



Review

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Bioactive constituents of animal-derived traditional Chinese medicinal materials for breast cancer: opportunities and challenges

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Abstract: Breast cancer is globally the most common invasive cancer in women and remains one of the leading causes of cancer-related deaths. Surgery, radiotherapy, chemotherapy, immunotherapy, and endocrine therapy are currently the main treatments for this cancer type. However, some breast cancer patients are prone to drug resistance related to chemotherapy or immunotherapy, resulting in limited treatment efficacy. Consequently, traditional Chinese medicinal materials (TCMMs) as natural products have become an attractive source of novel drugs. In this review, we summarized the current knowledge on the active components of animal-derived TCMMs, including *Ophiocordyceps sinensis*-derived cordycepin, the aqueous and ethanolic extracts of *O. sinensis*, norcantharidin (NCTD), Chansu, bee venom, deer antlers, *Ostrea gigas*, and scorpion venom, with reference to marked anti-breast cancer effects due to regulating cell cycle arrest, proliferation, apoptosis, metastasis, and drug resistance. In future studies, the underlying mechanisms for the antitumor effects of these components need to be further investigated by utilizing multi-omics technologies. Furthermore, large-scale clinical trials are necessary to validate the efficacy of bioactive constituents alone or in combination with chemotherapeutic drugs for breast cancer treatment.

Key words: Breast cancer; Traditional Chinese medicinal material (TCMM); Bioactive constituent; Anticancer effect

1 Introduction

As the most common female malignancy worldwide, breast cancer accounts for 32% and 12.8% of female malignancies in incidence and mortality, respectively (DeSantis et al., 2019; Lei et al., 2021; Sung et al., 2021). The optimal therapy for breast cancer depends on the tumor subtype, the cancer stage, and the patient preferences (Waks and Winer, 2019). Besides surgery, radiotherapy, endocrine therapy, chemotherapy, and immunotherapy play vital roles in the management of breast cancer (Hassan et al., 2010; Bhushan et al., 2021). Chemotherapy can be used as a treatment option in almost all stages of breast cancer, either alone or in combination with other therapies to inhibit tumor progression and thus

extend patient survival (Waks and Winer, 2019). In comparison, immunotherapy has proven more effective for cases of triple-negative breast cancer (TNBC) (Emens, 2018; Mediratta et al., 2020), an aggressive and lethal subtype of breast cancer, due to the high heterogeneity and the lack of treatment options (Zhu et al., 2021). However, some breast cancer patients are prone to drug-resistance to chemotherapy or immunotherapy, resulting in limited efficacy (Nedeljković and Damjanović, 2019).

Natural products comprise a valuable pool of drug sources for chemotherapy and immunotherapy (Dutta et al., 2019; Deng et al., 2020). For example, more than 60% of the approximately 200 drugs approved by the Food and Drug Administration (FDA) to treat different types of malignant tumors, such as taxol, doxorubicin, and vincasar, are derived from natural ingredients (Demain and Vaishnav, 2011; Cragg and Pezzuto, 2016). Therefore, refining, processing, and standardizing active constituents in natural products is an established approach for the development of novel chemotherapies or immunotherapy drugs.

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Traditional Chinese medicinal materials (TCMMs) as natural products are an attractive source of novel drugs. The *Shen Nong Ben Cao Jing* alone, which is the earliest pharmacological monograph of Chinese medicine written between about 200 and 250 CE, has a record of 365 kinds of Chinese medicinal materials. Among them, numerous active ingredients in Chinese herbal medicines have potential utility for the development of new drugs, with the examples of artemisinin, which exhibits anti-malarial (Adekunle et al., 2016; Guo, 2016) and anti-systemic lupus erythematosus effects (An et al., 2017; Mu and Wang, 2018), and thapsigargin A, which exerts anti-neuroinflammatory effects by acting directly on the inosine monophosphate dehydrogenase type II (IMPDH2) protein (Liao et al., 2017). More recently, Chinese herbal medicines have played an irreplaceable role in the fight against coronavirus disease 2019 (COVID-19) in China (Ni et al., 2020; Xiao et al., 2020; Zhao et al., 2021). These successes suggest that looking into the bioactive components derived from TCMMs may lead to the discovery of novel effective chemotherapeutic or immunotherapeutic agents.

Previous reviews have systematically analyzed and evaluated the anticancer effects of Chinese herbal medicines and their active ingredients (Zhang Y et al., 2020; Wang KL et al., 2021; Wang S et al., 2021). Animal-derived TCMMs are central components of traditional Chinese medicine, and many of them are key agents for well-known compound prescriptions presenting satisfactory efficacy. The active ingredients of animal-derived TCMMs are mainly allogeneic proteins or peptides and vitamins, which showed marked efficacy in treating rheumatoid arthritis, ankylosing spondylitis, cancers, and other diseases (Carpena et al., 2020; Li et al., 2021). However, according to our literature search, no systematic summary of the anti-breast cancer effects or clinical applications has been performed for animal-derived TCMMs.

Therefore, the objective of this review was to systematically summarize and analyze the relevant animal-derived TCMMs along with their active components and anti-breast cancer effects, and to provide inspiration for the development of novel anti-breast cancer chemotherapeutic or immunotherapeutic drugs.

2 Active ingredients of animal-derived TCMMs for breast cancer

2.1 Components of the aqueous and ethanolic extracts of *Ophiocordyceps sinensis*

Ophiocordyceps sinensis (or Chinese caterpillar fungus), generally called “Dong Chong Xia Cao,” is a well-known and valued natural TCMM, which has been used to treat a wide range of diseases for hundreds of years in China and other Asian regions (Chen et al., 2013). *O. sinensis* is made into a dried composite consisting of the stroma of the fungus (*O. sinensis* (Berk) Sacc.) parasitized on the larva of some species of insects and dead caterpillars (Chen et al., 2013). *O. sinensis* is a potential source of novel drugs and is known to have therapeutic effects, including immunomodulatory (Lee et al., 2020), anti-inflammatory (Qi et al., 2020), anti-tumor (Qi et al., 2020), antioxidant (Li et al., 2001), and anti-aging effects (Li XT et al., 2010).

