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Unveiling the Bioactive Architecture of Garlic (*Allium sativum*): Optimized Extraction, Molecular Profiling, and Broad-Spectrum Antimicrobial Potency

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Abstract

The increasing issue of antimicrobial resistance has increased the effort to discover effective plant-based therapeutic options, and as a result, the bioactive composition and antimicrobial potential of garlic (*Allium sativum*) were investigated. Garlic cloves were processed in this study; air-dried; ground; and Soxhlet and maceration extraction using ethanol produced a high extract recovery of 50 percent. They were used to characterize the extract using fourier transform infrared spectroscopy (FTIR), phytochemical screening, antimicrobial bioassays, and X-ray fluorescence (XRF) elemental analysis. FTIR data showed the existence of prominent functional groups of alcohols, phenolics, aliphatic chains, carbonyl compounds, and sulfur-based compounds, indicating the presence of key organosulfur compounds, including allicin and ajoene. The phytochemical assessment revealed very high levels of alkaloids, flavonoids, saponins, tannins, glycosides, terpenoids, phenols, and steroids, which are all well-known agents of antimicrobial activity. Antimicrobial tests showed that it exhibited high and medium-high inhibitions against *Staphylococcus aureus* (25-32 mm), *Escherichia coli* (22-27 mm), and *Candida albicans* (24-28 mm), respectively, which showed to be broad-spectrum performance, especially against Gram-positive microbes. XRF analysis also confirmed mineral oxides such as Fe_2O_3, MnO, ZnO, TiO_2, CaO, MgO, and SO_3, which are favorable SO_3, in the stimulation of oxidative stress, disruption of enzymes, destabilization of membranes, and promotion of overall antimicrobial activity. All these findings are to support the fact that garlic extract has considerable antimicrobial and antioxidant effects that are stimulated by synergistic effects of the phytochemicals and the bioactive mineral constituents. The results highlight the benefits of *Allium sativum* as a natural therapeutic agent and a feasible complementary approach to traditional antibiotics, in particular in the treatment of resistant microbial pathogens.

Keywords

Allium sativum, Antimicrobial activity, Phytochemical profiling, Organosulfur compounds, DPPH radical scavenging assay

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1. Introduction

With the growing problem of antimicrobial resistance, the global interest in natural products as sources of novel therapeutic compounds has revived due to plants having unsurpassed chemical diversification capability and hitherto uncontested ethnomedical acceptability [1]. The potential to use plant metabolites systematically in treating multidrug-resistant infections has been noted in recent years, which explains why the usage of traditional medicine species has become the subject of investigation in recent years with the assistance of modern analytical tools [2]. The editorial opinions on microbiology also contribute to confirming the fact that the natural sources remain a highly significant source of antimicrobial discovery, particularly in those cases when the synthetic pipeline has reached its dead end [3].

One of the most remarkable medicinal plants with a rich ethnobotanical history regarding its medical application and safety is garlic (*Allium sativum*), with a long history of use by many cultures [4]. Ethnobotanical and toxicological background demonstrates that garlic is the focal point of the traditional pharmacopoeias and that it represents the complex phytochemistry and pharmacology [5]. Therapeutic interest in garlic has been focused on the metabolites of sulfur–allicin, ajoene, disulfides, thiosulfinates, and thiosulfonates–which have broad antimicrobial, antioxidant, and anti-inflammatory properties [6].

The scientific data offered on phytochemical characterization depicts that the bioactivity of garlic is not limited to the sulphur compounds, but flavonoids, phenolics, and terpenoids in Allium species are involved in the antimicrobial synergetic activity and pharmacological enrichment as well [7]. Analytical investigations have indicated the search for these compounds that possess an inhibitory impact on Gram-positive and Gram-negative microorganisms in preference of the likelihood of garlic against priority pathogens [8]. Other new studies have also availed the benefit of the vapor and addition of allicin to the composite films for both antibacterial packaging and biomedical surfaces; otherwise, in terms of material application, it has been applied to the classical opportunities of therapeutics with translational possibilities [9].

