The Journal of Medicinal Plants and By-products

Original Article

From Systems Biology to Traditional Medicine: Unraveling Garlic's Pharmacological and Genetic Targets in Respiratory Infections

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Article History: Received 22 February 2026/Accepted in revised form 18 July 2026

ABSTRACT

Allium sativum L. (garlic) has been widely used in Traditional Persian Medicine (TPM) for the management of cough, fever, and respiratory infections. This study aimed to elucidate the molecular mechanisms underlying these traditional applications using a network pharmacology approach supported by literature-based validation. Garlic-derived bioactive compounds were collected from the Dictionary of Natural Products and published literature, and their chemical structures were verified using PubChem. Compound-target interactions were predicted using the STITCH database, while respiratory disease-associated genes were retrieved from DisGeNET. Protein-protein interaction (PPI) networks, topological analyses, Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using Cytoscape and related bioinformatics tools. A PRISMA-guided PubMed review was conducted to summarize experimental evidence supporting the predicted targets. Network analysis identified TNF, MMP9, IL6, CASP3, CASP9, AKT1, and XIAP as major hub genes involved in inflammatory, apoptotic, and immune-related pathways. GO and KEGG enrichment analyses highlighted the NF-κB, MAPK, apoptosis, and cytokine signaling pathways as key biological processes associated with garlic activity. Literature-based validation confirmed the central roles of TNF and MMP9 in respiratory inflammation and supported the predicted pharmacological actions of garlic-derived compounds. Garlic demonstrates a multi-target pharmacological profile that may contribute to the prevention or adjunctive management of respiratory infections through coordinated regulation of inflammation, immune responses, and apoptosis. These findings provide a molecular basis for the traditional use of garlic in TPM and identify potential therapeutic targets for future investigation. Further experimental studies are required to validate these findings.

Keywords: Garlic, *Allium sativum*, Respiratory infections, Organosulfur compounds, Inflammation

Highlights:

- Garlic exerts multi-target pharmacological effects in respiratory diseases.
- TNF, MMP9, IL6, CASP3, and CASP9 were identified as key hub genes.
- GO and KEGG analyses highlighted NF-κB, MAPK, apoptosis, and cytokine signaling.
- Literature-based validation confirmed the biological relevance of TNF and MMP9.
- Network pharmacology provides molecular support for Traditional Persian Medicine.

INTRODUCTION

Respiratory infections affect a large global population annually and are a significant cause of morbidity and mortality worldwide [1]. Common initial symptoms include persistent cough and fever. The cough reflex, mediated by the vagus nerve, is triggered by sensory nerve stimulation in the larynx or lower respiratory tract. Inflammation heightens laryngeal reactivity, leading to spontaneous or stimulus-induced coughing, such as from inhaling cold air [2]. Treatments often include antitussives, bronchodilators, antihistamines, mucolytics, corticosteroids, and leukotriene receptor antagonists. Fever, driven by cytokine production as a response to infection, is commonly managed with antipyretics; however, their use remains controversial, as suppressing fever may interfere with the body's natural defense mechanisms [2,3]. In parallel, the increasing prevalence of antimicrobial resistance further complicates the effective management of respiratory infections [4].

Rising antimicrobial resistance and the limitations of current antiviral drugs, including toxicity and viral resistance, underscore the urgent need for new therapeutic options [4,5]. Traditional medicine has gained growing global attention due to its holistic perspective on health and disease management. Herbal remedies, with their diverse bioactive molecules, provide polypharmacological benefits by targeting multiple disease pathways, offering synergistic, multifaceted treatment for complex conditions [6]. This approach supports holistic, individualized therapies.

Garlic (*Allium sativum* L.), traditionally used as both a functional food and medicine, is widely applied for preventing and relieving respiratory infections, such as colds, coughs, and rhinitis [7,8]. It contains pharmacologically active compounds with anti-inflammatory, antioxidant,

antiviral, antibacterial, and antifungal properties, aiding in the management of conditions like asthma, chronic fever, and tuberculosis. Garlic also modulates the immune system, inhibits microbial growth, reduces inflammation, and exhibits potential anti-tumor properties [9].
In traditional Persian medicine (TPM), garlic is recognized as an effective remedy for respiratory illnesses due to its hot and dry nature, making it suitable for treating conditions characterized by cold and wet qualities. In TPM, the therapeutic use of garlic is primarily based on its humoral properties rather than the isolation of specific phytochemicals. Classical sources describe garlic as a remedy for respiratory conditions, and Avicenna reported that cooked garlic exhibits antitussive effects and can help relieve chest discomfort, reduce excessive pulmonary mucus, and improve hoarseness [10,11]. TPM texts also indicate that garlic is often administered with adjunct ingredients such as honey or animal fat to improve its palatability and tolerability. In addition, combining garlic with other medicinal plants, such as caraway, is traditionally recommended to enhance its overall therapeutic effects. However, TPM cautions against potential side effects of improper or excessive garlic use, including hemorrhoids, headaches, and irritation of the eyes, lungs, and stomach. Therefore, garlic should be used cautiously under the guidance of a qualified healthcare practitioner to ensure safety and effectiveness [10,11].

MATERIALS AND METHODS

Herbal Constituents

Garlic-derived compounds were systematically compiled from the Dictionary of Natural Products (DNP) database [12]. To ensure structural reliability and computational compatibility, only compounds with available and valid SMILES (Simplified Molecular Input Line Entry System) notations were retained, and their structures were cross-verified using the PubChem database [13]. Compounds lacking defined chemical structures or SMILES representations were excluded from further analysis.

Target Prediction of Active Compounds

The curated compound set was subsequently submitted to the STITCH database (Search Tool for Interacting Chemicals, an online resource for identifying compound-protein interactions: http://stitch.embl.de/). The STITCH confidence score was set to 0.4 [14]. Additionally, “garlic oil” was included as a composite phytochemical entity due to its representation in STITCH and its relevance in previous interaction-based analyses. This multi-step curation and filtering strategy ensured the inclusion of structurally defined and biologically relevant compounds for subsequent network pharmacology analyses.

Collection of common targets of Garlic in treating pulmonary disease

The human pulmonary disease genetic markers were obtained from the Disgenet Database. The target genes for pulmonary disease were obtained upon searching the DisGeNET database using the following keywords: coughing, fever, acute respiratory infections, viral respiratory infection, upper respiratory infections, lower respiratory tract infection, respiratory tract diseases, respiratory tract infections [15]. Subsequently, a Venn diagram was used to identify the potential targets of garlic common compounds for the purpose of pulmonary infections treatment [16]. This identification involved the consideration of the overlap between the expected garlic common targets and the pulmonary disease-related targets that had been researched.

