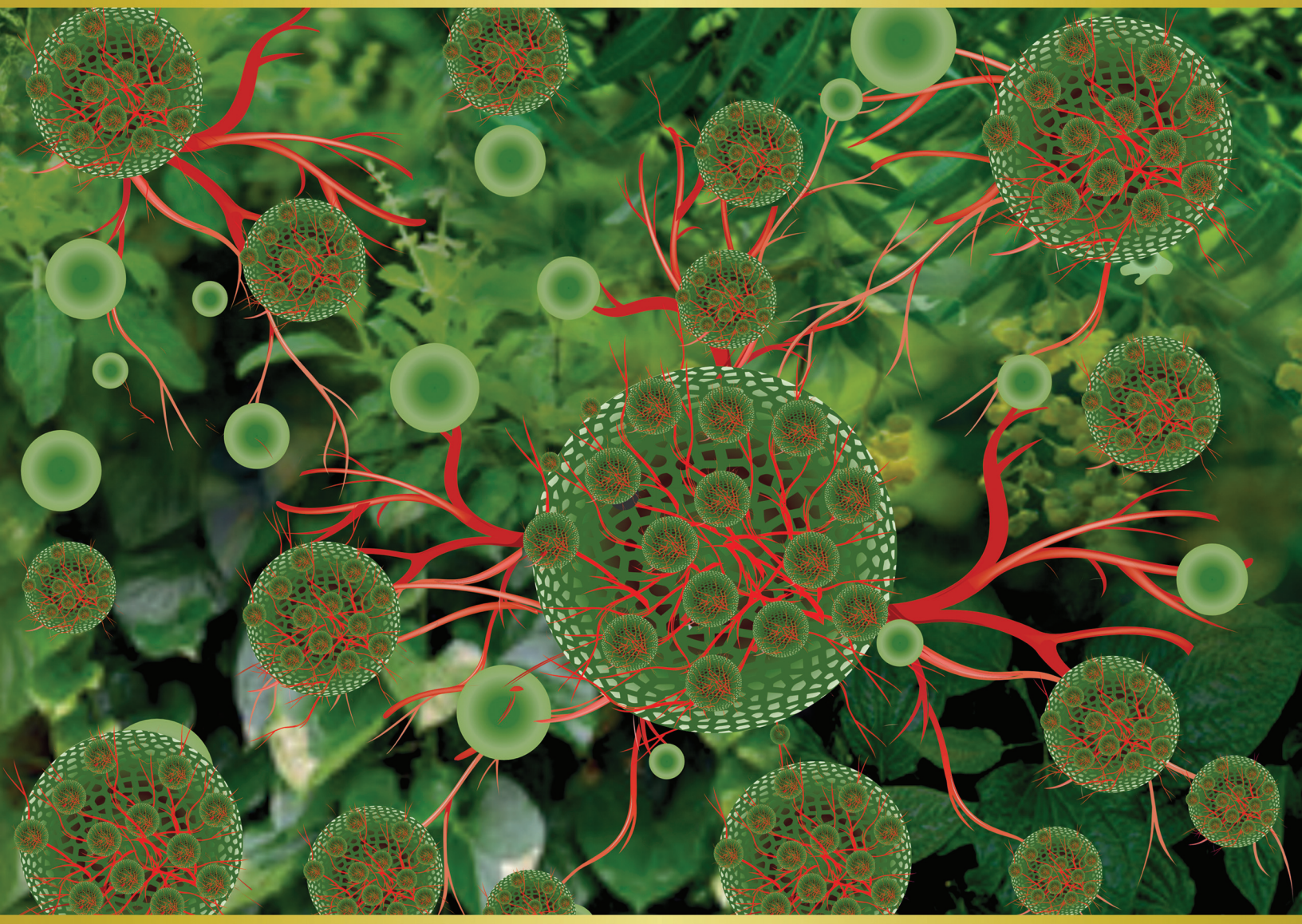
# THERAPEUTIC DRUG TARGETS AND PHYTOMEDICINE FOR TRIPLE NEGATIVE BREAST CANCER



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# Therapeutic Drug Targets and Phytomedicine For Triple Negative Breast Cancer

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# CONTENTS

# FOREWORD

Conventional drug therapies have greatly deteriorated the health of diseased individuals. Triple negative breast cancer is an aggressive form of breast cancer has led to mortality issues worldwide. Till now, no FDA-approved drugs are available in this regard. I certainly believe that herbal medicine can potentially revitalize human health affected due to triple negative breast cancer. Dietary intake of herbal products inadequate quantity may help in the prevention and treatment of disease. Herbal medicine may act as both preventive and therapeutic measures against the disease. In this regard, I would like to congratulate Acharya Balkrishna for designing this phenomenal piece of work to enlighten and enhance our understanding of TNBC. To create awareness amongst the diseased individuals & researchers, the present book would be of great help that will provide an overview of etiology, its treatment strategies, and prognostic marker to identify the outcome of standard therapies. In this book, herbal medicine exhibiting high potency to target TNBC has been enlightened to avoid the side effects associated with synthetic analogs used during chemotherapy, and also their ability to fight against chemoresistance was also represented. The proposed book will be of great importance for the wide spectrum of readers especially those working in the field of breast cancer, herbal medicine, traditional medicine, etc. The present piece of work will surely provide valuable hints to discover novel therapeutic regimens to fight against other cancers also. It will act as reference material as well for research scholars and both graduate & post-graduate students to explore novel therapeutic regimens against the disease without affecting the health of affected individuals.

**Swami Ramdev**

Founder of Patanjali Yogpeeth Trust
Panchayanpur,
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# PREFACE

In the 21st century, the current scenario of breast cancer was nightmarish and has greatly threatened millions across the world. Triple negative breast cancer, the most aggressive form of breast cancer has horrified people and seemed to kill the individuals with frightening certainty. Poor clinic-pathological attributes, prognostic markers, unavailability of efficient therapeutic approaches, higher chances of disease relapse along with metastasis to distant sites have worsened the clinical outcome of the disease. Prevalence and epidemiology trends of TNBC patients have induced a global catastrophic risk. This lucid work entitled 'Therapeutic Drug Targets and Phytomedicine For Triple Negative Breast Cancer' is an attempt to rapidly disseminate to oncologists and other members of the scientific community regarding updates of TNBC. The present work examines TNBC from basic definition to stratification of subtypes, genetic and transcriptional profiling, cellular and molecular diagnostic approaches, molecular signaling pathways involved in complications, preclinical and clinical evidence of conventional therapeutic regimens along with unveiling efficacy of herbal medicines to combat complications of TNBC. The present book deals with detailed etiological insights of TNBC including diverse subtypes, and practical information will help clinicians engaged in the determination of molecular and pathological cognizance of disease. Genetic, transcriptional, and clinical heterogeneity of disease has been discussed from multidisciplinary perspectives. The molecular complexity of signaling pathways and prognostic markers would help in the identification of therapeutic vulnerabilities. A paradigm of the therapeutic approach along with completed, ongoing, and terminated clinical trials were discussed to analyze overall survival, disease free survival, and distant metastasis free survival in TNBC patients. The impact of tumor microenvironment in facilitating the escape of TNBC cells from chemotherapeutic and immunological response were also highlighted. To combat drug resistance and efficacy issues, the potential role of natural moieties as dynamic, promising, and new therapeutic strategies to benefit TNBC patients was foreground. Druggability parameters of these phytochemicals including bioavailability, bio-absorption were discussed and nanosoldiers have been introduced to enhance their pharmacokinetic profile, distribution, and release rate. Combinational therapies comprising of conventional and herbal medicine approach to completely abolish complications of TNBC including their regulatory issues and potential role of herbal medicine in rejuvenating the health of affected individuals were uncovered. The present compendium will be of great interest to oncologists, clinicians, researchers, students, and the pharmaceutical sector to gain further insights into TNBC and to identify the potential role of herbal medicine in tackling the disease.

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# CHAPTER 1

# Etiological Insights into TNBC and their Related Catastrophic Risks

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**Abstract:** Triple-negative breast cancer is the most aggressive form of breast cancer that lacks expression of estrogen, progesterone, and human epidermal growth factor receptor 2. TNBC is characterized by poor clinic-pathological attributes, prognostic markers, unavailability of efficient therapeutic approaches, and higher chances of disease relapse along with metastasis to distant sites. Dysregulated epigenetic and transcriptional profiling was involved in cancer progression including histone modification, altered miRNA, DNA methylation, and long non-coding RNA signatures. This chapter will provide an insight into the molecular biology of TNBC including gene expression patterns and their subtypes. TNBC molecular spectrum was extensively studied to depict the distant metastasis-free survival and overall survival rate in affected individuals. Prevalence and epidemiology trends of TNBC patients across the globe were also studied to determine the impact of genetic predisposition and socioeconomic factors behind its aggressive behavior.

**Keywords:** Basal like, Epidemiology, Epigenetic profiling, Immune-modulatory, Intratumor heterogeneity, LncRNAs, Luminal androgen receptor, Mesenchymal stem-like, Metastatic, MiRNA, Molecular classification.