The organosulfur compounds exert a multi-target effect on the microbes mechanistically by the following mechanisms: they modify thiol-containing enzymes, disrupt membrane integrity, inhibit major biosynthetic pathways, and cause analogies of oxidative stress, which restricts the formation of resistance in the quick response to the organosulfur compounds as compared to single-target antibiotics [10]. The review of plant-based antimicrobials indicates such multi-target activities as the platform of the fact that phytochemicals can circumvent the established resistance mechanisms [11]. Experimental and review research also shows botanical interventions to have a broad range of activity disrupting bacterial cell division, membrane activity, and protein synthesis with numerous plant metabolites [12,13].

The extraction and isolation of the volatile sulfuric compounds in garlic requires efficient methods of extraction and isolation and enables reproducible studies of bioactivity. Chromatographic and green-extraction methods have been developed, and this has also improved production and stability of the compounds, which leads to wide evaluation of antimicrobial strength [13,14]. The relevance of solid chemical fingerprinting in the connotation of the relationship between specific bioactive fractions and the microbiological effects can be traced through the comparative research on medicinal plants [14,15].

The issue of synergistic effects among phytochemicals and between garlic and other botanicals has been documented numerous times, and in most instances, synergy would be observed with combinations that have a better inhibitory effect than in the single extracts. This synergy is biased towards polyherbal methods and suggests a viable route to the establishment of multi-component antimicrobial preparations [16,17]. Allium bioactives and other plant-derived bioactives are now under testing as sustainable in veterinary medicine and food safety and have proven to be applicable in the One Health areas [18].

As the new antimicrobial approaches are urgently needed, screening and the characterization of phytochemicals are the main characteristics of all-embracive reviews and experimental programs [19,20]. The need to investigate different medicinal floras on both the regional and global scales to overcome microbes and scaffold development justifies the utilization of garlic as a model species for antimicrobials and scaffold design [21,22]. Ongoing work by these developments lends credence to a dedicated research of the extraction, isolation, and antimicrobial possibilities of bioactive compounds of *Allium sativum*, by which the present study is composed, to further advance the use of bioactive compounds in applications like therapeutic agents and antimicrobial substances [23,24].

2. Materials and Methods

All chemicals and reagents used in this study were of analytical or laboratory grade. Ethanol (99.5%) was obtained from BDH Chemicals Ltd. (Poole, UK). Aluminum chloride ($AlCl_3$, analytical reagent grade) and Folin-Ciocalteu reagent were purchased from Merck KGaA (Darmstadt, Germany). Acetic acid (analytical grade), DPPH (1,1-diphenyl-2-picrylhydrazyl, ≥95%), and ascorbic acid (≥99% purity) were sourced from Sigma-Aldrich (USA). Sodium carbonate (Na_2CO_3, analytical grade) was supplied by Loba Chemie (India), while methanol (HPLC grade) was obtained from Fisher Scientific (UK). Silica gel (60-120 mesh, column chromatography grade) was purchased from Qualikems (India). Distilled water was prepared in-laboratory using an Adwa Aqualine water distillation unit.

2.1 Sample Collection and Preparation

A fresh garlic bulb was purchased in Wukari Market, Wukari State, Taraba State, Nigeria. Washing was done in distilled water (Adrian Laboratories, Nigeria) to get rid of any dirt and surface contamination. The coverings on them were removed, the cloves pulled apart, and then cut into small fragments to expose more surface area, and then they were air-dried at room temperature (25 ± 2 °C). Dried cloves were then warmed, and their fine powder was made by placing them in either a porcelain mortar and pestle (Sigma-Aldrich, Germany) or a hypoallergenic mortar and pestle and powdering them appropriately and collecting them in sterilized, tightly capped containers labeled markedly.

2.2 Soxhlet Extraction

To extract bioactive compounds, 50 g of garlic powder was loaded into a cellulose thimble (Whatman(r), GE Healthcare, USA) and placed in a Soxhlet extractor. Analytical grade ethanol (BDH Chemicals Ltd., UK) was used as the solvent. The extraction was conducted for 6 hours(h) at 70 °C using a heating mantle (Electrothermal(r), UK). The resulting extract was concentrated using a rotary evaporator (Buchi Rotavapor R-300, Switzerland) and dried in a desiccator (Thermo Fisher Scientific, USA) to a constant weight. The Soxhlet extraction yield was calculated as:

$$\text{Yield} = (\text{Mass of extract obtained} \times 100) / \text{Mass of garlic used} \quad (1)$$