Network Construction and Module Analysis

To construct the network, a compound-target network was generated by connecting garlic-derived phytochemical compounds with their predicted molecular targets. The network was visualized and analyzed using Cytoscape software (version 3.8.0). Gene-gene and protein-protein interaction analyses were performed using the GeneMANIA platform, which integrates multiple biological datasets, including protein interactions, genetic interactions, co-expression, co-localization, and pathway information [17].
Network topology analysis was performed to identify key hub genes based on centrality parameters, including degree, betweenness centrality, and closeness centrality. Functional enrichment analysis was conducted using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses [18,19]. The DAVID bioinformatics platform was used for enrichment analysis, and significantly enriched terms were identified based on Benjamini-adjusted false discovery rate (FDR), with statistical significance defined as FDR-adjusted $p < 0.05$ [20]. Enriched biological processes, molecular functions, cellular components, and pathways were subsequently interpreted in the context of respiratory inflammation and infection.

Literature Review

A comprehensive literature search was performed in the PubMed database to identify pharmacological studies investigating garlic (*Allium sativum*) and its bioactive constituents. The search strategy combined the terms garlic, *Allium sativum*, and the names of major garlic-derived phytochemicals with respiratory- and pharmacology-related keywords, including cough, fever, infection, anti-tussive, pulmonary, lung, immunomodulators, immunostimulants, antipyretics, antihistamines, anti-inflammatory, bronchodilators, mucolytic, and expectorant. Study selection was conducted in accordance with the PRISMA 2020 statement.

Quality Assessment of Included Studies

The methodological quality of the included studies was evaluated using a predefined qualitative checklist developed based on key methodological characteristics of pharmacological studies. The assessment included the presence of appropriate control groups, randomization, blinding, sample size justification, statistical analysis, dose or concentration specification, methodological clarity, and relevance to respiratory conditions. Based on the overall methodological characteristics, studies were classified as high, moderate, or low quality.

Traditional Persian Medicine Literature Analysis

A review of TPM manuscripts, including *Qanun* of Avicenna and *Makhzan al-advieh*, was carried out using the search word "*thūm*" (Arabic term for garlic). Relevant descriptions related to respiratory symptoms, cough, and therapeutic applications were extracted and compared with current pharmacological findings. In Traditional Persian Medicine, garlic is characterized as a hot and dry remedy *(Har wa Yabes*) and has been described as possessing multiple pharmacological properties, including a liquefying and resolving effect (*Mollatef va Mohallel*), regulation of thick pathological secretions (*Moghatti Akhlat-e Ghalizeh*), and a chest-clearing and respiratory facilitating effect (*Monaghi-e Sadr va Mosahhel-e Tanaffos*). Classical Persian medical texts have recommended garlic for respiratory conditions associated with cough *(Soal*), chest disorders (*Amraz-e Sadr*), asthma-like symptoms (*Rabow*), and difficulty in breathing (*Zigh-e Nafas*). Furthermore, garlic has traditionally been described as protective against harmful environmental influences, including epidemic air and putrefactive conditions (*Rafe Mazarrat-e Hava-ye Vabaei va Taaffon*), which may represent historical observations compatible with potential antimicrobial and immunomodulatory properties. Although these traditional pharmacological concepts cannot be directly translated into individual molecular pathways, they provide valuable hypotheses for modern biological investigation.

RESULTS

The workflow and principal findings of the network pharmacology analysis are presented below.

Study Selection

The study selection process is summarized in the PRISMA 2020 flow diagram (Fig. 1). The PubMed search identified 233 records. After removal of 21 duplicate records, 212 records remained for title and abstract screening. Following the initial screening, 54 records were excluded, and 158 full-text articles were assessed for eligibility. Of these, 125 articles were excluded after full-text review because they did not meet the predefined inclusion criteria, resulting in 33 studies included in the qualitative synthesis. The study selection process is summarized in figure 1.

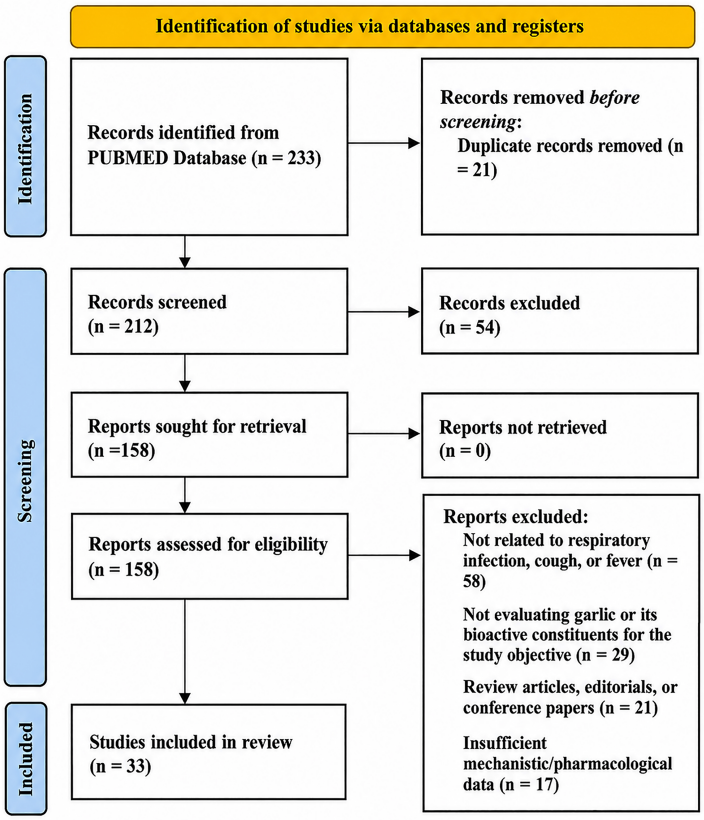

Fig. 1 PRISMA 2020 flow diagram for the identification, screening, eligibility assessment, and inclusion of studies investigating the pharmacological effects of garlic and its bioactive constituents on respiratory infections, cough, and fever.

Chemical Composition

Garlic contains a wide range of bioactive constituents, including organosulfur compounds (OSCs), polysaccharides, saponins, and phenolic compounds [21]. Thiosulfinates, particularly alliin, are key precursors of garlic's biological activity [22]. Upon mechanical disruption, alliin is converted by alliinase into allicin, which rapidly transforms into diallyl disulfide (DADS), diallyl trisulfide (DATS), dithiins, and ajoene, while γ-glutamyl-cysteine is converted into S-allylcysteine (SAC) [23]. Garlic consists of approximately 200 compounds, including water (65%), carbohydrates (28%), OSCs (2.3%), proteins (2%), amino acids (1.2%), fiber (1.5%), vitamins, and minerals, with OSCs primarily responsible for its pharmacological properties [24].

