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SAUSSUREA COSTUS A PLANT USEFUL IN AVERTING BREAST CANCER PROGRESSION: AN OVERVIEW

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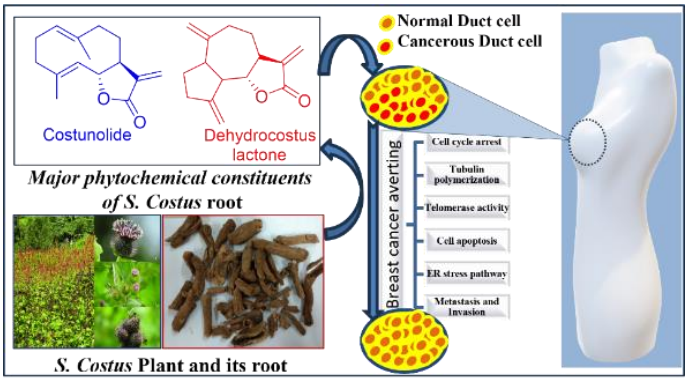
ABSTRACT

In the post COVID era, among the many incidences reported two are significant; increase in case report for breast cancer and significance of Ayurveda and natural medicine in modern era. In case of cancer, the currently available treatments chemotherapy and radiotherapy associate with huge collateral damages. So, one can look for phytochemical-based pharmaceutics as an alternative. *Saussurea costus* root extract has two important phytochemicals; Costunolide (CE) and Dehydrocostus lactone (DE), that have been reported for their anticancerous potential in isolation as well as in combination. Breast cancer; the most prevalent malignancy among women worldwide, characterized by complex nature and molecular heterogeneity across subtypes like luminal, HER2-enriched, and triple-negative. Cell lines (MCF-7, MDA-MB-231, MDA-MB-468, MDA-MB-453, etc.) serve as foundational *in vitro* models for elucidating disease mechanisms, drug responses, and therapeutic strategies. In present review we have tried to compile all the previously reported work of past two decades associated with anticancerous potential of *S. costus* especially in light of CE and DE, that may help in developing NCEs (New Chemical Entities) in future for breast cancer treatment.



KEYWORDS: *Saussurea costus, Costunolides, Dehydrocostus lactone, Breast cancer, Apoptosis.*

INTRODUCTION

Cancer is a leading cause of morbidity and mortality worldwide and have some unique features like; aberrant cells growth (i.e. not following the normal cell apoptosis process), spreading out of control (i.e. metastasis), heterogeneous phenotypes that exhibit dynamic plasticity, etc. Breast cancer, Lung cancer, Liver cancer (Hepatocarcinoma), Colorectal cancer (CRC) are among the most commonly detected cancer. In the post COVID era Breast cancer has surpasses lung cancer as most detected form of cancer (Itself in 2020 with more than 2.26 million new cases and this leads to approximately 6,85,000 death cases worldwide), also it is the second greatest cause of mortality for women globally and one of the most prevalent malignant diseases. In comparison to the conventional therapies: Chemotherapy and Radiotherapy, plant-derived products are selective; they damage cancer cells and have no significant effect on healthy cells. So, it may be a plausible alternative to conventional practice to treat breast cancer.[1]

Herbal medicine plays a significant role in the field of cancer treatment, both as a complementary therapy and in the development of new pharmaceutical drugs. Many herbs contain compounds with direct cytotoxic effects on cancer cells. For example, compounds like curcumin (from turmeric), epigallocatechin gallate (EGCG from green tea), and resveratrol (from grapes) have been shown in laboratory studies to induce apoptosis (programmed cell death) in cancer cells. Similarily, some herbal compounds may prevent the spread of cancer cells to other parts of the body. For instance, the herb *Tripterygium wilfordii*, used in traditional Chinese medicine, has been studied for its potential to inhibit tumor growth and metastasis.[2] Previous research suggests that phytochemicals like curcumin, can enhance cancer cell sensitivity to radiation and chemotherapy. Flavonoids, found in plants, can counteract multidrug resistance. Herbal remedies, like valerian and chamomile, are commonly used to treat anxiety and nausea, while ginger and peppermint help with chemotherapy-induced nausea. Modern anticancer drugs, like paclitaxel and vincristine, are derived from plants. Herbal medicine, including traditional systems like Ayurveda and TCM, is a rich source of new compounds with potential anticancer activity. Herbs like Astragalus, Echinacea, and Reishi Mushrooms are believed to boost the immune system and fight cancer more effectively.

The Indian subcontinent is gifted by the nature; one of the richest sources for diversified plant species. Eastern Himalayas and Western Ghats are two biodiversity hotspots in India. These regions contain a lot of medicinally important plant species and over the years they have been used traditionally for various ailments. As in the Indian Himalayan Region (IHR) approximately 1750 medicinally important plant species (~23.2% of India) has been found. *Saussurea costus* (Falc.) Lipschitz, syn *Saussurea lappa* C.B. Clarke is one of the most important and medicinally significant Angiosperm plant genera is Saussurea (family: Compositae/Asteraceae) consisting of ~ 400 species, in which a total of 62 species has been reported.[3] The major phytochemical constituents obtained from extract of *S. costus* root are Costunolide (CE) and Dehydrocostus lactone (DE), saussureal, saussureamines, lupeolpalmitates, flavone glycosides, betulinic acid, and guaianolides etc. Due to its versatile application and uses in different traditional medicine, this mini review is focused towards the two major phytochemicals found in root of *S.costus*: Costunolide (CE) and Dehydrocostus lactone (DE) and their anticancerous potential in breast cancer.