Cordycepin (Kato et al., 2021), found in the aqueous and ethanolic extracts of *O. sinensis* (Yong et al., 2016; Lin et al., 2021), and *O. sinensis* polysaccharides (Yang et al., 2021) have been demonstrated to exert the main therapeutic effects of *O. sinensis*. It is a nucleoside analog (3'-deoxyadenosine), which is considered as the most important bioactive constituent of *O. sinensis* with significant therapeutic potential (Paterson, 2008; Tuli et al., 2013). Lee et al. (2019) recently found that cordycepin significantly inhibited the cell viability of MCF-7 breast cancer cells, and its half-maximal inhibitory concentration (IC_{50}) value was found to be 9.58 $\mu\text{mol/L}$. Furthermore, cordycepin's potential antitumor targets and pathways were associated with Sonic hedgehog (Shh) signaling, apoptosis, p53, and estrogen pathways based on network pharmacological analysis (Lee et al., 2019). Some of the available evidence on the anti-breast cancer effects of cordycepin is discussed below. First, it has been demonstrated that cordycepin can cause apoptosis or persistent cell cycle arrest by inhibiting RNA synthesis and DNA double-strand breaks in breast cancer cell lines (Lee et al., 2012). Next, a pro-apoptosis effect from cordycepin was observed in MCF-7 breast cancer cells, which indicated that the apoptotic cell death induced by cordycepin treatment was related to caspase-dependent pathways (Song et al., 2016). In addition, a study demonstrated that cordycepin treatment induced

apoptotic cell death in MDA-MB 231 cells, triggered by the release of cytochrome C from mitochondria to the cytosol, leading to caspase activation and poly-(ADP-ribose) polymerase (PARP) cleavage (Kim et al., 2011). Finally, cordycepin caused autophagic cell death in MCF-7 cells irrespective of the estrogen receptor (ER) response, indicating that cordycepin could be used to treat ER-independent human breast cancers (Choi et al., 2011). However, the apoptosis pathway was only confirmed in vitro, and thus cordycepin's MCF-7 cell inhibitory effects that depend on estrogen signaling pathways require further confirmation in vivo.

In addition to the pro-apoptosis and pro-autophagic effects of cordycepin on human breast cancers, *O. sinensis* also possesses immunoregulatory functions. It was reported that the water extracts of *O. sinensis* significantly inhibited 4T1 cell viability and reduced lung metastasis without affecting body weight in a mouse model of breast cancer metastasis (Cai et al., 2018). Furthermore, metastasis-related cytokines including C-C motif chemokine ligand 17 (CCL17), matrix metalloprotein-9 (MMP-9), osteopontin (OPN), and interleukin-33 (IL-33) were reduced, indicating that the aqueous extracts of *O. sinensis* could inhibit breast cancer cell metastasis via downregulating metastasis-related cytokines (Cai et al., 2018). However, the exact mechanisms remained elusive. Additionally, the ethanolic extracts of *O. sinensis* could induce immunogenic and apoptotic cell death in breast cancer (Quan et al., 2020). It is widely accepted that M1 macrophages with pro-inflammatory activity can exert antitumor effects and that M2-polarized macrophages contribute to malignant tumor growth, angiogenesis, and migration (Liu et al., 2021; Mehta et al., 2021). Thus, increasing the proportion of M1 macrophages and inhibiting the M2 phenotypic differentiation of macrophages in the tumor microenvironment could be a promising approach for cancer treatment (Tariq et al., 2017; DeNardo and Ruffell, 2019). Recently, the aqueous extracts of *O. sinensis* were shown to inhibit TNBC progression by promoting the M1 phenotypic differentiation of macrophages and producing inflammatory cytokines via activating the nuclear factor- κ B (NF- κ B) signaling pathway in the tumor microenvironment (Li J et al., 2020). However, the aqueous and ethanolic extracts of *O. sinensis* may contain various active components; therefore, the

specific mechanisms of action of individual components in these extracts need to be further investigated.

Recent studies have demonstrated that *O. sinensis* polysaccharides can also inhibit cancer cell growth via inducing apoptosis and cell cycle arrest (Xu J et al., 2021), prompting autophagy flux blockage via the mammalian target of rapamycin (mTOR) signaling (Qi et al., 2020). Jeong et al. (2013) found that cordycepin-enriched *Cordyceps militaris* effectively reduced the regulatory T cell (Treg) population and increased the number and percentage of interferon- γ (IFN- γ)-expressing cluster of differentiation 8-positive (CD8⁺) T cells in a breast cancer mice model, which implied that *C. militaris* could enhance IFN- γ production and effector T cell function to exert antitumor effects. Another mechanistic hypothesis is related to epithelial-mesenchymal transition (EMT), that is, the biological changes of polarized epithelial cells to a mesenchymal cell phenotype, which plays a significant role in cancer metastasis and chemoresistance (Mittal, 2018). In particular, transforming growth factor- β (TGF- β) signaling plays a crucial role in mediating this process (Hao et al., 2019). Lin et al. (2016) found that MHP-1, an isolated polysaccharide from *Mortierella hepihali* (anamorph of *O. sinensis*), significantly inhibited breast cancer metastasis in the MDA-MB-231 xenograft mice model and restored sensitivity in toptecan-resistant MCF-7 cells via downregulating TGF- β signaling and EMT. Yang et al. (2015) purified a novel antitumor protein named *C. militaris* immunoregulatory protein (CMIP) from the fruiting body of *C. militaris* (Yang et al., 2015). They found that despite showing less ability to kill breast tumor cells, CMIP promoted macrophage proliferation, which indicated that it could activate the immune system. In addition, CMIP inhibited the lung metastasis of 4T1 breast cancer cells and extended the life span of tumor-bearing mice (Yang et al., 2015).

