CHAPTER 8

Summary and Conclusion

Cancer has emerged as a second leading cause of disease related death worldwide and since the existing therapies are inadequate to control this disease, pharmacological intervention by using naturally occurring non-toxic chemicals gained impetus in recent past. Due to continuous efforts over years, numbers of phytochemicals with chemopreventive and anticancer potentials have been isolated from plant sources, but the main problem with these phytochemicals are the associated toxicity and lack of specificity. Therefore, identification of novel phytochemical with chemopreventive and anticancer potential has gained impetus in recent past. With this background, in the present study, attempts was made to identify medicinal plant based on traditional and indigenous knowledge from the north eastern region of India. The works also aimed to validate and elucidate the ethno-claimed activity in experimental cancer models along with identification of active principle responsible thereof. The findings of present investigation are summarized as follows:

- Three medicinal plants were selected based on the traditional and indigenous knowledge from the plethora of plant diversity of north eastern region of India. The selected plants are *Nyctanthes arbor-tristis* Linn., *Phlogacanthus tubiflorus* Nees. and *Phlogacanthus thyrsiflorus* Nees.

- The root extract of *Phlogacanthus tubiflorus* Nees. (REPT) and flower extract of *Nyctanthes arbor-tristis* (FENA) exhibited significant protective functions against oxidative stress induced by H_2O_2 in the lymphocytes cultured *in vitro*. The biochemical mechanistic study indicated significant modulation in the activities/levels of xenobiotic metabolism enzymes, antioxidants and reactive species NO are involved in protective function.

- The FENA and LEPT were found to be prominent in directly scavenging the free radicals *viz.* ABTS, DPPH, superoxide, hydroxyl and inhibited the Fe^{2+} chelation activity in in vitro cell free chemical based reactions. The FENA and LEPT also exhibited reducing potentials of Fe^{3+} to Fe^{2+}.

- Quantitative phytochemical analysis revealed presence of total phenolic content 71.2 ± 0.6 and 19.8 ± 2.0 µg gallic acid equivalent (GAE) per mg FENA and LEPT respectively. The total flavonoid content in the FENA and LEPT were found to be 520.9 ± 16.2 and 220.2 ± 11.3 µg Epicatechin equivalent (ECE) per mg extract respectively.

- The oral feeding of FENA and LEPT caused significant increased the phase II xenobiotic metabolism enzymes, antioxidants and decreased the phase I xenobiotic metabolism enzymes along with toxicity related parameters in hepatic and extra hepatic tissues in *Swiss albino* mice.

- The FENA and LEPT inhibited the incidence and multiplicity of papilloma as well as papilloma yield and burden against DMBA induced and croton oil promoted skin papillomagenesis in *Swiss albino* mice. The chemopreventive efficacy of FENA and LEPT had strong correlation with differential modulation of enzymes of xenobiotic metabolism, antioxidants and reactive species in hepatic, extra hepatic as well as in papilloma bearing tissues.

- The FENA and LEPT also inhibited the multiplicity of papilloma as well as papilloma yield and burden against Benzo(a)Pyrene induced forestomach papillomagenesis in *Swiss albino* mice. There were evidences of significantly differential modulation in the activities/level of enzymes involved in xenobiotic metabolism, antioxidants and reactive species in hepatic, extra hepatic as well as in papilloma bearing tissues.

- Analysis of phytochemical content by GC-MS revealed the presence of 14 and 42 Nos. of phytochemicals in FENA and LEPT respectively. The prevailing compounds in FENA were Dodecanoic acid, 3-hydroxy- (26.41%), 2,5-Methano-furo[3,2-b]pyran, hexahydro (13.56%), Oleic acid acid (8.68%), 9-Oxa-bicyclo[3.3.1]nonane-1,4-diol (8.59%) and 9,12-Octadecadienoic acid, ethyl ester (8.54%) that constituted about 66% of total phytoconstituents in

FENA. The phytol (31.04%), n-Hexadecanoic acid (22.53%), Cyclopentaneundecanoic acid (20.04%) and Oleic acid (19.61%) are the major constituents of LEPT as ascertained from GC-MS analysis. ADME/Tox analysis validated these phytochemicals as druggable candidate.

- The free flavonoid fraction of *Nyctanthes arbor-tristis* (F^3NAF) was found to be most active fraction as it exhibited strong antiprolifeartive activity against human lung cancer (H1299 and A549) and prostate cancer cell lines (PC3 and LNCap). However, the inhibition of growth and proliferation by F^3NAF was more promising against prostate cancer cell lines used in this study.

- The treatment of F^3NAF to PC3 cells resulted arrest of cell cycle progression at G1 and G2 phase as evident from the flow cytometry analysis. The immunoblotting study indicated that the down regulation in the expression level of regulatory protein involved in G1 to S phase of cell cycle transition (CDK4, CDK6, cyclin D1, cyclin D3, cyclin E) and G2 to M transition (cdc25c and cdc2) could have played role in observed activity of F^3NAF in PC3.

- The F^3NAF remarkably inhibited multiple signaling molecules involved in growth, proliferation and survival. Phosphorylation of EGFR (Tyr1173), Akt (Ser473), p44/42 MAPK(ERK1/2) (Thr202/Tyr204), mTOR (Ser2448) were found to be downregulated along with rictor, as evident from the western blotting analysis, suggesting their probable role in inhibition of growth and prolifeartion by (F^3NAF) in human prostate cancer PC3 cells.

- Additionally, F^3NAF caused significant inhibition of migration and invasion of PC3 as evident from the wound healing and cell invasion assays. The treatment of F^3NAF inhibited the expression of level of MMP2 and MMP9 in mitomycin C treated quiescent PC3 cells, clearly demonstrated that inhibition of migration and invasion is through MMPs downregulation.

Further studies are warranted using different site specific animal models of carcinogenesis in order to establish the chemopreventive and anticancer efficacy of flower extract of *Nyctanthes arbor-tristis* (FENA) and leaf extract of *Phlogacanthus thyrsiflorus* Nees. From the findings of the present investigation, we conclude that the anticancer efficacy of free flavonoid fraction of *Nyctanthes arbor-tristis* (F^3NAF) against human prostate cancer is through inhibition of regulators of cell cycle and signaling component of cell proliferation. There is also need to identify and characterize the specific flavonoids responsible for inhibition of proliferation of prostate cancer cells and to precisely understand the molecular mechanisms using gene knock out/xenograft cancer models.

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