Active constituents of herbal medicines for breast cancer: Current status

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ABSTRACT

Breast cancer (BC) is the second most common cause of cancer-related deaths in women. The risk profile of BC and the choice of its treatment modalities depend upon the rate of incidence, treatment outcome, prognosis, and the extent of metastases. Though multiple modalities to treat BC exist, there is no single panacea available. The discovery of novel molecular targets and the availability of innovative dosage form to deliver drugs to the tumor microenvironment and an upsurge in the biologicals have revolutionized the treatment of BC. However, the cost-effectiveness of such therapies, their unavoidable adverse effects, and poor therapeutic outcomes underscore the need to search for still better therapeutic options. Against this backdrop, the striking anticancer efficacies of multiple phytoconstituents that have succeeded to enter the later phases of clinical trials present a renewed hope in treating BC. In this review, we have given the account of selected secondary plant metabolites that are posing as new options in treating BC. Their interactions with molecular pathways and therapeutic targets involved in the initiation and progression of BC are elaborated.

INTRODUCTION

Breast cancer (BC) and non-melanoma skin cancer are the most common types of cancers in women (Bray et al., 2018; Kumar et al., 2016). The incidence of BC is rapidly increasing and in the year 2020, around 2.3 million women were diagnosed with BC and there were 685,000 BC-associated deaths worldwide (Breast Cancer, 2021). BC affects women of any age group, and the prevalence is more amongst elderly women. BC represents multiple subcategories comprising of discrete cellular compositions, characteristic molecular perturbations, and specific clinical presentations. The prognosis and response to therapy depend upon multiple factors like size of the tumor, histological characteristics, metastasis, presence of hormone receptors—estrogen receptor (ER) and progesterone receptor (PR), and presence of human epidermal growth factor receptor 2 (EGFR-2 or ErbB2) (Eliyatkin et al., 2015).

The BC patients were conventionally categorized as non-specific ductal carcinoma and other specific types dependent upon the morphology, histological findings, etc. (Eliyatkin et al., 2015). The emerging details on the molecular and genetic characteristics are also being used for patient categorization. The ERα and ErbB2 (previously Her-2 or Her-2/neu) are the principal molecular
targets expressed on the BC cells (Figs. 1 and 2). Progesterone, another steroid hormone, is closely related to ERα signaling and acts through the PRs (Joshi and Press, 2018). About 70% of the patients with invasive BC express either ER or PR receptors in >1% cancer tissue cells (Hammond et al., 2010; Joshi and Press, 2018). Similarly, ErbB2 is also expressed on the BC cells. Triple-negative BC (TNBC) lacks the said molecular target (ER, PR, and EGFR-2) and roughly constitute 15% of the cases (Denkert et al., 2017). TNBC has a high prevalence of relapse during the first and third years after diagnosis and the average life expectancy after diagnosis of TNBC is 5 years (Sporikova et al., 2018). Also, germline mutation in BC gene 1 (BRCA1) and BRCA2 genes (responsible for the repair of DNA single-strand break) is associated with approximately 5% of BC cases (Robson et al., 2017).

ErbB2 is a receptor tyrosine kinase that activates several signaling pathways involving the Ras/c-Raf/MAPK-ERK kinase (MEK)/extracellular signal-regulated kinase (ERK), signal transducer, and activator of transcription (STAT), Phosphoinositide-3-Kinase (PI3K)/ protein kinase B (Akt)/mammalian target of Rapamycin (mTOR), Phospholipase C (PLC)-γ1, and c-Src pathways (Wang et al., 2018). Nearly 20% of BCs overexpress the ErbB2 gene (classified as ErbB2+/Her2+) and have a poor prognosis if lacking systemic therapy (Piccart-Gebhart et al., 2005; Wolff et al., 2013).

The foremost objectives of therapy for non-metastatic BC are eliminating tumors from the breast and neighboring lymph nodes and suppressing metastatic relapse. For non-metastatic BC, local therapy consists of surgical removal of breast tumor and/or axillary lymph nodes, with post-surgery radiation therapy. Systemic treatment could be adjuvant (post-surgery), neoadjuvant (pre-surgery), or both. BC subtype influences the standard systemic treatment provided, consisting of hormone therapy for all HR+ tumors (plus chemotherapy in certain cases), ErbB2-targeted therapy along with chemotherapy for all ErbB2+ tumors (plus hormone therapy in case of HR+ tumors), and only chemotherapy for TNBC (Waks and Winer, 2019).