Due to the vast market demand and limited natural resources, the price of natural Chinese *Cordyceps* has increased exponentially in the last decades in China. Thus, the artificial fermentation product of *O. sinensis*, namely *Hirsutella sinensis* fungus (anamorph of *C. sinensis*), has become a widely used substitute for *C. sinensis* (Dong et al., 2015). A recent study demonstrated that *H. sinensis* fungus could inhibit tumor growth in mice with spontaneous polyomavirus middle T antigen (PyMT) breast cancer and reduce

orthotopic lung metastasis by promoting the transcription factor Eomes to increase the production of IFN- γ and granzyme B, leading to enhanced cytotoxic capacity among effector T cells. In addition, *H. sinensis* fungus reduced programmed death 1 (PD-1) expression in T cells and decreased exhausted CD8⁺ T (Tex) cell formation via inhibiting the transcription factor T-bet (Jin et al., 2021).

In summary, the existing evidence suggests that the bioactive ingredients of *O. sinensis* exert anti-breast cancer effects via multiple approaches, including apoptosis, autophagy, metastasis, inflammation, and cell cycle signaling (Fig. 1). Therefore, *O. sinensis* may hold great promise for anti-breast cancer drug discovery. However, further scientific methods such as genomics, transcriptomics, proteomics, and metabolomics should be applied to identify more active constituents associated with the anti-breast cancer effects of this fungus. The wild resources of *O. sinensis* are decreasing due to global warming and ecological changes in its original area, the Tibetan Plateau, driving up the cost of natural *O. sinensis* medicinal materials and limiting the performance of related clinical trials. Therefore, manufactured products from *O. sinensis* are gradually becoming an alternative option due to the increasing demand in the pharmaceutical market (Dong et al., 2015). Thus, the validation of action mechanisms and safety and quality control of the manufactured products from *O. sinensis* warrant further attention. With the aid of the systems biology

research method and multi-omics technology platform, the anti-breast cancer mechanisms of *O. sinensis* can be studied further, and substances related to the formation and efficacy of *O. sinensis* may open a path for the artificial cultivation of the species. Additionally, clinical trials investigating the effects of artificial *O. sinensis* anamorphs of *C. sinensis* and supplementation therapy involving these anamorphs for breast cancer should also be established.

2.2 Chansu

Chansu, also known as toad venom, is a popular but expensive TCMM extracted from the skin or parotid venom glands of *Bufo gargarizans* Cantor (Chen et al., 2018). It is a Class II wild TCMM under national protection and is one of the 28 species of poisonous herbs that require unique management promulgated by the State Council of China (Zhang and Li, 2004). The chemical components of Chansu are divided into small- and large-molecule compounds (Cao et al., 2019). Thus far, more attention has been focused on the small-molecule compounds, including toad lactones, indole alkaloids, and sterols (Li et al., 2021). However, the qualitative analysis of Chansu has been mainly limited to bufadienolides (a group of the toad lactones, including bufalin, cinobufagin, and resibufogenin), which are cardiotoxic steroids isolated from Chansu (Zhang et al., 2005; Qi et al., 2011). Bufadienolides are Na⁺/K⁺ ATPase inhibitors

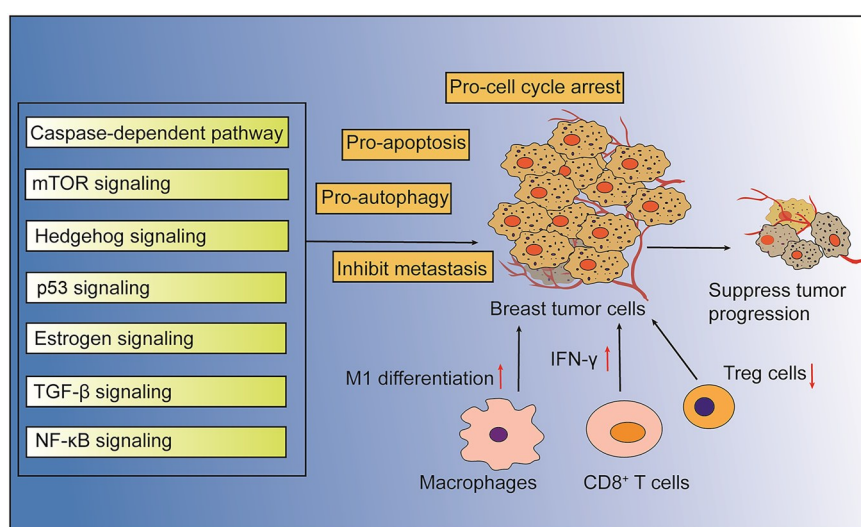


Fig. 1 Action pathways involved in the anti-tumor effects of bioactive components of *Ophiocordyceps sinensis*. mTOR: mammalian target of rapamycin; TGF- β : transforming growth factor- β ; NF- κ B: nuclear factor- κ B; IFN- γ : interferon- γ ; Treg: regulatory T cell; CD8⁺: cluster of differentiation 8-positive.

that can regulate renal sodium transport and have cardiotoxic, anesthetic, and blood pressure stimulation effects (Garcia et al., 2019).

