CHAPTER-1
GENERAL INTRODUCTION

1.1 Introduction

The history of our planet goes back to about 4,500 million years. It is believed that the plant kingdom and the forests evolved in this planet some 300 million years ago. Ever since his appearance in this planet, man depended on his immediate vegetational environment for fulfilling his primary needs. As civilization advanced, man used plants not only for food but also for, various other purposes like clothings, shelter, gums, resins, perfumes, cosmetics, oils, dyes and above all for alleviating pain and curing diseases. The poisonous and healing properties of the plants were discovered by man during his search for food. Later, man used a large number of wild plants and animal products for treating a variety of human ailments. The most effective remedies from among these were chosen by a process of trial and error, observations and reasonings and were passed on to subsequent generations in the form of oral instructions. In many eastern cultures like that of India, China and the Middle East, these experiences were systematically recorded and incorporated into the regular systems of medicine, which later became the Materia Medica of the respective cultures.

India has a long history of using herbal medicines for the prevention and treatment of diseases extending to several millennia. The promotive, preventive, and curative properties of herbs were recognized by the ancient sages and they gave sound theoretical and conceptual foundations to the classical Indian systems of Medicine. These systems of medicine are deeply rooted in the traditions, culture, civilization and religions of our land and people1.
Natural products from plants, animals and minerals have been serving as sources of drugs for the treatment of human diseases since time immemorial. Our ancestors derived therapeutic materials from thousands of plants. Considerable research on phytochemistry, pharmacology, toxicology and to a lesser extent, on clinical pharmacology is being carried out on medicinal plants”. Of late, many of the major pharmaceutical industries have renewed their interest in favour of plant drugs. Numerous drugs have entered international pharmacopoeia through the study of ethnopharmacology and traditional remedies.

Naturally occurring medicines are of great importance as a reservoir of chemical diversity aimed at new drug discovery and are screened for many pharmacological studies like antimicrobial, antihypertensive, immunomodulatory, anti-inflammatory, antioxidant, antidiabetic, anticancer activities etc. Around 80% of all such products are of plant origin. Herbal medicines are becoming very popular, not only in developing countries with a long tradition in the use of medicinal plants, but also in some developed countries like Germany, France, Italy and the United States. The future discovery of naturally occurring drugs depends mainly on the wise use of ancient and modem therapeutic skills in a complementary manner so that the community may be provided with maximum benefits. Lag phase for plant medicines is now changing rapidly. Problems with drug resistant micro-organisms, adverse effects of modern drugs, emerging diseases for which no proper medicines are currently available etc., have stimulated our renewed interest in plants as a significant source of new medicines. Pharmaceutical scientists are experiencing a lot of difficulty in identifying new lead structures and templates in the vast world of chemical diversity. As synthetic drugs have been known to have adverse and unwanted side effects, emphasis is
being made in the development of potent, safe and novel drugs from natural sources. The recent success in the field of plant based drugs is most notably from Chinese medicines.

A whole range of chronic diseases such as cancer, hypertension, diabetes, rheumatism, immuno deficiency syndromes etc. require novel drugs and most of the developing countries rely and will continue to rely on traditional medicines since the drugs of modern system of medicines are not only costly and urban oriented and hence not available to the common man but also possess a variety of side effects. Recent studies indicate that about 80% of people in developing countries still rely on traditional medicines, based largely on various species of plants. According to the WHO, because of poor economic conditions and also lack of access to modern system of medicines, about 65-80% of the world’s population in developing countries depend essentially on plants for their primary health care. It is reported that four out of every ten Americans used alternative medicine therapies in 1997 and the number of total visits made by Americans to practitioners of alternative medicine has increased by almost 50% from 1990 to till date.

1.2 Herbal drug development

Looking towards the arena of medicinal plant drugs, herbal drug development needs to include various steps, starting from collection of data on raw materials, pharmacognostic and phytochemical quality standardization, experimental and clinical pharmacology and randomized, controlled clinical trials. Lately, many international bodies, including the WHO, US Agency for Health Care Policy and Research and the Dept, of Indian System of Medicine have started creating new regulations to include and regulate quality control and standardization of medicines of
plant sources. Currently existing regulatory methods for herbal medicines include drug prescription, over-the counter drugs, traditional medicines and dietary supplements and hence the need for establishment of global and/or regional regulatory mechanisms for regulating herbal drugs seems obvious\textsuperscript{10}.

