SUMMARY

Naturally occurring compounds are of great importance as a reservoir of chemical diversity aimed at new drug discovery and are screened for various pharmacological activities such as antimicrobial, anti-inflammatory, cardiovascular, antioxidant, antidiabetic, anticancer etc. The development of plant based pharmaceuticals requires a multidisciplinary approach directed towards ultimate production of crude drugs, standardized extracts and pure compounds or intermediates for modern drugs. Development of these products requires sustained and often expensive effort. Hence the initial selection of plants is extremely important. The major criteria should include evidence of safe, effective use in the country or in the region, assured plant availability from wild or cultivated sources and export potential.

In our present study, Chapter I deals with the general introduction including the importance of herbal medicines and development of herbal drugs. It includes a detailed survey of literature on pharmacognostical, phytochemical and pharmacological aspects of various species of Dalbergia.

Survey of literature shows that some phytochemical work has been carried out on Dalbergia malabarica Prain and no such work is reported with Dalbergia rubiginosa Roxb. There is no record of pharmacognostical and pharmacological work so far on both the Dalbergia species selected for our study and hence an attempt has been made to carry out pharmacognostical, phytochemical and pharmacological investigations on D. malabarica and D. rubiginosa.
Chapter II deals with anatomical and histochemical studies of *D. malabarica* and *D. rubiginosa* belonging to the sub-family Papilionaceae of Leguminosae. Though the genus *Dalbergia* is well known for its wood value, some species of *Dalbergia* are often used much for their medicinal values throughout the world. Recent molecular biological and classical studies have found that the plants which are grown in wild contain a wide array of pharmacologically useful secondary chemical constituents.

Both the *Dalbergia* species, purely wild plants in nature were collected from two hilly regions of Tamilnadu. In general, the members of Papilionaceae are important sources of secondary metabolites which are very useful in pharmaceutical industries. The genus *Dalbergia* is a rich source of isoflavonoids and in addition, it contains other types of polyphenolic constituents and primary metabolic compounds.

In plants, the synthesis and accumulation of metabolites take place in an orderly fashion. It starts from seedling germination up to the end of fruit setting period. Furthermore, the maximum chemical syntheses are identified in the vegetative phase. The quality and quantity of the chemical constituents produced in the plant are directly proportional to their growth form as well as the internal tissue distribution pattern which is purely species specific.

The present work on both the *Dalbergia* species finds the relationship between the anatomical structures and the chemical constituents present in them by various histochemical methods. The internal tissue distribution of *Dalbergia*, made by six different types of tissues, is: transport tissue, functional tissue, mechanical tissue, storage tissue, secretory tissue, boundary tissue and synthetic tissue in the stem,
root and the leaves. As far as the histochemical localization is concerned, metabolites are accumulated and stored in two major sites i.e. cell wall and cytoplasm. In these *Dalbergia* species the cell wall is the main source of lignin, cellulose, pectin, total insoluble polysaccharides and wall bound (3-glucoside whereas the cytoplasm contains the dissolved form of total proteins, lipids, alkaloids, phenolic substances, flavonoids and peroxide. The histochemical and anatomical studies identified the distribution pattern of the major chemical constituents in the stem, root and leaves of the two *Dalbergia* species.

As a step to overcome the misidentification problem of the plants, in the present study a very meticulous methodology was followed to determine the pharmacognostical standards such as organoleptic characters, macroscopical, microscopical and histochemical studies, physicochemical standards, phytochemical screening, quantitative microscopy and fluorescent characteristics for both the species.

The quantitative microscopical constants like vein-islet number, veinlet termination number, stomatal number and stomatal index are more in *D. malabarica* leaves than in *D. rubiginosa*, except the palisade ratio. Organoleptic studies showed that the colour of the root, stem and leaf powder of *D. malabarica* is darker than *D. rubiginosa*. *D. malabarica* has a characteristic odour, while *D. rubiginosa* is odourless. The leaf and roots of both the species are bitter in taste and the stem of both the species are tasteless. The other parameters such as loss on drying and crude fibre content showed higher value for *D. malabarica* when compared to *D. rubiginosa*. The physico-chemical standards of ash values and extractive values are higher for *D. malabarica* than for *D. rubiginosa*. 

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The results of fluorescent characters of various extracts of both the species gave distinct colours in day light and UV light after treatment with specified reagents. The preliminary phytochemical screening of various extracts of root, stem and leaves of both the species showed the absence of saponins in all the extracts; quinones and anthroquinones in the extracts of stem; quinones, tannins and coumarins in the extracts of leaves. Flavonoids, glycosides, triterpenoids, steroids, reducing sugars and alkaloids are present in the root, stem and leaves of both the species. There was no major difference between *D. malabarica* and *D. rubiginosa* in their physico-chemical standards, quantitative microscopical constants and fluorescent characters.

