Nature is an excellent synthetic organic chemist. Using mild reaction conditions and a few elemental combinations a large variety of complex molecules are made in and around us. The atoms are put together in precise arrangements to enable to carry out different tasks with remarkable specificity. The treasure house of natural chemicals contain delicate perfumes, spectacularly coloured substances, medicines for numerous ailments including deadliest of poisons.

It is a familiar fact that the living world which surrounds us and of which we are an integral part consists of two kinds of organisms each of which has developed a pattern of living successfully in its own way. One is represented by the animal kingdom and the other developed by plants with a resulting diversity of quarter of a million species. The plant kingdom is a virtual goldmine of new chemical compounds waiting to be discovered. According to rough estimates, among the 7.5 lakh species of plants on earth, 5 lakh being classified as higher plants and 2.5 as lower plants, only 5-10% have been examined for their chemical constituents and / or biological activity. With the extermination of plant species progressing at an alarming rate in certain areas, even before the plant has been recorded, much less studied chemically, increased efforts directed towards the conservation of gene pools of locally used medicinal plants are urgent and most needed.

India with its vast area from Kashmir to Kanyakumari and varying soil and climatic conditions ranging from tropical to temperate, has one of the world's richest vegetations (Angiosperms, the largest of all the major groups of living plants, include about 300
families with 12,500 genera and 2,50,000 species), to be aptly called "Botanic garden of the World". India also has a rich heritage of indigenous drugs from the vedic times. The 'Ayurvedic system' of medicine is strictly Indian in origin and development. More than 2400 remedies have been known from Indian medicinal flora. The remarkable properties and therapeutic uses of about 700 plant drugs have been recorded by ancient Indian scholars - Sushruta, Charaka and Vagbhatta probably earlier than 1000 B.C. Sanskrit literature written by them contains information about the morphological features of many medicinal plants, their geographical distribution, condition of growth, the best season for their maximum potency as well as toxicity.

India like all other countries, has made significant progress by a systematic scientific study of these plant drugs from the pharmacological, chemical, pharmacognostical and clinical points of view during the past 65 years, bringing to the forefront a large number of herbs used in Indian indigenous system for their approved efficiency and administration in modern medicine. Recognising the importance of medicinal plants, collaborative teamwork for a complete study of plant drugs has been encouraged by the Indian Council of Medical Research (ICMR), Central Council for Research in Ayurveda and Siddha (CCRAS) and Council for Scientific and Industrial Research (CSIR). The results of various studies on Indian medicinal plants are available as research publications and a number of books and monographs.8-10 Central Drug Research Institute (CDRI), Lucknow initiated a programme from 1979 to investigate Indian plants which either had a reputation in folklore medicine or whose extracts showed consistent biological activity when put to a broad biological screen. So far under this programme over 8000 species have been screened and biological activity has been confirmed in about 650 plants.20
Phytochemistry,\textsuperscript{21-26} evolved from natural products chemistry is confined to the study of products elaborated by plants and it has developed as a distinct discipline between natural product organic chemistry and plant biochemistry in recent years. It deals with the study of chemical structures of plant constituents, their biosynthesis, metabolism, natural distribution and biological functions. The fact that only less than 6% of about 6 lakh species of plants on earth has been investigated, indicates the opportunity provided and challenges thrown open to phytochemists. The task of phytochemist is compounded in accomplishing the characterisation of very small quantity of the compounds isolable from plants. Phytochemistry also enjoys the application of modern research for the scientific investigation of ancestral empirical knowledge. It has found wide and varying application in about all fields of life and civilization. Its direct involvement in the field of food and nutrition, agriculture, medicine and cosmetics is well known for years. Its contribution even in seemingly remote areas such as plant physiology, plant pathology, plant ecology, paleobotany, plant genetics, plant systematics and plant evolution has been increasingly felt. One of the more encouraging trends as phytochemistry continues to grow and develop as a scientific discipline, is the wider and wider applications that are occurring in agriculture, horticulture and forestry.\textsuperscript{27}

Among the phytochemicals, the polyphenolics constitute a distinct group. They embrace a wide range of substances which possess in common an aromatic ring bearing one or more hydroxy substituents or their ether or glycoside derivatives. These compounds possess great structural diversity and are of widespread occurrence among the secondary metabolites. A further feature of this particular group of compounds is their ability to interact with primary metabolites such as polysaccharides and proteins.
Among the several thousands of naturally occurring phenolic compounds, the flavonoids are the largest and the most widespread. Though the flavonoids have been found along with alkaloids, steroids etc., not much attention was paid to their medicinal importance for a long time. Considerable interest has now been shown in plant flavonoids as human dietary components, therapeutic agents and as having significant activity in a variety of isolated animal cell system. Given the importance of flavonoids as components of the human diet (the daily diet consists approximately 1g of mixed flavonoids) the pharmacology of these compounds has been studied. The resurgence of interest in the study of traditional medicines together with the availability of better bioassays has seen renewed efforts to understand the interaction of various flavonoids with mammalian tissues and enzymes, and an extensive review\textsuperscript{28} is available on this topic.