Garlic-derived organosulfur compounds, including allicin and allyl sulfides, exhibit broad-spectrum antimicrobial activity, including against drug-resistant strains, by interacting with sulfhydryl groups and disrupting microbial cell integrity [25]. These compounds also demonstrate antiviral activity, with different extraction methods yielding distinct bioactive profiles. Garlic preparations vary considerably: fresh garlic is rich in alliin and allicin, while aged garlic extract (AGE) contains more stable compounds such as S-allyl cysteine (SAC) and S-allylmercaptocysteine (SAMC) with higher bioavailability and antioxidant capacity. Bioactivity is strongly influenced by processing conditions, as heat and storage reduce phenolic and flavonoid content, while allicin rapidly transforms into other sulfur-containing derivatives with distinct biological activities [24,26,27]. Garlic oil, although present in small amounts, is particularly rich in OSCs such as DADS and DATS [21].

Literature review

Pre-clinical and clinical evidence supports the antiviral potential of garlic and its OSCs against various human pathogenic viruses. Mechanisms include inhibition of viral entry, suppression of key enzymes (RNA polymerase, reverse transcriptase, DNA synthesis), blocking immediate-early gene 1 (IEG1) transcription, and downregulation of the ERK/MAPK pathway. Garlic and OSCs also exert immunomodulatory effects that reduce viral infections. Clinical trials confirm garlic's prophylactic role in lowering viral infection incidence in humans, primarily via enhanced immune responses [7]. The pharmacological effects of garlic and its bioactive metabolites are summarized in Table 1. Garlic bioactive compounds have been reported to attenuate inflammatory responses by modulating NF-κB and MAPK-dependent signaling pathways and reducing oxidative stress [28]. Notably, water-soluble garlic polysaccharides have been shown to attenuate inflammatory responses via inhibition of NF-κB and STAT3 signaling pathways, leading to reduced production of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β [29]. Detailed characteristics of included studies and the complete quality assessment process are provided in Supplementary Table S1. Quality assessment was based on predefined criteria including study design, control groups, dose specification, statistical analysis, methodological transparency, and relevance to respiratory inflammation or infection.

Table 1 Pharmacological evidence supporting the potential respiratory-related effects of garlic and its bioactive constituents

Effect	Mechanism	Compound(s)	Study Model	Quality Assessment	Ref.
Anti-inflammatory	Suppressed IL-6 and MCP-1; modulated gene expression	Alliin (100 μM)	3T3-L1 adipocytes (in vitro)	Moderate	[30]
	Modulated pro-inflammatory cytokines	Garlic extract (1–2 μg/mL)	Human PBMCs (in vitro)	High	[31]
	Reduced IL-2, IL-6, IL-8, IL-12, TNF-α; ↑ IL-10	Garlic extract (≥10 μg/mL)	Whole blood & PBMCs (in vitro)	High	[32]
	Reduced NF-κB activity	Garlic powder, DADS, allicin	HEK293 cells & human blood (in vitro)	Moderate	[33]
	Inhibited cytoskeleton formation	Garlic oil (1–10 μg/mL)	HL-60 cells (in vitro)	Moderate	[34]
	Inhibited STAT3 and COX-2	Ajoene	RAW264.7 macrophages (in vitro)	Moderate	[35]
	Reduced cytokines; decreased paw edema	DATS (50 mg/kg)	RAW264.7 (in vitro) + mice (in vivo)	High	[36]
	Suppressed NF-κB and STAT3	Garlic polysaccharides	RAW264.7 (in vitro)	Moderate	[24]
	Reduced airway inflammation; Nrf2/HO-1 activation	DADS	RAW264.7 + BALB/c mice	High	[37]
	Reduced TNF-α and IL-1β in BALF	Alliin	BALB/c mice (in vivo)	Moderate	[38]
	Regulated TLR4/MyD88/NF-κB	Allicin	Rats (in vivo)	Moderate	[39]
	Reduced lung inflammation	Garlic oil + DADS	Mice (in vivo)	Moderate	[40]
	COX-2 inhibition; HO-1 induction	S-allyl cysteine	C57BL/6 mice (in vivo)	Moderate	[41]
Immunomodulatory	Mitogenic activity	Garlic proteins	Human & murine immune cells	Moderate	[42]
	Reduced IL-6 levels	Aged Garlic Extract (AGE)	Human clinical study	High	[43]
	Balanced Th1/Th2	Garlic oil	Wistar rats (in vivo)	Moderate	[44]
	Increased TNF-α, IFN-γ, IL-12	Allicin	Malaria mice (in vivo)	Moderate	[45]
	Increased NO and cytokines	Allicin	Murine macrophages (in vitro)	Moderate	[46]
	Cytokine modulation	Garlic extract	Human placental explants	Moderate	[47]
	Reduced IL-17	Fresh garlic extract	PBMCs (in vitro)	Moderate	[48]
	Inhibited NF-κB signaling	DADS	SW480 cells (in vitro)	Moderate	[49]
	S-thioallylation of proteins	Allicin	Human T cells (in vitro)	Moderate	[50]
	Increased antibody production	Dietary garlic	Chickens (in vivo)	Moderate	[51]
	Increased IgA	Ajoene	Mice (in vivo)	Moderate	[52]
	Enhanced phagocytosis	Allicin	Mice (in vivo)	Moderate	[53]
	Increased NK activity	AGE	Mice (in vivo)	Moderate	[54]
	Increased CD4+/CD8+ ratio	S-allyl cysteine	Rats (in vivo)	Moderate	[55]

	Gene expression modulation	Raw garlic	Human clinical study	Moderate	[56]
	Reduced cold/flu severity	Aged Garlic Extract	Human clinical study	High	[57]
Antiviral	Inhibited viral entry (H1N1)	Garlic extract	A549 cells (in vitro)	Moderate	[58]
	Reduced viral load; increased IFN-β	DATS	A549 cells + mice	High	[59]
Antiallergic	Suppressed histamine	AGE	RBL-2H3 cells + mice	Moderate	[60]
	Reduced airway inflammation	AGE fraction	BALB/c mice	Moderate	[61]

Network Analysis Results

Network pharmacology analysis was performed to identify the molecular targets and biological pathways potentially involved in the respiratory effects of garlic. By integrating garlic-associated compound-target interactions obtained from STITCH with respiratory disease-related genes retrieved from DisGeNET, overlapping targets were identified using Venn analysis (Fig. 2).