## INTRODUCTION

Breast cancer is one of the most common malignancies among women globally [1]. In 2018, approximately 2.1 million cases of breast cancers were diagnosed globally which account for 11.6% of women's population, and a mortality rate of 6.6 has been reported across the world [2]. The heterogeneous nature of breast cancer might be responsible for such aggressive behavior. Identification of diverse cell phenotypes, their localization, and cell density may help in predicting the heterogeneous nature of the disease. A substantial difference in incidence and mortality rate has been observed so far depending upon the geographical location.

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According to GLOBOCAN reports, incidences of breast cancer are more prevalent in Australia, Northern Western Europe, and North America. Whereas the incidences of the disease are slightly lower in Africa, South America, and Asia. However, a sudden increase in the number of cases in the latter regions has also been observed in the past few years (Fig. **1**) [1, 3].

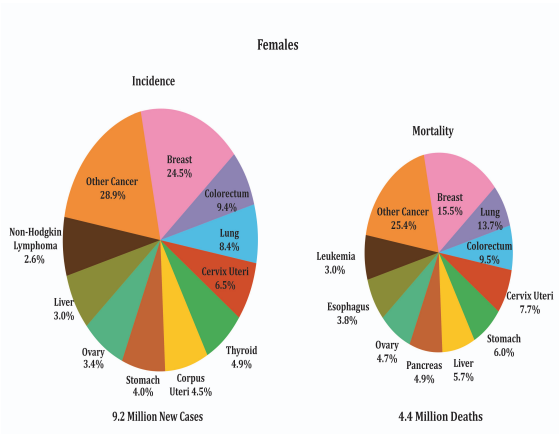


**Fig. (1).** Incidences and mortality rate amongst females due to different types of cancers according to GLOBOCAN reports.

Intertumor or intratumor heterogeneity is majorly responsible for the evolution of different subtypes within the same tumor. These subtypes can be further characterized by their morphology, molecular profiling, or hormone receptors which can be used as a specific biomarker of the disease, for example, estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER-2). Such a huge variation in individual tumors suggested that tumor cells may exhibit different phenotypes with diversified functions and multiple expression markers of the disease. Furthermore, this intratumor heterogeneity enhances the ability of tumor cells to adapt to near micro-environmental conditions. A tumor sample dissected from the patient's body during biopsy cannot be considered as a true representative of the real tumor. A single tumor may comprise different cancer cell populations with diverse phenotypes, and properties and may show resistance to drugs. Collectively all these factors contribute to the complication of the disease and thereby making it more cumbersome to treat [4, 5]. Triple-negative breast cancer (TNBC) is another subtype of breast cancer that was deprived of the expression of ER, PR, and HER2. TNBCs are highly heterogeneous in the name of morphology, presentation, and genetic aberration. TNBC is characterized by high tumor grade, early relapse of the disease, high proliferation rate, and decreased overall survival rate [6, 7]. Processes like angiogenesis, uncontrolled proliferation, invasion and

metastasis, and inhibition of apoptosis were the predominant pathways involved in complicating TNBC (Fig. **2**).

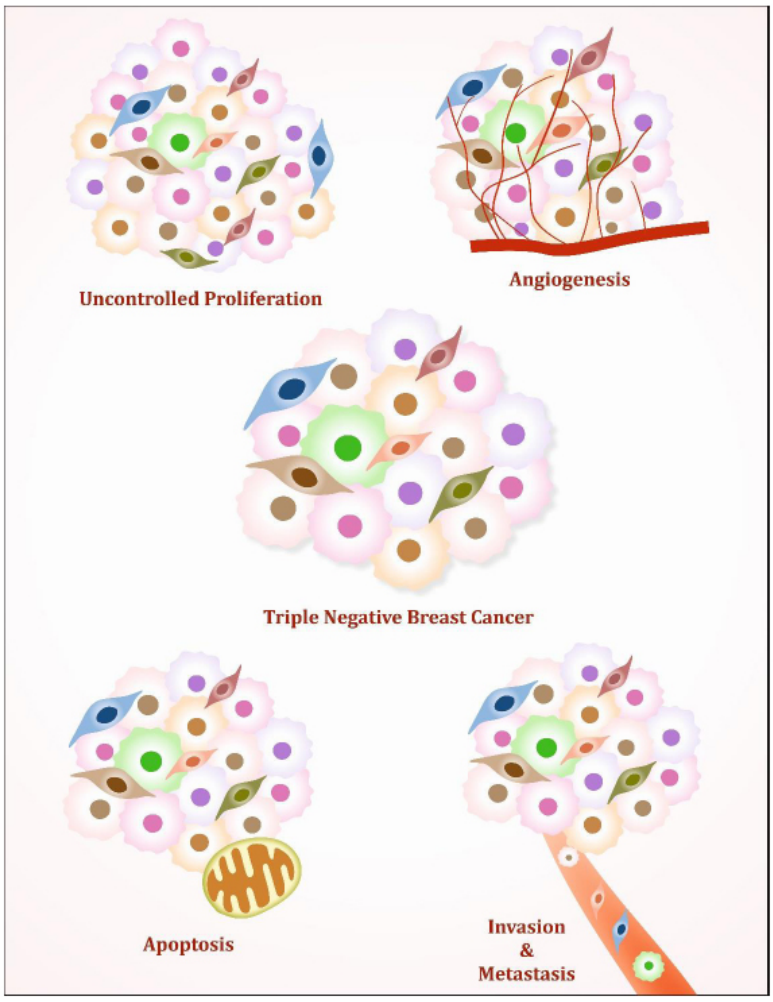


**Fig. (2).** Figure representing uncontrolled proliferation, angiogenesis, apoptosis, and invasion & metastasis in TNBC.

So far on the basis of transcriptional profiles, TNBC has been classified into 6 major molecular subtypes exhibiting differential responses to diverse chemotherapeutic regimens (Fig. **3**) [8, 9]. According to Lehmann *et al*, these 6 subtypes include; mesenchymal (M), basal-like (BL-1 & 2), mesenchymal stem-like (MSL1), immune-modulatory (IM), and claudin low.

**CHAPTER 2**

# A Clinical Cognizance of Molecular and Pathological Diagnostic Approach of TNBC

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**Abstract:** Genetic, transcriptional, and clinical heterogeneity of disease has remained to be a prominent obstacle to the development of a targeted therapeutic approach against TNBC. So far, based on tumor size, lymph node status, and histologic features TNBC subtypes were stratified. Insights into inter and intratumoral heterogeneity of TNBC were gained by next-generation sequencing, genomic, transcriptomic, proteomic, and clinicopathological characterization. To depict tumor response to neoadjuvant chemotherapy, radiological characterization may also a play significant role. Biomarkers for subtyping TNBC were highly needed to depict the survival outcome. This chapter discussed the available and possible molecular and pathological diagnostic approaches to TNBC. Furthermore, the integration of morphological and genomic data may emerge as a promising approach for the identification of new therapeutic and prognostic markers to predict the likely outcome of the disease. This chapter aims to highlight the molecular and pathological diagnostic approaches to depict both metastatic and non-metastatic TNBC.

**Keywords:** Core basal group, CtDNA, Dynamic contrast-enhanced MRI, Histology, Immunohistochemistry, Mammography, Medullary carcinoma, Metaplastic carcinoma, Molecular, Morphology, Mutation profiling, Neoadjuvant chemotherapy, Pathological, Prognosis, Proteomic, TCGA, Transcriptomic.

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## INTRODUCTION

Several clinical and pathological factors were generally considered to categorize breast cancer patients and thereby determine the appropriate treatment strategy and assess prognosis. These factors included tumor size, axillary lymph node status, patient age, histological features such as lymphovascular invasion or histological grade, hormonal receptor status, and many more. Rather than assessing these factors individually, it would be of great clinical importance to consider them collectively as a combined approach that would help to categorize patients into diverse risk categories for example [1, 2]:

(a) National Institute of Health Consensus Criteria

(b) St. Gallen criteria

(c) Nottingham prognostic index

(d) Adjuvant online


**Fig. (1).** Figure representing immunophenotypic and pathologic characterization of TNBC

Although these factors have been of great value to assess prognosis and risk factors but are simultaneously linked with certain limitations and a combination of these would yield different clinical outcomes. Therefore, we require better therapeutic and prognostic markers to select an efficient treatment strategy to fight against the disease (Fig. **1**).