The flavonoids, one the most numerous and widespread group of natural constituents, are important to man not only because they contribute to flower colour but also because many members (eg: coumestrol, phloridzin, rotenone) are physiologically active. Although the anthocyanins have an undisputed function as plant pigments, the raison d’etre the more widely distributed colourless flavones and flavonols still remain a mystery. It is perhaps the challenge of discovering these yet undisclosed functions which has caused the considerable resurgence of interest in flavonoids during the last few decades. Flavonoids exhibit a wide range of biological activities, and currently are of particular interest as potential anticancer agents,\textsuperscript{28} as insect antifeedants and as natural insecticides.\textsuperscript{29}

Over 4000 chemically unique flavonoids have been identified in plant sources and new structures are being reported at an ever increasing rate. These low molecular weight
substances, found in all vascular plants are phenyl-benzopyrones (phenylchromones) with an assortment of basic structures. Primarily recognised as the pigments responsible for the autumnal burst of hues and many shades of yellow, orange and red in flowers and food, the flavonoids are found in fruits, vegetables, nuts, seeds, stems, flowers as well as tea and wine. Flavonoids have definite advantage over alkaloids from the point of view of pharmacological evaluation, that they occur universally making a search for new active substances simpler and the overall uniformity in their chemical structure providing easier understanding of Structure Activity Relationship (SAR). The ready availability of the more common flavonoids in pure form is a definite advantage for the study of their biological activity and evaluation of their pharmacological properties. Flavonoids have attracted the attention of scientists of different disciplines and the systematic investigation on their occurrence, natural distribution, chemical nature and biological functions continues unabated.30-36

The term “flavonoid” was first applied about 47 years ago by Geissman and Hinreiner37-38 to embrace all those compounds whose structure is based on that of flavone (2-phenylchromone) (I) having a basic C₆-C₃-C₆ skeleton in common. When the heterocyclic ring is reduced, it becomes flavan (2-phenylchroman) (II). Flavone (I) consists of two benzene rings (A and B) joined together by a γ-ring (ring C). The various classes of flavonoid compounds differ from one another only by the state of oxidation of this carbon link. There is a limitation to the number of structures commonly found in nature, which vary in their state of oxidation from flavan-3-ols (catechin) (III) to flavonols (3-hydroxy flavones) (IV) and anthocyanins (V). Flavanones (VI), flavanonols or dihydroflavonols (VII) and the flavan-3, 4-diols (proanthocyanidins) (VIII) are also
included in the flavonoids. It should be noted that there are also five classes of compounds (dihydrochalcones or 3-phenylpropiophenones (IX) chalcones or phenyl styryl ketones (X) isoflavones or 3-phenyl chromones (XI) neoflavones or 4-phenyl coumarins (XII) and the aurones or 2-benzylidene-3-coumaranones (XIII) which do not actually possess the basic 2-phenyl chromone (I) skeleton, but are closely related both chemically and biosynthetically to other flavonoid types, that they are always included in the flavonoid group.

The individual compounds in each class are distinguished mainly by the number and orientation of hydroxy and methoxy groups in the two benzene rings. These groups are usually arranged in certain restricted pattern in the molecule, reflecting the different biosynthetic origin of the two aromatic nuclei. Thus, in the A ring (I) of the majority of flavonoid compounds, hydroxy groups are distributed at either C-5 and C-7 or only at C-7 (C-5 and C-7 of flavone become C-2' and C-6' in dihydrochalcones and chalcones and C-4 and C-6 in aurones) and generally are unmethylated. This pattern of hydroxylation follows from the acetate or malonate origin of the ring. The B-ring (I) of flavonoids on the other hand is usually substituted either by one, two or three hydroxy or methoxy groups. The rarely methylated position is C-4' with often methylation at C-3' and C-5'. The hydroxylation pattern of the B-ring thus resembles that found in commonly occurring cinnamic acid (XIV) and coumarins (XV) and reflects their common biosynthetic origin from prephenic acid and its congeners.
STRUCTURES OF SOME COMMON
FLAVONOID TYPES

