Natural Products and Their Combinatory Effects in Breast Cancer Treatment

By

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Bachelor of Pharmacy (Hons.)

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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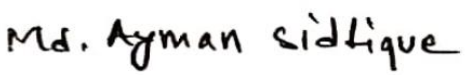

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Approval

The project titled “Natural Products and Their Combinatory Effects in Breast Cancer Treatment” submitted by Md. Ayman Siddique (17146045) of Spring, 2017 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.).

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Ethics Statement

This study does not involve any human or animal trial.

Abstract

Breast cancer is one of the major causes of mortality for women across the globe. As treatment options for breast cancer, hormonal therapy, surgery, chemotherapy and radiotherapy have long been used. Despite advances in cancer treatment, care, and management, there is still an ample space for cancer therapeutic research. The biggest downside to synthetic medicines and cytotoxic drugs is that they are non-selective and, thus, produce various adverse effects as well as multidrug resistance. However, the application of alternative treatments, such as the use of natural products, is becoming beneficial for treating breast cancer. Naturally occurring substances can combat breast cancer aggression, limit the growth of cancer-associated cells, and regulate pathways linked to cancer. This review discusses the potential of natural products and their combinatory effects in breast cancer treatment, along with their mechanisms of action.

Keywords: Natural products; Breast cancer; Combinatory effects; Multidrug resistance

Dedication

Dedicated to those who lost their battle and those who will not stop fighting breast cancer

Acknowledgement

This study could have not been completed without the aid of many people who are here warmly acknowledged.

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List of Acronyms

ABC	ATP Binding Cassette Transporters
AIF	Apoptosis Inducing Factor
AKT	Protein Kinase B
AMPK	Adenosine 5'-Monophosphate Activated Protein Kinase
BAX	Bcl-2 Like Protein 4
BCL-2	B- Cell Lymphoma 2
CDK2	Cyclin Dependent Kinase 2
CDK4	Cyclin Dependent Kinase 4
DRP1	Dynamin-Related Protein 1
ERK1/2	Extracellular Regulated Protein Kinase 1/2
EZH2	Enhancer of Zeste Homolog 2
FAK	Focal Adhesion Kinase
FAS	Fas Receptor
GR	Glucocorticoid Receptor
GST	Glutathione-S-Transferase
HER-2	Human Epidermal Growth Factor Receptor-2
iPLA2	Independent Phospholipase A2
IGF-1	Insulin Like Growth Factor

IKB	Inhibitor Of NF-KB Alpha
JNK	C-Jun N-Terminal Kinase
LEC	Lymph Endothelial Cell
LRP 6	Lipoprotein Receptor-Related Protein 6
MMP-9	Matrix Metalloproteinase-9
MPT	Mitochondrial Permeability Transfer
MTP	Mitochondrial Permeability
mTOR	Mammalian Target of Rapamycin
NAC	N-acetyl-L-cysteine
NF-KB	Nuclear Factor-KB
OPA1	Optic Atrophy Protein 1
PARP-1	Poly ADP-Ribose Polymerase
PMA	Phorbol 12-Myristate 13- Acetate
P-gp	P-Glycoprotein
PI3k	Phosphoinositide 3 Kinase
p38MAPKs	Phospho P38 Mitogen-Activated Protein Kinases
ROS	Reactive Oxygen Species
Skp2	S-phase Kinase Associated Protein
STAT3	Signal Transducer and Activator of Transcription 3

TPA	12-O-Tetradecanoylphorbol-13-Acetate
Twist 1	Twist-Related Protein 1

Chapter 1

Introduction

Cancer is a condition that influences the cells in the body, causing them to alter and evolve out of control. It is the world's second highest in the world and is a major health issue across the globe. Among a variety of cancer forms, breast cancer is a category of disease in which irregular cells proliferate and differentiate inside the breast tissues and create lumps or masses. The majority of breast cancers begin in the lobules or ducts associated with the nipple. Natural products obtained from different sources can facilitate more and more medicinal products for certain particularly aggressive illnesses, such as cancer (Cragg & Newman, 2005). According to Global Cancer Observatory 19.3 million new cancer cases were recorded in the year of 2020. Among them, 2.3 million people have been diagnosed and 684,996 people have died because of breast cancer (Sung et al., 2021). People living in western countries are more drawn to breast cancer than people living in developing countries due to the risk factors associated with breast cancer. Women living in developing countries like Bangladesh are vulnerable because of a lack of economic resources to combat the disease. Natural products or the combination of natural products and synthetic drugs in treating breast cancer might be the right alternative for economically deprived women living in emerging countries (Hossain et al., 2014). Surgery, hormonal therapy, chemotherapy and radiotherapy have been used for long time as a treatment for breast cancer. Their severe adverse effects and multidrug resistance make these therapeutic approaches increasingly ineffective. Nevertheless, it is possible to adopt a supplementary treatment approach since substances from natural sources are obviously active in cancer (Mitra & Dash, 2018). Natural products are made up of a vast number of different phytoconstituents that may each operate on a range of bodily targets in order to elicit pharmacodynamics reactions, and, combined;

they may lead to a clinical response. Natural products can also be used in different combination to treat a variation of ailments. As a result, these drugs have paved the way for strong combination therapies. The creation of multifunctional therapies, which may distort illness networks in particular ways, but with several facets, to exert powerful effects, like typical synthetic medication combinations with natural product extracts. Natural product-based combination medications have a variety of well-established pharmacokinetic and pharmacodynamics benefits, making them excellent candidates for innovative treatment leads (Isgut et al., 2018). Hence, current treatment options are natural products and combinational treatment with natural products and synthetic products to combat the disease. This approach to breast cancer treatment could serve as a model for people living in low-income areas who are infected with diseases.

1.1 Prevalence of breast cancer worldwide

In 2020, according to the World Health Organization (WHO) and Global Cancer Observatory 19.2 million diagnosed with cancer and 9.9 million people died. Among those 19.2 million cancer cases incidence rate of breast cancer is high 11.7% and then lung cancer 11.4%. However, lung cancer still leading in mortality 18% people died with lung cancer globally and breast cancer responsible for 6.9% of fatality (Sung et al., 2021). In the year 2020, in the USA alone, 253,465 breast cancer cases were observed, and 42,617 women died from these diseases. In the global scenario, 2,261,419 new instances of breast cancer were reported, along with 684,996 fatalities in 2020 (Sung et al., 2021). Based on a 2015-2017 NICRH cancer registry report, 1,354 females (29.5%) (with 880 females diagnosed at the ages of between 35 and 54 of this population) were verified with breast cancer. In 2017, 2,095 (31.1%) women with breast cancer (with 1,293 females diagnosed at the ages of between 35 and 54 out of this population) were registered (Based & Registry, 2021). GLOBOCAN 2020 recorded 13,985 breast cancer cases in Bangladesh, killing about 6,783 women (Country-

specific et al., 2020). In 2019, approximately 268,600 new IBC cases and 48,100 DCIS cases were diagnosed, with 41,760 women died. Invasive breast cancer may be detected throughout their lifetime in about 13 percent of women (1 in 8). The risk of a lifetime represents the danger to a woman of death from other causes that may exclude the detection of breast cancer. From 1975 to1989, the average annual breast cancer mortality rate increased by 0.4 percent, then decreased rapidly, totaling 40% in 2017. This decrease prevented 375,900 breast cancer deaths in the USA owing to further treatment (DeSantis et al., 2019).

1.2 Existing cancer treatment options and their limitations

Many types of cancer treatment are available. The kind of treatment you have depends on the type and the progress of the cancer you have. Some cancer patients will only receive one treatment. However, most people have a combination of treatments, like chemotherapy and/or radiation treatment. You can also receive immunotherapy, treatment or hormonal treatment (*Treatment for Cancer - National Cancer Institute*, 2015). The primary treatment for cancer is chemotherapy with cytotoxic agents. It is commonly utilized in a patient's care as a supplement to either surgery or radiotherapy. The latest molecularly targeted drugs are showing potential against gastrointestinal stromal tumors and chronic myeloid leukemia (Lind, 2008). High-grade leukemia and lymphomas can be cured with intensive treatment; low-grade tumors can be managed with medication doses designed to allow normal daily activities (Corrie, 2008). Chemotherapeutic agents such as anthracycline related compounds, topoisomerase inhibitors, tyrosine kinase inhibitors all help to combat the illness through different mechanisms (Lind, 2008). The easiest approach to combating cancer is to reduce its scale rather than battling cancer all over. Tumors contain both innate and inherited mutations that lead to the formation of cancer cells. The most significant limitation of chemotherapy is medication refusal (Corrie, 2008). Hormone therapy is a widely successful as well as non-toxic therapy for both breast and prostate cancer and several other cancers show a moderate

susceptibility to hormonal therapies. Hormone therapy is typically included as a method of functional care prior to more drastic treatment options such as radical surgery, radiotherapy, or even chemotherapy (Abraham & Staffurth, 2020). Surgical facilities are the center of healing for the cancer and plays the critical function for recovery of cancer patients (Dare et al., 2015). Surgical procedures are an integral part of the multidisciplinary treatment plans for breast cancer. Few patients with localized growth may have radiation treatment, but for patients with metastatic expansion, surgery is required (Corrie, 2008). Surgical treatment, which aims to treat tumors and reduce their mass, can actually encourage the formation of new tumors. If the factors that promote the capture and promotion of residual metastases can be addressed during the perioperative period, the immediate postoperative period may become a unique window for controlling residual malignant cells (Tohme et al., 2017). Over the last 100 years, radiotherapy has been the main treatment for cancer, with more than a third of patients completely receiving radiotherapy. The aim of the radiation therapist is to deliver the tumor tissue with a lethal dose of ionizing radiation damaging its DNA, accelerating cell death when they attempt to divide (Murray & Lilley, 2020). Furthermore, brachytherapy utilizes a radioactive source to specifically address the tumor. Commonly utilized nuclear products contain cesium-137, iridium-192 and iodine-125. This is the radiotherapy treatment procedure for breast and ovarian cancer (Robinson, 2008). Adjuvant therapy is any medication offered following an initial treatment aimed at helping to aggressively cure a tumor until it has been entirely eliminated. In most cases, patients are not diagnosed with a metastatic illness. Adjuvant treatment has demonstrated that the wellbeing and success of treatment for a range of cancer patients has improved. The reasoning for adjuvant therapy is focused on the assumption that certain patients harbor unavailable micro metastases that could not be identified at the time of tumor diagnosis. As adjuvant therapy targets occult metastases, or to reduce cancer mortality. Thus, cancer care can take a variety

of forms, including chemotherapy, hormone therapy, immunotherapy and radiotherapy (Parmar, 2007).

1.3 Natural products as cancer remedy

Natural plants and animal products have in the past been a source of almost every therapy and in the past they prototypes for pharmacologically active molecules, particularly in cancer and antimicrobial medications (Harvey et al., 2015). Despite the advances in cancer care and management that have been made, there is also ample space for more progress. The biggest downside to synthetic medicines is that they have related adverse effects. However, the application of alternative treatments, such as the use of natural foods or plant-related natural ingredients, is becoming beneficial for treating cancer. In the year 1950, the quest for anti-cancer agents from plants began with the isolation of *Vinca alkaloids* (VLBs) (Cragg & Newman, 2005). There were only 174 substances (drugs) licensed for use by the year 2014 (Newman & Cragg, 2016). In addition to their presence in *Catharanthus roseus, vinca alkaloids* are also found in *Vinca rosea*. The extracts of Sweet Violets are very useful for treating cancer patients. That was the first time *vinca alkaloids* were shown to have anti-tumor activity. Taxanes show peculiar cytotoxic activity by targeting cellular microtubules, rather than enabling microtubules to get disrupted, as *vinca alkaloids* do. They block microtubules from disassembling, thereby interfering with several cellular activities that need frequent microtubule assemblage and disassembly. Trabinectin has also received the most scientific research. In September of 2007, it was given marketing permission for the treatment of soft tissue sarcoma following the failure of conventional chemotherapy (Blay et al., 2005). Chemoprevention is a successful cancer therapy that decreases the incidence of cancer by preventing the occurrence of cancer. Curcumin is a cancer preventive drug. Curcumin has also been found to have anti-tumor efficacy in *in vitro* tumor models and in tumor animal models (Duvoix et al., 2005). Nutraceutical is a pure substance isolated from

foods, not typically correlated with foods. It was an original concept that merged diet and pharmaceuticals. Nutraceuticals ought to be shown to provide physiologic effects against chronic diseases (Foster et al., 2005). Cancer is a complex disease that requires a wide range of novel anti-cancer therapies. It is no longer cost-effective to concentrate on a specific aspect of the disease, such as cancer or metabolism. A multi-target approach is required today for the management of dynamic cancer biology, which is aimed at the integrated use of natural and synthetic cancer agents capable of synergizing various carcinogenesis signals (Salehi et al., 2018).Thus, we can use natural products because nature has a number of extremely potent chemicals that are all very effective at combating cancer.

1.5 Rationale of the study

In this era of growing demand for treating chronic conditions like cancer, natural compounds have started to employ as an affordable treatment option in to the oncology segment. Natural ingredients like curcumin, carvacrol, quercetin and many more have been employed in the cancer therapy and constitute a significant research field for developing and producing anti-cancer drugs to deliver an accessible treatment option in this sector of oncology paradigms. Historically, hormone therapy, surgery, chemotherapy, and radiation have been used to treat breast cancer. But these therapies are becoming ineffectual due to significant adverse effects and multi-drug resistance. The adoption of an additional therapeutic technique might nonetheless be an important solution to this circumstance since it is known that natural source chemicals show potential role in anti-cancer activity. Natural chemicals may combat breast cancer aggression, limit the multiplication of cancer cells, and alter the pathway associated with cancer. This approach has already started to receive attention since it is an affordable accessible alternative to breast cancer therapy. Furthermore, it remained one of the major reasons of cancer prevalence and fatalities with an estimated 2.3 million new cases and 684,996 deaths in 2020 which led to the study of the available therapeutic possibilities for

breast cancer. The goal of this study is to highlight and discuss the possible roles and effects of natural substances that will enhance substantially the survival rate of breast cancer patients in light of existing treatment choices for breast cancer.

1.6 Aim and objectives of the study

The aim of this review on 'Natural Products and Their Combinatory Effects in Breast Cancer Treatment' is to provide an unparalleled opportunity to understand natural compounds better as an accessible and inexpensive alternative therapy and to explore their chemotherapeutic uses that may lead to a new frontier for the treatment paradigm of breast cancer.

The objectives of the study:

- To compile information on the significance of different natural compounds in breast cancer treatment,
- To explain the potential of individual natural compound to treat breast cancer along with the mechanism of action,
- To delineate the therapeutic benefits of combination of natural compounds and combination of natural and synthetic drugs in breast cancer treatment with their mechanisms of actions,
- To address the potential of natural compounds in reversal of multi-drug resistance.

Chapter 2

Methodology

The current study included a comprehensive overview of the current treatment options for breast cancer with natural products, as well as combinational treatment with natural and synthetic products to improve the disease. The information for this review paper was collected from various primary sources, such as Nature, the American Cancer Society, The Lancet, Cells, NCBI, Springer, Google Scholar, etc. Information is also collected from secondary research articles such as Elsevier, PubMed, Medline, MDPI etc. After extracting necessary information from all the articles, an outline was created to present the information in an orderly fashion. At first, it was important to identify potential available natural compounds to combat against breast cancer and natural products for multi drug resistance breast cancer cells. Later, a further literature search was performed on their mechanism of action and outcomes utilizing anti-cancer natural compounds to treat the disease. It was given proper attention to the use of valid and reliable information as well as the appropriate citation of literature.

Chapter 3

Breast cancer

Breast cancer is the most common malignancy in women, accounting for more than one out of every ten new cancer diagnoses each year (Simon & Robb, 2014). Breast cancer is a malignancy that develops most frequently in the inner layer of the milk canals or lobules that provide milk to the ducts. This illness is currently the major cause of worldwide cancer, with an expected 2.3 million extra patients in 2020 and represents 11.7% of all cancer cases (Sung et al., 2021). The uncontrolled growth and development of cells in the breast tissue is defined as breast cancer. Two different tissue types are present in the breast: glandular and stromal. The milk-producing glands and ducts comprise glandular tissue, while fatty and fibrous breast connective tissues contain stromal tissue. The breast also comprises lymph tissue, which is an immune tissue that removes cell fluids and debris. In different areas of the breast, different types of cancers can occur. Benign breast alterations are the cause of most cancers. For example, fibrocystic transition is a non-cancerous disorder when people acquire cysts, fibrosis, lumpiness, thickening areas, tenderness or pain in the breast. Others come from cells lining the lobules (lobular tumors), whereas others begin with a small number (Sharma et al., 2010a).

3.1 Breast cancer types

Many breast cancer varieties exist with various means of describing them. Breast cancer is determined by each of the affected breast cells (American Cancer Society, 2017). The form of breast cancer can be also determined by site, by frequency, by the degree, affected cells etc. to which the breast tissue spreads and by the level of the hormone (American Cancer Society, 2017).

Breast cancer types include non-invasive breast cancer (NIBC), in which cells are confined to conducting and do not enter the fatty and connective tissue of the breast. The most prevalent kind of non-invasive cancer of the breast is ductal carcinoma in situ (DCIS). The occurrence of lobular in situ carcinoma (LCIS) is low and is expected to cause breast cancer. Invasive breast cancer (IBC) cells penetrate the fatty tissue of the breast when they breach the duct and lobular walls. The most frequent kinds are invasive ductal and lobular carcinoma. Invasive ductal carcinoma represents around 70-80% of all forms of breast cancer. Lobular carcinoma *in situ* (LCIS) is a common type of breast cancer. The LCIS is characterized by a significant increase in the number of cells in the milk glands (lobules) of the breast. Ductal carcinoma *in situ* (DCIS) is localized to the breast ducts as the most prevalent kind of non-invasive breast cancer. Invasive lobular carcinoma (ILC) begins in the milk glands of the breast (lobs) but can spread to other parts of the body. In all breast cancers, ILC accounts for 10 to 15%. Invasive ductal carcinoma (IDC) starts from the breast's milk ducts, penetrating the tube wall and invading the tissue of the breast and maybe other regions of the body. IDC represents 80 percent of all diagnoses, the most frequent type of breast cancer. Medullary carcinoma, a progressive breast cancer with distinct tumor-to-natal tissue boundaries, is a less common type of breast cancer. Only 5 per cent of all breast cancers are due to medullary carcinoma. Mutinous carcinoma is also characterized as colloid carcinoma, an uncommon kind of breast cancer due to mucosal cancer cells. Tubular carcinoma is a specific form of IBC. It represents around 2 percent of the diagnoses of breast cancer. Inflammatory breast cancer (IBC) is described as inflammatory breasts (red and warm) with pinches and thick ridges aggravated by cancer cells that obstruct lymph veins or ducts on the surface. It makes up from 1 to 5% of all breast cancers (Sharma et al., 2010a). Furthermore, angiosarcoma of the breast is an unusual form of cancer that begins in the cells that line the blood and lymph vessels. It is frequently a side effect of prior breast radiation therapy. It can occur 8-10 years after

receiving breast radiation therapy. Phyllodes breast tumors are rare breast cancers that begin in the breast conjunctive tissue. For women with Li-Fraumeni disease, the chances of phyllode tumors are higher (American Cancer Society, 2017). Positive hormone receptors are found in the three main subtypes of breast cancer. cancer, which is 2/3 of all breast cancers, requires the development and replication of female hormones (estrogen and/or progesterone). Estrogen receptor (ER) positive cells in this form of breast cancer have receptors that enable them to develop by using the hormone estrogen. Estrogen induces breast epithelial cell differentiation and proliferation by interacting with the estrogen receptor (ER) in the nucleus. Prolonged estrogen use is a concern for breast cancer (Dai et al., 2017). Progesterone receptor (PR) positive, this form of breast cancer is PR positive, and the cells have receptors that enable them to use this hormone to mature. Endocrine treatment is used to prevent cancer cell growth (Dai et al., 2017). Hormone receptor (HR) negative, since this form of cancer lacks hormone receptors, it will not respond to endocrine therapies that block hormones in the body. These types of patients have a lower chance of death following diagnosis than people with ER- and/or PR-negative illness (Dai et al., 2017).

