**Exploration of Therapeutic Potential of *Allium sativum L.* (Black Garlic) Against Breast Cancer Cells**

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**Abstract**

Breast cancer remains a leading cause of mortality among women globally. Natural compounds have garnered attention for their therapeutic potential with reduced side effects. This study investigates the phytochemical profile, antibacterial activity, and cytotoxic potential of *Allium sativum* (black garlic) ethanolic extract against MCF-7 breast cancer cells. Phytochemical screening revealed the presence of flavonoids, phenolics, alkaloids, steroids, and terpenoids. Antibacterial assays demonstrated dose-dependent inhibition of *E. coli* and *S. aureus*, with fermented black garlic showing enhanced efficacy against Gram-positive strains. The MTT assay indicated moderate cytotoxicity on MCF-7 cells, with an $IC_{50}$ value of 923.54 µg/mL. The findings suggest that black garlic exhibits promising antibacterial and anticancer potential, warranting further investigation for use as a functional food-based therapeutic adjunct.

Key words: Fermented (Black) Garlic, Bioactive compound, MTT, Breast cancer cells, Antimicrobial activity, Cytotoxicity

**Introduction**

Breast cancer remains the most commonly diagnosed cancer among women worldwide and a leading cause of cancer mortality despite advances in screening, molecular subtyping, and targeted therapies. In 2022, an estimated 2.3 million women were diagnosed with breast cancer, resulting in approximately 670,000 deaths globally, underscoring the persistent public health burden and the unmet need for complementary strategies that can prevent disease onset, curb progression, and overcome therapeutic resistance (Suthar, R., et al. (2023). Although the development of endocrine therapies, HER2-directed agents, and CDK4/6 inhibitors has improved outcomes in select subtypes, disparities in access and response persist across regions and demographics. Additionally, aggressive phenotypes such as triple-negative breast cancer (TNBC) lack broadly effective targeted therapies and are prone to early metastasis and chemo resistance. These challenges have intensified interest in bioactive food-derived agents that may modulate carcinogenic pathways with favorable safety and pharmacokinetic profiles, either as preventive nutraceuticals or as adjuvants to standard care.

Garlic (*Allium sativum* L.) has long been recognized for cardio metabolic, antimicrobial, and immunomodulatory properties attributed largely to its organosulfur constituents. “Black garlic” (BG) is produced by aging fresh garlic under controlled heat and humidity for several weeks, a process that triggers Maillard reactions and complex biochemical transformations (Zhang, W., et al. (2025). Compared with raw garlic, BG exhibits lower allicin but consistently higher levels of stable, water-soluble organosulfur compounds—most notably S-allyl-L-cysteine (SAC) and S-allylmercaptocysteine (SAMC)—alongside increased total phenolics, flavonoids, reducing sugars, and high-molecular-weight melanoidins formed during non-enzymatic browning (Geng, Z., Rong, Y., & Lau, B. (1997).

These compositional shifts correlate with enhanced antioxidant capacity and altered redox behavior relative to fresh garlic, suggesting a distinct pharmacology that may be advantageous for chronic disease applications including cancer prevention and therapy (Zhang, W., et al. (2025).

Process parameters (temperature, humidity, and duration) critically shape BG chemistry. Kinetic studies indicate that SAC rises markedly in the early phases of aging (e.g., around 7 days under specific fermentation/thermal conditions) and can decline with over-processing, highlighting the need for optimized manufacturing to standardize bioactive content (Zhang, W., et al. (2025).

Recent investigations of physicochemical quality show that higher temperatures and extended aging lower pH and elevate organic acids (with citric acid often predominant), while increasing total phenolic content and antioxidant indices; melanoidins isolated from BG demonstrate notable in vitro antioxidant and immunomodulatory activities (Wu, J.-F., et.al.,2023). These features, together with the aqueous solubility and oral bioavailability of SAC documented in pharmacokinetic studies, provide a rationale for exploring BG as a pragmatic dietary adjunct with translational potential in oncology (Sun, L., et al. (2025)..

