SUMMARY

The isolation and characterisation of four flavonoid constituents from the roots of *Dalbergia congesta*, not hitherto phytochemically examined, are presented in Chapter I. The petroleum ether extract, when subjected to column chromatography, yielded four compounds designated as compounds A, B, C and D.

Compound A (m.p. 179-181°C) was characterised to be Cearoin (2, 5-dihydroxy-4-methoxybenzophenone) from the PMR, 13C-NMR, DEPT NMR studies. Compound B (m.p. 184-185°C) was identified to be Dalspinin (5, 7-dihydroxy-6-methoxy-3\4',-methylenedioxyisoflavone). The colour reactions and UV data, suggested that compound B could be an isoflavone with a methylenedioxy substitution. A one-proton singlet at 5 6.52 ppm and a typical ABX pattern in B-ring observed in the PMR spectrum showed that compound B could be 5,7-dihydroxy-3\4'-methylenedioxyisoflavone with methoxyl substitution in A-ring. The chemical shift values of the carbons and protons suggested the presence of the methoxyl substitution at C-6 of the isoflavone which was further confirmed by 2D NMR studies like HMQC and HMBC. On the basis of the colour reactions, UV, IR, PMR, 13C-NMR, HMQC and HMBC 2D NMR and mass spectral studies, compound B was characterised as SJ-dOrydroxy-e-methoxy-SM'-methylenedioxyisoflavone (Dalspinin).
Compound C (m.p.162-163°C), from the colour reactions, was found to have an isoflavone nucleus with a free hydroxy at C-5. Characteristic UV absorption suggested the presence of an isoflavone and a neoflavanoid moiety in compound C. The use of diagnostic-shift reagents in the UV spectrum of compound C suggested the presence of a 5, 7-dihydroxyisoflavone unit. The IR spectrum showed the presence of two carbonyl groups at 1620 and 1690 cm⁻¹. The PMR spectrum showed the presence of a 5, 7 dihydroxyisoflavone unit with methoxylation at C-6. A typical ABX pattern in B-ring suggested substitution in C-4' and C-6'. Two one-proton singlets at 8 6.25 and 6.90 ppm and a five-proton multiplet between 8 7.40-7.55 ppm suggested the presence of a 5,7, 8-trisubstituted neoflavone unit. The PMR spectrum of compound C also showed the presence of an olefinic proton at 8 6.99 ppm. The $^{13}$C-NMR spectrum showed the presence of 36 carbons including two carbonyl groups at 8 181.06 and 161.03 ppm corresponding to C-4 (isoflavone) and C-2 (neoflavone) respectively. The presence of a neoflavone unit attached to C-6' of the isoflavone through a hydroxy ethenyl linkage was identified by the detailed analysis of the HMQC, HMBC 2D NMR and mass spectral studies. From the UV, IR, PMR, $^{13}$C-NMR, 2D NMR and mass spectral studies, compound C was identified to be $5,7$-dihydroxy-6, $4'$-dimethoxy-6'-$[2''$-hydroxy-2'']-(5,8-dimethoxyneoflavonyl)ethenyl]isoflavone. This

133
is the first report of the natural occurrence of an oligomeric isoflavonoid of an isoflavone and a neoflavone from *Dalbergia* species and is given the trivial name Dalcongestin.

