Chapter – 8

SUMMARY & CONCLUSION

Herbal medicine or Phytotherapy has a significant role in health care system. Now a days Indian pharmaceutical industries have focused their research and development on plant based drug discovery. Government of India has approved and established many alternative systems of medicines and organizations to encourage the practice of indigenous plant products for different ailments. Cancer is one of the deadly diseases next to cardiovascular disease causing maximum mortality and morbidity in large number of people. There is no satisfactory therapy for cancer due to less efficacy and more adverse drug reaction of the chemotherapeutic agents. The thirst area in the drug discovery for cancer is focused towards finding of lead drug molecule from the natural products. Sikkim is a state in the Sub-Himalayan region highly blessed with large number of valuable medicinal plants. The people of Sikkim believe on traditional medical practice, especially ethno-medical practice. The main objective of this research work based on the exploring of medicinal plants and their phytoconstituents for cancer treatment. The present studies evident that drugs with anticancer activity may have anti-inflammatory activity and vice versa. Therefore, it was thought to be worthy to isolate some phytoconstituents and screen them for their possible anticancer activities in a research program entitled ***"Evaluation of anti-inflammatory and anticancer activities of some plants of Sub-Himalayan region of Sikkim"***

Chapter-1 of this thesis dealt with the importance of herbal medicine and the practice of medicinal plants by the people of Sikkim. Inflammation and cancer epidemiology, etiology, types and treatments were discussed in elaborate manner. Special emphasis was given to the medicinal plants and their phytoconstituents used in the treatment of inflammation and cancer. Drugs used in the treatment of inflammation, targets enzymes like COX and LOX in the arachidonic acid pathway was thoroughly discussed. Dual inhibitors (both COX and LOX) found to be safe compared to non-selective NSAIDs and selective COX-2 inhibitors. Apoptosis pathway, a leading mechanism by which most of the chemotherapeutic agents act on cancer cells has been reported. Recent studies revealed the cross link between inflammation and cancer, which has been reproduced in this introductory chapter.

In the **Chapter-2** the selected plants *Bischofia javanica and Fraxinus floribunda* were reviewed for their distribution, traditional uses, and phytoconstituents which have been already reported. The different research works on the topic of anti-inflammatory activity of plant products was discussed with references. Here a special focus was given to the carrageenan induced paw edema, cotton pellet induced granuloma, Freund's adjuvant induced arthritis and arachidonic acid induced paw edema in rats. The literatures with anti-nociceptive and antipyretic studies were also discussed in brief manner under this section. The research works on the topic of anticancer activity of plant products with special emphasis to the apoptosis pathway was discussed in detail. The research articles dealing with *in vitro* antioxidant activities have also been thoroughly discussed.

Chapter 3 dealt with phytochemical studies of *B.javanica* and *F.floribunda*. Here the methanol extracts of both the plant were fractionated with petroleum ether, chloroform and water. The chloroform fraction was undertaken for the isolation purpose of triterpenoids using coloumn chromatography. In the separation process of the phytoconstituents silica gel was used as stationary phase and the combination of n-hexane: ethyl acetate in different ratio was used as mobile phase. The fractions obtained from the coloumn chromatography were studied by TLC using the Liebermann-Burchard spray reagent for confirmation of triterpenoids. The fractions from the first coloumn chromatography showing pharmacological activity was further purified to obtain a single component by eluting in smaller size coloumn using silica gel as stationary phase and n-hexane: chloroform: ethylacetate ratios as a mobile phase. The isolated and purified compounds of both the plants were subjected to structure elucidation with the help of IR, ^{1}H, ^{13}C NMR and DEPT-90, DEPT-135, ES-MS studies. The spectral data interpretation revealed that the isolated compound, LS-1 from *B.javanica* was **Friedelin-3α- acetate** (FA) and compound, LS-2 from *F.floribunda* was **β-amyrin.**

In the **Chapter 4** toxicity and anti-inflammatory studies of the methanol extracts of the leaves of *B.javanica* (BJ), *F.floribunda* (FF) and their isolated compounds were described with their experimental work on animals. Toxicity studies revealed that LD_{50} of methanol extract of leaves of *B.javanica* was 3400mg/kg b.w and for *F.floribunda* it was