Mechanistically, organosulfur compounds from garlic modulate multiple cancer-relevant signaling axes. SAC and related moieties have been shown to attenuate oxidative stress and inhibit activation of nuclear factor-κB (NF-κB), a master regulator of inflammation, survival, and metastasis; they also interface with PI3K/Akt and MAPK/ERK pathways, among others, thereby impacting proliferation, apoptosis, angiogenesis, and epithelial–mesenchymal transition (EMT) (Nguyen, T. T., et al. (2024). In preclinical cancer models, aged garlic extract (AGE)—a standardized preparation enriched for SAC—has exhibited anti-inflammatory and immunomodulatory effects (e.g., supporting NK-cell number and function), along with suppression of pro-inflammatory cytokines and pro-angiogenic mediators . BG's melanoidins further contribute antioxidant and potential immune-active functions, suggesting multi-constituent synergy that may overcome the limitations of single-target agents (Nguyen, T. T., et al. (2024).

Within the context of breast cancer, accumulating data indicate that BG and AGE can directly affect tumor cell phenotypes. Notably, aged/black garlic extracts suppress proliferation of estrogen receptor-positive (ER+) breast cancer cells, reduce migration and invasion, and enhance apoptosis—effects mechanistically linked to reactive oxygen species (ROS) generation, c-Jun N-terminal kinase (JNK) activation, and down regulation of the anti-apoptotic protein MCL-1 (Li, M., et al. (2023). In ER+ models (e.g., MCF-7 and MDA-MB-361), BG extract inhibited EMT markers and curtailed invasive behavior, aligning with transcriptional down-modulation of pro-survival and metastasis-associated pathways (Li, M., et al. (2023). Emerging investigations also point to anti-proliferative activity of *Allium sativum* fractions against TNBC cell lines (e.g., MDA-MB-231), a subtype with pressing therapeutic gaps [22].

While mechanisms are incompletely delineated in TNBC, plausible targets include NF-κB-driven inflammatory signaling, oxidative stress homeostasis, and PI3K/Akt-dependent survival circuits commonly hyperactive in this phenotype (MedNexus. (2024).

These anticancer properties align with broader evidence that BG and AGE counter inflammatory microenvironments conducive to tumor promotion. Preclinical and ex vivo data show reductions in TNF-α, IL-1β, and TGF-β–driven signaling, with downstream inhibition of NF-κB activation and related transcriptional programs (Chen, C,(2022). Such actions may be particularly relevant to breast tumorigenesis, where chronic inflammation, oxidative stress, and stromal remodeling foster immune evasion, angiogenesis, and metastatic competence. At the biochemical level, the redox-buffering and radical-scavenging capacity of BG's phenolics and melanoidins, together with modulation of glutathione-dependent defenses by organosulfur species, may rewire oxidative signaling thresholds that gate apoptosis versus survival in cancer cells (Choo, S., Lee, Y., & Lee, H. (2022).

An additional practical strength of BG-derived agents is the favorable pharmacokinetic and safety profile of SAC. Early and contemporary studies report high oral bioavailability with predictable metabolism (including N-acetylation and S-oxidation) and low toxicity across species, supporting its candidacy as a lead nutraceutical component and potential adjuvant (Choo, S (2022).

Standardization around SAC (and possibly SAMC) content could mitigate batch variability and enable dose–response work critical for translation. Moreover, preliminary reports suggest that garlic-derived constituents may sensitize cancer cells to chemotherapies (e.g., gemcitabine in other tumor types), raising the prospect of combination strategies to overcome resistance—an idea ripe for systematic testing in breast cancer models (Tesfaye A. (2021).

**Aim and Objectives:**

- To evaluate the cytotoxic effects of black garlic extracts on human breast cancer cell lines.
- To identify and quantify the bioactive compounds present in black garlic extracts.
- To compare the effectiveness of black garlic extracts with conventional chemotherapy agents.

## Materials and Methods

### Plant Material Collection and Extraction

Fresh cloves of *Allium sativum* L. (black garlic) were obtained from a certified supplier of commercially aged garlic. The botanical identity of the plant material was confirmed by a qualified botanist, and a voucher specimen was retained for reference. Only clean, uniform cloves were selected for processing. The cloves were rinsed thoroughly with distilled water, air-dried, and further dehydrated in a hot-air oven at 40 °C to remove residual moisture. Dried samples were coarsely ground using a mechanical grinder and stored in airtight containers, protected from light and humidity to preserve phytochemical integrity.