In metastatic BC, therapy objectives are extending life and palliative care as metastatic BC is presently terminal in almost all cases. However, similar elementary sets of adjuvant/neoadjuvant systemic treatments are practiced in metastatic BC. Local therapy procedures (surgery and radiation) are utilized only for palliative purposes. Also, targeted drugs like abemaciclib, palbociclib, and ribociclib (inhibitors of Cyclin-dependent kinases 4 and 6) to target cancer cells’ cell cycle received approval for adjuvant therapy in the management of metastatic BCs (Waks and Winer, 2019).

Notwithstanding the benefits of the available systemic therapies in different BC patients, these therapies have several
issues including adverse effects (Goss et al., 2016), recurrence of the disease (Waks and Winer, 2019), limited effect (von Minckwitz et al., 2017), cost, and no significant improvement in disease-free survival (Francis et al., 2018).

Therefore, new treatment approaches are needed with reduced adverse effects specifically during longer therapies, especially for patients with TNBC. Also, novel agents are required to reduce the number of chemotherapeutic agents needed for low-risk patients and novel agents that are effective in high-risk BC patients like trastuzumab in ErbB2+ BC (Waks and Winer, 2019).

Diet with high-fiber, low-fat, fruits, and vegetables is linked to a lower risk of cancer, according to studies (Soerjomataram et al., 2017), cost, and no significant improvement in disease-free survival (Francis et al., 2018).

In this review, we will discuss certain secondary metabolites of plant origin which are promising in BC treatment and have reached the clinical phase of the investigation.

**GROWTH FACTORS AND THEIR RECEPTORS**

**Insulin-like growth factor (IGF)**

A study was conducted to analyze the effect of green tea extract (GTE) containing 832 mg of Epigallocatechin Gallate (EGCG) on mammographic density (MD) in healthy postmenopausal women. After 12 months of oral administration, no significant change was observed in the percent or absolute MD of treated women. However, a significant reduction in the percent MD was observed in women aged 50–55 years. The GTE administration also significantly increased circulating estradiol in healthy postmenopausal women, but no significant change was observed in the plasma levels of IGF-1 and IGF binding protein.
(IGFBP)-3 (Samavat et al., 2017). The study did not assess the ER expression which increases in benign breast epithelium with age and postmenopause and positively correlated with BC risk. Other studies have reported mixed results on the effect of EGCG on BC risk and its association with the Catechol-O-methyltransferase (COMT) gene SNP rs4680, mainly in Asian-American women (Lazzeroni et al., 2017; Samavat et al., 2017).

**Fibroblast growth factor receptor 2 (FGFR2)**

FGFR2 is one of four receptors that control signaling from fibroblast growth factors (FGFs), a family of genes that play a role in development, cell growth, and death. FGFR2 signaling pathways are divided into two groups, one that relies on FGFR substrate 2α (FRS2α) and one that does not. These pathways include RAS-MAPK, PLCγ, PI3K, and Janus kinase (JAK)/STAT (Lei and Deng, 2017). Genetic changes in FGFR2 have been found in BC, leading to the activation of downstream FGFR2 signaling pathways, like the FGFR2 rs2981582T/C variant linked to BC in the Saudi population (AlRaddadi et al., 2021).

Soy intake for 7–30 days resulted in significant overexpression of the FGFR2 gene, protumorigenic growth factor receptor, in breast tumor, and no significant change was observed in Ki67 or caspase 3 levels (Shike et al., 2014). However, the study has not evaluated the clinical impact of increased expression of the FGFR2 gene and the short period of intervention might not be enough to produce significant changes in phenotypes. Also, heterogeneity in breast tumor and menopause status of subjects might have influenced the outcome of the study.

