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Garlic constituents for cancer prevention and therapy: From phytochemistry to novel formulations

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Chemical compounds studied in this article

γ-glutamyl-S-allyl-L-cysteine (PubChem CID: 11346811)
Ajoene (PubChem CID: 5386591)
Alliin (PubChem CID: 87310)
Allicin (PubChem CID: 65036)
Allyl methyl sulfide (PubChem CID: 66282)
Diallyl trisulfide (PubChem CID: 16315)
Diallyl disulfide (PubChem CID: 16590)
Diallyl sulfide ((PubChem CID: 11617)
N-trans-feruloyloctopamine (PubChem CID: 24096391)
Thiacremonone (PubChem CID: 539170)

Abstract

Garlic (*Allium sativum* L.) is one of the oldest plants cultivated for its dietary and medicinal values. This incredible plant is endowed with various pharmacological attributes, such as antimicrobial, antiarthritic, antithrombotic, antitumor, hypoglycemic, and hypolipidemic activities. Among the various beneficial pharmacological effects of garlic, the anticancer activity is presumably the most studied. The consumption of garlic provides strong protection against cancer risk. Taking into account the multi-targeted actions and absence of considerable toxicity, a few active metabolites of garlic are probably to play crucial roles in the killing of cancerous cells. Garlic contains several bioactive molecules with anticancer actions and these include diallyl trisulfide, allicin, diallyl disulfide, diallyl sulfide, and allyl mercaptan. The effects of various garlic-derived products, their phytoconstituents and nanoformulations have been evaluated against skin, prostate, ovarian, breast, gastric, colorectal, oral, liver, and pancreatic cancers. Garlic extract, its phytocompounds and their nanoformulations have been shown to inhibit the different stages of cancer, including initiation, promotion, and progression. Besides, these bioactive metabolites alter the peroxidation of lipid, activity of nitric oxide synthetase, nuclear factor-κB, epidermal growth factor receptor, and protein kinase C, cell cycle, and survival signaling. The cur-

rent comprehensive review portrays the functions of garlic, its bioactive constituents and nanoformulations against several types of cancers and explores the possibility of developing these agents as anticancer pharmaceuticals.

Keywords: Garlic, *Allium sativum*, diallyl disulfide, allicin, cancer, antineoplastic agents, molecular mechanisms, nano-formulations.

1. Introduction

Throughout history, natural products have played an important role in the treatment of human ailments and are becoming a significant resource for drug development research. Early diagnosis and tumor eradication by radiation therapy or surgical intervention provides the best promise for cancer patients. However, regardless of these developments, cancer remains a leading cause of death around the world [1]. There is a vital need for more efficient methods to counter the morbidity and mortality as well as the enormous economic burden associated with the illness. Another important solution to alleviating this overwhelming public health risk is the use of synthetic or natural products to prevent, retard, or reverse carcinogenesis [2]. Over 60% of the current anticancer drugs have been derived, in one way or another, from natural sources [3]. In addition to providing nutrition, many natural ingredients help to sustain health and therefore reduce the risk of multiple diseases, including cancer [4]. Phytochemicals have been thoroughly investigated and have demonstrated anticarcinogenic properties by affecting cancer initiation, promotion and progression by regulation of various pathways, including cell proliferation, differentiation, apoptosis, angiogenesis, invasion, migration, and metastasis [5-9].

Garlic (*Allium sativum* L.) is an important member of the family of plants called Alliaceae and they are named from the Celtic word “all”, which means powerful and nature’s boon to mankind. For the past 5,000 years, garlic has been utilized as a curative food-based plant for the treatment of several diseases [10]. It is used in Arabic, Egyptian, Chinese, Persian, and Indian

traditional medicinal systems and folk medicines to treat cardiovascular diseases, regulate blood pressure, lower the blood sugar, and cholesterol levels [10]. In the ethnomedicinal uses, garlic has been reported to be used in the management of diarrhea, prevention of bacterial, viral, fungal, and parasitic infections, improvement of the immune system [11-12]. The plant has been used for the treatment of hay fever, asthma, and the common cold in Asia (specifically in India and Pakistan) and Europe [13]. The plant also exhibits antitumor and anticancer activities [14].

Garlic consists of white to pink colored bulbs (size of 1.5 to 2.5 cm) with aromatic and distinctive odor. The taste of garlic is choking and aromatic. Multiple epidemiologic, preclinical and clinical studies have evaluated the therapeutic and preventive effects of garlic against cancer [15]. Preclinical studies provided multiple pieces of evidences for the suppressing effects of garlic in different types of cancers, including oral, gastric, pancreatic, colorectal, ovarian, endometrial, breast, prostate, and bone cancers. Clinical evidence also supports the therapeutic anticancer effects of garlic and its ability to reduce the risk of certain cancers [16-17]. According to the report of the American Institute for Cancer Research consumption of garlic frequently lowers the risk of colorectal cancers [16]. Laboratory studies have shown that garlic compounds help to repair DNA, delay cancer cell growth, and reduce inflammation [18]. Various sulfur-containing compounds and other phytoconstituents of garlic were extracted, purified, and extensively studied for their anticancer properties [19]. According to several reports, allicin is the major active sulfur compound and it forms many oil-soluble allyl sulfur compounds with anticancer activities [20-23]. Nanotechnology seeks to make medications more successful at reaching their intended targets in sufficient amount in the treatment of cancer [24]. Nanotechnology-based delivery systems have recently received a lot of recognition as a way to overcome problems with bioavailability, solubility, distribution, and toxicity. As a result, novel strategies encompassing the medicinal properties of garlic extract and its phytoconstituents alone and in combination with nanoparticles may lead to more efficient and effective anticancer activity.

Just a few previous reviews offer a detailed knowledge on the progress of this significant research area. One of the previous review articles presents a brief overview of compilation and exploration of the therapeutic properties of secondary garlic metabolites in cancer [25-27]. There are no publications on the overview of novel formulations of banana extract and its phytoconstituents in cancer. This review portrays, in detail, the functions of garlic, its bioactive constituents and nanoformulations against several types of cancers and explores the possibility of developing these agents as anticancer pharmaceuticals.

2. Anticancer phytoconstituents of garlic

Garlic contains numerous phytochemicals but, in this section, we only discuss compounds with anticancer activities. Whole intact garlic cloves contain two types of nonvolatile organosulfur molecules, such as γ-glutamyl-L-cysteine peptides [comprises of γ-glutamyl-S-allyl-L-cysteine (**1**)] and L-cysteine sulfoxides [containing S-allyl-L-cysteine sulfoxide] (**Figure 1A**) [28]. An estimated 80% of cysteine sulfoxides in garlic are S-allyl-L-cysteine sulfoxide, also known as alliin (**2**). An enzyme called alliinase is produced whenever raw garlic cloves are crushed, mashed, or chewed. Alliin is an amino acid that is converted to 2-propenesulfenic acid by the alliinase enzyme, along with the subsequent liberation of pyruvic acid and ammonia. 2-Propenesulfenic acid is unstable and extremely reactive at room temperature. Two molecules of 2-propenesulfenic acid react with each other spontaneously to produce allicin (**3**) with consequent elimination of water (**Figure 1B**) [29]. Allicin is a volatile compound found in garlic. Allicin breaks down to create a range of organosulfur compounds that are soluble in fat, such as diallyl trisulfide (DATS) (**4**), diallyl disulfide (DADS) (**5**), diallyl sulfide (DAS) (**6**), and allyl methyl sulfide (AMS) (**7**) (**Figure 1C**) [26, 30]. It also produces thiacremonone (**8**), a novel sulfur-containing compound [31]. When fermented with organic solvents, it also produces ajoene (**9**) [32]. Allicin can interact with L-cysteine in the body to form *S*-allyl mercapto cysteine (SAMC) (**10**)[33]. N-trans-feruloyloctopamine (FO) (**11**), a derivative of hydroxycinnamic acid, was isolated from garlic skin [34]. Garlic contains natural organoselenium molecules called Se-

methyl-L-selenocysteine (MSeC) (**12**) which is a derivative of *S*-methylcysteine. γ-Glutamyl-L-cysteine is a peptide which contain a water-soluble dipeptide γ-glutamyl-S-allyl-L-cysteine. S-allyl-cysteine (SAC) (**13**) and SAMC are water-soluble organosulfur molecules produced from γ-glutamyl-S-allyl-L-cysteine after long-term fermentation of crushed garlic in aqueous medium, as shown in the production of aged garlic extracts (**Figure 1D**). S-benzyl-cysteine (SBC) (**14**) (**Figure 1D**) is a water-soluble structural analog of SAC was isolated and characterized from aged garlic extract. S-propargyl-cysteine (SPRC) (**15**) (**Figure 1D**) is a derivative of SAC, an H_2S donor produced from garlic extract [35-36].

3. Anticancer effects of garlic extract, phytoconstituents, and their nano-formulations

Owing to the presence of different sulfur and organic compounds, garlic is known to possess possible anticancer effects. These compounds with effects on different pathways, such as cell cycle interruption, signaling pathway inhibition, induction of apoptosis, autophagy, and antioxidant activity, interact with various stages of cancer cell development, proliferation, growth, invasion, migration, and metastasis. Garlic consists of both water-soluble and fat-soluble sulfur compounds that exhibit anticancer properties through attenuation of oxidative stress, suppression of carcinogen metabolism, and improvement of immune function [37]. Nano-formulations have been utilized for the direct delivery of parent compounds to the critical sites of the malignant tissues to demonstrate their possible anticancer activities. Garlic nano-formulations exhibited better therapeutic potential than phytocomponent alone. For example, allicin nanoparticles exhibited elevated anti-angiogenesis effect than allicin alone [38].

Therefore, it is of considerable significance to analyze the anticancer functions of key garlic components, garlic extract and their nano-formulations. The antineoplastic effects and associate cellular and molecular mechanisms of action of various garlic extract, its phytocompounds and their nano-formulations are discussed in the following sections and highlighted in **Tables 1** and **2**.

3.1. Breast cancer

Cancer of the breast is the most prevalent type of neoplasm found in women of both developing and developed countries worldwide. It is estimated that 2,261,419 new female breast cancers and 684,996 cancer deaths occurred worldwide in 2020 [39,40]. It is the most commonly diagnosed and leading cause of cancer among women. Available treatments, such as hormone therapy, chemotherapy, surgery, and radiation therapy, are used to ameliorate proliferation and invasion of breast cancer [41].

Aqueous extract of garlic powder and allicin inhibited the proliferation of MCF-7 human breast cancer cells. Growth inhibition was accompanied by the accumulation of cells in the G_0/G_1 or G_2/M phases of the cell cycle. Allicin induced a decrease in the level of intracellular glutathione (GSH), which was very closely linked to allicin's growth inhibitory effect [42]. Both alliin and allicin decreased the cell viability of luminal A MCF-7 and triple-negative HCC-70 breast cancer cell lines, but allicin displayed more profound cytotoxicity and antiproliferative activity in both the cell lines by inducing apoptosis, decreasing mitochondrial membrane potential ($\Delta\Psi m$), and increasing the expression level of caspase-3, caspase-8, and caspase-9. It also downregulated the expression level of antiapoptotic protein (Bcl-xL) whereas, the expression level of proapoptotic proteins (p21, Noxa, and Bak) were upregulated [43]. Alliin induced senescence in both the cell lines at a concentration of 10 µM, whereas allicin did not exhibit the same activity [43].

In comparison to water-soluble organosulfur compounds of garlic, oil-soluble compounds, such as DAS, DADS, and DATS, markedly suppressed canine mammary tumor (CMT-13) cell growth [44]. DAS and DADS were cytostatic, while DATS was cytotoxic. In addition to the number of sulfur atoms, the cancer-suppressing potency increases with an increase in intracellular GSH [44]. In addition to CMT-13 cell growth suppression, DADS exhibited cytotoxicity against estrogen receptor (ER)-positive human breast cancer cell lines, MCF-7, KPL-1 (cell line

established from the malignant effusion of a breast cancer patient), and T47D (differentiated epithelial sub strain), and ER-negative human breast cancer cell lines MDA-MB-231 and MKL-F [45-47]. It induced apoptosis and cell cycle arrest at the sub G_1 phase in MDA-MB-231 cells, associated with an increase in the protein expression level of Bax, caspase-3, and a decrease in Bcl-xL expression level (**Figure 2**). DADS exhibited its cytotoxicity against MCF-7 carcinoma cells by inducing apoptosis with an increase in caspase-3 and PARP cleavages. It further inhibited the stress-activated protein kinase extracellular signal-regulated kinase (ERK)/MAPK pathway and also activated the stress-activated protein kinase, SAPK/JNK, and p38 pathways [45]. These results were further supported by Altonsy et al. [48] revealed that DADS exhibited its anticancer activities in MCF-7 carcinoma cells by inducing apoptosis and cell cycle arrest at sub-G_0 phase. DADS also triggered phosphatidylserine translocation to activate caspase-3 and modulated the protein expression levels of Bax, Bcl-2, Bcl-xL, and Bcl-W and ultimately induced H4 histone hyperacetylation. In a separate study, DADS displayed cytotoxicity against MCF-7 cells and inhibited proliferation by inducing apoptosis via intrinsic signaling pathway associated with increased expression of Bax, Bad, caspase-3, and caspase-9, and decreased expression of Bcl-2 [49]. DADS prevented the growth and metastatic capacity of three human triple-negative breast cancer cells (MDA-MB-231, MDA-MB-468, and BT-549) by inhibiting the β-catenin signaling pathway. It induced apoptosis and inhibited cell proliferation linked with an increase in the expression level of Bax, caspase-3, and caspase-9, and decrease in the protein expression level of Bcl-2, matrix metalloproteinase-9 (MMP-9), and β-catenin siRNA [50]. The expression of miR-34a in DADS-treated MDA-MB-231 cells was found to be upregulated. It further inhibited the SRC expression to ultimately inhibit the SRC/Ras/ERK signaling pathway [51]. DATS suppressed the carcinogenic action of benzo[α]pyrene (BaP) in MCF-10A cells (normal human mammary epithelial cells) by abrogation of cell proliferation, inducing cell cycle arrest at G_2/M phase and inhibition of ROS synthesis, and DNA damage [52].

DATS significantly decreased the viability of the MCF-7 breast cancer cell line and MCF-12A normal human breast epithelial cells by inducing apoptosis and cell cycle arrest at the G_2/M phase. Activation of apoptosis was linked with the upregulation of the protein expression level of Bax and p53 with an increased translocation of p53 from the cytoplasm into the nucleus. It further suppressed the protein expression level of Akt and Bcl-2, and increased the expression level of FAS and cyclin D1 [53]. DATS prevented the development of tumor spheres utilizing cancer stem cells (CSCs) from two human breast cancer cell lines, MCF-7 and SUM159, by decreasing the protein expression level of CD44, ALDH1A1, Nanog, and Oct4. Inhibition of cell proliferation was induced by apoptosis with a decrease in the expression level of proliferating cell nuclear antigen (PCNA), cyclin D1, and Bcl-2, and an increase in the expression level of Bax and caspases (caspase-8, cleaved caspase-9, and cleaved caspase-3). DATS blocked the Wnt/β-catenin signaling pathway (regulator of breast CSCs). DATS treatment has led to the decreased expression level of phospho-glycogen synthase kinase-3β (p-GSK3β), and β-catenin, and increased expression level of GSK3β and c-Myc [54]. Kim et al. [55] in support of these results further observed that DATS-mediated CSC inhibition was associated with a decrease in FoxQ1 protein expression level, decrease in aldehyde dehydrogenase 1 (ALDH1) activity, and a loss of Dachshund homolog 1 (DACH1) expression. DATS inhibited Trx-1 expressions and Trx-1 reductase enzyme function in breast cancer cells (MDA-MB-231, Hs578T, MCF-7, and ZR-75-1). Thioredoxin-1 (Trx-1) is a redox-active disulfide/dithiol-containing 12kD protein and its cysteine residues are transformed in a chemical reaction to an oxidized intramolecular disulfide bond configuration (Trx-1-S2). Trx-1 is responsible for accelerated tumor development and diminished patient survival when secreted by tumor cells. Trx1 overexpression in tumor cells resulted in the suppression of apoptosis signal-regulating kinase 1 (ASK1) expression associated with JNK/p38 signaling pathway, and apoptosis suppression [55]. In a separate study, DATS demonstrated growth inhibition of MDA-MB-231, MCF-7, and MCF10A-H-Ras breast epithelial cells by inhibiting the expression level of α secretases, such as ADAM-10 (a disintegrin and

metalloprotease-10) and ADAM-17 which are associated with the activation of the Notch signaling pathway. Furthermore, it suppressed the Notch ligands Jagged-1 and Jagged-2 associated with the activation of the Notch signaling pathway [56]. DATS suppressed the platelet-mediated hematogenous metastasis of MDA-MB-231 cells by preventing the activation and aggregation of platelet induced by platelet-activating factor (PAF) and ultimately reduced the formation of thromboxane B2 (TXB2) and TGF-β1 [57]. Another novel target of DATS in breast cancer was ER-α which was inhibited by DATS in MCF-7 and T47D breast carcinoma cells. The expression level of pS2 and cyclin D1 were decreased, along with downregulation in nuclear levels of ER-α protein, and ER-α mRNA suppression. DATS further inhibited the ERE2e1b-luciferase reporter activity associated with a decrease in the over expression level of peptidyl-prolyl cis-trans isomerase (Pin1) [58]. DATS intervention also inhibited leptin-induced cell proliferation, clonogenic cell viability, and migration and invasion potential in MCF-7 and MDA-MB-231 breast cancer cells by inhibiting mRNA expression levels of Bcl-2, Bcl-xL, cyclin D1, vascular endothelial growth factor, and MMP-2. DATS also exhibited potent antimetastatic activity against triple-negative breast cancer cells (MDA-MB-231 cells and HS 578t) by inhibiting the expression level of MMP-2 and MMP-9, which finally inhibited both the NF-κB signaling pathway and ERK/MAPK signaling pathways [59]. By way of ROS generation and subsequent activation of JNK and AP-1, DATS-induced apoptosis was mediated in MCF-7 cells in association with increased accumulation of ROS [60].