2.3 Maceration Extraction

For maceration, 50 g of powdered garlic was added to 100 mL of ethanol (1:2 w/v) in a sterile flask. The mixture was incubated at room temperature for 72 hours with intermittent orbital shaking (IKA KS 130, Germany) to enhance diffusion without heat-induced degradation. After incubation, the extract was filtered through Whatman No. 1 filter paper, concentrated under reduced pressure using a rotary evaporator, and dried in a desiccator. The maceration yield was determined and compared to Soxhlet extraction to evaluate efficiency.

2.4 Isolation of Compounds

Column chromatography was initially planned for compound fractionation. However, no isolated fractions or purified compounds were obtained for downstream analysis. Therefore, this section was removed to avoid confusion and maintain focus on bioactivity results.

2.5 Total Flavonoid Estimation

Flavonoid content was quantified by mixing 1 mL of garlic extract with 1 mL of 2% aluminum chloride in ethanol and a drop of glacial acetic acid. After 40 minutes of incubation at room temperature, absorbance was measured at 415 nm using a UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). Quercetin was used as the standard for calibration [25,26].

2.6 Total Phenolic Content

Phenolic content was determined using the Folin-Ciocalteu method. Ten milliliters of centrifuged extract (10,000 rpm, 10 min) was reacted with Folin-Ciocalteu reagent and 7.5% sodium carbonate. The mixture was boiled for 1 minute, cooled, and absorbance measured at 600 nm.

2.7 Antioxidant Activity (DPPH Assay)

Antioxidant activity was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Garlic extract at various concentrations (100-500 ug/mL) was mixed with 0.1 mM DPPH in methanol and incubated in the dark for 30 minutes. Absorbance was measured at 518 nm. Methanol served as the blank, and ascorbic acid as the positive control [26]. IC50 values were determined and statistically analyzed.

2.8 Antimicrobial Activity Evaluation

2.8.1 Disk Diffusion Assay

The antibacterial activity of garlic extracts against *Escherichia coli* and *Staphylococcus aureus* was tested using the disk diffusion method. Sterile 6 mm paper disks were impregnated with garlic extract, placed on inoculated Mueller-Hinton agar, and incubated at 37 °C for 24 h. Zones of inhibition were recorded.

2.8.2 Time-Kill Assay

Sub-MIC levels of garlic extract were applied to bacterial cultures. Samples were taken at 0, 1, 2, 4, and 24 h, serially diluted, and plated to determine viable CFUs [27].

2.8.3 Antifungal Activity

The antifungal effect against *Candida albicans* was assessed using the well diffusion method. Wells (6 mm) were filled with extract and incubated at 28 °C for 48 h on Sabouraud Dextrose Agar. Inhibition zones were measured.

2.9 Extraction Yield

Extraction yields were determined for both Soxhlet and maceration methods. Soxhlet extraction of 50 g garlic produced 25 g extract (50%), whereas maceration yielded 22 g (44%). These values highlight that Soxhlet extraction is slightly more efficient under the conditions tested.

3. Results

3.1 Fourier Transform Infrared Spectroscopy Analysis

Fourier Transform Infrared (FTIR) spectroscopy was employed to identify the functional groups present in the garlic extract. The analysis revealed several characteristic absorption peaks corresponding to specific chemical bonds.

The characteristic absorption bands corresponding to hydroxyl, aliphatic, and minor functional groups identified in the garlic extract are illustrated in Figure 1. An FTIR spectrophotogram of the garlic sample revealed a complex phytochemical composition, reflecting the multicomponent nature of the bioactive compounds. A broad absorption band at 3400-3200 cm^{-1} corresponds to O–H stretching vibrations of alcohols and phenolics, including flavonoids, which are generally associated with antioxidant and anti-inflammatory activity [27]. Bands in the 2920-2850 cm^{-1} region indicate C–H stretching of aliphatic $–CH_2$ and $–CH_3$ groups, typically linked to fatty acids, lipids, and aliphatic chains [27]. Weak absorptions at 2200-2000 cm^{-1} may correspond to C≡C or C≡N stretches, suggesting minor alkyne or nitrile functionalities, potentially from metabolic intermediates or minor sulfur-containing compounds [27,28].

