CHAPTER V

SUMMARY

The world health organization (WHO) has estimated that 80% of world population still relies on traditional medicine for primary health care. The use of drugs goes back to time immemorial, ever since primitive man restored to the world around him to derive remedies, which could alleviate pain and cure illnesses. The knowledge of drugs has developed together with evaluation of scientific and social program. There is an urgent need to combine the best elements of traditional medicine and modern medicine to improve the health care system of human kind.

Chapter 1 deals with a brief description of the use of plants as phytomedicine and the details on flavonoids with special reference to their detection, isolation and structural elucidation.

Chapter 2 deals with the photochemical investigation of four medicinal plants and their results are briefly presented here.

Phytochemical investigation of Rivea hypocrateriformis:

The flowers of R. hypocrateriformis, belonging to the Convolvulaceae, were to contain two polyphenolic compounds viz., RI-1 and RI-2. RI-1 (m.pt 122±1°C) answered all the colour reactions expected of a flavonoid glycoside. The UV studies indicated a flavone skeleton with a α-dihydroxy pattern in B ring and a substitution of OH at C-7. The hydrolytic studies revealed the nature of sugar as rhamnose. The 13C NMR data were helpful in the identification of prenyl group at C-6. Use of 2D NMR spectral technique like 'H-'H-COSY,
HSQC and HMBC in addition to \( ^1H \) and \( ^{13}C \) NMR data confirmed the substitution pattern in A and B rings. On the basis of data from \( R_s \), UV, \( ^1H \) NMR, \( ^{13}C \) NMR, 2D NMR and EI-MS studies, the RI-1 was characterized as luteolin 6-prenyl 7-0-rhamnoside.

RI-2. (m.pt. 118 + 2° C) gave positive colour reactions expected of the phenolic glycoside nature. The UV studies indicated a flavone skeleton with o-dihydroxy pattern in B ring and a substitution at C-5 and C-7. The hydrolytic studies and \( ^{13}C \) NMR revealed the nature of sugar as neohesperidoside. Use of 2D NMR spectral technique like \( ^1H-^1H\)-COSY, HSQC and HMBC in addition to \( ^1H \) and \( ^{13}C \) NMR data confirmed the nature of substitution in A and B rings and the attachment of coumaroyl at C-4" of the glucose unit. On the basis of evidences from \( R_s \), UV, \( ^1H \) NMR, \( ^{13}C \) NMR, \( ^1H-^1H\) COSY, HSQC, HMBC and EI-MS, the RI-2 was characterized as luteolin 5-methyl ether 7-0-(4" neohesperidoside).

**Phytochemical investigation of *Sarcostemma brevistigma* :**

The systematic investigation of ethyl acetate fraction ethanolic extract of *S. brevistigma* of Asclepiadaceae yielded SA, which was characterized by various chemical and spectral methods. UV studies of SA indicated 3, 7 disubstituted 5, 3', 4' trihydroxy flavonol glycoside with sugar unit attached to C-3 and C-7 positions. The hydrolytic studies revealed the nature of the sugar as glucose and rhamnose in the ratio of 2:1. The \( ^{13}C \) NMR data were helpful in the identification of prenyl group at C-5' and coumaroyl group at C-4" of neohesperidoside. Use of 2D NMR spectra technique like \( ^1H-^1H\)-COSY, HSQC and HMBC in addition to \( ^1H \) and \( ^{13}C \) NMR data confirmed the compound SA to be quercetin 5’ - prenyl,
3 O glucosyl, 7-0-(4’-p-coumaroyl) neohesperidoside. There has been no prior report on the occurrence of this flavonoid glycoside.

**Phytochemical investigation of *Ecbolium viride*:**

Three flavonoid glycosides, EC-1, EC-2 and EC-3 have been isolated from the ethyl acetate fraction of 80% ethanolic extract of roots of *Ecbolium viride* (Acanthaceae). The EC-1 was characterized as apigenin 8-C-glucoside (vitexin). The identity of EC-1 was confirmed by direct comparison with authentic sample of vitexin.

The UV studies of EC-2 indicated a flavone skeleton with catechol type nucleus in ring B. It also suggested C-7 OH was blocked. The hydrolytic studies revealed the nature of sugar as glucose and nature of acyl moiety to be sinapoyl. The $^{13}$C NMR data were helpful in confirming the presence of sinapoyl group at C-2’. On the basis of $R_n$, UV, $^1H$ NMR, $^{13}$C NMR and EI-MS spectral data, EC-2 was characterized as luteolin 7-0-(2’ sinapoyl) glucoside.

