PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 12(2): 1-5
https://doi.org/10.14719/pst.7499

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RESEARCH ARTICLE

Phytochemical analysis and anticancer potential of Iraqi *Allium sativum* on colon cancer cells

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OPEN ACCESS

ARTICLE HISTORY
Received: 29 January 2025
Accepted: 28 February 2025
Available online
Version 1.0 : 11 March 2025
Version 2.0 : 01 April 2025

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Amjed HK, Ali RJ. Phytochemical analysis and anticancer potential of Iraqi *Allium sativum* on colon cancer cells. Plant Science Today. 2025; 12(2): 1-5. https://doi.org/10.14719/pst.7499

Abstract

Allium sativum has been cultivated in different areas around the world and has been used as a food, condiment. Many sources mention *Allium sativum* as a potential treatment for different ailments. The Current study aimed to assess the anticarcinogenic and antitumor properties of *Allium sativum* and identify of the major phytochemicals present in Iraqi species. Specific and general tests were carried out for the qualitative analysis of different natural components present in the plant extract in addition to the assessment of the possible antitumor activity of *Allium sativum* by detecting the dose-response effect of the methanol extract. Serial extract concentrations were tested on a colon cancer cell line to determine the dose-response effect. The anticancer effects after 48 hours of exposure showed a significant inhibition rate in a dose -dependent manner on cancer cell growth. The IC50 value of the extract was found to be 35.13 µg/mL. The inhibition rates at various concentrations were as follows: at 200 µg/mL, the inhibition was 90.9 ± 0.03 %, at 100 µg/mL, it was 68.1 ± 0.05 %, at 50 µg/mL, the inhibition was 52.7 ± 0.09 %, at 25 µg/mL, it was 49.54 ± 0.03 %, at 12.5 µg/mL, it was 36.3 ± 0.05 % and at 6.25 µg/mL, the inhibition was 36.2 ± 0.05 %. Phytochemical analysis revealed the presence of several bioactive secondary metabolites in the methanol extract, including alkaloids, saponins, steroids, carbohydrates, flavonoids, terpenoids, anthraquinones and cardiac glycosides. These compounds are likely supporting the potential of *Allium sativum* as a natural anticancer treatment.

Keywords

Allium sativum; anticancer; colon cancer cell; phytochemistry

Introduction

Allium sativum belongs to the Amaryllidaceae family. It is an aromatic herbaceous plant known for its annual growth cycle, recognized as one of the oldest and most significant herbs historically utilized in traditional medicine (1). Since along time *Allium sativum* cultivated in different areas around the world and used as a food, condiment and folkloric medicine for thousands of years and widely researched plant (2). Dating back to approximately 1550 B.C., the Egyptian medical papyrus known as Codex Ebers contains 22 therapeutic formulas highlighting garlic as a potent remedy for various ailments like heart problems, headaches, worm's bites and tumors (3). According to biblical accounts, slaves in old Egypt were provided with allium vegetables, particularly garlic, believed to enhance their strength and productivity (4).

In old Greece, garlic was used to alleviate disorders of the intestines and lungs (5). The documents of Louis Pasteur refer to the antibacterial

properties of garlic as early as 1858 (3). For centuries garlic has been used in India as a disinfectant for cleansing wounds and ulcers. During World War II, garlic was applied to treat injuries sustained by soldiers (6). Numerous spices and herbs, such as garlic, are well-known for their medicinal attributes, which encompass hypolipidemic, anti-thrombotic and anti-hypertensive properties (7)

Garlic has been increasingly acknowledged for its effectiveness in managing conditions like cardiovascular diseases, owing to its capacity to enhance lipid profiles.

Bulbs of *Allium sativum* reportedly harbor numerous phytochemicals, including sulfur-containing compounds like ajoenes, thiosulfinates (allicin), vinyldithiins, sulfides (diallyl disulfide, diallyl trisulfide), comprising 82 % of the overall garlic sulfur content (8). Allinase enzyme facilitates the conversion of S-methyl cysteine-sulfoxide, S-propyl-cysteine -sulfoxide, and alliin into various molecules, including allyl methane thiosulfinates, methyl methanethiosulfonate and other corresponding thiosulfinates (9).