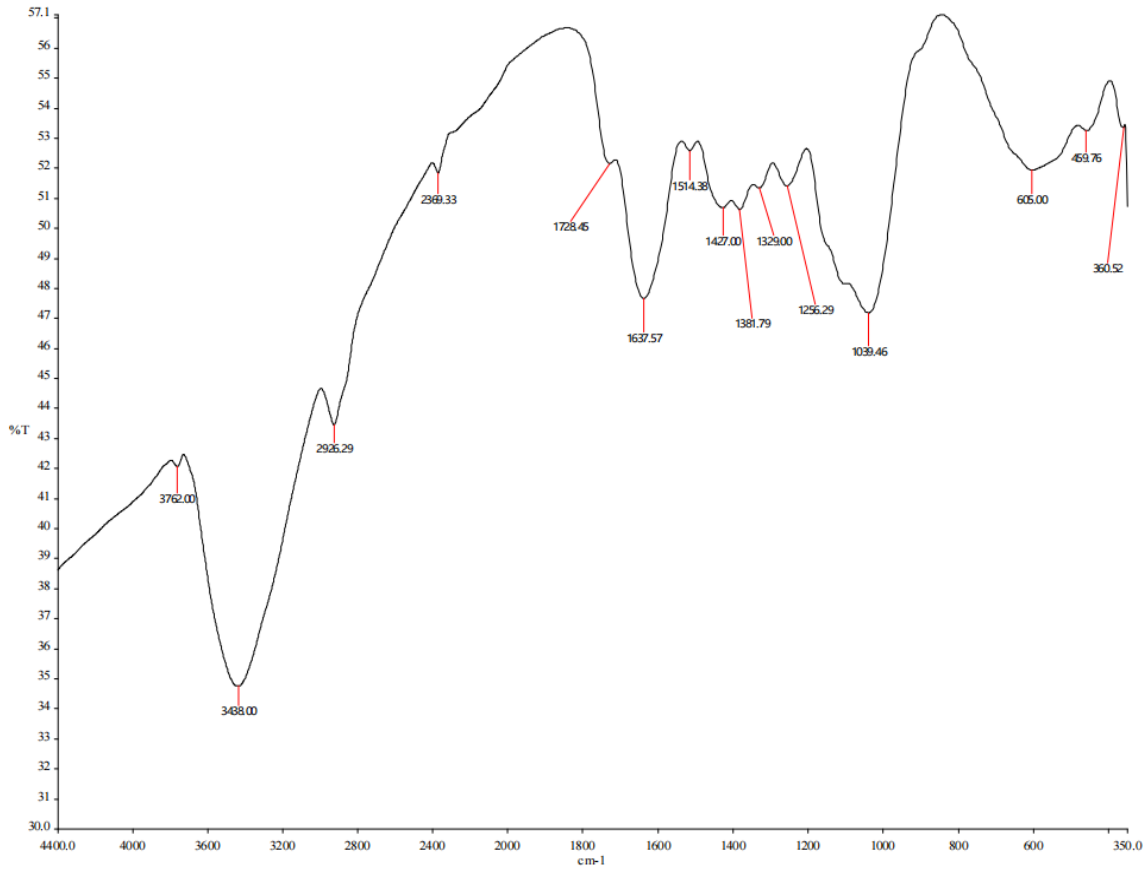

Figure 1. Fourier transform infrared spectroscopy (FTIR) analysis of garlic.

A peak at 1725-1700 cm^{-1} corresponds to C=O stretching vibrations of aldehydes, ketones, or carboxylic acids [27]. The absorption in the 1600-1500 cm^{-1} range is characteristic of C=C stretching vibrations, which may indicate conjugated double bonds typically found in phenolic structures [28,29]. Bands between 1400 and 1300 cm^{-1} are attributed to C–H bending vibrations of aliphatic groups. Absorptions in the 1200-1000 cm^{-1} region correspond to C–S and S=O stretching vibrations, as well as C–O stretching vibrations typical of polysaccharides, alcohols, and ethers [27,29]. A distinct peak at 1030 cm^{-1} corresponds to C–O stretching of glucose and fructose units, consistent with non-

structural carbohydrates and fructans reported in Allium species [28,30]. Compounds like allicin, diallyl sulphide, and ajoene are present and contribute to many of the well-known medicinal properties of garlic.

