Potential Use of Garlic for Control of Drug-Resistant Bacteria

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SUMMARY

This review paper provide a comprehensive elaboration about the antibacterial properties of Garlic and its derivatives. The antibacterial qualities of garlic, which has long been used in many traditional healing methods and is recognized for its therapeutic qualities. The study explores the bioactive substances found in garlic, including allicin, which has been shown to have antibacterial qualities against a variety of infections. The usefulness of garlic extracts and its organic compounds in preventing bacterial development is examined in a number of investigations and experiments that are discussed in this stud. It assesses the ways that garlic inhibits the growth of biofilms, interferes with the integrity of bacterial cell membranes, and modifies bacterial gene expression in order to determine how effective garlic is against bacteria. Garlic has the potential to improve the effectiveness of currently available medications and lessen the threat of antibiotic resistance to the world's health. The study also emphasizes the need for additional investigation to clarify the ideal circumstances and dosages for combining garlic with antimicrobial medications. To sum up, this study offers insightful information about the potential use of garlic as a natural remedy to manage bacterial infections. The results acknowledge the need for more investigation and clinical testing, but they also point to garlic's potential value as a supplement or replacement for traditional antibacterial medications. This presents a promising path for the creation of new and efficient treatment approaches to combat bacterial infections.

INTRODUCTION

Over recent years, there has been a substantial rise in the occurrence of infections stemming from multidrug-resistant microbes. Multidrug resistance (MDR) refers to the capacity of a microorganism to withstand the effects of a previously effective antimicrobial drug, posing a considerable challenge to public health, as virtually all existing antimicrobial agents are susceptible to this issue. While antimicrobial resistance evolves naturally over time, the widespread proliferation of MDR pathogens, particularly in patients with compromised immune systems, is often correlated with unfavorable outcomes (Magrys et al., 2021).

Antibiotic resistance occurs both in Gram-negative and Gram-positive bacteria. In 2017, the World Health Organization (WHO) filed the research and development of new antibiotics in their top priority list. The *Klebsiella pneumoniae* (3rd generation cephalosporin-resistant and carbapenem-resistant), *Pseudomonas aeruginosa* (carbapenem-resistant), *Enterobacteriaceae* noticeably *Escherichia coli*, *Enterococcus* (vancomycin-resistant), and *Staphylococcus aureus* (methicillin-resistant) are highly resistant against antibiotics. Aside from antibiotic resistance, these above-mentioned microorganisms are also nosocomial infections. So, these pathogens are listed on the top as serious hazards to public health that necessitate global attention and quicker action. Due to this alarming situation of antibiotic resistance, alternative treatment strategies are required to cure the patients (Magrys et al., 2021).

Garlic has long been recognized for its medicinal qualities, with references dating back to the era of King Tutankhamun in Egypt, around 1336–1327 BC. Before the development of

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antibiotics, garlic was a widely used remedy for various health issues, thanks to its extensive range of benefits (Petrovska and Cekovska 2010). It served as a nutritional supplement for Egyptian laborers, a cure for exhaustion and skin infections in India, and a remedy for colic and seasickness in Greece. The first account of garlic's antimicrobial properties dates back to the 1721 Marseilles plague in France. During that time, four individuals consumed a blend of crushed garlic and wine, known as 'vinaigre des quatre voleurs,' to prevent contracting the devastating illness (Harris et al., 2001). Garlic's antibiotic properties were utilized to provide medical treatment to wounded soldiers during World War II (Oosthuizen et al., 2018). However, as antibiotics became more accessible, interest in garlic's medicinal uses waned.

Garlic (*Allium sativum*), a well-known herb, that belongs to the *Liliaceae* family, has great benefits to humans. It is an aromatic plant which has much importance in traditional medicines. Species within the *Allium* genus and their constituents exhibit a variety of beneficial properties, such as antioxidant, antitumor, anti-inflammatory, immunoregulatory, antiviral, antimicrobial, and cardiovascular defense capabilities. They also hold potential for therapeutic applications, including treatments for respiratory ailments, pertussis, gastrointestinal issues, common colds, and ear pain. Garlic is known for its health benefits, particularly in relation to fighting cancer, reducing inflammation, and combating infections caused by fungi, viruses, and bacteria. Evidence from various studies conducted on cells, animals, and human populations has shown that garlic has the potential to suppress the growth of cancer cells, and this may be due to its ability to enhance metabolic processes, prevent DNA damage, and reduce the harmful effects of free radicals. Additionally, garlic's antibacterial and antiviral properties may make it useful in treating and preventing infections. Its immunoregulatory impact is mediated through modulating cytokine production and promoting immune responses by encouraging antibody production and immune cell activation (Circella et al., 2022).