I Flavone

II Flavan

III Flavan 3-ol

IV Flavonol

V Anthocyanidin

VI Flavanone

VII Dihydroflavonol

VIII Flavan 3,4-diol
A comparison of the nature and position of various substituents at different carbons of the flavonoid skeleton has been made use of in arriving at certain generalisations regarding structure and properties.
Most of the flavonoids occur naturally in conjugated form, usually bound to sugar, by a hemiacetal linkage. But their conjugation with inorganic sulfates or organic acids is not unusual. The sugar free compounds are referred to as aglycones and it is probable that in most cases they are formed as artefacts during the course of extraction, since most living tissues contain very active glycosides which can work even in the presence of high concentration of organic solvents. The presence of sugars in the molecule confers sap-solubility to the generally somewhat insoluble flavonoid compounds. In anthocyanins the sugar imparts stability to the aglycone. Stability conferred by glycosylation to flavonols is observed in 3-O-glycosides of quercetin and myricetin which are not susceptible to oxidation catalysed by phenolase unlike the corresponding aglycones, presumably because of steric reasons. More and more range of new glycosides are encountered in plants. An increasing number of flavonoid glycosides carrying sugars in B-ring hydroxyls have been reported. Conjugation of flavones and flavonols through glucose with organic acids like malonic acid and derivatives of cinnamic acids have also been reported. The number of acylated flavonoid compounds succeeded by terpenoid counterparts is on the increase. As a result of electrophoretic studies, a number of zwitter ionic anthocyanins with malonic acid and succinic acid linked to C-6 of glucose have been isolated and characterised.

The sugars found in flavonoid glycosides include simple pentoses and hexoses (monosides) and di- and tri-saccharides (biosides and triosides) mostly combined through oxygen at C-1 position of sugars, usually by a β-linkage. In many cases more than one phenolic hydroxyl group in the flavonoid molecule maybe glycosylated giving rise to diglycosides and so on. The common sugars encountered are D-glucose, D-galactose, L-
rhamnose, D-xylose, L-arabinose and D-glucuronic acid. D-allose and D-galacturonic acid are rare and D-apiose is an unusual and uncommon one.

The study of the distribution of flavonoids in plants is a continuing exercise and known flavonoids are being regularly discovered from new sources. Flavonoids are universal in vascular plants, but variation according to phyla, order, family and populational variation within species have been detailed by Harborne and Turner.

Only in 1985, the distribution of flavonoids in animals has been reported with the isolation of 4'-methoxy flavone from scent glands of Canadian beaver (castor feber) and in Lepidoptera. No doubt, the presence of flavonoids in butterflies has been earlier recognised.

A single plant may contain one or more aglycones with several glycosidic combinations. For this reason, it is better to examine the aglycones of acid hydrolysate of the plant extract before examining the nature of glycosides. Flavonoids being polyphenolic compounds give characteristic colour changes when treated with alcoholic Fe, ammonia vapour or alkalis. Positive Shinoda test (Mg-HCl) and formation of coloured complexes with heavy metal salts are characteristic of flavonoids.

Standard methods of extraction, separation and chemical characterisation of flavonoid compounds are described by Peach and Tracey as well as Harborne. Systematic procedure for their identification employing chromatographic methods of analysis and chemical and spectral methods of identification have been detailed by Geissman, Mabry et al, Markham, Jay et al, Harborne and Mabry and Linskens and Jackson.

The conventional chromatographic methods like column, paper and thin layer are still in use for separation and purification of the flavonoid compounds. Increase in speed and efficiency in the separation of mixtures by new techniques like centrifugal TLC (chromatotron)\(^6\) and high pressure liquid chromatography (HPLC)\(^{66-68}\) have been achieved. Among the modern separation techniques applied to flavonoids, HPLC has the advantage over other techniques in regard to sensitivity, rapidity and easy quantification. Hosttetman and Hosttetman\(^69\) reviewed the relevant literature on HPLC upto 1980. A few other publications in this field include \(R_s\) values by Daigle and Conkeston,\(^70\) use of Bondpak C\(_{18}\) with MeOH-HOAc-H\(_2\)O as developing system with two pumps by Casteele et al\(^71\), analytical problems in HPLC by Bankova et al\(^72\), Tamma et al\(^73\) and Barberan et al\(^74\). Recently the application of HPLC combined with FABMS has helped in structural elucidation of anthocyanin pigments.\(^75\) On line HPLC-UV spectroscopy, centrifugal thin layer chromatography\(^76,77\) using chromatotron and flash chromatography\(^78,79\) are also applied in the isolation of flavonoids. For difficult separations requiring very high resolution semipreparatory HPLC with automatic fraction collector is an ideal method. Reverse phase chromatography\(^80\) on chemically bonded phases gives better results for the separation of plant phenolics. The complication of irreversible adsorption and decomposition of the solute at the liquid-solid interface in all techniques employing a solid stationary phase and liquid phase is overcome by various support free liquid-liquid partition techniques. Among these the two most efficient all liquid chromatographic techniques, DCCC\(^81,82\) and RLCC\(^83\) have been employed profitably for quantitative and qualitative separation by Hosttetman and collaborators,\(^84\) Aritomi et al\(^85\) and Gunasegaran et al.\(^86\)
However the constant need in natural product chemistry to separate large and small quantities of complex mixtures efficiently and rapidly is unfortunately seldom satisfied by the use of any one chromatographic technique. The best results have been obtained by a combination of several techniques which are often complementary. Paper electrophoresis is a technique of limited application in flavonoid analysis, since to be mobile, a flavonoid must be in an ionised state at the pH of the electrolyte. Its useful application lies in the recognition and identification of flavonoid sulfates and in the distinction of glycuronides from glycosides. Relative mobilities of different flavonoid sulfates are listed by Hosttetman. Electrophoresis finds greater application in the field of anthocyanins and betacyanins.