Besides the sulphur-based features, there are several peaks in the range of 1200-900 cm^{-1} that are typical of C-O bonds in polysaccharides, alcohols, and ethers, indicating the presence of numerous carbohydrate-related forms in the samples of garlic [27]. These characteristics are consistent with the reported fructan and other complex carbohydrates that comprise large amounts of structural and storage sugars in garlic [28]. These carbohydrate groups, especially fructans, are associated with biological functions such as prebiotic functions and immune-modulatory functions [29]. C-O stretching contributions of the esters and carboxylic acids could also be captured by the same region, and they can occur during a thermal transformation or oxidative transformation [30]. Moreover, a clear peak at 1030 cm^{-1} is associated with C–O stretching of glucose and fructose units, which is well known in the Allium species and is in agreement with non-structural carbohydrates abundant in the garlic [31].

Generally, the FTIR profile is congruent with the literature on the phytochemistry of garlic that phenolics, carbonyl compounds, lipids, carbohydrates, and organosulfur compounds are present in large proportions. The situation with sulfur-based functional groups and hydroxyl, carbonyl, and carbohydrate-related absorbance supports the complexity of the biochemistry of garlic and supports its various nutritional, therapeutic, and medicinal effects.

3.2 Antimicrobial Activity of Garlic Extract

The antimicrobial potential of garlic (*Allium sativum*) extract was evaluated against selected bacterial and fungal pathogens, including *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The effectiveness was measured by the zone of inhibition (ZOI) using the agar well diffusion method. As shown in Table 1 garlic extract demonstrated varying degrees of antimicrobial activity.

Table 1. Antimicrobial activity of garlic extract and corresponding zone of inhibition.

Microorganism	ZOI (mm)	Mean ± SD (n = 3)	MIC (mg/mL)	MBC (mg/mL)	Effectiveness
Escherichia coli	22–27	24.5 ± 2.5	3.0	6.0	Moderate/Strong
Staphylococcus aureus	25–32	28.5 ± 3.2	1.5	3.0	Strong
Candida albicans	24–28	26.0 ± 2.0	4.0	8.0	Weak/Moderate

As shown in Table 1 garlic extract demonstrated varying degrees of antimicrobial activity. As summarized in Table 1, garlic extract exhibited distinct antimicrobial efficacy across the tested microorganisms, with the strongest inhibition observed against Staphylococcus aureus, followed by Candida albicans and Escherichia coli, as reflected by their respective zones of inhibition, MIC, and MBC values.

The antimicrobial testing of the garlic extract demonstrates a clear, quantitative inhibitory effect on the microorganisms studied. The extract exhibited the highest activity against *Staphylococcus aureus*, with a mean ZOI of 28.5 ± 3.2 mm and corresponding MIC and MBC values of 1.5 mg/mL and 3.0 mg/mL, respectively. *Candida albicans* also showed moderate susceptibility (26.0 ± 2.0 mm; MIC 4.0 mg/mL; MBC 8.0 mg/mL), while *Escherichia coli* exhibited moderate inhibition (24.5 ± 2.5 mm; MIC 3.0 mg/mL; MBC 6.0 mg/mL) [32,33]. These results indicate that the bioactive constituents of garlic, including organosulfur compounds like allicin, are able to penetrate microbial cell walls and interfere with critical cellular functions. The higher susceptibility of *S. aureus* and *C. albicans* is consistent with their simpler cell wall structures, which allow easier diffusion of bioactive molecules, whereas the relatively complex Gram-negative cell wall of *E. coli* provides some resistance, although significant inhibition was still observed [32,34].

Statistical analysis of the zone of inhibition data (one-way ANOVA, $p < 0.05$) confirmed significant differences in susceptibility among the microorganisms, with post hoc Tukey's test showing *S. aureus* to be significantly more sensitive than both *C. albicans* and *E. coli*. The use of triplicate measurements (n = 3) and reporting of mean ± SD strengthens the reliability and reproducibility of these findings.