Garlic is composed of various biologically active constituents that include both volatile and non-volatile compounds. These constituents consist of water-soluble, oil-soluble, and organosulfur compounds, such as Diallyl sulfide (DAS), Diallyl disulfide (DADS), and Diallyl trisulfide (DATS). Essential oils, sugars, dietary fiber, pectin, and flavonoids are also present in garlic. The majority of garlic's cysteine sulfoxides, specifically L-cysteine sulfoxides, are composed of alliin, which accounts for about 80% of these compounds. In contrast, DAS, DADS, DATS, DAS, vinyldithiins, and ajoene are examples of fat-soluble organosulfur compounds that are also present in garlic (Amagase 2006). Alliin, which is a solid substance, has a solubility of 1e+006 mg/L at 25 °C, a boiling point of 416.13 °C, and a melting point of 163 °C (Bijun Cheng and Tianjiao Li 2020). Glutamyl-L-cysteine peptides are water-soluble dipeptides, and their content in garlic remains stable even when crushed. Non-sulfur phytochemicals, such as saponins, flavonoids, organoselenium, steroids, and allixin, are also present in garlic. Thiosulfinates (e.g., allicin), vinyldithiins (e.g., vinyl-1,3-dithiin, vinyl-1,2-dithiin), sulfides (e.g., DADS, DATS), and ajoenes (e.g., E-ajoene, Z-ajoene) are some of the other compounds found in garlic (Bazaraliyeva et al., 2022).

A. sativum bulbs are known to possess a plethora of phytochemicals, with the majority being sulfur-based compounds (refer to Tab 1). Garlic-based formulations contain numerous organosulfur compounds, such as N-acetylcysteine (NAC), S-allyl-cysteine (SAC), and S-allyl-mercapto cysteine (SAMC), all of which originate from alliin. It is worth noting that SAC demonstrates antioxidant, anti-inflammatory, redox regulation, pro-energetic, antiapoptotic, and signaling properties, while SAMC exhibits anticancer activity by inhibiting cancer cell proliferation (Batiha et al., 2020). The majority of garlic's bioactive components can be traced back to sulfur-based compounds and their precursors. These include Allicin, DAS, DADS, DATS, 2-Vinyldithiins, ajoene which not only possess significant antioxidant properties but also contribute to garlic's essential biological activities (Nakamoto et al., 2020). Garlic contains a variety of hydrophobic antimicrobial compounds, which have been identified and their structures are depicted in Fig 1.

ALLICIN (S-ALLYL-2-PROPENE THIOSULFINATE)

In the realm of garlic's bioactive compounds, allicin is recognized as a key contributor to the plant's medicinal properties (Nakamoto et al., 2020). Upon crushing of garlic clove, a heat-sensitive enzyme called alliinase transforms alliin (S-allyl-L-cysteine sulfoxide) into allicin and ammonium pyruvate, producing garlic's characteristic pungent aroma (Nayab et al., 2021). Fresh garlic bulbs have been found to contain roughly 0.4% allicin and 0.9% alliin. Alliin is the main sulfur compound in both raw and powdered garlic, with about 8 g/kg present in garlic cloves (Espinoza et al., 2020). Allicin can compromise bacterial cell walls by destabilizing the peptidoglycan layer, leading to cell shrinkage, swelling, and eventual lysis. Additionally, allicin has been demonstrated to inhibit bacterial biofilm formation by regulating quorum sensing, which is a significant contributor to antibiotic resistance. Diallyl sulfide, diallyl, and diallyl trisulfide, which possess hydrophobic properties, can disrupt phospholipids in bacterial cell membranes, increasing permeability and causing cell lysis and death (Ezeorba et al., 2022). Antibiotic-resistant bacteria, including MRSA and various multidrug-resistant strains such as *Enterococcus*, *E. coli*, *S. flexneri*, *Shigella*