The flavonoid once isolated as a homogenous compound is characterised by the specific colour tests, physical constants, elemental analysis, $R_f$ values in various solvent systems, analysis of hydrolysis products, preparation of derivatives and comparison of these data with related compounds. Further support for confirmation of the structure of the flavonoid is achieved by the analysis of different spectral data (UV-Visible, IR, $^1$H and $^{13}$C NMR and MS).

The development in the general methodology of natural product chemistry is readily applicable to flavonoid compounds also. Ultra violet spectroscopy is still one of the oldest and useful techniques for flavonoid identification. The UV spectrum with MeOH and with diagnostic shift reagents can give information on the type of flavonoid as well as its substitution pattern. The use of AlCl$_3$ and AlCl$_3$/HCl UV spectra in the precise determination of the structure of flavonoid compounds was demonstrated by Voirin and the limitation of NaOAc spectra in flavonoid analysis has been reported by Rosler et
IR spectroscopy provides valuable information regarding the type of flavonoid as well as the nature of the substituents like methoxy, methylene dioxy and prenyloxy groups. It is also used as a 'finger-printing' devise for establishing the identity of two samples.

Over the past thirty years, chiroptical methods like ORD and CD spectral analysis have been employed for the determination of stereochemistry of chiral flavonoids. The exciton chirality method employing the application of coupled oscillators in determination of the chirality of natural products is receiving greater attention.

Mass spectrometry of flavonoids serves as a valuable aid in determining the molecular weight and probable structure even with very small sample size. The electron impact mass spectrometry (EIMS) is used for the volatile compounds, the non-volatile compounds are converted to suitable derivatives like trimethyl silyl ether, permethyl ether, methyl ester or similar derivatives. When two MS are linked in tandem, it has now become possible to employ the first as separator and the second as analyser to perform direct mixture analysis (Tandem-MS). This multiple stage MS has been reviewed by Roush et al. Field desorption mass spectrometry (FD-MS) employed for polar and thermolabile compounds like flavonoids has been reviewed by Schulten and Games. Desorption chemical ionisation mass spectrometry (DCI-MS) using electrically heated tungsten probe and fast atom bombardment mass spectroscopy (FAB-MS) using the sample solubilised in polar matrix (like glycerol, thioglycerol etc.,) deposited in a copper target which is bombarded with energised neutral atoms to induce ionisation and desorption appears to be the most advantageous one for the analysis of flavonol glycosides. In the chemical ionisation mass spectrometry (CI-MS)
the ions are formed in ion molecular collisions which include abstraction as primary process using weak gas phases like CH₄, NH₃, i-C₄H₁₀ in positive CI for protonation. In negative CI, OMe⁻ as reagent for proton abstraction or CI⁻ as an attachment reagent is employed. The MS analysis of permethyl ether of glycosides is widely employed for settling structural problems in C-glycosyl flavones.¹⁰⁷,¹⁰⁸ Pyrolysis chemical ionisation mass spectrometry of flavonoids under positive and negative ionisation conditions is observed¹⁰⁹ to yield data characteristic of both aglycone and sugar residues, providing an alternative for FD and FABMS techniques. The easy MS differentiation of flavanones and dihydroflavonols by the characteristic fragments has been reported.¹¹⁰ The application of GC-MS¹¹¹ analysis to perdeuteromethylated derivatives of flavonoids¹¹² has rendered the identification of certain methoxylated compounds easier. Online HPLC-MS¹¹³ will be readily accepted by flavonoid researchers. Solution phase secondary ion mass spectrometry¹¹⁴ has proved useful in the determination of molecular weight of complex flavonoid glycosides. Becchi and Fraisse¹¹⁴ have reported mass analysed ion kinetic energy (MIKE) and collision activated dissociation MIKE spectra of flavonoids providing characteristic fragment ions which permit differentiation of the 6- and / or 8-substituent location and the position of O-glycosylation. Electrospray MS¹¹⁴ has become quite versatile on account of the amanability of the technique to highly polar and large sized molecules including biomolecules like proteins.