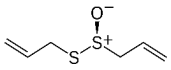
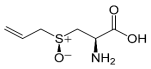
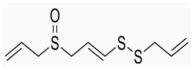
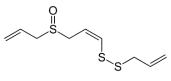
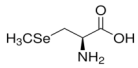
The bulbs are renowned not only for their distinctive sulfur compounds but also for containing non-sulfur compounds like fructans (acting as non-digestible fibers), steroid saponins and selenium. The organoselenium compound in garlic, Se-methyl selenocysteine, as shown in Table 1, metabolizes into methylselenol, exhibiting anticarcinogenic and antioxidant effects (10).

The chemo preventive efficacy of *Allium sativum* is linked to organosulfur compounds, and various bioactive compounds play a pivotal role in cytotoxic activity. Bioactive compounds such as allicin, alliin, diallyl disulfide and diallyl trisulfide function by up regulating p38 MAP kinase expression and cleaved caspase 3. The activated caspase-3, in turn, cleaves various downstream substrates, inducing characteristic morphological changes in apoptotic cells (11).

Allium sativum exhibits anticancer activity through bioactive compounds like allicin, which inhibit colon cancer cell proliferation and induce apoptosis. Compared to other medicinal plants like *Curcuma longa* and *Camellia sinensis*, garlic's anticancer effects are largely attributed to its sulfur compounds, while turmeric and green tea primarily rely on curcumin and catechins for their cancer-fighting properties (12).

Z-Ajoene functions by triggering apoptosis in human leukemic cells, stimulating peroxide production and activating caspase-3-like and caspase-8 activities (13). Furthermore, various studies have investigated the influence of garlic-derived organosulfur compounds on Glutathione-S-transferases, a prominent group of detoxifying enzymes involved in Phase II drug metabolism. These investigations indicate an elevation in the levels and/or activity of Glutathione-S-transferases, highlighting their role in detoxifying carcinogens (14).

Table 1. Compounds derived from *Allium sativum* include sulphur and non-sulphur containing verities (15)

Compound	Chemical formula	Chemical structure
Allicin	$C_6H_{10}OS_2$	
Allin	$C_6H_{11}NO_3S$	
(E)-Ajoene	$C_9H_{14}OS_3$	
(Z)-Ajoene	$C_9H_{14}OS_3$	
Se-methylselenocysteine	$C_4H_9NO_2Se$	

Materials and Methods

Plant material

Non-aged garlic samples from Iraq were obtained from local markets in Baghdad and authenticated by the Department of Pharmacognosy, College of Pharmacy, University of Baghdad. The experimental procedure involved using powdered garlic byproducts. Initially, the juice was extracted by pressing from whole garlic bulbs by applying mechanical force to squeeze out the liquid. At 25° C, this process is gentle, allowing the bioactive compounds, like allicin, to remain intact without being degraded by high heat. The resulting juice contains various sulfur compounds and other nutrients from garlic. This process followed by drying the residual material at 60 °C for 6 hr. Subsequently, 50g of garlic powder was mixed with 250 mL of 85 % methanol which is preferred for garlic extraction because it efficiently dissolves a wide range of bioactive compounds, including both polar and non-polar ones, while preserving their integrity. It also allows for easy evaporation, concentrating the extract without degrading sensitive compounds. The resulting mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator (LabTech, RE-500) at 40 °C, resulting in a dark viscous residue. This concentrate was then spread on aluminum foil and subjected to freeze-drying for subsequent experimentation (16).

Phytochemical screening

Phytochemical screening was performed on a small quantity of dried garlic extract to analyze secondary metabolites including alkaloids, saponins, carbohydrates, flavonoids and cardiac glycosides, among others, using established methods with minor adaptations, table 2 (17).

Cell lines

Colon cancer cell lines HCT116 Purchased from American type culture cell line (a human colorectal carcinoma cell line initiated from an adult male and adherent with an epithelial morphology, It is a widely used, highly aggressive cell line that exhibits mutations in key genes

like KRAS and p53, making it a suitable model for studying the molecular mechanisms of colon cancer and evaluating potential anticancer therapies) were cultured in RPMI1640 medium (Gibco, UK) supplemented with 10 % heat-inactivated fetal calf serum (Gibco, UK) and 1% penicillin/ streptomycin (Sigma Aldrich, Germany), as specified in the growth medium guidelines for each cell line. To prepare methanol extracts, sequential dilutions were made by dissolving samples in DMSO and further diluting them with the respective cell line medium. The final DMSO concentration in the medium was maintained at 1%. Control wells received medium containing the same final DMSO concentration, while triplicate samples were added to experimental wells. Cultures were then incubated at 37˚C with 5 % CO_2 for 48 hr. MTT assay was conducted by adding MTT to the cells and incubating for 4 hours, followed by measuring absorbance at 570 nm (18).

