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Anticancer Activities of *Allium sativum* L. Against MCF-7 and MDA-MB-231 Breast Cancer Cell Lines Mediated by Caspase-3 and Caspase-9

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BACKGROUND/AIMS
Allium sativum (garlic) has been used as a medicinal herb for centuries, and commonly among cancer patients as herbal supplements. The aim of this study is to explore the in vitro antitumoral effects of *Allium sativum* L. (ASB) on human breast cancer (BCa) cell lines, MCF-7 and MDA-MB-231.

MATERIAL and METHODS
Trypan blue assay and LDH assays were used to quantitatively determine the cytotoxicity effects of ASB extract on BCa cell lines. Cytotoxicity experiments were followed up by caspase-3 and -9 activity assays to get a mechanistic insight into the associated molecular pathways. The MTT assay was used to demonstrate the antiproliferative activity, whereas the lateral motility assay was used to gain insight into the migration potential of BCa cells upon incubation with the ASB extract. The bioactive molecules in the ASB extract were delineated through GC-MS analysis.

RESULTS
Exposure to ASB extract caused a significant cytotoxicity effect on MCF-7 and MDA-MB-231 cell lines. A significant activation of caspase-9 was observed in both tested cell lines, indicating that the cytotoxic activity is mediated in an apoptotic manner. The results of the MTT assay revealed a significant antiproliferative effect on both tested cell lines at all tested time points. The lateral motility experiments showed a significant reduction in BCa cell motility demonstrating the antimotility potency of the ASB extract. An abundance of bioactive molecules in ASB were revealed via GC-MS analysis, many of which have been previously associated with anticancer activities.

CONCLUSION
Overall, *Allium sativum* has significant antitumoral and antimotility effects on MDA-MB-231 and MCF-7 human breast cancer cells which are attributed to its bioactive molecules.

Keywords: *Allium sativum* L., anticancer, apoptosis, proliferation, cytotoxicity

INTRODUCTION

Globally, breast cancer is the most frequently diagnosed type of cancer and the main cause of cancer related mortality in women (1). Non-gender discriminatory data also suggests that breast cancer is the second main cause of cancer-related mortalities in both sexes, representing 11.6% of all cancer deaths, led only by lung cancer at 18.4% (1). The statistics representing cancer cases and cancer-related deaths are expected to rise by 70%, causing the annual cancer-related deaths to reach 17 million by 2030 (1). Although it has been established that current therapies used against cancer have reached some success at preventing or delaying cancer-related deaths, current statistics are indicative of the need for further research to improve the patients' quality of life and reduce the death rates. It is imperative to research novel molecules as well as commonly used resources to learn basic biological aspects and treatment/prevention of the disease and to reveal novel therapeutics.

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Several drugs that are currently used have originated from therapeutic plants (2). Furthermore, various bioactive molecules obtained from plants are used for the development of new medicines. Around 28.187 different species of plants are used for medicinal purposes or the development of new therapeutics (3).

In vitro and *in vivo* anticancer studies of garlic, *Allium sativum*, have revealed that molecules within the bulbs utilize several mechanisms to prevent cancer formation, such as the induction of drug metabolizing enzymes (4), by serving as an antioxidant agent (5) and by inhibiting tumor formation (6). Altering cell signaling mechanisms and inducing apoptosis (7) as well as boosting immune system cells against cancer (8) and inhibiting angiogenesis (9) were demonstrated as anticancer mechanisms associated with *Allium sativum. In vivo* research also revealed that the consumption of *Allium sativum* plays a role in significantly reducing the risk of some gastrointestinal cancers (10, 11).

Although various studies on *Allium sativum* and its effects on cancer have been conducted, the mechanistic details of its anticancer effects on breast cancer cells are yet to be investigated. To this end, the aim of this study is to mechanistically investigate the antiproliferative, cytotoxic, antimetastatic, and apoptotic effects of the ethanolic extracts of *Allium sativum* L. bulb on weakly metastatic MCF-7 and strongly metastatic MDA-MB-231 BCa cell lines.

MATERIALS and METHODS

Collection of garlic cloves

The fresh forms of *Allium sativum* L. were obtained from a local market in North Cyprus and the identification of garlic cloves was performed by Prof. Dr. Mehmet Koyuncu (CIU, Pharmaceutical Botany Dept).