Recently, several molecular techniques have gained enormous attention such as gene expression profiling to refine the strategy of breast cancer classification, and to depict therapeutic and prognostic markers [3 - 5]. TNBCs were heterogeneous with variable morphological and pathological factors and could be referred to as medullary, ductal, metaplastic, or adenoid cystic carcinoma. Unexpectedly, this adenoid cystic carcinoma presents an excellent prognostic value with an 87.5% of survival rate and 6.5 year follow-up period [6, 7]. Previously also breast cancers were classified on the basis of their cellular morphology or presence/absence of hormone receptors. To depict the molecular heterogeneity of TNBC, Perou and their colleagues studied the gene expression profile and revealed five distinct molecular subtypes as discussed in chapter 1. RNA expression arrays predicted that different subtypes would possibly have emerged from varying precursor cells with diverse progression pathways. Since TNBCs or basal-like subtype resembles normal breast epithelial cells, these were believed to be originated from myoepithelial cells which is the outermost layer of breast ducts. Upon staining, these tumors were found to be positive for basal cell cytokeratin, and EGFR and negative for ER, PR, and HER-2. Characteristic morphological features were also depicted in TNBC such as high grade, central necrosis, higher mitotic count, and lesions of invasion could also be seen. From molecular expression profiling, it was identified that TNBC demonstrated p53 nuclear expression which was further linked with prevailing TP53 gene mutations. TNBCs gradually express proliferation linked markers such as MIB-1 and TOP2A whereas cell cycle proteins such as Cyclin D1 and CCND1 were found to be downregulated [6, 8 - 10]. A similar expression pattern was also found in tumors originating from BRCA1 mutation carriers. But TNBCs and these subtypes cannot be considered synonymous with each other. TNBCs exhibited certain basal-like characteristic features but basal-like subtypes were not the same as TNBCs. On the basis of gene expression profiling, Rody *et al*. revealed that approximately 73% of TNBCs exhibited basal-like features. On the basis of immunological response, inflammation, angiogenesis, proliferation, and apocrine activity, the rest of the TNBC subtypes were classified. They also identified another subset that possessed elevated expression of B cell and downregulated IL-8 and was further considered as an efficient prognostic marker of disease. Similar evidence was also represented by Tan *et al*., in their study which included 245 TNBCs cases. 19.4% of TNBC cases were found to be negative for basal-like subtype markers whereas 73% of non-TNBC cases showed markers related to basal-like subtype.

CHAPTER 3

# Molecular Sub-Typing and Exploration of Key Signalling Pathways Involved in Complicating the Disease

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**Abstract:** Triple-negative breast cancer is characterized by distinct molecular profiles, unique metastatic patterns, aggressive behavior, lacks the targeted therapeutic approach, and caused significant mortality worldwide. The molecular complexity of angiogenesis, autophagy, apoptosis, and metastasis process in TNBC has fostered research efforts to unleash the molecular, pathological, and genetic drivers of their lethal cascade. This complex disease entity involves PI3k/Akt/mTOR, NF-kB, ERRs, and miRNA trafficking which has further worsened the clinical outcome. Due to their heterogeneous nature, none of the drugs were able to completely target the TNBC tumor spectrum. This chapter highlights the classification of TNBC on the basis of aberrated copy number, histology, proteomic, and mutational profiles to understand the aetiology of the disease. The identification of therapeutic vulnerabilities was also carried out by gaining insights into the above-mentioned signalling pathways and their role in further complicating the disease.

**Keywords:** Androgen receptors, Angiogenesis, Apoptosis, Autophagy, Copier overdrive, Invasion, Lymphoplasmacytic infiltration, Metastasis, MiRNAs, Monoclonal antibodies, MTOR, Mutational analysis, Oncogenes, PI3k/Akt, Pro-proliferative, Pro-survival, Tumor suppressor.

## INTRODUCTION

Triple-negative breast cancer was considered to be the most aggressive form of breast cancer across the globe. Perou and colleagues have distinguished diverse molecular subtypes of disease based on differential expressions of immune-histochemical markers such as ER, PR, and HER-2.Treatment strategy and

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prognostic markers significantly vary for different breast cancer subtypes. In comparison to other sub-types, TNBCs were highly heterogeneous with higher chances of distant metastasis [1, 2]. So far, TNBCs were observed to be highly prevalent amongst pre-menopausal women with high a proliferation rate. Therefore, a major concern in this regard was the early detection of TNBC using an efficient diagnostic approach which would further help in determining the appropriate therapeutic regimen. TNBCs were often diagnosed as hypoechoic masses with microlobulated margins [3]. However, in some cases, it was misdiagnosed as benign masses which led to an additional difficulty. The pathogenesis of TNBCs has always remained to be quite complicated and was greatly influenced by their hormonal environment. Alteration in hormonal levels could potentially disrupt the body's metabolism and may further enhance the complication associated with the disease. During menopause, drastic hormonal changes occur in the human body that would eventually reduce the level of estrogen and ultimately triggered the possibility of its occurrence. Alterations in hormonal levels could further stimulate tumor cells. Several pieces of evidence have supported the findings that estrogen can induce complications of breast cancer in postmenopausal women [4 - 6].

## Aetiology of Triple Negative Breast Cancer Subtypes

The heterogeneous nature of TNBC has significantly contributed to its aggressive behavior. For the past many decades, this heterogeneity has posed a challenge in front of clinicians. Multiple 'omics' platforms were required to gain an insight into the complex, dynamic, and heterogeneous molecular landscape of such tumors. Stratification of TNBC patients into differentiable, identifiable, stable, and actionable subtypes on account of their biomarker profile was highly needed to predict the optimal therapeutic, and prognostic marker of the disease. Several types of research have been carried out to identify different molecular subtypes of TNBC. Prat and their colleagues extensively studied, luminal A and 11 protein proliferation signatures to determine molecular subtypes on basis of the patient's response against TNBC. They concluded that TNBC patients may be referred to as basal-like breast cancer patients on account of their preferential response against NAC but all basal-like subtypes cannot be ascribed as TNBCs. Another research group postulated that loss of BRCA1/2 mutations has led to pathogenesis in a subset of TNBC and 10% of TNBC cases were carrying a BRCA1/2 germline mutation hence this sub-group was called "BRCAness". This subtype exhibited a bitter clinical response to combinational therapy comprising platinum agents and taxane/anthracyclines [7 - 14]. However, the evidence presented by Lehmann *et al*. was groundbreaking. They analyzed gene expression profiles of TNBC tumors and prepared subsequent seven unique clusters. Out of 7, 6 were observed to be stable clusters and 1 was unstable. These clusters were further characterized by

gene ontologies and specific canonical pathways and were named BL1 & BL2, IM, M, LAR, MSL, and UNS. Till now also, subtypes mentioned by Lehmann *et al*. were given preference and were considered a landscape to identify other molecular subtypes of TNBC. A detailed description of the above-mentioned subtypes has already been provided in chapter 1 of the book. After Lehmann *et al*., several other scientists re-classified molecular subtypes of TNBC. Burstein *et al*. adopted the IHC method to identify ER, PR, and HER2 expression, and further to classify TNBC on basis of these three receptors. When IHC screening for these three receptors was carried out, only five of these 6 subtypes were detected in TNBC tumor samples. Upon further exploration of DNA and mRNA expression profiles, 4 different subtypes were classified which were in tone with Lehmann classification named as luminal androgen receptor 2 subtype, mesenchymal (MES), basal-like immune-suppressed (BLIS), and basal-like immune activated (BLIA). LAR2 exhibited a resemblance to LAR proposed by Lehmann whereas the MES subtype showed similar characteristic features to MSL, BL1, and BL2. In both the studies, in BL1, BL2, MSL, and MES subgroups; the cell cycle, inherent breast cancer signaling, and DNA damage repair pathways were highly active. Genes related to osteocyte and adipocyte expression and IGF growth factor were found to be elevated in the MES sub-group. Members of the SOX transcription factor family were found to be upregulated in the BLIS subtype whereas natural killer cells, B & T cells, and cytokine pathways were found to be downregulated. Additionally, in the BLIS subtype; innate, and adaptive immunological response, antigen presentation, and immune cell differentiation pathway were observed to be suppressed. However, DFS and OS rate was worst and therefore may cause mortality. Contrastingly, STAT-mediated immune-regulatory pathways were overexpressed which may lead to a better prognosis. In comparison to the other four groups, BLIS revealed specific molecular expressions of cell signaling proteins. Furthermore, DNA copy number analysis showed elevated expression of AR, MUC-1, IGF-1, PTGF, VTCN1, and CTLA4 in LAR2, MES, BLIS, and BLIA tumors [15 - 17].

From gene expression analysis it was anticipated that approximately 6-8% of TNBCs expressed HER-2 and can be considered as a separate clinical entity. HER-2 enriched TNBC subtype represented common gene ontologies to Lehmann's BL2 and LAR subtypes [18]. Similar to LAR, HER2 enriched TNBCs possessed PIK3CA mutations and exhibited elevated expression of AR and on the virtue of this characteristic feature, these two were considered similar. p53 mutations were found to be highly prevailing in this sub-set and additionally expressed angiogenic factors for example VEGF. Although, the biological role of this subset could not be identified so far but possibly would be of great importance to determine the therapeutic target against the disease (Fig. **1**) [19 - 22].

CHAPTER 4

# Pre-Clinical and Clinical Evidence of Recent Therapeutic Trends and Spotting Possibility of Cure in Near Future

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**Abstract:** Substantial cohort studies, pre-clinical, clinical trials, and in-depth genomic and proteomic analysis underlie that several molecular alterations exist in TNBC that may be favorable or detrimental to cancer progression. Molecular heterogeneity in TNBC has shortened the disease-free survival rate in response to adjuvant and neoadjuvant therapies. To determine possible vulnerabilities in TNBC, several drugs were under investigation. This chapter highlighted the current paradigm of the therapeutic approach including surgery, radiotherapy, and chemotherapy. In this review, we also highlighted the clinical trials involved in the management of TNBC by targeting angiogenesis, apoptosis, androgen receptors, cell cycle, and pro-survival signalling pathways. To overcome the constraints associated with the mono-therapeutic approach, pre-clinical and clinical studies of combinational therapy have also been discussed to improve OS, DFS, and DMFS in TNBC patients.