Furthermore, an excess protein called human epidermal growth factor receptor 2 is seen on the surface of HER2-positive breast cancer cells. This additional HER2 receptor facilitates the growth of cancer cells. HER2-positive cells can be beneficial or harmful to hormone receptors. Herceptin (trastuzumab) has been shown to be especially useful in the treatment of HER2-positive breast cancer (Dai et al., 2017).

Triple negative breast cancer (TNBC) lacks all three of the receptors typically present on breast cancer cells. Approximately 10 to 15 percent of all breast cancers are represented by TNBC. It applies to cancer cells that lack ER and PR as well as a high level of the protein HER2 (All three experiments resulted in "negative" results for the cells.) These cancers are

more frequent in women under the age of 40, African-American women, and women with a BRCA1 mutation (American Cancer Society, 2017; Dai et al., 2017).

3.2 Types of cells lines responsible for breast cancer

In the modeling of breast cancer, breast cancer cell lines have been widely used to encompass a range of illnesses with different phenotypic correlations. Though cell lines are reasonably simple to culture and provide an unrestricted supply of homogeneous materials for tumor studies, they are known to accumulate mutations during the initial establishment and subsequent sequence of cultivation. Thus, if the variability of breast cancer cell lines resembles that of carcinoma, it remains a critical question to be resolved before making any credible conclusions at the tumor level utilizing cell lines (Dai et al., 2017). About 50 years ago, in a Baltimore laboratory, George Gey produced the first human cell line. The concept of Gey opens the way for cell culture, as we now know it, which makes it extensively employed in the scientific study of cancer. A key benefit of cancer research is that the cultivated cell lines have an unending supply of an adequately homogenous cell population capable of replicating themselves in the usual cell culture medium. BT-20 was the first cell line for breast cancer to be found in 1958. However, it took another 20 years for the establishment of breast cancer cell lines to become more mainstream, like the MD Anderson series and MCF-7, which is now the most widely used breast cancer cell line in the world, which was founded in 1973 by the Michigan Cancer Foundation. MCF-7's prominence stems largely from its exquisite hormone sensitivity through estrogen receptor (ER) expression, rendering it an ideal model for studying hormone response (Holliday & Speirs, 2011). The MDA MB-231 cell line is an epithelial human breast cancer cell line that is widely used in medical research labs. Aside from MCF-8, which is derived from pleural effusion and is one of the most commonly used cell lines in breast cancer worldwide, the Michigan Cancer Foundation (from which the name was derived) in 1973, and the MDA-MB-231 lines of other cells are frequently used as

breast cancer models. Other commonly used cell lines include 600MPE, Hs578T, AMJ13, BT-549, T-47D, SkBr3, and others (Burdall et al., 2003; Neve et al., 2006).

3.3 Diagnostic and treatment approaches for breast cancer

The drastic changes in screening procedures, early detection and advances in care are responsible for the increased survival in the breast cancer treatment paradigm (Nounou et al., 2015). Breast imaging covers all imagery methods utilized for breast cancer detection and diagnosis. The most popular method of breast imaging is an x-ray test of the breast, mammography (Bassett & Lee-Felker, 2018). Several diagnostic approaches of breast cancer are given below:

Mammography: In several breast-screening facilities, digital mammography (DM) has been substituted for traditional (film screen) mammography. DM's potential benefits involve the usage of machine-aided identification, algorithm-based computer programs, which warn the radiologist of suspected mammogram abnormalities and allow centralized movie readings. However, the common usage of mammograms requires careful monitoring of possible risks of radiation (Bassett & Lee-Felker, 2018).

Magnetic resonance imaging (MRI): It is a flexible imaging technology which produces pictures with a high resolution without the use of harmful radiation. In order to generate MRI images, this technique is close to nuclear magnetic resonance by studying a proton map of the tissue. Breast MRI is not often used in breast cancer diagnosis. The MRI's high sensitivity allows for early detection of breast cancer despite its poor selectivity (Bassett & Lee-Felker, 2018).

Molecular breast imaging (MBI): MBI employs a radiation tracer that illuminates cancerous breast tissues as seen by a nuclear medicine detector. This method is also known as the

Miraluma examination, sestamibi test, scintimammography, or specific gamma imaging. (Bassett & Lee-Felker, 2018).

Breast biopsy: Breast biopsies are the most conclusive means of diagnosing breast cancer. Breast biopsies are classified into many groups. Medical breast analysis, breast ultrasound, and biopsy are often done at the same time to improve diagnostic precision and remove as many false negative findings as practicable (Bassett & Lee-Felker, 2018).

Blood-based assay: CA 15-3, carcinoembryonic antigen (CEA), and CA 27-29 are breast biomarkers. All have poor sensitivity and accuracy, making them ineffective for early diagnosis of breast cancer. Only in metastatic environments can CEA, CA 15-3, and CA 27–29 be included, according to the American Society of Clinical Oncology (Nounou et al., 2015).

Available treatment options for breast cancer are given below**:**

Surgery: Breast conservation surgery is a common treatment option for locally advanced breast cancer. To reduce tumor bulk, neoadjuvant therapy is administered prior to surgery. Adjuvant therapy is often used in surgery to ensure full cure and to minimize metastases (Makhoul, 2018).

Radiotherapy: Cancer cells that are not visible during treatment may be destroyed by radiation, lowering the chance of local recurrence. In conjunction with CT, RT following treatment shrinks the tumor. However, there are certain side effects of RT, such as reduced sensitivity, soreness, swelling, redness, and the skin may become wet and weepy at the end of RT. Radiation therapy is a form of brachytherapy. It is also known as rapid partial breast irradiation (APBI). APBI only sends radiation to the region around the tumor. This will eliminate the need for total breast radiation. It also reduces the number of therapy sessions

needed. The long-term consequences remain unknown. Furthermore, which women would reap the most is also being researched (Makhoul, 2018).

Endocrine therapy: ET's role is to either balance or block hormones. ET is recommended for all patients with measurable ER expression, identified as 1% of invasive cancer cells, regardless of CT or targeted therapy (Nounou et al., 2015)**.**

Chemotherapy: CT has a larger effect on ER-negative cancers. CT is indicated for the vast majority of TNBC, HER2-positive malignancies and most high-risk malignancies. In ER-positive tumors, CT plays a function in the induction of ovarian failure, at least. Overall, the CT regimen based on anthracycline and taxanes decreases breast cancer deaths by around 1/3 (Makhoul, 2018). Monoclonal antibodies: They are being designed to find and attack cancer cells on their own or in conjunction with CT and RT. Normally, the immune mechanism of the body searches for a foreign invader, such as cancer. It will then develop antibodies to aid in the battle. Cancer cells are not recognized by the body as a kind of external invader. As a result, antibodies are not made. Trastuzumab (Herceptin) is an example of a monoclonal antibody that has been licensed by the FDA.

Targeted treatment: It may prevent the effect of an abnormal protein (like HER2) that promotes the development of breast cancer cells (Makhoul, 2018).

Bisphosphonates: Some evidence suggests that bisphosphonates have an anticancer effect, especially when used in a low-estrogen context. Bisphosphonates reduce the incidence of skeletal problems in patients with treatment-related bone loss (Nounou et al., 2015; Sharma et al., 2010b)**.**

Natural products to treat breast cancer

Natural chemicals may combat breast cancer aggressiveness and inhibit cancer cell development and alter cancer-related pathways. A broad array of clinical trials nowadays

focuses on natural and nutrient elements for novel and more efficient breast cancer therapeutic solutions. The current study discusses various key natural compounds which are able to combat breast cancer extremely effectively and to become much more active via careful adjustments and subsequent trials. Future studies with natural compounds may offer a new frontier for breast cancer (Shareef et al., 2016).

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Chapter 4

Natural products in breast cancer treatment

Plant-derived medications have been used to treat several pathological disorders. These drugs are used as compounded or concentrated plant extracts, in which the active components have not been isolated (Thomford et al., 2018). Certain plant components and their processes for being employed as anti-tumor medicines are quite valuable (Ouyang et al., 2014). Although these approaches have historically shown greater utilization, over the last two decades they have decreased because of technological (Harvey et al., 2015). In anticancer treatment, natural sources, such as plant extracts or fluids, operate as both a therapeutic and protective agent. Drugs of natural origin are one of the last hopes for preventing or reversing breast tumor growth (Levitsky & Dembitsky, 2015).

4.1 Curcumin

Curcumin, a long curcumin polyphenol molecule, has been extensively studied for its anti-cancer properties. It has been demonstrated to inhibit a range of tumor induction, growth and metastasis (Shanmugam et al., 2015). Kim et al, conducted a study on the cytotoxic impact of curcumin on H-Ras induced MCF10A epithelial cells. Downregulation of Bcl-2 and upregulation of Bax during apoptosis in the cells of H-Ras MCF10A is consistent with curcumin-induced cell death. Notably, caspase-3 and ROS are both involved in apoptosis in H-Ras-induced MCF-10A cells. Moreover, Mcl-1 another anti-apoptotic gene was than the controlled group after curcumin (Koohpar et al., 2015). Curcumin induces apoptosis through the signaling pathway PI3K/Akt. It causes phosphorylation of Akt, but the PI3K inhibitor combination treatment, LY290042, synergizes the apoptotic impact. Curcumin-induced apoptosis in the MCF-7 cells is impaired by blocking the PI3K/Akt pathway with LY290042 12 (Kizhakkayil et al., 2010). The miR-17-92 cluster overexpression is a significant

oncogenic occurrence for a number of cancers. The recognized endocrine disruptor bisphenol A (BPA) is correlated with breast cancer growth. Curcumin reduced oncogenic miR-19a/b expression in another sample of induced BPA-MCF-7 breast cancer cells. The expression of miR-19-related target proteins and proliferating nuclear cell antigen was enhanced by curcumin. The proliferation impact of BPA on MCF-7 cells was reduced by curcumin via modulation of the miR-19/PTEN/AKT/p53 axis (X. Li et al., 2014). It has also been shown to inhibit cyclin D1 expression. In many cancers, the p53 gene is inactivated, and the transcription factor NFB is activated, which has a significant impact on the progression of the disease. Curcumin increased p53 expression, enhanced DNA-binding function, and delayed effector Bax expression, hence apoptosis occurred. Curcumin, in short, improves growth suppressor factors like p57Kip2, TP53, and Rb, decreases proliferative related pathways like NFκB, AP1, PI3K, Sonic Hedge-hog, TGF beta, JAK STAT, MAPK, and Wnt-Beta-catenin, angiogenesis (VEGF) and enhanced apoptosis (BAX, BIM, PUMA, BCL2, and BCL-XL) (Shanmugam et al., 2015).

4.2 Carvacrol

Carvacrol is a significant part of *Origanum vulgare* and *Thymus vulgaris* (Kiskó & Roller, 2005; Lampronti et al., 2006). Arunasree et al, results showed a concentration dependent increase in the sub G0/G1 phase of the apoptotic cell cycle and a reduction in the S phase cells, signaling stimulation of apoptosis and DNA synthesis in the S phase. In addition, carvacrol treatment membranes potentially lead to the release into the cytosol of mitochondrial apoptosis initiator factors (AIFs), cytochrome-c and Apaf-1, which in turn activates the caspase-9, -3 and -7 enzymes, which leads to a decrease in this cytochrome-c membrane potential of the cells and membrane stability shifts. Also, poly ADP-ribose polymerase (PARP) cleavage observed also leads to DNA stand breakage. However, Bcl-2 is reduced while Bax is increased, resulting in a decreased Bcl2/Bax ratio and subsequent

apoptosis activation (Arunasree, 2010). Ahmed et al, study presented that carvacrol treatment revealed that the Bcl-2 gene is regulated and that the Bax gene is up-regulated depending on the dosage. The Bcl2/Bax ratio was therefore decreased, signaling the cells to apoptotic death. The result thus revealed that carvacrol could have caused apoptosis via p53 and mitochondrial processes. Carvacrol-induced apoptosis is regulated by mortal receptors exhibiting elevated p53, Bax, caspase-9, -6 and -3 expression levels (Al-fatlawi, Rahisuddin & Ahmad, 2014).

4.3 6- Shogaol

Shogaol, the active component of several spices in the family Zingiberaceae, is widely used and has an anti-cancer effect in many neoplasms (Ishiguro et al., 2007). Ray et al, conducted studies with 4 different monolayers and spheroids. 6-Shogaol therapy triggers the death of all breast cancer cells. The CD44+CD24-/low cell percentage comprising a reduction in the formation of secondary spheroids has also been decreased. 6-Shogaol-caused LC3 alteration of lipid chain and acidic vacuole development inside the cell are thus released to cell death via auto phagosomes. Also, 6-shogaol reduces the expression of cleaved notch-1, and notch 1 target proteins hes-1 and cyclin D1 in spheroids and further spheroid development is also reduced by the presence of the γ-secretase inhibitor (Ray et al., 2015). 6-Shogaol blocked MDA-MB-231 cell invasion when stimulated with PMA, accompanied by a decline in matrix metalloproteinase-9 (MMP-9) secretion. By inhibiting IkB phosphorylation and deprivation 6-shogaol inhibited NF-kB transcriptional activity, which resulted in the repression of NF-kB p65 phosphorylation and nuclear translocation (Ling et al., 2010).

4.4 Resveratrol

Resveratrol is a natural polyphenol found in grapes *Vitis vinifera*, berries, peanuts, and medicinal plants (Baur & Sinclair, 2006). Resveratrol stops the cell cycle in the G1 step,

phosphorylating pRb complexes in cyclin E/cdk2 (Alamolhodaei et al., 2017). Current cell cycle analysis, survival and apoptosis revealed that G1 triggered resveratrol was associated with lower survival and higher sensitivity to TRAIL-mediated apoptosis. The outcome was reduced survival and sensitized cells in G1 arrest induced by treatment with mimosine and p21 over expression (Fulda & Debatin, 2004). EZH2 in breast cancer is frequently over-expressed and is necessary for cell proliferation. Hu et al. discovered that expression of EZH2 was necessary in ER-positive breast cancer for estrogen-induced cell proliferation and it was monitored by ER and ERK1/2. Furthermore, resveratrol inhibited the development of ER-positive breast cancer cells and downregulated EZH2 (C. Hu et al., 2019). Tang et al, found that IGF-1 is a powerful stimulant of ER-negative cell migration. Resveratrol exerts its inhibitory role in part by inhibiting the activation of the PI3K/Akt signaling pathway. Additionally, resveratrol greatly inhibited the expression of MMP-2 which is also responsible for invasion and migration induced by IGF-1, concurrently with a change in cell invasion (Tang et al., 2008). Additionally, resveratrol alleviated PI3K/Akt/mTOR signaling by decreasing Akt phosphorylation and increasing PTEN expression (Khan et al., 2014). Gomez et al, showed that glycolysis requires the enzyme 6-phosphofructo-1-kinesase (PPFK), which is directly proportionate to the cells' glucose level of activity. In the human breast cancer cell line MCF-7, resveratrol was able to decrease lifespan, glucose consumption, and ATP content by inhibiting PFK. As a result, a new target has been identified in the mechanism by which resveratrol exerts its anti-tumor effects (Gomez et al., 2013).

4.5 Withaferin A

Withaferin A is a well-known substance in the *Withania somnifera* plant. The potential of Withaferin A to disrupt many signaling pathways and interact with different tumor cells shows the promise of anti-cancer effects (Sivasankarapillai et al., 2020). Withaferin-A works by inhibiting the expression of NF-κB and mTOR pathways in a dose-dependent manner.

These alterations remained associated with an increased expression of Bax and a decreased expression of Bcl-2 (X. Liu et al., 2019).Withaferin therapy may induce harmful alterations of mitochondrial dynamics in a manner that can establish and increase cellular volume. OPA1 and DRP1 have anti-apoptotic properties and are essential in late embryonic growth. Inhibition was found to cause apoptosis. The most interesting feature of the process of withaferin-induced apoptosis is that it includes the modifications of both complex III function and mitochondrial dynamics in cancer cells (Sehrawat et al., 2019). Treatment with just WA was found to be inhibitory on NF-kB activation, causing little to no ERK phosphorylation, but only dose-dependently inhibits HSP90 (H. C. Wang et al., 2019). These findings suggest that differences in the mitochondrial dynamics of withanolides and withaferin may be used to target withanolides and other breast cancer cell lines, effectively distinguishing them from P13 K/AKT inhibitors used to treat breast cancer (Mallipeddi et al., 2021).

4.6 Berberine

Berberine, as well as other isoquinoline alkaloid, found in both root and stem root of *Coptis Chinensis* as well as in the root, root bark, and stem bark of *Berberis aristata* (Singh & Mahajan, 2013; N. Wang et al., 2015). Both ER positive and negative cells inhibited the development of the cell cycle by berberine. Higher MMP-9 was suppressed by berberine in a negative cell sample via reduced AP1 activity (Jong et al., 2008). In cells treated with berberine, mitochondrial cytochrome c levels dramatically increased, indicating that mitochondrial release of cytochrome c was caused by berberine. It increased only the caspase-9 pathway while reducing the caspase-8 pathway. Also, berberine increased the expression of p53 and p27, indicating that it may have a pro-apoptotic effect on cancer cells (Patil et al., 2010). Kuo et al. have shown that, in addition to stimulating BBR mitochondria/caspase pathways, cyclin D1 and E expression suppression leads to a cell cycle arrest of the G1 stage. In addition, cellular proliferation suppression, and apoptosis promotion

are accomplished via reducing HER2/PI3K/Akt pathway expression (Kuo et al., 2011). Findings also suggested that berberine is a potent DNA damage agent for the treatment of TNBC (Zhao et al., 2017). In the case of breast cancer cells with a mutant TP53 gene, berberine has produced different effects on P53 expression. TPA reduces the expression of p53 mRNA and protein levels. Berberine inhibits TPA. Therefore, it causes cell cycle arrest of P53 genes and the impact of apoptosis (S. Kim et al., 2012).

4.7 Arctigenin

Arctigenin (ATG) is a bioactive lignan derived from *Arctium lappa* seeds that is part of the Asteraceae family. *Arctum lappa* is a vegetable plant consumed across the globe, commonly known as the biggest burdock (Chan et al., 2011). The research led by Hsieh et al, to explore the influence of phytoestrogens on breast cancer cells, first looked at the effects of phytoestrogens on cell proliferation. ATG significantly inhibited MDA-MB-231 proliferation. On the other hand, ATG had little impact on the proliferation of ER-positive cells, implying that ATG could have an antiproliferative effect on ER-negative breast cancer cells. This was confirmed by utilizing an active ER-negative breast cancer cell line that was also significantly inhibited by ATG. According to their studies, ATG induces programmed cell death by increasing superoxide anion and hydrogen peroxide to generate O2 and H2O2, which stimulates the mitochondrial caspase-independent apoptotic pathway. The translocation of AIF and Endo G from the mitochondria to the cytoblast is a crucial step in apoptosis. By activating the p38 MAPK pathway, ATG decreases Bcl-2 expression and stimulates the release of AIF and Endo G, resulting in apoptosis (Hsieh et al., 2014). Research by Shi et al, demonstrated that arctigenin inhibits STAT3 phosphorylation and β-catenin signaling by reduction of arctigenin-induced tumor promoting cytokines GM-CSF and TSLP blocked the progression of breast cancer cell proliferation, invasion, and stemness (Shi et al., 2020).