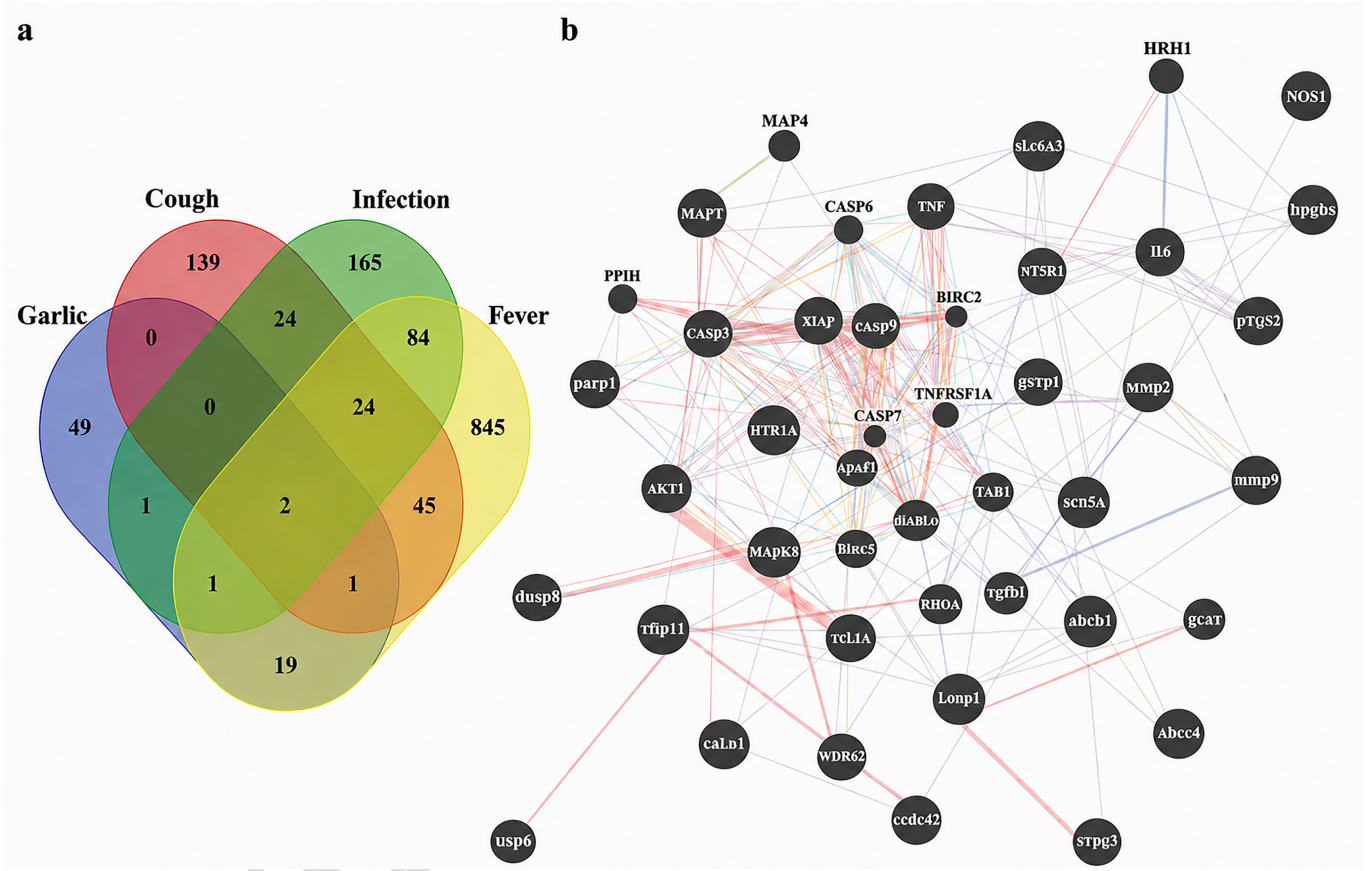

Fig. 2 Identification of overlapping garlic-associated targets and protein-protein interaction network. (a) Venn diagram showing the shared targets between garlic-associated compounds and respiratory disease-related genes (cough, infection, and fever). (b) Protein-protein interaction network of the overlapping target genes generated using GeneMANIA. Node size reflects the degree of connectivity, while edge thickness represents the strength of functional associations between interacting genes.

The PPI network consisted of the identified overlapping targets and demonstrated a highly interconnected functional module. Network topology analysis based on degree, betweenness centrality, and closeness centrality was subsequently performed to identify key hub genes.

Network Topology Analysis Identifies Hub Genes

The protein-protein interaction network included 44 overlapping target genes. Network topology analysis was performed based on degree, betweenness centrality, and closeness centrality to identify the most highly connected and influential nodes within the network. The network exhibited an average degree of 10.82, with node connectivity values ranging from 1 to 35. Among the identified targets, CASP3 showed the highest degree of connectivity (degree = 35), followed by CASP9 and CASP7, highlighting their central position within the interaction network. Furthermore, TNF and other inflammation-related genes demonstrated relatively high betweenness centrality values, suggesting that these targets may serve as important connectors linking distinct functional modules involved in inflammatory and apoptotic processes (Table 2).

Table 2 The top-ranked genes according to centrality parameters

Gene	Degree	Betweenness	Closeness
CASP3	35	0.073	0.551
CASP9	34	0.041	0.538

CASP7	33	0.05	0.566
BIRC2	27	0.027	0.531
XIAP	27	0.042	0.518
CASP6	23	0.021	0.512
APAF1	21	0.05	0.506
BIRC5	18	0.038	0.489
TNFRSF1A	17	0.049	0.506
TNF	17	0.078	0.531

KEGG

KEGG pathway enrichment analysis showed that the garlic-associated target genes were primarily involved in pathways related to inflammation, immune regulation, and cellular survival (Table 3). Among the identified pathways, the TNF signaling pathway showed the highest level of statistical significance, with eight associated targets, including TNF, IL6, MAPK8, PTGS2, AKT1, XIAP, CASP3, and MMP9 (FDR = 8.57×10^{-7}). Other significantly enriched pathways included apoptosis (FDR = 3.8×10^{-5}) and IL-17 signaling (FDR = 5.22×10^{-5}), suggesting that garlic-related targets may influence both inflammatory cascades and apoptosis-associated cellular responses. Several additional enriched pathways, such as cancer-related and neurodegeneration-associated pathways, were also identified. However, these pathways likely reflect shared molecular mechanisms, including regulation of apoptosis, oxidative stress responses, and intracellular signaling networks, rather than indicating direct disease-specific effects (Fig. 3 and Fig.4).