The continuing rapid development in instrumentation in particular those involving Nuclear Magnetic Resonance (NMR) over the last twenty years has given considerable impetus to structural elucidation in all fields of natural product chemistry. The proton magnetic resonance spectroscopy is the established non-destructive method of flavonoid
analysis. Use of high field magnets and computer assistance has made the recording of high resolution $^1$H NMR spectra of minute quantity of flavonoids.115-117 Typical application of $^1$H NMR includes determination of oxygenation pattern, number of methoxy group, distinction of isoflavones, flavanones and dihydroflavonols, number and nature of sugar present (whether α-linked or β-linked) and detection of hydrocarbon side chain. Shift reagents provide a method of spreading out PMR absorption signals. The use of lanthanide shift reagents in positioning of methoxyl groups of flavonoids was reported by Joseph Nathan et al.118

Of late $^{13}$C NMR spectroscopy119-125 has become the most useful technique for the structural determination of flavonoids. $^{13}$C resonance signals extending over 200ppm provide the nature of the carbon skeleton. Carbon relaxation time measurements being less difficult is very useful in differentiating otherwise non-discernible carbon atoms like C-6 and C-8 in flavonoids. Interglycosidic linkages in the case of disaccharides126 (rutinose, neohesperidose etc.,) type of linkage with aglycone and sugars and conjugation with sulfates and organic acids can also be determined. Homonuclear and heteronuclear correlation spectroscopy127 (HOMCOR and HETCOR) and the various decoupling experiments have reduced the difficulty in the interpretation of $^1$H and $^{13}$C spectra of complex molecules. Fourier transform NMR employing popular pulse sequences like off-resonance, heteronuclear decoupling, gated heteronuclear decoupling, inverse gated heteronuclear decoupling and selective proton decoupling has eased the task of assignment of peaks in a $^{13}$C NMR spectrum. Refocused INEPT with decoupling is an alternate of off-resonance decoupling for assigning carbon multiplets. J-modulated spin echo spectroscopy128 is yet another method of off-resonance decoupling which gives
positive signal for even number of hydrogen (\(\text{CH}_2\) and quaternary) and negative signal for carbon with odd number of hydrogen (\(\text{CH}\) and \(\text{CH}_3\)) attached. Usually a set of routine 2D experiments\textsuperscript{130} including COSY (and / or DQF cosy), relayed COSY [(HOHAHA), 2D NOE (NOESY/ROESY) and HETCOR (HMQC)] are used in structure elucidation exploiting a wealth of information inherent in the data. No doubt, considerably decreased sample size required for 2D NMR studies at present\textsuperscript{131,132} make it more acceptable to natural product chemist.

If the flavonoid compound can be obtained in a fine crystalline form, X-ray analysis can help in further confirming the structure as reported in the case of calycopterin.\textsuperscript{133}

Structures of organic compounds are usually determined by using data generated by chemical and instrumental methods. A chemist then invariably attempts to establish the proposed structure by unequivocal total synthesis. The conventional methods of synthesis of flavonoids from simple precursors by condensation methods have been proposed by Baker and Venkataramen,\textsuperscript{134} Algar and Robinson,\textsuperscript{135} and Algar and Flynn.\textsuperscript{136} These methods of synthesis have been modified by Farkas et al\textsuperscript{137} and Wagner et al\textsuperscript{138} as illustrated by the synthesis of a number of flavonoids and their methyl ethers. Many flavonoid compounds have been prepared by simple modification of the existing structure through nuclear oxidation, nuclear reduction, isomerisation, selective alkylation and dealkylation, selective glycosylation and partial hydrolysis. Farkas et al\textsuperscript{139} accomplished the synthesis of methoxylated flavones from the corresponding brominated methoxy chalcones. The synthesis of 5,6,7,3',4'-pentamethoxy flavone (sinensetin) by dehydrogenation of the corresponding flavanone with \(\text{SeO}_2\) has been achieved by Wagner
This flavone has been used as the starting material for the synthesis of a number of related flavones. Bose et al.\textsuperscript{140} have reported cyclisation and simultaneous dehydration of the hydroxy chalcone to the corresponding flavone by heating with palladium on charcoal. A short and facile synthetic route to hydroxylated flavones has been reported by Nagarathnam and Cushman.\textsuperscript{141}