Accumulating evidence suggests that Chansu can inhibit the proliferation and induce the apoptosis of various cancer cells (Qi et al., 2011; Lee et al., 2014; Lan et al., 2019). Among the components isolated from Chansu, bufalin and cinobufagin are the primary agents responsible for its antitumor effects (Zhang JH et al., 2020). One hypothesis regarding its antitumor mechanism is related to the tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a critical member of the TNF superfamily that triggers apoptosis upon attachment to death receptors (DRs) on the cell surface via the extrinsic caspase activation cascade (Rowinsky, 2005). TRAIL selectively kills cancer cells without causing damage to normal cells. Thus, it is considered an attractive novel anticancer agent (Yuan et al., 2018; Wong et al., 2019). In terms of breast cancer, Yan et al. (2014b) found that bufalin can enhance TRAIL-induced apoptosis in different breast cancer cells via redistributing DRs in lipid rafts. They further demonstrated that bufalin prompted enhanced TRAIL-induced apoptosis via the upregulation of DR4 and DR5, the activation of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38-mitogen-activated protein kinase (MAPK) signaling pathways, and the downregulation of Casitas B-lineage lymphoma-b (Cbl-b) (Yan et al., 2012). Consistently, another study also reported that the inhibition of signal transducer and activator of transcription 3 (STAT3)/myeloid cell leukemia-1 (Mcl-1) pathway contributed to enhanced TRAIL-induced apoptosis in MCF-7 and MDA-MB-231 breast cancer cells by bufalin (Dong et al., 2011). Another hypothesis was proposed by Chen et al. (2020), who demonstrated that bufalin inhibited cell proliferation by inducing G2/M cell cycle arrest, triggering apoptosis in the TNBC cell lines MDA-MB-231 and HCC-1937, and reducing the stemness of TNBC stem cells via suppressing the SRY-box transcription factor 2 (SOX2)/octamer-binding transcription factor 4 (OCT4) signaling pathway (Chen et al., 2020). Furthermore, bufalin can inhibit breast cancer tumorigenesis by inducing cell necroptosis via targeting the reactive oxygen species (ROS)-mediated receptor-interacting protein kinase-1 (RIP1)/RIP3/PARP-1 and RIP1/RIP3/phosphoglycerate mutase family member 5 (PGAM5) pathways (Li YL

et al., 2018, 2019). Another hypothesis was related to the steroid receptor coactivator-3 (SRC-3), which is overexpressed in multiple solid tumors. The overexpression of SRC-3 correlates with high levels of epidermal growth factor receptor (EGFR), ER, and human epidermal growth factor receptor 2 (HER2), and thus enhances tamoxifen resistance in human breast cancer (Osborne et al., 2003). Several studies have concluded that bufalin acts as a potent molecule inhibitor of SRC-3 to reduce tumor growth in breast cancer cells and animal models (Wang Y et al., 2014; Song et al., 2015). It has been demonstrated that bufalin can downregulate the activity of the protein kinase B (Akt) pathway to prompt the apoptosis of cancer cells (Yan et al., 2014a). In addition, the combinatorial regimen of bufalin and the histone deacetylase (HDAC) inhibitor suberanilohydroxamic acid inhibited Akt and reduced B-cell lymphoma-2 (Bcl-2) levels, indicating that the combination of HDAC and SRC-3 inhibition might be a novel and promising therapeutic approach for breast cancer (Zou et al., 2016).

Combination treatment with bufalin and chemotherapeutic drugs can synergistically induce the apoptosis of breast cancer cells. Yan et al. (2016b) found that bufalin and cisplatin could synergistically suppress the proliferation and apoptosis of MCF-7 breast cancer cells by downregulating the cisplatin-induced activation of EMT factor and SRC in a dose-dependent manner. Treatment with bufalin at 10 nmol/L plus paclitaxel at 40 nmol/L also increased the apoptotic fraction of cancer cells from 12.5% (paclitaxel alone) to 23.7%, indicating that the combination of bufalin and paclitaxel synergistically suppresses proliferation and induces apoptosis (Yan et al., 2016a). Further analysis showed that bufalin and paclitaxel treatment downregulated the activity of Akt and upregulated the activity of p38, thus inhibiting tumor growth (Yan et al., 2016a).

Cinobufagin is another water-soluble component of *B. gargarizans* Cantor with various biological effects, such as antineoplastic (Zhang C et al., 2019) and immunomodulatory roles (Xie et al., 2016). Cinobufagin has apparent cytotoxicity in cancer cells, including those of colorectal cancer (Li XW et al., 2019), liver cancer (Jin et al., 2020), and prostate cancer (Yu et al., 2008). Zhu et al. (2018) demonstrated that cinobufagin could induce cell apoptosis and cause G1 phase arrest in MCF-7 breast cancer cells in a dose-dependent manner based on an increase in the

Bcl-2-associated X (Bax)/Bcl-2 ratio, indicating that cinobufagin exerts an anti-breast tumor effect via the mitochondrial apoptotic pathway. Similarly, Ma et al. (2012) showed that cinobufagin induced apoptosis and cytotoxicity in MDA-MB-231 cells.

These findings imply that a wide range of signaling pathways correlated with cell proliferation, apoptosis, metastasis, and drug resistance participate in the anti-breast cancer effects of the active components of Chansu. It should be noted that Chansu has a complex chemical composition. However, the current research on its anti-breast cancer effects is mainly performed on bufadienolides; the content ratios of each component in Chansu and their contribution to the overall efficacy are still unknown. In addition, Chansu and bufadienolides as its active ingredients are highly toxic and can cause heart rate disturbance, tissue ischemia, and even hypoxia (Li M et al., 2020). The cardiotoxicity induced by bufadienolides was associated with membrane depolarization, binding to the retinoic acid receptor and γ -aminobutyric acid (GABA) receptor, the positive regulation of nuclear division, the negative regulation of genome replication, and cell cycle changes (Zhang et al., 2018). Therefore, it is necessary to explore the dose threshold for the efficacy and toxicity of this ingredient in depth, and to develop a more rigorous and practical extraction process as well as a quantitative determination method. With the continuous advancement of molecular biology, medicinal chemistry, and drug metabolism research, the active ingredients and toxicity mechanisms of Chansu can be more scientifically and accurately elucidated. Stable and quality-controlled Chansu-derived drugs with low toxicity and high efficiency should be developed to promote more widespread, safer, and feasible applications in clinical practice. In addition, due to the dwindling resources of wild Chansu, its supply cannot meet the rising demand. Meanwhile, it is challenging to discover satisfactory alternatives to Chansu because of its high efficacy and potent bioactive ingredients.

