Chapter 7

Chapter 7
Summary and Conclusion

7.1 Summary:

Breast cancer is associated with high morbidity and mortality rates in women worldwide. In the recent decade, several evidences suggest the role of chronic inflammation in Breast cancer inception and progression. Inflammation provides a survival benefit to tumor cells in tumor microenvironment which is enriched with various tumor promoting factors including proinflammatory cytokines, like TNF- α. TNF- α is one of the key regulators of inflammatory response which plays important role in tumorigenesis by immune activation and evading cell death signals. TNF- α induced ROS generation favours the tumor growth and survival which further causes mitochondrial dysregulation. Pro-inflammatory environment including TNF- α provides the cue for the tumor cells to acquire mutations, induce genomic alteration and leading to genomic instability promoting tumorigenesis and resistance to therapies. So, it is the need for an hour to explore new therapeutics which are effective against this deadly disease with minimal or no side effects. In the recent years, extensive efforts have been made to understand the role of phytocomponents present in various medicinal plants for different types of cancer, including breast cancer. The current study focuses on a medicinal plant, *Bauhinia variegata* L. which is known for plethora of potentials including anti-oxidant, anti-inflammatory and anti-cancer properties.

In the present study, *Bauhinia variegata* leaves extract were explored to check its effect on hormonally distinct breast cancer cell lines, ER/PR +ve, Luminal subtype MCF-7 and ER/PR -ve, Her2 -ve, Basal subtype MDA-MB-231. Efforts were being made to identify and understand the role of active phytoconstituents present in *Bauhinia variegata* L. leaves, affecting the tumorigenic potential of breast cancer cells. Further, the aqueous extract was explored for the TNF-α-regulated mitochondrial function and regulation of cell death. Here, the aqueous extract reveals its potential role as a tumor suppressor via regulating TNF- α induced cell death. The major findings of the study are summarized as below:

7.1.1 Aqueous extract of *Bauhinia variegata* L. leaves possess phyto-components with anti-cancer activity against breast cancer cell lines MCF-7 and MDA-MB-231

7.1.1.1 The extracts from *Bauhinia variegata* L. leaves are rich in phytocomponents with anti-oxidant property.

- The crude extracts were obtained from *Bauhinia variegata* L. leaves after extraction with different solvents (petroleum ether, n hexane, choloroform, ethyl acetate, methanol and water) in increasing polarity.

- Methanolic and aqueous extract showed maximum antioxidant activity with the strongest DPPH radical scavenging activity out of all the fractions.

7.1.1.2 Aqueous extract of *Bauhinia variegata* L. leaves showed anti-proliferative activity against breast cancer cell lines MCF-7 and MDA-MB-231.

- The aqueous extract showed good cytotoxicity against both the cell-lines and IC50 was found to 35μg/ml for MCF-7 cells and 1300μg/ml for MDA-MB-231 cells for 24 hours.

- Cytotoxicity assay of fractions obtained after flash chromatography showed the presence of probable anti-cancer compounds

7.1.1.3 HRLC-MS analysis revealed the presence of known anti-cancer compounds: Berbamine, 4'-Desmethylpapaverine and Rhapontin in the aqueous extract.

- HRLC-MS analysis was performed which revealed the presence of three known anti-cancer compounds: Berbamine, 4'-Desmethylpapaverine and Rhapontin in the aqueous extract.

- Different fractions of aqueous extract were collected by Flash chromatography. Eluents collected from flash chromatography were further separated for phytocompounds by TLC separation.

7.1.2 Aqueous extract suppresses the tumorigenic potential of breast cancer cell lines MCF-7 and MB-MB- 231.

7.1.2.1 Aqueous extract of *Bauhinia variegata* leaves have inhibited cell migration and clonogenic ability of ER/PR +ve MCF-7 and ER/PR -ve MDA-MB-231 breast cancer cells.

7.1.2.2 Aqueous extract of *Bauhinia variegata* leaves showed anti-proliferative and anti-migratory properties in MCF-7 spheroids (3D cell culture).

7.1.2.3 Aqueous extract of *Bauhinia variegata* leaves inhibited the cell proliferation of ER/PR +ve MCF-7 and ER/PR -ve MDA-MB-231 breast cancer cells in the presence of TNF- α. The aqueous extract of *Bauhinia variegata* leaves restricted the cell invasion in MCF-7 spheroids even in the presence of TNF-α.

7.1.3 Aqueous extract acts as a tumor suppressor by modulating TNF-α-regulated mitochondrial function and cell death in cancer cells

7.1.3.1 Aqueous extract impairs mitochondrial function in the presence of TNF- α in ER/PR +ve MCF-7 and ER/PR -ve MDA-MB-231 breast cancer cells

- Aqueous extract alters TNF- α induced ROS generation in MCF-7 and MBA MB 231.
- Aqueous extract induces loss in mitochondrial membrane potential in breast cancer cell lines MCF-7 cells and MDA-MB-231 cells in presence of TNF- α.

7.1.3.2 Aqueous extract might induce necroptosis type of cell death in breast cancer cells.

- PARP and Bcl2 levels remained unaltered indicating the absence of apoptotic type of cell death in both the breast cancer cell lines MCF-7 (hormonal positive and Her2 negative) and MDA-MB-231 (triple negative) breast cancer cells treated with aqueous extract of *Bauhinia* variegata L. leaves.
- Levels of Capsase-8 was altered in MCF-7 and MDA-MB-231. Caspasae-8 is known to be important for the necroptosis type of cell death

These results strongly suggest that the aqueous extract *B. variegata* L. acts as a potential tumor suppressor by modulating TNF- α induced ROS generation and TNF- α -regulated mitochondrial metabolism and cell death in breast cancer cells.

7.2 Conclusion

The present study shows the role of aqueous extract of *Bauhinia variegata* L. as tumor suppressor in ER/PR +ve MCF-7 and in highly invasive triple negative (ER/PR -ve, Her2 -ve) MDA-MB-231 breast cancer cell lines. Berbamine, Papaverine and Rhapontin were active phytocomponents present which may act in a synergistic manner for the anti-cancer activity. Understanding the role and benefits of phytocomponents with medicinal properties at molecular level, in combination with current FDA-approved therapeutics might help in developing a novel therapeutic approach with reduced side effects and potentially improved survival in breast cancer.

7.3 Future Prospects

It is important to validate the anti-tumorigenic potential of the aqueous extract in the in vivo models. Hence, DMBA induced mammary tumor model was developed in Sprague Dawley rats, to check how aqueous extract from *Bauhinia variegata leaves,* acts under normal physiological conditions in cancer model. After 28 days DMBA induction, the animals were palpated twice weekly to detect the presence and location of mammary tumors. The model was successfully generated using Sprague Dawley rats but unfortunately the work was not further pursued due to unavailability of animals due to corona pandemic. However it will be important to validate the finding in the animal model of breast cancer.

Hence, further studies are required to establish the role of aqueous extract of *Bauhinia variegata L.* leaves to explore its antioxidant and anti-tumorigenic property in animal model. Overall, *Bauhinia variegata* can be considered as a model herbal drug for free radical induced disorders like cancer. Experiments on systemic characterization of molecular targets and delineating the molecular mechanisms behind the anti-cancer property will help in exploring the therapeutic potential of this plant.