For extraction, 100 g of the powdered black garlic was macerated in 500 mL of 95% ethanol and heated at 70–80 °C for 24 h with intermittent stirring to ensure efficient extraction of phytoconstituents. Ethanol was selected for its high efficiency in extracting polar and semi-polar compounds, including phenolics, flavonoids, and organosulfur compounds (Harborne, 1998). The mixture was filtered through Whatman No. 1 filter paper, and the residue was re-extracted under the same conditions. Filtrates were pooled and concentrated under reduced pressure using a rotary evaporator at 45 °C. The crude extract was further dried in a vacuum desiccator and stored in amber-colored vials at 4 °C until use. Extraction yield was calculated as:

$$\text{Yield (\%)} = (\text{Weight of dried extract} / \text{Weight of initial plant material}) \times 100$$

### Phytochemical Screening

Qualitative phytochemical screening of the ethanolic black garlic extract was performed following standardized methods (Harborne, 1998; Trease & Evans, 2002). Tests were conducted in triplicate to ensure reproducibility.

- Alkaloids (Mayer's Test): Extract (1 mL) was treated with Mayer's reagent; cream-colored precipitate indicated alkaloids.

- Flavonoids (Lead Acetate Test): Extract (1 mL) mixed with lead acetate produced yellow precipitate.
- Tannins and Phenolics (Ferric Chloride Test): Extract treated with 5% ferric chloride produced blue-black/green coloration.
- Steroids (Salkowski Test): Extract mixed with chloroform and concentrated $H_2SO_4$ produced reddish-brown interface.
- Terpenoids (Liebermann–Burchard Test): Extract treated with acetic anhydride and conc. $H_2SO_4$ produced blue-green coloration.
- Carbohydrates (Molisch's Test): Extract mixed with Molisch's reagent and conc. $H_2SO_4$ produced purple ring.

**Proteins and Amino Acids:**

Biuret Test: Violet coloration after Biuret reagent addition indicated proteins.

Ninhydrin Test: Blue/violet coloration indicated amino acids.

**Antibacterial Activity Assay**

Antibacterial activity was evaluated using well-characterized reference strains of *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive), sourced from a certified microbiology laboratory. These strains were maintained on nutrient agar slants at 4 °C. For assay preparation, overnight cultures were generated by inoculating the bacterial strains into nutrient broth and incubating at 37 °C for 18 to 24 hours. The resulting cultures were adjusted for turbidity to match a 0.5 McFarland standard, corresponding to approximately 1×1081×108 CFU/mL. To assess antibacterial efficacy, normal-aged (NG) and fermented (FG) black garlic extracts were dissolved in either sterile distilled water or DMSO and tested at 25 µL, 50 µL, and 100 µL concentrations.

The experiment employed the agar well diffusion method on Mueller-Hinton Agar (MHA). MHA plates were prepared, each containing roughly 25 mL of medium. The standardized bacterial suspension (100 µL) was spread uniformly across the agar surface using sterile swabs. Aseptic technique was used to punch 6 mm diameter wells into the agar. Into these wells, the garlic extracts were dispensed in the specified concentrations. Controls were included: negative controls utilized DMSO or sterile water, while positive controls consisted of chloramphenicol for *E. coli* and clindamycin for *S. aureus*. The plates were incubated at 37 °C for 24 hours, after which zones of inhibition were measured in millimeters and compared against controls to determine the antibacterial effectiveness. Cytotoxicity assessment on MCF-7 cells followed appropriate cell viability protocols after the antibacterial assay

**Cell Lines and Culture**

Human breast cancer cell line MCF-7 was procured from the National Centre for Cell Science (NCCS, Pune, India). Cells were cultured in Eagle's Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution (Himedia, India). Cultures were incubated at 37 °C in a humidified atmosphere containing 5% $CO_2$ and routinely sub-cultured every 3 days.

**MTT Assay**

The cytotoxic effect of the black garlic extract on human breast cancer cells (MCF-7) was assessed using the MTT assay, as described by Mosmann (1983) with minor modifications. Briefly, MCF-7 cells were seeded at a density of 20,000 cells per well in 96-well culture plates and incubated for 24 h at 37 °C in a humidified 5% $CO_2$ atmosphere to allow cell attachment.