Genistein is a type of isoflavonoid found in plants such as soybeans, clovers, and lupins (Garbiec et al., 2022). Epidemiological studies have shown that a diet rich in soy is associated with cancer prevention in Asian populations (Ahnae-Jarvis et al., 2015; Dong and Qin, 2011). Meta-analysis studies have also found that consuming soy isoflavones can reduce the risk of BC before and after menopause (Boutas et al., 2022; Chen et al., 2014). The incidence of BC also increases in Asian women after they migrate to the United States, suggesting that lifestyle, diet, and environmental factors play a role in the development of these cancers (Shimizu et al., 1991). A study in Japan found that consuming miso soup and isoflavones was inversely associated with BC risk (Fujimaki et al., 2003). It is believed that the isoflavones in soy-containing foods are responsible for their chemopreventive effects.

The ambiguity about the estrogenic effects of genistein continued in clinical studies as well. On one side studies have reported the safety of genistein on breast tissue (Atteritano et al., 2008; Marini et al., 2008) whereas, on the other side, soy isoflavones stimulated breast proliferation in premenopausal women (Hargreaves et al., 1999; Khan et al., 2012; McMichael-Phillips et al., 1998). It is a possibility that genistein might have behaved differently in premenopausal and menopausal women. Interestingly, a phase II clinical study namely “Gemcitabine Hydrochloride and Genistein in Treating Women with Stage IV Breast Cancer,” has reported the closure of the study because of the lack of efficacy (Gemcitabine hydrochloride and genistein in treating women with stage IV breast cancer—study results, n.d.). Therefore, further studies with a large sample size are needed to ascertain the potential of genistein in BC and to clarify the mechanism of opposite effects in women before and after menopause.

**OXIDATIVE STRESS AND INNATE ANTIOXIDANTS**

**Nuclear factor erythroid 2 (NF-E2-p45)-related factor 2 (Nrf2)/antioxidant response element (ARE) signaling pathway**

Transducer KEAP1 controls the equilibrium between apoptosis and autophagy in addition to regulating the oxidative stress response through the downstream Nrf2-dependent signaling (Sajadimajd and Khazaei, 2018; Stepkowski and Kraszewski, 2011). In BC, dipeptidyl-peptidase 3 is substantially overexpressed and interacts with KEAP1 to increase Nrf2 expression (Lu et al., 2017). p62, an autophagy adapter protein, and KEAP1, an adaptor for the Cul3 E3 ubiquitin ligase for Nrf2, directly interact to regulate autophagy in large part (Jiang et al., 2015b). Sulforaphane prevents KEAP1 from promoting Nrf2 proteasome-mediated breakdown, however, KEAP1 can still network with LC3 and p62 under duress by employing p62 as a link between autophagosomes and ubiquitin aggregates (Fan et al., 2010). Therefore, KEAP1 is nearly entirely involved in directing cells into autophagy.

A study of eight healthy women showed that consuming broccoli sprouts increased sulforaphane in breast tissue (Cornblatt et al., 2007). Several trials found a significant decrease in Ki-67 protein expression in women with ductal carcinoma in situ (DCIS) after eating cruciferous vegetables or sprout extracts (Study to evaluate the effect of sulforaphane in broccoli sprout extract on breast tissue—study results, n.d.; Zhang et al., 2016). Short-term sulforaphane supplementation reduced Ki-67 and HDAC3 in benign tissue, but more research is needed to determine the effects on DCIS and invasive ductal carcinoma (Atwell et al., 2015). An open-label study is evaluating the efficacy of sulforaphane in combination with tamoxifen and fulvestrant (SFX-01 in the treatment and evaluation of metastatic breast cancer, n.d.). A current double-blind trial is examining the protective effects of sulforaphane on doxorubicin-related heart damage in BC patients and its impact on Nrf2- and SIRT1 expression (Protective effects of the nutritional supplement sulforaphane on doxorubicin-associated cardiac dysfunction—no study results posted, n.d.).

**Hypoxia inducible factor (HIF)-1α pathway**

Compared to normal cells, cancer cells are more hypoxic and subject to oxidative stress. Hypoxia and oxygen radicals work together to induce tumor angiogenesis (Brown and Bicknell, 2001) HIF-1, activated by hypoxia and encourages the production of vascular endothelial growth factor (VEGF) for angiogenesis regulation in BC (Ferrara et al., 2003). Oxygen radicals also elevate HIF-1 levels. Oxygen radicals also trigger the NF-κB activation, which boosts the production of VEGF. To accelerate the development of BC, VEGF also promotes the development and migration of cancer cells (Yang et al., 2011). By lowering VEGF and HIF-1 expression and reducing NF-κB activation, ECGG considerably reduces breast tumor angiogenesis and prevents the growth and metastasis of cultured mice and human BC cells without influencing healthy tissues like the heart and skeletal muscles (Braicu et al., 2013; Gu et al., 2013; Wei et al., 2018).