Tab 1. Active phytochemicals of garlic

Sulfur containing compounds		**Non-volatile**		**Non-sulfur phytochemicals**
Allicin (thiosulfinate)		γ-glutamyl-*S*-alk(en)yl-L-cysteine peptides γ -glutamyl-*S*-allyl-L-cysteine		Flavonoids
Diallyl sulfide (DAS)		γ -glutamyl-*S*-*trans*-1-propenyl-L-cysteine		Organoselenium compounds
Diallyl disulfide (DADs)		*S*-alk(en)yl-L-cysteine sulfoxides	*S*-allyl-L-cysteine sulfoxide (alliin)	Steroid saponins
Diallyl trisulfide (DATS)			*S*-(*trans*-1-propenyl)-L-cysteine sulfoxide (isoalliin) *S*-methyl-L-cysteine sulfoxide (methiin) S-allyl cysteine (SAC)	
Methyl allyl disulfide (MADS)				
Methyl allyl sulfide				
Ajoene				
Vinyl dithiins	2-vinyl-1,3-dithiin			
	3-vinyl-1,2-dithiin			

dysenteriae, and *S. sonnei*, have been found to be susceptible to allicin. Moreover, pure allicin has exhibited antifungal activity in vitro against organisms like *Cryptococcus*, *Candida*, *Epidermophyton*, *Microsporum*, and *Trichophyton*, with MICs ranging from 1.57 to 6.25 $\mu g\ mL^{-1}$. Allicin's antibiotic potency is noteworthy, with 1 mg of allicin equating to 15 IU of penicillin. Allicin is believed to exert its antimicrobial effects mainly by impeding the function of microbial enzymes containing thiol groups through a fast chemical reaction between thiosulfonates and thiols (Liang et al., 2022).

AJOENE (*E*-AJOENE, *Z*-AJOENE)

Ajoene is a collection of various organosulfur compounds that are associated with the properties of garlic. Chemically identified as (E, Z)-4,5, 9-trithiadodeca-1, 6, 11-triene 9-oxide, ajoene remains stable in water (Espinoza et al., 2020). Derived from three allicin molecules, both types of ajoene undergo this conversion (Nakamoto et al., 2020). Several theories have been proposed to explain the antibacterial properties of these compounds. Ajoene fights bacteria by inhibiting DNA, RNA, and protein synthesis, which stops bacterial growth and ultimately results in cell death (Ezeorba et al., 2022). Furthermore, ajoene, when isolated from garlic extracts, may obstruct adhesive interactions and the fusion of leukocytes (Fesseha and Goa, 2019).

POLYSULFIDES/ DAS$_N$

Allicin, produced by the conversion of alliin via the enzyme alliinase, is known to naturally break down into a variety of sulfides. These include Diallyl disulfide (DADS) and Diallyl trisulfide (DATS), which are primary constituents of garlic oil. During this process, allicin is converted into different types of sulfides, with DADS being the most prevalent. Sulfides with higher numbers of sulfur atoms, such as DATTS and DAPS, exhibit stronger antimicrobial properties than those with fewer sulfur atoms (Kim et al., 2009).

DASn compounds are significant components of garlic oil, formed from allicin during steam distillation. The quantity of sulfur atoms present in DASn, which is found in garlic oil, can vary from 1-9 depending on the way it is produced. The most commonly occurring sulfur compounds in this oil are tri- and tetra-sulfur compounds. DASn has been found to have some antimicrobial activity against Gram-positive bacteria, even those that are resistant to drugs. The degree of antimicrobial activity is dependent on the number of sulfur atoms present in the molecules, with DAS4 being the most effective, followed by DAS3, DAS2, and DAS1. Thus, DASn with more than 5 sulfur atoms may display increased potency against bacterial pathogens (Nakamoto et al., 2020).

Generally, organosulfur compounds like DAS, DADS, and DATS are classified as polysulfides and have minimal antioxidant capabilities. Additionally, these compounds lack the ability to generate sulfenic acid. DATS possesses antioxidant properties that can inhibit the formation of lipid hydroperoxide in LDL. This is due to unpaired electrons connected to sulfur's double bonds, which can enhance antioxidant activity. The number of sulfur atoms in the molecule impacts this property since disulfides have lower activity than trisulfides (Subroto et al., 2021).

VINYLDITHIINS

Vinyldithiins, which consist of 2-vinyl-4H-1,2-dithiin and 3-vinyl-4H-1,3-dithiin, are derived from a single allicin molecule. These substances are distinct sulfur-bearing compounds found in garlic oil macerate products. Vinyldithiins exhibit a variety of biological effects, including anti-obesity properties, yet they lack antimicrobial activity (Nakamoto et al., 2020).