In Ayurveda (a great oldest compilation of indigenous medicine system of India) cancer is referred to as either Granthi (small neoplasm) or Arbuda (large neoplasm) and is defined as inflammatory and non-inflammatory swelling.[4] Generally, Ayurveda is not the preferred primary treatment for cancer due to its slower therapeutic action compared to conventional biomedical approaches. However, in cases where biomedicine is not viable, Ayurveda may be employed as an alternative or complementary approach for cancer management.

Early detection of abnormalities, such as nodular or globular muscle accumulation, may indicate the preliminary stages of breast cancer, facilitating timely intervention and improved outcomes. In fact, Biochemically, above mentioned four subtypes have its own uniqueness. The subtypes were chosen for treatment in accordance with the Estrogen receptor (ER), Progesterone receptor (PR), and Human Epidermal growth factor receptor (HER2) because they are linked to variances in outcome. One of the forms of breast cancer, triple-negative breast cancer (TNBC), is deadly and difficult to cure. Breast cancer may metastasize—a process known as organ metastasis—through the blood or lymphatic system if it is not treated in its early stages. There are four distinct subtypes of breast cancer: luminal A, luminal B, HER2 and basal-like (which resembles TNBC). ER, PR, and HER2 are frequently linked to various cancer forms. Immunohistochemistry (IHC) of various receptor expressions, such as ER/PR positive or negative and HER2 positive or negative, is used to identify the subtypes of breast cancer.[5]As per the report of 'The International Agency for Research on Cancer' Breast cancer is the sixth most frequent type of cancer worldwide and the most prevalent cause of cancer death among women. Additionally, breast cancer became the most frequently diagnosed cancer type in the world in 2020 with more than 2.26 million new cases and this leads to approximately 6,85,000 death cases worldwide.

The International Agency for Research on Cancer (IARC) identifies breast cancer as the second leading cause of cancer-related mortality among women worldwide. Low and low-middle SDI (socio-demographic index) countries and regions have the highest rates of cancer diagnoses and fatalities. Researchers forecast that by 2040, low- and middle-income nations would bear the burden of more than two-thirds of all the cancer cases reported worldwide. Due to high prevalence of chemoresistance, there are only a limited number of treatments available for breast cancer, which highlights the requirement to find and create novel therapeutic strategies. Cancer screening has completely stopped in the last two years when the pandemic was raging.

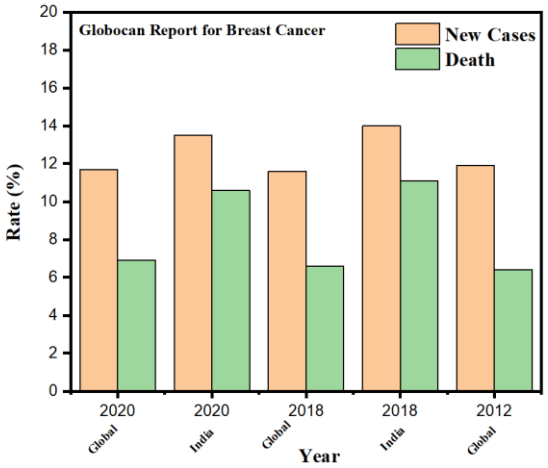


Figure 1: Bar diagram shows comparison of new breast cancer cases report and associated death cases at Global level and in India. (Data source: Globocan; WHOs International Agency for Research on Cancer).

As from the bar diagram (**Figure 1**) in comparison to global statistics in India death cases is more (like for year 2020: reported cases are 13.5% & death is 10.6% whereas for Global it is 11.7%& 6.90% only). Also, 'Down to Earth' statistics, shows India experienced an increase in cancer incidence at an average yearly rate of 1.2% to 2% in last decade. During the same time period, the average annual rate of cancer-related deaths in the nation increased by 0.1% to 1.0%. the paper showed. The ongoing novel corona virus disease (COVID-19) pandemic may have slowed progress in cancer care, according to the IHME study. The analysis shows main obstacles were likely to have been delays and decreases in cancer screening, diagnosis and treatment.

Chemical Composition and Major Phytochemicals

As per literature survey, it has been found that *S.costus* root extract in different solvent contains different phytochemical constituents. In which Terpenes, Sesquiterpenes, Flavanoids, Polyphenolics are very common. Following **table 1** shows the list of phytochemicals and some important of them has been depicted in **figure 2**.

Among all of these two important constituents: CE and DE shows great interest in past decades in area of various types of cancer treatment, like breast, ovarian, lung, colon, hepatic etc.

Table 1: Various Phytochemicals reported in *S.costus*.

Type	Name (composition in)	Ref.
Terpenes	Estragole, α--Thujene, Pinene, Camphene, Phellandrene, Anethole, Thymol, Citronellyl propionate, Myrcene, Sabinene, Limonene, 1,8 Cineol, Terpinen-4-ol, Ocimene, Linalool, Citronellal, Terpinen-4-ol, p-Cymene, Sitosterol, etc.	[6]
Sesqui terpenes	**Costunolide, Dehydrocostus lactone**, aluzanin C, 11β, 13-Dihydro-3-epizaluzanin C, Saussureamine B, Saussureamine C, Reynosin, β-CycloCE, α-Costol, Santamarine, Arbusculin-A, Colartin etc. and certain Germacranes type sesquiterpenelactones, etc.	[7]
Flavonoids	Acylated flavonoids Luteolin-7-O-D-glucoside, Rutin, Apigenin-7-O-D-glucoside Kaempferol 3- O-α-L-(2α', 3α'- (E)-di-p-coumaroyl) rhamnoside 7- O- (6'''-O- acetyl-β-D glucopyranosyl- (1→3)- [α- Lrhamnopyranosyl-(1→2)]-β-D-glucopyranoside. Rhein-8-O-dglucopyranoside, and Chrysophanol etc.	[7]

Following Table 2 summarises all the previously reported literature of S.costus root extract application for breast cancer treatment.