The antimicrobial, antioxidant, and phytochemical activities of *Allium sativum* have been reported in previous studies; the present work advances this knowledge by providing quantitative and statistically validated evidence. The observed differential susceptibility among microorganisms also reflects the effectiveness of the extraction technique employed, which may enhance the chemical profile and potency of the extract. These results support prior correlations between antimicrobial activity and the putative bioactive compounds, contributing to understanding of structure-activity relationships.

The specific phytochemicals responsible for these antimicrobial mechanisms and their corresponding biological roles are presented in Table 2. The antimicrobial potential of garlic arises from a multifactorial mechanism linked to its rich phytochemical composition. Alkaloids in garlic destabilize microbial protein production and inhibit the synthesis of essential enzymes and structural proteins, ultimately compromising cell viability [35,36]. Flavonoids provide antioxidant and anti-inflammatory effects, neutralizing reactive molecules and modulating biofilm formation, which enhances microbial susceptibility to external antimicrobial activity [37]. Saponins directly disrupt microbial cell

membranes, causing permeabilization, leakage of cellular contents, and subsequent cell death [38]. Tannins interact with microbial proteins and enzymatic pathways, thereby disrupting normal metabolism. Glycosides, such as alliin, interfere with microbial energy metabolism by inhibiting ATP production, suppressing cellular respiration, and suppressing growth. Terpenoids, including diallyl disulfide, modify microbial signaling pathways, interfere with quorum sensing, and inhibit growth cycles [39].

Table 2. Major phytochemicals in garlic and their antimicrobial roles.

Phytochemical	Biological Role	Impact on Antimicrobial Properties
Alkaloids	Antimicrobial, antifungal	Disrupt bacterial protein synthesis
Flavonoids	Antioxidant, anti-inflammatory	Neutralize toxins, inhibit biofilm formation
Saponins	Disrupt cell membranes	Increase permeability leading to cell lysis
Tannins	Antibacterial, antioxidant	Bind microbial proteins, inactivating enzymatic functions
Glycosides	Inhibit metabolism	Prevent ATP production
Terpenoids	Antimicrobial, anti-inflammatory	Disrupt quorum sensing and inhibit microbial growth

While the general antimicrobial and phytochemical properties of *Allium sativum* are established, this study advances current knowledge by demonstrating that the applied extraction strategy yields a chemical profile with enhanced or distinct antimicrobial activity. The work highlights comparative efficacy among major phytochemicals, correlating specific compounds with quantitative inhibition outcomes, thereby providing structure-activity insights that have not been explicitly reported in prior studies. This approach allows a more nuanced understanding of how optimized extraction enhances the synergistic antimicrobial effects of garlic, positioning it as a natural agent capable of targeting a broad spectrum of pathogens efficiently.

The confirmatory phytochemical analysis of *Allium sativum* revealed a diverse spectrum of bioactive compounds, each contributing to the antimicrobial properties of the extract. Alkaloids were detected, indicating their potential to inhibit microbial protein synthesis and disrupt essential cellular activities and growth. Steroids suggest the ability to compromise microbial membrane integrity, increasing permeability and causing leakage of cellular contents. Glycosides may impair microbial respiration and ATP production, limiting energy availability and growth. Tannins are likely to form complexes with microbial proteins, inhibiting enzymatic activities and disrupting cell integrity. Phenolic compounds reduce pathogen viability by denaturing enzymes and interfering with metabolic pathways. Terpenoids, including organosulfur derivatives, can destabilize lipid biosynthesis, interfere with microbial signaling, and inhibit growth cycles. Saponins enhance antimicrobial activity through increased membrane permeability, leading to lysis, while flavonoids contribute antioxidant activity, neutralizing reactive molecules, inhibiting microbial enzymes, and preventing biofilm formation [40]. The detected phytochemicals, the qualitative tests confirming their presence, and their corresponding antimicrobial functions are presented in Table 3.

Table 3. Confirmatory phytochemical analysis of garlic extract.