SAMC, a stable organosulfur compound of garlic, exhibited strong antiproliferative activity against MCF-7 breast carcinoma cells [61]. SAMC inhibited the cell growth of MDA-MB-231 and MCF-7 breast carcinoma cells by inducing apoptosis and cell cycle arrest at G_0/G_1 phase associated with increased expression of p53 and p21. Associated with the mitochondrial apoptotic pathway, the protein expression level of Bax, caspase-9, and caspase-3 were increased, whereas the expression level of Bcl-2 and Bcl-xL were decreased [62]. SAC significantly reduced the growth of MDA-MB-231 human breast tumor cells in a concentration and time-

dependent manner [64]. SAC treatment of MDA-MB-231 cells increased the E-cadherin expression level and reduced the MMP-2 expression level, suggesting inhibition of cell proliferation, adhesion, and invasion, contributing to suppression of metastasis [63].

A preclinical study revealed the use of garlic powder revealed successful tumor suppression activities utilizing the DMBA-induced mammary tumor model in rats [64]. It was further revealed that DADS has been more active than DAS and AMS [64]. Schaffer et al. [65] reported that garlic powder, SAC, and DADS supplementation suppressed the N-methyl-N-nitrosourea (MNU)-induced mammary carcinogenesis in rats by minimizing the incidence of mammary tumorigenesis. Garlic extract showed anticancer activity by inhibiting the growth of breast cancer cells 67NR, which are implanted in the mammary pads of BALB/c mice [66]. Additionally, garlic powder inhibited DMBA-induced mammary cancer tumor in rats by minimizing DMBA-DNA interaction to reduce the final mammary tumor incidence [67].

Oil-soluble garlic compounds have demonstrated potent anticancer activity than water-soluble compounds in rats among the various organosulfur compounds. DADS exhibited significant inhibition against DMBA-, MNU-, and 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP)-induced mammary cancer in rats [68]. DADS displayed anticancer and antiproliferative behavior in xenograft tumor models which were established by injecting MCF-7 and MDA-MB-231 breast cancer cells into nude mice subcutaneously. Treatment with DADS contributed to substantial downregulation of protein expression of urokinase-type plasminogen activator (uPA) and MMP-9 and stimulation of tristetraprolin (TTP) expression [69]. DADS inhibited the growth of human breast cancer cells not only through *in vitro* analysis but also through *in vivo* experimentation in mice, where DADS showed growth retardation by suppressing primary tumor weight in female nude mice (orthotopic transplantation of KPL-1 in mice) [45]. Another *in vivo* study revealed that DATS downregulated the expression level of phosphorylated STAT3 in breast cancer xenografts in rats [70]. DATS diminished the metastasis of breast cancer in xenografted MDA-MB-231 tumor model by lowering the expression level of MMP-2 and MMP-9

[71]. DATS, a natural histone deacetylase inhibitor, prevented the MDA-MB-231 hypoxia-inducing cell metastasis by inhibiting HIF-1α transcriptional activity in the embryonic zebrafish tumor model. Additionally, it decreased the expression of L1CAM, VEGF-A, and epithelial mesenchymal transition (EMT)-related proteins (Slug, Snail and MMP-2) [72]. DAS decreased the concentrations of diethylstilbestrol (DES)-induced lipid hydroperoxides (LPH) in female ACI rats (an inbred line originating from a cross between the strains of August and Copenhagen) [73].

The anticancer compounds in different extracts can form nanoconjugates which can help to prevent cancer cell lines from proliferating. Garlic clove extract-mediated silver nanoparticles (G-AgNPs) exhibited cytotoxic activity against MCF7 cell line associated with decrease in cell viability at 100 µg/mL. It further exerted nuclear morphological variations, such as cell clumping and membrane instability in MCF-7 cells [74]. G-AgNPs did not induce any toxicity or mortality in *Corylus cornuta* neonates. In a separate study, garlic extract-mediated silver nanoparticles (Ag-S2) induced cytotoxicity in MCF-7 carcinoma cell line by decreasing cell viability in a concentration-dependent manner [75]. However, garlic extract-mediated gold nanoparticles (G-AuNPs) did not exert any toxicity towards MCF-7 cells [76]. Garlic extract-based size controlled superparamagnetic hematite nanoparticles were prepared and investigated for their cytotoxicity against breast cancer MCF-7 cell line. The results suggest that the growth of the cell line was concentration-dependently arrested at an IC_{50} of 346.25 mg/mL [77]. Even ZnO-reduced graphene oxide nanocomposites (ZnO-RGO NCs) using garlic clove extract also showed enhanced cytotoxicity against MCF-7 cells [78]. DADS solid lipid nanoparticles exhibited higher cytotoxicity than DADS alone against MCF-7 carcinoma cells, where it inhibited the proliferation of carcinoma cells by triggering apoptosis through the intrinsic signaling pathway linked with elevated expression level of Bax, Bad, caspase-3, and caspase-9, and decreased protein expression level of Bcl-2 [49].

3.2. Cervical cancer

With an expected 604,000 new cases and 342,000 deaths globally in 2020, cervical cancer is the fourth most frequently diagnosed cancer and the fourth major cause of cancer mortality in women [39,40]. Allicin exhibited antitumor activity by decreasing the cell viability of a human cervical squamous cell carcinoma cell line (SiHa cells). It inhibited cell proliferation by inducing apoptosis through suppression of the expression level of nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase 1. Allicin also inactivated the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) signaling pathway [79]. Green synthesized silver nanoparticle from aqueous extract of elephant garlic exhibited cytotoxicity against HeLa cervical carcinoma cell line. It exhibited potent inhibitory activity against the carcinoma cell line with an IC_{50} <25 mg/mL [80]. Another study demonstrated that size controlled superparamagnetic hematite nanoparticles of garlic extract showed cytotoxic response against HeLa cells with IC_{50} value of 285 mg/mL [77]. Garlic extract-mediated silver nanoparticles (Ag-S2) demonstrated cytotoxic activity against HeLa cells in which it inhibited cell growth in a concentration-dependent manner [75]. Ag-S2 further induced cytotoxicity against Hep-2 cervical adenocarcinoma cells.

3.3. Colorectal cancer

In 2020, more than 1.9 million new instances of colorectal cancer (including anus) are estimated to occur with 935,000 deaths. Colorectal cancer is the third most common cancer, but it is the second most deadly in terms of mortality [39,40]. The driving forces behind CRC development are known to be obesity, lack of physical activity, red meat intake, alcohol, and tobacco. Early detection and treatment can reduce CRC mortality.

Dietary supplement use of garlic can reduce the risk as well as prevent colorectal cancer [81]. Aged black garlic extract suppressed the proliferation of HT29 colon cancer cells by inducing apoptosis and cell cycle arrest at the G_0/G_1 phase through regulation of the PI3K/Akt pathway. It upregulated PTEN expression and downregulated the expression level of Akt and p-Akt,

and p-70-kDa ribosomal protein S6 kinase 1 (p70S6K1) [82]. The crude extract of garlic triggered apoptosis in human colon cancer colo205 cells. It reduced the mitochondrial membrane potential and elevated the caspase-3 activity as well as expression level. The extract also elevated the expression levels of Bax, cyt. c, and caspase-3, whereas the expression level of Bcl-2 was reduced [83].

Allicin demonstrated its antiproliferative and cytotoxic potential against HCT-116, LS174T, HT-29, and Caco-2 colon cancer cell lines by inducing apoptosis. It elevated the expression level of Bax, and release of cyt. c into the cytoplasm, and decreased the expression level of Bcl-2. It also activated the translocation of Nrf2, which further triggered luciferase transactivation activity [84]. Allicin also increased the radiosensitivity of the colorectal carcinoma HCT116 cells by inhibiting the proliferation of the cells through elevated apoptosis [85]. Synergistically, allicin, in combination with 5-fluorouracil (5-FU), exhibited potent antitumor activity against colorectal carcinoma DLD-1 cells. Additionally, it induced apoptosis with a reduction of total caspase-3 [86].

Se-methyl-L-selenocysteine (MseC), an organoselenium compound extracted from garlic, inhibited the growth of colo205 cells by inducing apoptosis with the elevated expression level of Fas and FasL as well as increased cleavage of caspase-3, caspase-8, DNA fragmentation factor 45 (DFF45), and poly (ADP-ribose) polymerase (PARP) expression levels. It also increased the level of Baxprotein, but decreased the Bid and Bcl-2 proteins expression levels. It also downregulated the protein levels of ERK1/2 and PI3K/Akt and has optimized the protein levels p38 and JNK [87].

Thiacremonone, a sulfur compound extracted from garlic, induced apoptotic death of SW620 and HCT116 human colon cancer cells by modulating the tumor necrosis factor-α (TNF-α), TPA-induced NF-κB transcriptional activity, and the DNA binding activity. Moreover, it decreased the antiapoptotic proteins, such as Bcl-2, cIAP1/2, and XIAP, and inflammatory pro-

teins, such as iNOS and COX-2 but it increased the expression level of apoptotic proteins, such as Bax, cleaved caspase-3, and cleaved PARP [88-89].

Z-ajoene, is also a sulfur-containing compound extracted from garlic, inhibited colon cancer cell growth of SW480 cells by reducing the expression levels β-catenin, c-Myc, and cyclin D1. It increased the phosphorylation of β-catenin at Ser45 to modulate the casein kinase 1α (CK1α) activity and downregulated the Wnt/β-catenin signaling pathway [90].

SAMC displayed antiproliferative activity by inducing apoptotic cell death of human colon cancer cells (SW-480 and HT-29) via cell cycle arrest at the G_2/M phase in association with the elevation of the caspase-3 activity. It also activated the Jun kinase activity with significant elevation in the endogenous levels of the reduced GSH [91].

DAS inhibited the arylamine N-acetyltransferase (NAT) activity and expression of mRNA NAT in various colon cancer cells, such as colo205, colo320 DM, and colo320 HSR [92]. Anticancer action of DAS led to increased apoptosis of Colo320 DM colon cancer cells and induction of cell cycle arrest at G_2/M phase. Moreover, it increased the expression of NF-κB and caspase-3 and decreased ERK-2 activity with increased production of ROS [93].

DADS, an organosulfur compound of garlic, elevated Ca^{2+} mobilization in SW480 colon cancer cell line as an early signaling effect [94]. DADS induced cell cycle arrest at the G_2/M phase in HT-29 Glc(-/+) human colon adenocarcinoma cell line associated with increased L-glutamine oxidation [95]. DADS-induced apoptosis in colo205 cells was associated with increased expression of signal transducer and activator of transcription 1 (STAT1) [96]. DADS caused cell cycle arrest at the G_2/M phase in HCT-116 cells associated with increased cyclin B1 and p53 expression [97]. It also elevated the ROS production in HCT-116 carcinoma cells [98]. DADS altered ERK signaling pathway with increased ERK phosphorylation in human colon tumor cells (HCT-15) to cause cell cycle arrest at G_2/M phase [99]. The antiproliferative activity of DADS against Caco-2 and HT-29 cells was exhibited through cell cycle arrest at G_2 phase by inhibition of histone deacetylase (HDAC) activity, H4 histone hyperacetylation, and increase in

the protein expression levels of p21(waf1/cip1) [100]. The sustained hyperacetylation of histone H3 K14 was accomplished with repeated DADS exposure [101]. DATS caused cell cycle arrest at the G_2/M phase in HCT-15 and DLD-1 cell lines, linked with increased caspase-3 activity and inhibition of tubulin polymerization [102]. In a separate study, DATS exhibited similar cytotoxic activity against HCT-15 and DLD-1 cell lines by inducing apoptosis via disruption of microtubule network formation of the cells in which specific oxidative alteration of cysteine residues Cys12β and Cys354β, resulting in the formation of S-allyl-mercaptocysteines within the tubulin molecule [103].

DAS, DADS, and DATS targeted drug-resistant genes in human colon cancer cells (colo205). DATS elevated the expression level of multidrug-resistant 1 (Mdr1) and MRP-1, whereas DAS and DADS increased the expression level of multidrug resistance-associated protein-3 (MRP-3). DADS and DATS targeted and increased the expression of MRP4 and MRP6 genes [104]. DAS, DADS, and DATS inhibited the proliferation of colo205 cells by decreasing the expression levels of PI3K, Ras, MEKK3, MKK7, ERK1/2, JNK1/2, and p38 to inhibit MMP-2, MMP-7, MMP-9, NF-κB and COX-2 expressions [105].

DAS and AMS inhibited cytochrome p4502E1 (CYP2E1) expression in a nitrosamine induced colon carcinogenic animal model utilizing F344 rats [106]. Garlic extract inhibited the growth of tumors induced by 1,2-dimethylhydrazine (DMH) in rats by downregulating the expression of cyclin B1 and cdk1 through inactivation of NF-κB [107-108]. Allicin inhibited colonic tumorigenesis in mice via inhibiting the proliferation of HCT116 cells by suppressing the STAT3 signaling pathway. Treatment with allicin decreased the numbers and the size of the tumors associated with decreased expression level of Mcl-1, Bcl-2 and Bcl-xL [109]. *In vivo* study of allicin combined with X-ray radiotherapy revealed that growth and weight of transplanted tumor of CT26 cells in BALB/c mice were inhibited in greater extent in comparison with the X-ray radiotherapy alone [85]. *In vivo* analysis revealed that DAS reduced the incidence of colonic polyps in colon carcinogenesis utilizing engineered transgenic Apc Min/$^+$ mice [110]. DADS

inhibited colorectal tumorigenesis in a mouse model involving NF-κB signaling pathway in which the nuclear localization of NF-κB and its activity was lost. Moreover, DADS also inhibited the activation of GSK-3β [111]. DATS suppressed the growth and proliferation of xenografted CT26 colon carcinoma cells and induced apoptosis, resulting in a reduction of tumor volume and weight [112]. Similar observations were reported in an *in vivo* study utilizing human colon colo 205 cancer cells implanted in mice, where DADS and DATS reduced both tumor weight and size [104].

Size controlled superparamagnetic hematite nanoparticles prepared from aqueous garlic clove extract exhibited cytotoxic activity against colorectal carcinoma HCT-116 cell line. It inhibited the growth of the cell line in a concentration-dependent manner having an IC_{50} value of 230 mg/mL [77]. ZnO-reduced graphene oxide nanocomposites (ZnO-RGO NCs) using garlic clove extract exhibited elevated cytotoxicity against human colorectal cancer (HCT116) cells [78].

3.4. Gastric cancer

Gastric cancer is still a major health challenge in the globe, with over one million new cases expected in 2020 and a predicted 769,000 deaths, ranking fifth in incidence and fourth in fatality [39,40]. Various factors, such as the environment and genetics, may contribute to its etiology.