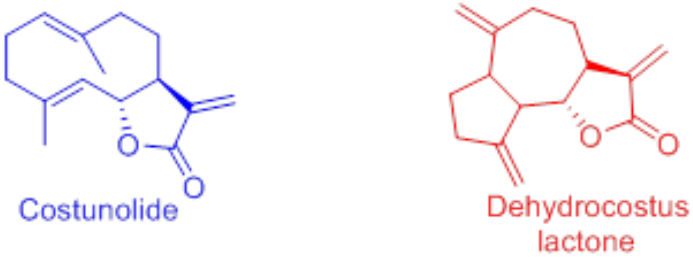


Figure 2: Structure of Costunolide($C_{15}H_{20}O_2$) and Dehydrocostus lactone ($C_{15}H_{18}O_2$).

Table 2: Summary of work done in past years by using *S. costus* root extract for their activity towards breast cancer.

Cell line	Dose	Treatment duration	Target genes, proteins or pathways	Molecular Target	Ref.
MCF-7 (wild-type p53), and MDA-MB-231 (mutant p53)	10 to 100 µM (CE)	48 hrs	hTERT, c-Myc, Sp1	Reduces hTERT (human telomerase reverse transcriptase gene) mRNA expression, telomerase activity, and transcription factor binding to hTERT promoters to inhibit the growth of tumors.	[8]
MCF-7, MDA-MB-231	8µg/mL (DE)	48 hrs	JAK/STAT, SOCS proteins	Stops the cell cycle and causes apoptosis Up-regulation of p53, p21 expression, Bax, cyclin D, cyclin A, cyclin B, cyclin-dependent kinase 2, cdc25A, cdc25C, and bcl-2 Bad and promotes chk1 activity, which inhibits colony formation and tumor progression.	[9]
MDA-MB-436, MDA-MB-157 and Bt-549	10 to 25 µM (CE)	6 hrs	Detyrosinated tubulin	Reduces the frequency of microtentacles (McTN), which inhibits tumor cell attachment (i.e. metastasis), by lowering detyrosinated tubulin independently of CE's NF-B inhibitory effects.	[10]
MDA-MB-231	20 µM (CE)	24 hrs or pretreated 1 hr	TNF-α, NF-κB	Inhibits tumor growth and metastases, By reducing TNF-induced tumor invasion and migration, it also reduces MMP-9 expression, NF-B transcriptional activity, and TNF-induced NF-B signaling activation.	[11]
MDA-MB-231	10, 20, 30, and 40 µM (CE)	24 hrs	NF-κB subunit proteins	Reduces overexpressed NF-B/p65, p52, and p100 and increases apoptosis. Inhibits tumor cell viability.	[12]
ER-α +ve and -ve human breast cancer MCF-7 and MDA-MB-231	10, 20, 40, 80 and 100 µM (CE)	24 hrs	Induces Apoptosis	Reduces the expression of caspase-3 and caspase-9, cyclin D1, D3, CDK-4, CDK-6, and CDK-4 in breast cancer cells. increases the expression of p18 INK4c, p21 CIP1/Waf-1, and p27 KIP1.	[13]
MCF-7, MDA-MB-231	0.4 - 2.8 µg/mL (CE,1/2, w/w)	48 hrs	c-Myc/p53 and AKT/14-3-3 pathway	Stops the cell cycle and causes apoptosis, enhances p53, p-14-3-3, and BAX/BCL-2, reduces c-Myc, p-AKT, and p-BID expression.	[14]
MDA-MB 231-Luc	3.92 µM 100.57 µM	-	Apoptotic inducer	Increased Bax and decrasedBcl levels	[15]
MCF- 7 HCC-70 MCF-12A	-7.33 -5.97	-	DHLC -4 DHLC-3	Protein kinase theta Protein kinase iota	[16]
MCF -7	20,40,60,80,100 µg/mL	48hrs	Apoptosis	Loss of mitochondrial membrane, DNA damage	[17]
SK BR3 MCF -7 TU7D MDA-MDA -MB-231 MCF -10 A	12.76,45.34,30. 16,27.90	48 hrs	Cell cycle arrest Tnf-α NF - KB	ROS depended metastasis, apoptosis, cellcycle arrest	[18]
MDA-MB -231	-	-	Apoptosis Cellcylcle arrest	FAS Caspase 8 caspase 3, degradatiob of PARP, decrease in cdc2, cyclin b1, increase in p2 /waf1	[19]

Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway

It's a manually drawn pathway map (**Figure 3**) representing the assumption made on the basis of various experimental data base showing molecular interaction, reaction and relation network. KEGG is a valuable resource for understanding the pathways involved in breast cancer development and progression. Here we mention few of them associated with breast cancer:

1. PI3K-Akt signaling pathway: This intracellular signaling system plays a crucial role in controlling the cell cycle. It frequently exhibits dysregulation in breast cancer and is crucial for cell survival, growth, and death. It involves the activation of PI3K (phosphoinositide 3-kinase) and downstream signaling through Akt (protein kinase B) to regulate cell growth and survival.

2. MAPK (mitogen-activated protein kinase) signaling pathway: Cell survival, differentiation, and proliferation

are all regulated by this route. Any disruption of this pathway, especially through the Ras-Raf-MEK-ERK cascade, is linked to the onset and spread of breast cancer.

3. Tumor suppressor protein (p53) signaling pathway: This is an important regulator for cell cycle arrest, DNA repair, and apoptosis; which is commonly observed in breast cancer, leading to uncontrolled cell growth and reduced DNA repair mechanisms.