Phytochemical	Test Used	Presence (+/-)	Antimicrobial Role
Alkaloids	Mayer's & Wagner's Tests	+	Inhibit microbial protein synthesis
Steroids	Conc. H_2SO_4 Test	+	Disrupt microbial membranes
Glycosides	Ferric chloride & benzene-ammonia solution	+	Inhibit microbial respiration and energy production
Tannins	Ferric chloride test in distilled water	+	Precipitate microbial proteins and disrupt cell integrity
Phenols	10% Ferric chloride test	+	Denature microbial enzymes and inhibit metabolism
Terpenoids	Chloroform + conc. H_2SO_4 Test	+	Disrupt lipid biosynthesis in cell membranes
Saponins	Froth and Foam Test	+	Increase microbial membrane permeability, causing lysis
Flavonoids	NaOH and dilute acid (lead acetate test)	+	Neutralize oxidative stress and inhibit microbial enzymes

Beyond confirming known antimicrobial properties, this study highlights that the applied extraction method yields a phytochemical profile with enhanced or distinct bioactive composition, including organosulfur compounds such as alliin and its derivative allicin. These compounds inhibit DNA/RNA synthesis, disrupt microbial cellular structures, and interfere with key metabolic pathways. The study also emphasizes synergistic interactions among phytochemicals, which may collectively enhance the broad-spectrum antimicrobial efficacy of garlic. These findings provide quantitative and qualitative insights into structure-activity relationships, offering a more detailed pharmacological understanding of garlic as a natural antimicrobial agent, which advances knowledge beyond mere confirmation of

previously reported effects.

Table 4. XRF elemental analysis of garlic extract and its functional implications.

Element	Function in Antioxidant/Antimicrobial Activity	Effect on Garlic Bioactivity
SiO_2	Enhances structural integrity, stress resistance	Protects bioactive compounds from degradation
Al_2O_3	Disrupts microbial enzyme activity	Boosts antibacterial performance
Fe_2O_3	Catalyzes redox reactions	Promotes ROS generation, damaging microbes
MnO	Co-factor for antioxidant enzymes (e.g., SOD)	Strengthens antioxidant defenses
CaO	Affects microbial enzymes and cell wall integrity	Enhances activity against Gram-positive bacteria
P_2O_5	Supports energy metabolism and ATP synthesis	Aids antibacterial and antifungal effects
K_2O	Regulates pH and osmotic balance	Prevents biofilm formation
TiO_2	Photocatalytic and DNA-damaging to microbes	Enhances DNA degradation in bacteria
SO_3	Main component of allicin and sulfur compounds	Central to antimicrobial potency
Na_2O	Osmotic balance disruption in microbes	Inhibits microbial growth
MgO	Cofactor for antioxidant enzymes	Enhances ROS scavenging
Cl	Regulates microbial pH and osmotic stress	Disrupts bacterial membranes
LOI	Measures organic content	Indicates high bioactive component levels
Rb_2O	Disrupts microbial ion transport	Impairs bacterial metabolism
ZnO	Enzyme disruption in microbes	Inhibits essential microbial functions
Cr_2O_3	Stress regulator and antimicrobial	Boosts antioxidant effects
SrO	Modifies microbial cell walls	Enhances bactericidal activity
NiO	Interferes with microbial respiration	Applies stress on microbial survival

XRF elemental analysis of the garlic extract demonstrates a complex mineral composition that contributes significantly to the stability, nutritional quality, and potential functional properties of the extract [41,42]. Major elements such as silicon (Si), magnesium (Mg), calcium (Ca), and potassium (K) are present in appreciable amounts and play important roles in structural and biochemical integrity. Silicon is known to support cell wall strength in plants and may contribute to the preservation of bioactive compounds by stabilizing the extract matrix. Magnesium and calcium serve as essential cofactors for numerous enzymatic processes and are critical for human nutrition, contributing to antioxidant enzyme functions and maintaining cellular homeostasis [41,42]. Potassium, being a key electrolyte, helps regulate osmotic balance and ionic strength, which can indirectly influence the stability of phytochemicals. The elemental composition identified by XRF analysis and its corresponding functional implications for antioxidant and antimicrobial activity are summarized in Table 4 above.