Table 3 KEGG pathway enrichment analysis of garlic-associated target genes

#	KEGG pathway	FDR (Benjamini)	Genes
1	TNF signaling pathway	8.57×10^{-7}	IL6, MAPK8, CASP3, XIAP, AKT1, PTGS2, TNF, MMP9
2	Apoptosis	3.8×10^{-5}	CASP9, MAPK8, PARP1, CASP3, XIAP, AKT1, TNF
3	Pathways in cancer	4.23×10^{-5}	CASP9, IL6, MAPK8, CASP3, GSTP1, MMP2, XIAP, AKT1, PTGS2, MMP9
4	Alzheimer disease / neurodegeneration pathways	4.23×10^{-5}	CASP9, IL6, MAPK8, CASP3, AKT1, MAPT, NOS1, PTGS2, TNF
5	Hepatitis B	4.23×10^{-5}	CASP9, IL6, MAPK8, CASP3, AKT1, TNF, MMP9
6	IL-17 signaling pathway	5.22×10^{-5}	IL6, MAPK8, CASP3, PTGS2, TNF, MMP9

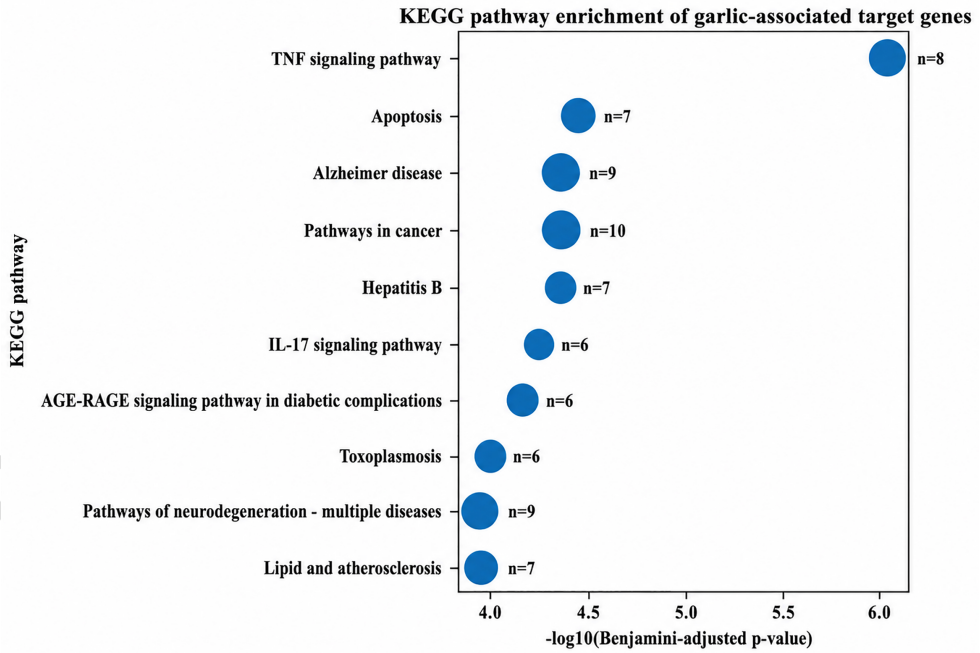

Fig. 3 KEGG pathway enrichment analysis of garlic-associated target genes. The bubble plot represents the top enriched pathways based on Benjamini-adjusted FDR values. Bubble size indicates the number of target genes involved in each pathway.

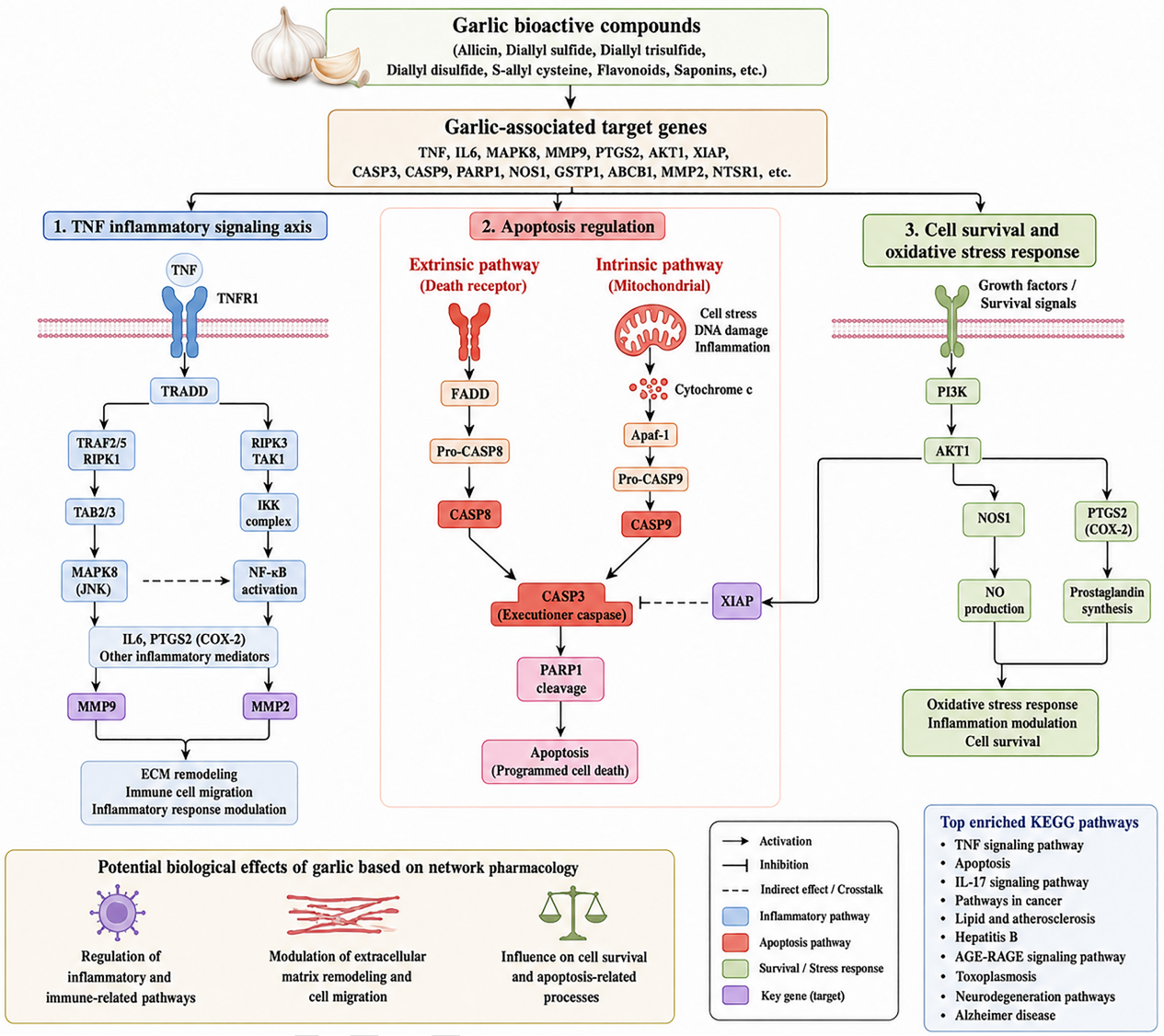

Fig. 4 Proposed molecular mechanisms underlying the pharmacological effects of garlic-associated targets in respiratory inflammation and infection. The figure was initially generated using artificial intelligence (AI) tools and subsequently reviewed, modified, and finalized by the authors.