Following incubation, the culture medium was replaced with fresh medium containing different concentrations of the black garlic extract. The treated cells were further incubated for 48 h under identical conditions. After treatment, the medium was carefully removed, and 20 µL of MTT solution (0.5 mg/mL in PBS) was added to each well. Plates were covered with aluminum foil to prevent light exposure and incubated for an additional 3 h to facilitate the formation of purple formazan crystals by metabolically active cells.

The MTT-containing medium was then aspirated, and 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. Complete solubilization was ensured by gentle shaking for 10 min. Absorbance was measured at 570 nm using a microplate reader (Epoch2, BioTek, USA). The percentage of viable cells was calculated according to the following formula:

%Viability= (OD of control cells / OD of treated cells) ×100

**RESULTS AND DISCUSSION**

**Phytochemical Screening**

Phytochemical screening is a crucial step in identifying the bioactive components present in plant extracts, which could contribute to their pharmacological properties such as antioxidant, antimicrobial, and anticancer activities. The ethanolic extract of black garlic (*Allium sativum* ) was subjected to standard qualitative tests to detect various classes of secondary metabolites.

Table : Phytochemical Constituents Detected in Ethanolic Extract of Black Garlic

| **Phytochemical Group** | **Test Used** | **Inference** |
|---|---|---|
| Alkaloids | Mayer’s test | + |
| Flavonoids | Lead acetate test | + |
| Tannins & Phenolics | Ferric chloride test | + |
| Steroids | Salkowski test | + |
| Terpenoids | Liebermann–Burc hard test | + |
| Carbohydrates | Molisch’s test | + |
| Proteins & Amino Acids | Biuret and Ninhydrin tests | + |

The qualitative phytochemical analysis of the ethanolic extract of *Allium sativum* (black garlic) revealed the presence of several bioactive secondary metabolites, each known to contribute significantly to the plant's therapeutic efficacy. Alkaloids were detected using Mayer’s test, indicated by the formation of a cream- colored precipitate. Their presence is significant as alkaloids possess strong biological activities, including antimicrobial, antimalarial, and cytotoxic effects, and many FDA- approved anticancer drugs are derived from alkaloid structures (Edeoga *et al.,*2005). These compounds are known to interfere with DNA and enzyme function in both microbial and cancer cells.

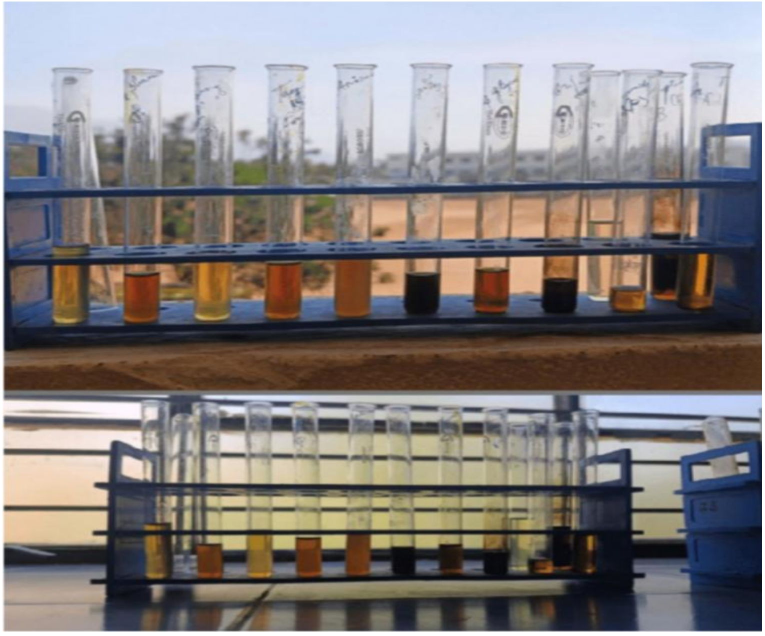


Fig. 1: Phytochemical screening

The extract also tested positive for flavonoids, as evidenced by the formation of a yellow precipitate in the lead acetate test. Flavonoids are well-documented antioxidants that neutralize reactive oxygen species (ROS), modulate cell signaling pathways such as PI3K/Akt, and exhibit anti-inflammatory and anticancer effects (Kumar & Pandey, 2013). Their presence in black garlic supports its reported health benefits, including its protective effects against oxidative stress and cancer.