4. Cell cycle pathway: It regulates the different phases of the cell cycle (viz. G1, S, G2 and M phase) progression, including DNA replication and mitosis. In case of cancer Dysregulation of this pathway is an universal phenomenon, including breast cancer.
5. Wnt signaling pathway: Dysregulation of this pathway particularly the activation of the canonical Wnt/β-catenin pathway, has been observed in the breast cancer initiation and progression.

6. Estrogen signaling pathway: It involves the binding of estrogen to its receptor, leading to the activation of downstream signaling cascades that regulate gene expression and cell proliferation. It has an important role in the hormone receptor-positive breast cancer.

7. Transforming growth factor-beta (TGF-β) signaling pathway: It is a multifunctional cytokine produce by lineages of white blood cells that belongs to the transforming growth factor family. It regulates cellular processes, like cell growth, differentiation, and apoptosis. Dysregulation of this pathway contributes to breast cancer progression, affecting tumor cell invasion, metastasis, and immune evasion.

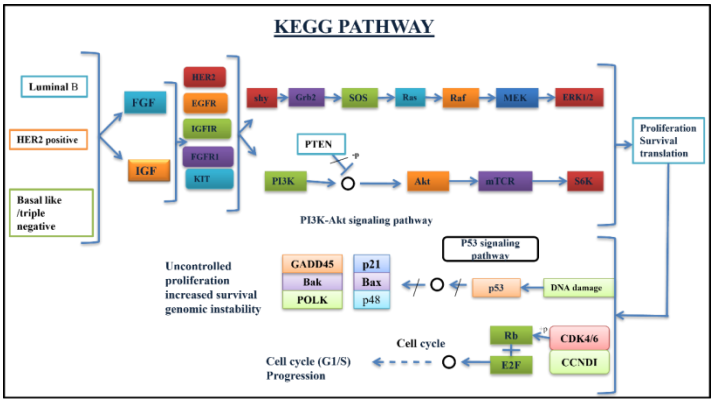


Figure 3: Schematic sketch for KEGG pathway for breast cancer.

Different mechanism through which it can work: Modulate Inhibition effect on Breast Cancer Cell Proliferation

Since diverse mechanisms are involved in the anticancer activity of phytochemicals, So a strong emphasis is placed on the anticancer pathways underlying their action.

1. Modulation of Cell Cycle Progression

Four key phases of Cell Cycle (G1, S, G2, & M phases) are highly regulated by both activators and inhibitors, as has been shown in the following **Figure 4**. Moreover, its dysregulation leads to features like loss of cell cycle control, uncontrolled cell division and tumor growth which is significantly correlated with cancer. Cyclin-dependent kinases (CDKs) are a family of enzymes that regulate the cell cycle, and CDK inhibitors can block the activity of these enzymes, preventing cell cycle progression. Similarly, Human epidermal growth factor receptor 2 (HER2) is a protein that can drive cell proliferation when overexpressed in breast cancer. According to previously published research, CE and DE greatly slowed down the cell cycle by increasing cell accumulation in the G2/M phase and reducing the G0/G1 phase (which prevents mitosis) in various cancer cells.[20]

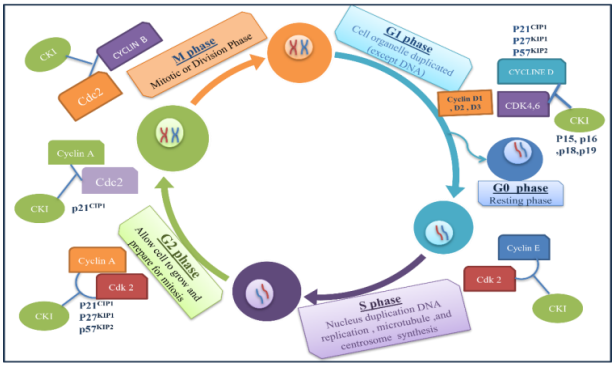


Figure 4: Phases in cell cycle along with involvement of proteins in different steps.

Lohberg et al. (2013), have demonstrated that DE reduced the expression of the cyclin-dependent kinase CDK2 and its inhibitor, p27Kip1, and caused the cell cycle to be arrested at the G2/M junction. Additionally, the number of cells at the G2/M phase transition junction significantly reduced cdc2 (CDK1) and cyclin B1.[21]

Kuo et al. (2009) find out the anticancerous effect of sesquiterpene DE on MCF-7 and MDA-MB-231 through cell cycle arrest and apoptosis. They described the mechanism, in which DE acts as a suppressor of cyclin D/A; or Cyclin dependent kinase 2 and cdc25A; and increases the quantity of p53 and p21, causing G0/G1-S phase arrest in MCF-7 cells. The pharmacological analysis of 45-days treatment period a 50% decrease in tumor volume.[9] Also, by measuring the levels of TNF-α and nitric oxide, Sunkara et al. (2010) showed the cytotoxicity tests of chloroformic extract of *S. lappa*. (costus) on breast cancer cell line (MDA-MB) and reported the comparable activity of this extract to a well-known standard medication doxorubicin, a well-known chemotherapeutic.

