CHAPTER 5

CONCLUSION

The fruitful investigations in the domain of Programmed Cell Death constantly adds new protein targets to the list of molecules involved in chemo-cytotoxic drugs. The diminishing pipeline of new molecules has become a limiting factor in drug discovery programme. Many pharmaceutical industries are constantly trying to obtain new molecules that may be more potent and less toxic than the existing drugs. Nature is the best source of material to embark upon the development of new chemical entities. High speed technologies and molecular biological approaches have made this possible. The aim of the present study was to investigate the biological properties of traditional Indian plants leading to the mechanism of their action, identification of active components and eventually development of new drugs, which can be promoted either as alternate or complementary medicines. In this study the knowledge of chemistry, cell biology, molecular biology and biotechnology was used to isolate and characterize the biologically active molecule from *Piper longum* which killed or arrested the growth of MDA-MB-231 Breast cancer cells and PC-3 Prostate cancer cells.

Bioassay-guided fractionation led to the isolation of pure compound. Their molecular structure was elucidated and the compound was chemically identified as 1-C 3’ 4’-Methylenedioxy phenyl dodec-1-en-10-one from *Piper longum*. Through a series of investigation of intracellular signalling cascades this study arrives at the mechanism of action of the extract and pure compound on MDA-MB-231 cells and PC-3 cells. The bioactivity based
screens used in this study involves measuring proliferation of cancer cells using $[^3]$H-Thymidine uptake and monitoring known key mediators of apoptotic pathways, namely Bcl-2, Bax, cytochrome c, caspases and growth factors at a secondary level, with a view to postulate the possible mechanism by which these extract and pure compound induce apoptosis leading to cell death.

The following aspects in the present study led to the conclusion that the ethyl acetate extract and pure compound of *Piper longum* induced apoptosis in MDA-MB-231 cells and PC-3 cells through a common mitochondria and caspase mediated pathway. In addition, G2/M phase of the MDA-MB-231 cells treated with ethyl acetate extract and pure compound from *Piper longum* was arrested. Parallely, generation of intracellular ROS was observed in PC-3 cells treated with MDPD isolated from *Piper longum*.

- Anti-proliferative study of extracts of *Piper longum, Cissuus quadrangularis*, and *Ficus racemosa* by $[^3]$H-Thymidine incorporation assay revealed that ethyl acetate extract of *Piper longum* possess higher inhibitory effect on MDA-MB-231 breast cancer cells and PC-3 prostate cancer cells.

- $[^3]$H-Thymidine incorporation assay performed in MDA-MB-231 cells and PC-3 cells treated with ethyl acetate extract and pure compound of *Piper longum* showed 50% of inhibition at a lower concentration compared to others.

- LDH release assay, DNA fragmentation assay, propidium iodide staining and flow cytometry analysis confirmed that the death of both MDA-MB-231 cells and PC-3 cells treated with ethyl acetate extract and pure compound of *Piper longum* was due to apoptosis and not due to necrosis.
Ethyl acetate extract and pure compound of *Piper longum* on treatment with MDA-MB-231 cells and PC-3, increased proapoptotic Bax expression and down regulated anti-apoptotic Bcl-2 thus regulating the apoptosis process in favor of cell death.

Ethyl acetate extract and pure compound of *Piper longum* on treatment with MDA-MB-231 cells markedly down regulated G2/M proteins involved in the G2/M Phase of cell cycle.

Ethyl acetate extract and pure compound of *Piper longum* followed a closely similar kinetics in both PC-3 cells and MDA-MB-231 cells, in inducing cytochrome c release due to loss in mitochondrial membrane potential leading to activation of caspase 9 and caspase 3 leading to apoptotic cell death.

Activation of caspase 8 that paralleled the activation of caspase 9 at similar time points on exposure of PC-3 cells to the ethyl acetate extract and MDPD of *Piper longum* indicated a possible involvement of death receptor signalling in the process of PC-3 cell death.

The current thesis, thus examines the effects of the ethyl acetate extract and pure compounds isolated from *Piper longum* plant on various intracellular targets and confirmed their efficacy on proapoptotic activity and inhibitory effect on anti-apoptotic marker by providing interesting results. This study provides important baseline data regarding the biological properties of these medicinal plants reported for usage as traditional medicines for treating many disorders including cancers. However in the face of emerging the side effects caused by anticancer drugs, these molecules isolated from *piper longum*, demonstrated in this study for their apoptotic induction effect in breast cancer cells and prostate cancer cells can be further considered for *in vivo* studies to
develop them as potent plant based chemo preventive agents for the treatment of cancer with less adverse effects and low toxicity levels.

5.1 SCOPE FOR THE FUTURE STUDY

Drug discovery efforts to harness novel anticancer targets with potential to provide more selective and safe anticancer drugs have advanced significantly. There is much excitement in the cancer drug discovery field at the moment, and as the first generation of these new agents enter clinical trials it remains to be seen whether the present optimism will be confirmed. The present study involving *Piper longum* and its active molecule showed promising antiproliferative activity in various cell lines tested and induced apoptosis, which is a prerequisite for an ideal anticancer drug. Pharmaceutical companies are still tapping the natural source for an ideal molecule that could cure cancer and from that prospective the active compound from *Piper longum*, can be considered for further validation on various molecular targets and further animal studies and clinical trials thereafter, if found successful, could help in realizing the bench to bedside concept.