Extraction of garlic cloves

Allium sativum L. bulb (ASB) samples were separated, sliced, and kept at the room temperature for drying. The dried plant material was then powdered and the extraction was done by mixing powdered material with 95% ethanol with a 1:10 w/v ratio. The sample was then macerated at room temperature for 24 h and filtered with Whatman No°1. Rotary-evaporator (Heidolph, Germany) was used for the concentration of samples at 40 °C. ASB yield was 0.344%. ASB extracts were labeled accordingly and stored at 4 °C for further analysis.

Main Points:

- Allium sativum L. induces cytotoxicity on MCF-7 and MDA-MB-231 breast cancer cell lines in a caspase-3, and caspase-9 mediated manner.
- Allium sativum L. inhibits the proliferation of MCF-7 and MDA-MB-231 breast cancer cell lines with a higher antiproliferative effect on the strongly metastatic MDA-MB-231 line in a concentration-dependent manner.
- Allium sativum L. exerts antimotility activity on both strongly and weakly metastatic breast cancer cell lines, MCF-7 and MDA-MB-231.
- The GC-MS analysis of Allium sativum L. bulb revealed several bioactive molecules with anticancer activities, including diallyl sulfide, diallyl trisulfide, dimethyl sulfide, 1,2-benzenedicarboxylic acid, and hexadecanoic acid.

Culture conditions for BCa Cell Lines

Strongly and weakly metastatic BCa cell lines, MDA-MB-231 and MCF-7, were grown at 37 °C, 5% CO_2, and 100% relative humidity in Dulbecco's Modified Eagle Medium (DMEM) (Gibco by Life Technologies TM, USA) supplemented with 4 mmol/l L-glutamine and 10% fetal bovine serum until 90-100% confluence as previously described by Fraser et al. (12). This study was conducted in accordance with the World Medical Association Declaration of Helsinki.

Trypan blue exclusion assay

The cytotoxicity effects of *Allium sativum* L. extracts on BCa cells were evaluated using trypan blue exclusion assay. Cells were plated with 3×10^4 /ml density and incubated overnight. MCF-7 and MDA-MB-231 breast cancer cells were incubated for 24, 48, and 72 h with *Allium sativum* extract. The percentage of alive cells was determined from 20 randomly selected areas under an inverted microscope (Leica, Germany) (13).

Lactate Dehydrogenase (LDH) Cytotoxicity assay

Lactate dehydrogenase (LDH) is a cytosolic enzyme released as a result of damaged plasma membrane which is directly proportional to cellular cytotoxicity. The LDH activity of ASB incubated cells (0.5×10^6 /ml cell density) was measured quantitatively using the LDH Cytotoxicity Assay Kit (Thermo Scientific, USA) in accordance with the manufacturers' instructions.

Caspase-3 and Caspase-9 activity

The caspase-3 induction by *Allium sativum* L. extract in breast cancer cell lines (0.5×10^6 /ml) was evaluated quantitatively using the Caspase-3 Colorimetric Activity Assay Kit (Merck Millipore, USA) in accordance with the manufacturer's directions. Similarly, caspase-9 activity measurements were determined quantitatively using the Caspase-9 Colorimetric Activity Assay Kit (Merck Millipore, USA) on MDA-MB-231 and MCF-7 cells (0.5×10^6) in accordance with the manufacturer's directions.

Methyl-thiazolyltetrazolium (MTT) assay

The change in the cell numbers was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Invitrogen, ThermoScientific) assay as previously explained by Isbilen et al. (13). Both BCa cell lines were plated (3×10^4 /ml cells) and kept overnight in an incubator followed by the *Allium sativum* L. bulb extract treatment (at 24, 48, and 72 h). All cells received 1 mg/ml MTT containing DMEM and were incubated at 37 °C for 3 h. Moreover, MTT was removed and replaced with 0.89 ml DMSO (Sigma-Aldrich) and 0.11 ml glycine buffer. Measurements to determine cell numbers were performed at 490 nm on a multi-well plate reader (ELx800, Biotek Instruments) (13). EXCEL software was used to calculate the IC_{50} value of the extract concentration that killed 50% of the cells.

Wound healing (lateral motility) assay

To determine the inhibition of motility on BCa cells, lateral motility assay experiments were performed. Breast cancer cells were plated at a density of 4×10^5 /ml and incubated overnight at 37 °C and 5% CO_2. Three wounds were produced with a width of 0.5–0.8 mm using 200 µl micropipette tips. Moreover, DMEM me-

dia was replaced with fresh media containing *Allium sativum* L. extracts, and wounds were visualized using a digital inverse microscope camera (Leica, Germany). At the end of the 24 h incubation with extracts, the wound widths were re-measured and re-evaluated via the ImageJ software (14).