**Keywords:** Androgen receptors, Anti-angiogenesis, Apoptosis, CDK inhibitors, Chemotherapy, Clinical trials, Combinational therapy, Immunomodulatory agents, Loco regional treatment, Monotherapy, Pre-clinical, Radiotherapy, Surgery.

## INTRODUCTION

Worldwide TNBC was classified as a highly aggressive form of breast cancer. On basis of the molecular profile, 5 distinct subgroups of TNBCs were identified. On the other hand, pathologically TNBCs were characterized by poor differentiation, larger tumor size, and higher proliferation index with significant involvement of lymph nodes. Studies revealed that the 5-year DFS rate amongst TNBC patients was worst in comparison to other breast cancer subtypes such as $ER^+/PR^+$, $HER^{2+}$

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or ERBB2 amplified sub-groups. Although the distant recurrence rate is higher in TNBC patients, approximately 33.9% in comparison to 20.4% of other subtypes, the mean survival time was predicted to be only 2.6 years than 5 years of other subtypes [1 - 3]. In spite of the development of diverse treatment strategies against TNBC, the median overall survival rate amongst the patients who have attained metastasis stage was only 13-18 months. From DFS, OS, and DMFS rates, the aggressive behavior of TNBC can be easily predicted [4]. Rather than considering TNBC as a clinical or biological entity, it should be classified on basis of its heterogeneous nature. Intratumor heterogeneity lying in TNBC has produced a discrepant response against the different therapeutic options in clinical therapy thereby limiting the treatment options against the disease. On the basis of integrated profiling, different researchers have classified TNBCs to determine their specific biological features and actionable targets. Apart from the determination of genomic aberrations for example BRCA1/2, different attempts were made to classify TNBC clusters and to identify specific therapeutic approaches against subgroups. Based on the classification strategy posed by Lehmann *et al*. of the 6 TNBC sub-groups and the observation by Jiang *et al*. of 4 sub-types, different treatment strategies were identified accordingly [5 - 8].

## Current Paradigm of Therapeutic Approach Against Triple Negative Breast Cancer

Globally, approximately 1 million cases of TNBC cases were found and around 12-20% constituting around 170,000 patients were diagnosed to be TNBC. For both researchers and clinicians, TNBC has remained to be a major area of threat because of:

1. Worst prognostic, OS, and DFS rate

2. None of the specific efficient therapeutic regimens were available

3. Amongst the premenopausal women and women of African descent, there exists a clustering of TNBC cases.

4. Overlapping of TNBC phenotypes and BRCA1 linked breast cancer

Hormonal or trastuzumab-based therapy was unable to provide benefit to TNBC patients due to the loss of targeted receptors such as ER, PR, and HER-2. Surgery and chemotherapy either individually or in combination have remained to be the only available treatment option against the disease. However, some studies have pointed out certain receptors as possible targets for the establishment of new therapeutic drugs. In this section of the chapter, we will briefly discuss the above-mentioned therapies against TNBC [9 - 11].

## Surgery in Triple Negative Breast Cancer Cases

Different studies were carried out to unpin whether TNBC patients preferred mastectomy over lumpectomy. Studies revealed that although its status was linked with higher tumor grades even at a younger age it did not influence the surgical treatment option. Keeping in view the aggressive nature of the disease, an option of surgery was often not opted rather traditional clinic-pathological strategies were preferred. Freedman and colleagues revealed that after breast conservative surgery the local recurrence rate was lesser in TNBC in comparison to other breast cancer subtypes. Therefore, in the case of TNBC, surgery could yield better outcomes for breast conservation. Since the early 1900s, surgical removal of tumor mass has remained to be a major strategy in the treatment of breast cancer [12 - 15]. However, metastasizing tumor lesions remained to be inoperative with technological advancement. In the past few years, efficient screening techniques have been made available which can diagnose the tumor mass even at early-stage without exhilarating much reliance on invasive biopsy techniques.

These procedures involved the introduction of the sentinel lymph node biopsy technique as a substitute for the conventional axillary dissection process. Additionally, the establishment of the MRI technique has further supported the use of image-guided biopsy and surgical excision of tumor lesions only detectable through MRI [16, 17]. These advancements have reduced our dependency on radical surgical procedures. BCS lumpectomy and quadrantectomy have also improved the perception of body image and especially life's quality amongst younger patients. But another prominent issue linked with BCS was that it required intensive counseling due to the increased risk of disease recurrence in affected individuals [18 - 20]. A considerable overlap of molecular signatures was observed between TNBC and BRCA1 mutated tumors, approximately 75-85% studies of such molecular profiling would further help in choosing optimal surgical procedures to remove these tumors. Mastectomy was generally preferred in patients with BRCA1 mutations, which might be due to lower risk factors associated with this surgical procedure. However, two publications presented conflicting outcomes on TNBC excision using surgical procedures [21, 22]. One of the studies induced BRCA mutation-positive TNBC patients. These patients exhibited a better recurrence-free survival rate in compassion to the patients with wild-type BRCA-positive breast cancer. A cohort of highly risked TNBC patients was evaluated to determine the rate of prognosis. From the study, it was observed that 50% of the population was positive for BRCA1/2 mutation and the overall prognosis did not differ much in both the cases within the first 5 years of diagnosis. In order to confirm the outcome of surgical procedures in the case of TNBC, extensive research needs to be carried out [11, 23, 24].

# CHAPTER 5

# Evaluating Fate of Emerging Resistance Hitting the Brakes on Conventional Treatment Approach

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**Abstract:** The tumor microenvironment of TNBC cells was associated with the induction of angiogenesis, proliferation, apoptosis inhibition, immune suppression, and drug resistance. TME creates a niche for the survival and interaction of cancer cells with surrounding cells. TME promoted epithelial to mesenchymal transition, stemness, and chemoresistance and ensured the escape of TNBC cells from the chemotherapeutic and immunological responses. This chapter highlighted the role of cancer stem cells, hypoxia, lysosomal biomass, tumor-associated macrophages, PTEN, PI3K/Akt/mTOR pathway, and ABC transporters in inducing resistance against standard therapeutic regimens. The possible role of miRNA, transcriptional signatures, and tumor-infiltrating lymphocytes as a predictor of chemoresistance was also depicted. The impact of drug repurposing and combinational therapeutic approach to overcome the obstacle of chemoresistance have been underlined in this chapter for the treatment of TNBC.

**Keywords:** ABC transporters, Cancer stem cells, Cardiotoxicity, Chemo-resistance, Chemo-sensitization, Clinical trials, Combinational therapy, Drug repurposing, Hypoxia, Immunosuppression, Iymphocytic infiltration, Iysosomes, MiRNAs, Monotherapy, Myelosuppression, Pgp, PI3k/Akt/mTOR, PTEN, TAM, Transcriptional signatures, Uprosertib.

## INTRODUCTION

Triple-Negative breast cancer represented only 15% phenotype of other breast cancer subtypes. In spite of not being highly prevalent, it has become the major target of research. TNBC has aggressive metastatic nature with fewer therapeutic regimens and poor prognostic markers [1, 2]. This was mainly due to the lack of molecular targets which have increased our dependency on the conventional

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approaches against TNBC. Diverse mono-therapeutic and combinational therapeutic approaches are available against the disease. Anthracyclines and a combination of taxanes and doxorubicin were commonly preferred against TNBC. Although related toxicity and issue of resistance have limited the application of these drugs, myelosuppression, dose cumulative cardiotoxicity, and immunosuppression were observed to be the major obstacles to treat TNBC patients using conventional chemotherapy strategies. However, the mechanism of chemoresistance and chemosensitivity was still unclear. Henceforth, it was highly needed to determine the regulatory pathways and specific targeted approach to optimize the clinical response against the treatment approach [3 - 5]. Cytotoxic chemotherapy, radiation, and tumor resection can benefit only 20-30% of TNBC patients and were further associated with a higher recurrence rate within 1-3 years after the diagnosis. Recently, from the results of clinical trials, it can be anticipated that TNBC patients with elevated expression of PD-L1 can get benefitted from combinational therapy comprising PD-L1 inhibitors named atezolizumab and conventional chemotherapeutic regimens. This particular combination was also approved by FDA against a specific subset of TNBC. Results obtained from the study also strengthened the fact that targeted therapy can efficiently combat TNBC and related complications and apart from this, precision therapy was also needed to tackle the disease [6 - 8].