2.3 Norcantharidin

The utility of the Chinese blister beetle and the method of concoction were first recorded in the *Shen Nong Ben Cao Jing* as early as a thousand years ago. Cantharidin (CTD) is the main active ingredient and toxic component of the Chinese blister beetle. It has been confirmed that CTD is an inhibitor of protein

phosphatase 2A (PP2A) (Li W et al., 2010), enabling the effective regulation of cell proliferation and protein transcription (Seshacharyulu et al., 2013). CTD exerts significant inhibitory effects in various cancer types, including gastric cancer (Song et al., 2020), breast cancer (Pan et al., 2019), prostate cancer (Nazim et al., 2020), and lung cancer (Hsia et al., 2014). However, the clinical application of CTD is greatly limited due to various adverse effects, including nausea, vomiting, intense burning sensation in the digestive tract, as well as urinary toxicity such as urinary pain, hematuria, proteinuria, and even renal failure (Liu and Chen, 2009).

Norcantharidin (NCTD, 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride) can be extracted from CTD or synthesized from furan and maleic anhydride to alleviate the side effects of CTD (Tu et al., 2014). It is a small-molecule synthetic compound with low cytotoxicity and few side effects after the 1,2-methyl groups on the chemical structure of CTD are removed (Dorn et al., 2009; Jiang et al., 2017). In addition, NCTD is an efficacious anticancer drug that has been applied for cancer therapy in China for many years. The underlying mechanisms of NCTD in various cancers have already been discussed in a previous review (Pan et al., 2020). Here, we mainly focus on its effects on breast cancer.

Accumulating evidence indicates that NCTD can inhibit the proliferation and induce the apoptosis of breast cancer cells. Liu et al. (2012) found that NCTD induced dose-dependent DNA damage and reduced the G1 peak in BCaP-37 breast cancer cells, accompanied by increased ROS levels and decreased mitochondrial membrane potential. In addition, NCTD can trigger cell senescence and cell cycle arrest by simultaneously downregulating the Akt and ERK signaling pathways in TNBC (He et al., 2019). Similarly, NCTD at concentrations of 6, 30, and 60 $\mu\text{mol/L}$ inhibited the Akt and NF- κB signaling pathways and reduced the Bcl-2/Bax ratio in a dose-dependent manner to induce cell apoptosis and cell cycle arrest, thus blocking tumor growth (Huang et al., 2009). The inhibition of Wnt/ β -catenin (Yin et al., 2019), STAT3, STAT5, ERK1/2, JNK, and p38 (MAPK) by NCTD also contributed to NCTD-induced breast cancer cell apoptosis (Shou et al., 2013). One hypothesis to explain this phenomenon is that both CTD and NCTD can inhibit the ability of MCF-7 breast cancer cells to adhere to

platelets through the downregulation of $\alpha 2$ integrin, an adhesion molecule on the surface of cancer cells involved in the interaction between cancer cells and platelets, thus repressing the metastatic potential (Shou et al., 2013). Furthermore, the repression of $\alpha 2$ integrin was observed through the downregulation of the protein kinase C (PKC) pathway (Shou et al., 2013). Huang et al. (2010) also found that NCTD suppressed the cell migration and invasion ability via inhibiting PKC signaling in several human breast cancer lines, including MCF-7, SKBR3, and MDA-MB231. These findings were consistent with a previous observation in pancreatic cancer (Xie et al., 2015). In addition, a growing body of evidence suggests that NCTD can enhance the antitumor effects or alleviate the resistance to chemotherapeutic drugs. Zhang XM et al. (2019) demonstrated that NCTD inhibited cell proliferation and tumor growth by regulating ER α signaling and tamoxifen resistance in breast cancer cells, wherein the microRNA-873 (miR-873)/cyclin-dependent kinase 3 (CDK3) axis mediated the increased tamoxifen mechanism sensitivity and the inhibition of cell proliferation.

Second mitochondria-derived activator of caspase (SMAC) mimetics have emerged as novel anticancer agents due to their potential for inducing cell apoptosis

in various types of cancer (Morrish et al., 2020; Zhao et al., 2020). However, resistance to SMAC mimetics was found to be widespread among cancer patients, which limits their more comprehensive clinical applications (Fulda, 2015; Noonan et al., 2016). Recently, it was found that the combination of SMAC mimetics with NCTD could enhance SMAC mimetic Birinapant-mediated apoptosis in breast cancer cells via the downregulation of cellular Fas-associated protein with death domain (FADD)-like IL-1 β -converting enzyme (FLICE)-like inhibitory proteins (c-FLIP), leading to the enhancement of caspase-8 activation and cell death (Zhao et al., 2017). In addition, NCTD treatment can reverse the resistance to doxorubicin and vinorelbine through inhibiting Shh and the downstream multidrug resistance 1 (MDR-1)/P-glycoprotein (P-gp) expression in human breast cancer cells (Chen et al., 2012).