One of the most recommended approaches to gastric cancer prevention is the consumption of garlic [113]. Mature black garlic extract showed cytotoxic activity in human gastric cancer cells as a result of antioxidant and immunomodulatory concentration-dependent apoptosis in SGC7901 [114]. Allicin exhibited chemo-preventive and growth inhibitory action against gastric cancer by inducing apoptosis through the induction of ROS and DNA damage through the caspase-dependent/-independent pathways and death receptor pathway [115]. Allicin suppressed the proliferation of two gastric carcinoma cell lines (HGC27 and AGS) by triggering apoptosis, which suppressed cell viability. The treatment further increased the expression of miR-383-5p,

whereas the expressions of ERBB4, p-PI3K, and p-Akt were decreased [116]. Allicin inhibited SGC-7901 gastric cancer cell proliferation by inducing apoptosis by activating the mitochondrial signaling pathways through the release of cyt. c into the cytoplasm, which subsequently activated caspase-3, caspase-8, and caspase-9. Treatment with allicin further increased the protein expression of Bax and Fas, and thereby regulating the extrinsic Fas/FasL-mediated cascade [117]. In a separate study, allicin exhibited similar anticancer activity by inducing apoptosis and cell cycle arrest at G2/M phase associated with inhibited telomerase activity in SGC-7901 cells [118]. It also showed growth-inhibitory activity against three human gastric carcinoma cell lines (MGC-803, BGC-823, and SGC-7901) by inducing apoptosis and increasing the expression levels of p38, and cleaved caspase-3 associated with an increase in Bax expression level and decreased level of Bcl-2. Moreover, it downregulated the p38 MAPK/caspase-3 signaling pathway [119].

DATS, the major volatile metabolite of garlic, inhibited the protein expression level of sulfiredoxin (Srx), MDA, and ROS in BGC823 gastric carcinoma cells. Overexpression of Srx in cancer led to carcinogenesis and tumor progression [120]. DATS inhibited the proliferation of SGC-7901 gastric cancer cells by inducing cell cycle arrest at the G_2/M phase which is evident from the increase in the expression level of the cyclin B1 and cyclin A2. Induction of apoptosis by DATS led to upregulation of proapoptotic factors Bax and downregulation of the antiapoptotic factor Bcl-2. It further increased the phosphorylation of ERK, JNK and p38, leading to downregulation of the MAPK signaling pathway. It also decreased the expression level of total Akt and increased the phosphorylated Akt level, activating the PI3K/Akt signaling pathway (**Figure 3**) [121]. DATS, also known as allitridi, exhibited cytotoxic effects by inhibiting the cell growth of human gastric cancer cells (MGC803 and SGC7901 cell lines). It induced cell cycle arrest at G_2/M or M phase by upregulating the expression levels of the p21(WAF1) gene [122]. DATS exhibited antiproliferative activity against gastric cancer cell lines, such as BGC823, SGC7901, AGS, and MT2A-BGC823, by inducing apoptosis and cell cycle arrest at the G_2/M phase. It

downregulated the expression of MT2A, which in turn improved the transcription of IκB-*α* to inhibit the activation of NF-κB, thereby regulating the MT2A/NF-κB signaling pathway [123]. DATS induced apoptosis in BGC823 cells (a gastric carcinoma cell line) by regulating the expression level of glutathione S-transferase-pi (GST-pi), voltage-dependent anion channel-1 (VDAC-1), Annexin I, Galectin, and S100A11, which are associated with mitochondrial apoptotic pathway [124]. DATS also triggered apoptosis in MGC803 cells, which can be prompted by the caspase-3 activation pathway [125]. DATS caused apoptosis and mitotic cell arrest by the ROS activation of the AMP-activated protein kinase in AGS cells. It triggered the cell cycle arrest at the G_2/M phase via activation of cyclin B1 and cyclin-dependent kinase p21(WAF1/CIP1) pathway [126]. DATS also activated the nuclear factor erythroid 2–related factor 2 (Nrf2) signaling pathway by interacting with Cys288 residue of Kelch-like ECH-associated protein-1 (Keap1) [127].

SAMC induced apoptosis in SGC 7901 human gastric cancer cells to inhibit cell proliferation through the JNK and p38 pathway [128]. SAMC displayed a similar cytotoxic effect on SNU-1gastric carcinoma cells by inducing apoptosis associated with the increased expression levels of p53, Bax, and caspase-9 (**Figure 4**). It further induced mitochondrial cyt. c release and subsequently activated caspase-3 [129].

DADS supplementation displayed growth inhibition of MGC-803 gastric cancer cells through checkpoint kinase-1 (Chk-1)-mediated G_2/M phase cell cycle arrest by activating the phospho-Chk1 and decreasing the CDC25C and cyclin B1 expression levels, thereby regulating the Chk1/CDC25C/cyclin B1 signaling pathway [130]. DADS exhibited a similar anticancer profile against MGC-803 cells through alteration of the ERK1/2 signaling pathway in which it reduced the phosphorylation of ERK1/2 [131]. DADS further inhibited the proliferation of the AGS human gastric adenocarcinoma cells by inducing apoptosis and increased production of ROS. In AGS cells, DADS raised the expression level of Fas, caspase-3, and Bax, and lowered the expression level of Bcl-2 [132]. DADS impaired the metastasis of human type II esophage-

al-gastric junction adenocarcinoma cells (OE19) through the suppression of the NF-κB and PI3K/Akt signaling pathways, in which it downregulated the expression level of MMP-2, MMP-9 and uPA [133]. DADS elevated the tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 mRNA levels and proteins expression levels and reduced the levels of claudin proteins (claudin-2, claudin-3, and claudin-4), the key components of tight junctions (TJs), thereby inhibiting the cell proliferation [134].

S-benzyl-cysteine (SBC), a water-soluble compound of garlic, displayed anticancer activity against SGC-7901 cells (another gastric carcinoma cell line) by inducing apoptosis and cell cycle arrest at G2 phase associated with loss of mitochondrial membrane potential ($\Delta\psi m$) and increased the activity of caspase-9 and caspase-3. Furthermore, it upregulated the expression level of p53, and Bax, and reduced the expression level of Bcl-2, thereby regulating the p53 and Bax/Bcl-2 signaling pathways linked with apoptosis [135].

SAC, a water-soluble metabolite of garlic, showed chemo-preventive activity against N-methyl-N'-nitro-N-nitrosoguanidine and sodium chloride-induced gastric carcinogenesis in Wistar rats by modulating lipid peroxidation (thiobarbituric acid reactive substances or TBARS) and increasing the GSH-dependent antioxidant enzymes [136]. Another *in vivo* study revealed that SAMC exhibited anticancer activity against SGC-7901 cells which were inoculated subcutaneously in BALB/c nude mice. It inhibited the proliferation of SGC-7901 carcinoma cells by activating the MAPK and inhibiting the PI3K/Akt signaling pathways in which the protein expression level of p-Akt was decreased and the expression levels of ERK, p38, and JNK were increased. It also reduced Bcl-2 expression, whereas the Bax expression was elevated [137]. Similar observations were observed, where SAMC exerted antitumor activity in mice bearing KMN-45 cells injected into the subcutaneous tissues. It inhibited the proliferation of the cancerous cells which is evident from the reduction in tumor volume by induction of apoptosis. It further increased the expression level of Bax and decreased the expression of Bcl-2 [138].

3.5. Liver cancer

With about 906,000 new cases and 830,000 fatalities expected in 2020, primary liver cancer is the sixth most frequently diagnosed cancer and the third major cause of cancer mortality globally [39,40]. It is mostly linked to infection with hepatitis C or B viruses and the consumption of alcohol. Among the various liver cancer, hepatocellular carcinoma (HCC) is the most prevalent one and it is often diagnosed in the advanced stage [139]. Chemotherapy and immunotherapy are the available options. However, new treatment options involving natural products can lead to better prognoses.

Garlic exhibited protective as well as anticancer therapeutic actions against liver cancer [140]. Allicin induced apoptosis of Hep 3B ($p53^{mutation}$) cell lines via p53-mediated autophagy induction [141]. Allicin synergistically sensitized hepatocellular cancer cells to 5-fluorouracil. The combination therapy inhibited the proliferation of HCC SK-Hep-1 and BEL-7402 cells [142]. DAS resisted oxidation, reduction, and metabolism of diethylstilbesterol (DES), thereby inhibited the formation of DNA adducts to prevent the risk of liver cancer [143]. DATS lowered the cell viability of J5 cells, causing cell cycle arrest at the G_2/M phase by decreasing the cyclin-dependent kinase-7(Cdk7) and increasing the expression level of cyclin B1 [144]. DATS also inhibited the proliferation of HepG2 cells by increasing the formation of H_2O_2, decreased the thiol level, and activated the caspase-3 activity [145].

SAMC induced apoptosis in HepG2cells by promoting the TGF-β1, TβRII, p-smad2/3, smad4, and smad7 signal proteins. It also increased the expression of Bim, caspase-3, and caspase-9 and decreased the expression of Bcl-2, thereby activating the TGF-β signaling and inhibiting the MAPK signaling pathway [146].

SAC possessed inhibitory action on proliferation and metastasis of MHCC97L HCC cells. This compound suppressed the expression of the proliferation marker (Ki-67 and PCNA) and caused cell cycle arrest at the S phase. It also reduced the expression of Bcl-xL, Bcl-2, cdc25c, cdc2, and cyclin B1, whereas the expression of caspase-3 and caspase-9 were increased [147].

FO, a hydroxycinnamic acid compound isolated from garlic skin, exhibited antiproliferative activity against Huh7 and HCCLM3 HCC cells by decreasing the phosphorylation expression levels of Akt and p38 MAPK. Furthermore, Slug expression was impaired and E-cadherin level was enhanced. Furthermore, it regulated the EMT-related signaling molecule (E-cadherin) as well as PI3K/Akt, and p38 MAPK signaling pathways [148].

Kay et al. [149] showed that ajoene from garlic stimulated Nrf2 in HepG2 cells, resulting in increased level of glutamate-cysteine ligase (GCL) and GSH in cells, decreased the interaction of Nrf2 with Kelch-like ECH-associated protein-1 and reduced the ubiquitination of Nrf2.

Combination therapy of allicin and 5-FU exhibited antitumor activity in subcutaneous xenograft tumor model by transplanting SK-Hep-1-GFP cells into nude mice. This treatment induced apoptosis through elevated levels of ROS, decreased mitochondrial membrane potential ($\Delta\Psi m$), activated caspase-3 and PARP, and decreased expression of Bcl-2 [142]. DAS inhibited the formation of DNA adducts, which is induced by diethylstilbesterol in male Sprague-Dawley rats. SAMC inhibited hepatic tumorigenesis in Huh-7 xenograft/orthotopic mouse model by reducing the overexpression of low-density lipoprotein receptor (LDLR)-related protein 6 (LRP6) and activating the LRP6/Wnt pathway [150]. SAC prevented N-nitrosodiethylamine (NDEA)-induced hepatocarcinogenesis in Wistar rats by reducing tumor incidence, increasing lipid peroxidation and the level of the antioxidants, and decreasing the glutathione S-transferase (GST) activity [151].

Silver nanoparticles synthesized using garlic extract induced cytotoxicity in HepG2 human liver carcinoma cells to inhibit its proliferation [152]. It induced apoptosis, DNA fragmentation and cell cycle arrest at G2/M phase in a concentration-dependent manner. It reduced the protein expression level of Bcl-2, increased the expression levels of Bax, caspase-3, caspase-9, and reslted in cleavage of PARP. It further reduced the expression levels of cyclin B, cdc2 and cdc25c, and increased the expression level of p21 and p53. In a separate analysis, alginate-based black garlic nanoparticles exhibited cytotoxicity against HepG2 cells. The percentage inhibition

for the above-mentioned cell line was found to be 85.6±4.5%, but the concentration of the nanoparticles was not mentioned [153]. In another liver cancer cell line Hep-2, the silver nanoparticles of garlic aqueous extract was applied to screen cytotoxic effect, where the nanoparticles was found to be capable of inhibiting the cell proliferation in a concentration-dependent manner associated with decrease in cell viability with IC_{50} value of 27.63±0.88 µg/mL [75]. Allicin-loaded polypyrrole (PPY) nanoparticles exhibited elevated cytotoxicity in HepG2 carcinoma cell line in comparision to allicin alone with significant decrease in cell viability of 30% at 100/500 µg/ml concentration [154].

3.6. Lung cancer

Lung cancer is the second most frequently diagnosed cancer and the major cause of cancer mortality in 2020, with an expected 2.2 million new cases and 1.8 million fatalities [39,40]. Allicin decreased the cell viability, proliferation, and migration of non-small cell lung cancer (NSCLC) cells (A549 and NCI-H460). It induced apoptosis and triggered the autophagy pathway in A549 cells by increasing ROS level and caused cell cycle arrest at S/G2-M phase. It was further reported that it exhibited cytotoxic activity through regulation of ROS/MAPK and ROS/JNK signaling pathway [155].

Garlic extracts mediated silver nanoparticles (Ag-S2) showed cytotoxicity against lung carcinoma cell line (A549) by inducing apoptosis associated with morphological changes in the cells [75]. During a study with alginate-based black garlic nanoparticles, it has been found that it possesses anticancer activity against lung carcinoma LU-1 (HTB-57^{TM}) cell lines with an inhibition of 47.8±2.7%, but the concentration at which it exhibited this inhibition was not mentioned [153].

3.7. Oral cancer

Oral cancer is becoming one of the most common form of cancer worldwide as a result of tumor invasion and several lymph node metastases. It is estimated that 377,713 new cases of cancers of the oral cavity and more than 177,757 deaths will occur in 2020 [39,40]. The exposure of tobacco to the oral epithelium surface to free nitrogen and oxygen radicals is one of the risk factors for oral cancer.

Garlic consumption can reduce the risk and growth of oral cancer [156]. Alliin activated the human salivary aldehyde dehydrogenase (hsALDH) enzymatic activity which could reduce the risk of oral carcinogenesis [157]. Allicin decreased the cell viability of oral squamous cell carcinoma (OSCC) at a concentration of 100 ng/mL. It inhibited the protein expression level of TNF-α, IL-8, and endothelin [158]. SAC treatment inhibited the growth of human oral squamous cancer CAL-27 cells by increasing the expression of E-cadherin and stabilizing the E-cadherin/β-catenin adherent junction complex. Therefore, it inhibited and regulated the MAPK/ERK signaling pathway by reducing the SLUG repressor protein expression [159].

Consumption of SAC inhibited the growth and proliferation of oral tumor cells in a mouse xenograft model bearing CAL-27 cells. It suppressed the N-methylpurine DNA glycosylase and osteopontin (OPN) level in the plasma associated with the prevention of phosphorylation of Akt, decrease the expression level of cyclin D1 protein with an increase in the level of cell-cycle inhibitor p16(Ink4) expression. In addition, SAC blocked the protein expression of COX-2, vimentin, and NF-κB p65 (RelA) [160].

In an experiment performed by Ai Thach *et al*. [153], cytotoxic effect of alginate-based black garlic nanoparticles was studied against human mouth epidermal carcinoma KB (CCL-17^{TM}) cell line. The black garlic nanoparticles showed significantly inhibition of 42.4±3.1%, but the concentration at which it exhibited this inhibition was not reported. Garlic extract-modified titanium dioxide nanoparticles exhibited cytotoxic activity against KB oral cancer cell line by decreasing the cell viability dependent on concentration. The percentage cell viability was found

to be 60.76% at 10 mg/mL concentration. Moreover, the production of ROS led to decrease in cell viability [161].

3.8. Ovarian cancer

It is expected that there are 313,959 new diagnosed cases of ovarian cancer and 207,252 cancer deaths worldwide in 2020 [39,40]. Because of limited success in surgery and short survival times after chemotherapy, it is important to boost its prevention or novel treatment.

Garlic and its components have shown inhibitory activity against multiple types of ovarian carcinoma cells. SAC exhibited antiproliferative, apoptosis-inducing effects, and G_1/S phase cell cycle arrest in A2780 human ovarian cancer cells in a concentration-dependent fashion [162-163]. SAC also downregulated the expression of PARP-1, pro-caspase-3, and Bcl-2, and increased in the expression levels of Bax and caspase-3. SAC suppressed the expression of c-Jun, p-Akt, and Wnt5a, the main proteins that are implicated in cancer cell proliferation metastasis [162]. SAC also elicited DNA 5-mC demethylation with a decrease in DNA methyltransferase (DNMT) activity where the mRNA expression level of DNMT1 was reduced markedly, whereas the mRNA levels of cyclin-dependent kinase inhibitor 1A (CDKN1A) was increased [163].

Allicin, one of the active compounds of garlic, possesses significant antiproliferative activity against the human ovarian cancer cell line SKOV3 by inducing apoptosis and JNK phosphorylation linked with JNK MAPK signaling pathway (**Figure 5**). It further increased the expression level of Bax and cyt. c [164]. By conjugation of garlic with other natural/synthetic compounds, the effectiveness of those natural/synthetic compounds can be enhanced in the treatment of ovarian cancer. For example, daidzein-alliinase conjugates with alliin demonstrated significant antiproliferative activity in various xenografted ovarian cancer models (ES-2 and OVCAR3) [165].

