et al., 1973), cysteine proteinase (Silva et al., 1995), and fatty acids (Misra et al., 1975) in the plant. The flavonoids in the wood of *Adenanthera pavonina* had been reported by Gennaro et al (1972). Recently a new 5-membered lactone called pavonoin was isolated by Ali et al (2004).

Regarding the phytochemical studies on the root of *A. pavonina*, only oleanolic and echinocystic acids and their esters have been reported (Ram P Rastogi and Mehrrotra, 1999).

### 1.3.3 *Ruellia tuberosa* Linn.

*Ruellia tuberosa* Linn. is an erect perennial herb growing upto a height of 6½” with a hairy stem, with elongated fleshy, tuberous roots, elliptic or ovate leaves and blue mauve flowers. The plant flowers only after the start of the rainy season. The herb possesses emetic properties. It is used as an anthelmintic, against joint pains and strained muscles. *Ruellia tuberosa* was screened for its possible antifertility activity (Andiwall et al., 1986). It is also used for the treatment of stones in bladder. A decoction of the leaves is given in chronic bronchitis (Wealth of India, 1972). The plant is used medicinally in West indies, Central America, Guina and Peru and it is distributed throughout India, Ceylon and East Africa (Kirtikar & Basu, 1994). It is grown as an ornamental plant (Ram P Rastogi and Mehrrotra, 1999).

The vernacular names of the plant are as follows:
Figure 1.6: Adenanthera pavonina Linn.

Figure 1.7: Ruellia tuberosa Linn
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The plant is grown in gardens, giving a fine display of colour during the summer months. Another form, plant bearing white flowers are also grown. The one having flowers with rosy throat is particularly attractive. The species has run wild at many places.

The plants are propagated by cuttings or tubers or through seeds produced in brownish black capsules. Capsules explode on ripening and the seeds disperse all around. The ripe fruits contain 7-8 seeds each in a pod.

Phytochemical reports on *R. tuberosa* show that the alkane fraction consists of triacontane, pentatriacontane, hentriacontane, lupeol and nonacosane as major constituents. The sterol fraction contains stigmasterol, β-sitosterol, campesterol and cholesterol (Rastogi and Mehrotra, 1993a). A flavonoid apigenin-7-β-D-glucoside was isolated from the plant (Rastogi and Mehrotra, 1991). Plant contains leucine, tyrosine, valine and glycine (Ram. P. Rastogi and Mehrrotra, 1995a).

1.4 Objectives of the present study

With the above views the present study was intended to evaluate the antioxidant properties of three medicinal plants on which no systematic biochemical and phytochemical research work has been so far carried out and to isolate the active principles from them. *Cochlospermum religiosum* which showed maximum antioxidant property was selected for detailed phytochemical and biochemical investigation. Most of the diseases including cancer and cataract are caused by oxidative stress which leads to cell death in our body. Hence, the therapeutic evaluation of the anticataractogenic and anticarcinogenic potential of the alcohol extract of *C.*
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religiosum and the flavonoid, isorhamnetin-3-glucoside were determined in in vitro models. The antioxidant effects of the samples were evaluated using in vitro and in vivo experimental models. This work assumes significance as it is the first report on the isolation and characterization of isorhamnetin-3-glucoside and isorhamnetin from the leaves of C. religiosum and the evaluation of their biochemical parameters.

The following studies were carried out:

1. Five plants on which no indepth phytochemical and biochemical studies carried out so far were selected.
2. A preliminary screening study for their flavonoid content was performed and three plants selected from the initial five.
3. In vitro studies on antioxidant activity of the three selected plants - Adenanthera pavonina, Cochlospermum religiosum and Ruellia tuberosa - were carried out.
4. Successive extractions using different organic solvents were carried out and their antioxidant potential assessed in vitro.
5. Identification of the fraction exhibiting maximum antioxidant activity.
6. Isolation and identification of the major flavonoid components from the active fractions of C. religiosum.
7. Structure-activity relationship studies on apigenin (R. tuberosa), robinetin (A. pavonina) and isorhamnetin (C. religiosum) for antioxidant activity in comparison with quercetin.
8. In vivo study to ascertain the toxicity of the selected plant materials.
9. In vivo dose response study to fix the minimum effective dose and toxicity study of the alcohol extract and the flavonoid isolated - isorhamnetin-3-glucoside - from C. religiosum.
10. In vitro studies to assess the antioxidant potential of the alcohol extract and the flavonoid, isorhamnetin-3-glucoside from C. religiosum.
11. *In vivo* studies to monitor the effects of these plant products on different biochemical parameters such as (i) activity of antioxidant enzymes (ii) levels of lipid peroxidation products (iii) glutathione metabolism (iv) activity of certain enzymes involved in liver function and (v) cholesterol metabolism

12. *In vitro* anticataractogenic property on selenite-induced cataract models in culture.

13. *In vitro* studies to assess the protective effect of the compound as an anticarcinogen.