Gas Chromatography–Mass spectroscopy (GC-MS) analysis

Gas chromatography–mass spectroscopy analysis was performed as previously described by Leikshmi et al. (15). *Allium sativum* L. extracts were filtered and analyzed using the GC-MS-QP2010 PlusSystem (Shimadzu). By using the MS data library WILEY7.LIB, the spectrum was analyzed, and the bioactive compounds were identified.

Statistical analysis

The experiments of this study were performed at least three times and in triplicates. The data obtained were presented as means±S.E.M. Experimental data were analyzed using Student's unpaired *t-test* and one-way ANOVA followed by Newman-Keuls post hoc analysis (INSTAT Software). Statistical significance was set at p<0.05(*) or p<0.01 (**).

RESULTS

Allium sativum L. induces cytotoxicity in MCF-7 and MDA-MB-231 breast cancer cell lines

Viability assay (trypan blue assay) was used to assess the toxicity of the ASB extract on MCF-7 and MDA-MB-231 breast cancer cells at different time points (24, 48, and 72 h). The ASB extract demonstrated a significant cytotoxicity on MCF-7 (at 24 h, 86.27% decrease in viability; at 48 h, 91.65%; at 72 h, 89.63%) (control *vs* 10000 µg/ml; p<0.01; n=3; Figure 1) and MDA-MB-231 breast cancer cells (at 24 h, 94.87% decrease in viability; at 48 h, 93.69%; at 72 h, 95%) (control *vs* 10000 µg/ml; p<0.01; n=3; Figure 1). Statistical analysis on the viability assay results revealed that starting from the 1250 µg/ml incubations of the ASB extract, a high cytotoxicity level on both MCF-7 and MDA-MB-231 cells was observed. In conclusion, the ASB extract caused a sharp

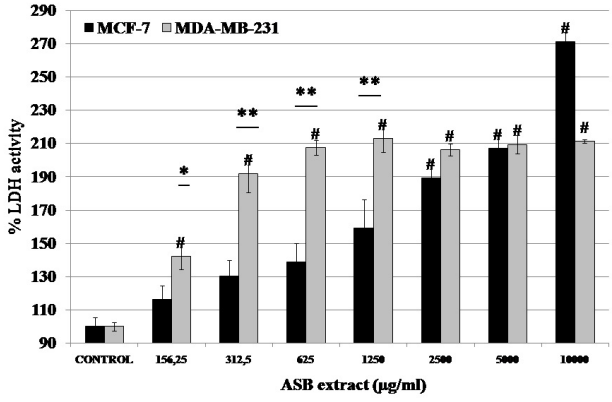

FIGURE 2. The Allium sativum bulb (ASB) extract induces LDH-related cytotoxicity in MCF-7 and MDA-MB-231 BCa cells. Data represents mean±S.E.M. #, p<0.05 vs control*, p<0.05 and **, p<0.01.