A basic requirement for preparation of a herbal drug of good pharmaceutical quality involves the use of optimum quality and quantity of it. The phytoconstituents of the herbal drugs significantly influence the quality of the herbal drug and thus the efficacy and safety of the resulting herbal medicines. For this reason, both the quality and reproduction of the total spectrum of constituents of the herbal drug are extremely important. However, since the herbal drugs are always composed of a complex mixture of different chemical constituents, it is not possible to define all of them and to determine them during quality control. According to the current state of scientific knowledge, only those constituents that are relevant to ensure efficacy and safety are used to characterize the quality of the drugs”.

Standardization of drugs is a very important aspect of the manufacture and the supply of herbal drugs. It is only in recent years, the importance of standardization of herbal products is realized and efforts are being made to satisfy the regulatory requirements. Many of the manufacturers export herbal extracts without proper standardization and the importers also accept them since it is very expensive to check the purity by modern methods of standardisation at their end. However this cannot go on for long and regulations are likely to be applied strictly to herbal products in all developed nations, though it may not be possible to apply the parameters of quality control and standardization to herbal
drugs as applicable to modern synthetic drugs. At present, it has been widely accepted that characterization of active constituents is mandatory for the standardization of herbal medicines, whether they are in the extract form or in the pure isolated form.

Use of active constituents, enriched by solvent extraction instead of powdered plant materials, will result in value addition, enhancement in the efficacy of products and the manufacture of value-added products. Isolation and characterization of active ingredients, wherever possible, will lead to value addition by 250-500 percent depending on the nature of the products. In addition to the drugs of plant origin, dry extracts, soft extracts, infusions, tinctures, volatile oils etc., containing partially purified secondary active principles are also being widely used to fight against diseases.

The isolates which are homogenous and pure are generally employed, only if active principles of extracts show strong and specific activities with a small therapeutic index and hence require an accurate dosage. Currently, the chemical entities extracted from various species of higher plants in the near pure form, are being used in the field of medicine throughout the world.

Progress achieved over the last four decades in natural products isolation has been made possible, partly due to the tremendous development of separation techniques during this period. Though adsorption column chromatography has remained the mainstay for the separation and purification of the constituents in plant extracts, a broad range of chromatographic techniques such as HPLC and Liquid partition chromatography etc. make the isolation more rapid and efficient at present. Though natural products isolation still remains largely
empirical and it requires both experience as well as patience, with the availability of modern spectroscopic techniques, characterization of the isolates from plant extracts has become relatively simple and easy.

Bio-assay guided separation techniques have been proved to be beneficial and are relatively simple, rapid, reproducible and inexpensive. The techniques are not only sensitive enough to detect the active principles present in low concentration in the extracts but also selective to avoid false positive and negative tests. Tannins, generally give such false positive tests and they have to be suitably removed before bio-assay is done. Once the active extracts are obtained, further fractionation is carried out to confirm the activity of pure active constituents. Though certain groups of compounds like flavonoids, triterpenoids etc. are considered to be ubiquitous in nature and hence pharmacologically unimportant, in recent years flavonoids have been found to be major constituents of many drugs of plant origin, where they contribute substantially to their curative effects. Pentacyclic triterpenoids like cx-amyrrin and lupeol are shown to exhibit distinct antibacterial activity\textsuperscript{15}. Oleanolic acid glycoside has been shown to be a new hypoglycemic agent\textsuperscript{6} and betulin and its derivatives as anti HIV agents\textsuperscript{17}. In the light of these findings, it becomes imperative that herbal plants used in the system of native medicines are to be examined thoroughly for their phytoconstituents of all types.

Pharmacological and clinical studies are important to evaluate the efficacy and safety of herbal drugs. Different approaches involved in the evaluation of medicinal plants include broad based biological screening, specialised biological evaluation (based on traditional use), selected tests based on chemical structures of isolated compounds and initial clinical
trials under controlled conditions of drugs used in traditional system of medicine. Normally clinical evaluation is done after collecting a lot of pre-clinical data. Molecular pharmacology provides a new interface between traditional and modern system of medicines\textsuperscript{18}.

Pharmacological studies of phytoconstituents have assumed a lot of significance in drug research, as combined efforts of pharmacognosists, phytochemists and pharmacologists have resulted in the development of newer drugs of varying structural features with promising therapeutic utility. Gupta has reviewed in detail the various perspectives of a number of medicinal plants\textsuperscript{19}. Opportunities for multidisciplinary research that links natural products chemistry, molecular and cellular biology, synthetic and analytical chemistry, biochemistry, pharmacognosy and pharmacology help to exploit the vast diversity of chemical structures and biological activities of natural products\textsuperscript{19}.

