Chapter 6

CONCLUSION

6. CONCLUSION

The undeniable significance of ethnomedicine in the primary healthcare system as well as its importance in the development of naturally occurring drugs has led to the massive increase in research on traditional herbal medicines. Sikkim being one of the less populated states of India with a rich flora and fauna, there is a high existence of ethnomedicinal practices. This study has covered the west part of the state i.e. the West district of Sikkim as it was the most neglected area in terms of survey and documentation of the traditional herbal medicine. This study covered the documentation and data collection of the traditional knowledge of herbal medicines practiced by the herbal healers of the area followed by the preliminary studies regarding standardization along with the estimation of bioactive phytochemicals present in them. It also assessed some of the pharmacological activities of the collected herbal formulations and eventually identification of the bioactive compounds responsible for the bioactivity was carried out. From the overall study and the results recorded, following conclusions could be drawn or could be considered important.

i. The traditional practices of herbal medicines in the West district of Sikkim is slowly depleting as the younger generations are least interested in learning the knowledge from their elders and also due to the availability of modern medicines. A comprehensive documentation as well as conservation strategies of traditional knowledge is required.
ii. Amongst 46 herbal formulations collected, 11 were selected for further analysis and 8 of them were finalized for the preliminary studies. The pharmacognostic study based basic standardization was established for the 11 herbal formulations.
iii. Out of 11 fomulations, 9 of them showed potential pharmacological activities and phytochemical content.
iv. The bark of *Fraxinus floribunda* which was used for the treatment of diabetes stood out from the rest of the formulations by exhibiting overall highest activities in the assays that were performed.
v. *F. floribunda* bark showed potent anti-inflammatory, anti-diabetic and hepatoprotective activity also in animal model.

SUMMARY

Ethnomedicine has been a vital part of human civilization. It has been a boon to primary healthcare specially in the rural sector where the modern facility could not reach. Ethnomedicine has not only contributed in the rural healthcare but it has also contributed in the development of naturally occurring modern drugs. Therefore, the documentation of traditional knowledge of herbal medicine is of high priority in the present scenario as there is a high possibility of depletion of this valuable knowledge due to various factors but the major factor being the lack of interest of the young generation in acquiring this knowledge from their elders. Other factors are the domination of modern medicine in the present world as well as due to the less scope of traditional medicine as a source of income.

Sikkim is a state of India where many ethnic communities still reside and practice the traditional system of healthcare. Studies have suggested existence of numerous medicinal plants and herbal formulation in the state. This study has focused on the collection of data regarding herbal medicines from the traditional practitioners from the West district of Sikkim since this district has been neglected as compared to the rest of the districts. From the survey, about 46 herbal formulations were collected which were prepared from 36 different plants. These plants were identified and authenticated in the NBU Herbarium and recorded. The survey data has revealed that most of the herbal practitioners were above the age of 50 years old and by the end of the completion of this work, it was found that 2 of the aged healers passed away due to old age health issues. Younger generations were not keen on learning this knowledge of herbal medicine from their elders.

From all the herbal formulations collected, 11 were selected on the basis of effectiveness and availability. A basic standard was established for all the same for their quality control and authentication on the basis of pharmacognostic studies such as organoleptic tests, powder microscopy, fluorescence analysis, physicochemical study and thin layer chromatography. Further preliminary phytochemical estimation was carried out on all the formulations after extracting them in 10 different solvent on the basis of their polarity. On the basis of this test, 9 formulations were subjected to further pharmacological analysis. Free radical scavenging activity was carried out on the basis of DPPH, ABTS scavenging activity along with metal chelating and

reducing powder assays. Almost all the extracts showed a potential activity with some of the solvent extracts showing extremely impressive bioactivity.

The *in vitro* pharmacological activity such as anti-hypertensive activity (angiotensin coveting enzyme inhibition, antidiabetic activity (α-glucosidase inhibition), cytotoxicty (human liver cell line, WRL-68) were also performed. Cytotoxic activity was average while very potential antidiabetic activities were observed from the extracts. From the overall study, bark of *Fraxinus floribunda* (BOFF) was selected for *in vivo* studies and purification depending on the overall highest activity exhibited by the same. Traditionally, BOFF was boiled in water and the water consumed for the treatment of diabetes. Before heading for further analysis, BOFF was subjected to four different extraction methods so as to compare their activity with each other and to find out the best extraction process for the same for the extraction of bioactive compounds. Four different methods of extraction were, normal boiling through reflux, soxhletion, autoclave pressure boiling and cold percolation. Antioxidant, quantitative phytochemical estimation and *in vitro* antidiabetic activity were performed on these extracts. The result of this showed that autoclave boiling is the best extraction method for BOFF for the bioactivity. Hence, the extract obtained through autoclave boiling was subjected to *in vivo* anti-inflammatory, hepatoprotective and antidiabetic assays. Anti-inflammatory activity was done with the rat paw oedema method and the result showed that the extract of BOFF significantly reduced the paw oedema with 55% percentage inhibition which was very close to the standard drug aspirin that showed 59% inhibition. Hepatoactivity was performed on the CCl_4 induced hepatic rats and the result was taken on the basis of biochemical parameters of liver such as SGOT, SGPT, ALP and bilirubin levels. The reduction of these in hepatic rats by the extract of BOFF showed its hepatoprotective activity. Similarly antidiabetic assay was also performed on the streptozoticin-induced diabetic rats. The parameters such as changes in body weight, oral glucose tolerance test, fasting blood glucose level, lipid profile and liver histopathology of diabetic rats were considered for the antidiabetic activity. The overall result revealed that the extract of BOFF at 400mg/kg b.w. exhibited a potential antidiabetic activity and was also able to reduce or repair the hepatic injury in diabetic rats.

The extract of BOFF was run for bioassay guided partial purification through column chromatography followed by GC-MS. The result of GC-MS showed the presence of 10 different compounds from the NIST library. The compounds with extablised bioactivity were flavones (antidiabetic, antimicrobial, anti-inflammatory, carcinogenic etc), Coumarin, 6-amino-3-phenyl (antibacterial, anti-neoplastic, anti-inflammatory, anti cancer), acacetin (anti cancer, antimicrobial) and some fatty acids such as Heptadecanoic acid, 10-Octadecenoic acid, methyl ester has antimicrobial activity. The most abundant compound revealed in the GC-MS study was 2(1H)-Quinolinone, hydrazone also known as 2-hydrazinoquinoline with 25.7 % in the extract. Various biological activities such as antithrombotic, anti-inflammatory, antidiabetic were observed in its derivatives. Some of the other derivatives were also helpful in the pathogenesis of circulatory disorders and arteriosclerosis. On the basis of its abundance and its biological activities, the extract used of GC-MS was futher purified through TLC and antidiabetic assay was performed on the TLC separated extracts. The separated extracts were subjected to H1 NMR analysis for the identification of the bioactive compound. On the NMR analysis it was revealed that there is a presence of 2-hydrazinoquinoline in the BOFF extract which could be the possible compound responsible for its bioactivity. Our study also supports the use of BOFF as an anti-diabetic agent in traditional system of medicine.