4.8 Scandenolone

Scandenolone is isolated from *C.tricuspidata* and has potential cancer cell inhibition of proliferation, migration and apoptosis properties (Y. Hu et al., 2017). MAPK signaling pathways, including JNK, P38, ERK and ERK have been demonstrated in many studies to trigger cell death (Ravindran et al., 2011). When activated, p38 proteins begin to migrate from the cytosol to the nucleus, which contributes to cellular responses by activating its transcription factors, such as caspase (Sui et al., 2014). p38 is likely to control mitochondrial activity, resulting in cytochrome being released, and caspase activation (Park et al., 2011). It plays a vital role in cellular development, DNA synthesis, transformation, and apoptosis (Woods & Vousden, 2001). P53 induces apoptosis by various apoptosis-inducing proteins (such as p53-AIP) and pro-apoptosis proteins in the Bcl-2/Bax pathway (Chipuk et al., 2004). Transcription factors and mitochondrial proteins may be regulated by the JNK (Radogna et al., 2015). Study have shown that scandenolone was unable to influence p-JNK concentration, and hence the levels of Bcl-2 and Bcl-xL remained unaffected. The ER's primary function is to regulate homeostasis, but ER stress is also important for apoptosis. The influence of stress depends on p38 MAPK activation (Hamamura et al., 2009). Jiang et al investigateds and found that scandenolone causes mitochondrial membrane partly disrupted (Jiang et al., 2019). However, p38 and ER stress-induced cell apoptosis were both activated by treatment with scandenolone, indicating that it's related to ER stress that means increased amount of p38 MAPK and ERK promotes cell death (Cao et al., 2010; Deng et al., 2010).

4.9 Epigallocatechin-3-gallate

It is a green tea polyphenol component that is produced by *Camellia sinesis* (Kanwar et al., 2012). Green tea catechins in breast cancer cells, particularly EGCG, have demonstrated that they inhibit proliferation, migration, and angiogenesis, while inducing programmed cell death and stoppage of cell cycles. The anti-cancer effects of EGCG, along with its selectivity for

tumor cells and lack of toxicity, make it a viable adjuvant therapy option for breast cancer. Moradzadeh et al. investigated and reported that the up-regulation of pro-apoptotic genes like p53, p21, caspase3, caspase9, Bax, and PTEN, as well as the down-regulation of survival genes like PI3K, AKT, and Bcl-2, triggered T47D breast cancer cell death. We also found that EGCG improved cellular ageing by reducing the telomerase gene (Moradzadeh et al., 2017). Besides, treatment with EGCG prevented the development, invasion, and survival of IBC cell lines.

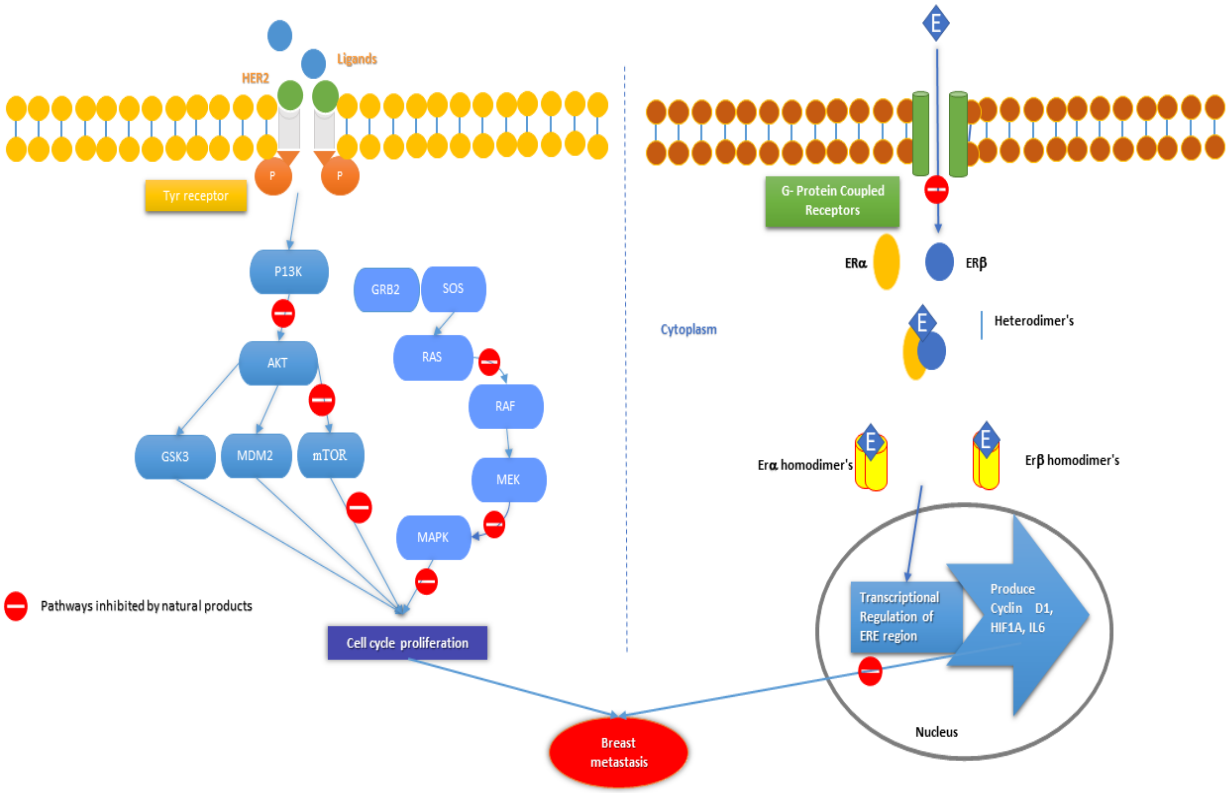

Figure 1: Different signaling pathways in breast cancer and their inhibition by natural products (Adapted from Feng et al., 2018)

4.10 Alisol B

Alisol B have anti-cancer and anti-complementary activities and it's chemical constitute is found in the rhizome of *Alisma orientale* (Law et al., 2010; J. W. Lee et al., 2010). It is well established that apoptosis activation is a promising form of cancer treatment. Caspases play

an important role in the importance of cells during the cell death process (Vaux & Korsmeyer, 1999). Mitochondria are stated to play an important role in apoptosis control (Brenner & Kroemer, 2000). Another explanation for the cytotoxic effects of anticancer medications is the activation of cell cycle arrest (Xavier et al., 2009). Amongst them, AKT is an effective cell growth and apoptosis regulator. PI3 K activates it, and phosphorylates a range of main pro-oncogenic targets that encourage cell growth and inhibit apoptosis. Zhang et al, conducted a study on alisol B in a cell line and found that alisol B demonstrated a significant cytotoxic effect. Their study found that cell cycle arrest, mitochondrial membrane potential (MMP) failure causes release of cytochrome-c and activation of caspase-3/-9, pathway p38 MAPK activation, by decreasing phosphorylation and ROS build-up, causes oxidative stress and causes apoptosis (Zhang et al., 2017).

Table 1 : List of natural products effective against breast cancer along with their mechanisms of actions

Phytochemicals with natural Origin	**Anticancer/cytotoxic effect (IC_{50} value and breast cancer cells)**	**Mechanism of action**	**Literature cited**
Curcumin; *Curcumin longa*	40 µM; MCF-7, MCF 10A and MD-MB-231	Downregulated Bcl-2, upregulated Bax. Generation of ROS. Block the PI3K/Akt pathway. Modulation of miR-19/PTEN/AKT/p53.	(M. S. Kim et al., 2001) (Kizhakkayil et al., 2010) (X. Li et al., 2014)
Carvacrol; *Origanum vulgare/ Thymus*	100 - 244.7 ± 0.71 µM; MCF-7 and MD-MB-231	DNA synthesis inhibition in the S phase. Cyto-C release, cleavage of PARP and increasing in Bax.	(Arunasree, 2010) (Al-fatlawi,

vulgaris		Elevated p53 dependent and Bcl-2/Bax pathway induce apoptosis.	Rahisuddin & Ahmad, 2014)
6- shogaol; *Zingiber officinale*	39.52±0.62 μM - 11.18±0.83 μM, 23.3 μM; MCF-7, MDA-MB-231 and T47D	Increase vacuole, LC3 formation. Reduce notch 1 expression and notch1 target proteins Hes-1 and cyclin D1. MMP-9 transcription is inhibited by inhibiting the NF-kB activation cascade.	(Ray et al., 2015) (Ling et al., 2010)
Resveratrol; *Polygonum cuspidatum* Siebold and Zucc.	100 μM; MCF-7, MDA-MB-431	Induced G1 arrest with decreased survivin expression and increased susceptibility to TRAIL-induced apoptosis. Inhibition of phospho-ERK1/2, and downregulated EZH2. Akt was dramatically decreased, improved processing of pro-caspase-9. Inhibiting the activation of the PI3K/Akt signaling pathway by inhibiting IGF-1 Downregulation of the FASN and HER2 genes. Inhibiting the PFK and reduce MCF-7 cell glucose and ATP consumption.	(Fulda & Debatin, 2004). (C. Hu et al., 2019) (Y. Li et al., 2006) (Alkhalaf, 2007) (Tang et al., 2008) (Khan et al., 2014) (Gomez et al., 2013)
Withaferin A; *Withania somnifera*	100 μg/mL; MD-MB-231 and MDA-MB-453	Inhibiting the expression of NF-κB and mTOR pathways, increased Bax, reduces bcl-2 levels. Generated ROS induce cell death.	(X. Liu et al., 2019). (Hahm et al., 2011)

		Alteration of mitochondrial dynamics and decreasing OPA1 and DRP1 cause apoptosis. Inhibition of P13/AKT leads to G2/M cell cycle arrest. Promote notch 2 and 4 cleavage and inhibit cell migration.	(Sehrawat et al., 2019) (H. C. Wang et al., 2019) (J. Lee et al., 2012)
Berberine; *Berberis aristata*	25 µM; MCF-7 and MD-MB-231	Increase cytoplasmic cytochrome c, caspase-9 activity, and PARP cleavage while lowering Bcl-2 levels. increased the expression of p53 and p27 cause pro apoptotic effect. Decreasing the expression of HER2/P13K/AKT signaling pathway leads to apoptosis	(Patil et al., 2010) (Kuo et al., 2011)
Arctigenin; *Arctium lappa*	100 - 200 µM; MCF-7, MDA-MB-231 And MDA-MB-431	ROS/p38 MAPK signaling pathway and epigenetic control of Bcl-2(decrease) through histone H3K9 trimethylation upregulation. Inhibit STAT3 disrupt the hydrogen bonding between STAT3 and DNA	(Hsieh et al., 2014) (He et al., 2018)
Scandenolone; *Cudrania tricuspidata*	38.5 ± 2.3 µmol/L; MCF-7	MMP reduced, caspase-3 cleavage increased, resulting in p53 phosphorylation. p38 and ERK are activated, leads to apoptosis	(Jiang et al., 2019)
Epigallocatechin-3-gallate; *Camelia*	14.17 µM; T47D cells	Suppressed HK, PFK, (LDH), and PK. inhibited HIF1 and GLUT1	(Wei et al., 2018)

sinesis		Up-regulation like p53, p21, caspase3/9, Bax, and PTEN, as well as the down-regulation of survival genes like PI3K, AKT, and Bcl-2.	(Moradzadeh et al., 2017) (Mineva et al., 2013)
Alisol B; *Alisma orientale*	13.96 µM; MDA-MB-231	G0/G1 arrest, MMP failure leads to cyto-c release and activation of caspase-3/-9, pathway p38 MAPK activation, AKT/mTOR/NF-kB suppression by decreasing phosphorylation, ROS build-up cause oxidative stress	(Zhang et al., 2017)

Chapter 5

Natural product in combination against breast cancer

Biochemical and molecular causes of carcinogenesis became better understood during the previous decade, leading to the justification of combining both therapeutic and chemo preventive medicines to attack various pathways (L. Chen & Malhotra, 2015). Due to the various impacts, the present cytotoxic therapies against breast cancer have many distinct adverse effects. While plant-derived products have little toxicity and side effects, they are chock-full of many medicinal properties. Formulated combination medications that use one or more entire natural components have showed additive or synergistic effects. Most of the natural product has medical characteristics that are previously recognized (Isgut et al., 2018).

5.1 Combination of quercetin & topotecan

Topotecan, a semi-synthetic camtothecin (CTP) analogue, suppresses the replication of DNA in cancer cells by inhibiting topoisomerase I nuclear enzyme (NTEI) (topo I). This inhibition raises the number of I-DNA complexes within the cells, and the association of these complexes with replication forks results in small numbers of permanent DNA splits (Kaufmann et al., 1996). Quercetin, a flavonoid, has a high inhibitory development in many human cell lines (Scambia et al., 1991, 1994). In the biological system, ROS are continuously generated and removed. The body of research shows that cancer cells have a higher level of intrinsic ROS tension. Increased ROS is a source of DNA-damaging agents, which foster genetic instability and drug resistance growth. The cellular apoptotic reaction to anticancer agents is often influenced by mitochondrial dysfunction (Pelicano et al., 2004). Since ROS are thought to play a role in drug-induced apoptosis, it's reasonable to assume that Quercetin, as an anti-oxidant, will prevent chemotherapeutic drugs from causing ROS generation and apoptosis (Gibellini et al., 2010). Akbas et al, study reviled that, Topotecan's IC50

concentration increased cytotoxicity 1.4-fold in MCF-7 cells and 1.3-fold in MDA-MB-231 cells after treatment with Quercetin. Following Topotecan application, substantial rise in ROS and nitrite concentrations were observed in breast cancer cell lines (Akbas et al., 2005).

5.2 Combination of resveratrol & quercetin

A small number of *in vivo* studies in conjunction with quercetin have also investigated the chemical preventive effects of resveratrol (Mouria et al., 2002). Mouria et al., a research showed that both cyclosporin A and the aristolochic acid (phospholipase A2 inhibitor inhibitor) reduced cell death in quercetin-mediated cancer cells, whereas cyclosporinA was required to block release and apoptosis of resveratrol-mediated cytochrome c. Cyclosporin Quercetine and resveratrol therefore had a synergistic role in the release and activation of the mitochondrial cytochrome (Mouria et al., 2002). Schlachterman et al, study revealed *in vitro* study of individual quercetin, resveratrol or catechin at concentration of 0.5 μM have no significant effect on ER positive breast cancer cell line. However, with same concentration but combination of quercetin, resveratrol and catechin demonstrated G2/M phase cell cycle blockade and minimize the growing cells. *In vivo* study demonstrated at 5mg/ kg and 25 mg/kg dose of combination of quercetin, resveratrol and catechin decrease the tumor advance in mice xenograft model (Schlachterman et al., 2008). These combination is also regulate PI3-K/Akt/mTOR pathways and induces MD-MB-231 breast cancer cell apoptosis (Castillo-Pichardo & Dharmawardhane, 2012).

5.3 Combination of luteolin & quercetin

Luteolin and quercetin are broadly dispersed plant flavonoids, including free radical scavenging and antioxidant function, with a range of chemical and biologic activities (Cantero et al., 2006). Shih et al, study found that in combination with luteolin and quercetin, AKT activation was synergistically inhibited. Nicotine mediated ERK activation, however,

was inhibited by Luteolin alone. Luteolin also inhibits NF-kB-mediated transcriptional activation of the a9-nAChR promoter. Hence, AKT signals perform a significant part in the carcinogenic process induced by nicotine (Shih et al., 2010). P13K kinase phosphorylated the IκBα, he promoter also helps for translocation of NF-kB (Ho et al., 2005). Thus, MDA-MB-231 cell proliferation inhibited combined treatment with Luteolin and Quercetin deeper than average breast epithelial cell (MCF-10A) proliferation (Shih et al., 2010).

5.4 Combination of theophylline & berberine

Synergistic approach is the best possible way to boost therapy reaction, decrease drug tolerance and adverse effects (K. Wang et al., 2016). According to breast cancer cell growth colony formation study theophylline increase the effectiveness of berberine cytotoxicity properties on MDA-MB-231 cells (Mantena et al., 2006). Combination of theophylline and berberine demonstrated cell cycle arrest G2/M phrase at higher concentration and G1/M phrase at lower concentration in cancer cells (Jantová et al., 2003). However, G1/M phrase cell cycle blockade only observed in MCF-7 cell (Wen et al., 2013). Hashemi-Niasari et al study found that treatment by berberine in a dose dependent manner with an IC50 value of 100 μM MD-MB-231 reduced cell proliferation, where combinational treatment showed that concentration is reduced to half at 50 μM and gave the same reduced cell proliferation effect (Hashemi-Niasari et al., 2018). Therefore, it was evident that theophylline and berberine follows intrinsic apoptotic pathway by activating ROS, Bcl-2, Cytochrome-c, cleaved the PRAP by the help of caspases and increased Bax expression (Hashemi-Niasari et al., 2018).

5.5 Combination of chrysin & apigenin

Chrysin and apigenin are abundant in a variety of fruits and herbs. They are highly present in Morinda citrifolia (Cimanga et al., 2006; Potterat & Hamburger, 2007). The HER2/neu oncogene encodes a tyrosine kinase for transmembrane receptor proteins and it is largely

found in breast cancers and is linked to low overall survival (Schnitt, 2001). Apigenin promotes cell death by decreasing HER2/neu protein through proteasomal degradation in breast cancer cells. Apigenin can impede cell growth and induce apoptosis by disrupting the PI3K/Akt-dependent pathway by suppressing HER2/HER3 signaling (Way et al., 2004). Thus, the PI3K-Akt pathway may be used by HER2/neu to control cellular cyclin D1, meaning that PI3K-Akt signaling is primarily involved in G1-to-S progression (R. Kim et al., 2002; Way et al., 2004). Wei et al, study also found that Apigenin treatment triggered a rapid activation of caspase-3 activity and stimulated proteolytic cleavage of DFF-45, resulting in apoptosis by cytochrome c release (Way et al., 2005). Chrysin therapy encouraged caspase activation, a community of cysteine proteases and caspase-3 and -9 cell initiation. The Bcl-2, Bcl-xl Mcl-1 anti-apoptotic proteins were suppressed after chrysin therapy. After chrysin therapy, the pro-apoptotic protein Bax was up regulated. These results suggest that chrysin selectively induces apoptosis via the intrinsic apoptotic pathway (Samarghandian et al., 2014). ROS are reactive radicals free of oxygen which cause various cellular responses such as ER stress and apoptosis (Redza-Dutordoir & Averill-Bates, 2016). Further research showed that chronic oxidative stress may be used to kill tumor cells (Moloney & Cotter, 2018). The increased ROS synthesis in chrysin and chrysin-induced ER stress, p-STAT3 inhibition and apoptosis were also found, indicating the critical function of ROS in the anti-tumor activity of chrysin (Xu et al., 2018). In conjunction with apigenin, chrysin further reduced the development of cancer in the human cells via the downregulation of ki-67 and Skp2 and synergistically induced apoptosis (Huang et al., 2016).