1.3 Plant Biodiversity in India

India is one among the 12 megadiversity countries of the world. Of the estimated 2,50,000-5,00,000 plant species on this planet, the flora of India comprises of about 45,000 species from the unicellular cyanobacteria to the flowering plants. This forms roughly about 11\% of the world population. India has about 17,000 flowering plants which comprises about 6\% of the world population. The biogeographic position of India is unique in the fact that we have diverse ecosystems ranging from the humid tropics of the Western Ghats to the Alpine zones of the Himalayas, from the mangroves of the tidal Sunderbans to the dry deserts of Rajasthan\textsuperscript{1}. Western Ghats of India is a chain of mountains, 1600 kms in length, running parallel to the west coast of Peninsular India from the river Tapti in the North to Kanyakumari in the South. This mountain
chain is part of the Indian plate of the Gondvana land. The flora of Western Ghat comprises of about 4000 species of flowering plants which forms roughly 27% of the Indian flora. It is estimated that 1500 species among these are endemic to the Western Ghats.

**L4 Challenges in isolation and characterization of bioactive compounds from Plants**

The paucity of compounds is one of the major challenges to phytochemists in their responsibility of assigning structures to the plant isolates and the continuous efforts taken by the scientists have led to the development of new refined techniques of separation such as various kinds of analytical and preparative chromatographic methods viz., column chromatography (CC), gas chromatography (GC), thin layer chromatography (TLC), high performance liquid chromatography (HPLC), paper chromatography (PC), electrophoresis, ion exchange and gel chromatography etc. These techniques have made it possible to isolate compounds present in extremely small quantities. In earlier days, structure elucidation was carried out mainly by chemical methods of structural elucidation, degradation of pure compounds into smaller fragments of known structures, combined with investigations of the reactivity pattern and elemental analysis of the compounds. With the recent advancement in spectroscopic techniques it is now possible to establish the structure of compounds by interpreting the spectral data. The various spectroscopic techniques used are UV, IR, $^1$H and $^{13}$C NMR, Mass and Electron Spin Resonance (ESR). Circular Dichroism (CD), Optical Rotatory Dispersion (ORD) and X-ray crystallographic data also help in structure elucidation.
Today we make use of about 425 bioactive molecules isolated from natural sources in modern medicine. These compounds are isolated from a limited number of plants. Among the available flowering plants, shrubs and trees in Earth, less than one half of 1% have been screened even cursorily for their potential therapeutic value. It is predicted that if the present trend in loss of tropical plant habitats continue, as many as 60,000 plants, could be extinct by the middle of the 21st century.

1.5 Polyphenolics - A distinct group among the galaxy of phytochemicals

Among the galaxy of phytochemicals, polyphenolics constitute a distinct group embracing a wide range of compounds that possess aromatic rings bearing one or more hydroxyl substituents. These oxygen heterocyclic polyphenolics are water soluble, since they occur most frequently as glycosides in combined forms with sugars. They are usually located in the cell vacuole. Flavonoids are a large family of 4000 secondary metabolites which occupy a prominent position among the polyphenols. A characteristic feature of this group of compounds is their ability to interact with primary metabolites such as proteins and polysaccharides. The pivot role played by nearly 8000 flavonoids, identified from vascular plants and their medicinal properties have been an important subject of research in recent years.

Compounds like flavonoids, chalconoids and auroids possess a 1,3-diphenyl propane skeleton, those isoflavonoids, 3-phenyl coumarins and pterocarpanoids possess a 1,2-diphenyl propane skeleton while compounds like neoflavonoids possess a 1,1-diphenyl propane skeleton.
1,3-diphenyl propane
1,2-diphenyl propane
1,1-diphenyl propane
Flavonoid
Flavanoid
Isoflavonoid
Isoflavanoid
3-phenyl coumarin
pterocarpanoid
neoflavonoid
The study of the distribution of flavonoids in plants is a continuing exercise and known flavonoids are being regularly derived from new sources. Flavonoids are universal in vascular plants, but variation according to the phyla, order and family have been detailed by Harborne and Turner. Only in 1985, the distribution of flavonoids in animals has been reported with the isolation of 4'-methyl flavone from the scent glands of Canadian beaver and in Lepidoptera.