Additionally, tannins and phenolic compounds were present, indicated by the blue-green coloration upon reaction with ferric chloride. These compounds play crucial roles in plant defense mechanisms and are known to inhibit microbial enzymes, chelate metal ions, and block oxidative chain reactions (Balasundram *et al.,*2006). Their ability to reduce oxidative stress and microbial virulence suggests their relevance in both antibacterial and anticancer contexts.

The Salkowski test confirmed the presence of steroids in the extract, observed by the formation of a reddish-brown ring. Steroids, including phytosterols, are known to stabilize cell membranes and possess anti-inflammatory properties. Certain plant steroids have shown estrogenic activity, which is particularly relevant in hormone-sensitive cancers such as breast cancer (Senthilkumar *et al.,*2020).

Furthermore, terpenoids were detected via the Liebermann–Burchard test, where a reddish-to-pink coloration was observed. Terpenoids are a diverse class of compounds that exhibit anticancer effects by promoting apoptosis and inhibiting angiogenesis and cell proliferation (Gutiérrez-del-Río *et al.,*2020).

The presence of these compounds may contribute to the cytotoxic effects observed in the MTT assay on MCF-7 cells.

Carbohydrates were confirmed by a violet ring at the interface in the Molisch's test. While carbohydrates are generally known as energy sources, certain complex polysaccharides in medicinal plants also exhibit immunomodulatory and anticancer activities by enhancing natural killer cell activity and stimulating cytokine production (Sofowora, 1993). Their presence may enhance the nutritional and therapeutic value of black garlic as a functional food.

Finally, proteins and amino acids were detected using Biuret and Ninhydrin tests, evidenced by the appearance of violet and blue colors respectively. These biomolecules play vital roles in cellular function, including enzyme activity, structural integrity, and immune response. In some cases, bioactive peptides derived from plant proteins exhibit anticancer and antimicrobial properties (Bhagya & Chandrashekar, 2020).

Overall, the phytochemical profile of black garlic confirms the presence of multiple classes of bioactive compounds with known pharmacological relevance. These constituents collectively contribute to its antioxidant, antimicrobial, and anticancer potential, supporting its use in traditional medicine and highlighting its relevance in modern therapeutic research. The combination of sulfur-containing amino acids, flavonoids, and phenolics in particular, offers a potent matrix of compounds that may act synergistically in preventing and combating chronic diseases, including breast cancer.

**MTT Assay Results**

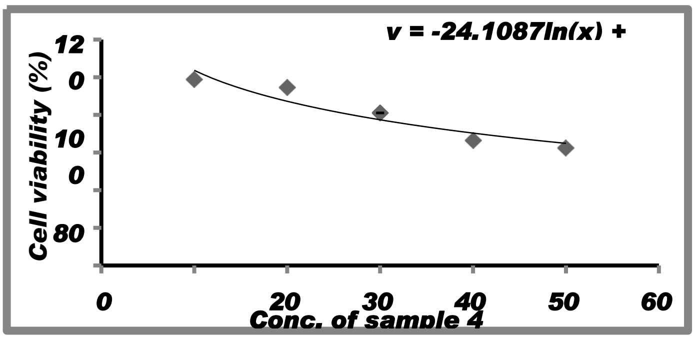
The cytotoxic effect of black garlic ethanol extract (Sample 4) on MCF-7 human breast cancer cells was assessed using the MTT assay. The assay evaluates cell metabolic activity as an indicator of cell viability, proliferation, and cytotoxicity. After 48 hours of exposure to varying concentrations of the extract, a dose-dependent decrease in cell viability was observed.