Compound D (m.p. 15 fr 157°C), from colour reactions and UV spectral studies, was found to be a 5, 7-dihydroxyisoflavone. A C-2 proton at 5 7.87, two one-proton singlets at 5 6.51 and 6.62 ppm due to H-6 and H-8, a one-proton singlet at 5 6.87 ppm due to the lone B-ring proton at C-4' and also the presence of four methoxyl signals in the PMR spectrum indicated a tetramethoxyl substitution in B-ring. The upfield shift of C-1' at 8 119 ppm and overlapping signals due to C-2' & C-6' at 5 143.12 and also due to C-3' & C-5' at 8 150.16 ppm confirmed the position of the four methoxyl groups at C-2', C-3', C-5' and C-6' in B-ring of the isoflavone nucleus. The mass spectrum of compound D showed m/z 221, corresponding to the RDA fragment of the tetramethoxylated B-ring. The mass spectrum also showed a RDA fragment at m/z 153 corresponding to 5, 7-dihydroxylation in A-ring. From the colour tests, UV, IR, PMR, $^{13}$C-NMR, DEPT, HETCOR 2D NMR and mass spectral studies, compound D was identified to be 5, 7-dihydroxy-2y, 5', 6Metramethoxyi8oflavone. This is the first report of the isolation of an isoflavone, from *Dalbergia* species with all the four methoxyl groups in the B-ring.
Chapter II deals with the phytochemical examination of the alcohol extracts of the roots of *D. horrida*, leaves of *D. sympathetica* and also the flowers of *Pterolobium hexapetallum*.

The crude from the alcohol extract of *D. horrida*, on column chromatography, yielded a solid, which was designated as compound E (m.p. 208-210°C). On the basis of the UV, IR and PMR spectral data, compound E was found to be a 5-hydroxy-6-memoxy-3',4'-methylenedioxyisoflavone glycoside, with sugar unit attached to C-7 in A-ring. The presence of two anomeric protons at $\delta$ 5.13 and 4.96 ppm in the PMR spectrum, the appearance of two anomeric carbon signals at $\delta$ 100.80 and 109.24 ppm and eleven aliphatic carbon signals between $\delta$ 64-78 ppm in the C-NMR spectrum indicated the diglycosidic nature of compound E. The proton signals at $\delta$ 7.61 (1H, d, J=16Hz), 7.17 (2H, d, J=2Hz), 6.34 (1H, d, J=16Hz) ppm and the carbon signals at $\delta$ 167.0, 147.13, 145.64, 123.07, 115.03, 109.38, 55.78 and 55.75 ppm showed the presence of a sinapoyl unit in compound E. Compound E, when subjected to alkaline hydrolysis, yielded an aglycone, an acid residue and two sugar moieties, which were identified as dalspinin, sinapic acid, glucose and apiose respectively, by co-TLC and PC comparison with authentic samples of them. The 1D and 2D NMR spectral data confirmed the substitution patterns in A and B-rings, the point of attachment of the sugar moiety to the
aromatic nucleus, the linkage of the apioglucose unit and the site of attachment of the sinapoyl unit to the glucose unit. On the basis of UV, IR, PMR, $^{13}$C-NMR, HMQC 2D NMR and mass spectral studies, compound E was identified to be DaIspinin-7-0-apiosyl-(1″,V>6″M4″-sinapoyl)glucoside. This was further confirmed by the PMR spectral data of the hepta-acetate formed by acetylation of compound E. This is the first report of an acylated apioglucoside of Dalspinin from Dalbergia species and it is given the trivial name Dalhorrinin.

The alcohol extract of D. sympathetica, on column chromatography, yielded a compound designated as compound F, (m.p. 194-196°C) and was analyzed for C$_6$H$_n$N$_3$O$_3$ (M* 145). The IR spectrum of compound F showed the presence of carbonyl and hydroxyl groups. The PMR, C and DEPT NMR spectral studies of compound F showed the presence of two methine, two methylene and one N-methyl groups. A quaternary carbon signal at 6 172.88 ppm was assigned to C-2 carbonyl of compound F. From all the above observations and also from the HMQC 2D NMR spectrum, compound F was identified to be 3, 6-dihydroxy-N-methyl-2-piperidone. This is the first report of the natural occurrence of a piperidone derivative from Dalbergia species.