Higher mitotic rate, increased lymphocytic infiltration rate, larger size and enhanced grade were the major characteristic features of TNBC. In 15% of TNBC cases, distant metastasis to the brain was also observed and was found to be linked with the worst prognosis and reduced survival time. In spite of complete resection of breast tumors, instances of brain metastasis after several years suggested that these disseminated cells carried special characteristic features which permitted them to metastasize to distant sites even in the absence of a primary tumor. Even after receiving the chemotherapeutic regimens, events of metastasis of TNBC cells to different organs were also found. In comparison to other tumor subtypes, a specific chemotherapeutic protocol has not yet been established. However, most of the therapeutic strategies have been planned based on the size, involvement of lymph nodes, and comorbidities but none of the therapies could assure the complete efficacy against the disease. Due to the absence of ER/PR/HER-2 expression, endocrine therapy along with aromatase inhibitors, tamoxifen, fulvestrant, and HER-2-based therapies could not benefit TNBC patients. Even TNBC tumor cells were found to be resistant to taxanes and anthracyclines [9 - 11]. Recently conducted studies revealed that TNBC cells were associated with elevated expression of receptor tyrosine. These receptors expressed a high affinity for diverse polypeptides such as hormones, cytokines, and growth factors. These RTKs were discovered approximately 25 years ago, different members of these cell surface receptor families have been identified so far and may play a pivotal

role in several regulatory pathways such as cell proliferation, cell differentiation, invasion, migration, and angiogenesis. RTKs displayed a similar molecular scaffold which was comprised of a ligand binding site situated in the extracellular domain, transmembrane helix, cytoplasmic region constituting protein tyrosine kinase along with carboxy-terminal and juxtamembrane region. EGFR, FGFR, PDGFR, VEGFR, and IGF1R were the most prominent RTKs expressed in TNBC cells. The mono-therapeutic strategy comprises selective inhibitors namely trastuzumab, imatinib, and bevacizumab which could not provide significant benefit to TNBC patients and even failed in reducing the progression of tumor cells (Fig. **1**) [12 - 14].

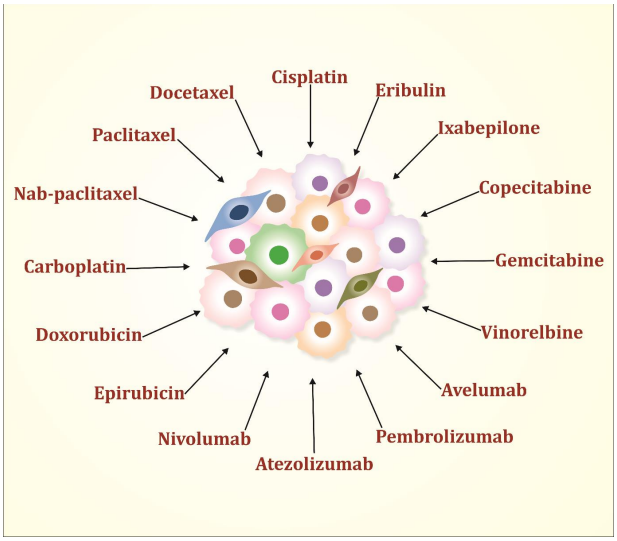


**Fig. (1).** Figure representing available conventional therapeutic regimens against TNBC.

However, precise interpretation of mechanistic insights involved in complicating events was highly essential for attaining the optimum results. Elevated expression of acute-phase proteins (APPs) has further enhanced the tumor recurrence rate by 2-fold. Increased expression of these proteins might lead to the overexpression of pro-inflammatory cytokines in TNBC cells. Different studies also unveiled that the elevated expression of IL-6 is directly linked with the worst prognostic rate in TNBC [15, 16]. Apart from this, prolonged consumption of NSAIDs could significantly reduce the risk of tumor initiation and further development. These data indicated that inflammation may also lead to the development of breast cancer. Pro-inflammatory cytokines named IL-6 and IL-8 can increase tumor growth and metastasis by hindering the tumor cell biology and by activating stromal cells such as tumor-initiated macrophages, vascular endothelial cells, and fibroblasts in the TNBC microenvironment [17, 18].

# CHAPTER 6

# Herbal Medicine: Prejudice to Realm of Reality Against TNBC

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**Abstract:** In triple-negative breast cancers, there exist tumor-specific vulnerabilities that can be targeted to avoid compensatory adaptation of cancer cells in response to standard pharmaceutical therapies. Natural moieties are well-known to possess a multitude of medicinal properties and deserve attention for TNBC prevention and therapy. To overcome drug resistance and efficacy issues, the exploration of natural moieties as targeting agents may emerge as dynamic, promising, and new therapeutic strategies to benefit TNBC patients. This chapter summarizes the role of polysaccharides, flavonoids, phenols, saponins, and taxanes in targeting TNBC. The potent role of herbal medicine in targeting molecular signalling pathways with special emphasis on their ability to target uncontrolled proliferation, metastasis, angiogenesis, and autophagy has also been discussed. Furthermore, the ability of herbal medicine in inhibiting PI3K/Akt/mTOR, STAT3, and Wnt/β-Catenin has also been explored. Combinational therapy comprising chemotherapeutic drugs and active plant constituents was also explored to overcome the complications of TNBC.

**Keywords:** Anti-proliferative, Artemisinin, Bruceine, Chemo-sensitization, Chrysosplenol, Ebushicao, Flavonoids, Guggal, Multidrug resistance, Oleuropein, Oroxylin A, Phenols, Plumbagin, Polysaccharides, Pro-apoptotic, Saponins, Taxanes.

## INTRODUCTION

TNBC exhibited highly invasive and malignant behaviour with a higher disease recurrence rate. TNBC can also be referred to as a collection of different breast cancer subtypes but at the molecular level, its characteristic features have not been fully understood. It lacks a selective therapeutic approach and efficient prognostic marker against the disease. For both early and advanced stages of TNBC, chemotherapy was observed to be the major systemic therapeutic approach.

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Because of all these reasons, TNBC has become a new hot spot of research in the scientific community. Multiple drug resistance was observed to be another major issue associated with TNBC which has led to severe mortality because of the disease [1 - 4]. Hence, it was highly needed to understand the molecular basis of drug resistance because of which it became almost impossible to combat complications of TNBC. Several other mechanisms were also observed to be linked with instances of drug resistance, for example, alteration of drug efflux membrane transporters such as MRP1, altered beta-tubulin, P-glycoprotein, BCRP, and MRP family comprising 9 members. Anthracycline-based drugs such as daunorubicin, mitoxantrone, doxorubicin, and epirubicin; taxane-based drugs such as docetaxel and paclitaxel, and capecitabine were observed to be resistant to TNBC patients. Cancer stem cells were also another major factor contributing toward disease reoccurrence on the virtue of their ability to exert immune resistance against therapeutic regimens and to maintain stemness in the environment. Many side effects were also linked with the usage of chemotherapy. A decline in the concentration of white blood cells and red blood cells was observed in patients undergoing chemotherapy which has further enhanced the risk of anaemia and infection. Cancer stem cells possessed the ability to populate tumor mass again with a subsequent increase in new cancer cell supply. Side effects of chemotherapy also include continuous hair loss, fatigue, nausea, loss of appetite, constipation, change in skin colour and altered hormonal levels are . Due to these reasons, the application of chemotherapy against TNBC cannot be carried out blindly. Therefore, there exists an urgent need for an alternative therapeutic approach for the proper management of disease and to minimize the side effects induced by conventional treatment strategies [5 - 7].

## NATURAL PRODUCTS AGAINST TRIPLE NEGATIVE BREAST CANCER

Herbal medicines have been extensively used for many centuries to treat several acute and chronic disorders. Natural compounds can be prominently considered valuable resources for the development of efficient anti-cancer agents to treat breast cancer. Active moieties present in plants may exert anti-tumorigenic properties and therefore may contribute to optimize possible therapeutic effects. Natural drugs obtained from the plant may play a beneficial role in the development of chemotherapeutic regimens. Approximately 60% of currently used anti-cancer drugs were either directly or indirectly procured from natural resources. Paclitaxel, camptothecin, and vincristine were derived from plants and are currently being used as an efficient therapeutic approach [8 - 10]. But the identification of only these 3 compounds cannot resolve the issue. Hence there exists an urgent need for the discovery of more therapeutic molecules to combat

the issue of breast cancer. There were four major classes in which plant-derived anti-cancer drugs can be categorized for their clinical utility named as:

(a) Vinca alkaloids (b) Taxanes (c) Epipodophyllotoxins (d) Camptothecin

Epipodophyllotoxins and camptothecins are currently under clinical trials against breast cancer and the results were quite promising. Different studies revealed that plant-derived compounds possessed the potency to exhibit pleiotropic anticancer effects. The development of a target-specific, effective, dynamic, and promising therapeutic approach using natural compounds against TNBC has remained to be a major challenge. The use of dietary sources and Chinese herbs may emerge as a promising therapeutic strategy. In the past few years, many natural compounds such as curcumin and resveratrol have been observed to play a significant role in targeting TNBC and other subtypes of breast cancer [11 - 13]. In this section of the chapter, we will briefly discuss the role of natural compounds in targeting triple negative breast cancer.