In summary, the anti-breast cancer effects of CTD and NCTD can be achieved via multiple targets and pathways, including the inhibition of proliferation, induction of apoptosis and cell cycle arrest, increase of cell sensitivity to chemotherapeutic drugs, and repression of metastasis (Fig. 2). However, other possible anti-breast cancer mechanisms of NCTD, such as anti-angiogenesis and immune regulation, have been less studied. The present evidence indicates that NCTD, a

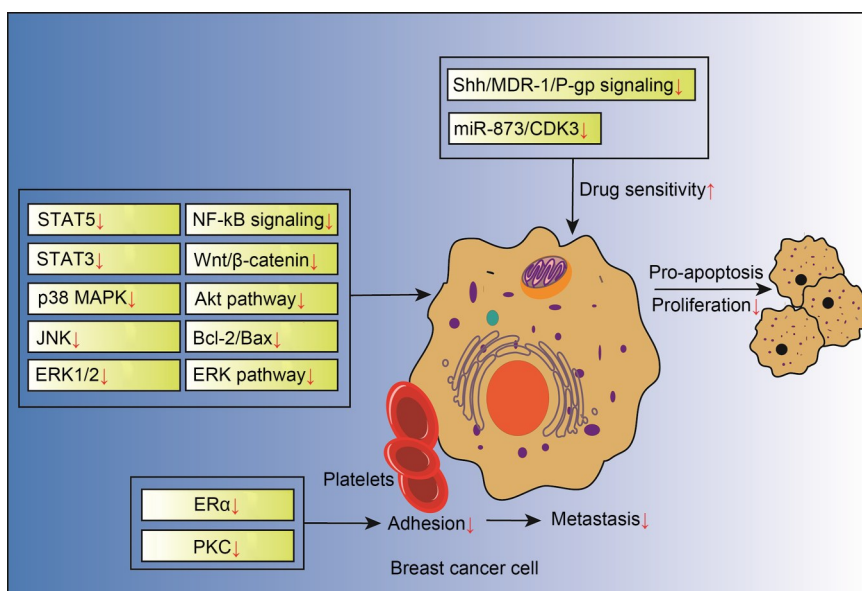


Fig. 2 Action pathways associated with the anti-breast cancer effects of NCTD. NCTD: norcantharidin; STAT: signal transducer and activator of transcription; MAPK: mitogen-activated protein kinase; JNK: c-Jun N-terminal kinase; ERK: extracellular signal-regulated kinase; NF- κ B: nuclear factor- κ B; Akt: protein kinase B; Bcl-2: B-cell lymphoma-2; Bax: Bcl-2-associated X; ER α : estrogen receptor α ; PKC: protein kinase C; Shh: sonic hedgehog; MDR-1: multidrug resistance 1; P-gp: P-glycoprotein; miR-873: microRNA-873; CDK3: cyclin-dependent kinase 3.

demethylation derivative of CTD, may be a complementary anticancer drug. Therefore, well-designed and large-scale clinical trials investigating the therapeutic effects of NCTD are necessary.

2.4 Bee venom

Bee venom is a complex mixture of compounds in an aromatic yellowish transparent venom secreted by the venom glands of worker bees, which has various pharmacological and biological activity (van Vaerenbergh et al., 2013). There is a long history of using bee venom in Chinese medicine to treat rheumatism, rheumatoid arthritis, and other diseases (Son et al., 2007). In recent years, the composition and chemical structure of bee venom have been gradually verified thanks to the advancement of separation and purification technologies. Function and action mechanism studies have revealed that bee venom exhibits potent antitumor effects (Oršolić, 2012). Bee venom contains several biologically active peptides, including melittin, apamin, adolapin, mast cell degranulating peptide, and enzymes (phospholipase A2 and hyaluronidase) as well as non-peptide components such as histamine, dopamine, and norepinephrine (Wehbe et al., 2019).

Melittin is the major peptide component, accounting for nearly 40%–50% of the total dry weight of bee venom (Ceremuga et al., 2020). A previous review summarized the evidence on the possible mechanisms of bee venom and melittin in various types of cancers (Rady et al., 2017). The anticancer effects were associated with the regulation of angiogenesis and necrosis, cell proliferation, motility, migration, metastasis, invasion, and cell apoptosis in many cancers, including ovarian, prostate, lung, and breast cancers as well as hepatocellular carcinoma, melanoma, leukemia (Rady et al., 2017). Moghaddam et al. (2020) reported that melittin exerted cytotoxic effects on 4T1 breast cancer cells involved in upregulated dynamin-related protein 1 (Drp1) and mitofusin-1 (Mfn1) expression. Another study reported that melittin inhibited tumor motility and invasion in vitro and in vivo by suppressing the phosphatidylinositol-3-kinase (PI3K)/Akt/mTOR signaling pathway and reducing focal adhesion kinase phosphorylation levels via the inhibition of mTOR/p70 ribosomal protein S6 kinase (p70S6K)/eukaryotic initiation factor 4E-binding protein-1 (4E-BP1) pathway (Jeong et al., 2014). Cho et al. (2010) found that melittin can inhibit MMP-9

expression in MCF-7 cells by suppressing NF- κ B via the p38 MAPK and JNK signaling pathways, and thus exerts anti-metastatic and anti-invasive effects. Furthermore, melittin can also shape the tumor microenvironment. It has been demonstrated that melittin treatment suppresses hypoxia-inducible factor-1 α (HIF-1 α) involved in tumor microenvironment formation via the downregulation of NF- κ B, leading to the decreased expression of vascular endothelial growth factor A (VEGFA) and lactate dehydrogenase A (LDHA), which participate in angiogenesis and anaerobic respiration, leading to the inhibition of tumor growth. Furthermore, melittin activates the extrinsic and intrinsic apoptotic pathways by upregulating TNF- α and Bax expression to exert pro-apoptosis effects (Mir Hassani et al., 2021). Another possible mechanism is related to IL-2, which holds great potential for tumor immunotherapy. High and low doses of IL-2 lead to apparent systemic adverse effects and less satisfactory anti-tumor efficacy, respectively (Boyman and Arenas-Ramirez, 2019). Liu et al. (2016) recently reported that the melittin-mutant recombinant IL-2 fusion protein could induce the more vital cytolytic activity of T cells and natural killer (NK) cells, promote the production of IFN- γ , and inhibit lung metastasis in breast cancer. In addition, it was found that bee venom and melittin induced potent cell death in HER2- and EGFR-overexpressing breast cancer cells through interfering with growth factor-dependent receptor tyrosine kinase (RTK) interactions, critical for receptor phosphorylation and the activation of PI3K/Akt signaling (Duffy et al., 2020). The sensitization of breast cancer cells to docetaxel treatment in vivo by melittin involved the downregulation of microtubule-associated protein tau (*MAPT*) and β -tubulin class III (*TUBB3*) genes, consistent with previous findings (Wang RP et al., 2014). The potential underlying mechanisms for bee venom and melittin to alleviate cancer cells' resistance to chemotherapeutic agents warrant further investigations.