The synthesis of flavonoid glycosides have been achieved using the $\alpha$-acetobromo sugars of pentoses, hexoses or disaccharides and the aglycones in the presence of catalysts. The selective glycosylation of 7-OH has been achieved by Zemplen and Farkas.\textsuperscript{142} Synthesis of other glycosides have been accomplished by trans acylation methods by Nogradi et al.\textsuperscript{143} and Wagner et al.\textsuperscript{144} The total synthesis of C-glycosyl flavones has been reported by Eade et al.\textsuperscript{145} A more general synthesis, exemplified by the preparation of 5,7,4'-trimethoxy vitexin\textsuperscript{146} and other complex ones has been provided by later workers.\textsuperscript{147} Thus the synthesis of almost all types of mono and di-C-glycosyl flavones and flavone C-glycosyl-O-glycosides has been accomplished,\textsuperscript{148} synthesis of some novel flavonoids has been illustrated by Rakosi et al.\textsuperscript{149} The chiron approach to the total synthesis of natural products\textsuperscript{150} might become a useful guide in the synthesis of chiral flavonoid compounds. Studies of the selective O-alkylation and dealkylation of flavonoids with anhydrous AlBr$_3$ were reported by Horie et al.\textsuperscript{151}

Flavonoids may have existed in nature for over one billion years\textsuperscript{152} and thus may have interacted with evolving organisms over the aeons. Clearly the flavonoids possess some important purposes in nature, having survived in vascular plants throughout evolution. Our ideas of the evolution of flavonoids is mainly based on our knowledge of
the overall modes of biosynthesis of the different classes of compounds. Tremendous progress has been made in the biosynthesis of flavonoids. Our present knowledge in flavonoid biosynthesis is based on a combination of earlier results from radioactive tracer studies in vivo and the more recent data obtained at the enzyme level in vitro. In the past few years the enzymology of flavonoid biosynthesis has made particularly rapid progress. Flavonoid biosynthesis can be considered in three stages. The first is the formation of the basic C₆-C₃-C₆ skeleton through acetate-malonate and shikimic acid pathway to aromatic compounds. The second stage is concerned with the ways by which the different classes of flavonoids are synthesised. The final stage embraces the elaboration of individual compounds within each flavonoid class, involving steps such as hydroxylation, glycosylation, methylation etc. Chalcone is considered as the common intermediate in the biosynthesis of all classes of flavonoids. Insight into three aspects of the problem of flavonoid biosynthesis has come in the past from comparative anatomy and chemical genetic studies and recently from feeding experiments with radioactive tracers. Major work on chemical genetic studies have been carried out by Grisebach and co-workers. Research has led to the isolation and characterisation of enzymes of the pathway of flavonoid biosynthesis. The use of young plant tissues and cell suspension cultures as source materials have also greatly facilitated the study of flavonoid biosynthesis at the enzyme level. Roux and Ferreira have highlighted the special role of α-hydroxy chalcone as the key intermediate in flavonoid biogenesis. A comprehensive report on the biosynthesis of flavonoids by Manitto and Heller and Forkman, a good account of biosynthesis of shikimate derived phenolic compounds by Harborne, biosynthetic studies in vivo with labelled precursors and biochemistry of
flavonoid biosynthesis by Heller are useful publications. The importance of flavonoids and other secondary metabolites in plant biochemistry has been detailed in "the Biochemistry of plants". Different aspects of mammalian metabolism of flavonoids have been reviewed by De Eds, Scheline, Griffiths and Middleton Jr and Kandaswami. Flavonoids are the constituents of the mammalian diet derived from plants. The ingestion of flavonoids by mammals in the diet or for therapeutic use, brings them in contact with both intestinal micro-organisms and mammalian tissues which are capable of biotransformation of flavonoid compounds. The available evidences indicate that the hydrolysis of the flavonoid glycosides to their corresponding aglycones, ring fission and oxidative and reductive transformations are mediated by intestinal micro-organisms. Though it is certain that the metabolic changes undergone by flavonoids occur within mammalian tissues, the relative contribution of individual tissues is not fully understood.

The flavonoids find exceptional use as taxonomic guide in the classification of plants. The reason for preferring flavonols to other secondary metabolites is their structural diversity, widespread distribution, comparative stability, easy detection and identification and the fact that this group of plant products is not actively concerned with cellular metabolic processes. Any particular flavonoid can be relied to be present in more or less constant amount in the same tissue of the same species so long as the plants are grown under normal physiological conditions. The conspicuous exception to this is the variation in the relative concentration of p-coumaric acid and caffeic acid (mono and dihydroxy phenyl propanoid precursors) as well as kaempferol and quercetin which are insignificant from the taxonomic point of view. Importance of flavonoids in
chemotaxonomy can be illustrated with a few examples. Phloretin or its 3-hydroxy derivatives occur in all species of Malus (Rosaceae).\textsuperscript{174} No other genus in Rosaceae contain these compounds. Caviunin, an isoflavone is a taxonomic marker in Dalbergia species.\textsuperscript{175} Quercetrin, the major constituent in the parasite Dendrophoe falcata (Loranthaeceae) growing on plants of different families projects its possible use as a chemotaxonomic marker\textsuperscript{176} of this species. The flavonoid profile characterised by C-glycosyl flavones of Mollugo species\textsuperscript{177} supported its separation from Aizoaceae of the Centrospermae and placement in Molluginaceae along with Caryophyllaceae in Caryophyllales. Among the numerous flavonoids of higher plants the rare and unusual ones find more acceptance in micromolecular taxonomy. Quite recently a survey of 2'-oxygenated flavonoids along with their possible biosynthetic pathways has been reported.\textsuperscript{178}

