CHAPTER 12

SUMMARY

The present investigation entitled “A Prospective Phytochemical and Pharmacological screening of the Fruit pulp of *Limonia acidissima* Linn” has been carried out and its therapeutic utility has been evaluated in the thesis.

*Limonia acidissima* Linn (*Feronia elephantum, Feronia limonia, Hesperethusa crenulata, Schinus limonia*) belongs to family Rutaceae (Citrus family). It is a monotypic genus *Limonia*, native to India, Pakistan, Srilanka and Southeast Asia east to Java. Common names in English include wood-apple, elephant-apple, monkey fruit, curd fruit and Kath bel. This plant is prescribed as a traditional medicine for the treatment of various ailments. *Limonia acidissima* is well-known for its medicinal properties. This species has numerous described medicinal uses as capably. It has a wide range of biological activities viz., adaptogenic activity, for blood impurities, for leucorrhoea, for dyspepsia, for jaundice and hepatoprotectant. In India the fruit is used as a liver and cardiac tonic, in diarrhoea and dysentery, the different parts of the plant have been investigated phytochemically by several workers and found to contain coumarins, furano coumarins, lignans, alkaloids, steroids and flavonoids.

The fruits of this tree were collected in suitable period and authenticated by the Botanist. The shade dried, coarsely powdered fruit pulp was subjected to extraction with solvents of increasing polarity. Methanolic
extract yielded higher yield than the other solvents, thereby it was selected for screening pharmacological and phytochemical activities.

A preliminary phytochemical testing for the methanolic extract was done to identify the phytoconstituents, which revealed the presence of flavonoids, tannins, terpenoids, phenolic compounds, glycosides and proteins.

Toxicity testing for the methanolic extract of fruit pulp of *Limonia acidissima* (MELA) was carried over as per the OECD guidelines 420 and the therapeutic doses were fixed to be 200 and 400 mg/kg. This dose was taken for the further pharmacological investigations such as hepatoprotective activity, anti-diabetic activity, wound healing activity and anti cancer activity.

Hepatoprotective activity of MELA was screened against CCl₄ induced hepatotoxicity in rats for a period of 10 days. The parameters analyzed were ALT, AST, ALP, GGT, TB, TG, TC and antioxidant parameters like GSH, CAT, SOD and LPO. The results were compared with that of the standard drug Silymarin 100mg/kg and there was a significant increase in the serum marker enzymes and a normal antioxidant enzyme levels after treatment with 200 and 400mg/kg of MELA. It was observed that MELA at a dose of 400mg/kg exhibited a better hepatoprotective activity than 200mg/kg dose level. The histopathological observations also coincided with the biochemical findings.

Anti-diabetic activity of MELA was screened in Alloxan induced diabetes for a period of 21 days in rats. Anti-diabetic activity was confirmed by a decrease in the increased blood glucose levels which was analyzed every week for three weeks continuously. In addition to blood glucose levels other biochemical parameters like serum creatinine, blood urea nitrogen, total protein, total cholesterol and the normal levels of antioxidant enzymes were also maintained after MELA 200 and 400 mg/kg treatment which was
comparable to that of the standard drug Glipizide 5mg/kg. In this study, MELA at a dose of 400 mg/kg elicited a better antidiabetic response when compared with the 200 mg/kg dose. Histopathological studies also confirmed the protective effect of MELA to pancreas in a dose dependent manner.

Wound healing effect of MELA was screened in rats topically by incision, excision and administered orally for dead space wound models. Wound healing activity was assessed in rats by measuring the wound contraction and tensile strength of the wound for incision model, epithelialization period for excision model, antioxidant parameters like SOD, CAT and histopathological studies for dead space wound model. All the parameters were altered significantly which was comparable with that of the standard drug Nitrofurazone.

The effectiveness of MELA against DLA induced tumor was evaluated in Swiss albino mice for a period of 14 days. Anticancer activity was assessed by analyzing the hematological parameters, life span of the animal, tumor cell count, tumor volume, total protein and antioxidant parameters. It was observed that there was a significant increase in the hematological parameters and life span and a decrease in the viable tumor cell count. Antioxidant enzyme levels were also found to restore to normal which was comparable with that of the tumor control group animals. The anti tumor activity afforded by MELA was in a dose dependant manner.

MELA was subjected to phytochemical isolation of active constituents by Column chromatography technique. Two compounds were isolated from MELA namely a triterpenoid glycoside-Acidissimin and the other being an unsaturated digalacturonic acid, structure of these two isolated compounds were confirmed by physio chemical properties and spectral analysis by IR, $^{1}$HNMR, $^{13}$CNMR, and GC-MS. Both the compounds were screened for invitro antioxidant activity by DPPH method and cytotoxic
activity by MTT assay against cervical cancer cell lines which showed a significant antioxidant activity at lower dilutions which was comparable to that of Ascorbic acid and exhibited a significant cytotoxicity comparable with the standard drug Doxorubicin respectively.

All these data obtained after this research work supported the traditional claim associated with the fruit pulp of the *Limonia acidissima* literature. The results afforded by the work also confirms the involvement of oxidative stress in the studies carried out, which was then brought back to normal levels after MELA treatment. However this study can be extended in a way to explain about the mechanism of the proved activities of the fruit part other than the antioxidant property. Therefore the study can be concluded that the fruit pulp of *Limonia acidissima* and the compounds isolated from it can be utilized therapeutically for the folklore medicinal claims or can be included as a main ingredient for antioxidant herbal preparations after proper formulation.