

Antimicrobial properties of hydrophobic compounds in garlic: Allicin, vinylldithiin, ajoene and diallyl polysulfides (Review)

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Abstract. *Allium* plants, such as garlic, onion and leek have long been known to be effective in the therapy of infectious diseases. In particular, garlic has a greater antimicrobial activity than other *Allium* plants as it contains several hydrophobic antimicrobial compounds, such as allicin, vinylldithiins, ajoenes and diallyl polysulfides. Allicin is a characteristic sulfur-containing compound found in raw garlic produced from alliin and exhibits antimicrobial activity against both Gram-positive and Gram-negative bacteria. In addition, allicin has been reported to inhibit the biofilm formation of bacteria, which is a major cause of bacterial resistance to the antibiotic treatment of infections, by regulating quorum sensing in microorganisms. Other hydrophobic compounds also have similar inhibitory effects on bacteria as allicin. These biological properties of garlic-derived hydrophobic compounds can be used to enhance the effects of existing drugs and may thus be used in the treatment of infections, such as by preventing drug resistance through the inhibition of biofilm formation. In this review, we summarize the effects of hydrophobic compounds of garlic on bacteria.

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Abbreviations: DAS_n, diallyl polysulfides; DAS₁, diallyl sulfide; DAS₂, diallyl disulfide; DAS₃, diallyl trisulfide; QS, quorum sensing

Key words: garlic, hydrophobic compound, antimicrobial activity, biofilm

1. Introduction

Recently, the spread of drug-resistant bacteria has become a serious global concern in the therapy of infectious diseases. A number of antibiotics have been developed and used to treat infectious diseases; however, the increased frequency in the use of such antibiotics has led to changes in bacterial characteristics, with bacteria acquiring drug-resistant ability through the mutations of drug-target molecules, the overexpression of efflux pumps, changes in the composition of the cell membrane, the production of metabolizing enzymes and biofilm formation (1). Among these, the biofilm comprises a large community and aggregation of bacteria and its formation protects microbial cells from antibiotics and immune cells. The composition of biofilm is mainly water and extracellular polymeric substances (EPS), such as proteins, DNA, RNA and polysaccharides (1,2). The production of EPS is regulated by quorum sensing (QS), which represents cell-to-cell communication in bacteria and is controlled by chemical signaling molecules, such as *N*-acyl-L-homoserine lactones (AHLs) (3,4). Thus, QS plays a crucial role in biofilm formation. The biofilm formation of bacteria has been reported to be associated with chronic infections; therefore, an increase in biofilm-forming bacteria is a serious issue, not only in the medical field, but also in a number of industrial fields and facilities. Therefore, the discovery and development of drugs to combat the formation of biofilm is an important approach for the fight against drug-resistant infections.

Garlic (*Allium sativum* L.) has been used as not only a food, but also as a remedy for several diseases, such as cardiovascular diseases and cancer (5-7). In addition, garlic has long been used in the treatment of infectious diseases, as described in the 9th century literary book entitled 'Bald's Leechbook'. A remedy termed Bald's eye salve for stye that is caused by *Staphylococcus aureus* (*S. aureus*) infection was prepared by the alcoholic extraction of garlic, onion or leek in a brass pot (7,8). Fuchs *et al* demonstrated the antimicrobial activity of Bald's eye salve against *S. aureus* and *Pseudomonas aeruginosa*, including the multidrug-resistant phenotype and identified allicin as the principal antimicrobial compound in Bald's eye salve formulation. Allicin may greatly contributed to the treatment of stye in that era (7). On the other hand, allicin is chemically unstable and rapidly disappears when it comes into contact with body fluids (9,10). Therefore, it is difficult for allicin to reach the infected sites of

the body as an intact form. Recent studies have demonstrated that sulfur-containing compounds derived from garlic, such as diallyl disulfide (DAS₂) and ajoene, inhibit biofilm formation and the QS of bacteria, even though the antimicrobial activities of these compounds are lower than those in medical antibiotics used in clinical settings (11-16). Furthermore, Slachmuylders *et al* and others have demonstrated that some natural products, which exert an inhibitory effect on biofilm formation, have antibiotic-potentiating activity (17-20).

In this review, we focus on the antimicrobial activity of sulfur-containing compounds derived from garlic and describe their chemical and biological properties, including their inhibitory effect on bacterial biofilm formation.