GO Enrichment Analysis

GO enrichment analysis of the 24 overlapping target genes showed significant enrichment of biological processes associated with hypoxia response, apoptosis regulation, inflammatory signaling, and cellular stress adaptation (Benjamini-adjusted FDR < 0.05). Among the enriched terms, response to hypoxia showed the highest fold enrichment (7 genes, 29.17%, FDR = 3.55×10^{-5}), followed by positive regulation of apoptotic process (8 genes, 33.33%, FDR = 3.55×10^{-5}) and negative regulation of apoptotic process (9 genes, 37.50%, FDR = 3.86×10^{-5}) (Table 4). Multiple testing correction was applied using the Benjamini-Hochberg false discovery rate (FDR) method, and enriched terms with an adjusted FDR value <0.05 were considered statistically significant.

Table 4 Gene Ontology (GO) biological process enrichment analysis of the 24 overlapping target genes

#	GO Term	*P*-value	Benjamini (FDR)	Fold Enrichment	Related Genes
1	Response to hypoxia	5.57E-08	3.55E-05	31.07	CASP9, ABCB1, CASP3, MMP2, LONP1, NOS1, TNF
2	Positive regulation of apoptotic process	8.13E-08	3.55E-05	19.37	CASP9, IL6, MAPK8, CASP3, MMP2, TNF, MMP9, NTSR1
3	Negative regulation of apoptotic process	1.33E-07	3.86E-05	13.24	IL6, MAPK8, GSTP1, XIAP, AKT1, PTGS2, TNF, MMP9, NTSR1

Key Target Genes

Among the identified hub genes, TNF and MMP9 were selected for literature-based validation because they were consistently identified as common targets in both the Venn analysis and the revised network analysis and have well-established roles in respiratory inflammation and tissue remodeling (Fig. 2).

Literature-based Validation of the Principal Hub Genes

Tumor necrosis factor (TNF-α)

Tumor necrosis factor (TNF), produced by macrophages, T cells, and mast cells, is a key cytokine in inflammation and immunity. Targeting TNF-α processing by CD8+ T cells may reduce viral infection-related lung damage [62]. TNF-α stimulates ROS, NF-κB, and cytosolic phospholipase A2, contributing to lung inflammation and injury [63]. In severe COVID-19, TNF-α drives inflammation and organ damage; anti-TNF drugs can block its harmful signaling [64]. The traditional Chinese formula NRICM101 protects lung cells from TNF-α/IL-1β damage by modulating kinase pathways [65]. In SARS-CoV-2 infection and cytokine shock syndromes, TNF-α and IFN-γ jointly induce tissue damage and inflammatory cell death via caspase-8 and kinase cascades; blocking this pathway may alleviate cytokine storm-driven diseases [66,67].

In tuberculosis, TNF and its receptors regulate immune responses. Active TB patients show higher TNFR1 expression on immune cells and lower soluble TNFR1 levels, reflecting disease severity [68]. TLR2 detects mycobacterial components, with TNF-α modulating its response [69]. IL-10 and TNF-α control M. tuberculosis survival in alveolar macrophages via STAT3 activation, influencing TB progression and defense [70]. Adalimumab, a TNF-α-neutralizing monoclonal antibody, reduces pulmonary inflammation in acute asthma models [71]. LIGHT, a TNF superfamily protein, promotes memory CD8+ T cell development in lungs and blood after respiratory virus infection, supporting long-term immunity [72]. In Legionella pneumophila pneumonia, TNF and ROS are crucial for bacterial clearance. ROS aids neutrophil killing, while TNF signaling through TNFR1 in alveolar macrophages promotes lysosome fusion with bacterial vacuoles and caspase activation to restrict bacterial growth [73]. IL-10, an anti-inflammatory cytokine, suppresses TNF-α and CXCL8 production in LPS-activated monocytes by inhibiting phosphorylation of a transcription factor and the activity of an enzyme controlling their transcription; this regulatory function may be impaired in COPD [74]. The microRNA miR-130a-3p reduces lung fibrosis and inflammation in mice with pulmonary fibrosis by lowering TNF-α and TGF-β1 levels [75]. TNF also contributes to fatigue and inflammation. LPS exposure in mice increases TNF and 5-HT2A receptor expression in the brain, producing depression-like symptoms [76].

Matrix Metallopeptidase 9 (MMP9)

MMP-9, produced by cells such as macrophages and neutrophils, can have protective or harmful effects depending on context and duration. An ultra-diluted SARS-CoV-2 spike protein (UDSP) reduces lung damage in spike protein-exposed mice by lowering ferritin and MMP-9 levels and maintaining the MMP-9/TIMP-1 balance [77]. The TRPV4 activator GSK-1016790A increases pulmonary barrier permeability in mice by upregulating MMP-9 and MMP-2 and reducing TIMP-2; SB-3CT inhibits these enzymes and prevents TRPV4-induced lung injury [78]. MMP-9 worsens lung ischemia-reperfusion injury (LIRI) by inducing pyroptosis and IL-1β release; SB-3CT reduces both damage and pyroptosis [79]. Angiotensin II (Ang II) induces macrophage secretion of MMP-9, contributing to extracellular matrix degradation and pulmonary inflammation [80]. MMP-9 also regulates IL-23p19 production in dendritic cells by targeting membrane stem cell factor, thereby influencing Th17 cell-mediated IL-17 synthesis in the lungs [81]. Elevated MMP-9 levels are seen in COPD, where it may drive disease progression through matrix breakdown, inflammation, and impaired tissue repair [82], and in connective tissue disease-associated interstitial lung diseases (CTD-ILD), where similar mechanisms may contribute to pathology [83]. Collectively, the published experimental and clinical evidence supports the biological relevance of TNF and MMP9 in respiratory diseases and is consistent with the network pharmacology predictions.