Sulfur, detected as SO_3, is a significant component of garlic's mineral profile and serves as a nutritional precursor for organosulfur compounds such as alliin, which are important for the overall bioactivity of garlic [41,42]. While XRF does not measure bioactive compounds directly, the presence of sulfur indicates the potential of the extract to provide essential nutrients required for human health. Trace elements including rubidium (Rb), zinc (Zn), chromium (Cr), strontium (Sr), and nickel (Ni) are detected at low concentrations and contribute primarily to the nutritional and compositional profile of the extract [43,44]. Zinc and chromium, in particular, are essential micronutrients involved in enzyme activation, carbohydrate metabolism, and immune function, highlighting the nutritional relevance of garlic beyond its well-known phytochemical properties.

Additionally, the XRF results provide an overview of the organic content of the extract (LOI), which correlates with the presence of bioactive phytochemicals such as organosulfur compounds, phenolics, and flavonoids. The synergy between mineral composition and organic constituents is important in maintaining extract stability, optimizing nutrient availability, and potentially supporting health benefits through dietary intake [41,44].

The antioxidant activity of the garlic extract, as measured by the DPPH radical scavenging assay, demonstrates a clear dose-dependent response. At the lowest tested concentration (25 ug/mL), the extract achieved 34.8 ± 1.9% radical scavenging, indicating that even at minimal doses, the bioactive compounds contribute to neutralizing free radicals. As the concentration increased to 50 ug/mL, the scavenging activity rose sharply to 56.2 ± 2.4%, showing that higher availability of phytochemicals significantly enhances radical neutralization. At 75 ug/mL and 100 ug/mL, the extract

reached 71.5 ± 2.0% and 88.1 ± 2.3% inhibition, respectively, suggesting that the antioxidant potential is maximized near the upper tested concentration. The dose-dependent antioxidant activity of garlic extract, including radical scavenging percentages at various concentrations, is summarized in Table 5. The calculated IC50 value of 49.3 ± 2.1 ug/mL reflects the extract's potent radical-scavenging capacity, as less than 50 ug/mL is required to neutralize half of the free radicals. The statistically validated differences among concentrations ($p < 0.05$) further confirm that the increase in antioxidant activity is significant and reproducible.

Table 5. Antioxidant activity of garlic extract.

Extract Concentration (μg/mL)	Radical Scavenging %	Mean ± SD (n = 3)
25	34.8	34.8 ± 1.9
50	56.2	56.2 ± 2.4
75	71.5	71.5 ± 2.0
100	88.1	88.1 ± 2.3

The observed dose-dependent response unequivocally establishes the garlic extract's efficacy in mitigating oxidative stress through radical neutralization. This proportional relationship between extract concentration and scavenging activity underscores the direct involvement of its bioactive components in electron or hydrogen donation, a fundamental mechanism in neutralizing free radicals [44]. The IC50 value further substantiates this potency, highlighting the extract's capacity to achieve significant antioxidant effects at relatively low concentrations. This robust antioxidant potential, demonstrated by the DPPH assay, indicates a promising candidate for applications requiring the stabilization of free radicals [45]. Further investigation into the specific phytochemicals responsible for this activity, such as allicin and its derivatives, could elucidate the precise molecular interactions mediating these effects [46,47].

5. Conclusion

This research showed that garlic extract has high antimicrobial and antioxidant properties, which can be explained by the fact that this substance contains abundant phytochemical and elemental compounds. Availability of bioactive compounds such as allicin, sulphur-based compounds, alkaloids, flavonoids, saponins, and terpenoids, among others, also plays an important role in its broad-spectrum effectiveness against pathogens like *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The presence of functionally active groups was proved by FTIR and phytochemical and antimicrobial assays revealed significant zones of inhibition, especially against Gram-positive bacteria. Also, the elemental analysis showed important metal oxides (e.g., Fe_2O_3, MnO, ZnO, TiO_2) that contribute to the antioxidant activity of garlic by the creation of ROS and microbial lysis. These studies confirm the reason behind people using garlic as a natural medicine in the past and conclude it has the potential to become a viable alternative to conventional antibiotics, particularly in the treatment of antibiotic-resistant disease. In general, the research supports the scientific grounds of the pharmaceutical development of garlic-based therapeutics and suggests the continuation of research of its synergistic effects with traditional antimicrobial agents.

Conflicts of Interest

The authors have no conflicts of interest.

Generative AI Statement

The authors declare that no generative artificial intelligence (Gen AI) was used in the creation of this manuscript.

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