5.6 Combination of apigenin and luteolin

The family of fork-head (FOXO) transcription factors is regulated by the proliferation, metabolism, cell differentiation, cell death, DNA repair and apoptosis (J. Y. Yang et al., 2008). The P13/Akt pathway's main focus is FOXO3a and this FOXO3a is phosphorylated

Akt, and the FOXO3a is resides in cytoplasm after expelled from nucleus (Sunters et al., 2003; Tzivion et al., 2011). It targets p21 and p27 transcriptional components and make cycle arrest and apoptosis (Rathbone et al., 2008). C. H. Lin et al, study found that, apigenin, luteolin and flavones inhibit cyclin B and cyclin D1 mediated cell cycle arrest in MCF-7 cell. Induction of Akt/FOXO3a/p27 and P21 expression inhibits cell proliferation, cell apoptosis observed in MD-MB-231 breast cancer cell lines (C. H. Lin et al., 2015). Goodarzi et al, investigation showed that combination of luteolin and apigenin generate ROS/ ER stress, mitochondria dysfunction by caspase signaling pathway and G2/M cell cycle arrest in cancer cell lines (Goodarzi et al., 2020).

5.7 Combination of silibinin & chrysin

Flavonolignan silibinine is one of the most promising natural anti-cancer compounds, deriving from Silybum marianum seeds (commonly known as milk thistle), part of the Asteraceae family (Abenavoli et al., 2018). Silymarin flavonoids are one of the best complementary additives for the treatment of viral hepatitis, cirrhosis induced by substance misuse, and disruption to liver cells caused by synthetic pesticides, and it has also been used for antitumor effects (Das et al., 2008).. Silibinin cause apoptosis by various method such as repression of Bcl-2, especially decrease in Bcl-2/Bax ratio in breast cancer cells. It also increase P53, activate pro caspase and caspase pathways and release cytochrome c as a result mitochondria dysfunction occurs (Tiwari et al., 2011). However, Maasomi et al, study showed that silibinin and chrysin at IC50 induces an anti-proliferative synergistic reaction in breast cells by down-regulation of cyclin D1 and hTERT gene (Maasomi et al., 2017).

5.8 Combination of curcumin & resveratrol

Curcumin and resveratrol are bioactive, multidisciplinary substances which have been shown to have therapeutic advantages in cancer treatments (Filomeni et al., 2007). Curcumin is more

successful inhibiting cell proliferation than resveratrol. However, Resveratrol also increase the activity of sirtuin-1 (SIRT1), which in term increase the AMPK signaling and suppress the mTOR transformation (J. N. Lin et al., 2010; Menendez et al., 2013). Curcumin exercises have been confirmed to have antiproliferative and/or apoptotic anti-cancer effects via ERK or NF-jB-mediated pathways and PI3K/Akt signaling pathways modulation (Kunnumakkara et al., 2008; Reuter et al., 2008). However, the activation of cellular anti-aging/stress genes, such as Sirtuin 1 and NRF2 is often associated with resveratrol and therefore contributes to the activation of AMPK, a mTOR suppressor (J. N. Lin et al., 2010; Menendez et al., 2013). Mohapatra et al, study revealed that the combination of resveratrol and curcumin induced apoptosis by increasing the Bax/Bcl-xL ratio, Cyto-c release, PARP cleaved substance and cell caspase 3 (Mohapatra et al., 2015).

5.9 Combination of arabinogalactan & curcumin

Curcumin is isolated from Curcuma longa have large quantity of biphenyl compound and arabinogalactan is extracted from larch wood. However, both of them often used for the treatment of different disease including antitumor effects against many human cancers (Anand et al., 2008; Kelly, 1999; Sha et al., 2016). ROS are a community of extremely reactive, intracellular antioxidants regulated chemicals. The oxidation-antioxidation equilibrium is crucial to the preservation of normal cell functions and any deficiency here may lead to a broad variety of conditions like cancer. There is a lot of proof that ROS can function as cancer suppressors (Pelicano et al., 2004). The content of glutathione (GSH) in cancer cells is especially important for regulating the development, development and multi-drug resistance of DNA. In certain cases, the resistance is correlated with higher GSH levels in malignant tumors, compared to usual tissues (Ortega et al., 2011). Moghtaderi et al, found that combination of arabinogalactan and curcumin raised ROS amounts in cancer cells to more than when one of the medications are used alone. Also, they decreased the GSH level

and this is also known as potential cancer treatment approach which causes GSH depletion of these cancer cells. This combination also activates p53 proteins. Hence, by all these pathways, these combination inhibit cell proliferation and apoptosis (Moghtaderi et al., 2017).

5.10 Combination of quercetin & curcumin

Combined quercetin and curcumin treatment significantly decrease the expression of MMP-9 compared to their solo action. Treatment with HDAC inhibitors has been found to decrease cell migration and invasion by reducing MMP-9 participation in TNBC cells (Garmpis et al., 2017). The research of Kundur et al, revealed that BRCA1 expression was enhanced by the dose-dependent combination of quercetin and curcumin. Moreover, they have been found to modulate the BRCA1 level and to hinder cell viability and migration of cell lines of TNBC. The BRCA1 promoter of histone acetylation was induced by Quercetin and curcumin. Noteworthy, this combination generates ROS induced apoptosis in TNBC cells (Kundur et al., 2019).

Table 2 : Combinatory effects of natural products against breast cancer cell lines

Natural product in combination	**Anticancer/cytotoxic effect (IC_{50} value and breast cancer cells)**	**Mechanism**	**Literature cited**
Quercetin and Topotecan	0.62 µM and 100 ng/mL; MCF-7 and MD-MB-231	inhibiting topoisomerase, I nuclear enzyme (NTEI) (topo I). raises in I-DNA complexes and cause permanent DNA splits. Increased ROS and nitrite are source of	(Kaufmann et al., 1996) (Akbas et al., 2005)

		DNA-damage.	
Resveratrol and Quercetin	0.5 μM and 5 μM; MD-MB-231	release cyto-c and activation of caspase-3. Regulate PI3K/Akt/mTOR pathways.	(Mouria et al., 2002) (Castillo-Pichardo & Dharmawardhane, 2012)
Luteolin and Quercetin	0.5 μM and 0.5 μM; MDA-MB-231, MCF-7, and BT483	Akt/mTOR/c-Myc signaling control. Inhibit DNA topoisomerases I and II and to cause DNA damage and chromosome damage	(Shih et al., 2010) (Cantero et al., 2006)
Theophylline and Berberine	50 μM and 50 μM; MDA-MB-231	activating ROS, Bcl-2, Cytochrome-c, cleaved the PRAP by the help of caspases and increased Bax expression.	(Hashemi-Niasari et al., 2018)
Chrysin and Apigenin	10 μM and 10 μM; MD-MB-231	lowering the expression of Skp2 and low-density LRP6. Via downregulation of MMP2, MMP9, fibronectin, and snail. downregulating ki-67 and Skp2 protein	(Huang et al., 2016)
Apigenin and Luteolin	20 μM and 20 μM; MD-MB-231	apoptosis controlled by members of the forkhead class O (FOXO). ROS/ ER stress, G2/M cell cycle arrest.	(C. H. Lin et al., 2015) (Goodarzi et al., 2020)
Silibinin and Chrysin	67.7/35.4 μM and 72.2 /43.4 μM;	down-regulation of cyclin D1 and hTERT gene.	(Maasomi et al., 2017)

	T47D cells		
Curcumin and Resveratrol	3 µM and 3 µM; MCF-7	apoptotic anti-cancer effects via ERK or NF-jB-mediated pathways and PI3K/Akt signaling pathways modulation. Activation of stress-like anti-aging/cellular genes, such as Sirtuin 1 and NRF2, which contribute to activation of AMPK, a mTOR suppressor. Due to increased Bax/Bcl-xl ratio, release of cytochrome C, PARP cleaved and cell caspase 3, apoptosis was induced.	(Kunnumakkara et al., 2008; Reuter et al., 2008) (J. N. Lin et al., 2010; Menendez et al., 2013) (Mohapatra et al., 2015)
Arabinogalactan and Curcumin	10 mg/ml and 75 µM; MDA-MB-231 cells	Raised ROS, decreased the GSH level and increase the amount of sub-G1 cells. reduced MMP, Bax/Bcl-2 ratio is increased and disrupt the mitochondria function, release of caspase-3. activate p53 proteins cause apoptosis.	(Moghtaderi et al., 2017)
Quercetin and Curcumin	20 µM and 10 µM; MCF-7 and MDA-MB-231	Improved the expression of E-cadherin in all cell's, inhibited MMP-9 levels of expression & improved BRCA1 expression. Generates ROS induced apoptosis.	(Garmpis et al., 2017) (Kundur et al., 2019)

Chapter 6

Combination of natural products and synthetic drugs in the treatment of breast cancer

Combination therapy has been a widely used technique in cancer care. Natural anticancer products are widely accessible and effective, and their interaction with anticancer medications can have a synergistic therapeutic impact, reducing chemotherapy dosage, toxicity, and drug tolerance (Chou, 2010).

6.1 Combination of curcumin and docetaxel

Taxanes are frequently utilized in women with recurrent or metastatic breast cancer. Docetaxel is a semisynthetic derivative of Taxus baccata 10-deacetylbaccatin III. Docetaxel facilitates microtubule assembly and stabilization, thus avoiding the depolymerization of the microtubules. At the G2/M transition, cells are arrested, resulting in aborted cell division and cytotoxicity (Fulton & Spencer, 1996). In a Phase I dosage escalation study in patients with recurrent and metastatic breast cancer, Bayet Robert, et al. have added curcumin to docetaxel. Curcumin's MTD was 8,000 mg/day and orally administered in this Phase I trial duration of one week when treated interval of three weeks for six cycles in amalgamation with docetaxel. There was no evidence of progressive illness in all of the 14 patients involved in this research. Nine patients were qualified for evaluation of tumor response. In the majority of patients, at least some change was found, both biologically reduction in the CEA tumor marker over the course of therapy and clinically reversion of non-measurable lesions. On the basis of these findings, we should anticipate a response rate of up to 50% in a population of all evaluable patients treated with the docetaxel/curcumin combination. Hence, amalgamation of curcumin and docetaxel has been proven for anti-tumor commotion (Bayet-Robert et al., 2010).

6.2 Combination of vanillin and doxorubicin

Anthracycline antibiotic, doxorubicin is commonly used as a chemotherapeutics t in breast cancer and a first line monotherapy (Kanno et al., 2014). Vanillin is a natural substance that have diverse array of beneficial biochemical and pharmacological properties. Vanillin is present in a number of essential plant oils, most notably those of *Vanilla planifolia, Vanilla tahitensis, and Vanilla pompon*, are used in drinks, pharmaceuticals, and perfumes. A variety of preventative chemical characteristics have been demonstrated, including antioxidant function, mutagenesis reduction, anticarcinogenic impact, a rise in the responsiveness of cells to chemical treatment and the inhibition of invasion and migration of cells (Ali et al., 2012; Bezerra et al., 2016). Elsherbiny et al, conducted study on synergistic effect of vanillin and doxorubicin both *in vivo* and *vivo*. Vanillin (1,2 µM), DOX (100 µM), or their mixture are used to treat the MCF-7 cell line. Vanillin's anticancer activity in vivo was regulated by apoptosis and antioxidant (ROS generation) ability. Furthermore, it exhibited an inhibitory effect on *in vitro* growth and cytotoxicity via apoptosis, elevated cas-9 and Bax, Bcl-2, as well as the anti-metastasis effect. This study also conducted on EAC breast cancer mice model at a dose of vanillin (100mg/kg), DOX (2mg/kg) for 21 days and exhibited significant anti-neoplastic behavior in the EAC breast cancer mouse model through apoptosis and antioxidant ability. Vanillin has shown to be a beneficial agent in itself and to protect rats from doxorubicin-induced nephrotoxicity. The lead molecule for the synthesis of less harmful agents for the treatment of breast cancer is proposed to have considerable promise (Elsherbiny et al., 2016).

6.3 Combination of forskolin and doxorubicin

Forskolin is diterpene by the typical Indian Plant *Coleus forskohlii*. Natural substance forskolin has been used in medicine for many years and was also proven in numerous research (Loftus et al., 2015). Forskolin is able to cause ERK1/2 activation inhibition by

responding to low dose doxorubicin, which is followed by increased doxorubicin efficacy in these cell models. While there are different views on the sequence of doxorubicin-induced ERK1/2 activation on cell mortality/cell survival, the forskolin-induced potential for doxorubicin susceptibility is followed by phosphorylation inhibition of ERK1/2, which is imitated by the ERK inhibitor PD98059 and protein kinase A (PKA) and adenylate cyclase inhibitor pre-treated. As a result, Forskolin inhibits ERK1/2 function via a PKA pathway and increases the resistance of TNBC cell lines to doxorubicin, emphasizing its potential for use in the development of novel and effective pro-survival, anti-apoptotic therapeutic methods to combat triple negative breast cancer (Illiano et al., 2018).

6.4 Combination of furanodiene combination doxorubicin

The co-treatment of furanodiene and doxorubicin greatly increased the sub-G1 phase. However, no significant difference in p-cdc2, cdc2, p-cyclin D1, cyclin D1, and p21 expression was found between doxorubicin treatment alone and doxorubicin combinatory treatments, implying that the enhanced effects of furanodiene or doxorubicin may include cell apoptosis rather than cell cycle arrest. Furanodiene improved doxorubicin pro-apoptotic activity and an amalgamation of doxorubicin and furanodiene improved considerable induction compared to doxorubicin therapy alone of normal PARP-1 cleavage, caspase 7 and 9 break down, Bax, and bad expression. These findings showed that furanodiene improving doxorubicin's anti-cancer effects can rely on the mitochondrial caspase pathway. Furanodiene can enhance the anti-cancer effects of doxorubicin through increased cells apoptosis through mitochondrial-caspase-pathway and reactive oxygen-independent pathways in ER alpha negative breast cancer cells without contribution of ER alpha positive breast cells to cytotoxicity (Zhong et al., 2016). Another research by Zhong et al. presented that *in vitro* furanodiene-doxorubicin treatment combinations suppressed invasion and migration of breast cancer cells. Furanodiene has suppressed paxillin/Src phosphorylation, FAK, P85, and

PI3K/AKT have downregulated the integrin αV and β-catenin expression when combined with doxorubicin. Additionally, combinational therapies decreased matrix metalloproteinase-9 expression (MMP-9) (Zhong et al., 2017).

6.5 Combination of luteolin and celecoxib

Celecoxib and luteolin collective treatment of breast carcinogenic cells has led to a concentration and duration based synergistic effect on cell death compared with a single drug for each treatment. *In vitro* findings were supported in vivo in celecoxib treated tumor-bearing mice. Celecoxib and luteolin individual therapy reduce cell viability and enhances cell death in both ER-positive and negative human breast cancer cells after 72 hours of care. When administrated alone celecoxib and luteolin prevent tumor expansion with increased programmed cell death by 45% and 20% respectively. Celecoxib and luteolin combination therapy greatly reduced cell viability and was effective to destroy tumors after 72 hours of care relative to just one medication or control therapy. The number of cells in apoptosis rose by 52 per cent and 50 per cent respectively from regulation. The quantity of Akt-phosphorylation (PAkt) was reduced after celecoxib and luteolin combination treatment. As the Akt/PKB has a regulating role in pro-oncogenic pathways, it is an acceptable explanation for medication to block the celecoxib COX-2 anti-cancer activity (Jeon & Suh, 2013). Further study conducted by Jeon et al, found that Celecoxib and luteolin were used in combination to greatly inhibit the development of breast cancer cells *in viv*o as opposed to either agent alone. Celecoxib and luteolin therapy mediated synergistic effects via inactivating Akt and ERK signaling. In SkBr3, ER-negative and HER-2 negative development of the breast tumor were inhibited by inactivation of AKT and stimulation of the ERK signaling (Jeon et al., 2015).

6.6 Combination of luteolin and paclitaxel

Luteolin, a naturally produced flavone, exhibits a variety of biological properties, including antitumor activity. Paclitaxel, combined with less toxic natural antitumor agents, can provide a valid molecular foundation for innovative anticancer approaches. Research led by Yang et al, found that when luteolin-paclitaxel was combined, apoptosis was increased relative to when paclitaxel was used alone. *In vitro* assessment, such as DAPI strain and Annexin-v based assay results, suggest that the combination of luteolin and paclitaxel increases apoptosis. Additionally, immunoblotting research revealed that concurrent administration of luteolin and paclitaxel triggered caspase-8 and caspase-3, induced PARP cleavage and enhanced FAS expression, which is responsible for extrinsic apoptosis. Additionally, it was determined that the enhanced expression of FAS as a result of co-administration is attributed to the inhibition of STAT3 by decreasing the phosphorylation of STAT3 remarkably, thus, enhancing apoptosis activity. An *in vivo* experiment demonstrated a mixture of luteolin and paclitaxel greatly decreased tumor size and weight in a nude mouse xenograft tumor model using MDA-MB-231 cells (M. Y. Yang et al., 2014).

6.7 Combination of hesperidin, piperine and bee venom combination with tamoxifen

Hesperidin, piperine, bee venom has anticancer properties but when combine with tamoxifen they demonstrated synergistic effects. Khamis et al, have investigated these three nature products combine with each other and tamoxifen in 67 different combinations in breast cancer cells. In MCF7 and T47D cells, administration of the natural products hesperidin, piperine, and bee venom alone or in conjunction with the anticancer medication tamoxifen decreased cell viability and caused apoptosis. Apoptosis was detected by a significant rise Bax gene expression also a significant reduction was observed in the Bcl2 gene expression.

The MCF7 and T47D treatment cells displayed maximum levels of Bax and lowest Bcl2, suggesting higher apoptotic rate and synergism combined four compounds. Additionally, significant reductions in the mRNA levels of the two-breast cancer-related receptors EGFR and ERα, with the greatest impact observed in combined groups, especially those containing the four compounds. Hence, reduction of EGFR and Erα inhibit the growth and progression of breast cancer. All combinations when treated in MCF7 and T47D cells demonstrated the G2/M process of the cell division arrested, with the largest number of arrested cells in the group comprising the four compounds. It has been identified within the 4-compound group combination only piperine and bee venom responsible for the most influential effects in case of cell cycle arrest (Khamis et al., 2018).

6.8 Combination of proanthocyanidins and 5-fluorouracil

Proanthocyanidins is extracted from *Uncaria rhynchophylla* have anticancer properties especially on breast cancer. Fluorouracil (5-FU) is an anti-metabolite that is currently being used clinically to combat a variety of cancer, including breast cancer but have serious side effects. Combining non-toxic phytochemical constituents with cytotoxic agents to increase potency thus minimizing toxicity is one successful strategy. Chen et al, investigated synergistic cytotoxic activity of 5-fluorouracil proanthocyanidins. Proanthocyanidins alone inhibited cell viability and capacity to migrate. Apoptosis induced by G2/M phase cycle arrest, ROS generation rise, reduced mitochondrial membrane potential, elevated Bax/Bc1-2 rate and caspase-3 disruption. It was observed that, the blend of proanthocyanidins and 5-FU has been shown to have synergistic cytotoxic effects and increase 5-FU activity (X. X. Chen et al., 2017).