Evaluation of Suppression of Cancer Cell Growth

The inhibition of cancer cell growth was measured using the MTT assay, following Mosmann's method (19). Various concentrations of the extract were applied to the cells for 48 hr. Then, a 20 µL MTT solution (5 mg/mL in PBS) was added to each well and the plates were incubated at 37°C with 5 % CO_2 for 5 hr.

Following incubation, the plates were retrieved and the liquid supernatant was carefully removed. To dissolve the dark blue formazan crystals, 200 µL of dimethyl sulfoxide (DMSO) was added to each well and the plates were vigorously shaken for one minute at room temperature. Absorbance readings were then recorded at 570 nm, with a reference wavelength set at 650 nm using an enzyme-linked immunosorbent assay. The absorbance values of cells cultured in control medium were considered as 100 % viability and the viability of treated cells was calculated as a percentage relative to this untreated control.

Each concentration was assessed in triplicate and the experiment was replicated twice. The initial cell density in each well was $1x10^4$ cells and the percentage inhibition of the cell line was determined as the mean ± standard deviation (SD), using the formula:

$$\% \text{ of inhibition} = 1 - \frac{(A0-A1)}{(A2-A1)} \times 100 \qquad \text{(Eqn. 1)}$$

A0 represents the absorbance of the sample, A1 represents the absorbance of the blank, and A2 represents the absorbance of the control.

IC_{50} values were determined using both linear and logarithmic regression equations (20).

Results and Discussion

Our investigation has unveiled the presence of phytochemicals recognized as active medicinal constituents in *Allium sativum*. The methanol extract indicated the existence of phenolics, alkaloids, flavonoids, steroids, glycosides, saponins, cardiac glycosides and more, as detailed in Table 2. The key bioactive compounds in garlic responsible for its anticancer effects include allicin, diallyl disulfide, diallyl trisulfide and S-allyl cysteine.

Table 2. Qualitative analysis of the phytochemicals of *Allium sativum* methanol extract

Secondary metabolite	Detection method	Methanol extract
Alkaloids	Mayer's test	+
Saponins	Foam test	+
Steroids	Libermann-Burchard test	+
Carbohydrates	Benedict's Test	+
Flavonoids	Shinoda's test	+
Tannins	ferric chloride test	-
Terpenoids	Salkowski test	+
Anthraquinone	Brontrager's test	+
Phenolics	Bromine Water test	-
Cardiac glycosides	Baljet's test	+

The assortment of secondary metabolites found in garlic bulbs provides an explanation for its widespread use globally as a carminative, anthelminthic, antiseptic, diaphoretic, expectorant, diuretic, antiasthmatic, stimulant, antiscorbutic and aphrodisiac. It is employed in treating various ailments such as croup, whooping cough, tuberculosis, bronchiectasis, gangrene and many others (21).

MTT assay

The results showed that cancer cell growth was significantly inhibited in a concentration-dependent manner following 48 hr of incubation. Table 3 outlines the various concentrations of the extract tested: 200, 100, 50, 25, 12.5 and 6.25 µg/mL. Each concentration was evaluated in triplicate and the experiments were replicated twice. Data are presented as mean ± standard deviation (SD).

Inhibition rates for colon cell proliferation were observed at 90.9 ± 0.03 %, 68.1 ± 0.05 %, 52.7 ± 0.09 %, 49.54 ± 0.03 %, 36.3 ± 0.05 % and 36.2 ± 0.05 % corresponding to the sample concentrations of the methanol extract. A systematic analysis indicated a significant increase in cancer growth with increasing concentration. The lower inhibition rate at lower concentrations can be attributed to the insufficient concentration of bioactive compounds in the extract to exert a significant effect on cancer cell proliferation. As the concentration of the methanol extract increased, more bioactive compounds (such as allicin, diallyl disulfide and other sulfur-containing compounds) became available to interact with the cancer cells, leading to higher rates of inhibition.