2400mg/kg b.w. In carrageenan induced paw edema model FF at 400mg/kg b.w.p.o and its isolated compound β-amyrin at 20mg/kg b.w.p.o inhibited edema better than BJ and its isolated compound, FA. In cotton-pellet induced granuloma FA gave better protection than the β-amyrin. In chronic model of Freund's adjuvant induced arthritis BJ and its isolated compound, FA provided more protection from arthritis than FF. In the study of dual inhibition by the extract, arachidonic acid induced paw edema was reduced in a greater way by the BJ than that of the isolated compound, FA, also the FF and its isolated compound β-amyrin did not inhibit arachidonic acid induced paw edema in significant manner. It is concluded that the methanol extract of *B.javanica* follows dual inhibition (both LOX and COX) than the other drugs i.e. FA, FF and β-amyrin studied. In antinociceptive studies BJ and FF inhibited thermal (Tail-immersion test) and chemical induced (Writhing test with 1% acetic acid) nociception in significant way. The studies revealed that both central and peripheral mechanism exist on anti-nociceptive activity. BJ at the dose of 400, 600 mg/kg b.w p.o, FF at the dose of 200, 400mg/kg b.w p.o and their isolated compounds at the doses of 10 and 20 mg/kg b.w.p.o showed significant reduction in Brewer's yeast induced elevated body temperature.

Chapter 5 described about the anticancer activities of leaves of the two plants. The methanol extract of *Bischofia javanica* (BJ) and *Fraxinus floribunda* (FF) at the dose of 5, 10, 15μg/ml and their isolated compounds, Friedelin 3α-acetate (FA) and β-amyrin at the dose of 5,10μg/ml were studied for cell viability, cytotoxicity (MTT assay), DNA fragmentation assay, fluorescent and confocal microscopic studies. The studies revealed that BJ and FA showed dose-dependent cytotoxicity. The pathway of apoptosis mediated anticancer activity was confirmed in DNA fragmentation assay by ladder formation for BJ at 10μg/ml. FF and its isolated compound β-amyrin have not shown the cytotoxicity activity in significant manner.

In the **Chapter 6** antioxidant activities of BJ and FF along with their isolated compounds by DPPH radical scavenging assay, lipid peroxidation assay and hydroxyl radical scavenging assays were described. The antioxidant activity was sensitive in the concentration range of test drugs at 20, 40, 80, 160 and 320μg/ml. DPPH radical was

scavenged by BJ in an effective way (IC_{50}-118.29µg/ml) compared to other compounds. In lipid peroxidation assay, β-amyrin (IC_{50}-137.92µg/ml) showed better action compared to other test compounds like BJ, FF and FA. In hydroxyl radical scavenging assay FF and its isolated compound showed better protection than the BJ.

On basis of the ethno-medicinal utility Bischofia javanica and Fraxinus floribunda were selected for this study. The phytochemical analysis leads to the isolation of ***Friedelin-3α-acetate*** *from* ***Bischofia javanica*** *and* ***β-amyrin*** *from* ***Fraxinus floribunda*** *for the first time. The anti-inflammatory studies concluded that methanol extract of both the plants and their isolated compounds significantly inhibited inflammation in acute, sub acute and chronic models. The mode of action of methanol extract of B.javanica follows dual inhibition (both COX and LOX). Anticancer studies revealed that BJ has significant anticancer activity and that follows apoptotic pathway. Antioxidant studies derived on an idea that all the test drugs have significant activity against free radicals. Finally it has been concluded that methanol extract of B.javanica has significant anti-inflammatory and anticancer activities that follows dual inhibition and apoptotic pathways.*

The foregoing studies have confirmed the safe use of the methanol extract of the leaves of the plants *B.javanica* and *F.floribunda* in inflammation and cancer and opened a scope for incorporation of these leaf extracts of these plants in designing a suitable dosage form for proper delivery of these phytoconstituents in such ailments.