6.9 Combination of berberine and cisplatin

Berberine is a cancer-fighting quinoline alkaloid derivative. Cisplatin is an essential and effective chemotherapeutic drug that works by damaging DNA and causes cell cycle arrest and death. Research by Zhao et al, showed that berberine and cisplatin treatment improved capase-3 production, broke capspase-3 and -9, thus reducing Bcl-2 expression. Notably, berberine reduced cellular PCNA production, and immunofluorescence μH2AX has shown that berberine improves cisplatin-induced DNA damage. The study therefore demonstrates that the DNA breaks caused by berberine and caspase-3 dependent deaths in MCF-7 (Zhao et al., 2016).

6.10 Combination of quercetin and doxorubicin

Doxorubicin is an effective treatment for breast cancer as a cytotoxic agent, but it often limits its therapeutic use because of its side effects on healthy cells. Quercetin has also been shown to have anticancer and cell-protective properties. Li et al., conducted studies *in vitro* to find out how quercetin reduces toxicity and enhances the effectiveness of doxorubicin. Results revealed that the intracellular aggregation of dox can be amplified by downregulating ABC (ATP-binding cassette transporters) efflux transporter expression, including P-GP, BCRP and MRP1, which can effectively exclude cancer cells, including BCSCs. As a result, these combinations can be used in the treatment of breast cancer, enhancing both the anti-tumor effects and the toxicity of doxorubicin (S. Li et al., 2018). Research carried out by Staedler et al, revealed the interaction between quercetin and cell metabolism, GST function, cytoskeleton and invasive, particularly in tumor breast cells, in contrast with non-tumor breast cells. Doxorubicin in tumor and non-tumor cells was induced to damage DNA; however, quercetin reduced the damage to non-tumor cells alone and thus had a protective effect on the cells. In addition, quercetin changes cell protein tyrosine phosphorylation differently for tumor cells compared to non-tumor cells. These changes have persisted with

doxorubicin which shows that quercetin plays an vital role in the pathway of PKs in tumor cells and other non-tumor cells (Staedler et al., 2011).

6.11 Combination of curcumin and mitomycin-c

Mitomycin-c is among the cytotoxic medicines used in the treatment of breast cancer. The combination of curcumin with mitomycin-c is a potential approach to overcome toxicity and enhance breast cancer treatment efficiency. Zhou et al. have carried out in vivo and in vitro investigations. The findings indicate that antiproliferative action is increased while the dosage needed for MMC alone is decreased. The combination caused the G1 procedure to be discontinued and cyclin D1, cyclin E, cyclin A, CDK2 and CDK4 to decrease. In addition, p21 and 27 that govern advancement of cell cycles at the G1-S control point were substantially upgraded during the G1 process arrest after administration of a mixed combination. The MAPK signaling pathway p38 inhibits phosphorylation and the proliferation of cells caused by combination therapy in MCF-7 cells by regulating cycline D1, cyclin E, CDK2, CDK4 and p21 (Q. M. Zhou et al., 2011). Zhou et al conducted another study on the MCF-7 breast cancer xenografts model and found that combining curcumin with mitomycin-c leads to inhibition of tumor development synergistically. The RT-PCR study showed Mapk1 (ERK) and Mapk14 (MAPK p38) had a greater number of cross-reactions with other genes and demonstrated an improvement in expression of 8.14 and 11.84-fold, respectively, when curcumin and mitomycin-c were combined. Curcumin can synergistically enhance the mitomycin-c tumor impact. Combinatorial therapy has significantly induced apoptosis and inhibited the ERK inhibitor (PD98059) significantly. Hence, apoptosis occurs via the ERK pathway by applying a combination of curcumin and mitomycin-c (Q. M. Zhou et al., 2014). Lastly, Zhou et al, investigate curcumin to suppress the population of BCSC for the sensitization of mitomycin C cells (MMC). Result discovered that respectively curcumin

has 5 and 15 fold sensitized to mitomycin-c. Curcumin sensitized BCSCs by reducing ATP-binding (ABC) transport ABCG2 and ABCC1 expression (Q. Zhou et al., 2015).

6.12 Combination of quercetin and docetaxel

Natural product such as quercetin with docetaxel is a unique combination to treat breast cancer cells and reduce toxicity. Safi et al, a docetaxel and quercetin combination *in vitro* research, greatly enhances gene P53 expression known as the tumor suppressor gene, enabling apoptosis. On p53 activation, Bax is up-regulated, whereas bcl-2 is down-regulated. It is the underlying mechanism of docetaxel and quercetin joint treatment action by increasing the Bax/Bcl-2 ratio. Additionally, many mediators of the PI3K/AKT, MAPK/ERK, and JAK/STAT3 signaling pathways are activated. These mediators function as transcription factors, contributing to the dysregulation of the death machinery and the development of breast cancer. Though docetaxel or quercetin alone have no significant impact on MDA-MB-231 cells, their combination minimizes AKT formation by phosphorylation. Moreover, this combination also reduces ERK1/2, which is responsible for chemoresistance and cell survival by activating the anti-apoptotic protein Bcl-2. Also, the combination of quercetin and docetaxel with a STAT3 inhibitor induces apoptosis through significantly decreasing bcl-2, mcl-1, and surviving while raising Bax mRNA levels (Safi et al., 2021).

Table 3 : Anti-breast cancer effects of combination of natural products and synthetic drugs

Combination of natural product with synthetic drugs	**Anticancer/cytotoxic effect (IC_{50} value and breast cancer cells)**	**Mechanism of action**	**Literature cited**

Curcumin and Docetaxel	% of growth inhibition - 40–68%; MD-MB-231	At the G2/M transition, cells are arrested, resulting in aborted cell division and cytotoxicity.	(Bayet-Robert et al., 2010)
Vanillin and Doxorubicin	100 mg/kg and 2mg/kg; MCF-7, MD-MB-231 and 4T1	Increased caspase-9 and Bax: Bcl-2 ratio, as well as an anti-metastasis effect.	(Elsherbiny et al., 2016)
Forskolin and Doxorubicin	10 μM and 0.1 μM; MDA-MB-231 and MDA-MB-468	Inhibition of ERK1/2 activation via PKA pathway.	(Illiano et al., 2018)
Furanodiene and Doxorubicin	5-20 μM and 0.1 μM; MDA-MB-231	Increased the sub-G1 phase. Furanodiene have suppressed paxillin/Src phosphorylation, FAK, p85 and downregulate integrin αV and β-catenin expression when coupled with doxorubicin. Additionally, combinational therapies decreased MMP-9.	(Zhong et al., 2017)
Luteolin and Celecoxib	10-60 μM and 15-80 μM; MCF-7 and MD-MB-231	Less Akt phosphorylation (PAkt) levels have been observed. Since Akt/PKB is regulated by pro-oncogenic pathways. Inactivation of the Akt and activation of an ERK signal reduced the development of ER-negative and HER-2 negative breast tumors.	(Jeon et al., 2015)

Luteolin and Paclitaxel	15 μM and 40 nM; MD-MB-231	Trigger's caspase-8 and caspase-3, induce PARP cleavage and enhanced FAS expression which is responsible for extrinsic apoptosis. Consequently, STAT3 by decreased the phosphorylation of STAT3 remarkably thus, enhances the apoptosis activity.	(M. Y. Yang et al., 2014)
Hesperidin, Piperine and bee venom combination with Tamoxifen	8.76 μg/mL, 21.34 μg/mL, 7.83 μg/mL, and 5.51 μg/mL; MCF7 and T47D cells	Maximum levels of Bax and lowest Bcl2, suggesting higher apoptotic rate and synergism combined four compounds. G2/M process of the cell cycle arrest.	(Khamis et al., 2018)
Proanthocyanidins and 5-Fluorouracil	40 μg/mL and 500 μg/mL; MDA-MB-231	G2/M phase cell cycle arrest, increased ROS production, decreased MMP, increased the Bax/Bc1-2 ratio and cleaved caspase 3.	(X. X. Chen et al., 2017)
Berberine and Cisplatin	(52.178±1.593) μM and (49.541±1.618) μM; MCF-7	Increases the expression caspase-3, cleaved caspase-3, and caspase-9, thus decreasing the expression of anti-apoptotic Bcl-2.	(Zhao et al., 2016)
Quercetin and Doxorubicin	(5 – 10) μM and (10- 100) nM; MCF-7 cells and MDA-MB-231 cells	Downregulating ABC efflux transporter expression, including P-GP, BCRP and MRP1	(Staedler et al., 2011)
Curcumin and Mitomycin-c	100 mg/kg and 1-2 mg/kg; MDA-MB-231	G1 process arrest and a decline in cyclin D1, -E, CDK2 and 4, all of which are involved in the development of the cell cycle from the G1 period to	(Q. M. Zhou et al., 2011), (Q. Zhou et al., 2015)

		the S phase. p38 MAPK signaling pathway is involved in the inhibition by phosphorylation and cell growth.	
Quercetin and Docetaxel	95 μM and 7 nM; MDA-MB-231 cells	Increases p53 gene expression Increasing Bax/Bcl-2 ratio. PI3K/AKT, MAPK/ERK, and JAK/STAT3 signaling pathways are activated.	(Safi et al., 2021)

Chapter 7

Natural product on multi-drug resistance breast cancer cells

Multi-Drug Resistance (MDR) development is a major obstacle to tumor treatments and an important factor for the low survival percentage in cancer patients. Reverse agents from natural products have lately proved a successful technique to overcome the MDR (Zong et al., 2019).

7.1 Jadomycin

Jadomycin is a natural product demonstrating to be potent against drug-resistant breast cancer cells. Hall et al, investigated jadomycin cytotoxicity in drug sensitivity MCF-7 control and paclitaxel resistant cells. They discovered that after administration of jadomycin, the generation of ROS increased and DNA disruption occurred in both control and resistance cells of MCF-7. However, they also revealed that the antioxidant enzyme pathways of superoxide dismutase 1, glutathione, and peroxiredoxin/thioredoxin remove intracellular ROS produced and suggested that it is a potential strategy by blocking these pathways and enhancing jadomycin cytotoxic activity (Hall et al., 2015). Further research conducted by Hall et al, paclitaxel resistance and drug sensitive control MDA-MB-231 TNBC. Researchers found that jadomycin selectively poisons the topoisomerase IIa (topoisomerase which is responsible for preventing DNA supercoiling), hence inducing damage to MDA-MB-231 proliferating DNA and causing apoptosis (Hall et al., 2017).

7.2 Fumitremorgin C

BCRP is an ABC transporter superfamily member, also known as an ATP-binding cassette, with clinical significance in cancer due to its multi-drug resistance properties. Due to overexpression, it results in a number of cytotoxic drugs such as methotrexate, doxorubicin, daunorubicin tolerance to the cell. The FDA also gave reorganization to the BCRP as the

most significant drug transport system because it can reduce various cytotoxic drug efflux to desired cells by inhibiting the ATP dependent cassette pump, hence reducing efficacy. Marine natural products such as Fumitremorgin C, Tryprostatin A, Harmine, Botryllamides, Lamellarin O, Secalonic Acid D, Naphthopyrones have the potential to inhibit BCRP (Cherigo et al., 2015). Research by Rabindran et al, present that fumitremorgin C is the first highly selective and potent BCRP inhibitor, and it has been established that it reverses topotecan, mitoxantrone, and doxorubicin resistance in S1-M1-3.2 cells overexpressing BCRP (Rabindran et al., 2000).

7.3 Toosendanin

Resistance to many drugs has been a significant impediment to breast cancer chemotherapy. A study conducted by Kai et al, on the Toosendanin reversal effect of doxorubicin/Adriamycin resistance in the in vitro MCF 7/ADM cell and the *in vivo* orthotopic mouse breast cancer model. Toosendanin was found to significantly increase adriamycin accumulation in MCF-7/ADM cells, especially in the nucleus. Notably, Toosendanin also reduces the expression of ABCB1 transport proteins. In addition to that, Toosendanin inhibited dox induced Akt phosphorylation, most likely by downregulation of the P110 α and P110 b and inhibition of DNA-PKcs (DNA-dependent protein kinase, catalytic subunits). Inhibiting Akt phosphorylation inhibits the activity of numerous downstream effectors or molecules involved in cell survival, proliferation, expansion, protein synthesis, and DNA damage repair, thus facilitating the death of adriamycin-induced cells. As a consequence, it was unsurprising that blocking the PI3K/Akt pathway with TSN in combination with adriamycin resulted in improved efficacy (Kai et al., 2018).

7.4 Ursolic acid

Ursolic acid (UA) have a variety of biological effects, including antitumor activity. On the other hand, Doxorubicin exerts its effects to tumors cells by inhibits DNA replication, generates free radicals, increases lipid peroxidation, and alters the membrane structure. Zong et al, studied on multidrug resistance MCF7/ADR cells found that ursolic acid significantly increase the effectiveness of Doxorubicin by increase the concentration in the cell nuclei. Further investigation of the synergistic influence of Ursolic acid and doxorubicin on the energy metabolism of MCF-7/ADR cells revealed that the mixture would inhibit energy metabolism and that the effects are primarily due to ursolic acid. These findings suggested that Ursolic acid reversal of multi drug resistance was mainly due to the inhibition of P-gp efflux, disturbance of energy metabolism, namely glycolysis, the TCA cycle, and glutamine metabolism, related amino acid metabolism. To summarize, inhibition of P-glycoprotein (P-gp) transport activity, disruption of energy and subsequent amino acid metabolism can be critical mechanisms by which ursolic acid reverses multidrug resistance breast cancer (Zong et al., 2019).

7.5 Quercetin

Multidrug tolerance significantly impairs the clinical effectiveness of cytotoxic drugs in multiple cancer cell. Liu et al, performed a research to develop a method for reducing sensitive delivery of targeted quercetin and doxorubicin to the cell want to control MDR. The experiment was carried out on MDA-MB-231/MDR1 cells, and micelles were produced using a reduction-sensitive hyaluronic acid-based conjugate (HA-SS-DOCA) and D-tocopheryl poly (ethylene glycol) (PEG) 1000 succinate (TPGS). After that, MDA-MB-231/MDR1 cells were treated with doxorubicin-loaded mixed micelles and quercetin-loaded mixed micelles, and intracellular uptake, cell death, and cytotoxicity were detected. Prior micelle quercetin-loaded treatment reduced P-gp expression, enabling greater doxorubicin

amounts to aggregate in MDR cancerous cells and activating mitochondria-dependent apoptotic pathways to enhance DOX-induced death (S. Liu et al., 2020).

Chapter 8

Conclusion

The efficacy and safety of natural-derived chemicals in the treatment of breast cancer is well established and cannot be disregarded. More attention should be placed on research aimed at optimizing these substances' actions. They may act in a variety of ways without causing any harmful effects. Due to the rapid development of resistance to chemotherapeutic treatments, the search for novel therapies remains a primary objective in breast cancer therapy. Combination treatments may generate more "stable network responses" than a single drug. Mixtures of phytomedicine or natural products with synthetic medications (i.e., drug interaction) are complex but yet may substantially enhance pharmacotherapy. Furthermore, a synergistic combination aimed against MDR is expected to be a viable way of preventing MDR. Combination treatment has been employed progressively to treat cancer because the simultaneous use of several medications may overcome genetic heterogeneity and signaling system complexity.

8.1 Future aspect

With significant changes in the cancer therapy landscape, this study provides information to comprehend natural chemicals as a cheap and accessible alternative treatment and explore their therapeutic benefits to open up a new frontier in breast cancer treatment. Having completed this review study, we expect that natural chemicals and their combinations will be better adopted and integrated into the treatment of breast cancer. Continued research studies would also help to drive the uptake of natural compounds in the oncology sector to broaden accessibility to affordable breast cancer treatment options.

References

Abenavoli, L., Izzo, A. A., Milić, N., Cicala, C., Santini, A., & Capasso, R. (2018). Milk thistle (*Silybum marianum*): A concise overview on its chemistry, pharmacological, and nutraceutical uses in liver diseases. *Phytotherapy Research*, *32*(11), 2202–2213. https://doi.org/10.1002/ptr.6171

Abraham, J., & Staffurth, J. (2020). Hormonal therapy for cancer. *Medicine (United Kingdom)*, *48*(2), 103–107. https://doi.org/10.1016/j.mpmed.2019.11.007

Akbas, S. H., Timur, M., & Ozben, T. (2005). The effect of quercetin on topotecan cytotoxicity in MCF-7 and MDA-MB 231 human breast cancer cells. *Journal of Surgical Research*, *125*(1), 49–55. https://doi.org/10.1016/j.jss.2004.11.011

Al-fatlawi, Rahisuddin, A. A., & Ahmad, A. (2014). Cytotoxicity and pro-apoptotic activity of carvacrol on human breast cancer cell line MCF-7. *World Journal of Pharmaceutical Sciences*, 2–7.

Alamolhodaei, N. S., Tsatsakis, A. M., Ramezani, M., Hayes, A. W., & Karimi, G. (2017). Resveratrol as MDR reversion molecule in breast cancer: An overview. *Food and Chemical Toxicology*, *103*, 223–232. https://doi.org/10.1016/j.fct.2017.03.024

Ali, S. M. M., Abul Kalam Azad, M., Jesmin, M., Ahsan, S., Rahman, M. M., Khanam, J. A., Islam, M. N., & Shahriar, S. M. S. (2012). In vivo anticancer activity of Vanillin semicarbazone. *Asian Pacific Journal of Tropical Biomedicine*, *2*(6), 438–442. https://doi.org/10.1016/S2221-1691(12)60072-0

Alkhalaf, M. (2007). Resveratrol-induced growth inhibition in MDA-MB-231 breast cancer cells is associated with mitogen-activated protein kinase signaling and protein translation. *European Journal of Cancer Prevention*, *16*(4), 334–341.

https://doi.org/10.1097/01.cej.0000228413.06471.4c

American Cancer Society. (2017). Breast Cancer What is breast cancer ? *American Cancer Society. Cancer Facts and Figures Atlanta, Ga: American Cancer Society*, 1–19.