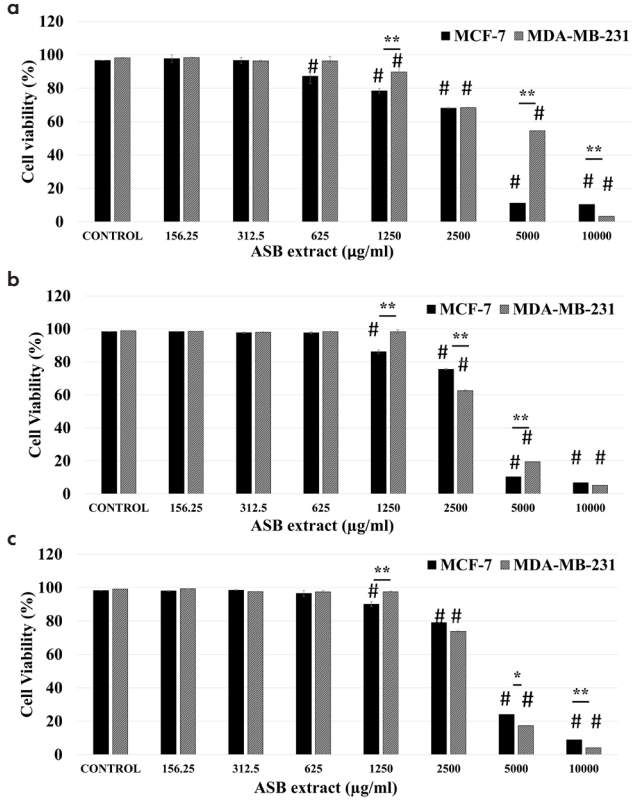

FIGURE 1. a-c. The Allium sativum bulb (ASB) extract decreases the viability of MCF-7 and MDA-MB-231 cells.% viability of BCa cell lines for 24 h (a), 48 h (b), and 72 h (c) periods. Data represents mean±S.E.M. #, p<0.05 vs control*, p<0.05 and **, p<0.01.

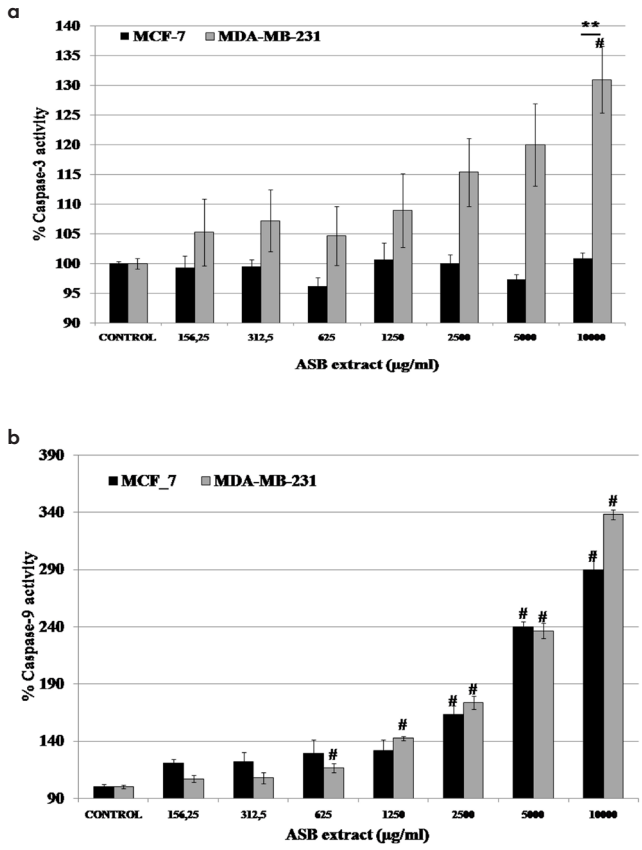

FIGURE 3. a, b. The Allium sativum bulb (ASB) extract induces caspase-3 (a) and caspase-9 (b) activities in MCF-7 and MDA-MB-231 BCa cells. Data represents mean±S.E.M. #, p<0.05 vs control*, p<0.05 and **, p<0.01.

decrease of cell viability specifically at high concentrations at all tested time points in both breast cancer cell lines.

Allium sativum L. induces LDH activity in both MCF-7 and MDA-MB-231 breast cancer cell lines

The LDH-related cytotoxic effects of ASB on both MCF-7 and MDA-MB-231 cells were evaluated. Control experiments were taken as 100%. The experimental results revealed that the incubations of ASB caused a significant LDH release in MDA-MB-231 cells (24 h: 211.57±1.05%; control *vs* 10000 mg/ml; p<0.05; Figure 2). Similarly, MCF-7 cells were incubated with ASB extracts for 24 h where ASB extract caused a significant induction of LDH release in MCF-7 cells as well (24 h: 271.45±5.63%; control *vs* 10000 mg/ml; p<0.05; Figure 2).

