CHAPTER 9

CONCLUSIONS

Medicinal plants from the foot hills of Western Ghats are screened for its pharmacological value and the following plants are found to be used to treat various ailments traditionally: Pavonia zeylanica, Hyptis suaveolens, Rhynchosia capitata, Trichodesma indicum, Crotalaria globosa, Delonix elata and Syzygium hemisphericum. Based on the literature survey, three plant species C. globosa, D. elata and R. capitata are selected for the study. The HPTLC analysis revealed that all the plants exhibited presence of flavonoids and maximum number of flavonoid compounds are found in EtOAc fraction of plant extracts. Analysis of total phenolic and total flavonoids in the EtOH extracts followed by DPPH’ assay revealed that R. capitata leaves possess higher flavonoid content than its roots. Further higher phenolic content especially tannins and proanthocyanidins are found in D. elata flowers followed by flavonoids. C. globosa leaves exhibited lowest amount of phenolic compounds including flavonoids, proanthocyanidins and tannins.

Hence R. capitata leaves are subjected to further analysis. To identify the medicinally active compounds, bioactivity guided fractionation using solvents of varying polarity led by in vitro antioxidant capacity assay by DPPH’, FRAP, NO’ and antihemolytic activity are carried out. It is observed that of all the solvents (n–hexane, C₆H₆, EtOAc and EtOH), EtOAc exhibits good activity in all assays. Therefore, EtOAc fraction is selected to investigate the nature of flavonoids present.
The EtOH extract is subjected to GC/MS analysis and the chromatogram displayed nine peaks at different R, indicating the presence of nine compounds. The chemical compounds identified are oleic acid, octadecanoic acid, phytol, hexadecanoic acid, C-11-hexadecenoic acid, Z,Z,Z-4,6,9-nonadecatriene, tetradecanoic acid, 5-azulenemethanol and 1-butanol.

Presence of phenolics such as gallic acid, ferulic acid, caffeic acid, rutin and quercetin has been investigated by RP–HPLC method for EtOH extract of *R. capitata*. The chromatographic separations occur as follows: gallic acid (R,=5.917), caffeic acid (R,=9.217), rutin (R,=10.258), quercetin (R,=12.342) and ferulic acid (R,=24.108).

Separation of flavonoids from EtOAc by refrigeration yielded two EtOAc(s) and EtOAc(l). The DPPH· scavenging activity for EtOAc(s) is 16.97±0.04 and EtOAc(l) is 44.91±0.21. The bioassay guided fraction revealed EtOAc(s) to be the most active DPPH· scavenger. Isolation of compounds from this solid is carried out by a series of steps: initially spot identification by color is carried out followed by acid hydrolysis which proved the mixture to be C-glucosides. LC–MS/MS analysis in negative ion mode produced fragment ions at four LC retention times (R,=5.2, R,=12.5, R,=23.2 and R,=29.1) at [M–H]−, m/z 447 and m/z 431 respectively. By comparison with the data available, structures of flavonoids are predicted to be vitexin, isovitexin, orientin and isoorientin.

Further confirmation of the compounds is done by TLC co-elution with standards. Separation of the C-glucosides is carried out by prep–TLC and the yields are vitexin (4.3 mg/g), isovitexin (5.1 mg/g), orientin (6.7 mg/g) and isoorientin (14.1 mg/g) respectively. Experimental radical scavenging behavior of the above said compounds are carried out by DPPH· assay and the order is found to be: vitexin<isovitexin<orientin<isoorientin.
The pharmacological activity of the C–glucosides vitexin, isovitexin and orientin present in the bioactive fraction has been carried out using *in silico* approach. PASS results indicates that the free radical scavenging activity is found to be significant in all the analyzed C–glucosides (0.955 for orientin, 0.948 for vitexin, and 0.845 for isovitexin) which indicates that the substance is very likely to exhibit the activity in experiment and the chance of the substance being the analogue of a known pharmaceutical agent is high.

The same compounds exhibit moderate antibacterial behavior (0.540 for orientin, 0.540 for vitexin and 0.511 for isovitexin) revealing that the substance is likely to exhibit activity in experiment, but the probability is less. The same is confirmed by experimental evaluation.

Theoretical calculations to determine the most probable site of radical attack on C–glucosides are carried out using DFT/B3LYP/6–311G(d,p) level of theory which led to the following conclusions:

- The 4′–OH is most favored site for homolytic –OH bond breaking in vitexin and isovitexin, whereas 3′–OH is preferred for orientin. These preferences are the same in the gas and solvent phases (both polar and non–polar)

- Intramolecular hydrogen bond formation between the 5–OH and the carbonyl group of compounds explains the higher BDE and PA values over 4′–OH, 3′–OH and 7–OH groups. Thus DFT results indicate that the 5–OH group is not/partially involved in the antioxidant mechanism

- The presence of additional functional groups like C=C and C=O group causes electron delocalization and hence increases the radical scavenging ability of C–glucosides
• The lowest energy gap value ($\Delta E$) for orientin C–glucoside suggests it to be a potent radical scavenger compared to isovitexin and vitexin. This is supported by its low BDE value and the generation of a more stable phenoxy radical.

• The existence of ortho dihydroxy structure in B–ring (catechol moiety) favoring the antioxidant activity. The presence of the 2,3 double bond in conjugation with a 4–oxo function (1,4–pyrone moiety) in C–ring, contributes to the antioxidant activity by increasing $\pi$–electron conjugation.

• The computed values of IPs, BDEs and PDEs indicates that one step HAT is the mechanism that best explains the antioxidant activity of isovitexin in the gaseous phase rather than ET–PT or SPLET.

• Presence of a sugar at the C6 or C8 position in apigenin or luteolin enables the solubility in water compared to its aglycones and thus enhances the radical scavenging ability, which is contrary to the behavior of the corresponding O–glycosyl flavonoids. Thus vitexin flavonoid acts as a better radical scavenger than apigenin.

• The sugar substitution considerably modifies the Mulliken charges on the O–atoms of the hydroxyls particularly the hydroxyls present in A–ring.

• The position of glycosylation of flavonoid C–glucosides influences its radical scavenging behavior. This is evidenced in the present study by comparing vitexin (C8–glycosylated) and isovitexin (C6–glycosylated).

• The occurrence of multiple –OH groups attached to the aromatic B–ring enhances the radical scavenging behavior in
C–glucosides as multiple –OH groups can give rise to several radicals depending on the group to be radicalised

- When the basic flavonoid nucleus is substituted by a bulkier glucoside unit, the internal rotations around the C3–C2 and C1'–C2' bonds are restricted as it results in very significant destabilizations. This in turn results in non–planarity and hence potential energy surface scan cannot be carried out for the compounds under study as the results are not reliable.

Combined information from various descriptors: BDE, IP, EA, \( \chi \), \( \eta \), S, \( \omega \), FMO, MEP and NBO analysis supports the above conclusions in predicting the radical scavenging efficiency of C–glucosides. Further the activity order of C–glucosides are vitexin<isovitexin<orientin as per theoretical model which is in line with the experimental data obtained.

The exploration of application of plants rich in polyphenolics in textile industry as a natural dye is studied using \( R. \ capitata \) leaves and \( D. \ elata \) flowers which yielded variety of shades depending on the nature of the mordant used and pad dyeing yielded better shade fastness than traditional. All the dyed fibers exhibited moderate fastness to wash and light on both cotton and silk.

Further scope of the work focuses on the reactions of the compounds separated with the biological molecular targets in real systems.