Chapter 7

Summary and Conclusion
7. Summary and Conclusion

The present work is a comprehensive compilation of findings in the evaluation of traditionally known medicinal plants for their hepatoprotective activity. To achieve this, following goals were set:

- Standardization and evaluation of selected medicinal plant material as per WHO guidelines
- Marker based identification of phytoconstituents in successive extracts of selected medicinal plants using HPLC
- *In-vitro* evaluation of standardized extracts of selected medicinal plants for antioxidant activity
- *In-vivo* evaluation of standardized extracts of selected medicinal plants for hepatoprotective and antioxidant activity
- Anti-inflammatory and immunomodulatory activity of bioactive extracts of selected medicinal plants
- Isolation of possible phytoconstituents from bioactive extracts

The work was accordingly planned and executed systematically to achieve the above set goals. A thorough literature survey was done using all the possible resources to select the plants for the study. Based upon earlier reported data like pharmacological activities, phytochemical composition and availability, and ethnomedical uses, two plants namely, *F. microcarpa* (Syn: *F. retusa*) and *L. acutangula* var. *amara* were selected.

The selected plants were collected from wild and authenticated by a botanist Dr. D. A. Patil, Professor and Head SVPM College, Dhule, Maharashtra, India. The bark of *F. microcarpa* and fruits of *L. acutangula* var. *amara* of the selected medicinal plants were first standardized as per WHO guidelines. In the next phase, plants were subjected for the extraction by using the solvents of increasing polarity using Soxhlet extraction technique. The prepared extracts were subjected for preliminary phytochemical evaluation followed by estimation of total phenolic and total flavonoid content. Identification of various markers, namely oleanolic acid, betulinic acid, lupeol, β-sitosterol, quercetin, rutin, catechin and chlorogenic acid was done by using High Pressure Layer chromatography (HPLC).
It is documented in the literature that oxidative stress is involved in the pathogenesis of liver injury. The plants with antioxidant potential have been reported to have hepatoprotective activity. With this view, the plants selected for the present study initially evaluated for their antioxidant potential by employing suitable in-vitro antioxidant models. Both the selected plants i.e. *F. microcarpa* bark and *L. acutangula* var. *amara* pericarp showed good antioxidant activity especially the ethyl acetate and ethanol extracts, respectively. A comparative assessment revealed that the *F. microcarpa* showed slightly better activity than the *L. amara*. The extracts were subjected for acute toxicity study according to OECD guideline 425 and found to be safe. Hence, all extracts were subjected for in-vivo hepatoprotective studies. In both paracetamol and CCl₄ induced acute liver toxicity models, the extracts showed good hepatoprotection when compared to toxic control. The activity of ethyl acetate extract of *F. microcarpa* and ethanol of *L. amara* extract was found to be superior, while petroleum ether (60-80°) extract of both plants showed significant activity when compared to other extracts. It may be probably due the presence of various antioxidant and cytoprotective principles. Based on acute hepatotoxicity study, the PE-FMB, EA-FMB and PE-LAP and EO-LAP extracts were evaluated for ethanol induced chronic hepatitis, ethyl acetate extract of *F. microcarpa* and ethanol of *L. amara* extract was found to be best compared to petroleum ether extract of both the plants. These extracts were further evaluated for anti-inflammatory and immunomodulatory study. At higher dose level plant shown good anti-inflammatory and immunomodulatory study.

In the final stage, focus was given on the isolation of possible phytoconstituents. Promising results from in vitro and in vivo studies for the candidate plants encouraged us for the isolation of possible phytoconstituents from ethyl acetate extract of *F. microcarpa* and ethanol extract of *L. amara*.

These extracts were portioned on silica gel with the combination of different polarities solvents to yield several fractions. Total eight fractions were prepared from both plant bioactive extracts. These fractions were evaluated for in vitro antioxidant activity using DPPH assay. The fractions with good antioxidant capacity were selected for further isolation and purification. The column was eluted with solvents of varying polarity. The column fractions were monitored with TLC and similar fractions were combined and further processed to yield the isolation of four different compounds.
These isolated compounds were characterized by using suitable spectral techniques in order to confirm their structure. The oleanolic acid, catechin and \( p \)-hydroxycinnamic acid were confirmed from \( F. \) microcarpa ethyl acetate extract. While one of the fraction yield mixture, revealed by GC-MS study. The mixture composed of three components with molecular weight 501, 319 and 589, however; the final structure could not be established for the same. Similarly, from bioactive ethanol extract of \( L. \) amara, luteolin and gallic acid were confirmed. Two bioactive fraction yields mixture, one of them showed the presence of mixture of three components with molecular weight of 561, 534 and 550 while other fraction contains two components with molecular weight of 120 and 592, structures were not confirmed.

In conclusion, the results of the present investigation infer that these plant extracts possess potent antioxidant and hepatoprotective property, the former being probably responsible for the latter. Thus, the extracts can be beneficial in treating liver damages caused due to chemical or xenobiotic exposure.
7.1 Future Prospects

Our finding suggest that the plant *F. microcarpa* and *L. amara* possesses hepatoprotective potential against acute and chronic hepatotoxicity study in Wistar albino rats. However further studies are required to explore the exact mechanism of action. At the same time the above mentioned plants can be formulated in a suitable dosage form individually or as a polyherbal formulation, its pharmacological evaluation by using suitable animal models followed by clinical trials in human volunteers are proposed.