Silver nanoparticles synthesized using garlic extract exhibited antiproliferative activity by inducing cytotoxicity in Skov3 ovarian cancer cell line**.** It exerted apoptosis, DNA fragmentation

and induced cell cycle arrest at G2/M phase in concentration-dependent manner. It also decreased the expression level of Bcl-2 and upregulated the expressions of Bax, caspase-3, caspase-9 with PARP cleavage. The expression levels of cyclin B, cdc2 and cdc25c were reduced, whereas the expression levels of p21 and p53 were increased [152].

3.9. Pancreatic cancer

Because of its dismal prognosis, pancreatic cancer is the seventh largest cause of cancer mortality in both sexes worldwide, accounting for nearly as many deaths (466,000) as diagnoses (496,000) [39,40]. Available treatment options include chemotherapy, radiation therapy, and surgery. Targeted therapy with natural products can be an alternative option.

Garlic showed potential anticancer activity by inhibiting the proliferation of various pancreatic carcinoma cell lines [166]. Allicin exhibited cytotoxicity against pancreatic cancer cells (MIA PaCa-2 cells) associated with a decrease in cell viability and activation of apoptosis. It further elevated the expression level of caspase-3 and $p21^{Waf1/Cip1}$. Moreover, it induced cell cycle arrest at G1 phase with increased ROS level and decreased intracellular glutathione (GSH) content [167]. DATS activated apoptotic pathway to inhibit the proliferation of human pancreatic cancer cells having wild-type p53 genes (Capan-2) and promoting cell cycle arrest at the G_2/M phase. It increased the protein expression level of Fas, p21, p53, Bax, and cyclin B1 and decreased the protein expression level of Akt, cyclin D1, MDM2, and Bcl-2 [168].

S-propargyl-L-cysteine (SPRC) exhibited anticancer activity against pancreatic ductal adenocarcinoma (PDA) by inhibiting the proliferation and inducing apoptosis and cell cycle arrest at the G_2/M phase in PDA cell line. It also inhibited tumor growth in Panc-1 xenograft models through the activation of the JNK signaling pathway [169].

Silver nanoparticle of elephant garlic aqueous extract was green synthesized in order to evaluate its cytotoxic activity against pancreatic tumor (insulinoma) βTC-3 cell line. The result

demonstrated a concentration-dependent potent inhibitory action against the above-mentioned cell line with an IC_{50} value < 25 μg/mL [80].

3.10. Prostate cancer

Prostate cancer is the second most common cancer in males and the fifth greatest cause of cancer mortality in 2020, with an expected 1.4 million new cases and 375,000 deaths globally [39,40]. Cancer of the prostate may spread to several parts of the body, specifically lymph nodes and bones. The available therapeutic options involve diagnosis, surgery, radiotherapy, and chemotherapy at the early stages [170]. It is particularly important to identify alternative approaches towards successful prevention and treatment of prostate cancer [171].

The garlic component DATS induced cell cycle arrest at the G_2/M phase in DU145 prostate carcinoma cell line which is correlated with the decrease in the expression level of the Cdc25C (cell division cycle 25C) protein during mitotic arrest and delayed nuclear translocation of cyclin-dependent kinase 1 (cdk1) [172]. Similar observations were reported where PC-3 and DU145 carcinoma cells treated with DATS which exhibited G_2/M phase cell cycle arrest with a reduction in the protein expression level of Cdc25C which is phosphorylated on Ser^{216} and it is caused by ROS [173]. DATS-induced ROS generation in human prostate cancer cells was mediated by an increase in labile iron due to the degradation of ferritin through the c-Jun NH_2-terminal kinase (JNK) signaling pathway which plays a critical role in the regulation of apoptosis and cell survival (**Figure 6**) [174]. DADS inhibited the proliferation of the PC-3 by modulating the insulin-like growth factor (IGF) signaling pathway. It blocked the phosphorylation of the Akt and thus restricted the progression of the cell cycle and cell survival. An increase in cell apoptosis is linked with downregulation of the protein expression level of cyclin D1, NF-κB, XIAP, and Bcl-2 and upregulation of the expression level of Bad, Bax, FAS mRNA, caspase-8, and caspase-9 [175]. There is an increase in the protein expression level of cyclin B1 and decrease in CDK1 expression level [176].

Additional analysis has been conducted to elucidate other possible mechanisms through which garlic derivatives exert their cytotoxic effects on prostate carcinoma cells. In androgen-independent prostate cancer cell lines (PCa and DU145), SAC and SAMC, water-soluble garlic derivatives, suppressed the proliferation and invasion ability of prostate cancer cells via restoration of E-cadherin expression and inhibiting the expression level of E-cadherin suppressor, Snail [177].

DADS suppressed the proliferation of PC-3 cells, an androgen-independent human prostate cancer cell line, in a concentration-dependent manner by induction of cell cycle arrest at the G2/M phase transition [174]. SAC, another major component of garlic, is an inhibitor of NF-κB activation in the NF-κB signaling pathway [178]. DATS-induced apoptosis in human prostate cancer cells (PC-3 and DU145) was triggered by Akt inactivation, leading to mitochondrial translocation of BAD followed by activation of caspase-3 and caspase-9 [179]. DATS activated both the checkpoint kinase 1 (Chk1) and checkpoint kinase 2 (Chk2), which acts as the mediator of DNA damage response (DDR). Chk1 plays an important role in Ser216 phosphorylation of Cdc25C in human prostate cancer cells (PC-3 and DU145) [180]. DADS inhibited the growth of androgen-dependent prostate cancer cell line LNCaP by inducing apoptosis with a decrease in the activity of prostatic acid phosphatase (PAcP) and the level of prostate-specific antigen (PSA) [181]. Similarly, SAMC induced growth arrest of androgen-dependent LNCaP human prostate cancer cells and caused a decreased PSA secretion [182].

In an *in vivo* study, SAC inhibited the growth and proliferation of CWR22R tumor, a human androgen-independent prostate cancer xenograft, in nude mice, in which a decrease in the serum PSA level was observed with concurrent restoration of the protein expression level of E-cadherin and γ-catenin. The suppressing effect was associated with the downregulation of Bcl-2 and upregulation of caspase-3 [183]. Similarly, SAMC acted as an antimetastatic agent in the therapy of androgen-independent prostate cancer as revealed from an *in vivo* study in CB-17 SCID/SCID mice [184]. Administration of DATS inhibited the growth of androgen-independent

PC-3 human prostate cancer xenograft (implanted to athymic mice), which correlated with the induction of Bax and Bak apoptotic proteins *in vivo* [185].

In a clinical study, the effects of aqueous garlic extract supplementation in the patients with benign prostatic hyperplasia (BPH) and prostate cancer, the mass of the prostate in the BPH category has been found to decrease substantially. Total and free PSA values have been substantially decreased following the extract intake in the prostate cancer category. Urinary frequency declined and the median urinary flow volumes increased sharply after the trial [186]. So, garlic intake is useful in the prevention and treatment of prostate cancer, which is revealed through various mechanisms [187].

3.11. Skin cancer

Cancer of the skin is one of the most common cancers worldwide and it is classified into melanoma and non-melanoma. Over one million new instances of nonmelanoma skin cancer are diagnosed each year (excluding basal cell carcinoma), with 64,000 fatalities worldwide [39,40]. Several factors are responsible for the initiation of cancer of the skin and this includes high exposure to ultraviolet radiation, depletion of the ozone layer, inflammation, suppression of the immune system, genetic factors, dietary and lifestyle patterns, and reactive oxygen species (ROS) [188]. Hence, prevention and treatment of skin cancer with natural products can be one of the alternative options.

Reports showed that the capacity of garlic to abrogate cancer of the skin is correlated to the organosulfur molecules present in the garlic. These include SAC, allicin, ajoene, and allyl sulfides [189-192]. DATS decreased the cell viability of A375 and basal cell carcinoma (BCC) cells. But the viability of the human keratinocyte HaCaT cell line (normal cell line) did not change. Moreover, DATS inhibited the cell growth of A375 human melanoma and BCC cells via activation of multiple target pathways [193-194]. DATS induced cell cycle arrest at the G_2/M phase by triggering apoptosis associated with decreased protein expression level of

Cdc25c and Cdc2 and increased protein expression level of Wee 1, cyclin B1, and caspase-3. It increased the intracellular ROS generation, activated the mobilization of cytosolic Ca^{2+}, and decreased the mitochondrial membrane potential ($\Delta\Psi m$). The level of expression of DNA damage markers, such as phosphorylated protein, H2A histone family member X (γ-H_2AX), phospho-p53 (Ser 15), and p21, has been substantially enhanced by DATS treatment [192]. DATS also disrupted the $\Delta\Psi m$ which is associated with the decrease of the antiapoptotic protein Bcl-2, Bcl-xL, and increase of proapoptotic protein Bax, cytochrome c (cyt. c), and apoptotic protease activating factor 1 (Apaf-1) (**Figure 7**) [178]. Recent studies have shown that allyl sulfides induced G_2/M phase cell cycle arrest in several human skin cancer cells [195-196]. The overexpression of antiapoptotic Bcl-2 protein was directly related to enhanced cell survival [197].

In an *in vivo* study, both oral and topical application of garlic oil or allyl sulfides exhibited protection against skin papilloma genesis induced by various chemical carcinogens, such as 7,12-dimethyl benz(a)anthracene (DMBA), 12-*O*-tetradecanoylphorbol-13-acetate (TPA), and phorbol myristate acetate (PMA), reduced tumor incidence, and increased the survival rates of tumor-bearing mice [189-192, 198-199]. Apoptosis induction and regulation of tumor suppressor p53 were considered to be the possible modes of action of antitumor activity of allyl sulfides in DMBA-induced mouse skin tumorigenesis [200-201]. Accordingly, the p53 pathway can serve an important role in allyl sulfide-induced apoptosis, which led to cell death of skin carcinoma cells. DAS inhibited DMBA-induced skin tumors by decreasing the expression level of Ras oncoprotein, and inhibiting Ras-mediated phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway. DAS also reduced the level of p38 mitogen-activated protein kinase (MAPKs) protein expression [202]. DATS is an effective antitumor agent in suppressing TPA-induced cyclooxygenase-2 (COX-2) overexpression. DATS also blocked the DMBA-initiated and TPA-promoted COX-2 and activator protein 1 (AP-1) expression level by inhibiting the JNK or Akt signaling pathway in mouse skin [203].

The topical application of ajoene onto BCC tumors in nodular or superficial basal cell carcinoma patients reduced the tumor size through induced apoptosis and decrease in the expression level of Bcl-2 protein [204]. In the A375 and BCC cancer cell lines, the p53 signaling pathway plays an important role associated with apoptosis. The phosphorylation of p53 protein at serine 15 performs a function in response to the damage of cellular DNA and hence the incidence of apoptosis induced by p53 occurs [205-206]. Garlic restores the p53 by activating the p53 pathway in A375 and BCC cancer cells. DAS exerted its antitumorigenic effects through several mechanisms of action, such as activating carcinogen metabolism, suppressing carcinogen-induced damage to DNA, promoting the cell defense mechanism, and inducing apoptosis in carcinogen-induced skin tumors [200-202, 206-210].

Alginate-based black garlic nanoparticles showed cytotoxicity against skin cancer SK-Mel 2 (HTB – 68^{TM}) cell line, where it exhibited 54.3±8.4% inhibition, but the concentration was not reported [153].

3.12. Renal cancer

It is estimated that 431,288 new cases and 179,368 deaths globally occurred in 2020 due to renal cancer [39,40]. Hence, prevention and treatment with garlic phytoconstituents and their nano-formulations can be alternate options. Allicin exhibited cytotoxicity against human renal clear cell carcinoma RCC-9863 cells by inducing apoptosis with decreased cell viability, colony formation, and cell migration. It significantly decreased the protein expression level of hypoxia-inducible factor 1-α (HIF-1α), thereby it increased the expression of Bax and decreased the expression of vascular endothelial growth factor (VEGF) and Bcl-2 [211].

DADS exhibited inhibitory activity against N-diethylnitrosamine-induced renal carcinogenesis in male F344 rats. Treatment with DADS reduced the kidney tubular lesions as well as lowered the incidence of nephroblastomas (212).

4. Clinical trials of garlic products and constituents

Garlic intake inhibited the progression of various cancers in humans, which have been summarized in Table 3. A clinical study conducted on 17 volunteers revealed that 5 g raw, crushed garlic upregulated the expression level of aryl hydrocarbon receptor (AHR), aryl hydrocarbon receptor nuclear translocator (ARNT), hypoxia-inducible factor 1α (HIF1A), proto-oncogene c-Jun (JUN), nuclear factor of activated T cells (NFAT) activating protein with immunoreceptor tyrosine-based activation motif 1 (NFAM1), oncostatin M (OSM), and V-rel avian reticuloendotheliosis viral oncogene homolog (REL) to inhibit proliferation of cancer cells by inducing apoptosis [213]. Similar observations were reported in a clinical trial where raw garlic, along with allicin exhibited cytotoxicity against various cancer types conducted on 20 cancer patients [214]. Garlic supplementation was also found to be useful in the treatment of patients with benign breast disease in which it induced regression of breast fibromatosis [215]. In a randomized controlled trial among breast cancer survivors, health can be improved by increasing the consumption of garlic [216]. In patients with advanced colon, liver, and pancreatic cancer, aged garlic extract greatly improved the level of natural killer (NK) cells and NK cell activity to boost immune functions after given a dose of 4 capsules per day for a period of 12 weeks. Each capsules contained 500 mg of AGE, 727 mg of crystalline cellulose, and 11 mg of sucrose fatty acid ester. The doses were stopped after 12 weeks and a follow up was conducted for another 12 weeks to observe the level of NK cells and NK cell activity [217]. Aged garlic extract suppresses the development of human colorectal adenomas in order to decrease the incidence of cancer and reduce its growth and proliferation through different pathways [218-219]. In a randomized controlled trial conducted on 57 560 patients bearing colorectal adenoma, raw garlic intake of 5 pyramid servings/day for a period of 12 months followed by a follow up of 12 months revealed that it reduced risk of colorectal adenoma [220]. Long-term garlic supplementation was associated with a statistically relevant decreased risk of death due to gastric cancer [221-222]. A dose containing 200 mg aged garlic extract and 1 mg steam distilled garlic oil was given twice daily

for a period of 7 years. 2×2 factorial design was adopted with 3,365 participants to analyze the gastric cancer incidence and mortality in this randomized intervention trial. Initially the follow up time is 7.3 years and, later on, the follow up time is increased to 22.3 years [221-222]. A greater protective effect of garlic supplementation on gastric cancer (GC) prevention was correlated with not consuming alcohol [223]. Smoking was linked to an elevated risk of GC incidence and death in this randomized clinical study involving 3,365 individuals. Garlic supplementation had a higher anti-GC impact when the participants did not consume alcohol with a follow up period of 22.3 years [223]. Garlic preparations inhibited the progression of precancerous gastric lesions, and it is further revealed that the concentration of S-allyl cysteine (SAC) was substantially higher among subjects in the participating treatment groups, based on serum sample measurements [224]. In a separate study of a randomized controlled trial involving 80 patients, allicin was administered via gastroscopy to the lesion region of patients bearing gastric carcinoma. It was administered 48 h before the patient underwent surgical operations. The gastric carcinoma tissue collected from gastrectomy were subjected to laboratory analysis, which revealed the antiproliferative potential of allicin based on its ability to induce apoptosis. Additionally, it triggered cell cycle arrest at G0/G1 phase by increasing the expression level of Bax, Fas and decreasing the expression of Bcl-2 [225].

Garlic phytoconstituents, such as allin and allicin, exerted protective effects in patients against post-chemotherapy complications, such as febrile neutropenia. This was commonly observed in patients with hematological malignancies receiving chemotherapy. Two doses of 450 mg (containing allin 9.9 mg and allicin 4.5 mg) per day were administered to patients enrolled in a randomized controlled trial on 101 patients. The patients were analyzed by continuous evaluation of complete blood counts daily till the resolution of neutropenia [226]. In a clinical study, aqueous garlic extract exhibited protective effects against prostate cancer by improving the urinary frequency and urinary flow. The trial was conducted on 27 patients with a dose of 1 ml/kg body weight per day for 1 month, although the follow up period was not reported [186]. Ajoene,

the active constituent of garlic demonstrated antitumor activity against skin cancer, which was conducted on 21 patients. Topical application of 0.4% ajoene cream (400 mg ajoene, 0.3 mL polysorbate 80, and 0.3 mL sorbitonoleate in 100 ml 1% carbomeric gel) reduced the tumor size which is evident by induction of apoptosis and decreased expression of Bcl-2 [204].