1.6 Methods of isolation and characterisation of polyphenolics

Methods of extraction, separation, purification and chemical characterization of the flavonoidal compounds have been discussed by Harborne J.B, Peach K. and Tracey M.V. Conventional column, paper and thin layer chromatographic techniques are still widely used for separation, purification and identification of flavonoids. Improved separation and identification of flavonoids had been achieved by gas chromatography on phenyl methyl silicone co-polymers. High performance liquid chromatography (HPLC) is an emerging useful tool because it involves short analysis period, better resolution and easy quantification. Both reverse phase and normal phase HPLC have been reported to be useful in the separation of the flavonoidal compounds. Other methods of separation and purification of flavonoids like liquid partitioning followed by flash column chromatography, droplet counter current chromatography (DCCC) and gel chromatography have been reported. Separation and analysis of flavonoids by miceller electro kinetic capillary chromatography is also in current practice. Efficient screening of the extracts is currently being performed using combined liquid chromatography hyphenated techniques such as HPLC coupled to UV photodiode array detection. Mass spectrometry and to NMR. HPLC with electro spray MS and HPLC in conjugation with
UV diode array and electro spray ionization mass spectrometry (LC/UV/.M.S) with negative ion detection as well as direct MS injection served as useful tool in analyzing flavonoidal compounds. Paper electrophoresis technique is also used in flavonoidal analysis\textsuperscript{44-45}.

Flavonoids, a large family of naturally occurring poly phenolic compounds give characteristic color changes when treated with neutral ferric chloride solution (dark blue or green), ammonia vapour and alkali (yellow). Certain other chemical tests are also used to find out the nature of flavonoids in the given extract. Development of blue to green color with the addition of 2,6-dichloro benzoquinone chlorimide in borate buffer (Gibb’s test\textsuperscript{46}) indicates the presence of 5-hydroxy flavonoid having no substitution at C-8. Development of red or magenta color by the addition of Mg/HCl (Shinoda test\textsuperscript{47}) to a polyphenol in ethanol reveals the presence of isoflavone or dihydroflavonol moiety. Development of pink color by the addition of Na/Hg in HCl (Wolfrom test\textsuperscript{48}) and yellow color by the addition of concentrated nitric acid (Durham test\textsuperscript{49}) indicates the presence of flavonone moiety. Development of blue or green color by the addition of alcoholic gallic acid in H\textsubscript{2}SO\textsubscript{4} (Labat’s test\textsuperscript{50}) shows the presence of methylenedioxy substituent in the given flavonoidal nucleus. Flavonoid glycosides give deep violet color with 20% alcoholic solution of a-naphthol and conc.H\textsubscript{2}SO\textsubscript{4} (Molisch’s test\textsuperscript{51}).

1.7 Spectral studies of Flavonoidal compounds

Spectroscopic data from UV, IR, 'H, \textsuperscript{13}C NMR, MS etc. are very useful in structure elucidation of these compounds. Infrared and ultraviolet spectroscopic measurements were the first to be developed and still constitute the primary proofs for the detection of functional groups and electrons in a molecule. In contrast, NMR spectroscopy is used to
establish the environment and nature of the different carbon and hydrogen nuclei present.

Various substituents present in the given flavonoid molecule can be identified by their characteristic IR absorption bands\(^{12}\). Using UV-Visible spectroscopy, the substitution of hydroxyl groups in various positions can be determined from \(A_{\text{max}}\) values and the characteristic shifts produced in them by the addition of diagnostic shift reagents\(^{33-08}\).

Proton magnetic resonance spectroscopy has been widely employed as a method in elucidation of structures of flavonoids. The NMR study of ether derivatives was experimented by Waiss \textit{et al}\(^{9}\). Batterham and Highet\(^{60}\) introduced DMSO-\(d_6\) as a solvent for more polar compounds. The importance of nuclear parameters such as the chemical shift (\(\delta\)) and the coupling constant (\(J\)) cannot be underestimated\(^{61}\) and these parameters can readily be obtained with great precision using off-line data processing, optimized window functions such as Lorentzian to Gaussian line shape conversion or reference deconvolution\(^{62}\) aided basic 2D and soft pulse I D experiments\(^{63}\).

\(^{13}\)C NMR spectroscopy, that exhibit carbon resonance signals extending over 200 ppm has become a vital tool in providing informations about the intricate nature of carbon skeleton of a flavonoid such as the total number of carbons, number of oxygenated carbons and the number of carbons present in the sugar moiety etc\(^{64-73}\).

Recent developments in NMR techniques such as ID and 2D NMR, which require considerably decreased sample size, make it more acceptable to natural products chemists. Various NMR techniques used in characterization of flavonoids include SFORD\(^{74}\) (Single frequency
Off-Resonance Decoupling), SEFT\textsuperscript{73,76} (Spin-Echo Fourier Transform NMR Technique), APT\textsuperscript{77} (Attached Proton NMR Technique), SPT\textsuperscript{78}\textsuperscript{79} (Selective Population Transfer NMR Technique), INEPT\textsuperscript{80} (Insensitive Nuclei Enhanced by Polarization Transfer NMR Technique), DEPT\textsuperscript{81}\textsuperscript{83} (Distortion-less Enhancement by Polarisation Transfer NMR Technique), NOE\textsuperscript{84} (Nuclear Overhauser Enhancement NMR Technique) and Gated decoupling NMR Technique\textsuperscript{84}\textsuperscript{86}.

