Conclusion and future prospects

6.1. Conclusion

The demographics of breast cancer incidences in India as well as in the world is frightening and there is an urgent need to fight against this situation. Notwithstanding the presence of various anti-cancer treatment options available to patients, the incidences of breast cancer and related mortalities are increasing. Breast cancer is a top cause of cancer-related mortality in women in India. Lack of awareness, social taboo, insufficient facilities in the rural areas are a few reasons for late diagnosis of the disease. Breast cancer is one prime example of cancer where metastasis is prevalent and late diagnosis of the disease aggravates the problem of metastasis onset in many patients. Chemotherapy is one of the key strategies to combat breast cancer which is preferred often over other available options such as surgery, radiation or hormone therapy. Chemotherapeutic drugs from natural origin are very low in number; most of these drugs being synthetic in nature either cause multiple side-effects in patients or fails to work in various types of cancers. Anti-cancer compounds from natural origin may overcome this problem owing to their high abundance, low cost and non-toxic nature. Moreover, a well-characterized anti-metastatic drug is yet to occupy the market. The current study is aimed at finding plant-derived anti-cancer compounds with potential of inhibiting metastatic properties.

Three plants based on traditional knowledge for their medicinal value were collected from the state of Assam, India. Different parts of these plants were used for extraction in 50% ethanol and screened for their cytotoxic efficacy against two breast cancer cell lines, i.e. MCF-7 and MDA-MB-231. Two plant extracts viz *Ricinus communis* L. fruit extract (RCFE) and *Amorphophallus paeoniifolius* tuber extract (APTE) were selected for further studies and their *in vitro* antioxidant activity was assessed. As expected lower antioxidant activity in tubers extract was found as compared to the fruit extract which was comparable to standards. Two breast cancer cells frequently used in this study are MCF-7, a less metastatic, estrogen positive cell and MDA-MB-231, a highly metastatic triple-negative cell.

In cancer, cells demonstrate uncontrolled proliferation that is influenced by internal stimuli or environmental factors. These cells are capable of activating/ deactivating different molecular pathways or machineries as a result of alteration at genetic as well

as protein levels. A logical strategy to diminish the activities of cancer cells will be to target these pathways or signaling mechanisms.

Our study demonstrated the efficacy of the fruit extract of common castor plant *R. communis* L. against two breast cancer cells of different characteristics. The extract inhibited the aggressiveness of the cancer cells by inhibiting properties of metastasis such as cell motility, adhesion and invasion *in vitro* and reduced MMP-2 and 9 expressions in MCF-7 and MDA-MB-231 cells. Treatment with the extract induced apoptosis in the cells which were shown by increased Bax/Bcl-2 ratio in extract-treated cells. This ratio is known to induce caspases through release of cytochrome c and subsequent cleavage of PARP. The pathways that attenuated MMP-2 and 9 and induced Bcl-2 may be centrally regulated by STAT3 which was dephosphorylated by the extract. This extract inhibited tumor progression *in vivo* up to approximately 80% in syngeneic mouse model. The extract RCFE was effective against other breast cancer cells MDA-MB-453 and ZR-75-1 as well as cancer cells of different origin i.e. HT-29 colon cancer cell and A549 lung cancer cells. The extract showed robust anti-oxidant activity and HPLC fingerprint analysis followed by ESI-MS suggested presence of four compounds viz. ricinine, p-coumaric acid, epigallocatechin and ricinoleic acid that might be responsible for the anti-cancer character of the extract. The effect of the individual purified compounds was also studied. This study contributes to the ever-increasing repertoire of plant-derived therapeutic strategies for the treatment of breast cancer.

Anti-cancer activity of the other extract from the edible tuber of *A. paeoniifolius* (Dennst.) was studied against MCF-7 and MDA-MB-231 cells. Our result demonstrated that this extract (APTE) is a promising anticancer agent against the less metastatic MCF-7 cell line. It induces apoptosis in both cell lines in a caspase-7 dependent and p53 independent manner. The treatment with APTE induced Bax/Bcl-2 ratio and cleavage of PARP as a result of caspases activation. On the other hand, the extract was highly potent against aggressive breast cancer cell line MDA-MB-231. It induced apoptosis in these cells in similar mechanism as MCF-7, however its effect to prevent metastatic activities in the cells were significantly high. HR-LCMS analysis of the active fractions of this extract reveals twelve tentative compounds with well-documented anti-cancer properties. The anticancer effect of APTE in MCF-7 and

MDA-MB-231 cells may be attributed to the presence of these compounds which may exert their effect either individually or in combination.