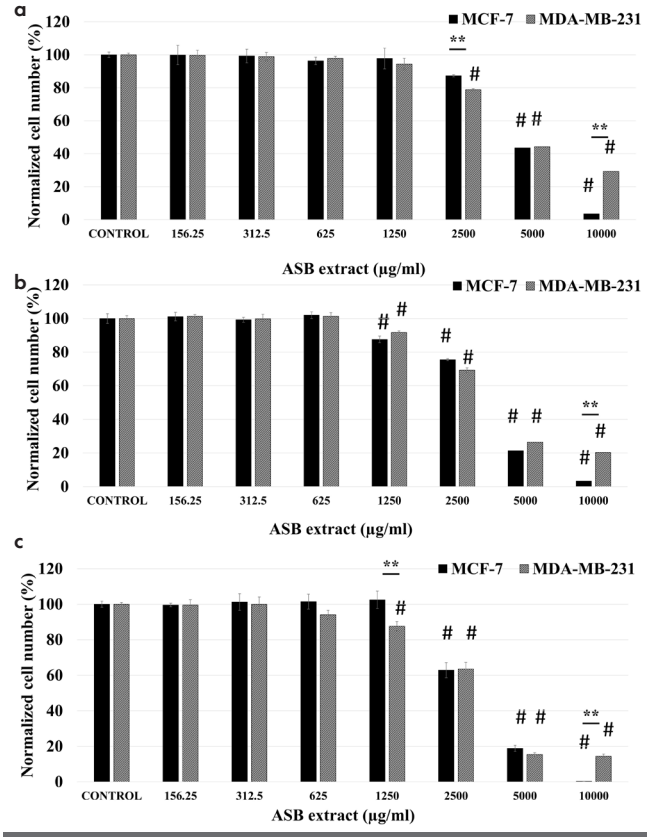

FIGURE 4. a-c. The Allium sativum bulb (ASB) extract exerts significant antiproliferative effect on MCF-7 and MDA-MB-231 cells. Data represents mean±S.E.M. #, p<0.05 vs control*, p<0.05 and **, p<0.01.

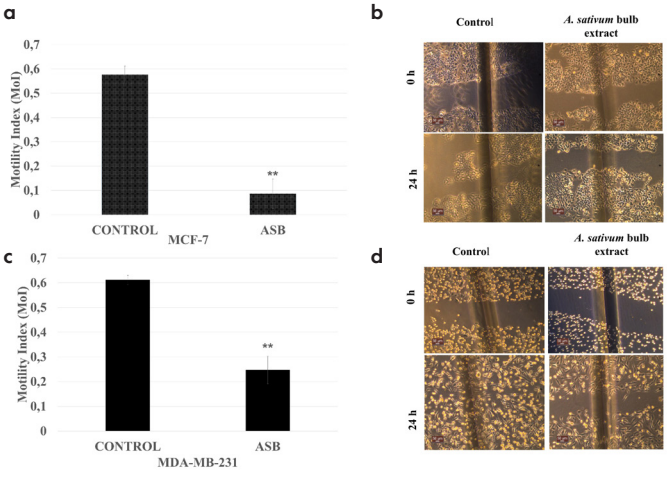

FIGURE 5. a-d. The Allium sativum bulb (ASB) extract inhibits the lateral motility of MCF-7(a) and MDA-MB-231(c) cells. *, p<0.05 and **, p<0.01 relative to control. Images of ASB incubation of MCF-7(b) cells and MDA-MB-231(d). Scale bars: 50 µm.

Allium sativum L. induces caspase-9 in both MCF-7 and MDA-MB-231 breast cancer cell lines

Caspase-3 activity experiments were performed on MCF-7 and MDA-MB-231 cells upon incubations with ASB extract for 24 h. Control experiments were taken as 100%. Experimental results revealed that incubations with ASB increased the caspase-3 activity significantly only at a concentration of 10000 mg/ml in MDA-MB-231 cells (24 h: 130.36±5.64%; control *vs* 10000 mg/ml; p<0.05; Figure 3a). On the other hand, treatments with ASB on MCF-7 cells did not show any significant increase in caspase-3 activity (24 h: 100.81±1.01%; control *vs* 10000 mg/ml; p>0.05; Figure 3a).

The caspase-9 activities of MCF-7 and MDA-MB-231 BCa cells incubated with ASB extracts were evaluated. Control experiments were taken as 100%. Experimental results showed that the incubation of MDA-MB-231 cells with ASB caused a significant increase in caspase-9 activity compared to the control experiments (24 h: 337.9 2±4.47%; control *vs* 10000 mg/ml; p<0.05; Figure 3b). Similarly incubations of MCF-7 cells with ASB caused a significant increase in caspase-9 activity at 24 h (24 h: 289.67±16.46%; control *vs* 10000 mg/ml; p<0.05; Figure 3b).

