

List of Content

Sr. no.	CONTENT	Page no
CHAPTER 1: INTRODUCTION		
	1.1 Cancer: The global burden	4
	1.1.1 Breast cancer statistics worldwide	5
	1.1.2. Breast cancer prevalence in India	6
	1.2. Tumor heterogeneity of breast cancer	8
	1.2.1 Tumor microenvironment as a premetastatic site	10
	1.2.2 Tumor microenvironment (TME) and breast cancer progression	12
	1.2.3 Tumor associated inflammation in breast cancer	13
	1.2.4 Cytokines induced by tumor microenvironment	14
	1.2.5 TNF-α: Significance of tumorigenesis	16
	1.2.6 TNF-α: an intricate link between inflammation and cell death	17
	1.2.7 The paradoxical role of TNF-α in breast cancer	18
	1.2.8 TNF-α influences ROS production in cancer	20
	1.2.9 Mitochondria: Regulator of cell death	21
	1.3 Phytocomponents as a source for potential therapeutic approach	23

	1.4 Potential phytochemicals against breast cancer and their mode of action	28
	1.5 Effect of various plant-derived bioactive substances and their mode of action in the process of mammary carcinogenesis	32
	1.5.1 Impact on inflammation leading to carcinogenesis	32
	1.5.2 Induction of apoptosis leading to carcinogenesis	34
	1.5.3 Inhibition of proliferation leading to protection against metastasis	34
	1.5.4 Impact on metastasis and angiogenesis	36
	1.5.5 Impact on breast cancer stem cells	37
	1.6 Plant derived Drugs in pipeline for breast cancer	39
	1.7 Cancer therapy approaches	44
CHAPTER 2: AIMS AND OBJECTIVES		47
	2.1 Rationale and Hypothesis	47
	2.2 Objectives of the study	48
CHAPTER 3: MATERIALS AND METHODOLOGY		50
	3.1 Materials and Methods for Phytochemical Investigations	50
	3.1.1 Documentation of biological material	50
	3.1.2 Extraction of Phytochemicals	51
	3.1.3 Phytochemical studies	53
	3.1.3.1 Qualitative Analysis	53
	3.1.3.2 Anti-oxidant activity	54

	3.1.4: Identification of phytocomponents	54
	3.1.4.1 Thin Layer Chromatography	54
	3.1.4.2 Flash Chromatography	54
	3.1.4.3 HRLC-MS analysis	55
	3.1.4.4 FTIR analysis	55
	3.2 Materials and Methods for <i>in vitro</i> cell migration and cell invasion studies	55
	3.2.1 Cell culture conditions	55
	3.2.2. Spheroid generation by Hanging drop method	56
	3.2.3 Trypan blue exclusion assay	56
	3.2.4 Cytotoxicity Assay	56
	3.2.5 Microscopic analysis for morphology studies	57
	3.2.6 Cell Migration Assay	57
	3.2.6.1 Wound Healing Assay	57
	3.2.6.2 In-house assay developed for cell migration assay	57
	3.2.7 Clonogenic cell survival assay	58
	3.3 Materials and Methods for <i>in vitro</i> cell death studies	58
	3.3.1 Quantification of Reactive oxygen species (ROS)	58
	3.3.2 Determination of Mitochondrial membrane potential	58
	3.3.3 Apoptotic assays	58

	3.3.4 Protein expression studies using Western blot	59
	3.4 Statistical Analysis	59
	CHAPTER 4: EXTRACTION, ACTIVITY GUIDED FRACTIONATION AND CHARACTERIZATION OF THE CRUDE EXTRACT FROM <i>Bauhinia variegata</i> L	61
	4.1 Introduction	61
	4.1.1 Classification of <i>Bauhinia variegata</i>	62
	4.1.2 Vernacular names of <i>B. variegata</i>	62
	4.1.3 Morphology of leaves	62
	4.1.4 Phytochemicals present in <i>B. variegata</i> leaves	63
	4.1.5 Phytochemical Analysis of <i>B. variegata</i> leaves extract	65
	4.1.6 Qualitative analysis of phytocomponents from <i>B. variegata</i> leaves extract	65
	4.2 Evaluation of Antioxidant Activity	66
	4.3 To check the cytotoxic effect of <i>B. variegata</i> L. extracts on MCF-7 and MDA-MB-231 cells in time and dose dependent manner	67
	4.4 Isolation and characterization of the phytocomponents from aqueous extract/fraction	69
	4.4.1 Characterization of aqueous extract of <i>B. variegata</i> L. using High Resolution Liquid Chromatography- Mass Spectrometry (HRLC-MS) analysis	69
	4.4.2 Thin Layer Chromatography (TLC) of the aqueous extract of <i>B. variegata</i> leaves	75