**CELL PROLIFERATION AND APOPTOSIS PATHWAYS**

**p53 Pathway**

The tumor suppressor protein i.e., p53 is often altered in a variety of human cancers and regulates a diverse range of pathways.
that include cell proliferation, DNA repair, and apoptosis (Kandoth et al., 2013; Muller and Vousden, 2014). Apoptosis regulator proteins of the Bcl-2 family influence p53 which activates Bax and leads to cell death through mitochondrial rupture and release of cytochrome c (Marzo et al., 1998; Olval et al., 1993). This activates caspase-3 and, poly (ADP-ribose) polymerase (PARP), two major mediators of apoptosis (Kluck et al., 1997; Reed, 1995). EGCG increases p53 expression and decreases Bcl-2, leading to a higher Bax/Bcl-2 ratio and increased apoptosis (Huang et al., 2017; Roy et al., 2005). Silibinin boosts proapoptotic gene expression such as IFN-stimulated gene (ISG)15, p53, CASP9, and BAX, while curcumin reduces BC growth through mechanisms such as cell cycle arrest and p53-mediated apoptosis, modulation of signaling pathways, Hedgehog/Glial-associated oncogene homolog-1 (Gli1) signaling pathway, down-regulation of transcription factors, and halting angiogenesis (Hossainzadeh et al., 2019; Khan et al., 2022; Song et al., 2019; Zhang et al., 2022a).

The p53 gene is involved in regulating cell growth and apoptosis in BC, and mutations in the gene are observed in 20% of cases (Hallman et al., 2017). P53 promotes the expression of p21, which arrests cells in the G1 phase for DNA repair, and upregulates apoptotic target genes such as PUMA (p53 upregulated modulator of apoptosis) and Bax if DNA repair fails (Takaoka et al., 2003). EGCG triggers apoptosis through mitochondrial dysfunction and stimulates autophagy by increasing ATG5 (autophagy-related protein 5), Beclin1, and autophagy marker light chain 3-B expression (Baliga et al., 2005; Wei et al., 2018). These proteins are major autophagy markers and play a role in the formation of autophagosomes membrane and interaction with lysosomes ( Mizushima and Yoshimori, 2007; Tanida et al., 2005; Wirth et al., 2013).

Wnt/β-catenin pathway

Wnt/β-catenin pathway is known to play a crucial role in the self-renewal of cancer stem cells (CSCs) in BC (Zhang and Wang, 2020). The pathway is activated by β-catenin through T cell factor/lymphoid enhancer factor to regulate the expression of downstream genes such as c-Myc and stem cell markers (Zhang and Hao, 2015). Glycogen synthase kinase 3 (GSK3) promotes the degradation of β-catenin via ubiquitin-mediated degradation and modulates the Wnt pathway (Takahashi-Yanaga, 2013). Curcumin therapy has been shown to inhibit the Wnt/β-catenin pathway by reducing GSK3, β-catenin, and c-Myc (Li et al., 2018). Sulforaphane also inhibits the Wnt/β-catenin pathway by activating GSK3 and promoting the degradation of β-catenin, potentially making it effective against breast CSCs (Li et al., 2010).

The Wnt/β-catenin pathway is essential for the growth and differentiation of mammary glands, angiogenesis, and hormone signaling, all of which are significant risk factors for BC (Amado et al., 2011). This pathway is activated by the interaction of Wnt proteins with the lipoprotein receptor-related protein5/6 and Frizzled receptors and inhibited by the tumor suppressor adenomatous polyposis coli-Axin-GSK3β-casein kinase 1 complex in the absence of Wnt (Barker and Clevers, 2006; MacDonald et al., 2009; Polakis, 2007). In BC cells, substances like curcumin, sulforaphane, EGCG, and silibinin have been shown to inhibit the Wnt/β-catenin pathway and its downstream gene c-Myc, leading to reduced cancer cell growth and self-renewal (Hong et al., 2017; Loh et al., 2013; Lu et al., 2012).