By following the ROS generation method, Choi et al. (2012) demonstrated that CE can act as a promising anticancer medication, particularly for ER-negative breast cancer. They got to the conclusion that CE causes an extrinsic mechanism that causes G2/M cell cycle arrest and apoptotic cell death in MDA-MB-231 cells. The CE-induced increase in p21WAF1 expression was associated with protein stability and ROS production among the p21WAF1 induction mechanisms studied.[19]

Li et al. (2020) discussed the role of key active part, α-methylene-γ-butyrolactone of CE and DE and their derivatives on MCF-7 and MDA-MB-231 (breast cancer) and leukemia by cell cycle arrest in G2/M phase, intrinsic and extric mechanism for apoptosis, telomerase activity and others related mechanism that involved in the breast cancer and metastasis suppression. Moreover, they mentioned the derivative product obtained by arylation of α-methylene-γ-lactone moiety in DE, through Heck reaction that shows high inhibitory potency to doxorubicin resistant cell line HL-60/A (IC50 = 6.2−19 μM). Similar derivative products obtained by Diastereoselective Michael addition in CE and DE also shows moderate cytotoxic activity.[23]

Recently Choi et al. (2023) has reported that CE induces apoptosis via reactive oxygen species (ROS) generation in various types of cancer cells. They assessed the impact of CE on the survival of breast cancer cells and discovered that SK-BR-3 cells, which overexpress the Her2 (Neu/ErbB-2) gene product, were more effectively cytotoxic than MCF-7 cells. Only in SK-BR-3 cells did CE treatment result in a mechanically significant increase in ROS levels, which triggers cathepsin D release and lysosomal membrane permeabilization (LMP), which in turn triggers apoptotic pathway by permeabilization of mitochondrial outer membrane.[18]

2. Influence of Tubulin Polymerization

Microtubules are cytoskeleton protein commonly found in all eukaryotic cells, that involved mainly in cell signaling and cell division. **Figure 5** shows the different stages involved in tubulin polymerization that finally leads to apoptosis. A unique feature associated with Microtubules are its ability to polymerize and depolymerize stochastically within a cell (microtubule dynamicity).[15] Since microtubule-binding substances can either stabilize them (promoting growth while preventing the microtubule filament from shrinking) or destabilize them (promoting shrinkage while preventing the microtubule filament from growing). Thus, controlling this process of microtubule stabilization and destabilization can arrest cancer cell in mitosis which eventually lead to cell death.[24]

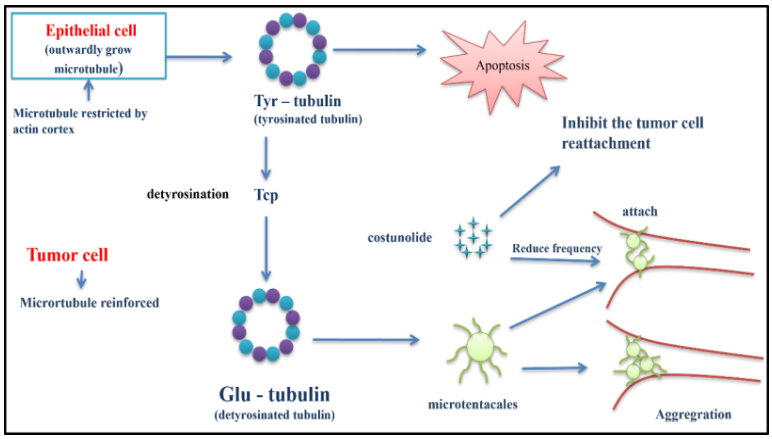


Figure 5: Correlation between tubulin polymerization and arrest of the cell cycle in breast cancer.

Bocca et al. (2004) demonstrated that CE, a microtubule-interacting medication, exhibits dose-dependent antiproliferative action in MCF-7 (human breast cancer) cells. From their analysis it has been found that after treatment with 100 nM of CE, cell size changes and the microtubules take on the appearance of a fine network of dense and misaligned fibers. Thus, it alters microtubule activity which leads to cell cycle arrest.[25] Nuclear

factor-kappa B (NF-κB) activation is a necessary step to defend the cell from TNF-induced apoptosis, and CE inhibits NF-κB activation to stop breast cancer growth and metastasis.[26]

According to Whipple et al. (2013), CE preferentially reduces detyrosinated tubulin without inhibiting NF-κB activation. CE treatment of suspended cells with live-cell scoring reveals a decrease in the frequency of microtentacles and an inhibition of reattachment. The microtubule network is indiscriminately disrupted by paclitaxel and colchicine, and they also show how CE can selectively target detyrosinated tubulin to lower microtentacles frequency and prevent tumor cell reattachment. In fact, inhibition of NF-κB is independent of all these actions, which is a promoter for TNFα-induced apoptosis. So, it can be used as anticancerous as well as reducing metastatic with less toxicity.[10]

3. Inhibition of Telomerase Activity

High telomerase activity is one of the characteristics of the cancerous cell leading to unlimited replication. Telomerase or terminal transferase a ribonucleoprotein that adds a species-dependent telomeres (repeated DNA sequences) to the 3' end (by adding TTAGGG repeats). The essential enzyme of telomerase is telomerase reverse transcriptase (hTERT), a specific ribonucleoprotein that reinforces the telomeres with TTAGGG repeats to guard against problems with end-replication. It plays a significant role in cell proliferation.[27]

Choi et al. (2005) earlier reported on the use of CE for the inhibition of telomerase activity, and they also investigated its function in slowing the growth of breast cancer in addition to suppressing telomerase activity. Also, down-regulated the hTERT; c-Myc and Sp1 (transcriptional factors) in breast cancer cell lines MCF-7 and MDA-MB-231.[8]

4. Induction of Cell Apoptosis

The effectiveness of CE and DE for apoptosis (or programmed cell death) against MDA-MB231 and MCF-7 cell line (breast cancer) through this mechanism has already been reported. It is a widely used and tightly controlled mechanism that involves an energetically dependent chain of molecular actions.[28] cell shrinkage and convolution, karyorrhexis, and pyknosis, as well as the retention of the entire cell membrane and cytoplasm in apoptotic bodies, are all morphological characteristics of apoptosis.[29]