Chapter III of the thesis deals with the phytochemical investigation of *Dalbergia rubiginosa* and *Dalbergia malabarica*, taken up as a part of our study to isolate and characterize the polyphenolic phytoconstituents present in *Dalbergia* species. Roots and leaves of *D. rubiginosa* and *D. malabarica* were extracted successively with various solvents and the concentrated crude extracts were kept in a refrigerator.

The solids separated as well as isolated by column chromatographic separation of the crude extracts of *D. rubiginosa* are designated as DR-1, DR-2, DR-3, DR-4 and DR-5 and those from *D. malabarica* are designated as DM-1, DM-2, DM-3, DM-4 and DM-5 and taken up for characterization both by chemical as well as spectral methods of characterization.

The compound DR-1, from the petroleum ether extract of *D. rubiginosa* roots did not answer the Shinoda test for flavonoids and Wolfrom test for isoflavonoids, but it gave a positive Libermann-
Burchard test for steroids. It was identified to be 3-sitosterol from its melting point and spectral characteristics.

The compound DR-2, isolated from the benzene extract of roots of *D. rubiginosa* was found to be 3-sitosterol. Compound DR-3 from the benzene extract of roots did not answer the test for flavonoids and isoflavonoids and it did not give any colour with alcoholic ferric chloride. The UV absorption maximum of the compound at 256 nm indicated the isoflavone nature of the compound. It formed a monoacetate and from the chemical and spectral data, DR-3 was identified to be 7-hydroxy-4-methoxy isoflavone (formononetin).

The compound DR-4, isolated from benzene extract of the leaves of *D. rubiginosa* showed colour reactions of an isoflavone with a chelated hydroxyl at C-5. Use of diagnostic shift reagents like AlCl₃, AlCl₃/HCl, NaOAc, NaOAc/H₂B₂O₂, and NaOEt in the UV spectral studies of the compound showed it to be a 5,7-dihydroxyisoflavone. The PMR spectrum of DR-4 showed four three protons singlets indicating the presence of four methoxyl groups. Three one proton singlets at 6.87, 6.63 and 6.53 ppm were assigned to the protons at C-4’, C-6 and C-8 respectively.

The $^{13}$C spectrum of DR-4 showed 17 carbons signals, 13 due to the isoflavone skeleton with signals for C-3’ and C-5’ & C-4’ and C-6’ overlapping. From the $^1$H, $^{13}$C and mass spectral data, the compound DR-4 was identified as SJ-dihydroxy$^{1,3,5,7}$-tetramethoxyisoflavone.

The compound DR-5 isolated from the acetone extract of leaves of *D. rubiginosa*, behaved similarly as DR-4 and hence it was identified to be 5,7-dihydroxy-2’,3’,5’,6’-tetramethoxyisoflavone.
The isolate from the petroleum ether extract of leaves of *D. malabarica*, DM-1 was found to be a pterocarpan from its UV spectral behaviour. The 'H NMR of DM-1 revealed the presence of one methylenedioxy and three methyl substituents apart from aromatic and aliphatic protons. On the basis of 'H, $^{13}$C and mass spectral data DM-1 was identified to be nitiducarpin.

The compound DM-2, isolated by preparative tic of a mixture of two components, separated from the benzene extract of leaves of *D. malabarica* was identified to be a neoflavone from its colour reaction with Mg/HCl. On the basis of its m.p. and co-tlc comparison with an authentic sample of it, DM-2S was identified to be 6-hydroxy-7-methoxy-4-phenylcoumarin (dalbergin).

The compound DM-3, isolated from the alcoholic extract of leaves of *D. malabarica* was identified to be a 5,7-dihydroxy isoflavone from its UV spectral behaviour. The PMR spectrum of DR-3 showed the presence of one methoxyl group. The compound was identified to be 5,7,4'-trihydroxy-6-methoxyisoflavone (tectorigenin) from its spectral and chemical behaviour.

The compound DM-4 from the benzene extract of the roots of *D. malabarica* was identified to be netiducarpin.