ANTIBACTERIAL ACTIVITY OF GARLIC IN DIFFERENT PREPARATIONS

Various garlic formulations are created to assess the antibacterial qualities of garlic, such as water-based garlic extract (AGE), powdered or natural garlic extract, alcohol-based garlic extract (using methanol or ethanol), raw or unprocessed garlic extract (FGE), and garlic paste.

Aqueous garlic extract (AGE)

For the aqueous extract of garlic, 70 grams of raw garlic cloves were meticulously mixed with 35 milliliters of uncontaminated distilled water. The mixture was then centrifuged at a speed of 5000 rpm and purified using a 0.45-micron membrane filter. The resulting aliquots were carefully preserved at a temperature of -20°C and used within a maximum period of two weeks (Hatem 2021).

Powdered or organic garlic extract

For the powdered or organic extract of garlic, the sun-dried plants under consideration are finely ground using a blender. A 40-gram portion of this powdered material was subjected to cold maceration in 100 cm3 of 96% methanol. The mixture was then stirred at four-hour intervals and maintained at room temperature for a total of 48 hours. The solvent was subsequently filtered using Whatman's No. 1 filter paper, sterilized with a 0.22-µm filter membrane, and then oven-dried at 40 °C for 24 hours. The methanolic extract is stored in a sterile, light-proof, airtight container at -4 °C in a refrigerator to prevent microbial contamination until needed for further examination (Ismail et al., 2020).

Nonetheless, research has shown that garlic's water-based extract is more potent than its organic counterparts. The aqueous extraction process releases a variety of enzymes, such as phenolases and hydrolases, which may contribute to the modulation of active compounds in plant material extracts (Ismail et al., 2020).

Methanolic or ethanolic garlic extract

In order to create the extract, 100 grams of garlic cloves pulverized using a mortar and pestle. The crushed cloves were then divided into two equal portions, with one part mixed with 50 milliliters of 20% ethanol and the other with 50 milliliters of 20% methanol. These mixtures are covered with aluminum foil and refrigerated at 4°C for a 48-hour period. Following this time, both solutions were subjected to centrifugation at 5500 rpm for 10 minutes using sterilized 50 milliliter Falcon tubes to separate solid debris from the liquid. The resulting supernatants transferred to a new 50 milliliter Falcon tube and stored at 4°C (Nayab et al., 2021).

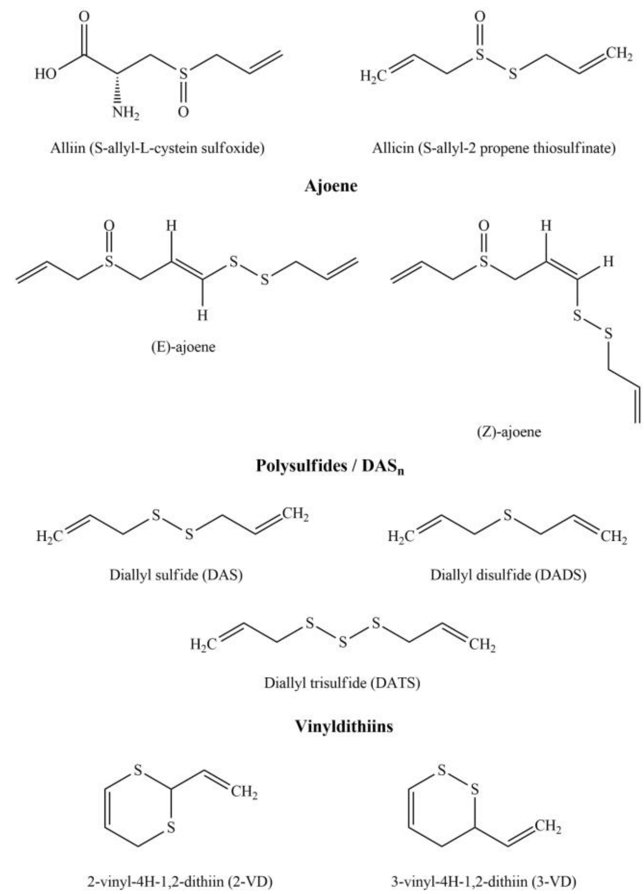

Fig 1. Compounds structures.

Further research has shown that a 70% ethanol extract of crude *A. sativum* exhibits a notably stronger inhibitory effect on methicillin-resistant *Staphylococcus aureus* than its aqueous counterpart (Mohammed et al., 2021).

