Chapter- 6

Summary and Conclusions
Plant-based medicines or plant-derived natural compounds are becoming popular now a days over allopathy or synthetic medicines because of less toxic effects and low cost.

Synthetic drugs have therapeutic activities against liver disease but in the meantime they also have adverse side effects. Natural antioxidants are the compounds can be used to overcome this problem as they maintain the homeostasis of cell and redox potential of ROS because of the concentration of ROS crosses the threshold it activates stellate cell by activating various cytokines and ultimately it will damage hepatocytes. Medicinal plants have natural antioxidant activity and majority of the world’s population are using for the treatment of various diseases and, antioxidant property of any plant is the backbone for the treatment of various ailments.

*Ficus* species are used as traditional medicinal system and it belong to Moraceae family. Hence, in the current study, we evaluated and compared the antioxidant, anti-inflammatory and hepatoprotective activities of selected *Ficus* species of Karnataka namely *F. parasitica, F. tsiela, F. microcarpa, F. heterophylla, F. dalhousiae, F. drupacea and F. mollis* because not much studies on the bioactivities have been done.

**Summary and conclusions of the present investigation are as follows:**

1. The total phenolic contents was higher in methanolic extracts compared with other solvents.

2. Preliminary screening of *Ficus* species for *in vitro* antioxidant and anti-inflammatory activity by using DPPH, Dot blot analysis, ABTS, hydroxyl and nitric oxide scavenging activity and LOX and COX-2 inhibitory activities respectively revealed that methanolic extracts of *F. dalhousiae* and *F. tsiela* possessed promising activity compared to other samples.

3. FTIR data revealed the similarity in wavelength of methanolic extracts corresponding to phenolic group. Among all, *F. dalhousiae* is the first candidate.
4. Further, methanolic leaf and bark extracts of *F. dalhousia* and *F. tsiela* were screened for *in vivo* hepatoprotective activity. All four extracts have activity against CCl₄-induced hepatotoxicity in rats but FdBM extract not only restored the level of hepatic specific serum biomarkers AST, ALT, ALP and LDH but also upregulated the activity of intra-cellular antioxidant enzymes SOD, CAT and GSH compared to other selected extracts. So, *F. dalhousiae* bark methanolic (FdBM) extract was selected for the purification and identification of potent molecule.

5. *F. dalhousiae* bark methanolic (FdBM) extract was first subjected for thin layer chromatography (TLC) for standardization of solvent mixture and that was dichloromethane: ethyl acetate: methanol (7:3:1) with 0.1% formic acid. This solvent mixture was used to perform column chromatography for partial purification of FdBM.

6. Fractions having good antioxidant activity were pooled together and subjected for preparatory high performance liquid chromatography (HPLC) with designed solvent system, methanol: water (84:16) with 0.1% formic acid. Collected fractions were again checked for antioxidant activity by using DPPH and fractions with retention time of 5.483 possessed very less IC₅₀ value i.e. 5.17 µg were further selected for identification of compound and further confirmation of the hepatoprotective activity of isolated compound. Purity of this fraction was confirmed by analytical HPLC.

7. Selected active fraction was further subjected to UV-Vis, FTIR, HRLC-MS and NMR spectrometer for elucidation of the structure and results showed that naringenin was the active molecule. For the very first time our study reports the presence of naringenin a flavonoid from *F. dalhousiae*.

8. Isolated compound was again subjected for hepatoprotective activity against CCl₄ and paracetamol-induced hepatotoxicity in rats. Pre-treated naringenin reduced the liver specific serum biomarkers such as AST, ALT, ALP and LDH in rats which were administrated with CCl₄ and paracetamol.
9. The effect of pre-treated naringenin on liver antioxidant enzymes such as GSH, SOD and CAT also revealed the protective role of naringenin because it restored the activity of these antioxidant enzymes. Further, it also balanced the concentration of ROS and inhibited oxidative stress by inhibiting lipid peroxidation.

10. The activity of two pro-inflammatory cytokines such as IL-6 and TNFα were also checked from serum because these cytokines enhance the pathogenesis of liver injury and it was found that naringenin inhibits the activity of IL-6 and TNF-α. A very first time result suggested the oral administration of naringenin influencing the level of cytokines which are responsible to activate stellate cells.

11. A very first report on effect of naringenin on paracetamol-induced hepatotoxicity on rats and histological studies also supported the obtained results. High dose of paracetamol damaged the liver tissue severely but pre-treated naringenin showed protective role against paracetamol and cellular architecture of tissue was normal.

12. The study of molecular docking suggested the mode of action of naringenin in the liver disease.

13. Thus, the present studies undertaken to screen the antioxidant, anti-inflammatory hepatoprotective potential of selected Ficus species used in traditional practices. Based on the results, F. dalhousiae bark methanolic extract was selected for purification, and the results showed that naringenin is the bioactive molecule from F. dalhousiae possessed very good hepatoprotective activity. So, it can be alternative drug against liver cirrhosis.

14. Further pharmacological, pharmacodynamics and molecular studies and validations are needed for determining the actual therapeutic potential including development of pre-clinical trials. So, our results suggested that F. dalhousiae can be used as traditional medicinal practice against liver diseases.