SUMMARY AND CONCLUSION

The history of medicine and surgery dates back perhaps to the origin of the human race. In recent times, focus on plant research has increased all over the world and a large evidence has collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last decade (Dahanukar et al., 2000). Plant tissue culture techniques offer available solution for the production of standardized quality phytopharmaceuticals through mass production of consistent plant material for physiological characterization and analysis of active ingredients. Tissue culture protocols have been developed for species, which are over exploited in pharmaceutical industries and need conservation.

The thesis presents an account of investigation on in vitro and in vivo studied of Flaveria trinervia. The thesis is organized into three chapters.

Chapter I presents general introduction with brief survey of literature on the tissue culture work on F. trinervia. In addition the importance, distribution and conventional methods of propagation of the selected medicinal species are discussed.

Chapter II deals mainly to establish a protocol on in vitro callogenesis on hormone free medium to carry out antioxidant and antimicrobial activities. Different media such as MS medium (Murashig and Skoog, 1962), B-5 medium (Gamborg et al., 1968) and White’s medium (White, 1943) were used to test the response of leaf explants of F. trinervia. The media were supplemented with various concentrations
of coconut milk. But in MS medium callus growth was maximum compared to other media and it was supplemented with 20% coconut milk. Further the primary callus was subcultured on the same medium composition on which the callus were dried and powdered and used for the other studies. In the present study free radical scavenging activities were studied and chloroform extract showed potent antioxidant properties against DPPH radical with IC 50 values of 125+-4.4 ug / ml and nitric oxide radical with IC 50 values of 640+- 1.2ug/ml. The MIC values of both the extracts were ranged from 5 to 40mg /ml. Aqueous extract of *F. trinervia* showed significant inhibition at MIC 10mg/ml against *Staphylococcus aureus* and *Salmonella paratyphi*, whereas, chloroform extract showed maximum activity against *Salmonella typhi* and *E coli* at MIC 10mg/ml.

Chapter III deals with hepatoprotectivity and anti inflammatory activities. Hepatotoxic liver sections were studied and shows parenchyma with focal effaced architecture and few of the periventral hepatocytes and focal medzonal hepatocytes showed macrosteatosis and microsteatosis. Some of the central veins and sinusoids show dilation with focal congestion, mild stromal inflammatory and macrophages. Micro view of chloroform extract of 150mg/kg showed parenchyma with partially effaced architecture. Some of the perivenular hepatocytes and focal midzonal hepatocytes show macrosteatosis and microsteatosis. These are seen moderate mixed inflammatory infiltration comprising of neutrophils and lymphocytes.

The isolated liver from the toxicant treatment animals exhibited increase in their physical parameters like wet liver weight and wet liver volume. In case of toxicant treated groups there will be rise in serum
marker enzymes such as SGPT, SGOT, ALP, direct and total bilirubin, cholesterol and triglycerides and decrease in the level of proteins. The same is observed in liver diseases in clinical practice and hence are having diagnostic importance in the assessment of liver function. In the present study, the chloroform extract significantly reduced the toxicant elevated levels of enzymes and increase in the level of protein. Hence at this point it was concluded that the chloroform extract possess hepatoprotective activity. Treatment of chloroform extract decreased thiopentone induced sleep time, an indirect evident of their hepatoprotective effect. In toxicant treated animals there will be severe histopathological disturbances in the cytoarchitecture of the liver. The same is observed in case of humans who are suffering from major liver disorders. In the present study, chloroform extract treated groups exhibited minimal hepatic damage and intact cytoarchitecture of the liver was maintained, indicating hepatoprotection.

Adminstration of carrageenan (1%, 0.1ml) significantly increased the paw edema but oral administration of chloroform extract in the dose of 250mg/kg significantly inhibited the carrageenan induced paw edema at 30, 60, 120, 180 and 240min interval as compared to carrageenan control and showed 31% at four hours, where as standard drugs showed 51% of inhibition.

Chapter IV deals with phytochemical analysis and it is evident that only chloroform fraction contains carbohydrates, glycosides and phytosterols which forms the main constituent in the callus. For further characterization of compounds, chloroform fraction was considered. The analysis of the F1 was done in high performance liquid chromatographic system (HPLC) equipped with LC8A pump, SPD-M
10A software with Shimadzu, Japan. The chromatographic conditions for the analysis were as follows: mobile phase; methanol: water 90: 10 v/v. Column: ODS (Octadecyl silane) Zorbax SB C 18 (4.6x 2250mm) 5 µm Detecor, wave length : 254nm, flow rate : 0.2ml/ min, injection volume : 5µl. The HPLC analysis of FT1 fraction shows that the peak eluted at 3 min is predominant showing the adsorbance maximum at 210nm. The same fraction was then passed to the MS to check the molecular weight of the compound. The mass analysis of FT1 was studied in LCMS- 2010 A Shimadzu system equipped with run mass hunter software. The sample was run in ESI position scan mode and monitored using single ion monitoring mode. FT1 fraction gave major peak at m/Z 400 indicating a molecular weight of 399 and molecular formula C₃₀H₅₅O. The IR spectrum exhibits absorption bands at 3400 cm -1 for (OH group), and at 1109 cm -1 for (C-O) groups. In the H- NMR data the peaks at delta 1.03 and 1.19 shows the presence of methylene groups and the peaks at delta 2.08, 2.49 may be due to methylene protons present adjacent to double bonds. Further in the 13 C – NMR spectrum only 5 peaks are given at 119.53, 129.29, 130.75, 134.45, 154.64 shows the presence of at least 6 unsaturated carbon atoms an hydroxyl group was attached. The compound isolated FT1 from leaf callus of F. trinervia have closely related structure of (8E, 10E, 12 Z ) –Triconta – 8,10,12 – trien 13 ol belong to terpenoids as analysed by spectral analysis.

In the present era, medicinal herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization (Vogel, 1991). Although a significant number of studies have used to obtain purified plant chemical, very few screening programmes have been initiated on crude plant materials. It has also been widely observed and accepted that the medicinal values of plants lies in the bioactive phyto
components present in the plants (Veermuthu et al., 2006). Scientists have realized an immense potential in natural products from medicinal plants to serve as alternate source of combating infections in human beings which may also be of lower cost and lesser toxicity. This investigation has opened up the possibility of the use of this plant in drug development for human consumption possible for the treatment of Jaundice and wound infections.