5. Adverse effects of garlic constituents

Garlic exerts various beneficial pharmacological activities, but considerations are needed to be paid towards its possible adverse effects. Safety and efficacy documentation is important in the identification of medications and nutritional supplements used for therapeutic purposes. In general, commercial garlic preparations are classified into four main types, such as fresh garlic, garlic oil, garlic powder, and aged garlic extract. Among the different components of garlic, allicin is known to exhibit toxic side effects. The intestinal epithelium was compromised using enteric coated garlic preparation, and the intestinal microflora was affected whenever allicin was administered directly into the intestines in rats [227]. Higher amounts of garlic extract in mice have proved to be clastogenic or mutagenic agent. Feeding of rats with raw garlic for extended period of time led to anemia and excessive weight loss due to red blood cell lysis [228]. The surviving rats displayed liver swelling, spleen and adrenal gland hypertrophy and a decline of the number of erythrocytes with different morphological modifications. The treatment with aqueous garlic extracts manifested liver damage as evidenced from elevated aspartate aminotransferase enzyme levels [229]. The adverse effects, metabolism, synergistic drug activity, interference with enzymes, and impact on natural microflora should be taken into consideration while utilizing garlic and its components for the treatment of cancer.

6. Conclusions

Garlic has been utilized for its nutritional and medicinal properties. Garlic is a plant used worldwide as an herb for the prevention and management of several disease conditions, including cancer. Among the different phytoconstituents of garlic, several compounds which exhibited

significant antineoplastic activities against various cancer models are allicin, SAC, SAMC, DAS, DADS, and DATS. Bioactive compounds found in garlic displayed significant anticarcinogenic actions via several mechanisms, including apoptosis of cancer cells, arrest of cell cycle, and activation of angiogenic cascade. Garlic nanoparticles exhibited better bio-efficacy than garlic alone in the treatment of cancer. Garlic, its active metabolites and their nano-formulations induce a change in expression level of various commonly known genes to regulate numerous well-known signaling networks, such as JNK signaling pathway, EMT-related signals, PI3K/Akt and p38 MAPK signaling pathways, LRP6/Wnt pathway, p53-mediated autophagy, hyperacetylation of histone H3 K14, ERK signaling pathway, NF-κB signaling pathway, Wnt/*β*-catenin signaling pathway, STAT3 signaling pathway, ERK1/2 signaling pathway, Chk1/CDC25C/cyclin B1 signaling pathway, Nrf2 signaling pathway, and MT2A/NF-κB signaling pathway. By modulating these pathways, bioactive phytochemicals from garlic and their nano-formulations confer inhibition of cell proliferation, apoptosis evasion, adhesion, invasion, migration, and metastasis of skin, prostate, ovarian, breast, gastric, ovarian, colorectal, oral, liver, and pancreatic cancer cells in various preclinical models (**Figure 8**). While various researchers have suggested many signaling pathways for the anticancer activity of garlic phytochemicals, more studies need to be performed to thoroughly understand the molecular targets of these bioactive phytochemicals and their metabolites within different organ systems.

The majority of known reports of the antineoplastic effects of the constituents of garlic and their nano-formulations are based on *in vitro* cancer models with only minimal *in vivo* research. More *in vivo* experiments should be performed since most of the available studies are based on *in vitro* analysis. With encouraging preclinical mechanistic results on anticancer effects of garlic phytochemicals, the need for further clinical research is increased by the lack of well-designed, prospective clinical studies and in-depth safety evaluation. The garlic nano-formulations highlighted some effective delivery systems with improved bioavailability and efficient targeting. Additional *in vivo* studies are also required to determine if garlic nano-formulations exert any

toxicity or not. In addition to conducting well-controlled clinical studies, more research on novel molecular targets and signaling mechanisms of garlic extract, its constituents and their nano-formulations will improve its therapeutic potential in the management of many forms of cancer. In the last several decades, despite the success of drug discovery initiatives, future efforts confront numerous obstacles. In order to maintain pace with other efforts in drug discovery, the quality and quantity of phytochemicals which come into drug development will have to be constantly improved by natural product scientists and the pharmaceutical sector. The drug discovery process is predicted to take 10 years on average and cost billions of dollars. A great deal of effort and resources are invested on numerous leads which were later rejected in the process of drug discovery. Only one in 5,000 lead substances is anticipated effectively to be used and authorized by clinical studies. The lead optimization process, involving pharmacology, toxicology, pharmacokinetics and drug delivery as well as clinical studies are all time consuming. In conclusion, our in-depth review of existing studies has shown the promise of garlic-based phytoconstituents and their nano-formulations as valuable nutraceuticals and pharmaceutical products for prevention and treatment of cancer.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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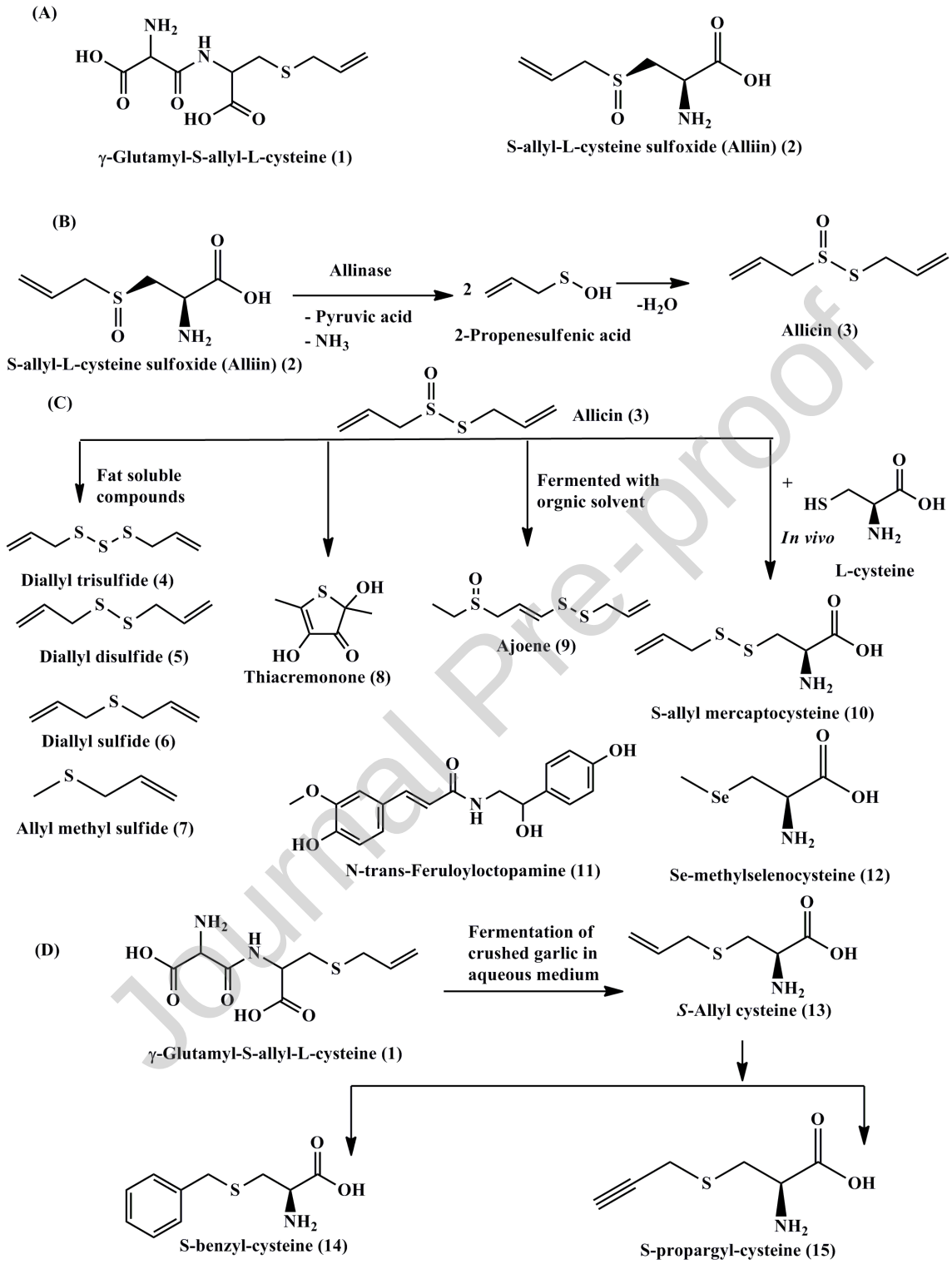

Figure 1. Anticancer phytoconstituents of garlic. **(A)** Non-volatile sulfur-containing compounds present in intact garlic. **(B)** Conversion of allicin from alliin. **(C)** Organosulfur analogs of allicin in the process of garlic preparation. **(D)** Water-soluble analogs of γ-glutamyl-L-cysteine peptides.

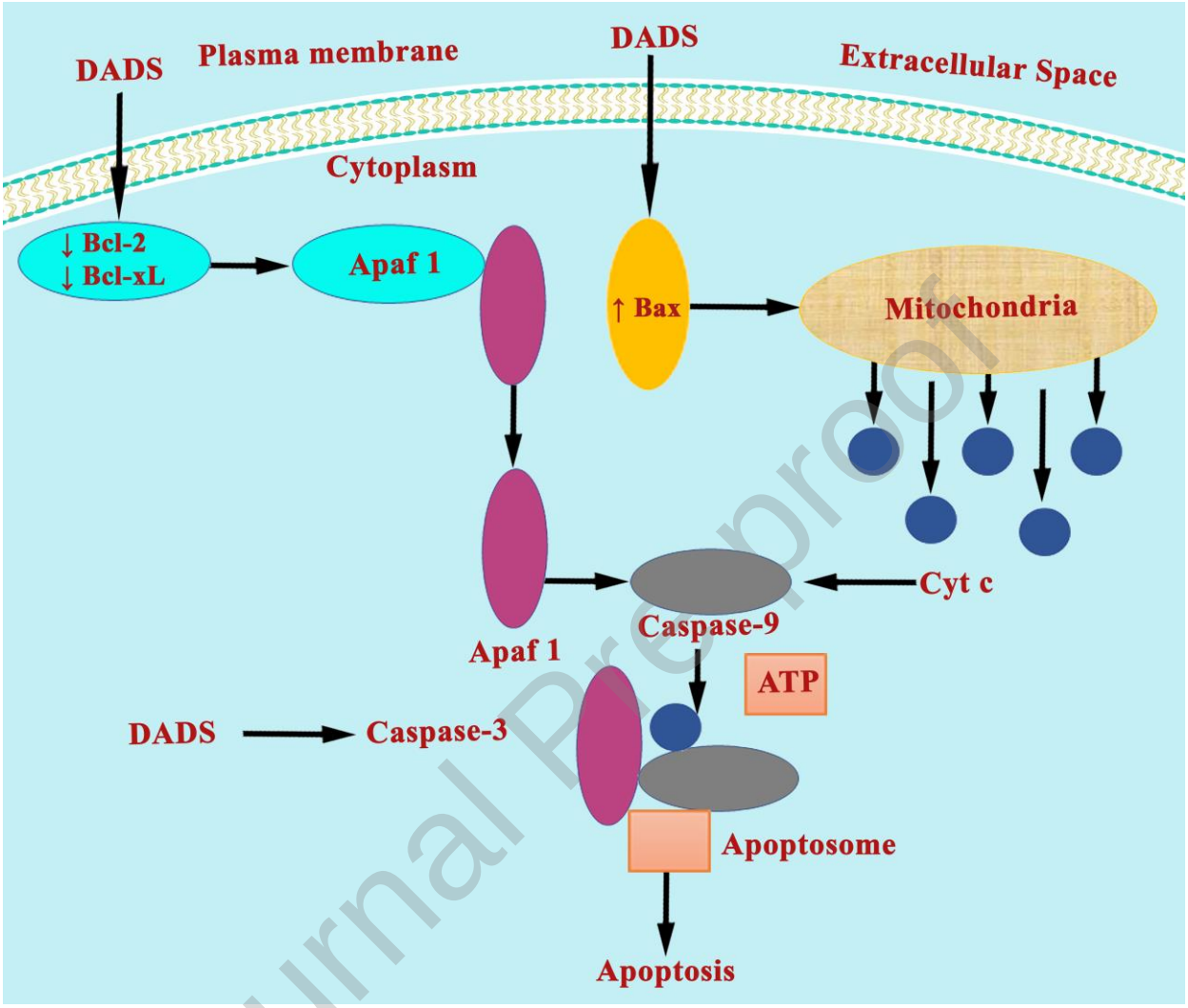

Figure 2. Proposed pathways for DADS-induced cell cycle arrest in breast cancer. Color reproduction only for online version.

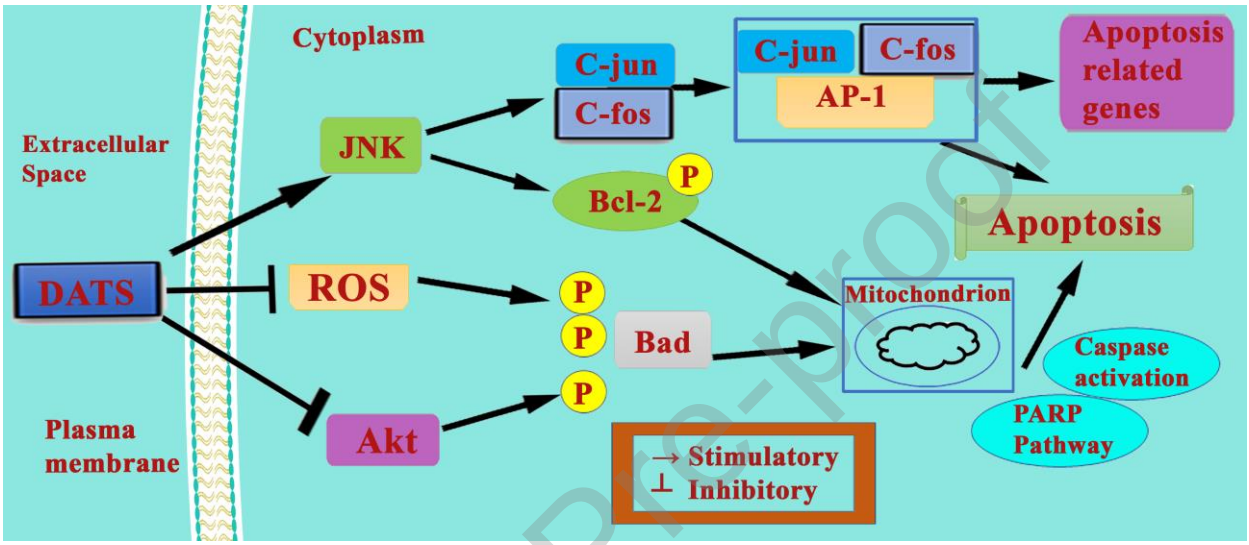

Figure 3. Proposed pathways for DATS-induced anticancer effects against gastric cancer cells. cancer. Color reproduction only for online version.