EC-3 (m.pt 184 ± 2° C) gave positive colour reactions for phenolic glycoside. UV spectral studies with characteristic diagnostic agents suggested EC-3 to be a 5, 3’, 4’-trihydroxy flavone. Hydrolytic studies indicated the glycoside to be an acylated bioside. $^1H$ NMR studies of EC-3 revealed the presence of neohesperidoside. $^{13}$C NMR confirmed the position of attachment of acyl moiety viz., senecioyl and the position of attachment of acylated sugar moiety. Based on these evidences the EC-3 was characterized as luteolin 7-0-(4’-senecioyl) neohesperidoside. The presence of vitexin, isovitexin, orientin, isoorientin have been reported by previous researcher from this plant.
The isolation of vitexin and flavone glycosides from *E. viride* lends support to the earlier observation that flavone are characteristically present in the Acanthaceae.

Phytochemical investigation of *Clerodendrum philippinum*:

Shade dried flowers of *C. philippinum* belonging to the family Verbenaceae, on column chromatography yielded a pure compound designated as CL.

CL (m.pt. 137 + 1° C) tested positively for flavonoid glycoside but did not answer Wilson’s boric acid test. UV studies revealed the presence of 4’ OH group and absence of OH groups at C-5 and C-7. 'H NMR and 13C NMR suggested the presence of methoxyl and prenyl substitution at C-5 and C-3. Hydrolytic studies revealed the nature of the sugar as rhamnose. Based on Rf, UV, ‘H NMR, 13C NMR and EI-MS studies the structure of CL has been identified as apigenin 3-prenyl, 5-methyl ether 7-O-rhamnoside. This is the first report of a flavonoid glycoside in this plant.

**Pharmacological studies:**

Chapters III and IV deal with pharmacological screening of the four chosen plants viz., *R. hypocrateriformis, S. brevistigma, E. viride* and *C. philippinum*.

Experiments were performed to determine LD50 and ED50 values of the test extracts. It was found out that all these extracts had LD50 values greater than 5 g/kg.

Anti-nociceptive activity of the test extracts was evaluated by hot plate method and acetic acid induced abdominal constriction assay. It was found that
the plant extract of RHF, SBF, EVR and CPF produce significant analgesic activity in dose dependent manner.

The ethyl acetate traction of RHF, SBF, EVR and CPF were evaluated for anti-inflammatory activity by carrageenin induced rat paw edema method as well as by cotton pellet granuloma method. The results showed that these drugs produced anti-inflammatory effect in a dose dependent manner. The SBF extract showed higher level of activity than other test drugs.

It was found that extracts of RHF, SBF, EVR and CPF showed greater hepatoprotective effect when evaluated using CC14 induced model. At a dose of 250mg/kg, the test drugs showed significant effect and the levels of the marker enzymes were comparable to those of silymarin, the standard drug used in the study. A significant reduction in liver weight supported this finding. Histopathological studies offered direct evidences for the efficacy of these drugs as hepatoprotective agents. The lesions developed on CC14 administration were found to be normalized with near normal histoarchitecture of liver cells in drug treated groups.

The ethyl acetate fractions of RHF and SBF were evaluated against Dalton’s ascites lymphoma (DAL) and Ehrlich ascites carcinoma (EAC). These plant extracts were found to be capable of prolonging the life of the tumour bearing mice when compared with tumour control. In both the types of tumour bearing mice the altered haematological parameters such as haemoglobin content, erythrocyte count, total leucocyte count and total protein were brought back to normal when treated with RHF and SBF like in the case of the treatment with standard drug viz., 5-fluorouracil.
An attempt was made to find out the possible biochemical mode of action of the flavonoid glycosides isolated from the four chosen medicinal plants. All the seven flavonoid glycosides Rl-1, RI-2, SA, EC-1, EC-2, EC-3 and CL were tested by *in-vitro* method on lipid peroxidation activity. The compound SA showed highest lipid peroxidation inhibition activity than the other glycosides.

The flavonoid glycosides isolated from the four chosen plants were also tested for anti microbial activity using the Gram-positive and Gram-negative test organisms. Results indicated that these plant drugs were active against Gram-positive organisms in a dose dependent manner and were selectively toxic to Gram-positive organisms.