Bee venom, which exhibits diverse pharmaceutical properties, is an attractive therapeutic agent in China for treating pain, rheumatoid arthritis, atherosclerosis, etc. (Zhang et al., 2018). In addition, its effects on tumors and neurodegenerative diseases have been highlighted (Carpena et al., 2020). However, even though the effects of the main components of bee venom, such as melittin, have been extensively elucidated, the mechanisms of action and metabolic pathways of its

other ingredients, including phospholipase A2, mast cell-degranulating peptide, and apamin, are barely known. Therefore, there is still a long way to go before bee venom can be applied for breast cancer therapy. Furthermore, clinical studies investigating the incidence of adverse events, doses, or administration forms have not yet been well established or critically assessed.

2.5 Deer antler

The deer antler, as a periodically regenerable mammalian organ, is composed of cartilage, bone, blood vessels, nerves, and skin tissue (Li, 2012). The process of deer antler regeneration is based on the proliferation and differentiation of stem cells. Deer antlers grow extremely quickly without canceration, and therefore can be used as a biomedical model for regeneration science (Wang et al., 2019). Antler polypeptides and polysaccharides are the main active components of deer antler, which possess the characteristics of neuroprotection, the promotion of reproductive function, the protection of cartilage tissue, the enhancement of immunity, the protection of cardiomyocytes and vascular endothelial cells, the improvement of liver and kidney damage, and anti-inflammation (Huo et al., 2014; Sui et al., 2014). Recently, it was discovered that antler polypeptides and polysaccharides also exhibited antitumor effects. It was reported that antler velvet extract reduced proliferation, inhibited migration, and promoted apoptosis in glioblastoma cell lines, indicating that it may serve as a new therapeutic strategy for glioblastoma treatment (Chonco et al., 2021). Using liquid chromatography-mass spectrometry (LC-MS), Zheng et al. (2020) identified several bioactive polypeptides with potential tumor-suppressing properties from the water-soluble extract of pilose antler, including extracellular matrix repair proteins, intercellular adhesion proteins, and angiogenesis inhibitors. Furthermore, it was demonstrated that pilose antler water-soluble polypeptides inhibited the growth and metastasis of 4T1 breast cancers mainly by activating the cleaved caspase-3, downregulating the expression of platelet-endothelial cell adhesion molecule, suppressing angiogenesis and EMT, inhibiting MMP-2 and MMP-9, and increasing the ratio of cadherin-1/cadherin-2 (Zheng et al., 2020). Similarly, Xu GG et al. (2021) reported that antler extract could inhibit the invasion and EMT of human breast cancer cells in a dose-dependent manner, and this antitumor

effect was mediated by the inhibition of NF- κ B signaling. Hu et al. (2015) also found that polypeptides from sika antler inhibited rat breast cancer cell proliferation and telomerase activity.

To date, no in-depth studies have been conducted on the effects and mechanisms of action of deer antler or its active components in breast cancer. In China, the clinical use of deer antlers has seen a gradual decline due to the strict animal ethics policy and increased price. The chemical components and standards for the quality control, identification, or pharmacodynamics of deer antler have not been validated, thus limiting its use for cancer therapy. In future work, the identification and screening of the active components of deer antler for their antitumor effects based on fingerprint analysis combined with a quantitative analysis of multi-components and multi-omics may inspire the development of novel antitumor chemotherapeutic agents.

2.6 Others

In addition to the materials mentioned above, a number of other animal-derived TCMMs have been attributed antitumor effects. For instance, oyster is an edible shellfish commonly found in coastal countries worldwide, which is rich in glycogen, taurine, amino acids, B vitamins, polysaccharides, low-molecular active peptides, Fe, Zn, Se, and other trace elements. In China, the use of oysters to treat breast lumps was recorded as early as a millennium ago. Chintalapati et al. (2009) reported that ceramide methylaminoethylphosphonate (CMAEPn), isolated from eastern oyster, exerted antitumor effects via anti-angiogenesis, inducing autophagic cell death and inhibiting cell migration as well as the invasion in MCF-7 and MDA-MB-435s breast cancer cell lines. However, no in-depth research has focused on the effects of oyster extract or its active ingredients on breast cancer. In another example, scorpions have been used in China to treat epilepsy, stroke, and physical mobility disorders for a long time. Scorpion venom, extracted from the scorpion, is the main component exhibiting a multitude of biological effects. The scorpion sting can cause pain and may lead to various complications such as hypotension, cardiac arrhythmia, and respiratory distress (Ahmadi et al., 2020), which results in the inhibition of the Na⁺/K⁺-ATPase pump, thus paralyzing the sympathetic and parasympathetic nervous systems

