Chapter 6

Summary

The present study had postulated that the metabolites from selected medicinal plants with its profound cytotoxicity might possess the ability to specifically annihilate human breast cancer cells through caspase-3 mediated apoptosis.

The selected medicinal plants, *Oroxylum indicum* and *Rheum emodi* had yielded 20 crude extracts upon soxhlet extraction and cold maceration with petroleum ether, chloroform, ethyl acetate, methanol and water.

The cytotoxicity of all the 20 extracts was scrutinized initially on both tumoral (MDA-MB-231) and non-tumoral (WRL-68) cell lines. In MDA-MB-231 cells, all extracts (except ACO and AHO) have exhibited a significant (P<0.05) cytotoxicity, and in WRL-68 cells, all other extracts except CCO, CCR, PHR, CHR and MHR have shown significantly (P<0.05) lesser/no cytotoxicity.

The extracts were then ranked based on their IC\textsubscript{50} values in MDA-MB-231 and WRL-68 cells. Based on their cumulative ranking, MHO, PHO, ECO, MCO, EHR, AHR, CHR, PHR, ACR, CCR, PCR and MCR from *O. indicum* and *R. emodi* were selected for further analysis.

The extracts had unveiled significant (P<0.05) levels of apoptosis induction in MDA-MB-231 cells but not in MCF-7 cells (except ACR and AHR).

In accordance, extracts were again ordered with their mean OD values and the four top ranking extracts PHO, MCO, PHR and MCR were selected for caspase-3 activation analysis in MDA-MB-231 and MCF-7 cells.

MCO had exhibited significantly (P<0.05) high percentage caspase-3 activation followed by PHR, MCR and PHO in MDA-MB-231 cells, and the extracts
did not show any caspase-3 activation in MCF-7 cells, as it naturally lacks procaspase-3. Thus the most effectual extract, MCO was designated to elute its bioactive components.

- The isolation process employed TLC and column chromatography techniques to yield a total of 5 compounds. Bioactivities of which were again confirmed through a series of analysis.

- All the 5 compounds demonstrated significant cytotoxicity (P<0.05) on MDA-MB-231 cells. Based on their IC_{50} ranking, top 3 compounds C1, C3 and C4 were then tested for its apoptosis induction capability.

- All the 3 compounds had exhibited a significant (P<0.05) dose dependent increase of apoptotic DNA fragments in MDA-MB-231 cells. Moreover, C1, C3 and C4 had demonstrated significant (P<0.05) caspase-3 activation in MDA-MB-231 cells, of which; C4 had shown the highest percentage caspase-3 activation.

- Compound C4 was further characterized by UV-Visible spectrum, HPLC, MS, NMR and FTIR techniques and was finally identified to be Benzoic acid 2-ethyl-nonyl ester (C_{18}H_{28}O_{2}).

- The isolated compound, Benzoic acid 2-ethyl-nonyl ester was thus proved for its specific chemotherapeutic properties against human breast cancer cells and could potentially serve as a key in the development of anti-breast cancer drug.
Chapter 6

Conclusion

The study had stated an insight on the type of solvent extraction, where it proved that soxhlet extraction resulted in high yield of extracts and they exhibited high cytotoxicity when compared to macerated extracts. In their cytotoxic potential, the extracts were found to exhibit significantly (P<0.05) better activity against cancer cells in comparison with normal cells. While studying the apoptosis induction potential of the extracts, MCF-7 cells were found to be resistant to apoptosis when compared to MDA-MB-231 cells. More interestingly, extracts/compounds with high cytotoxicity have shown comparatively lesser caspase-3 activation potential and vice versa. Thus the study had incurred a point that cytotoxicity alone cannot confirm the anticancer activity of an extract, as the target specific activities varied with the test system studied. The study has productively yielded 5 compounds with specific chemopreventive properties against MDA-MB-231 cells. Benzoic acid 2-ethyl-nonyl ester, isolated from the methanolic cold extract of O. indicum was proved to be an efficient cytotoxin, which annihilated the cells through caspase-3 mediated apoptosis induction. The compound could thus possibly find an abode in designing therapeutic drugs against breast cancer. The present study had also derived a mechanism-based strategy to screen and identify anti-breast cancer metabolites from natural products.