Biological Relevance of Additional Hub Genes

Alliin from garlic shows favorable docking scores with EGFR and AKT1 [84]. MAPK signaling is central in lung pathology: endothelial MAPK1 (ERK2) protects against hyperoxia-induced bronchopulmonary dysplasia and pulmonary hypertension in neonates [85]; TPL2 activates ERK1/2 via TLRs in bacterial infection, a target in cystic fibrosis [86]; Qingfei Formula inhibits MAPK to reduce RSV-induced inflammation/lung injury [87]; MAPK8 activates in influenza A and tuberculosis [88,89]; flavonoids (quercetin, luteolin, kaempferol) mitigate oxidative stress via IL-6, COX-2, MAPK8 [90]. IL-6, a key pro-inflammatory cytokine, is elevated in severe COVID-19, driving cytokine storm, ARDS, and mortality. IL-6 inhibition may prevent or treat lung failure [91-95]. It promotes pulmonary hypertension [96,97] and serves as an infection marker in critically ill patients [98]; IL-6 antagonists reduce ventilation needs in COVID-19 [99]. Ajoene-enriched garlic extract matches IL-6 receptor antibodies in anti-inflammatory effects for COVID-19 [100].

Caspase-9 (CASP9) initiates apoptosis by activating CASP3 [101,102]. Fresh garlic juice induces apoptosis in KB cells via increased CASP3 and Bax/Bcl-2 ratio [103]. CASP3 is elevated in SARS-CoV-2 infection, potentially marking severity [104]; elevated CASP3/FasL contributes to alveolar damage in endotoxemia [105]; MK2 (via p38 MAPK) regulates vascular permeability/apoptosis through cleaved CASP3 nuclear translocation [106]. SARS-CoV-2 activates NF-κB, upregulating XIAP (apoptosis suppressor) and TNF; proteasome inhibitors may modulate NF-κB to limit infection [107]; XIAP/cIAP inhibition promotes mesenchymal apoptosis in lung fibrosis [108].

Garlic fatty acids (myristic, lauric) inhibit *M. tuberculosis* by binding Akt [109]. COVID-19 elevates TGF-β, AKT1, α-SMA, MMP-9 for ECM remodeling/fibroblast differentiation [110]. PI3K/AKT inhibition reduces thrombosis in severe COVID-19 [111]. Akt drives airway smooth muscle hypertrophy in asthma [112]. Akt1 suppression boosts macrophage antiviral function in hypercapnia [113]; PI3K/AKT regulates pulmonary arterial smooth muscle survival/differentiation [114]; BYHWD modulates PI3K-Akt-eNOS to improve vascular remodeling in pulmonary hypertension [115]; triciribine (Akt inhibitor) prevents vascular pruning in fibrosis [116]; peimine targets 23 cough-related proteins, including MAPK1, AKT1, PRKCB [117].

DISCUSSION

Recent studies published in the field of medicinal plants have further emphasized the importance of phytochemical characterization, biological evaluation, and mechanistic investigation of plant-derived compounds, supporting the growing role of medicinal plants as sources of multi-target therapeutic agents [118-121]. The present study suggests that traditional respiratory applications of garlic may be partially interpreted through modulation of inflammatory signaling pathways (TNF, IL6, MAPK8). Also, regulation of extracellular matrix remodeling (*Tahlil-e Oram*) and tissue responses may be relevant to MMP9 and cellular stress-response pathways.

Garlic as a Multi-Target Therapeutic for Pulmonary Health

Pulmonary infections, often presenting with symptoms such as cough and fever, are typically managed through symptom-based monotherapy. Increasing evidence supports the use of natural medicines for polypharmacological interventions, as herbal compounds contain diverse bioactive molecules that can simultaneously modulate multiple disease-related pathways. Garlic enhances immune responses by activating macrophages, natural killer cells, and T lymphocytes, which improves the body's capacity to combat infections and tumors. It may serve as a complementary or alternative therapy to conventional antipyretics, providing immune support with minimal adverse effects, though further research is necessary to validate efficacy and safety across populations. In addition, potential sources of bias, including industry involvement and variability in garlic formulations, may influence study outcomes. Therefore, these findings should be interpreted cautiously. We have now emphasized that, despite promising results, the current clinical evidence remains limited and heterogeneous, and further large-scale, well-controlled trials with standardized preparations are required to confirm efficacy.

Our network pharmacology analysis suggests that the biological effects of garlic may involve the coordinated regulation of inflammatory signaling, extracellular matrix remodeling, and apoptotic processes. Among the identified pathways, the TNF signaling pathway showed the strongest enrichment, highlighting inflammation modulation as a potential central mechanism of garlic-associated targets. TNF, together with IL6, MAPK8, PTGS2, and MMP9, formed an important inflammatory regulatory axis. TNF is a key mediator of immune activation and can regulate downstream cytokine production and matrix metalloproteinase expression. The involvement of MMP9 suggests a possible role in extracellular matrix remodeling and immune cell migration, which are critical events during inflammatory responses. In addition to inflammatory pathways, our findings revealed significant enrichment of apoptosis-related signaling. The presence of CASP3, CASP9, XIAP, and AKT1 indicates that garlic-associated targets may influence the balance between apoptotic activation and cellular survival. This interaction between inflammatory and apoptotic pathways suggests a multi-target mechanism rather than a single molecular action.

Overall, these findings support the hypothesis that garlic may exert biological effects through simultaneous regulation of inflammatory mediators, stress responses, and cell fate-related pathways. However, these results are based on computational predictions and require further experimental validation to confirm the specific contribution of individual compounds and molecular targets. This study provides a systems-level insight into garlic's potential mechanisms against respiratory symptoms (cough, fever, and infection). Enrichment analysis revealed significant involvement of garlic-related genes in response to hypoxia, regulation of apoptosis, and inflammatory pathways (FDR < 0.05), with TNF-α, IL-6, and MMP-9 as key overlapping targets.

These results suggest that garlic's organosulfur compounds may help reduce inflammation, oxidative stress, and tissue damage in respiratory diseases through multi-targeted actions.

Despite promising findings, limitations including bioavailability issues and the need for clinical validation remain. Further experimental and human studies are warranted to confirm garlic's therapeutic potential. Overall, integrating traditional knowledge with network pharmacology opens new avenues for multi-target respiratory treatments.

Bioactive Compounds and Mechanisms

Key phytochemicals in garlic, such as allicin, DADS, S-1-propenyl cysteine (S1PC), and alliin, modulate key inflammatory nodes (e.g., NF-κB, ERK, IL-6 signaling) [122]. DADS has been reported to inhibit TNF-α-induced CCL2 secretion in in vitro models by reducing phosphorylation of ERK and IKKε, suppressing chemokine-mediated inflammatory signaling [123,124]. S1PC downregulates IL-6 production and mRNA expression, mitigating cytokine-driven inflammation [125]. Alliin exhibits high affinity for EGFR, a receptor implicated in tumor growth and respiratory pathology, highlighting potential anti-proliferative effects [126]. Diallyl trisulfide (DATS) decreases tumor cell proliferation and downregulates EGFR expression, promoting apoptosis without hepatotoxicity [127].