## Role of Polysaccharides in Targeting Triple Negative Breast Cancer

Keeping in view the aggressive behaviour of cancer cells, lack of targeted therapies, and involvement of cancer stem cells in inducing resistance; polysaccharides were extensively explored for their potency to exert anti-breast cancer effect. Huaier polysaccharide (HP), an active moiety extracted from *Trametes robiniophila* Murr, commonly known as mushroom was widely used clinically for the treatment of cancer in the major portions of China. Studies revealed that HP could potentially inhibit breast cancer stem cells in TNBC which ultimately led to the reduction in mammosphere formation and declined the expression of stem related genes and decreased the concentration of aldehyde dehydrogenase as evident from *in vitro* studies and it also retarded the xenograft tumor formation under the *in vivo* conditions. Additionally, HP decreased the expression of ERα-36 which is commonly known as another subtype of ER-α. Furthermore, HP exhibited an antagonistic effect against ERα-36 mediation Akt/β-catenin signalling in TNBC. Henceforth, it can be predicted that HP may target breast cancer stem cells and can potentially emerge as an effective therapeutic strategy. Even the impact of HP on stemness of TNBC was also evaluated and was also compared with the expression of related transcription factors inducing OCT4, BMI1, and Nanog. Treatment with HP significantly reduced mRNA expression of transcription factors in SUM159 and MDA-M--436 cells in a dosage-dependent manner. $ALDH1^+$ cells belong to the subpopulation of breast cancer stem cells henceforth the inhibitory potential of HP was also evaluated against $ALDH1^+$. Results revealed that upon treatment with HP, the $ALDH1^+$ population significantly declined in a concentration-dependent

# CHAPTER 7

# Unveiling the Potency of Phyto-Constituents to Target TNBC: Mechanism to Therapeutics

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**Abstract:** The development of an effective therapeutic approach against TNBC is a formidable challenge at present. Efficacy and drug resistance issues in response to adjuvant and neoadjuvant chemotherapy have prompted the development of new therapeutic regimens. In this concern, the scientific community has started exploring natural sources including medicinal plants exhibiting anti-cancer activity for their potent inhibitory potential against TNBC. The comprehensive analysis underlying the molecular mechanism of action of these natural bio-compounds provided substantial evidence to subject a few of these for clinical application. This chapter highlighted the momentous phytoconstituents as a genetic or epigenetic modulator by inducing demethylation and histone deacetylation in TNBC. Bioactive phytoconstituents including berberine, luteolin, cantharidin, saikosaponin D, wogonoside, and others targeted cell proliferation, metastasis, angiogenesis, autophagy, and induced apoptosis in TNBC. Furthermore, combinational therapy comprising phytoconstituents and chemotherapeutic drugs was explored to improve the clinical outcome of the disease. Additionally, drug ability parameters including bioavailability and bio-absorption of these phytoconstituents were also discussed.

**Keywords:** Absorption, Berberine, Bioavailability, Cantharidin, Chemo-sensitization, Combinational therapy, Curcumin, Demethylation, DNMT1, Epigenetic modulator, Gambogic acid, Histone deacetylation, hTERT, Liposome, Luteolin, Microencapsulation, Phytochemicals, Saikosaponin, Solubility, Synergistic effect, Wogonoside.

## INTRODUCTION

Traditional and complementary medicine, natural medicine was well-known for its potential therapeutic effect against breast cancer. Rapid progression and worst prognostic outcomes of TNBC have restrained the utility of conventional therapeutic regimens against the disease. TCM and herbal medicine were commonly used as an adjunct to enhance the immunological response.

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Based on this evidence, the anti-cancerous effect of herbal medicine was also explored against TNBC. Different clinical trials also supported the ability of herbal medicines to exhibit inhibitory potential against TNBC. Natural medicine and TCM comprising several active moieties have always been considered rich sources of drug development by exerting multiple targeted effects. It has been observed that herbal medicine exhibited this effect by targeting multiple signaling pathways but the exact mechanism behind this was still not clear. It was essential to identify active constituents present in medicinal plants to move ahead with further evaluation. Isolated active moieties do not represent the plant itself. Apart from pharmacological effects, the bioavailability and metabolism of the anti-cancer agent also needed to be investigated. Due to the side effects, drug resistance, reoccurrence issue, natural medicine, and TCM may bring new hope for TNBC patients and can also be preferred as long-term medication. Hence it was highly needed to investigate the associated damage of these agents over the metabolic organs including the kidney and liver. In the previous chapter, we studied the role of plant extracts in inhibiting cell proliferation, angiogenesis, invasion, and metastasis. In this chapter, we will further discuss the role of phytochemicals in targeting TNBC [1].

## Phyto-Constituents as Genetic or Epigenetic Modulator of TNBC

Along with genetic alterations, epigenetic modulations were equally important for the development of sporadic breast cancer, tissue homeostasis, and other key mechanisms including DNA methylation processes which were highly important. Modulation of DNA methylation may lead to several pathological processes including the development and progression of cancer cells. Different studies demonstrated that dietary plant products were highly efficient in inhibiting the growth and progression of cancer cells. Nutri-epigenetics centered their study on the evaluation of the potency of dietary agents to modulate epigenetics. Contrarily to genetic modification, epigenetic alterations exhibited a reversible effect in the early stage of carcinogenesis. Even in the case of breast cancer, the role of the dietary product was observed to be important. Phytochemicals can also induce epigenetic alterations in case of breast cancer. Several clinical pieces of research were conducted to identify the epigenetic modulations in high-risk breast cancer individuals. Epigenetic profiling will also help in the development of much-needed clinical strategies for the prevention of primary and advanced metastatic breast cancer [2].

## Phytochemicals Induced Demethylation in Triple Negative Breast Cancer

Although there is a lack of clinical evidence depicting the role of phytochemicals or plant extracts in modulating epigenetic responses in breast cancer patients.

From *in vitro* studies, it was evident that curcumin can significantly reduce the promoter methylation of RASSF1A and enhanced the related miRNA and protein expression in MCF-7 cells [3]. Curcumin downregulated mRNA and protein levels of DNMT1 and reduced DNA methylation activity in nuclear extract. Hence it can be stated that curcumin hindered the NF-Kβ/Sp1 complex which was bound to the DNMT1 promoter region. Conclusively, it can be said that curcumin may function as a hypo-methylating agent without inducing any side effects. Mirza and colleagues anticipated that genistein, EGCG, curcumin, guggulsterone, withaferin A, and resveratrol may act as significant demethylator. It was observed that these compounds profoundly altered the methylation status of genes and DNMTs expression in MDA-MB-231 cells. A decline in the transcription levels of DNMT1, DNMT3A, and DNMT3B was observed upon treatment with polyphenols. Even the expression of epigenetic regulators named MeCP2 and histone deacetylase 1 was reduced upon treatment with polyphenols. *In vitro* studies, unveiled the importance of sulforaphane as an epigenetic modulator in breast cancer. Lewinska *et al*. showed that in MDA-MB-231 cells, sulforaphane acted as a functional epigenetic modulator. Sulforaphane also induced cell cycle arrest and senescence in MDA-MB-231 breast cancer cells. It also exhibited oncostatin characteristic features including DNA hypomethylation, reduced DNMT1, and DNMT3B expression, and decreased m6A RNA methylation [4 - 7].

## Phytochemical Induced Histone De-acetylation in TNBC

EGCG, a prominent constituent of green tea was known to decrease telomerase activity by promoting apoptosis and retarding cell proliferation in breast cancer cells. But the bioavailability and stability issues have greatly reduced their clinical application. A study was carried out to evaluate the potency of EGCG to modulate epigenetic mechanisms in MDA-MB-231 cells. In a dosage and time-dependent fashion, the pro-drug of EGCG decreased the proliferation of MDA-MB-231 cells without altering breast epithelial cells named MCF-10A. Additionally pro-EGCG and EGCG hindered the transcription of the telomerase catalytic subunit named hTERT by modulating epigenetic mechanism in $ER^-$ MDA-MB-231 cells. Previously also it was observed that DNA methylation and histone acetylation can alter the hTERT expression [8 - 10]. In a time-dependent manner, pEGCG and EGCG inhibited HAT's activity without disturbing HDAC's function in MDA-MB-231 cells. Reduced HATs activity was linked with histone hypo-acetylation in the promoter region of hTERT which was further related to transcriptional suppression of hTERT expression [11, 12]. Hence it can be concluded that pretreatment with pEGCG and EGCG can induce histone modification in MDA-MB-231 cells. This compound also leads to the acetylation of transcriptionally active chromatin markers such as H3 at lysine 9, acetylated histone H3, and ac-H4

# CHAPTER 8

# Phyto-nanotechnology: Enhancing Plant Based Chemical Constituent Mediated Anticancer Therapies

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**Abstract:** 95% of anti-cancer agents were associated with the worst pharmaceutical and pharmacokinetic properties including poor targeted cellular uptake, shorter half-life, toxicity, and many more. In this regard, nanotechnology including nano-medicines, nano-carriers, and nanomaterials may emerge as a beneficial tool to facilitate an efficient delivery of therapeutic regimens by adapting active or passive targeting mechanisms. The nanotechnology-based delivery system of phytoconstituents can efficiently battle against recalcitrant TNBC. This chapter highlighted the nanotechnology-based therapeutic approach including smart nanoparticles, cell membrane-coated nanoparticles, and immunological cell-based nano-systems for the treatment of TNBC. Furthermore, the role of nano-soldiers in improving bioavailability and targeted drug delivery was highlighted. Nano conjugates of curcumin, anacardic acid, EGCG, betulinic acid, gambogic acid, and resveratrol were also evaluated to enhance the pharmacokinetic profile, distribution, and the release rate of respective compounds and ultimately their ability to target TNBC.