There are basically three routes that CE and/or DE cause apoptosis by, according to already published literature: the mitochondrial-dependent "intrinsic" cytochrome C/caspase-9 pathway, the death receptor-mediated "extrinsic" caspase-8 pathway, and the endoplasmic reticulum (ER) stress pathway.[30-31] As chemicals in one pathway might have an impact on another, the three pathways are interconnected.[32]

4 (A). The Mitochondria -Dependent Intrinsic Pathway

The broader idea that apoptosis resistance is a characteristic of cancer. Mitochondrial dependent intrinsic pathway in apoptosis can be summarized in nutshell:

Activation of the pathway in which cellular stresses such as DNA damage can trigger the activation of pro-apoptotic proteins like Bax and Bak; which are Bcl-2 protein family member (which in normal condition remains inactive). After activation, these proteins go through conformational changes that cause the mitochondrial outer membrane to become permeable, that leads to release of several proapoptotic factors like cytochrome-c release in cytoplasm.

Apoptosome, which is made up of cytochrome C, apoptotic protease activating factor 1 (Apaf-1), and ATP, activates pro-caspase 9 and starts the procaspase cascade. After being activated by an apoptosome, caspase-9 cleaves and activates downstream effector caspase like caspase-3 and caspase-7, which then target and cleave a number of cellular proteins to cause apoptotic cell death.[20]

(B)The Death Receptor-Mediated Extrinsic Pathway

This is basically trans-membrane receptor-mediated interactions. It is triggered by the binding of death ligands to specific death receptor on the cell surface leading to activation of a cascade of molecular events that ends with cell death. A death-inducing signaling complex (DISC) is assembled at the cytoplasmic domain of the death ligand receptor upon binding. The death receptor, adaptor molecules (such as receptor-interacting protein (RIP), TNF receptor-associated death domain (TRADD), and Fas-associated death domain protein [FADD]), and initiator caspases (such as caspase-8 and caspase-10) often make up the DISC. Through proteolytic cleavage, this complex formation causes the activation of initiator caspases. When caspase-8 or caspase-10 are activated, downstream effector caspases like caspase-3 are immediately activated to start the execution phase of apoptosis.[33-34]

Choi et al. (2012) has reported that CE causes apoptosis in MDA-MB-231 cells that lack the estrogen receptor by activating the enzymes Fas, caspase-3, and caspase-8, degrading PARP without impairing mitochondrial membrane potential and altering the expression of the proteins Bcl-2 and Bax.[19]

5. The Endoplasmic Reticulum (ER) Stress Pathway

This is a cellular signaling mechanism that responds to disturbances in ER homeostasis, such as the accumulation of misfolded proteins or disruption of calcium levels within the ER. One of the key regulators of the ER stress pathway is the unfolded protein response (UPR), which is induced in response to this pathway. The following **Figure 6** shows the three main branches for UPR which includes: protein kinase RNA-like ER kinase (PERK), the inositol-requiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6) pathways.[35]

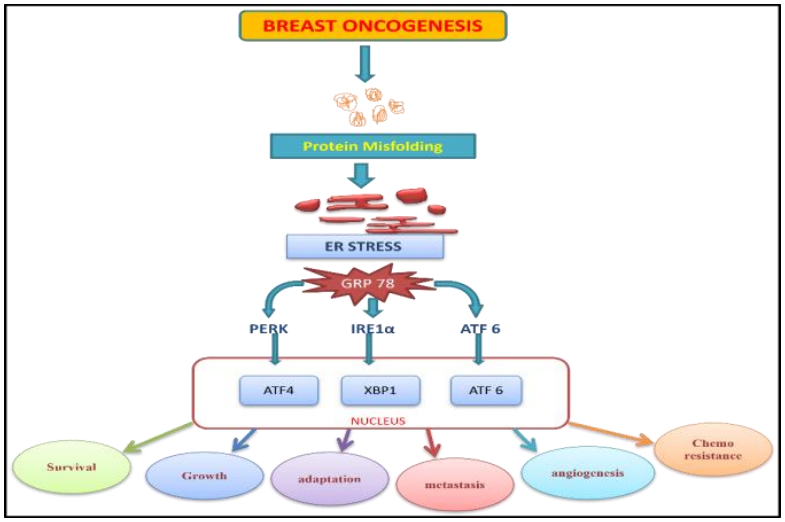


Figure 6: Depiction of ER stress pathway and its impact on different path of breast cancer mechanism.

In breast cancer, aberrant activation of the UPR has been observed in various contexts Activation of PERK can induce cell cycle arrest and promote apoptosis under prolonged ER stress conditions. ER stress can activate the UPR as well as the NF-B (nuclear factor kappa-light-chain-enhancer of activated B cells) and MAPK (mitogen-activated protein kinase) pathways in breast cancer cells. Moreover, it also has been mentioned that, CE-induces ROS generation that played a critical role in this process as pretreatment of cells with the ROS scavenger N-acetyl cysteine abolished the CE-induced ER stress and apoptosis.

7. Metastasis and Invasion for Anti-cancer Approach

One of the characteristics of cancer is recurrence of tumor even after conventional therapies like radiotherapy and chemotherapy; and metastasis i.e. spread of cancer cells from the primary tumor to distant sites in the body (**Figure 7** represents the spread of breast cancer to bone cancer); is the main cause of death in cancer patients.