Anand, P., Sundaram, C., Jhurani, S., Kunnumakkara, A. B., & Aggarwal, B. B. (2008). Curcumin and cancer: An "old-age" disease with an "age-old" solution. *Cancer Letters*, *267*(1), 133–164. https://doi.org/10.1016/j.canlet.2008.03.025

Arunasree, K. M. (2010). Anti-proliferative effects of carvacrol on a human metastatic breast cancer cell line, MDA-MB 231. *Phytomedicine*, *17*(8–9), 581–588. https://doi.org/10.1016/j.phymed.2009.12.008

Based, H., & Registry, C. (2021). *Hospital Cancer Registry Report, NICRH, Dhaka 2015-2017. December 2020*. https://doi.org/10.13140/RG.2.2.10796.08320

Bassett, L. W., & Lee-Felker, S. (2018). Breast imaging screening and diagnosis. In *The Breast: Comprehensive Management of Benign and Malignant Diseases* (Fifth Edit). Elsevier Inc. https://doi.org/10.1016/B978-0-323-35955-9.00026-X

Baur, J. A., & Sinclair, D. A. (2006). Therapeutic potential of resveratrol: The in vivo evidence. In *Nature Reviews Drug Discovery* (Vol. 5, Issue 6, pp. 493–506). Nat Rev Drug Discov. https://doi.org/10.1038/nrd2060

Bayet-Robert, M., Kwiatkowski, F., Leheurteur, M., Gachon, F., Planchat, E., Abrial, C., Mouret-Reynier, M. A., Durando, X., Barthomeuf, C., & Chollet, P. (2010). Phase I dose escalation trial of docetaxel plus curcumin in patients with advanced and metastatic breast cancer. *Cancer Biology and Therapy*, *9*(1), 37–41. https://doi.org/10.4161/cbt.9.1.10392

Bezerra, D. P., Soares, A. K. N., & De Sousa, D. P. (2016). Overview of the role of vanillin

on redox status and cancer development. In *Oxidative Medicine and Cellular Longevity* (Vol. 2016). Hindawi Limited. https://doi.org/10.1155/2016/9734816

Blay, J. Y., Judson, I., Van Oosterom, A., Verweij, J., Radford, J., Lorigan, P., Rodenhuis, S., Ray-Coquard, I., Bonvalot, S., Collin, F., Jimeno, J., Di Paola, E., Van Glabbeke, M., Nielsen, O. S., & Cesne, A. Le. (2005). Phase II study of ET-743 in advanced soft tissue sarcomas: A European Organisation for the Research and Treatment of Cancer (EORTC) Soft Tissue and Bone Sarcoma Group trial. *Journal of Clinical Oncology*, *23*(3), 576–584. https://doi.org/10.1200/JCO.2005.01.180

Brenner, C., & Kroemer, G. (2000). Mitochondria - The death signal integrators. In *Science* (Vol. 289, Issue 5482, pp. 1150–1151). https://doi.org/10.1126/science.289.5482.1150

Burdall, S. E., Hanby, A. M., Lansdown, M. R. J., & Speirs, V. (2003). Breast cancer cell lines: Friend or foe? *Breast Cancer Research*, *5*(2), 89–95. https://doi.org/10.1186/bcr577

Cantero, G., Campanella, C., Mateos, S., & Cortés, F. (2006). Topoisomerase II inhibition and high yield of endoreduplication induced by the flavonoids luteolin and quercetin. *Mutagenesis*, *21*(5), 321–325. https://doi.org/10.1093/mutage/gel033

Cao, X. hong, Wang, A. hua, Wang, C. ling, Mao, D. zhi, Lu, M. fang, Cui, Y. qian, & Jiao, R. zhi. (2010). Surfactin induces apoptosis in human breast cancer MCF-7 cells through a ROS/JNK-mediated mitochondrial/caspase pathway. *Chemico-Biological Interactions*, *183*(3), 357–362. https://doi.org/10.1016/j.cbi.2009.11.027

Castillo-Pichardo, L., & Dharmawardhane, S. F. (2012). Grape polyphenols inhibit Akt/mammalian target of rapamycin signaling and potentiate the effects of gefitinib in breast cancer. *Nutrition and Cancer*, *64*(7), 1058–1069. https://doi.org/10.1080/01635581.2012.716898

Chan, Y. S., Cheng, L. N., Wu, J. H., Chan, E., Kwan, Y. W., Lee, S. M. Y., Leung, G. P. H., Yu, P. H. F., & Chan, S. W. (2011). A review of the pharmacological effects of Arctium lappa (burdock). In *Inflammopharmacology* (Vol. 19, Issue 5, pp. 245–254). Birkhauser Verlag AG. https://doi.org/10.1007/s10787-010-0062-4

Chen, L., & Malhotra, A. (2015). Combination Approach: the Future of the War Against Cancer. *Cell Biochemistry and Biophysics*, *72*(3), 637–641. https://doi.org/10.1007/s12013-015-0549-0

Chen, X. X., Leung, G. P. H., Zhang, Z. J., Xiao, J. B., Lao, L. X., Feng, F., Mak, J. C. W., Wang, Y., Sze, S. C. W., & Zhang, K. Y. B. (2017). Proanthocyanidins from Uncaria rhynchophylla induced apoptosis in MDA-MB-231 breast cancer cells while enhancing cytotoxic effects of 5-fluorouracil. *Food and Chemical Toxicology*, *107*, 248–260. https://doi.org/10.1016/j.fct.2017.07.012

Cherigo, L., Lopez, D., & Martinez-Luis, S. (2015). Marine natural products as breast cancer resistance protein inhibitors. In *Marine Drugs* (Vol. 13, Issue 4, pp. 2010–2029). MDPI AG. https://doi.org/10.3390/md13042010

Chipuk, J. E., Kuwana, T., Bouchier-Hayes, L., Droin, N. M., Newmeyer, D. D., Schuler, M., & Green, D. R. (2004). Direct Activation of Bax by p53 Mediates Mitochondrial Membrane Permeabilization and Apoptosis. *Science*, *303*(5660), 1010–1014. https://doi.org/10.1126/science.1092734

Chou, T. C. (2010). Drug combination studies and their synergy quantification using the chou-talalay method. *Cancer Research*, *70*(2), 440–446. https://doi.org/10.1158/0008-5472.CAN-09-1947

Cimanga, R. K., Kambu, K., Tona, L., Hermans, N., Apers, S., Totté, J., Pieters, L., & Vlietinck, A. J. (2006). Cytotoxicity and in vitro susceptibility of Entamoeba histolytica

to Morinda morindoides leaf extracts and its isolated constituents. *Journal of Ethnopharmacology*, *107*(1), 83–90. https://doi.org/10.1016/j.jep.2006.02.010

Corrie, P. G. (2008). Cytotoxic chemotherapy: clinical aspects. *Medicine*, *36*(1), 24–28. https://doi.org/10.1016/j.mpmed.2007.10.012

Country-specific, I., Country-specific, M., Cervix, B., Lip, O. G., Lip, O., Cervix, B. L., Computed, P., & Index, H. D. (2020). *164 689 383*. *745*, 9–10.

Cragg, G. M., & Newman, D. J. (2005). Plants as a source of anti-cancer agents. In *Journal of Ethnopharmacology* (Vol. 100, Issues 1–2, pp. 72–79). Elsevier Ireland Ltd. https://doi.org/10.1016/j.jep.2005.05.011

Dai, X., Cheng, H., Bai, Z., & Li, J. (2017). Breast cancer cell line classification and Its relevance with breast tumor subtyping. *Journal of Cancer*, *8*(16), 3131–3141. https://doi.org/10.7150/jca.18457

Dare, A. J., Anderson, B. O., Sullivan, R., Pramesh, C. S., Yip, C.-H., Ilbawi, A., Adewole, I. F., Badwe, R. A., & Gauvreau, C. L. (2015). Surgical Services for Cancer Care. In *Disease Control Priorities, Third Edition (Volume 3): Cancer* (pp. 223–238). The World Bank. https://doi.org/10.1596/978-1-4648-0349-9_ch13

Das, S. K., Mukherjee, S., & Vasudevan, D. M. (2008). Medicinal properties of milk thistle with special reference to silymarinñ an overview. *Natural Product Radiance*, *7*(2), 182–192.

Deng, Y.-T., Huang, H.-C., & Lin, J.-K. (2010). Rotenone induces apoptosis in MCF-7 human breast cancer cell-mediated ROS through JNK and p38 signaling. *Molecular Carcinogenesis*, *49*(2), 141–151. https://doi.org/10.1002/mc.20583

DeSantis, C. E., Ma, J., Gaudet, M. M., Newman, L. A., Miller, K. D., Goding Sauer, A.,

Jemal, A., & Siegel, R. L. (2019). Breast cancer statistics, 2019. *CA: A Cancer Journal for Clinicians*, *69*(6), 438–451. https://doi.org/10.3322/caac.21583

Duvoix, A., Blasius, R., Delhalle, S., Schnekenburger, M., Morceau, F., Henry, E., Dicato, M., & Diederich, M. (2005). Chemopreventive and therapeutic effects of curcumin. In *Cancer Letters* (Vol. 223, Issue 2, pp. 181–190). Elsevier Ireland Ltd. https://doi.org/10.1016/j.canlet.2004.09.041

Elsherbiny, N. M., Younis, N. N., Shaheen, M. A., & Elseweidy, M. M. (2016). The synergistic effect between vanillin and doxorubicin in ehrlich ascites carcinoma solid tumor and MCF-7 human breast cancer cell line. *Pathology Research and Practice*, *212*(9), 767–777. https://doi.org/10.1016/j.prp.2016.06.004

Feng, Y., Spezia, M., Huang, S., Yuan, C., Zeng, Z., Zhang, L., Ji, X., Liu, W., Huang, B., Luo, W., Liu, B., Lei, Y., Du, S., Vuppalapati, A., Luu, H. H., Haydon, R. C., He, T. C., & Ren, G. (2018). Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. *Genes and Diseases*, *5*(2), 77–106. https://doi.org/10.1016/j.gendis.2018.05.001

Filomeni, G., Graziani, I., Rotilio, G., & Ciriolo, M. R. (2007). Trans-resveratrol induces apoptosis in human breast cancer cells MCF-7 by the activation of MAP kinases pathways. *Genes and Nutrition*, *2*(3), 295–305. https://doi.org/10.1007/s12263-007-0059-9

Foster, B. C., Arnason, J. T., & Briggs, C. J. (2005). NATURAL HEALTH PRODUCTS AND DRUG DISPOSITION. *Annual Review of Pharmacology and Toxicology*, *45*(1), 203–226. https://doi.org/10.1146/annurev.pharmtox.45.120403.095950

Fulda, S., & Debatin, K. M. (2004). Sensitization for Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand-Induced Apoptosis by the Chemopreventive Agent

Resveratrol. *Cancer Research*, *64*(1), 337–346. https://doi.org/10.1158/0008-5472.CAN-03-1656

Fulton, B., & Spencer, C. M. (1996). Docetaxel: A Review of its Pharmacodynamic and Pharmacokinetic Properties and Therapeutic Efficacy in the Management of Metastatic Breast Cancer. *Drugs*, *51*(6), 1075–1092. https://doi.org/10.2165/00003495-199651060-00011

Garmpis, N., Damaskos, C., Garmpi, A., Kalampokas, E., Kalampokas, T., Spartalis, E., Daskalopoulou, A., Valsami, S., Kontos, M., Nonni, A., Kontzoglou, K., Perrea, D., Nikiteas, N., & Dimitroulis, D. (2017). Histone deacetylases as new therapeutic targets in triple-negative breast cancer: Progress and promises. In *Cancer Genomics and Proteomics* (Vol. 14, Issue 5, pp. 299–313). International Institute of Anticancer Research. https://doi.org/10.21873/cgp.20041

Gibellini, L., Pinti, M., Nasi, M., De Biasi, S., Roat, E., Bertoncelli, L., & Cossarizza, A. (2010). Interfering with ROS Metabolism in Cancer Cells: The Potential Role of Quercetin. *Cancers*, *2*(2), 1288–1311. https://doi.org/10.3390/cancers2021288

Gomez, L. S., Zancan, P., Marcondes, M. C., Ramos-Santos, L., Meyer-Fernandes, J. R., Sola-Penna, M., & Da Silva, D. (2013). Resveratrol decreases breast cancer cell viability and glucose metabolism by inhibiting 6-phosphofructo-1-kinase. *Biochimie*, *95*(6), 1336–1343. https://doi.org/10.1016/j.biochi.2013.02.013

Goodarzi, S., Tabatabaei, M. J., Mohammad Jafari, R., Shemirani, F., Tavakoli, S., Mofasseri, M., & Tofighi, Z. (2020). Cuminum cyminum fruits as source of luteolin- 7-O-glucoside, potent cytotoxic flavonoid against breast cancer cell lines. *Natural Product Research*, *34*(11), 1602–1606. https://doi.org/10.1080/14786419.2018.1519824

Hahm, E. R., Moura, M. B., Kelley, E. E., van Houten, B., Shiva, S., & Singh, S. V. (2011).

Withaferin a-induced apoptosis in human breast cancer cells is mediated by reactive oxygen species. *PLoS ONE*, *6*(8). https://doi.org/10.1371/journal.pone.0023354

Hall, S. R., Blundon, H. L., Ladda, M. A., Robertson, A. W., Martinez-Farina, C. F., Jakeman, D. L., & Goralski, K. B. (2015). Jadomycin breast cancer cytotoxicity is mediated by a copper-dependent, reactive oxygen species-inducing mechanism. *Pharmacology Research and Perspectives*, *3*(2). https://doi.org/10.1002/prp2.110

Hall, S. R., Toulany, J., Bennett, L. G., Martinez-Farina, C. F., Robertson, A. W., Jakeman, D. L., & Goralski, K. B. (2017). Jadomycins inhibit type II topoisomerases and promote DNA damage and apoptosis in multidrug-resistant triple-negative breast cancer cells. *Journal of Pharmacology and Experimental Therapeutics*, *363*(2), 196–210. https://doi.org/10.1124/jpet.117.241125

Hamamura, K., Goldring, M. B., & Yokota, H. (2009). Involvement of p38 MAPK in regulation of MMP13 mRNA in chondrocytes in response to surviving stress to endoplasmic reticulum. *Archives of Oral Biology*, *54*(3), 279–286. https://doi.org/10.1016/j.archoralbio.2008.11.003

Harvey, A. L., Edrada-Ebel, R., & Quinn, R. J. (2015). The re-emergence of natural products for drug discovery in the genomics era. In *Nature Reviews Drug Discovery* (Vol. 14, Issue 2, pp. 111–129). Nature Publishing Group. https://doi.org/10.1038/nrd4510

Hashemi-Niasari, F., Rabbani-Chadegani, A., Razmi, M., & Fallah, S. (2018). Synergy of theophylline reduces necrotic effect of berberine, induces cell cycle arrest and PARP, HMGB1, Bcl-2 family mediated apoptosis in MDA-MB-231 breast cancer cells. *Biomedicine and Pharmacotherapy*, *106*(July), 858–867. https://doi.org/10.1016/j.biopha.2018.07.019

He, Y., Fan, Q., Cai, T., Huang, W., Xie, X., Wen, Y., & Shi, Z. (2018). Molecular

mechanisms of the action of Arctigenin in cancer. *Biomedicine and Pharmacotherapy*, *108*(May), 403–407. https://doi.org/10.1016/j.biopha.2018.08.158

Ho, Y. S., Chen, C. H., Wang, Y. J., Pestell, R. G., Albanese, C., Chen, R. J., Chang, M. C., Jeng, J. H., Lin, S. Y., Liang, Y. C., Tseng, H., Lee, W. Sen, Lin, J. K., Chu, J. S., Chen, L. C., Lee, C. H., Tso, W. L., Lai, Y. C., & Wu, C. H. (2005). Tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) induces cell proliferation in normal human bronchial epithelial cells through NFκB activation and cyclin D1 up-regulation. *Toxicology and Applied Pharmacology*, *205*(2), 133–148. https://doi.org/10.1016/j.taap.2004.09.019

Holliday, D. L., & Speirs, V. (2011). Choosing correct breast cancer cell line for breast cancer research. *Breast Cancer Research*, *13*(4), 1–7.

Hossain, M. S., Ferdous, S., & Karim-Kos, H. E. (2014). Breast cancer in South Asia: A Bangladeshi perspective. *Cancer Epidemiology*, *38*(5), 465–470. https://doi.org/10.1016/j.canep.2014.08.004

Hsieh, C. J., Kuo, P. L., Hsu, Y. C., Huang, Y. F., Tsai, E. M., & Hsu, Y. L. (2014). Arctigenin, a dietary phytoestrogen, induces apoptosis of estrogen receptor-negative breast cancer cells through the ROS/p38 MAPK pathway and epigenetic regulation. *Free Radical Biology and Medicine*, *67*, 159–170. https://doi.org/10.1016/j.freeradbiomed.2013.10.004

Hu, C., Liu, Y., Teng, M., Jiao, K., Zhen, J., Wu, M., & Li, Z. (2019). Resveratrol inhibits the proliferation of estrogen receptor-positive breast cancer cells by suppressing EZH2 through the modulation of ERK1/2 signaling. *Cell Biology and Toxicology*, *35*(5), 445–456. https://doi.org/10.1007/s10565-019-09471-x

Hu, Y., Li, Z., Wang, L., Deng, L., Sun, J., Jiang, X., Zhang, Y., Tian, L., Wang, Y., & Bai,

W. (2017). Scandenolone, a natural isoflavone derivative from Cudrania tricuspidata fruit, targets EGFR to induce apoptosis and block autophagy flux in human melanoma cells. *Journal of Functional Foods*, *37*, 229–240. https://doi.org/10.1016/j.jff.2017.07.055

Huang, C., Wei, Y. X., Shen, M. C., Tu, Y. H., Wang, C. C., & Huang, H. C. (2016). Chrysin, abundant in Morinda citrifolia fruit water-EtOAc extracts, combined with apigenin synergistically induced apoptosis and inhibited migration in human breast and liver cancer cells. *Journal of Agricultural and Food Chemistry*, *64*(21), 4235–4245. https://doi.org/10.1021/acs.jafc.6b00766

Illiano, M., Sapio, L., Salzillo, A., Capasso, L., Caiafa, I., Chiosi, E., Spina, A., & Naviglio, S. (2018). Forskolin improves sensitivity to doxorubicin of triple negative breast cancer cells via Protein Kinase A-mediated ERK1/2 inhibition. *Biochemical Pharmacology*, *152*(February), 104–113. https://doi.org/10.1016/j.bcp.2018.03.023

Isgut, M., Rao, M., Yang, C., Subrahmanyam, V., Rida, P. C. G., & Aneja, R. (2018). Application of Combination High-Throughput Phenotypic Screening and Target Identification Methods for the Discovery of Natural Product-Based Combination Drugs. *Medicinal Research Reviews*, *38*(2), 504–524. https://doi.org/10.1002/med.21444

Ishiguro, K., Ando, T., Maeda, O., Ohmiya, N., Niwa, Y., Kadomatsu, K., & Goto, H. (2007). Ginger ingredients reduce viability of gastric cancer cells via distinct mechanisms. *Biochemical and Biophysical Research Communications*, *362*(1), 218–223. https://doi.org/10.1016/j.bbrc.2007.08.012

Jantová, S., čipák, L., čerňáková, M., & Košt‘álová, D. (2003). Effect of berberine on proliferation, cell cycle and apoptosis in HeLa and L1210 cells. *Journal of Pharmacy and Pharmacology*, *55*(8), 1143–1149. https://doi.org/10.1211/002235703322277186

Jeon, Y. W., Ahn, Y. E., Chung, W. S., Choi, H. J., & Suh, Y. J. (2015). Synergistic effect between celecoxib and luteolin is dependent on estrogen receptor in human breast cancer cells. *Tumor Biology*, *36*(8), 6349–6359. https://doi.org/10.1007/s13277-015-3322-5

Jeon, Y. W., & Suh, Y. J. (2013). Synergistic apoptotic effect of celecoxib and luteolin on breast cancer cells. *Oncology Reports*, *29*(2), 819–825. https://doi.org/10.3892/or.2012.2158

Jiang, X., Cao, C., Sun, W., Chen, Z., Li, X., Nahar, L., Sarker, S. D., Georgiev, M. I., & Bai, W. (2019). Scandenolone from Cudrania tricuspidata fruit extract suppresses the viability of breast cancer cells (MCF-7) in vitro and in vivo. *Food and Chemical Toxicology*, *126*(December 2018), 56–66. https://doi.org/10.1016/j.fct.2019.02.020

Jong, B. K., Lee, K. M., Ko, E., Han, W., Jeong, E. L., Shin, I., Bae, J. Y., Kim, S., & Noh, D. Y. (2008). Berberine inhibits growth of the breast cancer cell lines MCF-7 and MDA-MB-231. *Planta Medica*, *74*(1), 39–42. https://doi.org/10.1055/s-2007-993779

Kai, W., Yating, S., Lin, M., Kaiyong, Y., Baojin, H., Wu, Y., Fangzhou, Y., & Yan, C. (2018). Natural product toosendanin reverses the resistance of human breast cancer cells to adriamycin as a novel PI3K inhibitor. *Biochemical Pharmacology*, *152*, 153–164. https://doi.org/10.1016/j.bcp.2018.03.022

Kanno, S. I., Yomogida, S., Tomizawa, A., Yamazaki, H., Ukai, K., Mangindaan, R. E. P., Namikoshi, M., & Ishikawa, M. (2014). Combined effect of papuamine and doxorubicin in human breast cancer MCF-7 cells. *Oncology Letters*, *8*(2), 547–550. https://doi.org/10.3892/ol.2014.2218

Kanwar, J., Taskeen, M., Mohammad, I., Huo, C., Chan, T. H., & Dou, Q. P. (2012). Recent advances on tea polyphenols. *Frontiers in Bioscience - Elite*, *4 E*(1), 111–131. https://doi.org/10.2741/363

Kaufmann, S. H., Peereboom, D., Buckwalter, C. A., Svingen, P. A., Grochow, L. B., Donehower, R. C., & Rowinsky, E. K. (1996). Cytotoxic effects of topotecan combined with various anticancer agents in human cancer cell lines. *Journal of the National Cancer Institute*, *88*(11), 734–741. https://doi.org/10.1093/jnci/88.11.734

Kelly, G. S. (1999). Larch arabinogalactan: Clinical relevance of a novel immune-enhancing polysaccharide. In *Alternative Medicine Review* (Vol. 4, Issue 2, pp. 96–103).

