CHAPTER 10

SUMMARY AND CONCLUSION

The major aim of the investigation was to explore two popular ethnomedicines used by traditional healer of Sikkim in their health care system since time immemorial. The medicinal plants used by the ethnic community of Sikkim are neither systemically documented nor properly tested by scientific approaches. The systemic scientific studies of these ethnomedicines can lead to promising phytochemicals (drugs) for many health problems. In view of the above, it was thought to be much worthy to take up the present research project i.e. "chemical and pharmacological evaluation of *Colebrookea oppositifolia* Smith and *Heracleum nepalense* D.Don DC" so as to explore the therapeutic values and development of some novel drugs from those indigenous plants.

The Chapter 1 of this thesis deals with the importance and development of herbal medicine in the present scenario. Herbal medicines include herbs, herbal preparations and finished herbal products that contain active ingredients, parts of plant, or other materials, or their combination. Traditional use of herbal medicines refers to the long historical use of these medicines. Their use is well established and widely acknowledged to be safe and effective, and accepted by national authorities. They contain pharmacologically active compounds. Furthermore, sometimes these are recommended as dietary supplements or valuable nutraceuticals. The actions of herbal preparation are often different from those of pharmaceuticals containing isolated single compound of the original plant preparation. The ultimate objective in drug discovery and drug development should be the production of safe and effective remedies. In the herbal preparations the molecules accompanying the active compound may help in stabilization, reduced toxicity or enhance the therapeutic value of active component. Further, different approaches used for the development of drug from natural sources, methods of drug evaluation, scope and aim of phytomedicine in the near future has thoroughly been discussed in this chapter.

In Chapter 2, various reports on phytochemical and pharmacological analysis of *C.oppositifolia* and *H.nepalense* have been presented. The current updated status of natural origin used as bioavailability enhancer, immunostimulators, antioxidant and antimicrobials have been elaborated in the form of comprehensive review of literature. Though some reports on phytochemical and pharmacological analysis of *C.oppositifolia*

and *H.nepalense* exist in the literature, the present study revealed the presence of additional constituents with significant pharmacological activities.

In Chapter 3, the modern methodologies for extraction and isolation of bioactive compounds from *C.oppositifolia* and *H.nepalense* along with phytochemical group tests have been presented. The isolated bioactive compounds were analysed by using physical spectroscopic methods like UV, IR, ^{1}HNMR, ^{13}CNMR, Mass and melting point determination etc. The evidence presented conclusively proved that the isolated bioactive compounds isolated were (+) – catechin-7-O-β-rhamnopyranoside from *C.oppositifolia* leaf and quercetin-3-O-β-D-glucopyranoside from *H.nepalense* root. The isolations bear much significance in the present context because both the isolated phytoconstituents have not been reported from the plants under the study till date.

In Chapter 4, toxicological investigation of methanol extract of *C.oppositifolia* leaf and *H.nepalense* root are described. The MLD of methanol extract of *C.oppositifolia* leaf was found to be 3.0 g/kg body weight and 4.5 g/kg body weight in intraperitoneal and oral route respectively. On the other hand the MLD of the methanol extract of *H.nepalense* root was to be 4.5 g/kg body weight and more than 5.5 g/kg body weight in intraperitoneal and oral route respectively. The MLD values of both the extracts were found to be much higher indicating the wide safety range of action.

Chapter 5 deals with the effects of methanol extract of *C.oppositifolia* and *H.nepalense* root and their isolated compound on bioavailability of antibiotics like amoxycillin, cefixime and rifampicin. A sensitive, rapid and precise High Performance Liquid Chromatographic (HPLC) method was used to measure the concentrations of antibiotics in plasma samples collected for 24 h following different administration of drugs in rabbits. Pharmacokinetic parameters, including peak plasma concentration (C_{max}), time to reach peak concentration (t_{max}), area under curve (AUC), plasma half-life ($t_{1/2}$) elimination rate constant (k_{el}), relative bioavailability (RB %) were calculated following the one compartment open model. When *C.oppositifolia* was either coadministered or preadministered with amoxycillin, and rifampicin, C_{max}, AUC, $t_{1/2}$, RB % of amoxycillin

and rifampicin were increased significantly than that of the control used. But the preadministration or coadministration of the extract with cefixime failed to show any change in all the pharmacokinetic parameters. Further study with compound I at dose of 50 mg/kg body weight had significantly increased the bioavailability of amoxycillin and rifampicin. However, preadministration of compound I 30 min before the administration of cefixime increases all the pharmacokinetic parameters of the antibiotic.