The 2D NMR techniques include the resolution of hetero or homo coupling into two dimensions or correlation of connectivity (HETCOR / HOMCOR). Various 2D NMR techniques\textsuperscript{87}\textsuperscript{91} include

1. Correlation Spectroscopy NMR technique (COSY) which include 'H-'H and 'H-\textsuperscript{13}C COSY.
2. Inverse NMR technique which includes
   a. Heteronuclear Multiple Quantum Coherence (HMQC).
   b. Heteronuclear Multiple Bonds Connectivity (HMBC)
3. Total Correlation Spectroscopy NMR Technique (TOCSY)

Mass spectrometry is another valuable tool in the determination molecular weight and probable structures of flavonoids. Electron Impact Mass Spectrometry\textsuperscript{13,92} (EIMS) is useful for volatile compounds. For non-volatile compounds, derivatisation must be essential in order to render the most volatile compounds for analysis. Some of the mass spectral techniques used for structure elucidation are

1. Field Desorption Mass Spectrometry\textsuperscript{93} (FD-MS)
2. Desorption Chemical Ionisation Mass Spectrometry\textsuperscript{94}
3. Fast Atom Bombardment Mass Spectrometry\textsuperscript{95} (FAB-MS)
4. Chemical Ionisation Mass Spectrometry\textsuperscript{96} (CI-MS)
5. Laser Desorption Mass Spectrometry<sup>96</sup> (LD-MS)
6. Plasma Desorption Mass Spectrometry<sup>96</sup> (PD-MS)
7. Secondary Ionization Mass Spectrometry<sup>96</sup> (SI-MS)
8. Pyrolysis Chemical Ionization Mass Spectrometry of Flavonoids<sup>96</sup>

The application of GC-MS analysis and solution phase mass spectrometry<sup>98</sup> has been proved useful in the determination of molecular weight of complex flavonoidal glycosides. Mass analyzed in kinetic energy<sup>99</sup> and collision activated dissociation MIKE spectra of flavonoid provide characteristic fragment ions that permit differentiation of C-6 and/or 8 substituent location and the position of O-glycosylation. ORD and CD spectral analysis have been employed for the determination of stereochemistry of chiral flavonoids<sup>100,102</sup>.

Not very much is yet known about the large number of individual steps through which the biosynthesis proceeds but it is well established that the rings A & B are formed by different routes. Ring A is produced by acetate pathway and ring B arises from shikimic acid pathway. The general belief is that the two fragments join together to form a complex polyketide which forms chalcone and then to individual flavonoid class and biosynthesis of flavonoids is a complex enzymatic, genetic and molecular biological work and hence is the subject of several reviews<sup>103,106</sup>.

1.8 Flavonoids as phytomedicines

Flavonoids exhibit a wide range of functions in biochemistry, physiology and ecology of plants. Some evidences have been presented for the flavonoid compounds having a function in the sexual process of plants. The inability of two varieties of Forsythia to cross pollinate is
associated with the presence of rutin in the pollen of one and quercetin in the other\textsuperscript{107}. They contribute to the fermentation and germination of pollen and the activation of bacterial modulation genes, which are involved in the nitrogen fixation process\textsuperscript{108,109}. Flavonoids can act as a growth regulators\textsuperscript{110}, enzyme inhibitors, insect anti-feedants as well as ovipositor stimulants for some butterflies\textsuperscript{111}. The presence of flavonoids in \textit{Spina} chloroplasts and the isolation of kaempferol 3-O-arabinoside from the chloroplast of \textit{hnpatiem balsamina} have suggested that the flavonoids may play a pivotal role in the photosynthesis of plants\textsuperscript{112}. Flavonoids find exceptional use as a toxonomical markers\textsuperscript{113-115}.

Epidemiological studies suggest that the consumption of diet containing fruits and vegetables, a major source of phytonutrients may reduce the risk of developing chronic diseases. The protective role of phytonutrients may be due to the variety of constituents including vitamins, minerals, fiber and numerous phytochemicals such as flavonoids\textsuperscript{116}. The human diet contains a complex mixture of plant phenols and it is believed that human individuals may consume as much as one gram of plant phenols per day in their diet. The protective role of flavonoids against many diseases has been evidenced by several \textit{in-vitro} and animal studies.