ErbB2/ErbB3/PI3K/Akt/mTOR pathway

The PI3K/AKT/mTOR pathway is a common signaling route in human cancer, triggered by the loss of phosphatase and tensin homolog (PTEN) activity, and stimulation of growth factor receptors (IGF-1R and ErbB2) (Liu et al., 2009). EGCG, an adenosine triphosphate (ATP)-competitive inhibitor, has been shown to inhibit PI3K and mTOR in BC cells and reduce levels of EGFR, ERK, and phospho-ERK p42/44, as well as cell migration and extracellular matrix (ECM) metalloproteinase inducer expression (Farabegoli et al., 2011). Curcumin has been found to block TGF-β and PI3K/AKT pathways and improve the inhibitory effect of doxorubicin in TNBC cells (Guan et al., 2016; Kunihiro et al., 2022; Mao et al., 2019). ErbB2 activates several pathways, including the PI3K/Akt/mTOR pathway, and is overexpressed in 20% of BCs (Piccart-Gebhart et al., 2005). Integrins are transmembrane receptors that link the ECM to intracellular actin and activate focal adhesion kinase, which leads to the activation of downstream proteins including Ras, Rac, and Cdc42 (Burcelo et al., 2004; Dovas and Couchman, 2005; Hood and Cheres, 2002; Machacek et al., 2009). Silibinin has been shown to affect the β1-integrin signaling pathway and the Ras-Raf-MEK-ERK pathway (Dastpeyman et al., 2012; Kolch et al., 2002; Lee et al., 2007; Lin et al., 2009).

ERK1/2-MAPK pathway

Raf, a serine/threonine protein kinase, directly phosphorylates or enhances protein phosphorylation by stimulating MEK/ERK signaling pathway (Chang et al., 2003; Roberts and Der, 2007). The Raf/MEK/ERK cascade regulates the growth and progression of several cancers, including BC (Li et al., 2016; De Luca et al., 2012). ARAF, BRAF, and CRAF are examples of Raf family members. Recent research has demonstrated that ARAF dimerization increases MAPK pathway activation and cell metastasis (Mooz et al., 2014). Sulforaphane hindered MEK and ERK phosphorylation via interacting with Raf family members such as ARAF, BRAF, and CRAF and reduced BC cell metastasis by preventing actin stress fiber production (Zhang et al., 2022b).

BRCA1 pathway

The BRCA1 gene is a pertinent DNA repair gene that is crucial for DNA repair, transcription, and regulation of the cell cycle. The cases of hereditary BCs are significantly associated with germine mutation of the BRCA1 gene and sporadic incidences of BC are linked with aberrant methylation of the promoter region of the BRCA1 gene that renders the gene nonfunctioning (Al-Moghribi et al., 2015; Gupta et al., 2014; Iwamoto et al., 2011; Wong et al., 2011). Further, the breast cancer-specific gene 1 (BCSG1) also called the SNCG gene expresses the γ synuclein protein of the synuclein family (Ji et al., 1997). The SNCG gene, a proto-oncogene, is related to metastasis and decreased duration of disease-free survival in stages III and IV of breast ductal carcinomas (Wu et al., 2007). The demethylation of Exon 1 of SNCG is incidental to an errant expression of SNCG in BC (Lu et al., 2001). Curcumin induced the expression of the BRCA1 gene by decreasing methylation in DNA promoter methylation in ER/PR+ and TNBC cells. Further, curcumin-induced methylation in the promoter region of the SNCG gene in the ER+/PR+ BC cell line (T47D cells) (Al-Yousef et al., 2020).
Apoptosis
miRNAs are small noncoding RNAs that control cellular processes by inhibiting messenger RNA (mRNA) translation or assisting mRNA degradation (Curatle, 2018; O’Bryan et al., 2017; Zadeh et al., 2016). miRNAs can have either tumor-suppressive or oncogenic impacts on cells (Garzon et al., 2010; Paranjape et al., 2009). Silibinin has been shown to inhibit the production of carcinogenic miRNAs such as miR-125b and miR-182 and cause autophagic cell death by increasing the expression of Bel-2 adenovirus E1B 19-kDa-interacting protein 3 (BNIP3), a pro-apoptotic protein, and elevating reactive oxygen species formation (Hossainzadeh et al., 2019; Jiang et al., 2015a). Inhibiting matrix metalloproteinases (MMPs) has been shown to protect against tumor advancement due to its effect on cyclooxygenase-2 (COX-2), an enzyme involved in inflammation and wound healing that can also promote carcinogenesis (Chun and Surh, 2004; Surh et al., 2001). Silibinin has been shown to suppress 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced COX-2 expression and MMP-9 expression in BC (Kim et al., 2009).