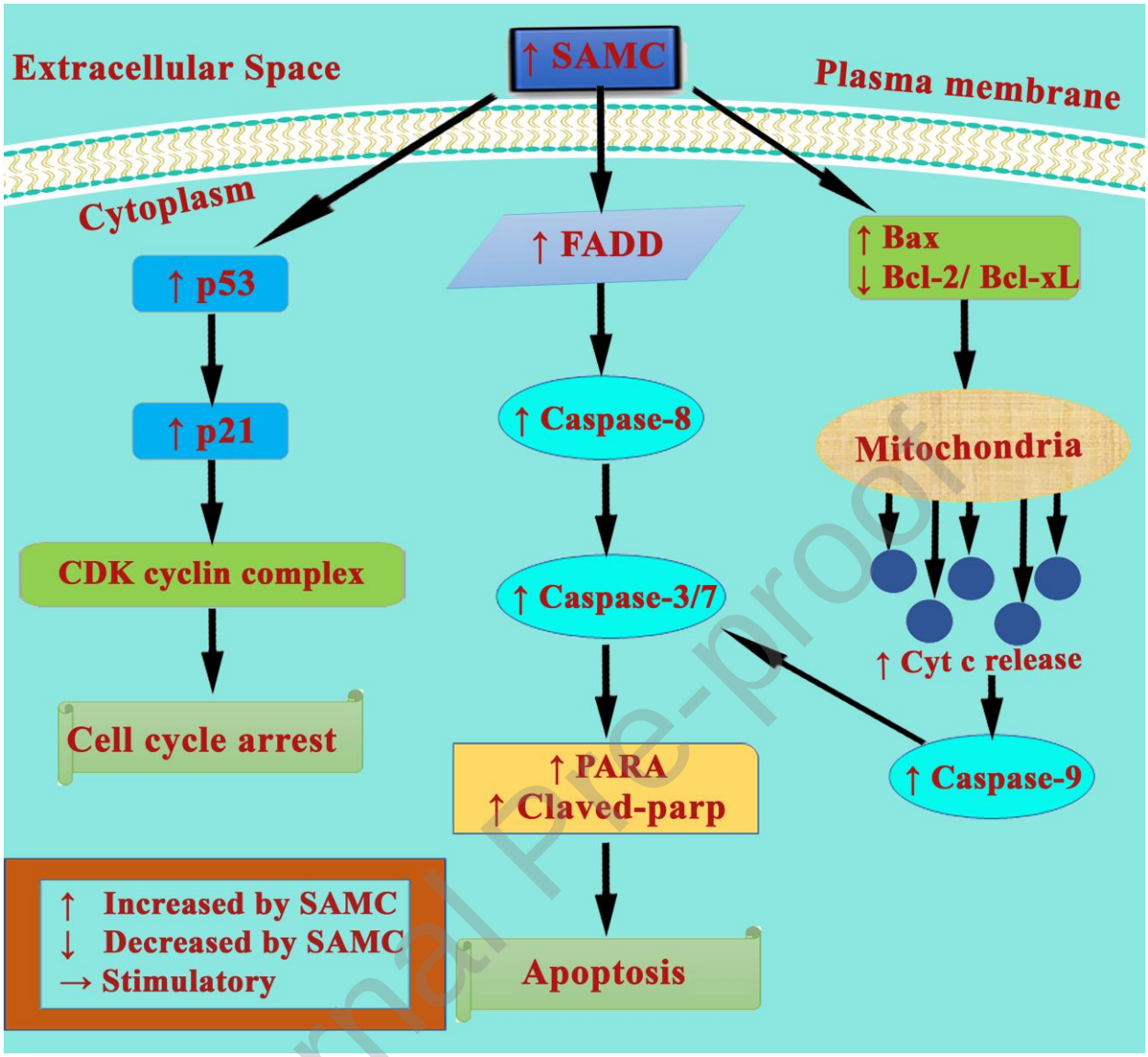

Figure 4. Proposed pathways for SAMC-induced cell cycle arrest and activation of apoptosis in gastric cancer cells. cancer. Color reproduction only for online version.

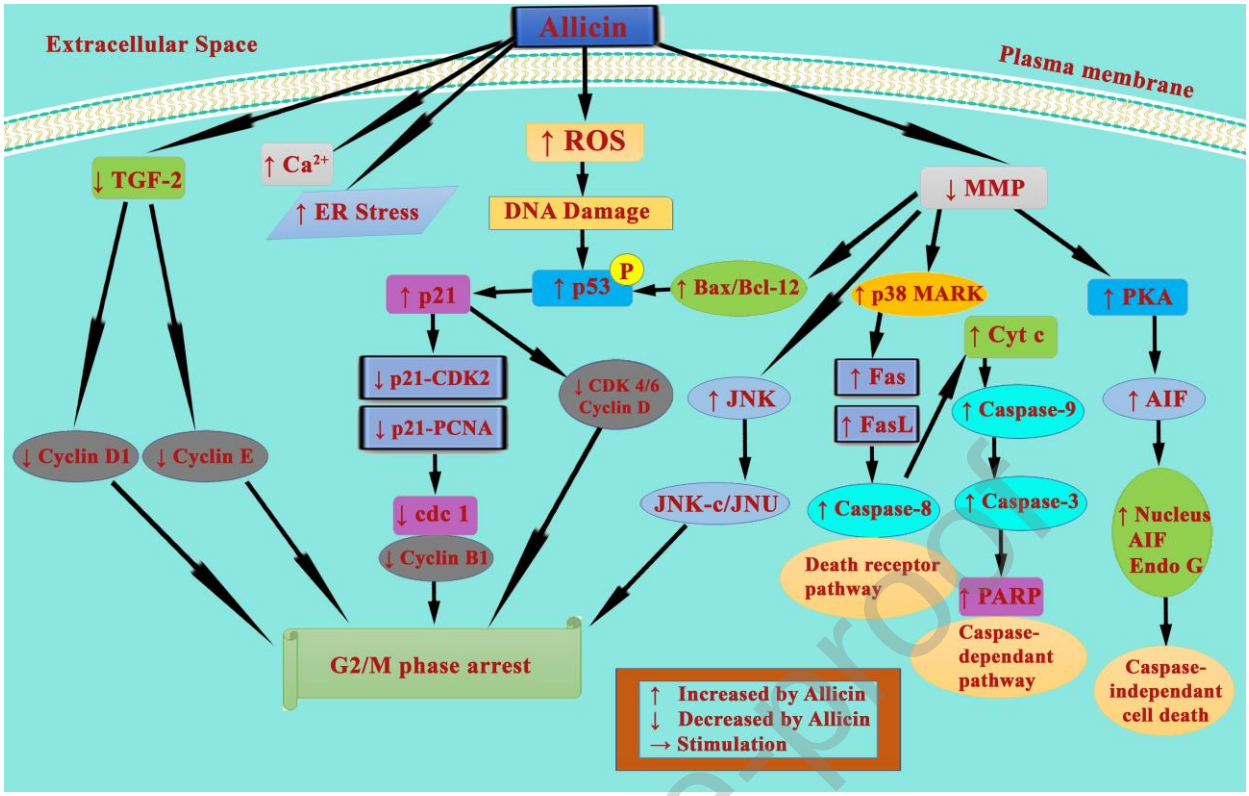

Figure 5. Proposed pathways for allicin-induced cell cycle arrest in ovarian cancer. cancer. Color reproduction only for online version.

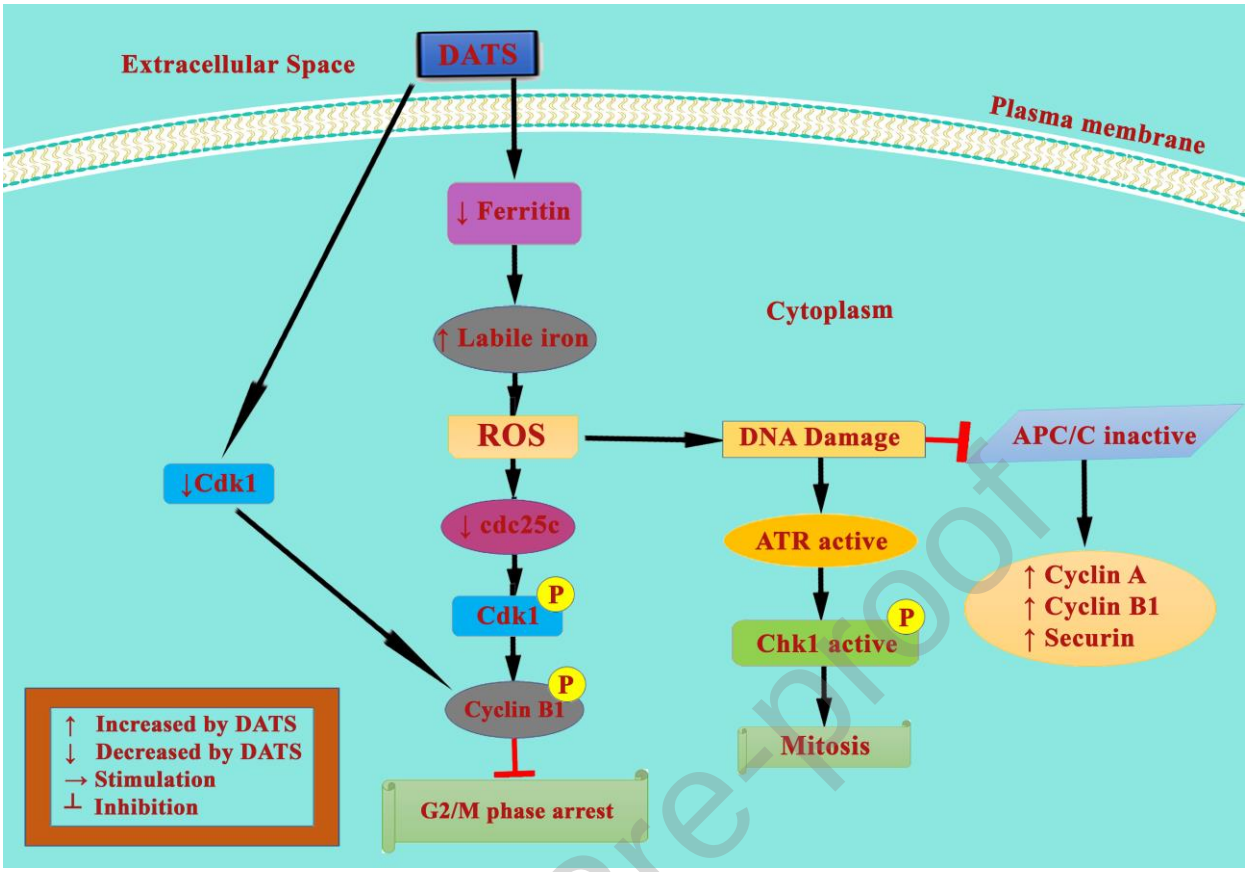

Figure 6. Proposed pathways for DATS-induced anticancer effects against prostate cancer cells. cancer. Color reproduction only for online version.

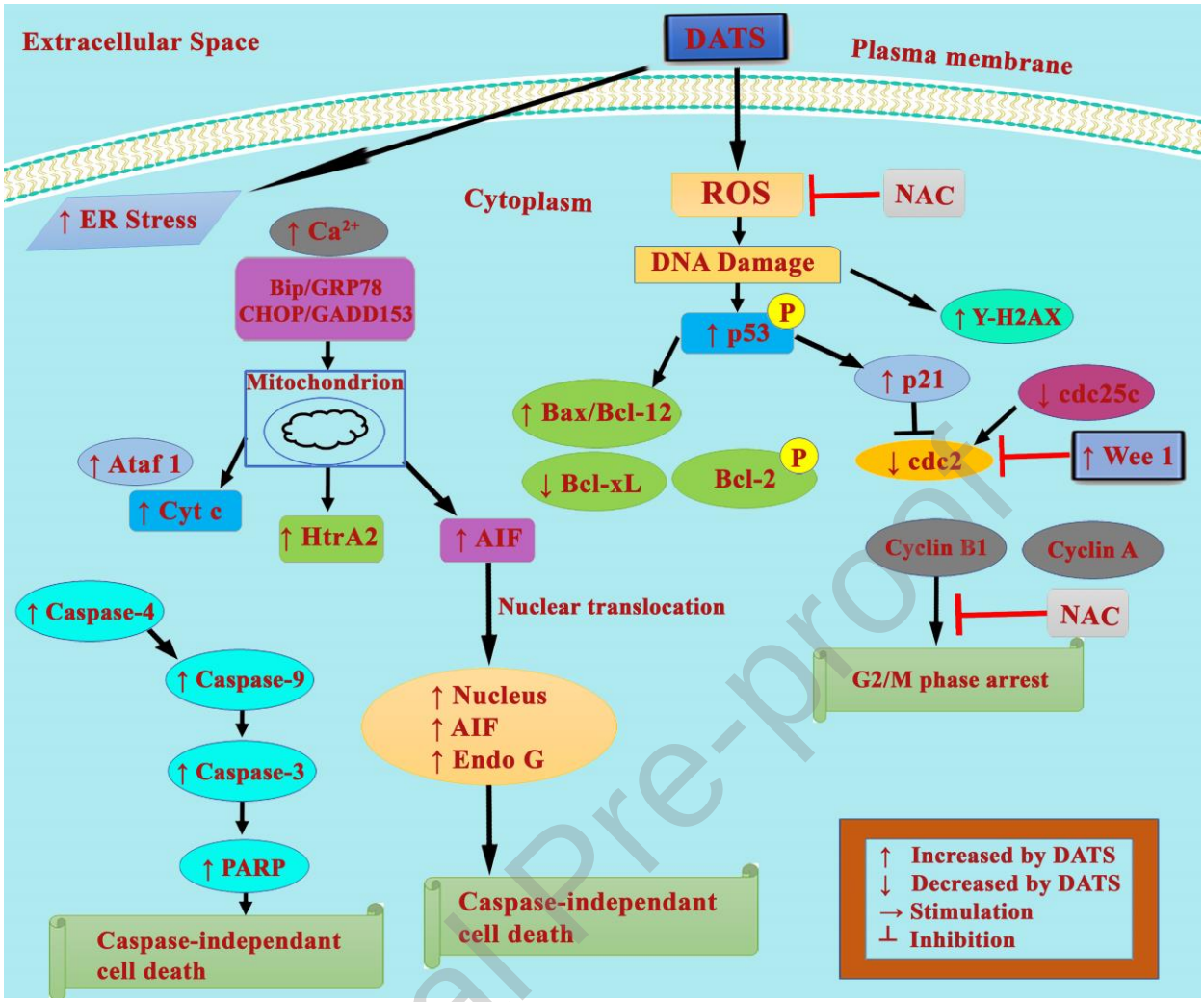

Figure 7. Proposed pathways for DATS-induced cell cycle arrest and activation of apoptosis in skin cancer cells. cancer. Color reproduction only for online version.

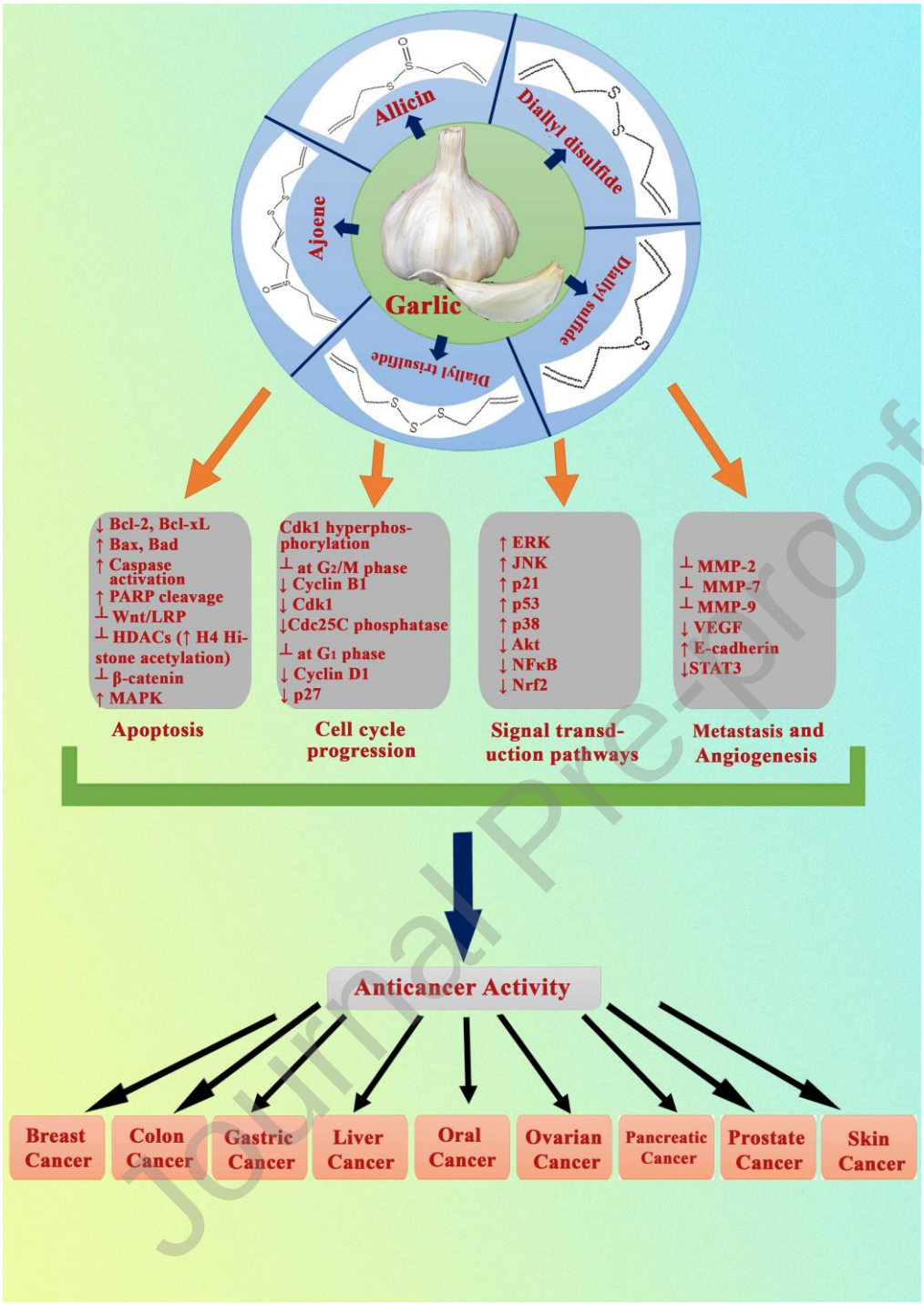

Figure 8. Summary of anticancer effects and associated molecular targets and cellular pathways of garlic phytochemicals. cancer. Color reproduction only for online version.