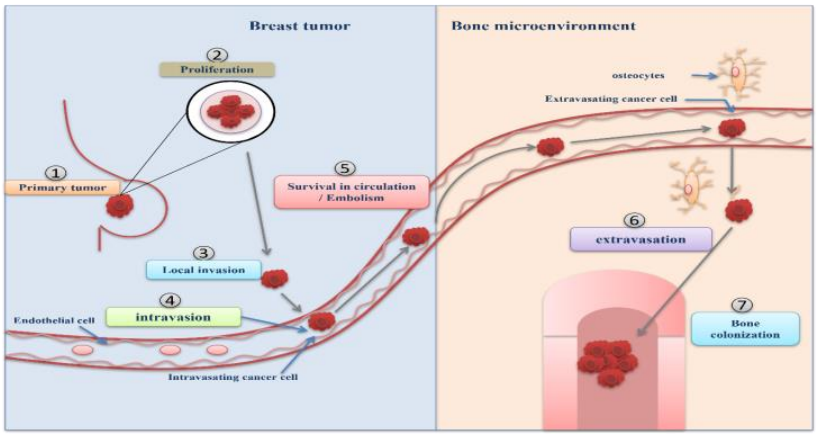


Figure 7: Representation of metastasis of breast cancer to bone cancer.

Some familiar signaling pathways like PI3K/Akt pathway, NF-κB, p53, and survivin, can influence metastasis and invasion in breast cancer. As it has been also reported the effectiveness of CE and DE for reduction of metastasis and invasion potential of cancer cell. In fact anti-metastasis mechanism of CE and DE are different.[21]Choi et al. (2013), shows the inhibitory effect of CE on TNF-induced breast cancer MDA-MB-231 cell migration and invasion. They also discussed a potential mechanism linked to a decrease in P-IKB, P-IKK and Nuclear p65 in the NF-B signaling pathway.[11]

Choi et al. (2013) shows the CE, obtained from *S. lappa* Clarke (SLC), inhibits TNF α-induced NF-κB activation and thereby suppress tumor growth and metastases of MDA-MB-231 highly metastatic human breast cancer cells, while having no effect on body weights in In vivo mouse ortho topic tumor growth assays. In vitro migration and invasion of MDA-MB-231 cells caused by TNF were likewise stopped by CE. In fact, suppression of TNFα-induced NF-κB signaling activation, leads to reduction in MMP-9 expression, a well-known NF-κB-dependent gene to mediate breast cancer cell growth and metastases.[11]

Some other derivative compounds have also been reported which shows enhanced activity towards breast cancer treatment. In this sequence, Kemboi et al. (2022)

reported the application of Michael addition reactions to synthesize DHLC (1-4) (13-amino derivatives of dehydrocostus lactone). three cancer cell line, viz. HCC70 (triple-negative breast cancer), MCF-7 (hormone receptor positive breast cancer) and MCF-12A (non-tumorigenic mammary epithelial) were chosen for the investigation, and they reported the DHLC's IC50 values were 1.11 (selectivity index (SI) = 0.06), 24.70 (SI = 0.01), and 0.07 M for the cell line HCC70, MCF-7 and MCF-12A respectively. All of the amino derivatives, with the exception of DHLC-3, had low micromolar IC50 values against both breast cancer cell lines (ranging from 0.07 to 4.24 M), and they were less hazardous to MCF-12A (SI= 6.00 to 126.86). The best selectivity for MCF-7 cells over MCF-12A cells was demonstrated by DHLC-1 and DHLC-2, which had SI values of 121 and 126.86 respectively. With 100-fold to 12 000-fold greater SI values than of MCF-12A, which indicates an improvement for breast cancer. In silico study shows the high docking score of -7.33 and -5.97 Kca/mol for DHLC-4 and DHLC-3, respectively than the parent DHLC (-5.34 Kca/mol). They claim that high binding affinities of these derivatives were enhanced by the addition of –NH2 group on C-13, which were seen to form hydrogen bonds and pi interactions with the enzyme.[16]

Along with above discussed mechanism a lot works carried out to show the importance of phytochemical extract-based cancer treatment mechanisms. Some Investigation have shown that, the anticancerous properties of DE and CE for human breast cancer cell lines MCF-7 and MDA-MB-453; obtained from hexane extract of *Saussurea lappa*. The characterization of the compound carried out by using techniques like, Mass Spectrometry, Infra-Red Spectroscopy, 1H and 13C NMR. On the basis of analysis, they reported that DE and CE inhibit proliferation of MCF-7. As shown, the hexane fraction is MCF-7 and MDA-MB-453 two cell lines 83.2% and 89.9%, respectively, at a treatment concentration of 50 µg/m/Growth was inhibited, $CHCl_3$ (52.6%, 52.4%) and l-BuOH (13.6%, 31.3%) Excellent growth inhibitory effect on cancer cells has been found.[21]

Robinson et al. (2008) has carried out the detailed analysis of hexane extract of *S. lappa* and found a new sesquiterpene Isodihydro CE, and investigated the In vitro cytotoxicity on MCF-7 (along with other cell line). They reported that exo-methylene group on lactone part of the sesquiterpenes is significant for cytotoxicity and good activity on cancer cell lines.[22]

By using the methyl thiazolyltetrazolium assay, Choi et al. (2010)'s excellent work on DE demonstrated its effectiveness as a potential anti-cancerous property for human breast cancer (MDA-MB-453, SK-BR-3 and MDA-MB-231) as well as ovarian cancer (SK-OV-3 and OVCAR3). They conducted a dose-dependent investigation of DE that revealed a reduction in cell proliferation. The IC50 values in the MDA-MB-231, MDA-MB-453, SK-BR-3 were 21.5, 43.2, 25.6, whereas, for SK-OV-3, and OVCAR3 cells were 15.9, and 10.8 M, respectively.[36]