Allicin's methanolic and ethanolic extracts are favored over water-based extracts due to the instability of allicin in water. This instability arises as hydrogen in water reacts with oxygen atoms in allicin, compromising the molecular structure. Allicin's interaction with water leads to the formation of diallyl disulfide, a compound lacking the desired antimicrobial potency (Nayab et al., 2021).

Numerous investigations have assessed the antimicrobial potential of different garlic formulations, including Raw garlic extract (RGE) and garlic paste. Researchers have reported the antimicrobial effects of these garlic preparations against both commensal and harmful gut bacteria, including various strains of *Escherichia coli, Salmonella, Shigella, Vibrio, Campylobacter, Listeria monocytogenes, Enterobacter, Enterococcus, Lactobacillus acidophilus, Staphylococcus aureus, Streptococcus,* and *Clostridium difficile*. RGE was found to be effective against multidrug-resistant Shiga-toxin producing *E. coli* (STEC) strains in clinical and food specimens. Additionally, both raw and aqueous garlic extracts showed anti-adhesion properties against the standard strain of *Streptococcus mutans*. An allicin-rich raw extract demonstrated superior antimicrobial action against *Mycobacterium phlei*, *Mycobacterium smegmatis*, and *Mycobacterium tuberculosis* when compared to isoniazid and ethambutol (Bhatwalkar et al., 2021). Furthermore, prior research revealed that combining fresh garlic (*Allium sativum*) extracts with antibiotics resulted in enhanced antimicrobial sensitivity, which could be crucial in addressing infections caused by virulent, drug-resistant bacterial strains that currently pose significant public health challenges globally. One study found that garlic extract exhibited a stronger inhibitory effect against pathogens than Ampicillin, and when combined with liquid garlic extract, the bacteriostatic activity of Ampicillin significantly increased, displaying effective synergism against various pathogens such as *P. aeruginosa, E. coli, S. aureus,* and *S. typhi*. This suggests that the primary compound allicin might boost the activity of ampicillin against pathogens in both in vitro and in vivo experiments (Ismail et al., 2020).

The antimicrobial properties of garlic's allicin compound have been demonstrated to be three times more potent against Gram-positive bacteria than Gram-negative bacteria (Putri et al., 2021). For instance, allicin was found to be more effective against the Gram-positive pathogen MRSA compared to E. coli (Choo et al., 2020). The presence of lipids in the membrane of Gram-positive bacteria like *Staphylococcus aureus* aids the penetration of allicin compounds, allowing them to impact bacterial RNA and protein synthesis (including through disulfide compound exchanges) by suppressing enzyme-free thiol group activity, resulting in bacterial damage or death. This confirms that allicin primarily targets RNA as an antibacterial agent (Putri et al., 2021).

Allicin, despite being chemically unstable and degrading quickly upon contact with bodily fluids, has the ability to easily penetrate the bacterial cell membrane due to its hydrophobic properties. This allows it to enter the bacterial cellular compartment and react with free thiol groups in a rapid manner (Nakamoto et al., 2020). However, Gram-negative bacteria, such as E. coli, possess a membrane with a lipid content ten times greater than that of *Staphylococcus aureus*. This makes it difficult for allicin compounds to reach their target, as they get trapped in the lipid content (Putri et al., 2021).

The impact of allicin on the enzyme thiol group's activity in bacteria makes it difficult for the bacteria to alter or modify the involved enzyme, significantly reducing the likelihood of bacterial resistance to allicin and garlic (Putri et al., 2021).

Recent findings have shown that allicin, extracted through an aqueous process, can effectively hinder the growth of various drug-resistant bacteria strains, including *S. aureus, E. coli, K. aerogenes,* and *S. enterica goldcoast,* with the most significant impact on the first pathogen (Choo et al., 2020). Moreover, allicin has demonstrated potential in inhibiting the growth of both drug-sensitive and drug-resistant *Mycobacterium tuberculosis* strains in vitro and in vivo without harming the host. In addition to inhibiting *M. tuberculosis*, allicin was also found to enhance the host's immune system. The researchers suggested that allicin could potentially be combined with standard antibiotics for TB treatment, though the results remain uncertain (Choo et al., 2020). A recent research study demonstrated that the administration of garlic extract significantly decreased the quantity of bacteria in mice. After being treated with either allicin or garlic extracts, no *M. tuberculosis* was detected in the mice even after a 60-day period. When combined with the standard *M. tuberculosis* drug isoniazid, the bacteria were eliminated in just 45 days, compared to over 60 days for isoniazid alone. This finding suggests the potential to shorten the duration of DOTS (Directly Observed Treatment, Short-course) treatment, which could help reduce the emergence of drug resistance. In terms of the immunological impact of garlic derivatives combined with DOTS treatment, garlic extract had a minimal effect on CD4+ and CD8+ T cell prevalence but still outperformed isoniazid treatment alone. According to a study, isoniazid treatment triggers the apoptosis of activated CD4+ T cells. However, the current research has validated that garlic has the potential to counteract these harmful effects, thus reducing the adverse reactions of DOTS therapy. This could be advantageous for patients undergoing DOTS treatment, or those who are recommended to consume garlic throughout their therapy to enhance their immune system continually and decrease the probability of TB reactivation or recurrence, a significant limitation associated with DOTS therapy (Fatima and Dwivedi 2020).