Table 1.

Potential *in vitro* anticancer effects and mechanisms of action of various secondary metabolites and nano-formulations of garlic.

Cancer types	Material tested	Cell lines used	Mechanism of action	Concentrations	References
Breast cancer	Allicin	MCF-7	⊥Proliferation; ⊥cell cycle at G_0/G_1 and G_2/M phase	10-25 µM	[42]
		MCF-7 and HCC-70	⊥Proliferation; ⊥clonogenicity; ↓cell viability; ↑apoptosis; ↑NOXA; ↑p21; ↑Bak; ↓Bcl-xL; ↑caspase-3; ↑caspase-8; ↑caspase-9	45 µM and 20 µM	[43]
	Alliin	MCF-7 and HCC-70	Senescence	10 µM	[43]
	DADS	CMT-I3	⊥Tumor growth	10.9 µM	[44]
		MCF-7	⊥ Proliferation; ↑apoptosis; ⊥ERK; ↑SAPK/JNK; ↑p38	200 µmol/dL	[45]
		MCF-7	⊥Cell cycle at sub-G_0 stage; ↑caspase-3; ↑apoptosis; ⊥ERK; ↑SAPK/JNK; ↑p38 pathway	200 µmol/dL	[48]
		MCF-7	⊥ Proliferation; ↑apoptosis; ↑Bax; ↑Bad; ↑caspase-3; ↑caspase-9; ↓Bcl-2	1.562-100 µM	[49]
		MCF-7 and MDA- MB- 231	⊥ Proliferation; ↓uPA; ↓MMP-9; ↑TTP	100-400 µM; 200 µmol/dL	[69]
	DADS solid lipid nanoparticles	MCF-7	⊥ Proliferation; ↑apoptosis; ↑Bax; ↑Bad; ↑caspase-3; ↑caspase-9; ↓Bcl-2	1.562-100 µM	[49]
	DATS	MCF-7	↑Apoptosis; ⊥cell cy-cle at G_2/M stage; ↓Akt	100 µM	[53]
		MCF-7 and SUM159	⊥proliferation; ↑apoptosis; ↓CSCs markers (CD44, ALDH1A1, Nanog and Oct4); ↓Wnt/β-catenin pathway		[54]
			⊥ proliferation; ↓ FoxQ1	2.5 and 5 µM	[55]
		MDA-MB-231, MCF-7 and MCF10A-H	⊥proliferation; ⊥ADAM10; ⊥ADAM17; ⊥Notch ligands Jagged-1 and Jagged-2;	30 and 40 µmol/L	[56]
		MCF-7 and T47D	⊥cell viability; ↑apoptosis; ⊥ER-α; ↓ER-α mRNA expres-sion; ⊥ERE2e1b-	20 µM	[58]

			luciferase;		
		MDA-MB-231 and HS 578T	⊥MMP2/9; ⊥NF-κB and ERK/MAPK signaling pathways	10 μM	[59]
	SAMC	MCF-7and MDA-MB-231	⊥Cell cycle at G_0/G_1 phase; ↑p53; ↑p21; ↑apoptosis; ↑Bax; ↓Bcl-2; ↓Bcl-xL; ↑caspase-9; ↑caspase-3	148 μM; 207 μM	[60-62]
	SAC	MDA-MB-231	↑E-cadherin; ↓MMP-2	1 mM	[63]
	G-AgNPs	MCF-7	⊥Proliferation; ↓cell viability; ↑cell clumping; ↑membrane instability	100 μg/mL	[74]
	Garlic extract mediated silver nanoparticles (Ag-S2)	MCF-7	⊥Proliferation; ↓cell viability	19.94 μg/mL	[75]
	Garlic extract-based size controlled superparamagnetic hematite nanoparticles	MCF-7	⊥Proliferation	346.25 mg/mL	[77]
	ZnO-reduced graphene oxide nanocomposites (ZnO-RGO NCs) using garlic clove extract	MCF-7	⊥Proliferation		[78]
Cervical cancer	Allicin	SiHa	⊥Proliferation; ↓cell viability; ↑apoptosis; ↓ Nrf2 and ↓heme oxygenase 1; inactivated PI3K/Akt pathway	5, 20 and 50 nM	[79]
	Green synthesized silver nanoparticle from aqueous extract of elephant garlic	HeLa	⊥Proliferation	< 25 mg/mL	[80]
	Size controlled superparamagnetic hematite nanoparticles of garlic extract	HeLa	⊥Proliferation	285 mg/mL	[77]
	Garlic extract mediated silver nanoparticles (Ag-S2)	HeLa and Hep-2	⊥Proliferation	16.75 and 27.63 mg/mL	[75]
Colon cancer	Allicin	HT-29	⊥Proliferation; ⊥cell cycle at G_0/G_1 and G_2/M phase (MCF-7 cells)	10-25 μM	[42]
		HCT-116, LS174T, HT-29 and Caco-2	⊥Proliferation; ↑Bax; ↓Bcl-2; ↑apoptosis; ↑translocation of Nrf2	6.2 to 310 μM	[84]

		HCT116	↑Radiosensitivity of HCT116 cells; ⊥proliferation; ↑apoptosis	10 μg/mL	[85]
	Allicin+ 5-FU	DLD-1	⊥Proliferation; ↓total caspase-3	1.625-100 μM	[86]
	Se-methyl-L-selenocysteine (MSeC)	Colo 205	↑Apoptosis; ↑Fas; ↑FasL; ↑Bax; ↑cleavage of caspase-3 & caspase-8, ↑DFF45; ↑PARP; ↓Bid; ↓Bcl-2; ↓ERK1/2; ↓PI3K/Akt; ↓p38; ↓JNK	200 μM	[87]
	Thiacremonone	SW620 and HCT116	⊥ NF-κB; regulates TNF-α and DNA binding activity; ↓Bcl-2, ↓cIAP1/2; ↓XIAP; ↓iNOS; ↓COX-2; ↑Bax, ↑cleaved caspase-3; ↑cleaved PARP	100 μg/mL	[88-89]
	Ajoene	SW480	⊥ β-catenin; ↓c-Myc; ↓cyclin D1; ↑phosphorylation of β-catenin	10 μM	[90]
	SAMC	SW-480 and HT-29	↑Apoptosis; ⊥ proliferation; ⊥Cell cycle at G_2/M phase; ↑caspase-3 activity; activates the jun kinase activity	50-250 μm	[91]
	DAS	Colo 205, colo 320 DM and colo 320 HSR	↓NAT mRNA	50 μM	[92]
		Colo 320 DM	⊥ cell cycle at G2/M phase; ↑apoptosis; ↑NF-κB; ↑caspase-3; ↓ERK-2; ↑ROS		[93]
	DADS	SW480	⊥ cell cycle at G_2/M phase; ↑apoptosis; ↑Ca2+ mobilization	232 μM	[94]
			↓NF-κB; ⊥ GSK-3β	20 μM	[111]
		HT-29 Glc(-/+)	⊥Cell cycle at G_2/M phase; ↑apoptosis; ↑L-glutamine oxidation	100 μM	[95]
		Colo 205	↑STAT1; ↑apoptosis	50 μM	[96]
		HCT-116	⊥ cell cycle at G_2/M phase; ↑ cyclin B1 expression	50-400 μM	[97]
			↑Apoptosis; ⊥cell cycle at G_2/M phase; ↑p53, ↑ERK phosphorylation	200 μM	[98]
		HCT-15		25-100 μmol/L	[99]
		Caco-2 and HT-29	⊥Proliferation; ⊥cell cycle at G_2phase; ↑p21(waf1/cip1) expression; ⊥HDAC; H4	200 μM	[100]

			histone hyperacetylation		
			⊥Proliferation; hyperacetylation of histone H3 K14		[101]
		Colo 205	⊥Proliferation; ↑MRP-3; ↑MRP; ↑MRP6	25 μM	[104]
	DATS	HCT-15 and DLD-1	⊥Cell cycle at G_2/M phase; ↑apoptosis; ↑caspase-3	11.5 μM and 13.3 μM	[102]
		Colo 205	↑Apoptosis; ↓PI3K; ↓Ras; ↓MEKK3; ↓MKK7; ↓ERK1/2; ↓JNK1/2; ↓p38; ⊥MMP-2, MMP-7, and MMP-9; ⊥NF-κB; ⊥COX-2;	25 μM	[105]
		HCT-15 and DLD-1	↑Apoptosis; ↑caspase-3; cell cycle arrest at G2/M phase; ↑disruption of microtubule network formation; ↑oxidative alteration of cysteine residues Cys12β and Cys354β; ↑formation of S-allylmercaptocysteines	20 μM	[103]
	Size controlled superparamagnetic hematite nanoparticles prepared from aqueous garlic clove extract	HCT-116	⊥ Proliferation	230 mg/mL	[77]
	ZnO-reduced graphene oxide nanocomposites (ZnO-RGO NCs) using garlic clove extract	HCT-116	⊥ Proliferation	Not specified	[78]
Gastric cancer	Allicin	Gastric cancer cells (cell line not specified)	⊥Cell cycle at G_2/M phase; ⊥proliferation; ↑apoptosis; ↑ROS; ↓MMP;	Not specified	[115]
		HGC27 and AGS	⊥Proliferation; ↑apoptosis; ↑ miR-383-5p; ↓ERBB4, ↓p-PI3K; ↓p-Akt; ↑Bax; ↓Bcl-2	10 μg/mL	[116]
		SGC-7901	⊥ Proliferation; ↓cell viability; ↑apoptosis; ↑caspase-3; ↑caspase-8; ↑caspase-9; ↑Bax; ↑Fas	15-120 μg/mL	[117]
		SGC-7901	⊥Proliferation; ↑apoptosis; ⊥cell cycle	0.016-0.1 mg/mL	[118]

			at G_2/M phase; ⊥telomerase activity		
		MGC-803	↑Apoptosis; ↑p38; ↑caspase-3	0.1-10 μg/mL	[119]
	DATS	SGC-7901	⊥Proliferation; ⊥cell cycle at G_2/M stage; ↑apoptosis; ↑cyclin A2; ↑cyclin B1; ↓Bcl-2	50-200 μM	[121]
		MGC803 and SGC7901	⊥Cell cycle at M phase; ⊥proliferation; ↑p21(WAF1) genes	6.4 μg/mL and 7.3 μg/mL	[122]
		BGC823, SGC7901, AGS, and MT2A-BGC823	⊥Cell cycle at G_2/M stage; ↑apoptosis; ↑MT2A; ↑IκB-α; ↓NF-κB activation; ⊥Srx	25-100 μM	[120, 123]
		BGC823	↑Apoptosis; regulation of GST-pi, VDAC-1, annexin I, galectin and S100A11	50 mM	[124]
		MGC803	↑Apoptosis; ↑caspase-3	12 mg/L	[125]
		AGS	⊥Cell cycle at G2/M phase; ↑apoptosis; ⊥proliferation; ⊥AMPK, ↑cyclin B1; ↑p21(WAF1/CIP1)	50 μM	[126]
			↑Nrf2	20 μM	[127]
	SAMC	SGC 7901	↑Apoptosis; ⊥proliferation; regulation of the JNK and P38 pathway; ↑p53; ↑Bax; ↑caspase-9; ↑caspase-3; ↑cyt c	300 μM	[128]
		SNU-1		100-400 μM	[129]
		MGC803	⊥Cell cycle at G2/M phase; ↓CDC25C and ↓cyclin B1	30 mg/L	[130]
			⊥ ERK1/2 signaling pathway; ↓phosphorylation of ERK1/2		[131]
	DADS	AGS	⊥ Proliferation; ↑apoptosis; ↑ROS	400 μM	[132]
		OE19)	⊥Cell viability; ↓MMPs; ↓u-PA	10 μg/mL	[133]
		AGS cell	⊥Proliferation; ↑TIMP-1; ↑TIMP-2; ↓claudin-2; ↓claudin-3;↓claudin-4	Not specified	[134]
	SBC	SGC-7901	⊥Cell cycle at G2-phase; loss of $\Delta\psi m$; ↑caspase-9 and ↑caspase-3; ↑apoptosis; ↑p53, ↑Bax; ↓Bcl-2	5-15 mM	[135]
Liver cancer	Allicin	Hep 3B ($p53^{mutation}$)	p53-mediated autophagy; ↑apoptosis; ↓MMP; ↑Bax; ↓Bcl-2; ↑ROS	IC_{50}: 35 μM	[141]

	Allicin + 5-FU	SK-Hep-1 and BEL-7402	Allicin synergistically sensitized hepatocellular cancer cells to 5-fluorouracil; ↓proliferation;	IC_{50}: 10.389 µg/ml and 10.004 µg/ml	[142]
	Allicin loaded polypyrrole (PPY) nanoparticles	HepG2	⊥ Cell proliferation; ↓cell viability	100/500 µg/mL	[154]
	DATS	J5	⊥ Cell cycle at G_2/M phase; ↓Cdk7; ↑cyclin B1	100 µM	[144]
		HepG2	⊥ Cell proliferation; ↑caspase-3; ↑H_2O_2; ↓thiol level		[145]
	SAMC	HepG2	↑Apoptosis; ↑TGF-β1; ↑TβRII; ↑p-smad2/3; ↑smad4 and ↑smad7 signals; ↑Bam; ↓Bcl-2; ↑caspase-3; ↑caspase-9	800 µmol/L	[146]
	SAC	MHCC97L	↑Apoptosis ↑E-cadherin; ↓VEGF; ↓Bcl-xL; ↓Bcl-2; ↓Ki-67; ↓PNCA; ↓cdc25c; ↓cdc2; ↓cyclin B1; ⊥cell cycle at S phase; ↑caspase-3; ↑caspase-9	IC_{50}: 33 mM	[147]
	FO	Huh7 and HCCLM3	↓ Akt and p38 MAPK; ⊥Slug; ↑E-cadherin	1.99 mM	[148]
	Ajoene	HepG2	↑ Nrf2; ↓interaction of Nrf2 with ECH-associated protein-1; ↑ GCL and GSH; ↓Nrf2ubiquitination; ↑Pd.D.	30 mmol/L	[149]
	Silver nanoparticles synthesized using *Allium sativum* extract	HepG2	⊥Proliferation; ↑apoptosis; ↑DNA fragmentation; cell cycle arrest at G2/M phase; ↑Bax, ↑caspase-3; ↑caspase-9; ↑cleavage of PARP; ↓Bcl-2; ↓cyclin B; ↓cdc2; ↓cdc25c; ↑p21; ↑p53	LC_{50}: 31.25 ng/mL	[152]
	Alginate based black garlic nanoparticles	Hep G2 (HB–8065™)	⊥Proliferation		[153]
	Silver nanoparticles of garlic aqueous extract	Hep2	⊥Proliferation	27.63±0.88 mg/mL	[75]
Lung cancer	Allicin	A549 and NCI-H460	↓Cell viability; ↓proliferation; ↓migration; ↑apoptosis; ↑autophagy; ↑ROS; ↑DNA damage; cell	10 and 40 µg/mL	[155]