The defatted flowers of P. hexapetallum were extracted with ethyl acetate, concentrated and chilled for 12 hours and the solid obtained was
designated as compound G. Compound G (m.p. 111-112°C), from its colour reactions was found to be a gallic acid derivative. The UV, IR, PMR and $^{13}$C-NMR spectral data of the compound G suggested it to be a trigalloyl derivative of a polyalcohol. Hydrolysis of compound G, using 2N HCl, gave gallic acid and mannitol, both identified by TLC comparison with authentic samples of them. The digalloyl substitution at C-1 and a galloyl substitution at C-5 of mannitol was shown by the chemical shifts of the carbon signals of mannitol. From the UV, IR, PMR, $^{13}$C-NMR spectral data, compound G was identified to be 1-O-digalloyl-5-O-galloylmannitol. This is the first report of the natural occurrence of a trigalloyl ester of mannitol from *Pterolobium* species.

Chapter III deals with the screening of biological activities of the ethyl acetate and alcohol extracts of *D.horrida* roots as well as of compound G, a gallotarmin isolated from *P.hexapetallum* flowers. The details of pharmacological studies carried out and the results observed are presented below.

1. Toxicity studies showed that the safe dose of administration of the test drugs was 5 mg/kg and 2.5 mg/kg B.W of rats in all the cases.

2. The analgesic activity in rats, assessed using acetic acid induced writhing method, showed that all the test drugs showed significant analgesic activity. The effect was maximum at 45 minutes and the
results were compared with standard drug diclofenac sodium at different time intervals. The activities were significant, when analyzed statistically using ANOVA and Dunnett's t-test.

3. The locomotor activity in rats was assessed using actophotometer and the results were compared with that of the standard drug diazepam. It was found that all the test drugs showed significant reduction in locomotor activity when statistically analyzed using paired t-test and the results were comparable with that of the standard drug.

4. The anti-inflammatory activity in rats was assessed by carrageenan-induced rat paw oedema method and the results were compared with standard drug ibuprofen at different time intervals. It was found that all the test drugs showed significant inhibition of paw oedema when analyzed using Dunnett's t-test and the effect was maximum at a time interval of 120 minutes after drug administration, when statistically analyzed using ANOVA.

5. The anti-bacterial activity of the test drugs was carried out using the diffusion method by using *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* as the test microorganisms and the results were compared with the standard drug Streptomycin. It was found that all the test drugs exhibited good anti-bacterial property.
Chapter IV deals with the bioassay studies, isolation, characterisation and HPLC assay of apigenin in the flowers of Clerodendron infortunatum. The aqueous alcohol extract of C. infortunatum flowers was assessed for its pharmacological properties. The test drug (at a dose of 15mg/kg B.W) showed a significant increase in locomotor activity when assessed for its CNS activity using actophotometer and was comparable with the standard drug caffeine. The anti-convulsant activity of the test drug (at a dose of 15 mg/kg B.W) was studied using maximal electroshock (MES) induced convulsions in rats through optical stimulation. It was observed that the test drug was found to reduce the time spent by the animal in the tonic extensor phase. The test drug exhibited significant anti-convulsant property when compared with the standard drug phenytoin. It was found that the test drug (at a dose of 15 mg/kg B.W) showed significant analgesic activity when evaluated in mice using hot plate method and compared with the standard drug morphine sulphate.

The ethyl acetate extract of C. infortunatum yielded a solid (compound H). From the UV, PMR, $^{13}$C-NMR and SEFT NMR studies, compound H was identified to be 5, 7, 4'-trihydroxy flavone (apigenin). The bioactivity of the aqueous alcohol extract of C. infortunatum may be attributed to the presence of apigenin and its
derivatives and hence, the quantification, and standardisation of apifeenin content in the alcohol extract of *C. infortunatum* flowers by reverse phase HPLC was taken up in the present study.

From the HPLC analysis, the unhydrolyzed and hydrolyzed extracts were found to contain 1.46% and 2.04% of apigenin respectively in the free form. This study further showed the presence of 0.1006% apigenin in the free form and 0.04% apigenin as hydrolysable derivatives in the flowers of *C. infortunatum*. 