Recent research has revealed that compounds containing sulfur, originating from garlic, like diallyl disulfide (DAS2) and ajoene, can hinder bacterial biofilm development and quorum sensing, despite having less potent antimicrobial properties than conventional antibiotics used in healthcare settings (Nakamoto et al., 2020). Studies involving *Salmonella sp*. and *Shigella sp*. have shown that unadulterated garlic extract (*Allium sativum*) exhibits limited antibacterial efficacy. The high lipid content (11-22%) and complex, multilayered cell walls of these two bacteria make it challenging for antibacterial agents to infiltrate the target bacteria (Javier et al., 2023).

Further research supports the traditional knowledge of *A. sativum* antimicrobial potential against group B *Streptococcus* (GBS). While the antimicrobial properties of this plant species are well-established, there is limited documentation of its effects against the pathogen (*S. agalactiae*), which is responsible for neonatal sepsis as a result of vertical transmission from mother to child before or during birth. This study identified that two peptides and a mixture of ajoene (E and Z) were the most effective compounds derived from *A. sativum* for combating *S. agalactiae*. These findings hold great significance, as they pave the way for the potential development of a novel topical medication to treat *S. agalactiae* infections during pregnancy, rather than relying on prophylactic measures during childbirth. The creation of such treatments could aid in reducing the overuse of antibiotics and the consequent rise in bacterial resistance (Torres et al., 2021).

Investigations into *E. coli* cultures have revealed that aged garlic extract, S-allyl cysteine, diallyl sulfide, and diallyl disulfide neither hinder the antibiotic effectiveness of gentamycin nor enhance its nephrotoxicity. Additionally, aged garlic has demonstrated the ability to counteract nicotine-induced oxidative damage in rat experiments. Further research could establish garlic as an exceptional option for reducing the harmful consequences of therapeutic drugs (Fesseha and Goa, 2019).

CONCLUSION

The antimicrobial properties of garlic are largely credited to allicin, a compound known for its sulfhydryl-modifying activity and its capacity to inhibit sulfhydryl enzymes. Raw garlic does not contain allicin, but the enzyme allinase quickly produces it. Allicin's main antimicrobial effects are thought to result from its rapid interaction with free thiol groups via thiol-disulfide exchange, specifically with enzymes containing thiol groups like cysteine proteases and alcohol dehydrogenases. These enzymes are essential for bacterial nutrition and metabolism, making resistance to allicin less likely to occur compared to certain antibiotics. Resistance to allicin is speculated to be 1,000 times less frequent than with some antibiotics. Moreover, garlic and its derivatives have regained attention as potential natural remedies due to the growing trend towards alternative medicine and natural products. Consumed worldwide in various forms, ranging from crushed to encapsulated, garlic boasts numerous benefits and potential applications in the prevention and treatment of various ailments. Fresh and powdered garlic remains popular for food flavoring and should continue to be utilized. Currently, bacterial resistance is a growing concern, and the future of antibacterial drugs remains uncertain. Despite the pharmaceutical industry's development of numerous new antibiotics in recent decades, bacteria's resistance to these drugs has escalated. Garlic serves as a valuable source of novel and biologically active compounds with antibacterial properties, either through direct action against bacteria or synergism with antibiotics. The antifungal, antibiotic, and potential anticancer effects of garlic are widely acknowledged, and supported by an extensive body of scientific literature. Moreover, advancements in analytical techniques, refined bioassays, and biotechnological exploitation should enable these essential plants to maintain their critical role in promoting human and animal.

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