Colour in flowers is the ultimate step in the synthesis of large bioorganic molecules, which are quite exclusively to be found among flavonoids, carotenoids and quinones. In contrast to carotenoids and quinones, which always absorb light in the visible range, flavonoids can exist in both coloured and colourless forms. Therefore, flavonoids may contribute directly or indirectly to the visual effect produced, not only on humans, but also on animals, especially insects, birds and herbivores. Thus, flavonoids are multifunctional and the function varies according to the need and stress placed upon the plant and depending on its stage of growth and development. The anthoclor pigments (chalconses and aurones) usually give yellow to orange colours to the tissues in which they are located. However, bright yellow colours are generally related to the presence of carotenoids.\textsuperscript{179} Moreover those flavonoids which are barely detectable to the human eye
may well be clearly visible to insects, especially to those which feed on plants and which are frequently involved in the pollination process. Naturally occurring chalcones and aurones have been reviewed from the point of view of their structure. Analytical chemical methods most useful in the structural determination of the faintly coloured to colourless flavones, flavonols and their glycosides have been reported. No doubt, the anthocyanins are the most important flavonoid plant pigment and their expression is achieved in a unique way when they are present in flowers. Major advances in the past decade concern the determination of anthocyanin primary structure and the aptitude of anthocyanins to give molecular complexes with a large variety of natural and artificial molecules. Primary structures are the forms recognised during anthocyanin identification i.e. the flavylium cations are zwitter ionic or not. Anthocyanin-flavone glycoside copigment complexes are stabilised by chelation with metal ions or by H-bonding. These copigments are found to protect the anthocyanin in vivo from nucleophilic attacks resulting in colour loss. Plant species now known to contain malonated anthocyanins in their flowers have been listed by Harborne. Being zwitterionic under suitable conditions, malonated anthocyanins are easily recognised by use of electrophoretic surveys. Several speculations about the role of anthocyanins on the perception or filtration of light and response to stress factors, including microbial attack, await further thorough studies. Anthocyanins may also be important factors with other flavonoids in the resistance of plant in insect attack. Several physiological functions for anthocyanins in the general metabolism of plants described in the literature are still rather obscure.
The important function of flavonoids in plants are their protective role as light screen against damaging UV radiation, as feeding deterrents and protection from herbivores and as allelopathic agents. In biological systems, the stimulation of protein degradation as well as protein formation by antibiotics resulted in flavonoid accumulation implicating the importance of flavonoids in protein synthesis.\textsuperscript{192,193} Other important functions include their role as anti-oxidants, enzyme inhibitors, precursors of toxic substances and as photosensitising and energy transferring compounds, in control of plant growth and development, in respiration, photosynthesis, morphogenesis and sex determination in plants. In general, flavonoids show potential as environmentally less harmful insecticides, especially when applied systematically, and knowledge of their properties in plants to deter insects can be used to breed more resistant crops. Thus, if flavonoids are used in integrated pest strategies, and as long as agronomists and plant breeders are aware of the properties of these compounds and other classes of secondary substances, they have a real potential for crop protection in the future.

Apart from the physical and morphological means, chemical means are a major method of plant defence. A significant range of flavonoids have been encountered as antifungal agents. A number of flavonoids are being induced in plants following fungal invasion. Some of the flavonoids affect the behaviour, growth and development of insects due to their toxicity, while flavone glycosides are feeding stimulants.\textsuperscript{194} The metabolic challenges of the plant flavonoids including the condensed tannins to the insects consists mainly of the variety of phenolic groups. This activity is destroyed when the phenolic OH group is methylated in flavonoids. The structure-activity effects have been studied for a number of flavonoids for anti-growth and anti-bacterial activity and it
has been found that growth inhibiting activity depends on the presence of ortho
dihydroxy group in ring A and B and not the functional group of ring C and the position
of ring B (at C-2 or C-3 as in flavones or isoflavones). The glycosylation of flavonoids
also show marked variation in growth inhibition; 3-O-glycosylation inhibits growth but
not 7-O-glycosylation. The enhanced activity of the flavanones compared to the
analogue showed that the coplanarity of the flavonoid rings of flavones
might hinder their biological efficacy. Different types of bioassay involving several
types of organisms conducted with isoflavonoid phytoalexins by Smith revealed that
they possessed fungitoxicity and limited antibacterial activity. The introduction of
iso-flavonoid phytoalexins in plants causes phytotoxicity such as inhibition of
respiration, reduced growth of suspension cultures, repressed seed germination, retarded
root growth and electrolytic leakage.