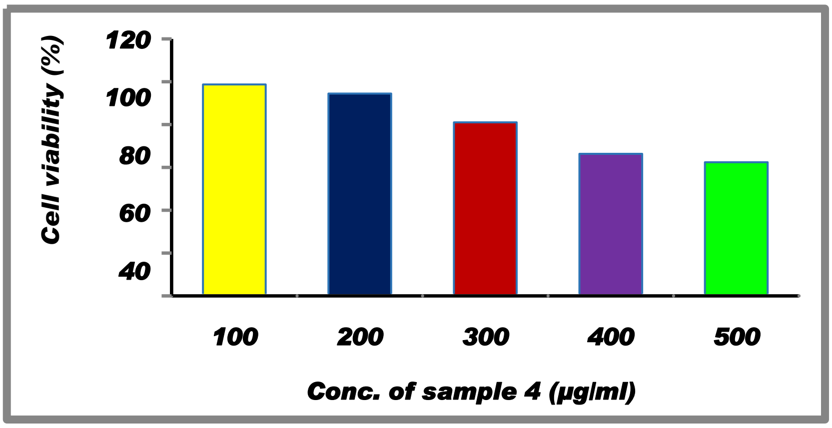
Cell Viability Data

| Concentration (µg/mL) | % Cell Viability |
|---|---|
| Control (Untreated) | 100.00 |
| 100 µg/mL | 98.74 |
| 200 µg/mL | 94.48 |
| 300 µg/mL | 81.04 |
| 400 µg/mL | 66.34 |
| 500 µg/mL | 62.41 |

The calculated $IC_{50}$ value was 923.54 µg/mL, indicating moderate cytotoxicity. The ethanolic extract of *Allium sativum* (black garlic) was tested for cytotoxic potential against MCF-7 human breast cancer cells using the MTT assay. The results revealed a dose-dependent decrease in cell viability, indicating that black garlic extract exerts a measurable cytotoxic effect on cancer cells. At the lowest tested concentration of 100 µg/mL, the extract maintained high cell viability (98.74%), while at 200 µg/mL and 300 µg/mL, cell viability dropped to 94.48% and 81.04%, respectively. More pronounced cytotoxicity was observed at 400 µg/mL (66.34%) and 500 µg/mL (62.41%). The calculated $IC_{50}$ value was 923.54 µg/mL, suggesting moderate cytotoxic potential.

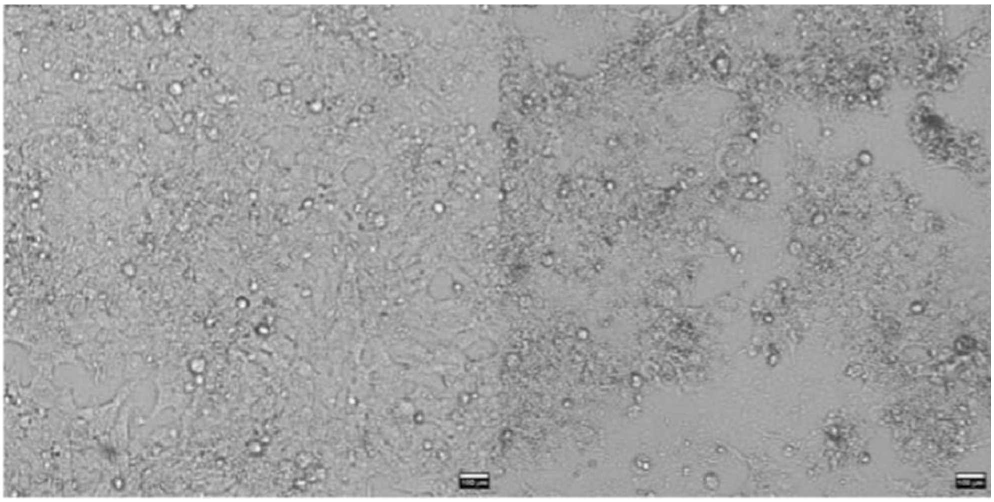
**Fig.2** : In vito anticancer activity of ethanolic extract of *Allium sativum* against breast cancer cell line MCF-7