**Keywords:** Absorption, BCSCs, Bioavailability, Curcumin, Drug efflux, EGCG, Lymphatic drainage, Metabolism, Nano-medicine, Nano-soldiers, Pharmaceutics, Phyto-nanotechnology, Rapid elimination, Resveratrol, SDDS, Systemic release, Targeted delivery.

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## INTRODUCTION

Phytochemicals are known to play an important role in preventing and protecting against the disease. From ancient times, these phytoconstituents were used to treat different ailments such as inflammation, diabetes, cancer, neurological disorders, and skin-related diseases [1 - 3]. Diverse epidemiological studies revealed that phytoconstituents can efficiently target cancer also. Curcumin, EGCG [4, 5], genistein, quercetin, naringenin [6 - 8], sulforaphane, silibinin, resveratrol, kaempferol [9 - 11], and many more have been identified to inhibit breast carcinoma through the alteration of several signalling transduction pathways and by modulating genes and their product. By inducing apoptosis, and inhibiting cell proliferation, these phytochemicals can potentially exert anti-breast cancer activity. Furthermore, they can inhibit invasion and metastasis, angiogenesis, and other migratory pathways in breast cancer. They can also overcome drug resistance issues and induce sensitivity against radiation-based therapy [12 - 14]. CSCs involved in the abnormal proliferation of cancer cells, disease recurrence, and drug resistance can also be efficiently targeted by using phytochemicals. Hedgehog, PI3k/Akt, Notch, and Wnt/β-catenin were also associated with the renewal capacity of CSCs and these can also regulate $CD44^{+}$/CD24/CSCs stemness [15, 16]. Dandawate and colleagues unveiled the potential role of phytochemicals in inhibiting BCCs and CSCs [17]. Petric *et al*. also showed that phytochemicals can modulate the signalling pathways involved in complicating breast cancer [18]. Evidence provided by Siddiqui *et al*. also stands in support of the anti-breast cancer potential of phytochemicals [19]. Several limiting factors were observed to be associated with current treatment strategies including lack of selective toxicity which can reduce the therapeutic benefits. Because of their non-specific nature, they adversely affected the healthy tissues and impaired the medical diagnostic approach. Preferably the doses of anti-cancer medicines have been decreased to reduce the associated toxicity to normal tissues. Poor drug penetration, bio-distribution, and heterogenic vessels have increased the extravasation of drugs. In comparison to the tumor site, a 10-20% increase in drug deposition was observed in healthy tissues. Sometimes chemotherapeutic agents were unable to penetrate vasculature greater than 40-50 mm which could lead to drug resistance and ultimately may lead to drug failure. The emergence of MDR in tumor cells upon treatment with one therapeutic strategy may induce resistance against other drugs also and eventually cause overexpression of proteins related to drug efflux. Conventional chemotherapeutic strategies were also linked with other several drawbacks including selective toxicity, and many more which have raised a need for the development of drug delivery agents to enhance the anti-cancer potential of these phytochemicals [20 - 23].

Nanoparticles were observed as an efficient agent for cancer diagnosis and as a therapeutic carrier for the delivery of anti-cancer agents. They may facilitate cell-specific delivery and can significantly reduce the cytotoxic effect. Primarily, these technological interventions were aimed to reduce residual tissue toxicity and increase drug solubility. By using this approach, cell-specific delivery can be attained based on permeability and retention effects. This has further facilitated the delivery of drugs in the cancer vicinity but failed to induce therapeutic benefits probably due to solubility issues [24 - 27]. To overcome the limitation associated with phytochemical-based anti-breast cancer strategies, nanotechnology may play a pivotal role in enhancing their efficacy. For imaging, diagnosing, monitoring, and delivery of chemotherapeutic drugs, nanotechnology can increase the anti-breast cancer potential of phytochemicals. The nano-medicinal approach is an integrated strategy comprising pharmaceutics, engineering, information technology, material science, and medicine. This integrated approach has provided a way to understand biological systems in an efficient manner and to analyze the mechanism linked with them. These nano-carriers can penetrate through the cell membrane and can overcome other barriers also thereby enhancing the permeation and transport of drugs. Stability, prolonged circulation duration, and biocompatibility can be significantly improved with the help of nano-medicine. Nano-medicines including micelles, liposomes, and drug-loaded nanoparticles exhibited specific characteristic features that may enhance the ability of drug candidates to pass through biological membranes and to deliver the entrapped drug at the targeted site. Biodistribution, cellular uptake, particle size and shape, and surface chemistry were the major factors affecting the efficacy of the drug that can be overcome through this approach. Clinical trials of nano-particulate chemotherapeutic delivery have been employed to evaluate their potency against different subtypes of breast cancer [28, 29].

## NANOTECHNOLOGY-BASED THERAPEUTIC APPROACH FOR TRIPLE NEGATIVE BREAST CANCER

Nanoparticle-based drug delivery systems have been employed to overcome the limitations linked with hydrophobic drug molecules. These nano-material-based drug carriers can resolve several issues associated with solubility, stability, and toxicity by protecting them from degradation, ensuring controlled release, biodistribution, and enhancing bioavailability *via* a targeted drug delivery approach. The preparation of these biodegradable polymer-dependent nano-carriers has gained enormous attention recently. Polymeric micelles offered a promising approach on the virtue of their ability to self-assemble in nano-sized structures. Amphiphilic copolymers comprising hydrophobic and hydrophilic segments can get assembled into polymeric micelles and this can be achieved at a concentration higher than the critical micelle concentration. These nano-

**CHAPTER 9**

# Novel Implications of Prognostic Markers to Monitor the Disease: An Overview

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**Abstract:** Apart from the absence of targeted therapies, poor prognosis in TNBC has affected the clinical outcome of the disease and has led to cause high mortality rate amongst diseased individuals. The discovery of potential biomarkers to determine the prognostic and predictive value may play a vital role in the development of an effective therapeutic approach and may improve the OS, DFS, and DMFS. This chapter highlighted the role of histological subtyping, lymph node status, lymphovascular invasion, miRNA, Ki-67, TILs, and BRCAness as prognostic markers of diseases. Nevertheless, patient selection and choice of treatment strategy will greatly impact the clinical efficacy of these prognostic markers but will remain to be a matter of further exploration.

**Keywords:** Kaplan-Meier, Ki-67, Lymph-node, Lymphovascular, MiRNA, Prognosis, Tumor.

## INTRODUCTION

Surveillance and Health service reports presented by the American cancer society revealed that there will be approximately 252710 new cases of breast cancer every year and 40610 patients may also die due to the disease which may account for 14% of related deaths. Although the 5-year survival rate of breast cancer cases was higher than 90% and many patients were observed to be experiencing chances of disease recurrence with a higher possibility of metastatic events [1, 2].

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Due to the complications caused due to the metastatic event, oncologists are unable to provide an efficient therapeutic approach to the disease. Henceforth, the determination of promising markers or approaches for the prediction of disease reoccurrence and evaluation of the efficacy of conventional treatment strategies are necessary so that they can be tailored accordingly to benefit the patients. Prediction of efficient prognostic markers may emerge as an efficient technique in reducing the chance of disease relapse and related death risk [3, 4]. Prognostic markers may prove to be of great importance, especially against TNBC which was linked with a higher reoccurrence rate because of its heterogeneous nature. Histology, lymph node status, and tumor size were also analyzed so far to depict the risk prediction linked with TNBC. Tumor size is an independent variable that can significantly influence the prognosis. Smaller tumor size was linked with better DFS in TNBC patients. To validate the same, Rakha and colleagues analyzed the TNBC tumor sample of 282 patients. They observed that in node-positive patients when tumor size was larger than 1.5 cm, then the patient is at higher risk of disease recurrence. Contrary to the above-mentioned study, few other types of research revealed that the prognostic importance of tumor size was independent of the lymph node status in TNBC-affected individuals. Moreover, lymph node positivity cannot be linked with poor prognosis and higher chances of disease recurrence rather it depends upon the biology and microenvironment of TNBCs. Initially, during the 3-5-year follow-up period, there are higher chances of distant reoccurrence in comparison to loco-regional metastasis. Hence, it can be said that there exists a sharp decline in survival rate after the first 5 years of the emergence of the disease and its diagnosis whereas distant relapse time varies from patient to patient. Few studies also investigated the relevancy of lymphovascular invasion as a prognostic marker against the disease. The vascular invasion might also help in the stratification of TNBC into diverse prognostic groups. TNBC and basal subgroups exhibited higher increased lymphatic or microvessel density which was further linked with higher reoccurrence and poor prognosis rate. But further research is required to fully explore the role of lymphovascular invasion as a prognostic marker of the disease. In this chapter, we will highlight the importance of prognostic markers to monitor the disease, OS, DFS, and DMFS in diseased individuals [5 - 7].