Khamis, A. A. A., Ali, E. M. M., El-Moneim, M. A. A., Abd-Alhaseeb, M. M., El-Magd, M. A., & Salim, E. I. (2018). Hesperidin, piperine and bee venom synergistically potentiate the anticancer effect of tamoxifen against breast cancer cells. *Biomedicine and Pharmacotherapy*, *105*(February), 1335–1343. https://doi.org/10.1016/j.biopha.2018.06.105

Khan, A., Aljarbou, A. N., Aldebasi, Y. H., Faisal, S. M., & Khan, M. A. (2014). Resveratrol suppresses the proliferation of breast cancer cells by inhibiting fatty acid synthase signaling pathway. *Cancer Epidemiology*, *38*(6), 765–772. https://doi.org/10.1016/j.canep.2014.09.006

Kim, M. S., Kang, H. J., & Moon, A. (2001). Inhibition of invasion and induction of apoptosis by curcumin in H-ras-transformed MCF10A human breast epithelial cells. *Archives of Pharmacal Research*, *24*(4), 349–354. https://doi.org/10.1007/BF02975105

Kim, R., Tanabe, K., Uchida, Y., Emi, M., Inoue, H., & Toge, T. (2002). Current status of the molecular mechanisms of anticancer drug-induced apoptosis: The contribution of molecular-level analysis to cancer chemotherapy. In *Cancer Chemotherapy and Pharmacology* (Vol. 50, Issue 5, pp. 343–352). Cancer Chemother Pharmacol. https://doi.org/10.1007/s00280-002-0522-7

Kim, S., Han, J., Kim, N. Y., Lee, S. K., Cho, D. H., Choi, M. Y., Kim, J. S., Kim, J. H.,

Choe, J. H., Nam, S. J., & Lee, J. E. (2012). Effect of berberine on p53 expression by TPA in breast cancer cells. *Oncology Reports*, *27*(1), 210–215. https://doi.org/10.3892/or.2011.1480

Kiskó, G., & Roller, S. (2005). Carvacrol and p-cymene inactivate Escherichia coli O157:H7 in apple juice. *BMC Microbiology*, *5*(1), 36. https://doi.org/10.1186/1471-2180-5-36

Kizhakkayil, J., Thayyullathil, F., Chathoth, S., Hago, A., Patel, M., & Galadari, S. (2010). Modulation of curcumin-induced Akt phosphorylation and apoptosis by PI3K inhibitor in MCF-7 cells. *Biochemical and Biophysical Research Communications*, *394*(3), 476–481. https://doi.org/10.1016/j.bbrc.2010.01.132

Koohpar, Z. K., Entezari, M., Movafagh, A., & Hashemi, M. (2015). Anticancer activity of curcumin on human breast adenocarcinoma: Role of Mcl-1 gene. *International Journal of Cancer Management*, *8*(3). https://doi.org/10.17795/ijcp2331

Kundur, S., Prayag, A., Selvakumar, P., Nguyen, H., McKee, L., Cruz, C., Srinivasan, A., Shoyele, S., & Lakshmikuttyamma, A. (2019). Synergistic anticancer action of quercetin and curcumin against triple-negative breast cancer cell lines. *Journal of Cellular Physiology*, *234*(7), 11103–11118. https://doi.org/10.1002/jcp.27761

Kunnumakkara, A. B., Anand, P., & Aggarwal, B. B. (2008). Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins. In *Cancer Letters* (Vol. 269, Issue 2, pp. 199–225). Elsevier Ireland Ltd. https://doi.org/10.1016/j.canlet.2008.03.009

Kuo, H. P., Chuang, T. C., Yeh, M. H., Hsu, S. C., Way, T. Der, Chen, P. Y., Wang, S. S., Chang, Y. H., Kao, M. C., & Liu, J. Y. (2011). Growth suppression of HER2-overexpressing breast cancer cells by berberine via modulation of the HER2/PI3K/Akt signaling pathway. *Journal of Agricultural and Food Chemistry*, *59*(15), 8216–8224.

https://doi.org/10.1021/jf2012584

Lampronti, I., Saab, A. M., & Gambari, R. (2006). Antiproliferative activity of essential oils derived from plants belonging to the Magnoliophyta division. *International Journal of Oncology*, *29*(4), 989–995. https://doi.org/10.3892/ijo.29.4.989

Law, B. Y. K., Wang, M., Ma, D. L., Al-Mousa, F., Michelangeli, F., Cheng, S. H., Ng, M. H. L., To, K. F., Mok, A. Y. F., Ko, R. Y. Y., Lam, S. K., Chen, F., Che, C. M., Chiu, P., & Ko, B. C. B. (2010). Alisol B, a novel inhibitor of the sarcoplasmic/endoplasmic reticulum Ca2+ ATPase pump, induces autophagy, endoplasmic reticulum stress, and apoptosis. *Molecular Cancer Therapeutics*, *9*(3), 718–730. https://doi.org/10.1158/1535-7163.MCT-09-0700

Lee, J., Sehrawat, A., & Singh, S. V. (2012). Withaferin A causes activation of Notch2 and Notch4 in human breast cancer cells. *Breast Cancer Research and Treatment*, *136*(1), 45–56. https://doi.org/10.1007/s10549-012-2239-6

Lee, J. W., Kobayashi, Y., Nakamichi, Y., Udagawa, N., Takahashi, N., Im, N. K., Seo, H. J., Jeon, W. B., Yonezawa, T., Cha, B. Y., & Woo, J. T. (2010). Alisol-B, a novel phyto-steroid, suppresses the RANKL-induced osteoclast formation and prevents bone loss in mice. *Biochemical Pharmacology*, *80*(3), 352–361. https://doi.org/10.1016/j.bcp.2010.04.014

Levitsky, D. O., & Dembitsky, V. M. (2015). Anti-breast Cancer Agents Derived from Plants. *Natural Products and Bioprospecting*, *5*(1), 1–16. https://doi.org/10.1007/s13659-014-0048-9

Li, S., Yuan, S., Zhao, Q., Wang, B., Wang, X., & Li, K. (2018). Quercetin enhances chemotherapeutic effect of doxorubicin against human breast cancer cells while reducing toxic side effects of it. *Biomedicine and Pharmacotherapy*, *100*(February),

441–447. https://doi.org/10.1016/j.biopha.2018.02.055

Li, X., Xie, W., Xie, C., Huang, C., Zhu, J., Liang, Z., Deng, F., Zhu, M., Zhu, W., Wu, R., Wu, J., Geng, S., & Zhong, C. (2014). Curcumin modulates miR-19/PTEN/AKT/p53 axis to suppress bisphenol A-induced MCF-7 breast cancer cell proliferation. *Phytotherapy Research*, *28*(10), 1553–1560. https://doi.org/10.1002/ptr.5167

Li, Y., Liu, J., Liu, X., Xing, K., Wang, Y., Li, F., & Yao, L. (2006). Resveratrol-induced cell inhibition of growth and apoptosis in MCF7 human breast cancer cells are associated with modulation of phosphorylated Akt and caspase-9. *Applied Biochemistry and Biotechnology*, *135*(3), 181–192. https://doi.org/10.1385/ABAB:135:3:181

Lin, C. H., Chang, C. Y., Lee, K. R., Lin, H. J., Chen, T. H., & Wan, L. (2015). Flavones inhibit breast cancer proliferation through the Akt/FOXO3a signaling pathway. *BMC Cancer*, *15*(1), 1–12. https://doi.org/10.1186/s12885-015-1965-7

Lin, J. N., Lin, V. C. H., Rau, K. M., Shieh, P. C., Kuo, D. H., Shieh, J. C., Chen, W. J., Tsai, S. C., & Way, T. Der. (2010). Resveratrol modulates tumor cell proliferation and protein translation via SIRT1 -dependent AMPK activation. *Journal of Agricultural and Food Chemistry*, *58*(3), 1584–1592. https://doi.org/10.1021/jf9035782

Lind, M. J. (2008). Principles of cytotoxic chemotherapy. *Medicine*, *36*(1), 19–23. https://doi.org/10.1016/j.mpmed.2007.10.003

Ling, H., Yang, H., Tan, S. H., Chui, W. K., & Chew, E. H. (2010). 6-Shogaol, an active constituent of ginger, inhibits breast cancer cell invasion by reducing matrix metalloproteinase-9 expression via blockade of nuclear factor-κB activation. *British Journal of Pharmacology*, *161*(8), 1763–1777. https://doi.org/10.1111/j.1476-5381.2010.00991.x

Liu, S., Li, R., Qian, J., Sun, J., Li, G., Shen, J., & Xie, Y. (2020). Combination Therapy of Doxorubicin and Quercetin on Multidrug-Resistant Breast Cancer and Their Sequential Delivery by Reduction-Sensitive Hyaluronic Acid-Based Conjugate/ d -α-Tocopheryl Poly(ethylene glycol) 1000 Succinate Mixed Micelles. *Molecular Pharmaceutics*, *17*(4), 1415–1427. https://doi.org/10.1021/acs.molpharmaceut.0c00138

Liu, X., Li, Y., Ma, Q., Wang, Y., & Song, A. L. (2019). Withaferin - A inhibits growth of drug-resistant breast carcinoma by inducing apoptosis and autophagy, endogenous reactive oxygen species (ROS) production, and inhibition of cell migration and nuclear factor kappa B (Nf-κB)/mammalian target of rapamycin (. *Medical Science Monitor*, *25*, 6855–6863. https://doi.org/10.12659/MSM.916931

Loftus, H., Astell, K., Mathai, M., & Su, X. (2015). Coleus forskohlii Extract Supplementation in Conjunction with a Hypocaloric Diet Reduces the Risk Factors of Metabolic Syndrome in Overweight and Obese Subjects: A Randomized Controlled Trial. *Nutrients*, *7*(11), 9508–9522. https://doi.org/10.3390/nu7115483

Maasomi, Z. J., Soltanahmadi, Y. P., Dadashpour, M., Alipour, S., Abolhasani, S., & Zarghami, N. (2017). Synergistic anticancer effects of silibinin and chrysin in T47D breast cancer cells. *Asian Pacific Journal of Cancer Prevention*, *18*(5), 1283–1287. https://doi.org/10.22034/APJCP.2017.18.5.1283

Makhoul, I. (2018). Therapeutic strategies for breast cancer. In *The Breast: Comprehensive Management of Benign and Malignant Diseases* (Fifth Edit). Elsevier Inc. https://doi.org/10.1016/B978-0-323-35955-9.00024-6

Mallipeddi, H., Thyagarajan, A., & Sahu, R. P. (2021). Implications of Withaferin-A for triple-negative breast cancer chemoprevention. In *Biomedicine and Pharmacotherapy* (Vol. 134). https://doi.org/10.1016/j.biopha.2020.111124

Mantena, S. K., Sharma, S. D., & Katiyar, S. K. (2006). Berberine, a natural product, induces G1-phase cell cycle arrest and caspase-3-dependent apoptosis in human prostate carcinoma cells. *Molecular Cancer Therapeutics*, *5*(2), 296–308. https://doi.org/10.1158/1535-7163.MCT-05-0448

Menendez, J. A., Joven, J., Aragonès, G., Barrajón-Catalán, E., Beltrán-Debón, R., Borrás-Linares, I., Camps, J., Corominas-Faja, B., Cufí, S., Fernández-Arroyo, S., Garcia-Heredia, A., Hernández-Aguilera, A., Herranz-López, M., Jiménez-Sánchez, C., López-Bonet, E., Lozano-Sánchez, J., Luciano-Mateo, F., Martin-Castillo, B., Martin-Paredero, V., … Segura-Carretero, A. (2013). Xenohormetic and anti-aging activity of secoiridoid polyphenols present in extra virgin olive oil: A new family of gerosuppressant agents. In *Cell Cycle* (Vol. 12, Issue 4, pp. 555–578). Taylor and Francis Inc. https://doi.org/10.4161/cc.23756

Mineva, N. D., Paulson, K. E., Naber, S. P., Yee, A. S., & Sonenshein, G. E. (2013). Epigallocatechin-3-gallate inhibits stem-like inflammatory breast cancer cells. *PloS One*, *8*(9), e73464. https://doi.org/10.1371/journal.pone.0073464

Mitra, S., & Dash, R. (2018). Natural Products for the Management and Prevention of Breast Cancer. *Evidence-Based Complementary and Alternative Medicine*, *2018*. https://doi.org/10.1155/2018/8324696

Moghtaderi, H., Sepehri, H., & Attari, F. (2017). Combination of arabinogalactan and curcumin induces apoptosis in breast cancer cells in vitro and inhibits tumor growth via overexpression of p53 level in vivo. *Biomedicine and Pharmacotherapy*, *88*, 582–594. https://doi.org/10.1016/j.biopha.2017.01.072

Mohapatra, P., Satapathy, S. R., Siddharth, S., Das, D., Nayak, A., & Kundu, C. N. (2015). Resveratrol and curcumin synergistically induces apoptosis in cigarette smoke

condensate transformed breast epithelial cells through a p21Waf1/Cip1 mediated inhibition of Hh-Gli signaling. In *International Journal of Biochemistry and Cell Biology* (Vol. 66). Elsevier Ltd. https://doi.org/10.1016/j.biocel.2015.07.009

Moloney, J. N., & Cotter, T. G. (2018). ROS signalling in the biology of cancer. In *Seminars in Cell and Developmental Biology* (Vol. 80, pp. 50–64). Elsevier Ltd. https://doi.org/10.1016/j.semcdb.2017.05.023

Moradzadeh, M., Hosseini, A., Erfanian, S., & Rezaei, H. (2017). Epigallocatechin-3-gallate promotes apoptosis in human breast cancer T47D cells through down-regulation of PI3K/AKT and Telomerase. *Pharmacological Reports*, *69*(5), 924–928. https://doi.org/10.1016/j.pharep.2017.04.008

Mouria, M., Gukovskaya, A. S., Jung, Y., Buechler, P., Hines, O. J., Reber, H. A., & Pandol, S. J. (2002). Food-derived polyphenols inhibit pancreatic cancer growth through mitochondrial cytochrome c release and apoptosis. *International Journal of Cancer*, *98*(5), 761–769. https://doi.org/10.1002/ijc.10202

Murray, L. J., & Lilley, J. (2020). Radiotherapy: technical aspects. *Medicine (United Kingdom)*, *48*(2), 79–83. https://doi.org/10.1016/j.mpmed.2019.11.003

Neve, R. M., Chin, K., Fridlyand, J., Yeh, J., Baehner, F. L., Fevr, T., Clark, L., Bayani, N., Coppe, J. P., Tong, F., Speed, T., Spellman, P. T., DeVries, S., Lapuk, A., Wang, N. J., Kuo, W. L., Stilwell, J. L., Pinkel, D., Albertson, D. G., … Gray, J. W. (2006). A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. *Cancer Cell*, *10*(6), 515–527. https://doi.org/10.1016/j.ccr.2006.10.008

Newman, D. J., & Cragg, G. M. (2016). Natural Products as Sources of New Drugs from 1981 to 2014. In *Journal of Natural Products* (Vol. 79, Issue 3, pp. 629–661). American Chemical Society. https://doi.org/10.1021/acs.jnatprod.5b01055

Nounou, M. I., Elamrawy, F., Ahmed, N., Abdelraouf, K., Goda, S., & Syed-Sha-Qhattal, H. (2015). Breast cancer: Conventional diagnosis and treatment modalities and recent patents and technologies supplementary issue: Targeted therapies in breast cancer treatment. *Breast Cancer: Basic and Clinical Research*, *9*(Suppl 2), 17–34. https://doi.org/10.4137/BCBCR.S29420

Ortega, A. L., Mena, S., & Estrela, J. M. (2011). Glutathione in Cancer Cell Death. *Cancers*, *3*(1), 1285–1310. https://doi.org/10.3390/cancers3011285

Ouyang, L., Luo, Y., Tian, M., Zhang, S. Y., Lu, R., Wang, J. H., Kasimu, R., & Li, X. (2014). Plant natural products: From traditional compounds to new emerging drugs in cancer therapy. *Cell Proliferation*, *47*(6), 506–515. https://doi.org/10.1111/cpr.12143

Park, G. Bin, Kim, Y. S., Lee, H. K., Song, H., Kim, S., Cho, D. H., & Hur, D. Y. (2011). Reactive oxygen species and p38 MAPK regulate Bax translocation and calcium redistribution in salubrinal-induced apoptosis of EBV-transformed B cells. *Cancer Letters*, *313*(2), 235–248. https://doi.org/10.1016/j.canlet.2011.09.011

Parmar, M. K. B. (2007). *Adjuvant therapy*. 38–40.

Patil, J. B., Kim, J., & Jayaprakasha, G. K. (2010). Berberine induces apoptosis in breast cancer cells (MCF-7) through mitochondrial-dependent pathway. *European Journal of Pharmacology*, *645*(1–3), 70–78. https://doi.org/10.1016/j.ejphar.2010.07.037

Pelicano, H., Carney, D., & Huang, P. (2004). ROS stress in cancer cells and therapeutic implications. In *Drug Resistance Updates* (Vol. 7, Issue 2, pp. 97–110). Churchill Livingstone. https://doi.org/10.1016/j.drup.2004.01.004

Potterat, O., & Hamburger, M. (2007). Morinda citrifolia (Noni) fruit - Phytochemistry, pharmacology, safety. In *Planta Medica* (Vol. 73, Issue 3, pp. 191–199). Planta Med.

https://doi.org/10.1055/s-2007-967115

Rabindran, S. K., Ross, D. D., Doyle, L. A., Yang, W., & Greenberger, L. M. (2000). Fumitremorgin C reverses multidrug resistance in cells transfected with the breast cancer resistance protein. *Cancer Research*, *60*(1), 47–50.