Flavonoids can act as potent antioxidants by either trapping the initiating radical, propagating lipid peroxy radicals or recycling \textit{a-tocoferol} and/or deactivating the photosensitizer etc\textsuperscript{120-127}. Bores \textit{et al}\textsuperscript{128} and Frankel\textsuperscript{129} elaborated the structural criteria that this activity is attributed to the phenolic hydroxyls, particularly if \textit{3’,4’-dihydroxyl} substitution is present in ring B and double bond between C-2 and C-3 of ring C and the activity increases with the number
of hydroxyl groups in rings A and B. Several flavonoids have been identified to elicit one or more of these mechanisms\textsuperscript{130-134}.

A possible protective role against coronary heart disease (CHD) of flavonoids has been reported in four out of six epidemiological studies\textsuperscript{135-137}. Reports are available on the hepatic protective nature of flavonoids\textsuperscript{138-143}. About half a dozen products containing silymarin, a naturally occurring flavonoid as an hepatoprotective agent are also marketed in India\textsuperscript{144,145}. Flavonoids also display antiviral activities\textsuperscript{146}, especially anti-HIV\textsuperscript{147} and the structural activity correlation of some related flavonoids is evaluated\textsuperscript{148,149}.

Intake of polyphenolics such as flavonoids or lignans in the diet has been correlated with low incidence of colon cancer and breast cancer\textsuperscript{150-152}. Apoptosis or programmed cell death is a highly organized physiological process to eliminate damaged or abnormal cells and modulating apoptosis may be useful in the management and therapy or prevention of cancer. Curcumin, a phenolic compound that has been identified as the major pigment in turmeric induces apoptosis in transformed rodent and human cell in culture\textsuperscript{153-156}. Flavone, flavonol, flavanone and isoflavone classes of flavonoids possess antiproliferative effect in different cancer cell lines\textsuperscript{157}. Experiments conducted with these flavonoids suggest that flavonoids induced apoptosis is stimulated by the release of cytochrome-C to the cytosol, by procaspase-9-processing and through a Caspase-3-dependant mechanism\textsuperscript{158}. Further more the potency of flavonoids for inducing apoptosis may be dependent on the number of hydroxyl groups in the two phenyl rings and on the absence of hydroxyl group at C-3.
Reports on the antiinflammatory\textsuperscript{159-161}, analgesic\textsuperscript{162-164}, antimicrobial\textsuperscript{165,166}, antibacterial, antifungal\textsuperscript{167,168}, antiallergic\textsuperscript{169,170}, antitumor\textsuperscript{171}, immuno modulator, antidiabetic and neuron protective\textsuperscript{172} activities of novel flavonoids continue to flash in the literature.

1.9 Phytochemical investigations of \textit{Dalbergia} and \textit{Derris} species

Among higher plants, the family Leguminosae is a large one having more than 600 genera and 12,000 species. Plants belonging to this family vary in size from small shrubs and creepers to large trees. \textit{Dalbergia} and \textit{Derris}, are important genus of trees, shrubs and climbers, belonging to the family of Leguminosae and sub-family Papilionaceae, with about 120 species is scattered in the tropics and sub-tropics and also in the temperate parts of South East Asia and North Australia.

Out of the \textit{Dalbergia} species available in the world, only 35 of them are reportedly present in India\textsuperscript{173-175}. \textit{Dalbergia} species have already been reported to possess a wide range of medicinal properties\textsuperscript{176}. Thorough phytochemical examination of various species of \textit{Dalbergia}\textsuperscript{177} has resulted in the isolation, characterization of a large number of compounds like flavonoids, isoflavonoids, neoflavonoids, steroids, terpenoids, etc and a number of reviews have been published on the phytochemical investigations of \textit{Dalbergia} species\textsuperscript{178,179}.

\textit{Derris} species\textsuperscript{180-185} have been exhaustively studied and a large number of compounds like flavonoids, isoflavonoids, rotenoids, pterocarpans and coumarins etc have been isolated and they are also reported for their varied pharmacological activities\textsuperscript{186-188}.
1.10 Pharmacological Investigations of *Dalbergia* and *Derris* species

It is estimated that at least 25% of the population of the United States will face a cancer problem during their lifetime, with one million new cancer patients diagnosed every year. Less than a quarter of these patients will be cured solely by surgery and/or local radiation and most of the remaining patients may receive systemic chemotherapy at sometime or the other during their illness. *Dalbergia* species with cancer chemopreventive activity have been studied in detail and five such cinnamyl phenols (1,3-diphenyl propenes) are reported to be isolated from *Dalbergia* species. 189

Cheng et al reported the antioxidant property of butein, isolated from *Dalbergia odorifera* and butein serves as a powerful antioxidant against lipid as well as LDL peroxidation by its versatile free radical scavenging actions and metal ion chelation. Three flavonoids isolated from the heartwood of *Dalbergia odorifera* were reported to possess anti-allergic and anti-inflammatory activities191. Prostaglandins and leucotrienes are released by a host of mechanical, thermal, chemical, bacterial and other insults and they contribute mainly to the genesis of the signs and symptoms of inflammation192194. The methylene chloride extract of *Dalbergia odorifera* is reported to be a potent inhibitor of LTC4 formation in mastocytoma cells195.