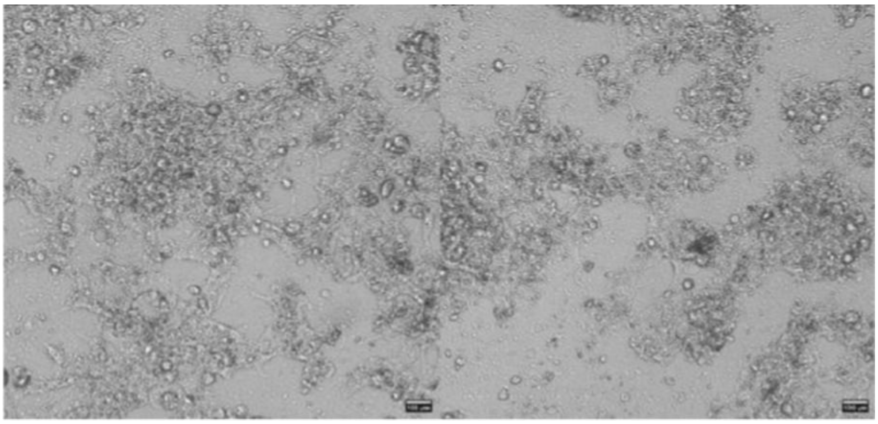

These results align with earlier reports on black garlic's anticancer properties, which are attributed to its enriched composition of bioactive compounds such as S-allyl cysteine, diallyl disulfide, polyphenols, and flavonoids (Kimura *et al.,*2017). The fermentation of garlic increases the concentration and bioavailability of these compounds, enhancing their pharmacological properties including antioxidant, anti- inflammatory, and anticancer effects (Sung *et al.,*2021). The observed reduction in MCF-7 cell viability can be attributed to the interference of these compounds with cellular pathways responsible for proliferation and survival. For instance, S-allyl cysteine has been reported to induce apoptosis via mitochondrial pathways and oxidative stress modulation (Amagase, 2006).

Although the $IC_{50}$ value was higher than that of potent chemotherapeutic drugs, the moderate cytotoxicity of the black garlic extract suggests that it may serve as a potential adjunct in breast cancer therapy, especially given its low toxicity and dietary origin. These findings are in agreement with a study by Zhang et al. (2018), which reported that black garlic extract selectively induced cytotoxicity in breast cancer cells while exhibiting minimal effects on normal mammary epithelial cells. This selective cytotoxicity is beneficial in reducing the collateral damage often associated with chemotherapy.



100 μg 200 μg

The MTT results also suggest that the whole extract may be less potent than isolated bioactive compounds. Therefore, future studies involving fractionation and purification could enhance efficacy. Moreover, combining black garlic extract with standard anticancer agents could potentially produce synergistic effects, reduce required dosages, and minimize side effects (Lee *et al.,*2012). The regression analysis of the dose-response curve in the present study showed a strong inverse correlation between extract concentration and cell viability ($R^2$ = 0.8892), further supporting the reliability of the data.



300 μg 400 μg

While the extract demonstrated moderate efficacy in vitro, it is essential to corroborate these findings through additional in vitro mechanistic studies and in vivo experiments. Assessments of apoptosis markers, oxidative stress indicators, and cell cycle analysis would provide greater insight into the pathways modulated by black garlic constituents (Choi *et al.,*2023). The overall findings suggest that black garlic may hold therapeutic potential as a functional food-based adjuvant in the management of breast cancer, promoting health through bioactive nutritional compounds with minimal toxicity

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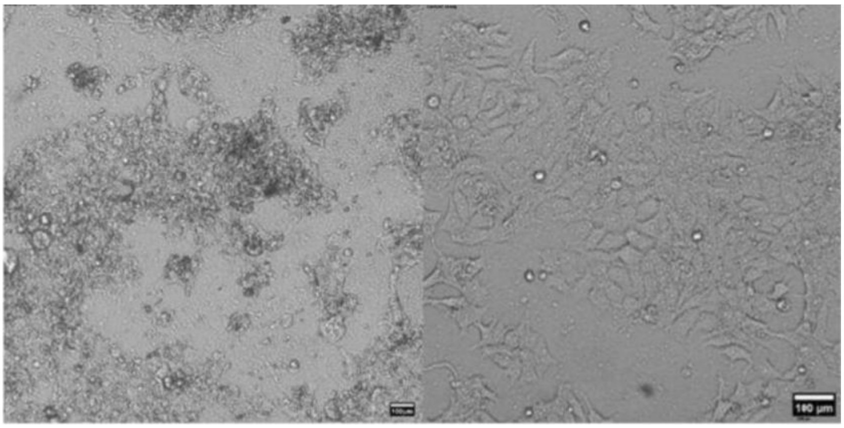