We propose a model depicting the mechanism of anti-cancer efficacy of RCFE and APTE in breast cancer cells **(Fig 6.1.)**.

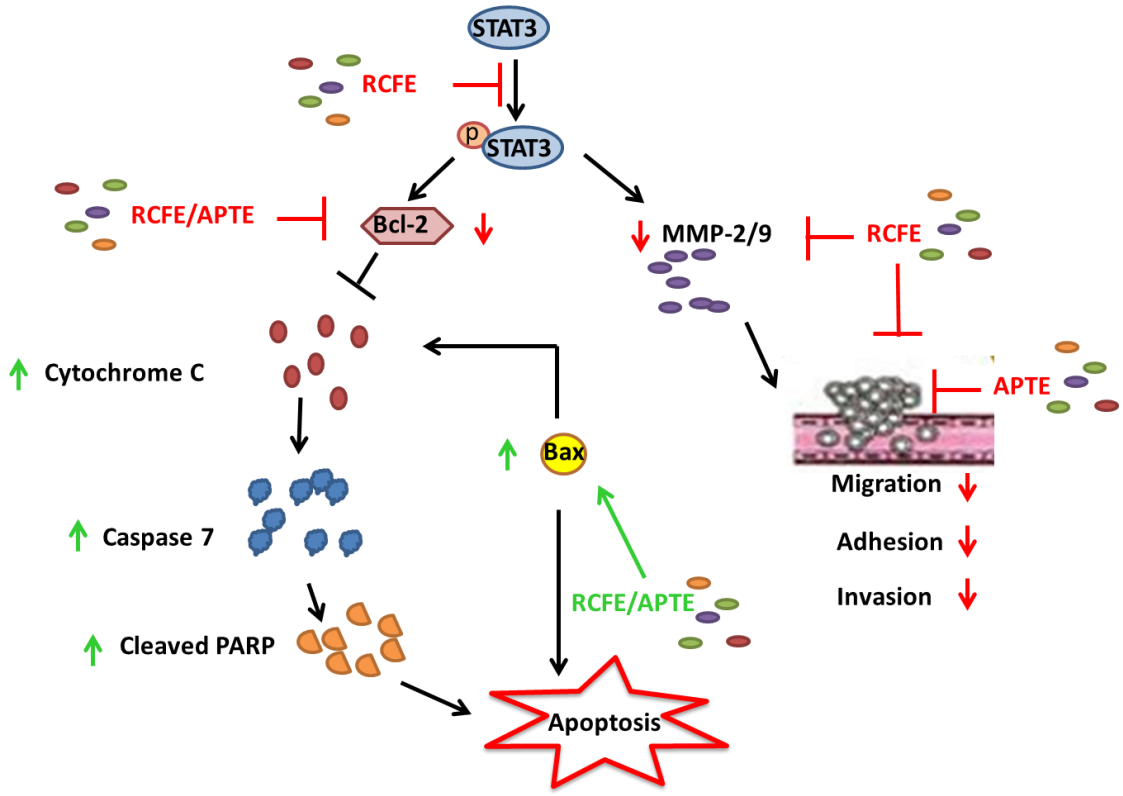

Figure 6.1.: Schematic representation of the effect of RCFE and APTE in MCF-7 and MDA-MB-231 cells

6.2. Future prospects

Based on the results of the current study, we propose the following areas that can be taken up for further studies

1. The anti-metastatic properties of the plant extracts have been studied using cell culture models. However, the efficacy of the extracts to prevent *in vivo* metastasis may be studied using well-characterized animal models.
2. The current study describes the effect of RCFE on diminishing tumor volume in the 4T1-injected syngeneic mouse model. A similar study can be performed to understand the *in vivo* efficacy of APTE.

3. The role of immune response elicited by the extracts on tumor volume reduction can also be studied in future.
4. Several bioactive compounds were identified from both these extracts. Clinical studies of active compounds identified from RCFE and APTE may be taken up for the development of anti-cancer drugs.