Radogna, F., Dicato, M., & Diederich, M. (2015). Cancer-type-specific crosstalk between autophagy, necroptosis and apoptosis as a pharmacological target. In *Biochemical Pharmacology* (Vol. 94, Issue 1, pp. 1–11). Elsevier Inc. https://doi.org/10.1016/j.bcp.2014.12.018

Rathbone, C. R., Booth, F. W., & Lees, S. J. (2008). FoxO3a preferentially induces p27Kip1 expression while impairing muscle precursor cell-cycle progression. *Muscle and Nerve*, *37*(1), 84–89. https://doi.org/10.1002/mus.20897

Ravindran, J., Gupta, N., Agrawal, M., Bala Bhaskar, A. S., & Lakshmana Rao, P. V. (2011). Modulation of ROS/MAPK signaling pathways by okadaic acid leads to cell death via, mitochondrial mediated caspase-dependent mechanism. *Apoptosis*, *16*(2), 145–161. https://doi.org/10.1007/s10495-010-0554-0

Ray, A., Vasudevan, S., & Sengupta, S. (2015). 6-shogaol inhibits breast cancer cells and stem cell-like spheroids by modulation of notch signaling pathway and induction of autophagic cell death. *PLoS ONE*, *10*(9), e0137614. https://doi.org/10.1371/journal.pone.0137614

Redza-Dutordoir, M., & Averill-Bates, D. A. (2016). Activation of apoptosis signalling pathways by reactive oxygen species. In *Biochimica et Biophysica Acta - Molecular Cell Research* (Vol. 1863, Issue 12, pp. 2977–2992). Elsevier B.V. https://doi.org/10.1016/j.bbamcr.2016.09.012

Reuter, S., Eifes, S., Dicato, M., Aggarwal, B. B., & Diederich, M. (2008). Modulation of anti-apoptotic and survival pathways by curcumin as a strategy to induce apoptosis in cancer cells. In *Biochemical Pharmacology* (Vol. 76, Issue 11, pp. 1340–1351). Biochem Pharmacol. https://doi.org/10.1016/j.bcp.2008.07.031

Robinson, M. H. (2008). Radiotherapy: technical aspects. In *Medicine* (Vol. 36, Issue 1, pp. 9–14). Elsevier. https://doi.org/10.1016/j.mpmed.2007.11.002

Safi, A., Heidarian, E., & Ahmadi, R. (2021). Quercetin Synergistically Enhances the Anticancer Efficacy of Docetaxel through Induction of Apoptosis and Modulation of PI3K/AKT, MAPK/ERK, and JAK/STAT3 Signaling Pathways in MDA-MB-231 Breast Cancer Cell Line. *International Journal of Molecular and Cellular Medicine*, *10*(1), 1–12. http://ijmcmed.org/article-1-1501-en.html

Salehi, B., Zucca, P., Sharifi-Rad, M., Pezzani, R., Rajabi, S., Setzer, W. N., Varoni, E. M., Iriti, M., Kobarfard, F., & Sharifi-Rad, J. (2018). Phytotherapeutics in cancer invasion and metastasis. *Phytotherapy Research*, *32*(8), 1425–1449. https://doi.org/10.1002/ptr.6087

Samarghandian, S., Nezhad, M., & Mohammadi, G. (2014). Role of Caspases, Bax and Bcl-2 in Chrysin-Induced Apoptosis in the A549 Human Lung Adenocarcinoma Epithelial Cells. *Anti-Cancer Agents in Medicinal Chemistry*, *14*(6), 901–909. https://doi.org/10.2174/1871520614666140209144042

Scambia, G., Ranelletti, F. O., Panici, P. B., De Vincenzo, R., Bonanno, G., Ferrandina, G., Piantelli, M., Bussa, S., Rumi, C., Cianfriglia, M., & Mancuso, S. (1994). Quercetin potentiates the effect of adriamycin in a multidrug-resistant MCF-7 human breast-cancer cell line: P-glycoprotein as a possible target. *Cancer Chemotherapy and Pharmacology*, *34*(6), 459–464. https://doi.org/10.1007/BF00685655

Scambia, G., Ranelletti, F. O., Panici, P. B., Piantelli, M., Bonanno, G., de Vincenzo, R., Ferrandina, G., Pierelli, L., Capelli, A., & Mancuso, S. (1991). Quercetin inhibits the growth of a multidrug-resistant estrogen-receptor-negative MCF-7 human breast-cancer cell line expressing type II estrogen-binding sites. *Cancer Chemotherapy and Pharmacology*, *28*(4), 255–258. https://doi.org/10.1007/BF00685531

Schlachterman, A., Valle, F., Wall, K. M., Azios, N. G., Castillo, L., Morell, L., Valance Washington, A., Cubano, L. A., & Dharmawardhane, S. F. (2008). Combined resveratrol, quercetin, and catechin treatment reduces breast tumor growth in a nude mouse model. *Translational Oncology*, *1*(1), 19–27. https://doi.org/10.1593/tlo.07100

Schnitt, S. J. (2001). Breast cancer in the 21st century: Neu opportunities and neu challenges. In *Modern Pathology* (Vol. 14, Issue 3, pp. 213–218). Nature Publishing Group. https://doi.org/10.1038/modpathol.3880288

Sehrawat, A., Samanta, S. K., Hahm, E. R., St. Croix, C., Watkins, S., & Singh, S. V. (2019). Withaferin A-mediated apoptosis in breast cancer cells is associated with alterations in mitochondrial dynamics. *Mitochondrion*, *47*(September 2018), 282–293. https://doi.org/10.1016/j.mito.2019.01.003

Sha, J., Li, J., Wang, W., Pan, L., Cheng, J., Li, L., Zhao, H., & Lin, W. (2016). Curcumin induces G0/G1 arrest and apoptosis in hormone independent prostate cancer DU-145 cells by down regulating Notch signaling. *Biomedicine and Pharmacotherapy*, *84*, 177–184. https://doi.org/10.1016/j.biopha.2016.09.037

Shanmugam, M. K., Rane, G., Kanchi, M. M., Arfuso, F., Chinnathambi, A., Zayed, M. E., Alharbi, S. A., Tan, B. K. H., Kumar, A. P., & Sethi, G. (2015). The multifaceted role of curcumin in cancer prevention and treatment. In *Molecules* (Vol. 20, Issue 2). https://doi.org/10.3390/molecules20022728

Shareef, M., Ashraf, M. A., & Sarfraz, M. (2016). Natural cures for breast cancer treatment. *Saudi Pharmaceutical Journal*, *24*(3), 233–240. https://doi.org/10.1016/j.jsps.2016.04.018

Sharma, G. N., Dave, R., Sanadya, J., Sharma, P., & Sharma, K. K. (2010a). Various types and management of breast cancer: An overview. In *Journal of Advanced Pharmaceutical Technology and Research* (Vol. 1, Issue 2, pp. 109–126). Wolters Kluwer -- Medknow Publications.

Sharma, G. N., Dave, R., Sanadya, J., Sharma, P., & Sharma, K. K. (2010b). Various types and management of breast cancer: An overview. In *Journal of Advanced Pharmaceutical Technology and Research* (Vol. 1, Issue 2, pp. 109–126). Wolters Kluwer -- Medknow Publications. /pmc/articles/PMC3255438/

Shi, H., Zhao, L., Guo, X., Fang, R., Zhang, H., Dong, G., & Fu, J. (2020). *Arctigenin Attenuates Breast Cancer Progression through Decreasing GM-CSF / TSLP / STAT3 / β - Catenin Signaling*.

Shih, Y. L., Liu, H. C., Chen, C. S., Hsu, C. H., Pan, M. H., Chang, H. W., Chang, C. H., Chen, F. C., Ho, C. T., Yang, Y. I. Y., & Ho, Y. S. (2010). Combination treatment with luteolin and quercetin enhances antiproliferative effects in nicotine-treated MDA-MB-231 cells by down-regulating nicotinic acetylcholine receptors. *Journal of Agricultural and Food Chemistry*, *58*(1), 235–241. https://doi.org/10.1021/jf9031684

Simon, A., & Robb, K. (2014). Cancer: Breast. In *Cambridge Handbook of Psychology, Health and Medicine, Second Edition* (pp. 577–580). Cambridge University Press. https://doi.org/10.1017/CBO9780511543579.131

Singh, I. P., & Mahajan, S. (2013). Berberine and its derivatives: A patent review (2009-2012). In *Expert Opinion on Therapeutic Patents* (Vol. 23, Issue 2, pp. 215–231). Taylor

& Francis. https://doi.org/10.1517/13543776.2013.746314

Sivasankarapillai, V. S., Madhu Kumar Nair, R., Rahdar, A., Bungau, S., Zaha, D. C., Aleya, L., & Tit, D. M. (2020). Overview of the anticancer activity of withaferin A, an active constituent of the Indian ginseng Withania somnifera. *Environmental Science and Pollution Research*, *27*(21), 26025–26035. https://doi.org/10.1007/s11356-020-09028-0

Staedler, D., Idrizi, E., Kenzaoui, B. H., & Juillerat-Jeanneret, L. (2011). Drug combinations with quercetin: Doxorubicin plus quercetin in human breast cancer cells. *Cancer Chemotherapy and Pharmacology*, *68*(5), 1161–1172. https://doi.org/10.1007/s00280-011-1596-x

Sui, X., Kong, N., Ye, L., Han, W., Zhou, J., Zhang, Q., He, C., & Pan, H. (2014). P38 and JNK MAPK pathways control the balance of apoptosis and autophagy in response to chemotherapeutic agents. In *Cancer Letters* (Vol. 344, Issue 2, pp. 174–179). Elsevier Ireland Ltd. https://doi.org/10.1016/j.canlet.2013.11.019

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209–249. https://doi.org/10.3322/caac.21660

Sunters, A., Fernández De Mattos, S., Stahl, M., Brosens, J. J., Zoumpoulidou, G., Saunders, C. A., Coffer, P. J., Medema, R. H., Coombes, R. C., & Lam, E. W. F. (2003). FoxO3a Transcriptional Regulation of Bim Controls Apoptosis in Paclitaxel-treated Breast Cancer Cell Lines. *Journal of Biological Chemistry*, *278*(50), 49795–49805. https://doi.org/10.1074/jbc.M309523200

Tang, F. Y., Su, Y. C., Chen, N. C., Hsieh, H. S., & Chen, K. S. (2008). Resveratrol inhibits migration and invasion of human breast-cancer cells. *Molecular Nutrition and Food*

Research, *52*(6), 683–691. https://doi.org/10.1002/mnfr.200700325

Thomford, N. E., Senthebane, D. A., Rowe, A., Munro, D., Seele, P., Maroyi, A., & Dzobo, K. (2018). Natural products for drug discovery in the 21st century: Innovations for novel drug discovery. *International Journal of Molecular Sciences*, *19*(6). https://doi.org/10.3390/ijms19061578

Tiwari, P., Kumar, A., Balakrishnan, S., Kushwaha, H. S., & Mishra, K. P. (2011). Silibinin-induced apoptosis in MCF7 and T47D human breast carcinoma cells involves caspase-8 activation and mitochondrial pathway. *Cancer Investigation*, *29*(1), 12–20. https://doi.org/10.3109/07357907.2010.535053

Tohme, S., Simmons, R. L., & Tsung, A. (2017). Surgery for cancer: A trigger for metastases. In *Cancer Research* (Vol. 77, Issue 7, pp. 1548–1552). American Association for Cancer Research Inc. https://doi.org/10.1158/0008-5472.CAN-16-1536

Treatment for Cancer - National Cancer Institute. (2015). https://www.cancer.gov/about-cancer/treatment

Tzivion, G., Dobson, M., & Ramakrishnan, G. (2011). FoxO transcription factors; Regulation by AKT and 14-3-3 proteins. In *Biochimica et Biophysica Acta - Molecular Cell Research* (Vol. 1813, Issue 11, pp. 1938–1945). Biochim Biophys Acta. https://doi.org/10.1016/j.bbamcr.2011.06.002

Vaux, D. L., & Korsmeyer, S. J. (1999). Cell death in development. In *Cell* (Vol. 96, Issue 2, pp. 245–254). Cell Press. https://doi.org/10.1016/S0092-8674(00)80564-4

Wang, H. C., Hu, H. H., Chang, F. R., Tsai, J. Y., Kuo, C. Y., Wu, Y. C., & Wu, C. C. (2019). Different effects of 4β-hydroxywithanolide E and withaferin A, two withanolides from Solanaceae plants, on the Akt signaling pathway in human breast

cancer cells. *Phytomedicine*, *53*, 213–222. https://doi.org/10.1016/j.phymed.2018.09.017

Wang, K., Zhang, C., Bao, J., Jia, X., Liang, Y., Wang, X., Chen, M., Su, H., Li, P., Wan, J. B., & He, C. (2016). Synergistic chemopreventive effects of curcumin and berberine on human breast cancer cells through induction of apoptosis and autophagic cell death. *Scientific Reports*, *6*(1), 1–14. https://doi.org/10.1038/srep26064

Wang, N., Tan, H. Y., Li, L., Yuen, M. F., & Feng, Y. (2015). Berberine and Coptidis Rhizoma as potential anticancer agents: Recent updates and future perspectives. In *Journal of Ethnopharmacology* (Vol. 176, pp. 35–48). Elsevier Ireland Ltd. https://doi.org/10.1016/j.jep.2015.10.028

Way, T. Der, Kao, M. C., & Lin, J. K. (2004). Apigenin Induces Apoptosis through Proteasomal Degradation of HER2/neu in HER2/neu-overexpressing Breast Cancer Cells via the Phosphatidylinositol 3-Kinase/Akt-dependent Pathway. *Journal of Biological Chemistry*, *279*(6), 4479–4489. https://doi.org/10.1074/jbc.M305529200

Way, T. Der, Kao, M. C., & Lin, J. K. (2005). Degradation of HER2/neu by apigenin induces apoptosis through cytochrome c release and caspase-3 activation in HER2/neu-overexpressing breast cancer cells. *FEBS Letters*, *579*(1), 145–152. https://doi.org/10.1016/j.febslet.2004.11.061

Wei, R., Mao, L., Xu, P., Zheng, X., Hackman, R. M., MacKenzie, G. G., & Wang, Y. (2018). Suppressing glucose metabolism with epigallocatechin-3-gallate (EGCG) reduces breast cancer cell growth in preclinical models. *Food and Function*, *9*(11), 5682–5696. https://doi.org/10.1039/c8fo01397g

Wen, C. J., Wu, L. X., Fu, L. J., Yu, J., Zhang, Y. W., Zhang, X., & Zhou, H. H. (2013). Genomic screening for targets regulated by berberine in breast cancer cells. *Asian Pacific Journal of Cancer Prevention*, *14*(10), 6089–6094.

https://doi.org/10.7314/APJCP.2013.14.10.6089

Woods, D. B., & Vousden, K. H. (2001). Regulation of p53 function. *Experimental Cell Research*, *264*(1), 56–66. https://doi.org/10.1006/excr.2000.5141

Xavier, C. P. R., Lima, C. F., Preto, A., Seruca, R., Fernandes-Ferreira, M., & Pereira-Wilson, C. (2009). Luteolin, quercetin and ursolic acid are potent inhibitors of proliferation and inducers of apoptosis in both KRAS and BRAF mutated human colorectal cancer cells. *Cancer Letters*, *281*(2), 162–170. https://doi.org/10.1016/j.canlet.2009.02.041

Xu, Y., Tong, Y., Ying, J., Lei, Z., Wan, L., Zhu, X., Ye, F., Mao, P., Wu, X., Pan, R., Peng, B., Liu, Y., & Zhu, J. (2018). Chrysin induces cell growth arrest, apoptosis, and ER stress and inhibits the activation of STAT3 through the generation of ROS in bladder cancer cells. *Oncology Letters*, *15*(6), 9117–9125. https://doi.org/10.3892/ol.2018.8522

Yang, J. Y., Zong, C. S., Xia, W., Yamaguchi, H., Ding, Q., Xie, X., Lang, J. Y., Lai, C. C., Chang, C. J., Huang, W. C., Huang, H., Kuo, H. P., Lee, D. F., Li, L. Y., Lien, H. C., Cheng, X., Chang, K. J., Hsiao, C. D., Tsai, F. J., … Hung, M. C. (2008). ERK promotes tumorigenesis by inhibiting FOXO3a via MDM2-mediated degradation. *Nature Cell Biology*, *10*(2), 138–148. https://doi.org/10.1038/ncb1676

Yang, M. Y., Wang, C. J., Chen, N. F., Ho, W. H., Lu, F. J., & Tseng, T. H. (2014). Luteolin enhances paclitaxel-induced apoptosis in human breast cancer MDA-MB-231 cells by blocking STAT3. *Chemico-Biological Interactions*, *213*(1), 60–68. https://doi.org/10.1016/j.cbi.2014.02.002

Zhang, A., Sheng, Y., & Zou, M. (2017). Antiproliferative activity of Alisol B in MDA-MB-231 cells is mediated by apoptosis, dysregulation of mitochondrial functions, cell cycle arrest and generation of reactive oxygen species. *Biomedicine and Pharmacotherapy*,

87, 110–117. https://doi.org/10.1016/j.biopha.2016.12.088

Zhao, Y., Jing, Z., Li, Y., & Mao, W. (2016). Berberine in combination with cisplatin suppresses breast cancer cell growth through induction of DNA breaks and caspase-3-dependent apoptosis. *Oncology Reports*, *36*(1), 567–572. https://doi.org/10.3892/or.2016.4785

Zhao, Y., Jing, Z., Lv, J., Zhang, Z., Lin, J., Cao, X., Zhao, Z., Liu, P., & Mao, W. (2017). Berberine activates caspase-9/cytochrome c-mediated apoptosis to suppress triple-negative breast cancer cells in vitro and in vivo. *Biomedicine and Pharmacotherapy*, *95*, 18–24. https://doi.org/10.1016/j.biopha.2017.08.045

Zhong, Z. F., Qiang, W. A., Wang, C. M., Tan, W., & Wang, Y. T. (2016). Furanodiene enhances the anti-cancer effects of doxorubicin on ERα-negative breast cancer cells in vitro. *European Journal of Pharmacology*, *774*, 10–19. https://doi.org/10.1016/j.ejphar.2015.11.039

Zhong, Z. F., Tan, W., Tian, K., Yu, H., Qiang, W. A., & Wang, Y. T. (2017). Combined effects of furanodiene and doxorubicin on the migration and invasion of MDA-MB-231 breast cancer cells in vitro. *Oncology Reports*, *37*(4), 2016–2024. https://doi.org/10.3892/or.2017.5435

Zhou, Q. M., Chen, Q. L., Du, J., Wang, X. F., Lu, Y. Y., Zhang, H., & Su, S. B. (2014). Synergistic effect of combinatorial treatment with curcumin and mitomycin C on the induction of apoptosis of breast cancer cells: A cDNA microarray analysis. *International Journal of Molecular Sciences*, *15*(9), 16284–16301. https://doi.org/10.3390/ijms150916284

Zhou, Q. M., Wang, X. F., Liu, X. J., Zhang, H., Lu, Y. Y., & Su, S. B. (2011). Curcumin enhanced antiproliferative effect of mitomycin C in human breast cancer MCF-7 cells in

vitro and in vivo. *Acta Pharmacologica Sinica*, *32*(11), 1402–1410. https://doi.org/10.1038/aps.2011.97

Zhou, Q., Ye, M., Lu, Y., Zhang, H., Chen, Q., Huang, S., & Su, S. (2015). Curcumin improves the tumoricidal effect of mitomycin C by suppressing ABCG2 expression in stem cell-like breast cancer cells. *PLoS ONE*, *10*(8), 1–12. https://doi.org/10.1371/journal.pone.0136694

Zong, L., Cheng, G., Liu, S., Pi, Z., Liu, Z., & Song, F. (2019). Reversal of multidrug resistance in breast cancer cells by a combination of ursolic acid with doxorubicin. *Journal of Pharmaceutical and Biomedical Analysis*, *165*, 268–275. https://doi.org/10.1016/j.jpba.2018.11.057