The effects of the methanol root extract of *H.nepalense* and compound II were also studied for their effect on bioavailability of amoxycillin, cefixime and rifampicin in rabbits. The results revealed that the methanol extract failed to produce any effect on the bioavailability of all the three antibiotics. However the coadministration of compound II has moderately increased the bioavailability of these drugs.

In conclusion, the presence of flavonoid could be attributed to the enhancement of bioavailability of these antibiotics. However, the role of microsomal enzyme systems and efflux pump inhibitor P-glycoprotein (Pgp) cannot be ruled out. Based on the results acquired from the rabbit model it is proposed that further *in vitro* and *in vivo* studies are required with Caco-2 cell, isolated microsomal enzymes and by using human volunteers. If these studies show positive results of enhancement of bioavailability of antibiotics by *C.oppositifolia* and *H.nepalense* along with their compounds will be a great breakthrough in the field of bioenhancers and its application in pharmaceutical industry and therapeutics.

In Chapter 6, immunostimulatory activity of methanol extract of *C.oppositifolia* leaf as well as compound I has been presented. The immunostimulatory potential was investigated by *in vitro,* phagocytic index and lymphocyte viability tests, using interferon-α-2b, a known immunostimulant drug, as standard. Other tests such as carbon clearance, antibody titer and delayed type hypersensitivity were studied in mice, using levamisole as the standard. The results revealed that the leaf extract at higher dose (1000 mg/kg) moderately increased the rate of carbon clearance, humoral antibody titer and delayed type hypersensitivity in mice. The extract also showed moderate increase in

phagocytic index at higher concentration (1000 µg/ml), but failed to produce any stimulation of PHA activated mononuclear cells.

The immunostimulatory activity of the methanol root extract of *H.nepalense* and compound II also has been studied by different *in vitro* and *in vivo* test models. To investigate immunostimulatory potential, phagocytic index and lymphocyte viability tests were performed *in vitro* using interferon-α-2b, a known immunostimulant drug as standard while *in vivo* carbon clearance, antibody titer and delayed type hypersensitivity parameters were studied in mice using levamisole as standard drug. The results demonstrated that: (1) at a concentration of 1000 µg/ml dried root extract and isolated quercetin glycoside at 50 µg/ml increased significantly i*n vitro* phagocytic index and lymphocyte viability in all assays. (2) The root extract at oral doses of 1000 mg/kg and the isolated quercetin glycoside at 50 mg/kg showed a significant increase of *in vivo* antibody titer, carbon clearance and delayed type hypersensitivity response in mice. The immunostimulatory effect was pronounced in dose dependent manner.

In conclusion, the immunostimulant effect of *C.oppositifolia* leaf and *H.nepalense* root could be attributed to the flavonoid content or due to the combination with other component (s). The present finding provides scientific evidence to the ethnomedicinal use of this plant by the tribal group of Sikkimese as antimicrobial and aphrodisiac. The plant *H.nepalense* may thus find new therapeutic application in the future as immunostimulant.

