Chapter - 5
Summary
5. SUMMARY

The present thesis entitled “Phytochemical Investigation and Hepatoprotective Screening of *Embelia Basaal* (Roem. & Schult.) DC.” deals with the exploration of phytoconstituents and hepatoprotective activity of the plant *E. basaal* (Myrsinaceae) which is traditionally used by local people and tribals in Kolli hills, Tamil Nadu, for the treatment of liver disorders and the dried fruit of the plant is used as anthelminthic. The plant was authenticated and stem bark was taken up for the present study.

The stem bark of the plant was subjected to continuous hot maceration by using Soxhlet extractor for the preparation of ethanol extract. The evaluation of preliminary phytochemical studies of ethanol extract revealed the presence of phytoconstituents like carbohydrates, proteins and amino acids, flavonoids, phytosterols, tannins and phenolic compounds and diterpenoids. Elution of column by gradient elution technique with different mobile phase in the order of increasing polarity led to the isolation of two phytoconstituents, one was orange crystalline compound from chloroform: methanol (95: 5) fraction and another one was white solid mass from methanol: ethanol (85: 15) fraction. The compound I yielded 0.82 g of orange crystalline compound which showed positive test for Embelin. After purification the melting point of the compound was found to be 144-146°C. When the compound was subjected to thin layer chromatography with the mobile phase of n-propanol: n-butanol: 4N ammonia (7: 3: 7) the R_f value was found to be 0.73. It was identified as a pink spot visible in day light and when viewed under UV 254 nm it was seen as blue color. The compound II yielded 1.12 g of white solid which showed positive test for tannins. After purification the melting point of the compound was found to be 248-250°C. When the compound was subjected to thin layer chromatography with the mobile phase of toluene: ethyl acetate: formic acid:
methanol (3.5: 2.5: 0.8: 0.1) the Rf value was found to be 0.64. It was identified as a brown spot under iodine chamber. The purified compounds were subjected to spectral studies like UV, IR spectroscopy, $^1$H NMR, $^{13}$C NMR and High Resolution Mass Spectroscopy [HRMS] and were confirmed to be 2,5-dihydroxy-3-undecyl-2,5-cyclohexadiene-1,4-dione (Embelin) and 3, 4, 5-trihydroxybenzoic acid (Gallic acid) respectively.

To assess the short term toxicity of the extract, acute oral toxicity study was carried out as per the guidelines No: 423 given by the Organization for Economic Co-operations and Development (OECD), Paris and it revealed that the extract was safe up to the dose level of 2000 mg/kg body weight of animals as no mortality was observed among the animals used. To assess the long term toxicity of the extract, sub-acute toxicity study was carried out. The results of the study indicated that the extract at the selected dose levels did not show any toxicity and was safe. The non-toxic nature of the extract was further supported by the histopathological examination of liver and kidney.

The hepatoprotective activity was screened in CCl$_4$ induced Albino rats of Wistar strain models. The various biochemical parameters like serum glutamic oxaloacetic transaminase, serum glutamate pyruvate transaminase, serum alkaline phosphatase, serum gamma glutamyl transpeptidase, sugar, total bilirubin, total protein, triglyceride, total cholesterol, high density lipoprotein-cholesterol, very low density lipoprotein-cholesterol and low density lipoprotein-cholesterol were estimated to determine the functional state of the liver. Estimation of immunological parameters like tumor necrosis factor - alpha and interleukin - 6 were also studied to further support the activity. The hepatoprotective activity was further strongly strengthened by the histological studies of the liver. With the liver homogenate the
antioxidant studies like estimation of lipid peroxidation, superoxide dismutase, catalase, glutathione peroxidation and glutathione transferase were carried out. The outcome of the study strongly supports the antioxidant property of the extract.

The results of screening of hepatoprotective activity and antioxidant property indicated that the ethanol extract possesses significant hepatoprotective activity and antioxidant property at the dose levels of 200 mg/kg and 400 mg/kg body weight.

From the above results, it may be concluded that the ethanol extract of *E. basaal* stem bark is non-toxic and is safe. As the results indicated that the extract possess significant hepatoprotective activity, after carrying out a thorough study of clinical trials, the plant can be considered as a low cost, potent, herbal medicine for liver disorders.