HORMONAL PATHWAY AND METABOLIC PATHWAY

Estrogen and progesterone
ER-α levels are higher in ER-positive cancers, and hyperplastic lesions, and linked to an increased likelihood of invasive breast disease (Frech et al., 2005; Poola et al., 2005). Estrogen-regulated genes (insulin receptor substrate 1, pS2, & cyclin D1) are suppressed when PR-β expression is high in BC cells, indicating PR/PR-B affects ER-α gene transcription (De Amicis et al., 2013; Khan et al., 1994). EGCG has been found to have co-estrogenic effects at lower concentrations and anti-estrogenic effects at higher concentrations (Belguise et al., 2007; Farabegoli et al., 2007). It may regulate the nuclear translocation of PR and the binding of PR-B onto the ER-alpha promoter, leading to transcriptional repression of genomic and non-genomic activity of ER-alpha, slowing the development of ER+ PR+ BC cells (De Amicis et al., 2013). Studies have shown that higher levels of EGCG are achieved in BC tissues with phosphatidylcholine formulation of EGCG (Lazzeroni et al., 2017). However, more research is needed to confirm the potential of EGCG in reducing BC risk, especially in the Asian population with the less active COMT genotype. Early exposure to genistein in immature rats may promote the differentiation of mammary gland cells and reduce BC susceptibility (Lamartiniere et al., 1998). However, genistein-containing foods may be harmful to ER+ BC in postmenopausal women (Ju et al., 2006). Low doses of genistein promote cell proliferation and overexpression of estrogen response element (ERE) in ER+ BC cells and can act like an estrogen molecule (Hsieh et al., 2006; Seo et al., 2006). It can also inhibit the tumor preventive and antiproliferative effects of tamoxifen and hydroxytamoxifen (Liu et al., 2005; Seo et al., 2006).

Silibinin stimulates apoptosis in MCF-7 cells by downregulating ERα and upregulating ERβ, and by promoting nuclear translocation of AIF. The apoptosis is reversed by AIF knockdown using siRNA (Liu et al., 2020; Zheng et al., 2015, 2016).

Lipid metabolism
Deregulation of the metabolism is thought to be a characteristic of cancer (Nicot et al., 2004; Pizer et al., 1996). Fatty acid synthase (FAS) inhibition has little effect on non-cancerous cells, but a recent study showed that FAS is expressed in TNBC patient samples and that FAS inhibition is beneficial for TNBC preclinical models (Pizer et al., 2000). EGCG has been shown to have antiproliferative effects on MDA-MB-231 TNBC cells and has been reported to inhibit FAS, trigger apoptosis, and reduce tumor growth without concurrent carnitine palmityltransferase-1 stimulation or weight reduction (Chisholm et al., 2004; Puig et al., 2008b, 2008a; Roy et al., 2005).

Leptin, a hormone produced by adipose tissue, has been shown to promote proliferation, transformation, and migration in healthy and cancerous mammary epithelial cells through the leptin receptor i.e., ObR (Dieudonne et al., 2002; Laut et al., 2002). Studies have shown that leptin stimulates the proliferation and transformation of T47D BC cells and various BC cell types express both leptin and its receptor (Hu, 2002). Silibinin has been found to suppress the development of T47D cells by reducing leptin expression through ERβ activation (Nejati-Koshki et al., 2012; Zheng et al., 2016).

Glutamine metabolism
Ferroptosis, or iron-dependent programmed cell death, plays a critical role in cancer-related immune evasion and drug resistance (Friedmann Angeli et al., 2019; Shin et al., 2018; Sun et al., 2016). Glutamine metabolism can promote ferroptosis by increasing the accumulation of oxidizable lipids (Gao et al., 2015; Xu et al., 2019). Soluble carrier family 1 member 5 (SLC1A5), a glutamine uptake transporter, has been linked to tumor progression. Curcumin may inhibit tumorigenesis by increasing SLC1A5 expression in BC (Cao et al., 2022; Jiang et al., 2020).