As an anti-cancer, the synergistic effect of CE and DE (obtained from the volatile oil of *S. lappa*) has been studied on breast cancer MCF-7 xenografts by Peng et al. (2014). Pharmacological studies on athymic nude mice were done by taking the doses of 30 mg/kg/2 days and 15 mg/kg/2 days for MCF-7 xenograft tumor growth and Paclitaxel standard respectively. The dynamic changes of serum and urine metabolism have been shown and correlated with the attenuated metabolic perturbation in tumor growth.[37]

Kim et al. (2014) investigated thoroughly the anti-cancerous efficacy of a 70% ethanolic extract of S. costus on breast cancer. For these different concentrations 1, 2, 5, 10, or 30 µg/mL were used to demonstrate the suppression of 12-o-tetradecanoylphorbol-13-acetate (TPA)-induced MMP-9 production and cell invasion in MCF-7. RT-PCR, zymography, and Western blot analysis showed that the compound decreased the rise of MMP-9 caused by TPA. Plant extract's role was further elucidated by its inhibitory impact on TPA-induced NF-κβ and AP-1 activation in MCF-7 cells.[37]

Patel et al. (2020) Investigated the aqueous extract of *S. lappa* root for Breast Cancer Cells (MCF-7) treatment. It shows cytotoxic activities with IC50 values ranging from 2.5 mg/ml to 0.85 mg/ ml. The result shows the ability to up-regulate the expression of TGF, BAX, and IKaB in MCF-7 while the expression of the P53 and Bcl2 genes down-regulated. On the other hand, the treated Caco2 cells (colorectal adenocarcinoma cancer cell) showed down regulation for P53, Bcl2 and IKaB genes.[38]

Hornal et al (2021) have synthesized MgO NP by two different methods, that were used in pharmaceutical and also shows that, the metal oxide is nontoxic and environment friendly in nature.[39]

Some other applications CE and DE obtained from many species of medicinal plants, including *S. lappa* Decne and *Laurus nobilis* L, have been reported by Lin et al. (2015). It shows numerous biological benefits other than Anticancerous are antiviral, antibacterial, antifungal, anti-inflammatory, antioxidant, antidiabetic, antiulcer, and anthelmintic properties.[21]

Elgharabawy et al. (2021) has find out from their study that costus (obtained from *S.lappa*) treated Ehrlich solid tumor (EST: a transplantable poorly differentiated malignant tumor originally appear as a spontaneous breast carcinoma in a mouse) has shown improved apoptosis, and induced a significant decrease in ki-67 expression, Na+ and high-density lipoprotein (HDL) levels.[40]

Manello et al. (2011) discussed about the members of matrix metalloproteinases (MMPs) and its role in breast carcinogenesis. They discussed about the MMP-1 (Collagenase-1) as a prognostic marker in patients with invasive or metastatic breast cancer. They correlate the MMP-1 as a risk factor as per say high levels of MMP-1 mRNA expression as well as aplotypes and polymorphisms in the MMP-1.[41]

Peng et al. (2017) mentioned about CE and DE obtained from *S. lappa* root and the apoptotic activity of breast cancer is inhibited through AKT/14-3-3 and c-Myc/p53 pathway.[14] Sesquiterpene lactone obtained from the dried roots of S. lappa acts as an anti-tumor agent against the MCF-7 cell line and IC50 value of 35.05 ±9.37 µg/mL for the same. Similar comparative results have also been mentioned in the above comparative table 1. In fact, it is well understood that CE suppresses the MDA-MB-231 cell growth and metastasis by inhibiting TNFα-induced NF-kB activation.

Some Metal Based Modification in Extract

In recent years some work has been carried out which shows some metal-based modification in these plant-based extract which were used for the breast cancer treatment.

According to Amina et al. (2020), biomasses of S. costus roots were used in the manufacture of MgO nanoparticles (MgONPs). Two S. costus varieties—Qustal hindi and Qustal bahri—were employed in the green synthesis of MgONPs to achieve this. To determine the morphological characteristics, SEM and TEM microscopy were used. The results suggest that the particle size for Qustal hindi and Qustal bahri, respectively, falls in the range of 30 and 34 nm. Six pathogenic strains were used to test the produced MgONPs' antibacterial activity. MgONPs shown a potential lethal effect as anticancer materials against MCF-7 breast cancer cell lines. When compared to Qustal hindi, MgONPs made by Qustal bahri exhibit high cytotoxicity against the breast cancer cell line MCF-7. In MCF-7 cells, MgONPs from S. costus (Qustalhindi and Qustalbahri) biomasses had IC50 values of 67.3% and 52.1%, respectively.[17]

N.S.Al-Radadi et al. (2022) has synthesize Palladium nanoparticles (PdNPs) by using S. costus extract as a stabilizing and reducing agent. they investigated for the possible use of the nanoparticles, which have an average grain size of 17.6 1. 2 nm, as anticancer, antioxidant, anti-Alzheimer's, and antibacterial agents. Using the breast adenocarcinoma (MCF-7) cell lines, PdNPs were produced that had anti-tumor effects and shown strong anti-cancerous activity.[42]

CONCLUSIONS

Despite of not availability of permanent cure of Cancer till date, one can be more optimistic for cure by using natural Plant based medicine. Convention medication has an averting effect in due time but health cost is high. Herbal medicine can offer many benefits; it is crucial to consider potential interactions with conventional cancer treatments. Some herbs may interfere with the metabolism of chemotherapy drugs or enhance their toxicity. Plant extract-based compounds like CE and DE (specially their α-methylene-γ-butyrolactone moiety) has a significant anticancerous potential specially in breast cancer progression. Also, both of these two phytochemicals in combination shows synergistic antitumor effect, thus shows significant importance in developing New Chemical Entities. Moreover, they can be used along with conventional anti cancerous drug for faster recovery and better tackling of cancer progression.

Conflicts of Interest

There is no conflict to declare.

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