Allium sativum L. inhibits the proliferation of MCF-7 and MDA-MB-231 breast cancer cell lines

MTT assay was used to determine the antiproliferative effects of the ethanolic *Allium sativum* L. extract on MCF-7 and MDA-MB-231 BCa cells at different tested time points (24, 48, and 72 h). *Allium sativum* was previously shown to have effects on MCF-7 cell lines (16). To investigate the antiproliferative effects of the ASB extract, different concentrations of the ASB extract were tested on both BCa cell lines. A significant antiproliferative effect was determined in MDA-MB-231 cells at a concentration of 2500 µg/ml at 48 and 72 h (Figure 4), suggesting a stronger effect on highly metastatic BCa cell line (MDA-MB-231 cell line) based on the IC_{50} calculations (Table 1).

Allium sativum L. inhibits the lateral motility of MCF-7 and MDA-MB-231 breast cancer cell lines

The antimotility potency of *Allium sativum* L. bulb in both MCF-7 and MDA-MB-231 cells was evaluated using the lateral motility assay. An extract concentration of 156.25 µg/ml was selected wherein no significant cytotoxic or antiproliferative activity was observed. During the control experiment, the MoI of MCF-7 cells was 0.57±0.03, whereas during the ASB extract treatment (24 h), the MoI of MCF-7 cells decreased to 0.086±0.06 (p<0.01) (Figure 5a). Moreover, during the control experiment, the MoI of MDA-MB-231 cells was 0.40±0.01, whereas during the incubation with ASB extract at 24 h, the MoI of MDA-MB-231 cells decreased to 0.24±0.05 (p<0.01) (Figure 5c). The changes in MoI during the incubations with ASB extract strongly indicate an inhibitory effect of ASB on the lateral motility of MCF-7 and MDA-MB-231 breast cancer cells.

GC-MS analysis of *Allium sativum* L. bulb extract

The bioactive compounds of ethanolic *Allium sativum* bulb extract were analyzed using gas chromatography–mass spectroscopy. The results obtained from the analysis showed the presence of acetic acid (2.53%), dimethyl trisulfide (0.30%), diallyl disulfide (1.05%), methyl allyl trisulfide (0.76%), diallyl trisulfide (1.6%), diallyl sulfide (0.13%), guanosine (5.78%), 1,2-benzenedicarboxylic acid (3.58%), hexadecanoic acid (1.58%), heptadecanoic acid (0.48%), 1H-purin-6-amine (2.16%), octadecamethylcyclononasiloxane (6.03%), tetramethylcyclotetrasiloxane (6.95%), octadecamethylcyclononasiloxane (6.03%), 4-hydroxy-1H-purine (2.2%), hexacontan (2.22%), 2, 4, 6, 8-tetramethyl-cyclotetrasiloxane (1.25%), and flavone 4'-OH,5-OH,7-di-O-glucoside (1.47%) (Figure 6; Table 2). These findings also support the data from Leikshmi et al. (15). and Park et al. (17) who previously performed a GC-MS analysis of the *Allium sativum* L. extract (15, 17).

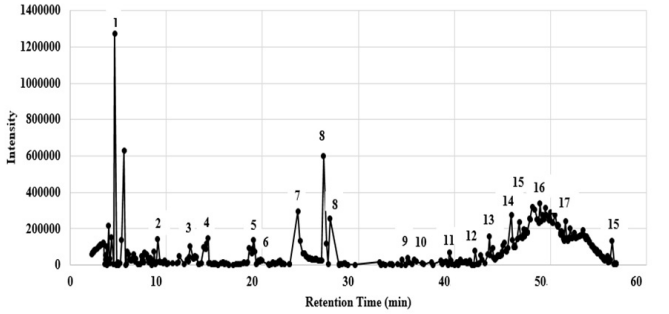

FIGURE 6. Chromatogram of gas chromatography–mass spectroscopy analysis: bioactive compounds obtained from ethanolic extracts of Allium sativum bulb (ASB). Numbers on peaks correspond to the molecules in Table 2.

TABLE 1. IC50 values of MCF-7 and MDA-MB-231 cells incubated with Allium sativum L. bulb (ASB) extract. Data represents mean ±S.E.M.

	IC_{50} concentration µg/ml	
	ASB	
	MCF-7	MDA-MB-231
24h	4822.2±325	4664.3±457
48h	4646.5±338	2874.2±201
72h	3924.5±298	2771.9±178

DISCUSSION

Breast cancer affects 2.1 million women every year (18). The mortality rates among breast cancer patients depend on the type of breast cancer and nature of the cancer tissue. Moreover, highly metastatic breast cancers, specifically triple negatives, cause an increase in the percentage of mortality (19) implicating a need for development of novel therapies and preventatives with antitumoral and antimetastatic activities.