Glycolysis
Glycolysis is a metabolic pathway used by cancer cells to produce anabolic substrates such as nicotinamide adenine dinucleotide phosphate and ATP from glucose, allowing them to adapt to fluctuating oxygen supply (Jose et al., 2011). Myc, a cancer metabolism regulator, suppresses the Thioredoxin-interacting protein (TXNIP) to increase glycolysis, essential for tumor development (Lim et al., 2016; Lunt and Vander Heiden, 2011). Silibinin has been found to lower glucose consumption and inhibit critical metabolic processes in TNBC, resulting in decreased cell growth, biomass, and stem cell features and improved anti-TNBC effectiveness when combined with paclitaxel. It also reverses the results of ectopic EGFR, Myc silencing, and TXNIP overexpression in TNBC (Iqbal et al., 2021; Lim et al., 2016).

INFLAMMATORY PATHWAY
C-X-C Chemokine receptor type 4 (CXCR4) signaling pathway
CXCR4 overexpression is linked to an increased risk of lung and bone metastasis in metastatic BC (Felix et al., 2010; Furusato et al., 2010). Silibinin at a concentration of 80 μM can prevent C-X-C Motif Chemokine Ligand 12-induced migration in MDA-MB-231 cells and CXCR4 stimulation by suppressing CXCR4 signaling, potentially explaining its anti-tumor activity (Wang et al., 2014).
Akt and nuclear factor–kappa B (NF-κB) pathway

The nuclear NF-κB pathway controls cell survival, inflammation, differentiation, and proliferation. Tumor necrosis factor-alpha (TNF-α) activates inhibitor of kappa B kinase (IKK), leading to phosphorylation and destruction of inhibitors of NF-κB (IκB), resulting in the transcription of downstream targets genes that control pro-inflammatory and anti-apoptotic processes (Park and Hong, 2016; Poma et al., 2017; Yu et al., 2009). EGFR and Akt also activate NF-xB. Genistein has been found to induce apoptosis and inhibit growth in BC cell lines by inhibiting Akt and NF-κB pathways when combined with other anticancer agents (Liu et al., 2005; Ma et al., 2022).

Intra-ductal administration of curcumin and its nano-formulation in the mammary duct of SD rats, treated with N-methyl-N-nitrosoarene to induce mammary carcinoma, significantly lowers the incidence of mammary tumors by decreasing activation of NF-κB (Chun et al., 2012). In T47D BC cells, curcumin therapy inhibits growth hormone-triggered invasion-metastasis, and epithelial mesenchymal transition (EMT) activation by blocking the NF-xB pathway and promoting apoptotic cell death by modifying the Bcl-2 family of proteins (Coker-Gurkan et al., 2018; Zhang et al., 2022a).

Different kinases such as NIK, NAK, MAPK, and Akt control the IKK-NF-xB signaling in different ways (Hwang et al., 2006). IKK enzyme is composed of two catalytic subunits (IKKα and IKKβ) and a regulatory component (IKKγ) (Li et al., 2001). Sulforaphane suppresses TPA-induced NF-xB activation and COX-2 expression in MCF-10A cells by inhibiting the ERK1/2-IKKα pathway and NAK-IKKβ signaling (Kim et al., 2014a; Taoqi Zhou et al., 2022).

Inflammation can aid in the development of malignant cells by suppressing the immune system (O’Callaghan et al., 2010). Reduced accumulation of Myeloid-derived suppressor cells (MDSCs) may enhance immune surveillance and antitumor immunity (Cuenca et al., 2011). In mice, Silibinin increases tumor-infiltrating T cells, and reduces tumor development and the number of MDSCs (Forghani et al., 2014).

STAT3 pathway

The transcription factor STAT3 transmits signals to the nucleus and its constitutive activation is linked to tumor growth and poor prognosis in BC (Turkson and Jove, 2000; Zhong et al., 1994). Studies that show STAT3 activation is a key factor in tumor invasion and activation of BC stem cells (Azare et al., 2011; Dauer et al., 2005). EGCG modifies STAT3-NF-xB signaling pathways to decrease the number of cancer stem-like clusters of differentiation 44-positive cells (Chung and Vadgama, 2015).