(Díaz-García and Varela, 2020). On the other hand, despite its neurotoxicity, scorpion venom has beneficial components that may inspire drug discovery (Ortiz et al., 2015). It has been demonstrated that scorpion venom has the potential for inhibiting several cancers, including leukemia (Song et al., 2012), breast cancer (Kampo et al., 2019), and colorectal cancer (Al-Asmari et al., 2018). A previous mini-review discussed potential anticancer peptides from scorpion venom and the action mechanisms of scorpion venom in treating breast cancer (Sarfo-Poku et al., 2016). It was suggested that scorpion venom exhibited anti-breast cancer functions mainly via increasing nitric oxide production, promoting apoptosis, and inhibiting DNA synthesis. A recent study reported that *Buthus martensii* Karsch antitumor-analgesic peptide, a scorpion venom peptide, can inhibit cancer cell stemness, EMT, migration, and invasion by downregulating pentraxin 3 (PTX3) through the NF- κ B and Wnt/ β -catenin signaling pathways in breast cancer cells and a xenograft tumor mouse model (Kampo et al., 2019). Additionally, scorpion venom induced cell apoptosis, reduced cell invasion and IL-6 cytokine levels, and downregulated several proteins, including Ras homolog gene family member C (RhoC), ERK1/2, and STAT3, associated with cancer survival in MDA-MB-231 cell lines (Al-Asmari et al., 2018). Although the anti-breast cancer effects of these animal-derived active ingredients have been illustrated, their pharmacological mechanisms remain undiscovered. Moreover, their hepatic or renal toxicity has not been well established.

3 Future perspectives and approaches

As highlighted above, despite the growing volume of scientific evidence suggesting that the bioactive components of these animal-derived TCMMs may possess attractive anti-breast cancer effects both in vivo and in vitro, several issues remain in this area that require urgent consideration. Firstly, studies investigating the mechanisms of anti-breast cancer effects of active ingredients have mainly focused on a single signaling pathway associated with apoptosis, proliferation, metastasis, cell cycle, or drug resistance, without considering associations with other biological processes, which may undermine their conclusions to an extent. The genes, proteins, metabolites, and signaling

pathways involved in inhibiting tumor progression should be studied in depth using state-of-the-art bioinformatics analysis and multi-omics technologies, including genomics, transcriptomics, proteomics, and metabolomics, which may broaden the understanding of the mechanisms of action of these components. Secondly, these TCMMs show various levels of toxicity, and the complexity of their composition makes their identification, quantification, and standardization more difficult. Therefore, analytical methods have become indispensable for identifying marker components. In this regard, the fingerprint analysis and quantitative analysis of components using high-performance liquid chromatography (HPLC), mass spectrometry (MS) coupled to electrospray ionization (ESI), or matrix-assisted laser desorption/ionization (MALDI) techniques will increase the current knowledge of these animal-derived TCMMs and promote drug discovery for breast cancer. Besides, combinations with developed chemotherapeutic drugs to reduce drug resistance or produce synergistic antitumor effects may also be considered. Thirdly, drug delivery and transportation systems technologies, such as microspheres (Huang et al., 2019), nanoparticles (Garbayo et al., 2020), liposomes (Vahed et al., 2017), and microemulsions (Callender et al., 2017), also hold great potential for new formulations involving these components. Drug delivery and transportation systems can enhance drug stability, prolong drug action, reduce drug dosage and administration frequency, and promote sustained and slow drug release rate while reducing the side effects (Singh et al., 2017). For example, Tian et al. (2014) designed a co-delivery system comprising bufalin-loaded biotinylated chitosan nanoparticles, which exhibited the sustained and slow release of bufalin, as well as superior intracellular uptake. This drug vehicle system significantly enhanced the cytotoxicity on breast cancer cells compared to native bufalin, with no or few apparent side effects. In a further example, melittin delivered by citric acid-functionalized Fe₃O₄ magnetic nanoparticles (Hematyar et al., 2018), carbon nanoparticles (Daniluk et al., 2019) and perfluorocarbon nanoparticles (Soman et al., 2009), or an estrone-appended polyion complex micelle exhibited substantially greater antitumor efficacy against several breast cancer cells (Raveendran et al., 2020). Furthermore, an NCTD-loaded RGD (Arg-Gly-Asp)-decorated lipid-polymer hybrid

nanoparticle inhibited TNBC tumor growth and metastasis via attenuating the Wnt/ β -catenin pathway more effectively than free NCTD (Li YF et al., 2019). These findings highlight that drug delivery and transportation systems possess great potential for the clinical application of these bioactive components derived from TCMMs.

In the future, methods for the screening, identification, and standardization of active compounds with higher efficiency and lower toxicity should be further developed. It should also be noted that the optimization of drug carrier materials to improve the encapsulation and drug delivery rates requires a multidisciplinary collaboration among the areas of Chinese medicine, oncology, pharmacology, toxicology, and material science.

4 Conclusions

In summary, the active components of animal-derived TCMMs, including *O. sinensis*, NCTD, Chansu, bee venom, deer antler, *O. gigas*, and scorpion venom, exert marked anti-breast cancer effects that include the regulation of cell cycle arrest, proliferation, apoptosis, metastasis, cell cycle, and drug resistance. In the future, the underlying mechanisms for the antitumor effects of these constituents need to be further investigated by utilizing multi-omics technologies. In addition, the development of appropriate drug delivery and transportation systems to increase the stability of these drugs in vivo, to prolong the duration of drug action, to reduce drug dosage, and to simultaneously alleviate toxic side effects, is also an important direction for further research. Furthermore, large-scale clinical trials are required to validate the efficacy of bioactive constituents alone or in combination with chemotherapeutic drugs in breast cancer treatment.

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Author contributions

Chaoyao YU conceived the main ideas and wrote this paper. Yi LI and Guopeng CHEN helped search the literature.

Chaoyao WU and Xiuping WANG illustrated figures. Yingwen ZHANG revised the manuscript. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Chaoyao YU, Yi LI, Guopeng CHEN, Chaoyao WU, Xiuping WANG, and Yingwen ZHANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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