500 µg control

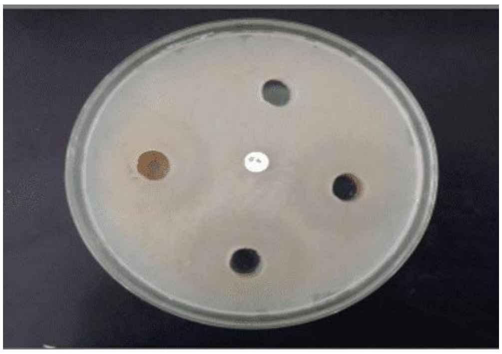
Antibacterial Activity

The antibacterial efficacy of two different black garlic extracts—NG (normal-aged garlic) and FG (fermented black garlic)—was evaluated against two clinically significant bacterial strains: *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). The antimicrobial assay was conducted using the agar well diffusion method, and the zones of inhibition were measured at three concentrations (25 µL, 50 µL, and 100 µL).

Table : Zone of Inhibition (in mm) of Black Garlic Extracts Against *E. coli* and *S. aureus*

| **Bacteria** | **Control (No zone)** | **Antibiotic Disc (12 mm)** | **25 µL** | **50 µL** | **100 µL** |
|---|---|---|---|---|---|
| | | | | | |
| | | **NG Extract** | | | |
| *E. coli* | No zone | Chloramphenicol – 12 mm | 14 mm | 18 mm | 20 mm |
| *S. aureus* | No zone | Clindamycin – 12 mm | 15 mm | 16 mm | 17 mm |
| | | | | | |
| | | **FG Extract** | | | |
| *E. coli* | No zone | Chloramphenicol – 12 mm | 15 mm | 16 mm | 17 mm |
| *S. aureus* | No zone | Clindamycin – 12 mm | 16 mm | 17 mm | 19 mm |

The results clearly indicate that both NG and FG extracts of black garlic possess dose-dependent antibacterial activity against *E. coli* and *S. aureus*. However, the spectrum and magnitude of inhibition varied between the two extracts and the tested bacterial specie.



Zone of inhibiton (mm)

The results clearly indicate that both NG and FG extracts of black garlic possess dose-dependent antibacterial activity against *E. coli* and *S. aureus*. However, the spectrum and magnitude of inhibition varied between the two extracts and the tested bacterial species.

For *E. coli*, the normal-aged black garlic (NG) showed a stronger antibacterial effect, with zones of inhibition increasing from 14 mm at 25 µL to 20 mm at 100 µL. In contrast, the fermented garlic extract (FG) showed slightly lower activity, with a maximum inhibition zone of 17 mm at the highest concentration. This suggests that while both extracts are effective, the NG extract may contain active compounds in higher concentration or with greater efficacy against Gram-negative organisms.

In the case of *S. aureus*, however, the FG extract exhibited superior activity, reaching up to 19 mm at 100 µL compared to 17 mm for NG extract. This observation may be attributed to the fermentation process, which enhances the concentration and bioavailability of specific sulfur-containing compounds such as S-allyl cysteine and organosulfur metabolites known for their bacteriostatic properties (Kimura *et al.,*2017).

Interestingly, both extracts exceeded the inhibition diameters of the standard antibiotic discs used for comparison (chloramphenicol and clindamycin, both 12 mm), particularly at higher doses. This highlights the potential of black garlic as a natural antimicrobial agent with activity comparable or superior to conventional antibiotics in certain contexts.

The mechanism of antibacterial action of black garlic is thought to involve disruption of bacterial cell walls, interference with DNA and protein synthesis, and oxidative stress induced by reactive sulfur species and polyphenolic compounds (Gutiérrez-del-Río *et al.,*2020). Moreover, the antimicrobial potential of garlic has been reported to be influenced by the extraction solvent, storage conditions, and processing methods, with aged and fermented garlic often exhibiting increased stability and activity (Kim *et al.,*2021).

The differential response between *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) may also be explained by differences in cell wall structure. Gram-negative bacteria have an outer membrane that can hinder the penetration of certain phytochemicals, while Gram-positive bacteria, lacking this barrier, may be more susceptible to these compounds (Sánchez *et al.,*2018).

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