In Chapter 7, antioxidant activities of methanol extract of *C.oppositifolia* and *H.nepalense* root as well as compound I and II has been presented. The antioxidant activity was studied by *in vitro* ferrous sulphate induced lipid peroxidation DPPH (1,1-diphenyl –2 picryl hydrazyl) free radical, hydroxyl radical, superoxide radical scavenging assay using vitamin E (5mM) and mannitol (50 mM) as standard drugs. The percentages of inhibitions were calculated as compared with standard drugs. It was observed that the methanol extracts of both the plants exhibited a considerable inhibition of lipid peroxidation and possessed DPPH radical, hydroxyl radical and superoxide radical scavenging activity. The percentage of inhibition was on a concentration dependent

manner in all the models. Further study with compound I and compound II at 25 and 50 µg/ml concentration showed significant antioxidant effects in concentration dependent manner in all the models tested. In conclusion, the presence of flavonoid isolated from the plants could responsible for observed antioxidant activity.

In Chapter 8, antimicrobial activity of methanol extract of *C.oppositifolia* leaf and compound I has been studied both *in vitro* and *in vivo* model system. The *in vitro* test was carried out by agar dilution and disc diffusion method. The *in vivo* study was performed by determining the protection offered to Swiss albino mice against the virulent strain of *Salmonella typhimurium* NCTC 74 and the mode of actions were determined in Oxoid brand nutrient broth at 2, 4, 8 and 18 h. The crude methanol extract of *C.oppositifolia* leaf was found to be active against 257 bacterial isolates comprising of 12 genera of both Gram-positive and Gram-negative organisms including multiresistant *Staphylococcus* (MRSC) strains. The minimum inhibitory concentration (MIC) ranges were found to be from 128-1000 µg/ml for most of the bacteria. The endogenous cfu counts in mouse spleen, liver homogenate and heart blood on 18th post-bacterial challenge hour with crude methanol extract demonstrated significant antibacterial effect. Inhibitory activity was based on bactericidal action and viable cell number reduced significantly after 18h incubation with the extract. The bactericidal activity was observed at the MIC value. Compound I showed similar activity against *Escherichia coli*, *Staphylococcus spp*, *Salmonella spp* and *Vibrio spp* including three MRSC, most of them being inhibited at 128 µg/ml concentration of the agent.

The antimicrobial activity of *H.nepalense* root and compound II were also studied by *in vitro* and *in vivo* model system. These are found to be active against several pathogenic strains of *Escherichia coli*, *Klebsiella spp*, *Shigella spp*, *Citrobacter spp*, *Pseudo aeruginosa*, *Bacillus spp*, *Streptococcus spp*, *Staphylococcus spp*, *Salmonella spp* and *Vibrio spp* including three MRSC strains. The minimum inhibitory concentration (MIC) ranges from 128-512 µg/ml for most of the bacteria. The endogenous cfu counts in mouse spleen, liver homogenate and heart blood on 18th post-bacterial challenge hour with crude methanol extract demonstrated significant antibacterial effect. Inhibitory activity was

based on bactericidal action and viable cell number reduced significantly after 18h of incubation with the extract. The bactericidal activity was observed at the MIC value. Compound II showed similar activity against *Escherichia coli*, *Staphylococcus spp*, *Salmonella spp* and *Vibrio spp* including three MRSC, most of them being inhibited at 128 μg/ml concentration of compound II. The study confirms the possible antimicrobial potentiality of *C.oppositifolia* and *H.nepalense*. The present finding provides scientific evidence to the ethnomedicinal use of this plant by the tribal group of Sikkimese in skin infection and diarrhoea. However, further studies are required with different isolates of fungus to confirm the spectrum of antimicrobial activity.

All the experiments were performed both by using *in vitro* and *in vivo* model to established that the plants of the Himalayan region of Sikkim investigated provide valuable proof of use of these plants by the people of this region as aids in their health care system in form of indigenous ethnomedicines since time immemorial. In the context of combinatorial chemistry and phytomedicine and herbal preparations (wherein large numbers of compounds are present as a mixture) may lead to the development of novel drugs of 21st century. Many drug discoveries resulted the key components and lead molecules, which are identified as fingerprints obtained from the studies of herbal medicines as well as ethnomedicines. The studies undertaken in this thesis was also an attempt towards the search of such lead molecules from the plant drugs, which could contribute a little in the development of some newer molecules, having therapeutic value. Most of the work presented in this thesis has been authenticated with concomitant publications in national and international journals.