## PROGNOSTIC AND PREDICTIVE POTENTIAL OF HISTOLOGIC SUBTYPING IN TRIPLE NEGATIVE BREAST CANCER

Predominantly TNBCs were high-graded invasive carcinomas without any specialized characteristic features such as central necrosis, marked nuclear pleomorphism, lymphocytic infiltrate, and also displayed pushing invasive borders. There also exist numerous rare histologic subtypes that were following the TN phenotype [8, 9]. Apocrine and medullary features, high grade, and

metaplastic carcinoma are the major characteristic features of the TN phenotype. TNBC carcinomas exhibiting apocrine features were likely to be androgen receptor-positive and exhibited a high similarity index to that of the LAR gene expression profiles [10]. Hence their identification may also lead to the development of anti-androgen agents that can significantly trigger AR testing but their role as a prognostic marker against TNBC is not defined yet certain studies represented contradictory evidence in response to the prognostic role of TNBC. According to the World Health Organization classification, medullary carcinomas possessed histologic special types including syncytial growth patterns, brisk lymphocytic infiltrates, and well-circumscribed borders. However high mitotic activity was the worrisome cytological feature of this subtype [11 - 13]. Therapeutic protocols have remained to be the same for these carcinomas similar to that of TNBC. Certain reports postulated that brisk lymphocytic infiltrate may act as prognostic markers in TNBC patient but concrete evidence regarding the same has not been attained yet. In metastatic breast cancer, low-grade spindle and adenosquamous carcinomas are linked with lesser aggressive clinical outcomes. In TN conditions exhibiting low graded neoplasm, two subsets have been identified by histological subtyping named as:-

1. Carcinomas with patho-genomic alterations, genetic instability, and exhibiting salivary gland-like morphological features.

2. Carcinomas with atypical lesions, benign hyperplastic proliferation, and low-grade morphology.

Hence from the above-mentioned evidence, it can be concluded that histological features of TNBC may also function as a prognostic marker of the disease and might help in predicting the clinical outcome of proposed therapies [14].

## PROGNOSTIC AND PREDICTIVE POTENTIAL OF LYMPH NODE STATUS IN TRIPLE NEGATIVE BREAST CANCER

Different studies were carried out to analyze the association of lymph node status and size of TNBC lesions by utilizing SEER data based on the population of affected individuals. Lymph node$^{+}$ TNBC groups showed larger tumor size in comparison to the $LN^{-}$ group. These results stand in support of the previous findings which stated that along with increasing $LN^{+}$ status, the size of tumors also increased gradually [15, 16]. However, the Sidak adjustment method was implemented to make a pairwise comparison, and a substantial difference was identified in the prognosis rate of N1 and N0, N3. To further evaluate the impact of tumor size on the prognosis of the disease, Cox proportional regression model was implemented. BCSS and OS rates between the nodal status groups were predicted upon stratification on the basis of tumor size. In T1 and T3 cohorts,

CHAPTER 10

# Stumbling Blocks in Reinvigorating the Health of Diseased Individuals Through Herbal Medicine

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**Abstract:** Natural products exhibited a profound effect as a template or direct treatment strategy against TNBC. Implementation of natural products as a chemotherapeutic or chemo-preventive strategy faces diverse problems and challenges. Several constraints including selection, identification, and screening of bioactive components furthermore, preclinical and clinical evaluation, and approval from regulatory bodies are other hurdles to the application of phytochemicals in targeting TNBC. Although the natural metabolites possessed the substantial potential to target the disease along with reinvigorating the health of affected individuals. This chapter has highlighted the perspectives and controversies associated with herbal medicines such as consumer preferences, bio-pharmaceutics consideration, HM-HM & HM-CM interactions, and drug regulations, and discussed the need to introduce natural moieties as an alternative therapeutic approach against TNBC.

**Keywords:** Biopharmaceutics, Challenges, Chemo-preventive, Phyto-constituents, Provisions, Regulations.

## INTRODUCTION

In the literature, several natural products have been highlighted that can potentially retard the initiation and development of diverse tumors. In the previous chapters also, we have studied the role of phytoconstituents in targeting TNBC. But several complications were observed to be associated with the clinical utility of these natural metabolites. Although advancements in nanotechnology and other related technologies have enhanced the large-scale clinical application of these natural metabolites [1].

Epidemiological, pre-clinical, and clinical data have supported the dietary intake of these phytochemicals for the prevention and treatment of TNBC. From differ-

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ent research works, it was evident that these phytochemicals can target multiple signalling pathways including uncontrolled proliferation, angiogenesis, invasion, and metastasis. Despite possessing anti-cancerous potential, several developmental challenges are associated with their clinical application. More than 1000 anti-cancer clinical studies have been registered to evaluate the efficacy of these phytoconstituents some of which are still active, completed, or have been terminated. Curcumin, resveratrol, flavonoids, luteolin and many more are in clinical trial. But the major challenge for the pharmaceutical scientists regarding their establishment as an anti-cancerous agent against TNBC is their overall quality, efficacy, safety, drug regulatory board approval, consumer's preference, biopharmaceutics, herbal medicine-herbal medicine interaction, herbal medicine-conventional medicine interaction, toxicity, and several other side effects. Claims often regarding their remarkable healing properties cannot be dealt with just by providing scientific evidence. In a few countries like the United States, these herbal products were recognized as dietary supplements and need to follow several regulatory protocols before being introduced into the market. In spite of *in vitro* and *in vivo* evidence, these phytoconstituents cannot be used directly. There are several perspectives and controversies in terms of their utility as a natural product for the treatment of TNBC. In this chapter, we will deal with those stumbling blocks that have restricted the use of previously mentioned phytoconstituents in reinvigorating the health of TNBC-affected individuals [2, 3].

Several factors were observed to be responsible for the resurgence of public interest in these remedial procedures comprising natural metabolites. These factors include: -

1. Diverse claims regarding the effectiveness of these plant-based medicines.

2. Consumer preferences for these herbal medicines.

3. Superiority of these herbal medicines in comparison to manufactured products.

4. Imprecise belief that herbal medicines may play a vital role in the treatment of several diseases where conventional therapeutic approaches remained ineffective.

5. Highly expensive nature of these orthodox pharmaceuticals.

6. Consideration of herbal medicines as an alternative therapeutic approach.

7. Approach to self-medication.

The use of herbal medicines is more emphasized when the body's tendency for self-healing is more preferred. In ancient times also, these plant-based medicines

were an indispensable source of alleviating several acute and chronic diseases. Even WHO has estimated that approximately 20,000 medicinal herbs are currently in practice. Around 2/3rd of the world's population especially in developing countries prefer these herbal medicines where these conventional therapeutic approaches were not affordable [4 - 6]. In industrialized countries also, cancer patients preferred conventional therapeutic regimens for their treatment. Different difficulties exist in the application of herbal medicine for the treatment of TNBC: -

## DIFFICULTY IN THE IDENTIFICATION OF POTENTIAL DRUG CANDIDATES AGAINST TNBC

Natural metabolites for their biological potential have been screened in different studies. Around 7000 known structures have been identified on the basis of polyketide metabolites that have led to the development of around 20 commercial drugs which exhibited a hit rate of 0.3%. High-throughput screening of these natural sources presented several issues. Problems related to the access and supply of these natural metabolites have raised an alarming situation [7 - 9].

Season or changing environment can greatly influence the constituents of living organisms that can exert an impact on the identification of active constituents. There also exists a possibility regarding the loss of source. In plants also, the extinction rate ranged between 100-1000. It has been predicted that approximately 15,000 medicinal plants out of 50,000-70,000 are currently facing the challenge of extinction [10]. Even if the issue of supply is resolved then another major constraint is that the plant extract is comprised of a complex mixture even after the fractionation. Although these extracts may contain a small fraction of bioactive compounds which may be either structurally related or differ significantly from each other. The initial concentration of the compound of interest primarily may be detected by using the HTS technique and other related procedures. Furthermore, the key compound may remain unstable either in the mixture or in the purified state. These compounds may also exhibit synergistic or antagonistic activity which may get disappear after their separation from the respective mixture. A substantial time is also needed to characterize these molecules. In spite of the availability of these phytochemicals, their examination has remained to be a major problematic issue that has affected the clinical utility of these phytoconstituents for the treatment of TNBC [11 - 13].

## CONSUMER'S PREFERENCE TOWARDS IMPLICATIONS OF HERBAL MEDICINE AGAINST TNBC

Usage of herbal medicines (HM) is highly common in all cultures, especially in China and Japan where it has been used as part of an integrated medicinal

# SUBJECT INDEX

## A



## B

## C



## D

## E



## F



## G

## H



## I



## J



## K



## L

## M



## N

## O



## P



## R

## S



## T

## V



## W

## Acharya Balkrishna

Acharya Balkrishna, Chairman & MD of Patanjali Ayurved and Founder Secretary of Patanjali Research Institute has carried out enormous work in revealing the potential therapeutic role of Natural Medicine and especially phytochemicals to resolve the complications linked with several acute and chronic disorders. He is a flag bearer of the ancient healing & lifestyle traditions of India and has made Ayurveda credible in the modern world. He has proposed several natural medicine to combat several diseases with their scientific evidences and their validated proofs have been published in several renowned journals. With the vision of universal health for over last two decades, he effectively cured more than 1.5 million patients with a number of stubborn, chronic and non-communicable diseases and is continuing the same. To reveal new ways to solve key health problems, fill gaps in knowledge & strengthening of health system he is guiding research works to create and improve preventive, diagnostic, and therapeutic interventions of diseases through herbal medicine.