Targeting Respiratory Disease Mechanisms

Our analysis showed that genes overlapping between garlic and respiratory symptoms (cough, fever, and infection) are strongly enriched in key biological processes such as response to hypoxia, regulation of apoptosis, and response to inflammatory triggers like lipopolysaccharide (FDR < 0.05). Central players including TNF-α, IL-6, and MMP-9 appear prominently. These molecules drive airway inflammation, fever, cough reflex, and lung tissue remodeling. The significant enrichment in hypoxia response and apoptotic regulation suggests that garlic may help protect lung tissue from oxygen deprivation and uncontrolled cell death during respiratory infections. Additionally, modulation of inflammatory pathways (e.g., LPS response) aligns well with garlic's known anti-inflammatory properties. Overall, these findings provide mechanistic evidence that garlic exerts its beneficial effects on respiratory symptoms through multi-targeted suppression of inflammation, oxidative stress, and tissue damage.

Clinical Translation and Recommendations

Garlic may provide a multi-target pharmacological profile compared with single-target therapeutic approaches. It contains a rich mix of bioactive compounds especially organosulfur substances like allicin, ajoene, DADS, DATS, SAC, and SAMC. These compounds work together to influence multiple connected systems in the body at once, helping to reduce inflammation, fight oxidative stress, balance immune

responses, combat microbes, and support metabolic health. In this sense, garlic can serve as a helpful complement to standard treatments rather than a direct replacement.

For example, while antipyretics mainly ease fever symptoms and corticosteroids primarily dampen inflammation (sometimes at the cost of weakening immunity), garlic-derived compounds can simultaneously calm inflammatory signals (such as IL-1, TNF-α, and NO), activate antioxidant defenses (like the Nrf2-ARE pathway), and support immune function. This broader action gives garlic promising potential as a supportive option for issues like respiratory infections, heart disease, diabetes, obesity, and even cancer. That said, most of the supporting data comes from laboratory experiments, animal studies, and smaller human trials. We still need well-designed, large-scale clinical studies before garlic can be routinely recommended alongside or instead of proven medical treatments [128].

Safety and Drug Interactions

Garlic is generally recognized as safe by the U.S. FDA for dietary consumption, although high doses or concentrated preparations may cause gastrointestinal discomfort, body odor, headache, dizziness, and mild hypotension, particularly in susceptible individuals. Owing to its antiplatelet activity, garlic may increase bleeding risk when combined with anticoagulant or antiplatelet drugs, and discontinuation 7-10 days before surgery is generally recommended. Toxicity appears to be dose-dependent; in animal studies, a dose of 50 mg/kg produced no significant hepatic or pulmonary toxicity, whereas daily doses of 250-1000 mg/kg induced liver injury, oxidative stress, and reduced antioxidant defenses. Similarly, garlic bulb extract at 300-600 mg adversely affected growth and tissue biomarkers in rodents. Compared with raw garlic, aged garlic extract (AGE) has a more favorable safety profile because it contains fewer irritating compounds. Therefore, garlic supplements should be used cautiously, particularly in individuals with gastrointestinal disorders or those receiving multiple medications [128].

Practical Dosage Guidance

Among the available formulations, aged garlic extract (AGE) is the most extensively studied and exhibits superior bioavailability and tolerability due to its stable water-soluble organosulfur compounds. Standardized preparations are preferred because the composition of garlic powders and oils varies with processing methods. For general health, commonly recommended daily intakes include approximately 4 g of fresh garlic, 7.5 g of aged garlic extract, or one tablet of dried garlic powder taken 3-4 times daily. In therapeutic applications, dosage should be individualized, with supplementation initiated cautiously, particularly in pregnant women, patients with comorbidities, or those receiving concomitant medications [128].

Future Directions

Future research should aim to experimentally confirm the molecular interactions and signaling pathways predicted by network pharmacology through well-designed in vitro and in vivo respiratory disease models. Further investigation of key hub genes, such as TNF, MMP9, IL6, AKT1, CASP3, CASP9, and XIAP, will be important to better understand their contribution to the therapeutic effects of garlic. Moreover, detailed pharmacokinetic, bioavailability, and safety assessments of major garlic-derived compounds are needed to support their potential clinical application. Ultimately, standardized garlic preparations should be evaluated in well-controlled clinical trials to determine their efficacy and safety as complementary approaches for the management of respiratory infections.

Limitations

Several limitations should be acknowledged when interpreting the findings of this study. Although network pharmacology provides valuable insights into potential molecular mechanisms, the results are primarily based on database-derived compound-target relationships and may not fully reflect the complexity of biological systems, including tissue-specific responses, pharmacokinetic properties, and dose-dependent effects. In addition, while the identified hub targets and enriched pathways are supported by available experimental evidence, the predicted interactions require further confirmation through targeted molecular experiments and well-designed in vivo and clinical studies. Another important consideration is the variability among garlic preparations, as differences in extraction methods, processing conditions, and phytochemical composition may influence biological activity and contribute to inconsistent findings across studies. Finally, current clinical evidence remains limited and heterogeneous; therefore, future randomized controlled trials using standardized garlic formulations and clinically meaningful outcomes are needed to better define its therapeutic potential.

CONCLUSION

This study highlights the potential of garlic as a multi-target therapeutic candidate in respiratory infections through the modulation of key biological processes, including inflammation, apoptosis, and oxidative stress. Network pharmacology analysis provides valuable insights into the possible molecular mechanisms underlying the traditional use of garlic. Nevertheless, these computational findings require further experimental and clinical validation to determine the biological significance of the identified targets and to clarify the therapeutic potential of garlic in respiratory disorders.

Consent for Publication

Not applicable.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interests

The authors have not declared any conflict of interests.

Funding

There is no financial support for this study.

Authors' Contributions

Gh.M: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Visualization, Writing-original draft, Writing-review and editing
Sh.H: Conceptualization, Data curation, Formal analysis, Methodology
M.SH: Investigation

Acknowledgements

The authors would like to express their gratitude to Shiraz University of Medical Sciences, Shiraz, Iran, for institutional support.
No specific funding was received for this study.
During the preparation of this manuscript, AI-based tools were used solely for language editing to improve clarity and readability. All outputs were critically reviewed and substantially revised by the authors, who take full responsibility for the final content.

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