The IC_{50} value for the methanol extract, calculated using linear regression (Y = percentage of inhibition, X = concentration), was determined to be 35.13 µg/mL, as depicted in Fig. 1. Various sulfur compounds derived from *Allium sativum*, such as diallyldisulfide, diallylsulfide, S-allylcysteine, diallyltrisulfide and S-allylmercapto-L-cysteine, have demonstrated anticarcinogenic properties. These compounds influence drug-metabolizing enzymes, exhibit antioxidant and free radical scavenging activities, inhibit tumor initiation and promotion and affect cell cycle progression and apoptosis induction in cancer cells. Recent reviews highlight significant advancements in understanding how garlic and its organosulfur compounds suppress cancer development, underscoring *Allium sativum*

Table 3. Dose-dependent inhibition of colon cancer cell proliferation for methanol extract

Methanol extract concentration (µg/ml)	mean ± standard deviation (SD)
200	90.9 ± 0.03%
100	68.1 ± 0.05%
50	52.7 ± 0.09%
25	49.54 ± 0.03%
12.5	36.3 ± 0.05%
6.25	36.2 ± 0.05%

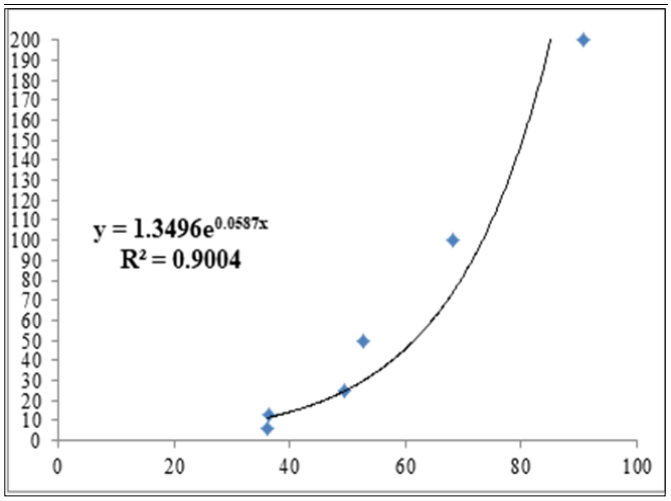


Fig. 1. Dose response curve for inhibition of colon cancer cell line.

potential as a potent anticancer agent (22).

Perchellet et al. demonstrated that garlic extract enhances glutathione peroxidase activity and counteracts the reduction in the reduced to oxidized glutathione ratio induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) in epidermal cells. Essential oils from *Allium sativum* also inhibit lipoxygenase, a crucial enzyme involved in the TPA-stimulated metabolism of arachidonic acid (23).

Furthermore, the methanol extract of garlic exhibited potent anti-angiogenic activity, potentially linked to mechanisms such as antioxidant effects rather than cytotoxicity. Other suspected mechanisms include inducing apoptosis, inhibiting cell proliferation and reducing oxidative stress and inflammation. Western blot analysis revealed its mechanism of action, which includes the suppression of heme oxygenase-1 expression and the activation of caspases-3, -8 and -9 (24).

Conclusion

In conclusion, accumulating evidence indicates that *Allium sativum* and its extracted compounds exhibit significant anti-proliferative effects on human cancer cells, particularly in colon cancer. Key findings highlight that compound such as allicin, diallyl disulfide and diallyl trisulfide induce apoptosis, regulate cell cycle progression and influence key signal transduction pathways involved in cancer cell growth. Additionally, garlic derivatives appear to modulate nuclear factors associated with immune function and inflammation, making them valuable candidates for cancer therapy. These findings suggest that garlic compounds may serve as potential adjuncts in colon cancer therapy by enhancing immune responses and reducing cancer cell proliferation. Further research is recommended to explore the molecular pathways through which these compounds exert their effects, including their interactions with key cancer-related proteins and enzymes. Additionally, investigating the synergistic effects of garlic derivatives with existing chemotherapeutic drugs could enhance therapeutic efficacy. Finally, bioavailability studies are crucial to determine the optimal dosage and formulation for maximizing the anticancer benefits of garlic in clinical settings.

Acknowledgements

The authors express their gratitude to the University of Baghdad for offering the essential facilities needed to conduct this study.

Authors' contributions

Study conception and design, data collection, analysis and interpretation of results were carried out by AHK. Draft manuscript preparation was done by ARJ. All authors reviewed the results and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical issues: None

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