Antiandrogens like finasteride inhibit 5-oc reductase that converts testosterone into its metabolite 5-<x dihydrotestosterone (5-cc DHT)196. Four new compounds, in addition to the eight known phenolic compounds, isolated from the stem of *Dalbergia cochinchinensis*, showed potent inhibitory effects towards 5-oc DHT197. Peters and Guerra198 carried out embryo fetotoxicity studies of stem bark decoction of
*Dalbergia subcymosa* using Wistar rats and Dalsaxini, a glycoside isolated from the root of *Dalbergia saxatilis* has been reported to cause rat uterine muscle contraction by mobilizing Ca\(^{2+}\) through predominantly a voltage-dependent Ca\(^{2+}\) channel\(^{199}\).

Yu et al\(^{200}\) reported that butein, isolated from *Dalbergia odorifera* caused endothelium-dependent relaxation of rat aorta that was precontracted with phenylephrine. Isoliquiritigenin, isolated from *Dalbergia odorifera* is a novel soluble guanylate-cyclase activator and is reported to induce vasorelaxant effect on rat aorta that was precontracted with phenylephrine\(^{201,202}\). Hot aqueous extract of *Dalbergia monetaria* reportedly exhibits very high CSF-inducing activity\(^{203}\).

The results of antimicrobial activity of *Dalbergia melanoxylon* extract reported by Gundidza and Gaza\(^{204}\) have shown that the ethanolic extract of leaves of *Dalbergia melanoxylon* showed significant antibacterial effect. Four flavonoids isolated from the heartwood of *Dalbergia louvelii* showed significant antiplasmodial activity against *Plasmodium falciparum*\(^{205}\).

The use of extracts of *Dalbergia* species is reported for the treatment of arthritis, gonorrhoea and rheumatism\(^{206,207}\). *Dalbergia sissoo, Dalbergia lanceolaria, Dalbergia nigra, Dalbergia parviflora* and *Dalbergia volubilis* are reported, in general to possess a number of medicinal properties including antimicrobial activity\(^{208}\). The leaves of *Dalbergia lanceolaria* are used in the treatment of arthritic disabilities\(^{209}\) and that of *Dalbergia nigra* are claimed to possess antibiotic potency\(^{210}\). The aqueous extract of *Dalbergia parviflora* is reported to be a blood tonic and secretolyte, whereas the oil obtained from *Dalbergia parviflora* is found to be curative in ulcerated wound\(^{211}\). The extracts of
leaves of *Dalbergia volubilis* showed significant anti-inflammatory and antiarthritic activity in rats\textsuperscript{212}.

The isolates from the heartwood of *Dalbergia odorifera*, inhibited the prostaglandin biosynthesis as well the platelet aggregation induced by arachidonic acid\textsuperscript{213,214}. Medicarpin, an isolate from the wood of *Dalbergia monetaria* was shown to possess high antifungal, antibacterial activities and also high anti-feedon activity against cotton-ball weevil\textsuperscript{215}. The binary flavonoids isolated from the roots of *Dalbergia odorifera* exhibited antilipidemic properties and reduced cholesterol level in serum\textsuperscript{216}.

The heartwood of *Dalbergia odorifera* has been used in traditional Chinese medicine to treat blood disorders, ischemia and inflammation\textsuperscript{217}. The methanol extract of *Dalbergia odorifera* heartwood has been shown to be effective against hypercholesterolemia\textsuperscript{218,219}. A patented pharmaceutical formulation of *Dalbergia hancei* with other plant drugs is available for transdermal application in the treatment of coronary heart disease\textsuperscript{220}.

The toxicity of ethanol:water (1:1) extract of *Dalbergia rubiginosa* was studied by Bhakuni *et al*\textsuperscript{221} who carried out only the assessment of toxicity quantitatively and the LD\textsubscript{50} was reported as 750 mg/kg. Ethanolic extract of dried aerial parts of *Dalbergia rubiginosa* was screened for insecticidal activity and it was found inactive\textsuperscript{222}.

Biochanin-A, an isoflavone isolated from the flowers of *Dalbergia sissoides* has been reported from our laboratories to possess significant antitumour activity against Dalton's ascitic lymphoma\textsuperscript{223} and larvicidal activity\textsuperscript{224}. 