The STAT3 and STAT5b members of the STAT family are oncogenes involved in the development, growth, and spread of malignant BCs (Garcia et al., 2001, 1997; Siveen et al., 2014). These proteins are activated by JAK and phosphorylation, resulting in the transcription of target genes (Darnell, 1997; Imada and Leonard, 2000). Silibinin at a 200 M concentration reduces Jak2 expression and phosphorylation and prevents Jak2 from phosphorylating STAT3, preventing TNBC cells from proliferating, migrating, and invading by inhibiting MMP2 gene-specific transcriptional activation (Byun et al., 2017).

Fibronectin (FN) is a glycoprotein in the ECM that aids in cell adhesion, proliferation, migration, differentiation, and neoplastic transformation (Fernandez-Garcia et al., 2014). FN expression is higher in TNBC instances and is linked with a worse clinical outcome in BC patients (Bae et al., 2013; Balanis et al., 2013) Additionally, FN can prevent apoptosis from conventional chemotherapy drugs. Silibinin has been found to suppress EG- induced FN expression in MDA-MB468 BC cells by suppressing STAT3 activity (Kim et al., 2014b).

Transforming growth factor-beta (TGF-β), a class of polypeptides, induces EMT, resistance to chemotherapy and cell death, and escape from immune surveillance (Thiery et al., 2009; Xu et al., 2009). Silibinin can inhibit the metastasis of TNBC cells in vivo and reduce FN and MMP-2 expression levels, which are induced by TGF-β (Kim et al., 2016).

LONG NONCODING RNAS (lncRNAs)

lncRNAs have been demonstrated to act as major transcriptional regulators in a variety of biological activities and disease processes, including cancer (Peng et al., 2017). Also, lncRNA H19 plays a significant role in BC growth, metastasis, chemoresistance, and endocrine resistance (Peng et al., 2017; Zhou et al., 2017). According to a recent study, H19 is significantly elevated in tamoxifen-resistant (TAMR) BC and is also linked to tamoxifen resistance (Basak et al., 2018). Several investigations have shown that H19 dysregulation promotes tumor metastasis by influencing tumor cell EMT (Wu et al., 2019; Ye et al., 2020; Zhou et al., 2017).

H19 upregulation promoted EMT, invasion, and migration in MCF7/TAMR cells through upregulating Snail. Curcumin reduced the expression of H19 in a dose-dependent manner. Curcumin significantly reduced the expression of H19-induced epithelial marker E-cadherin while significantly enhancing the expression of the mesenchymal marker N-cadherin in MCF7/TAMR cells (Cai et al., 2021).

CONCLUSION

The modern paradigm of drug discovery has provided promising therapies for the treatment of BC but with some drawbacks like severe toxicities, dose limitation, and cost of therapy. The phytoconstituents which are reported to exert anticancer effects provide certain advantages like multi-targeted actions, lesser toxicity, and a unique bioactivity profile that warrants further systematic clinical investigation of these phytoconstituents. Notwithstanding the advantages, the phytoconstituents have their own sets of limitations such as low bioavailability, varied effects in different populations, and mechanistic interference of available systemic therapies. The bioavailability can be improved with the help of novel delivery systems and chemical derivatization. The variable effects and mechanistic interference can be overcome with the meticulous designing of therapeutic regimens. Further investigations in these directions will add promising adjuvants to the existing chemotherapy for BC.

LIMITATIONS

Although several phytoconstituents with various underlying processes have been studied in preclinical settings, only a small number of them have been studied in clinical settings to see whether they may be used to treat BC. In this study, only those phytoconstituents were reviewed which have reached the clinical phases of the investigation. The author has consulted the PubMed database and the clinical trial registry of the US for this study.
AUTHOR CONTRIBUTIONS
SK, CRP conceptualized the review; SK, CRP, SB, RJJ prepared the first draft; Himangini, MM, VJ, SY prepared the artwork, tabulated data and critically evaluated the manuscript. All authors critically revised the draft. SK, CRP and SB approved the final version.

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ETHICAL APPROVALS
This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY
All data generated and analyzed are included in this research article.

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