			cycle arrest at S/G2-M phase; modulation of ROS/MAPK and ROS/JNK signaling pathway		
	Garlic extracts mediated silver nanoparticles (Ag-S2)	A549	⊥Proliferation; ↑apoptosis	13.26 mg/mL	[75]
	Alginate-based black garlic nanoparticles	LU-1 (HTB-57^{TM})	⊥Proliferation	1 mg of black garlic extract/mL alginate solution	[153]
	Allicin	CD133+	↓Cell viability; ⊥TNF-α; ⊥IL-8; ⊥endothelin	100 ng/mL	[158]
	SAC	CAL-27	⊥Proliferation; ⊥ MAPK/ERK signaling pathway; ↓SLUG repressor protein; ↑E-cadherin	20 mM	[159]
	Alginate based black garlic nanoparticles	KB (CCL-17^{TM})	⊥Proliferation		[153]
	Garlic extract modified titanium dioxide nanoparticles	KB	⊥Proliferation; ↓cell viability; ↑ROS	10 mg/mL	[161]
Ovarian cancer	SAC	A2780	⊥Proliferation; ⊥cell cycle G_1/S phase; ↓pro-caspase-3L ↓Parp-1; ↓Bcl-2; ↑caspase-3 ; ↑Bax; ↓Wnt5a; ↓p-Akt; ↓c-Jun	25 mmol/L	[162]
			⊥Cell cycle at G_1/S phase; ↓5-methylcytosine; ↓DNMT activity; ↓DNMT1 protein levels; ↓cdc2	16.25 mmol/L	[163]
	Allicin	SKOV3	⊥Proliferation; ↑apoptosis; ↑JNK phosphorylation; ↑Bax and ↑cyt. c	25 µg/mL	[164]
	Silver nanoparticles synthesized using *Allium sativum* extract	SKOV3	⊥Proliferation; ↑apoptosis; ↑DNA fragmentation; cell cycle arrest at G2/M phase; ↑Bax; ↑caspase-3; ↑caspase-9; ↑cleavage of PARP; ↓Bcl-2; ↓cyclin B; ↓cdc2; ↓cdc25c; ↑p21; ↑p53	LC_{50}: 31.25 ng/mL	[152]
Pancreatic cancer	Allicin	MIA PaCa-2	↓Cell viability; ↑apoptosis; ↑caspase-3	89.43 µM	[167]

			and ↑$p21^{Waf1/Cip1}$; cell cycle arrest at G1 phase; ↑ROS; ↓ GSH		
	DATS	Capan-2	⊥Cell cycle at G2/M phase; ↑apoptosis; ↑Fas; ↑p21; ↑p53; ↑Bax; ↑cyclin B1; ↓Akt; ↓cyclin D1; ↓MDM2; ↓Bcl-2	100 μmol/L	[168]
		Benzo(a)pyrene-induced precancerous MCF-10A cells	⊥Proliferation; ⊥Cell cycle at G_2/M phase	60 μM	[52]
	SPRC	PDA	⊥Proliferation; ↑apoptosis; ⊥cell cycle at G_2/M phase	Not specified	[169]
	Silver nanoparticle of elephant garlic aqueous extract	βTC-3	⊥Proliferation	< 25 mg/mL	[80]
Prostate cancer	DATS	DU145	⊥Cell cycle at G2/M phase; ↓Cdc25C protein; ⊥kinase activity of cdk1/cyclin B1 complex	20 and 40 μM	[172]
		PC-3 and DU145	⊥Cell cycle at G2/M phase; ↑Tyr^{15} phosphorylation of Cdk1; ⊥Cdk1/cyclinB1 kinase activity; ↓Cdc25C protein; ↑Ser216 phosphorylation	40 and 80 μM	[173]
		PC-3	↑Apoptosis; ↓cyclin D1; ↓NF-κB; ↓Bcl-2; ↑Bad; ↑Bax; ↓Akt	10-50 μM	[175]
		PC-3 and DU145	↓Ser^{473} and Thr^{308}; ⊥Akt; ↑apoptosis; ↓Ser^{155} and Ser^{136}; ↓phosphorylation of BAD	40 μM	[179]
		PC-3 and DU145	⊥Cell cycle at G2/M phase; ⊥ cyclin-dependent kinase 1 activity; ⊥hyperphosphorylation of Cdc25C at Ser^{216}		[180]
	DADS	PC-3	Antiproliferative activity; ⊥cell cycle arrest at G2/M; ↓CDK1 expression	25 and 40μM	[176]
		LNCaP	↓Proliferation; ↑Bax; ↓Bcl-xL; ↑caspase-3	25-100 mM	[181]
	SAC	PCa	↓Proliferation; ↓invasion ability; ↑E-	IC_{50}: 2.16 mM	[177]

			cadherin mRNA expression; ↓Snail expression		
	SAMC	PCa	⊥Proliferation; ↓invasion ability; ↑E-cadherin mRNA expression; ↓Snail expression	IC_{50}: 86.34 µM	
		LNCaP		500 µM	[182]
Skin cancer	Ajoene	TE354T	↓Apoptosis-suppressing protein; ↑mitochondria-dependent route of apoptosis; ↓tumor size	Not specified	[204]
	DATS	A375 and BCC	⊥Cell cycle at G_2/M stage; ⊥proliferation; ↑p53	25 µM	[193]
		BCC	↑Apoptosis; ↑phospho-p53; ↑Bax; ↓Bcl-2; ↓Bcl-xL; ↑Bip/GRP78and CHOP/GADD153; ↑caspase-4	25-100 µM	[194]
	Alginate based black garlic nanoparticles	SK-Mel 2 (HTB – 68™)	⊥Proliferation	1 mg of black garlic extract/mL alginate solution	[153]
Renal cancer	Allicin	RCC-9863	↓Cell viability; ↓colony formation; ↓cell migration; ↑apoptosis; ↓ HIF-1α, ↑Bax; ↓VEGF; ↓Bcl-2	0.016, 0.05, and 0.1 mg/mL	[211]

Various symbols (↑, ↓ and ⊥) indicate increase, decrease and inhibition in the obtained variables, respectively.

Table 2.
Potential *in vivo* anticancer effects and mechanisms of action of various secondary metabolites of garlic.

Cancer types	Material tested	Animal models	Effects and mechanism of action	Dose	References
Breast cancer	Garlic powder, SAC, and DADS	MNU-induced breast cancer in female Sprague-Dawley rat	⊥Tumor growth	57 μmol/kg	[66]
	DADS	orthotopic (right thoracic mammary fat pad) transplantation of KPL-1 cells in female nude mice	⊥Tumor growth; ↓tumor weight	1 or 2 mg/kg	[45]
	DADS	2- amino- l- methyl- 6- phenyIimidazo[4,5- b] pyridine (PhlP)- induced mammary carcinogenesis in SD rats	↓Tumor incidence	200 ppm	[68]
	DADS	Xenograft tumor models established by injecting MCF-7 and MDA-MB-231 breast cancer cells into nude mice	⊥Proliferation, ⊥invasion and ⊥migration; ↓ uPA; ↓MMP- 9; ↑TPP	50 mg/kg	[69]
	DATS	breast cancer xenografts in mice	↓ pSTAT3	2 mg/kg	[70]
	DATS	Xenografted MDA-MB-231 tumor model	↓Tumor weight; ↓tumor volume; ↓metastasis; ↓ MMP-2; ↓MMP-9; ↓Trx-1	25 and 50 mg/kg	[71]
	DATS	MDA-MB-231 cells in hypoxia-induced embryonic zebrafish, xenograft, and orthotopic tumors	⊥ Metastasis; ⊥ L1CAM, ⊥VEGF-A; ⊥EMT-related proteins (Slug, Snail, MMP-2); ⊥ HIF-1α transcriptional activity	Not specified	[72]
	DAS	Diethylstilbestrol-induced breast cancer female ACI rats	↑Lipid hydroperoxides; ⊥production of ROS	Not specified	[73]
Colon cancer	Allicin	Colorectal cancer mouse model having HCT116 cells	⊥Proliferation; ↓STAT3 signaling pathway; ↓number and size of tumors; ↓Mcl-1; ↓Bcl-2; ↓Bcl-xL	7.5 mg/kg	[108]
	Allicin+ X-ray	Transplanted tumor of CT26 cells in BALB/c mice	↓Tumor volume and weight	5 mg/kg	[85]
	DAS	nitrosamine induced colon carcinogenic	⊥ CYP2E1	50 mg/kg	[106]

		animal model utilizing F344 rats			
		Colon carcinogenesis utilizing engineered transgenic ApcMin/$^{+}$ mice	↓Incidence of colonic polyps by 32%	100 and 300 ppm	[110]
	AMS	Nitrosamine induced colon carcinogenic animal model utilizing F344 rats	⊥CYP2E1	50 mg/kg	[106]
	DADS	Colorectal tumorigenesis in a mouse model of colitis-induced colorectal cancer	NFκB nuclear localization and activity was diminished; ⊥ GSK-3β	60 mg/kg	[111]
	DATS	Mouse colon tumor in mouse CT-26 cells xenograft model	⊥Cell cycle at sub-G1; ↑apoptosis; ↓tumor volume and weight	10 and 50 mg/kg	[112]
	DADS and DATS	Human colon colo 205 cancer xenograft cells implanted in mice	↓Tumor weight; ↓tumor size	6 mg/kg	[104]
Gastric cancer	SAC	N-methyl-N'-nitro-N-nitrosoguanidine and sodium chloride-induced gastric carcino-genesis in Wistar rats	Suppression of carcinomas; lipid peroxida-tion; ↑GSH-dependent antioxidant enzymes;	200 mg/kg	[136]
	SAMC	Human gastric cancer SGC-7901 cells inoculated subcutaneously in BALB/c nude mice; KMN-45 cells injected into subcuta-neous tissues of nude mice	⊥Proliferation; ↑caspase-9, ↑caspase-3; ↑Bax; ↓pAkt; ↓Bcl-2; ↑ERK; ↑p38; ↑JNK; ↓tumor volume	40-80 mg/kg; 100 and 300 mg/kg	[137-138]
Liver cancer	Allicin +5-FU	Subcutaneous xenograft tumor model was established by transplanting SK-Hep-1-GFP cells into nude mice	↑Apoptosis; ↑ROS; ↓mitochondrial membrane po-tential (ΔΨm); activated caspa-se-3 and PARP; ↓Bcl-2	5 mg/kg/day (allicin)+ 20 mg/kg/day (5-FU)	[142]
	DAS	Diethylstilbesterol induced liver cancer in male Sprague-Dawley rats	⊥ DNA adducts	50 and 400 mg/kg	[143]
	SAMC	Hepatic tumorigenesis in Huh-7 xeno-graft/orthotopic mouse model	↓LDLR-related protein 6 (LRP6)	300 mg/kg	[150]
	SAC	NDEA-induced hepatocarcinogenesis	⊥Tumor inci-dence; ↑lipid peroxidation and antioxidants level; ↓GST activity	200 mg/kg	[151]
Pancre-atic can-	SPRC	Panc-1 xenograft	⊥Proliferation; ↑apoptosis;	Not speci-fied	[169]

cer			↑JNK signaling pathway		
Prostate cancer	DATS	PC-3 human prostate cancer xenograft implanted in male athymic mice	↓CDK1 and Cdc25C protein levels; ↑cyclin B1; ⊥cell cycle at G2/M phase	6 μM	[185]
	SAC	Human androgen-independent prostate cancer xenograft implanted with CWR22R cells	↑Apoptosis; ↓Bcl-2; ↑caspase-3	1 g/kg	[183]
	SAMC	Androgen-independent prostate cancer implanted in CB-17 SCID/SCID mice	Antimetastatic effect; ↓tumor growth; ↓Number of circulating tumor cells	100 and 300 mg/kg	[184]
Skin cancer	DAS	DMBA-initiated and BPO-promoted Sencar mouse skin tumorigenesis	⊥Tumor promotion	20 μmol/mouse	[191]
		Inhibited the promotion of DMBA-initiated and TPA-promoted SENCAR mouse skin tumorigenesis	⊥Proximate and ultimate carcinogens produced by biotransformation of DMBA; ↑GST activity	1 mg/100 μL of acetone	[192]
		DMBA-induced H-ras mRNA level in mouse skin tumors;	↑Cytosolic p21/ras; ↓p21/ras protein; ↓tumor size	10 mg/kg	[207]
		DMBA-induced skin carcinogenesis in female Swiss albino mice	↓Skin papilloma or preneoplastic benign growth; ↓p53; ↓caspase-3	250 mg/kg	[208]
		DMBA induced mouse skin mutagenesis	⊥DNA strand breaks	2.5-10 mg/kg	[209]
		DMBA-induced skin carcinogenesis in Swiss albino mice	↑p53; ↓p21/waf1; ↓ras oncoprotein; ↓PI3K/Akt; ↓p38MAPK	5-10 mg/kg	[210]
		DMBA-induced mouse skin tumors	↓Tumor cell proliferation; ↑apoptosis; ⊥cell cycle at sub-G1 stage	10 mg/kg	[200]
		DMBA-induced skin tumors in Swiss albino mice	↓Mutant p53 expression	Not specified	[201]
	DADS	DMBA-initiated and TPA-promoted SENCAR mouse skin tumorigenesis	⊥Proximate and ultimate carcinogens produced by biotransformation of	1 mg/kg	[192]

			DMBA; ↑GST activity		
	DATS	TPA-induced mouse skin tumors	↓TPA-induced expression of c-Jun and c-Fos; ⊥ activation of JNK and Akt; ⊥AP-1 DNA binding; ⊥COX-2	5 and 25 μmol	[203]
Renal cancer	DADS	**N**-diethylnitrosamine induced renal carcinogenesis in rats	↓Kidney tubular lesions; ↓incidence of nephroblastomas	50 and 200 mg/kg	[212]

Various symbols (↑, ↓ and ⊥) indicate increase, decrease and inhibition in the obtained variables, respectively.

Table 3.
Clinical trials of garlic and its constituents on various types of cancer.

Garlic and its constituents	Cancer type	Design	No. of participants	Doses	Duration	Follow-up time	Major findings	References
Raw, crushed garlic	All types	Randomized controlled trial	17	5 g	10	Not reported	↓AHR, ↓ARNT, ↓HIF1A, ↓JUN, ↓NFAT, ↓NFAM1, ↓OSM, ↓REL	[213]
Raw garlic and allicin		Not reported	20	3-80 g/day; 4.40 mg of allicin/g of garlic	3 weeks to 2 months	Not reported	↑Cell cytotoxicity	[214]
Garlic powder	Breast cancer	Randomized double blind placebo-controlled trial	66	150 mg twice a day	6 months	Not reported	Regression of breast fibromatosis	[215]
Raw garlic		Randomized controlled trial	125	Not specified; ≥ 4 times/week	6 months	6 months	Improved health of breast cancer patient	[216]
Aged garlic extract	Colon, liver, and pancreatic cancer	Randomized controlled trial	50 (42 patients with liver cancer, 7 patients with pancreatic cancer, and 1 patient with colon cancer	4 capsules/day (500 mg of AGE, 727 mg of crystalline cellulose, and 11 mg of sucrose fatty acid ester)	12 weeks	24 weeks	↑NK Cell number; ↑improvements in the NK cell activity	[217]
Aged garlic extract	Colorectal adenomas	Preliminary double-blind, randomized clinical trial	51	2.4 mL/day	12 months	12 months	↓Size and number of colon adenomas	[218-219]

Raw garlic		Randomized controlled Trial	57 560	5 Pyramid servings/day	12 months	12 months	↓Risk of colorectal adenoma	[220]
Aged garlic extract and steam distilled garlic oil	Gastric cancer	2×2 factorial design	3365	200 mg garlic extract and 1 mg garlic oil twice daily	7 years	7.3 years	↓Gastric cancer mortality	[222]
					7.3 years	22.3 years		[221]
Garlic supplementation		Randomized controlled trial	3365	Not specified	8 years	22.3 years	↓GC incidence and ↓GC mortality	[223]
Garlic extract + steam distilled garlic oil		Randomized, double-blinded, 23-factorial intervention trial	3599	1 pill/day (800 mg of garlic extract plus 4 mg steam distilled garlic oil)	39 months	39 months	⊥Progression of precancerous gastric lesions	[224]
Allicin		Randomized controlled trial	80	Data not available	Administered 48 h before operation	Till operation	↑Apoptosis; cell cycle arrest at G0/G1 phase; ↑Bax; ↑Fas; ↓Bcl-2; ⊥proliferation	[225]
Garlic extract	Leukemias and lymphomas	Randomized controlled trials	101	Two doses of 450 mg (allin 9.9 mg, allicin 4.5 mg) per day	Till the complete resolution of neutropenia	Not reported	Complete resolution of neutropenia	[226]
Aqueous garlic extract	Prostate cancer	Not reported	27	1 ml/kg weight per day	1 month	Not reported	Urinary frequency, maximum and average rates of urinary flow improved	[186]
Ajoene	Skin cancer	Not reported	21	0.4% ajoene cream (400 mg	Not reported	Not reported	↓Tumor size; ↓Bcl-2; ↑apoptosis	[204]

				ajoene, 0.3 mL polysorbate 80, and 0.3 mL sorbitonoleate in 100 ml 1% carbomeric gel)				

Various symbols (↑, ↓ and ⊥) indicate increase, decrease and inhibition in the obtained variables, respectively.

Graphical abstract