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*Dalbergia malabarica* showed significant CNS depressant activity\(^ {225}\) and antiinflammatory activity\(^ {226}\). Two isoflavonoids, namely Olibergin A and B isolated from the stem bark of *Dalbergia olivari* by Chihiro Ito et al\(^ {227}\) have been reported to possess antitumour activity. The significant antiulcer activity of the ethanolic extract of *Dalbergia sissoides* pods by reducing the ulcer index and preventing the gastric mucous erosion against pylorus ligated model\(^ {228}\) has been reported recently by us.

Infection with *Helicobacter pylori* is now recognized as the major cause of chronic active gastritis and peptic ulcer disease and eradication of the infection will prevent recurrence of the majority of such ulcers. Junko et al\(^ {229}\) rotenoids from the roots of *Derris malaccensis* showed antibacterial activity against *Helicobacter pylori*. In Thailand the roots of *Derris scandens* is used for antidysentrie, diuretic and for relief of muscular pain and the isoflavones from the stembark of this plant have been reported to possess anti-dermatophyte activity\(^ {230}\). The roots of *Derris laxiflora* possess insecticidal and piscicidal activities\(^ {231}\). *Derris robusta* have been used for pest control in horticulture and in poultry\(^ {232}\). Chulabhorn et al\(^ {233}\) have reported the cytototoxic activity of the flavonoids from the stem of *Derris reticulata* also this *Derris* species have been used as an expectorant for the relief of thirst in Thailand. The anti-oxidant and wound healing activity of *Derris benthamii* have been reported by us\(^ {234}\).

### 1.11 Corrosion inhibition action of *Dalbergia* and *Derris* species

Corrosion is the deterioration of metals and materials due to their reaction with the environment. Rusting of steel with the formation of iron oxide is a typical example for corrosion.
Metals and its alloys are exposed to the action of acids in industry\textsuperscript{235}. The exposure can be most severe but in many cases, corrosion inhibitors are widely used in industry to prevent or to reduce the corrosion rates of metallic materials in these acid media\textsuperscript{236,237}. Because of the toxic nature and the high cost of some chemicals currently in use, it is necessary to develop environmentally acceptable and less expensive chemicals as corrosion inhibitors. Natural products can be considered as good sources for this purpose\textsuperscript{238} as their phytoconstituents have been found to be effective as corrosion inhibitors in acid media. Studies on the relations between the organic structure of inhibitors and their effectiveness in acid systems have been made by many workers. It has been found that the corrosion inhibitors have O, N or S atoms in their structures donate electrons for bonding with the metal surface. The inhibition by organic compounds is due to the adsorption on the metal surface. The adsorption may be electrostatic or chemisorptive adsorption resulting from $n$ orbital interaction with the metal.

The possible replacement of some expensive chemicals as corrosion inhibitors for metal in acid cleaning process by naturally occurring substances of plant origin have been reported by Hosary and Salem\textsuperscript{238}. Natural products of plant origin contain different organic compounds (e.g. alkaloids, tannins, flavonoids, pigments, organic amino acids) are known to have inhibitive action. By understanding the principles and mechanism of corrosion, the country would be able to save at least 15-20% of the total loss, amounting to nearly about Rs. 15,000 crores annually\textsuperscript{239}.

1.12 Aim and scope of the present study

Flavonoids, synthesized in plants nearly to an extent of 0.5 to 1.5%, are reported for their diversified biological effects. *Dalbergia* and *Derris* species are reported to be potent sources of flavonoids and isoflavonoids.
Survey of literature shows that *Dalbergia malabarica* has been partly phytochemically examined and the isolation and characterization of nitiducarpin, luteolin neohespericloside has been reported by Vanagamudi *et al*. Pharmacological screening of *Dalbergia malabarica* for CNS depressant and anti-inflammatory activity has been reported by us. Detailed literature survey shows that no phytochemical and pharmacological examination has so far been reported for *Dalbergia rostrata* and *Derris benthamii*.

On the basis of the above considerations, the current focusing of world’s attention towards sphere of alternative system of medicine and the importance of flavonoids as curative measures in diverse ailments and in order to reduce the loss due to corrosion by use of phytoconstituents we have taken up three plant species namely, *Dalbergia rostrata*, *Dalbergia malabarica* and *Derris benthamii* with the idea of carrying out detailed phytochemical, pharmacological and corrosion inhibition studies for them. In our study, we have carried out the phytochemical examination of the three species, isolation and characterization of the phytoconstituents present in them using chemical and spectral methods, pharmacological screening of the plant extracts as well as an isolate for activities such as locomotor, anti-inflammatory, analgesic, antibacterial, antioxidant, hepatoprotective, antidiabetic, diuretic and antipyretic activities and also the corrosion inhibition properties of the acid extracts of these plants.
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