Allium sativum, also known as common garlic, is believed to have anticancer effects and therefore is consumed frequently by cancer patients as well as the general public as a means to avoid cancer. In recent decades, antitumoral activities of *Allium sativum* were widely studied in different *in vitro* and *in vivo* models. Experimental studies suggest that direct exposure of different cancer cells to garlic extract is more effective than the oral administration of the extract suggesting that bioactive molecules might be losing their anticancer potency through absorption via the epithelial lining of the gastrointestinal tract

TABLE 2. Compounds determined through GC-MS analysis of Allium sativum L. bulb (ASB) extract and their bioactivities

	Compound name	*Allium sativum* L. bulb% compound*	Biological activity
1	Acetic acid	2.53	Antibacterial and antifungal (26)
2	Trisulfide, dimethyl	0.30	Antiproliferative (26) and antimicrobial (27)
3	Diallyl disulfide	1.05	Inhibiting tumor cell proliferation, tumor cell apoptosis promoter, antimetastatic, anti-inflammatory (17), antibacterial (28)
4	Methyl allyl trisulfide	0.76	-
5	Diallyl trisulfide	1.60	Anticancer, antioxidant, blood pressure lowering, platelet aggregation (29,30,31)
6	Diallyl sulfide	0.13	Anticancer, antimicrobial, anti-angiogenic, and immunomodulatory (32)
7	Guanosine	5.78	Neuroprotective and neuromodulator (33)
8	1,2-Benzenedicarboxylic acid	3.58	Anticancer (34), antimicrobial, antifungal, anti-malarial (35,36)
9	Hexadecanoic acid	1.58	Antitumoral (37), antimicrobial, antioxidant, decreases blood cholesterol, anti-inflammatory (35)
10	Heptadecanoic acid	0.48	Antibacterial and antioxidant (38)
11	1H-Purin-6-amine	2.16	Anti-inflammatory and cytoprotective (39)
12	Tetramethylcyclotetrasiloxane	6.95	-
13	Octadecamethyl cyclononasiloxane	6.03	Antifungal (40)
14	4-Hydroxy-1H-purine	2.20	-
15	Hexacontan	2.22	-
16	Cyclotetrasiloxane, 2,4,6,8-tetramethyl-	1.25	-
17	Flavone 4'-OH,5-OH,7-DI-O-glucoside	1.47	Larvacidal (41)

*% peak area

(20). Previously, it was shown that the intraperitoneal treatment of sarcoma 180 and EL4-induced lethal ascites with raw garlic extract diminished the cancer formation in C57BL/6 mice (20). However, the oral administrations of the extract did not cause any significant anticancer effects in the in vivo model. Furthermore, this study revealed that the direct injection of *Allium sativum* in tumors is a promising candidate for the development of new applications of *Allium* species (20).

In line with the above *in vivo* findings, our study showed that the direct application of *Allium sativum* extract on tumor cells exerts significant anti-tumoral effects on both highly metastatic and weakly metastatic human breast cancer cell lines. The cytotoxicity effects exerted by *Allium sativum* on both MCF-7 and MDA-MB-231 breast cancer cell lines in a time- and concentration-dependent manner (Figure 1) are potentially due to the presence of diallyl trisulfide (1.6%) and diallyl disulfide (1.05) (Figure 6, Table 2). Diallyl trisulfide was previously shown to cause the induction of cytotoxicity in U937 leukemia cells (17). Diallyl trisulfide and diallyl disulfide which are organosulfur compounds found in *Allium sativum* were previously shown to inhibit PI3K/Akt/mTOR and induce apoptosis in colon, gastric, prostate, and breast cancers (21). The LDH cytotoxicity experiments of the ASB extract on MCF-7 and MDA-MB-231 BCa cells were consistent with the trypan blue exclusion assay experiments wherein a similar LDH cytotoxicity level was observed after incubations with the ASB extract at 24 h (Figure 2). Incubations with the ASB extract caused a significantly increased LDH release in both MCF-7 and MDA-MB-231 cell lines which presents supportive data on the cytotoxicity effects of the ASB extract on breast cancer cell lines.