	4.4.3 Flash Chromatography of aqueous extract of <i>B. variegata</i> L. Leaves	75
	4.4.4 Fourier-transform infrared spectroscopy (FTIR) of aqueous extract of <i>B. variegata</i> L. leaves	78
	4.4.5. Cytotoxicity assay of the probable anti-cancer compounds found in aqueous extract by HRLC-MS	79
	4.4.6. Cell cytotoxicity assay performed on breast cancer cell lines from the fractions of aqueous extract after flash chromatography	81
	4.5 Discussion	82
CHAPTER 5: TO ANALYSE THE EFFECT ON BREAST CANCER CELL MIGRATION, INVASION AND TNF-α REGULATED CELL GROWTH IN RESPONSE TO ACTIVE EXTRACT/ FRACTION/ ISOLATED PHYTOCOMPONENT/S OF <i>Bauhinia variegata</i> L.		
	5.1 Introduction	89
	5.2 Effect of TNF-α and Estradiol on breast cancer cell proliferation and apoptosis	91
	5.3 Effect of TNF-α and estradiol on cell migration in breast cancer	93
	5.4 To assess the effect of aqueous extract on cell migration in breast cancer cell-lines	94
	5.4.1 To assess the effect of aqueous extract on cell migration in breast cancer cell-lines by wound healing assay	94
	5.4.2 Development of in-house assay for cell migration study in breast cancer cell lines	96

	5.5 To study the effect of <i>Bauhinia variegata</i> aqueous extract on cell proliferation on MCF-7 spheroids	98
	5.5.1: Formation of spheroids by hanging drop method in breast cancer cell lines	99
	5.5.2: Effect of aqueous extract of <i>Bauhinia variegata</i> leaves on cell proliferation of MCF-7 spheroids	100
	5.5.3: To assess the effect of aqueous extract on cell invasion in MCF-7 spheroids	101
	5.6: Aqueous extract regulates tumorigenic potential of breast cancer cells	104
	5.7 To evaluate the effect of aqueous extract of <i>Bauhinia variegata</i> leaves on cell proliferation in absence and presence of TNF-α in breast cancer cell lines	105
	5.8 To evaluate the effect of aqueous extract of <i>Bauhinia variegata</i> leaves on cell invasion in absence and presence of TNF-α and estradiol in MCF-7 spheroids	107
	5.9 Discussion	114
CHAPTER 6: TO ANALYSE THE EFFECT OF ACTIVE EXTRACT/FRACTION /ISOLATED PHYTOCOMPONENT/S OF <i>Bauhinia variegata</i> L. ON CELL DEATH PARAMETERS IN BREAST CANCER CELL LINES.		
	6.1 Introduction	119
	6.2 TNF-α-regulates mitochondria and apoptosis in cancer	120
	6.3 Mitochondria and ROS: Important regulators of tumorigenesis	122

	6.4 TNF-α-induced ROS generation in cancer	123
	6.5 Morphological changes in the MCF-7 and MDA-MB-231 cells when exposed to different compounds present in the aqueous extract of <i>B. variegata</i> L.	125
	6.6 To study the effect of aqueous extract on ROS levels in breast cancer cell lines	127
	6.7 Analysis of mitochondrial transmembrane potential in breast cancer cell lines in response to <i>B. variegata</i> L. aqueous extract	130
	6.8 Effect of aqueous extract on the cell death in breast cancer cell lines	135
	6.8.1 To assess the effect of aqueous extract on PARP, Caspase 8 and Bcl2	141
	6.9 Discussion	143
CHAPTER 7: SUMMARY AND CONCLUSION		148
CHAPTER 8: REFERENCES		153
List of PUBLICATIONS		174