The isolate, DM-5 from the alcoholic extract of the roots of *D. malabarica* was identified to be a diglucoside of an isoflavone with one methoxyl group and a free hydroxyl group at C-5 from its spectral behaviour. The glycoside, on hydrolysis gave tectorigenin as aglycone and glucose as the only sugar. Permethylation followed by hydrolysis of
DM-5 gave 2,3,4,6-tetra-O-methyl-D-glucopyranose and 2,3,4-tri-O-methyl-D-glucopyranose showing the presence of two glucose units in the glycoside in the pyranose form with \(1\rightarrow6\) inter sugar linkage and hence the compound was identified to be tectorigenin-7-O-gentiobioside.

Chapter IV deals with the screening of biological activities of various extracts of the roots and leaves of *D. rubiginosa* and *D. malabarica*. Chapter V deals with the screening of the test compound 5,7-dihydroxy-2', 3', 5', 6'-tetramethoxy isoflavone isolated from the benzene extract of the leaves of *D. rubiginosa*. The studies carried out and the results obtained are:

1. Free radicals cause cell damage by lipid peroxidation resulting in lowering of serum glutathione (GSH). Naturally occurring antioxidants protect us from free radical induced injury, which is demonstrated by the elevation of GSH levels. In our study, the liver damage in rats induced by chronic ethanol administration is seemingly protected by the acetone, alcoholic and aqueous extracts of DRL and DML by restoring the balance between depletion and formation of GSH and thus the extracts demonstrate free radical scavenging activity. A similar free radical scavenging effect was observed with the test compound.

2. Ethylene glycol gets oxidized to oxalic acid resulting in hyperoxaluric renal stone formation. Urinary magnesium is an inhibitor of urolithiasis and the alcoholic and aqueous extracts of DRL and DML showed an increase in the urinary magnesium with considerable reduction in the urinary calcium, oxalate and phosphate levels and thereby reducing the severity of urinary lithiasis. The isoflavone
isolated from the benzene extract of DRL also exhibited antilithiatic effect by increasing urinary magnesium levels.

3. Hepatic fibrosis was achieved by feeding rats with CC1₃₄ chronically and serum biochemical parameters were determined including hydroxyproline (HP) which show a several fold increase under physiological and pathological stress conditions such as liver fibrosis. The alcoholic and aqueous extracts of DRR and DMR reduced the HP content and other parameters such as AST, ALT, ALP and TBL. The test compound also reduced all the biochemical parameters including the HP levels indicating a reduction in the lipid peroxidation associated with hepatic fibrosis.

4. Hepatoprotective activity of alcoholic, aqueous extracts of DRL and DML and the test compound was studied using rats intoxicated by CC1, administration and estimating serum transaminases and bilirubin and subsequent protection by the test extracts/test compound. After treatment with the alcoholic, aqueous extracts and the test compound, there was a significant reduction of serum transaminases and total bilirubin indicating the restoration of the functional status of liver.

5. The antidiabetic activity of the alcoholic and aqueous extracts of DRR and DMR was carried out using alloxan induced diabetic rats as animal models. Seven days after treatment with alloxan, the rats were administered with the test extracts and the blood samples collected at 5th and 10th hour of test extracts treatment were subjected to glucose determination. Though the extracts showed a reduction in the 5th hour sample, which was more pronounced than in the 10th hour sample, the antidiabetic effect of DRR and DMR was not comparable to that with the standard tolbutamide.
6. Diuretic potential of the chloroform, alcoholic and aqueous extracts of DRR and DMR were studied with rat model using frusemide as standard. The extracts were found to induce diuresis comparable to that of frusemide which is a high ceiling diuretic.

7. Subplantar injection of carrageenin in rats produced paw edema that was maximal after 3 hours and hence is given in result presentation. The chloroform, alcoholic and aqueous extracts of DML administered an hour before carrageenin injection showed a reduction in paw edema which is presented as percentage of inhibition of the increase in paw volume as compared to the positive carrageenin control. Carrageenin induced inflammation occurs in two phases. The first phase due to histamine/serotonin release lasts approximately for an hour and is followed by the release of bradykinin and prostaglandins. As the extracts of DML showed maximum reduction of paw edema at the third hour after carrageenin administration, it is clear that the extracts are effective in the second phase of inflammation.

8. The antipyretic activity of the chloroform, alcoholic and aqueous extracts of DRR was studied using rats, against yeast induced pyrexia. The experiments showed the test extracts to possess very weak antipyretic effect, as compared to aspirin.

9. Analgesic activity of the chloroform, alcoholic and aqueous extracts of DMR was evaluated by Eddy’s hot plate method and Rat’s caudal compression model. The extracts were found to prolong the ‘basal reaction time’ under both the methods, however the degree of analgesia was slightly lesser than that of the standard pentazocine.