Caspase-3 and caspase-9 activity experiments were conducted to investigate the apoptotic effects of the ASB extract on both breast cancer cell lines. Incubations with the ASB extract at a time period of 24 h showed a significant increase in caspase-9 activity in MCF-7 and MDA-MB-231 BCa cells, whereas this activity was observed to be higher in the highly metastatic breast cancer cell line MDA-MB-231 (Figure 3). This finding suggests a slow initiation process for apoptosis in MCF-7 cells as the initiator caspase (caspase-9) was significantly elevated at the 24 h period in both cell lines in which the only significant increase in the executioner caspase levels (caspase-3) was observed in MDA-MB-231 cells at this time period (Figure 3). The increase in caspase-3 activation in MDA-MB-231 cells suggests a faster apoptotic response in this strongly metastatic cell line.

Our cells have an infinite capacity to divide until they enter into a dormant state or cell death mechanisms are activated, whereas cancer cells have an unlimited capacity to proliferate (22). Currently, an approach to inhibit or reduce cell division in the process of tumorigenesis is being developed. To this end, the antiproliferative effects of the ASB extract were evaluated using MTT assays. Incubations with the ASB extract for both MCF-7 and MDA-MB-231 BCa cell lines revealed that the extract has a significant antiproliferative effect in a time- and concentration-dependent manner at designated time periods. Specifically, at a concentration of 2500 µg/ml, no cytotoxic effect was observed in MDA-MB-231 cells with the trypan blue assay, but significantly lower numbers of BCa cells were observed with the MTT assay, suggesting that at this concentration, instead of inducing cell death, the extract inhibits cancer cell proliferation. A similar effect was also observed at 48 h incubations with a concentration of 2500 µg/ml. For the 72 h incubations, the antiproliferative impact on MDA-MB-231 cells was assessed at a concentration of 1250 µg/ml wherein no significant decrease in viability was observed using the trypan blue viability assay, indicating that a longer exposure at lower concentrations also causes the inhibition of cancer cell proliferation.

Breast cancer is the leading cause of cancer-associated mortalities in women as well as one of the most diagnosed cancer types. Primary tumor treatment approaches have significantly improved over time, although current treatments for the inhibition of metastasis, which is the underlying reason for most of the mortalities attributed to cancer, are highly limited (23). Lateral motility assays that were performed to determine the antimotility effect of the ASB extract revealed that the incubation of MCF-7 and MDA-MB-231 cells with the ASB extract demonstrated a significant antimotility potency ($p<0.05$) on both tested cell lines. Experimental data revealed that the ASB extract significantly inhibited the motility for both MCF-7 and MDA-MB-231 cells, in which a more robust effect was observed in MCF-7 cells. This result is expected as MCF-7 is a model for lowly metastatic breast cancer cells. The antimotility effect of the ASB extract is potentially due to the presence of both hexadecanoic acid (1.58%) and diallyl disulfide (1.05%) in which both bioactive compounds were previously shown to exhibit antimetastatic properties (24, 17). Diallyl disulfide which is one of the major components of *Allium sativum* can block the NF-kB signaling pathways to suppress metastasis and the invasion of breast cancer cells (25). Furthermore, it has been reported that *Allium sativum* extracts induce the suppression of cyclooxygenase (COX)-2 (25). Since COX-2 plays an important role in several cellular processes such as cancer cell migration, metastasis, and tumor-associated angiogenesis, the suppression of COX-2 by Allium extracts is a significant modulator for the inhibition of tumor development (25).

Our GC-MS experiments were performed comparatively with those on well-known bioactive molecules and showed the presence of important bioactive molecules with eminent anticancer activities such as diallyl sulfide, diallyl trisulfide, dimethyl sulfide, 1,2-benzenedicarboxylic acid, and hexadecanoic acid (26, 33, 38).

Overall, the results of this study indicate the cytotoxic, antiproliferative, apoptotic, and antimotility effects of the ethanolic extract of *Allium sativum* bulb on weakly and strongly metastatic breast cancer cell lines MCF-7 and MDA-MB-231, revealing the mechanistic nature of the observed cell death and highlighting the anticancer potency of bioactive molecules found in *Allium sativum* L.

Ethics Committee Approval: N/A.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - O.I., E.V.; Supervision - E.V.; Resource - O.I.; Materials - O.I., E.V.; Data Collection and/or Processing - O.I.; Analysis and/or Interpretation - O.I., E.V.; Literature Search - O.I., E.V.; Writing - O.I., E.V.; Critical Reviews - O.I., E.V.

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Conflict of Interest: Authors have no conflicts of interest to declare.

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