The occurrence and concentration of flavonoids in foodstuff have been reviewed
by Hermann. The chemically modified anthocyanins have found increasing use as
approved colours in formulated food and beverages. Although different groups of
flavonoids are consumed, the diversity is removed by intestinal micro-organisms
hydrolysing the glycosides. The flavonoids combine with dietary proteins and
carbohydrates and with digestive enzymes and cellular components of the digestive tract.
A small fraction of the flavonoid is absorbed into the blood stream and the unabsorbed
compounds affect many aspects of digestion and health.

An ever-increasing number of pharmacological effects of flavonoids have become
known over the years through the discovery of new plant flavonoids and their
derivatives. The spasmolytic, antianginal, antiulcer, antihepatotoxic,
antiinflammatory, antiallergenic, antimicrobial and antiviral effects of flavonoids are noteworthy. In recent years a number of flavonoids like baicalin, taxifolin, gossypin, proanthocyanidins, nepetin, diosmin, fisetin, sophoricoside, (+)-catechin, (-)-epicatechin and 5,7-dimethoxy flavone have been reported to have anti-inflammatory effects.\textsuperscript{201-203} Recently the effect of flavonoids on arachidonate metabolism was reported by Ferrandiz et al.\textsuperscript{204} Many semisynthetic flavonoid derivatives also show this effect of which (O-β-hydroxy ethyl) rutin and various quercetin derivatives are important.\textsuperscript{205} Studies of compounds with flavone skeleton were stimulated by recognition of antiallergic effects. Thus, orally effective anti-allergic chromone drugs (kellin, hypolaetin-8-glycoside disodium chromoglycosate etc.) are in use.\textsuperscript{205} Antiviral activity of a few flavonoids were investigated by Pusztai et al.\textsuperscript{206} and observed that hydroxylation in 3-position appears to be a prerequisite for this activity. Antirhinovirus and antipicornovirus activity of certain flavonoids have also been reported.\textsuperscript{207} Anti-cancer activity of a few flavonoids (flavones, isoﬂavones, ﬂavanones, and ﬂavonols) are also reported.\textsuperscript{208,209} Recently 7,8-di-O-substituted flavans, biflavans and flavones showed cytotoxic activity\textsuperscript{210} and it has been established that the activity is due to methoxy and / or hydroxy group in the structure.

Inhibition of human immunodeficiency virus reverse transcriptase is currently considered a useful approach in the prophylaxis and intervention of Acquired Immuno Deficiency Syndrome (AIDS) and natural products have been extensively explored as inhibitors of this enzyme, to discover drugs active against AIDS. Recently 150 pure natural products have been examined\textsuperscript{211} and polyphenolic compounds were found to be responsible for the activity; among flavonoids tested quercetin exhibited moderate activity.
The anti-hepatotoxic effect of flavonoids was first demonstrated by Halm et al\textsuperscript{212} with the flavanolignan, siliybin and its isomers. About 13 flavonoids and coumarins have been demonstrated\textsuperscript{213} to be anti-hepatotoxic or liver protective agents. The screening of plants, recorded in Indian folk medicines, for liver injury has established\textsuperscript{214} that the active principles are flavonoids. Anti-ulcerogenic property of 3-O-methy1 (+)-catechin, apigenin, and luteolin, anti-diabetic effects of hispidulin and nepetin and analgesic effects of puerarin are also reported.\textsuperscript{215,216}

Several dihydrochalcone derivatives are used as sweeteners. The bitter taste of flavanones and their neohesperidosides, sweet taste of dihydrochalcone neohesperidoses, reduction of the bitter taste of flavanone glycosides by flavones and the tastelessness of the flavone glycosides irrespective of the nature of the sugars etc were elaborately studied by Horowitz and Gentili.\textsuperscript{217} Although flavonoids have been extensively studied in medicinal research for diverse physiological effects, their role in plants from which they are derived has not been unambiguously established.

Work on different aspects of flavonoids including discovery of new compounds, biological activity, medicinal properties etc., are reviewed regularly\textsuperscript{31-36} and the knowledge is updated through proceedings of regular international symposia on flavonoids.\textsuperscript{218} Considerable work on the chemistry and pharmacological properties of a number of flavonoids has been accomplished in the laboratories of our department.\textsuperscript{219-224}
REFERENCES


1605.


Plants with Special Reference to Flavonoids”, Ph.D Thesis, University of Madras.


224. Publications from our laboratory in the field of flavonoids cited in references 29 & 32-34.