2. Antimicrobial activity of hydrophobic compounds in garlic

Various hydrophobic antimicrobial compounds have been isolated from garlic and their structures are illustrated in Fig. 1. Among these compounds, allicin is considered to play a central role in the biological activity of garlic. However, allicin is unstable and tends to be converted into various compounds, such as ajoenes and diallyl polysulfides (DAS_n), which have been reported to exhibit antimicrobial activity. In this section, we describe the chemical and biological properties of hydrophobic compounds in garlic and its preparation, exhibiting antimicrobial activity.

Allicin. Allicin is the most abundant and characteristic sulfur-containing compound in raw garlic. It is produced from alliin (21). Allicin has been shown to exhibit broad-spectrum antimicrobial activity against Gram-positive and -negative bacteria, including multidrug-resistant bacteria (22-26). In addition, allicin has been shown to possess antiviral, anti-fungal and anti-parasitic activity (27,28). It has been reported that allicin exhibits antimicrobial activity by the S-allylmercapto modification of thiol-containing proteins in bacteria, which leads to lethal events, including the reduction of glutathione levels, the induction of protein aggregation and the inactivation of crucial enzymes (29). Reiter *et al* reported that allicin vapour exhibited antimicrobial activity against lung pathogenic bacteria (30). Additionally, topical treatment with allicin has been shown to improve skin infection caused by methicillin-resistant *S. aureus* (MRSA) (31). However, allicin is unstable and has been shown to be decomposed or metabolized within a few seconds in the blood (10). Therefore, the use of allicin may be limited to direct inhalation or external medicine due to its instability.

Vinyldithiins. Vinyldithiins that contain 2-vinyl-4*H*-1,2-dithiin and 3-vinyl-4*H*-1, 3-dithiin are converted from one allicin molecule (32,33). These compounds are characteristic sulfur-containing compounds in garlic oil macerate products (33). Vinyldithiins are known to have several biological activities, such as anti-obesity activity (34); however, they have no antimicrobial activity (35).

Ajoenes. Ajoenes (Z-ajoene and E-ajoene) are also characteristic sulfur-containing compounds in garlic oil macerate products. Both ajoenes are converted from 3 allicin molecules (21). The antimicrobial activity of ajoenes has been

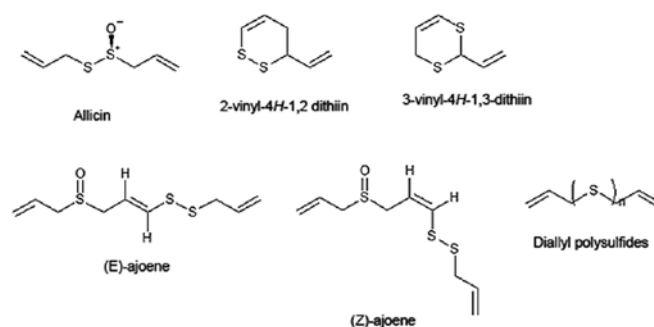


Figure 1. Hydrophobic compounds in garlic.

evaluated by several groups. Yoshida *et al* examined activity of ajoenes against Gram-positive and -negative bacteria and found that MIC values were 5-20 μ g/ml for Gram-positives and 100-160 μ g/ml for Gram-negatives. They also indicated that Z-ajoene had a slightly greater activity than E-ajoene (36). An additional antimicrobial study of ajoenes against 3 strains of *H. pylori* demonstrated that the antimicrobial activities of both forms were similar i.e., 15-20 μ g/ml for the Z-form and 25 μ g/ml for the E-form (37). Ajoenes are also active against fungi, such as *Aspergillus niger* and *Candida albicans* (38,39). Thus, ajoenes seem to be potent antimicrobial compounds; however, these compounds rapidly disappear after being mixed with the blood, as the case with allicin (10).

DAS_n. DAS_n are major components of garlic oil, which are produced from allicin during the processing of garlic oil by the steam distillation method (40). Sulfur atom numbers of DAS_n in garlic oil vary from 1 to 9, depending on the production conditions. Generally, tri- and tetra-sulfur compounds are abundantly present (40). DAS_n have limited antimicrobial activity against Gram-positive bacteria, including drug-resistant bacteria (41). Their antimicrobial activities depend on the number of sulfur atoms in the molecules and are in the order of diallyl tetrasulfide (DAS₄) > diallyl trisulfide (DAS₃) > DAS₂ > diallyl sulfide (DAS₁) (12). Therefore, DAS_n containing a higher number of sulfur atom than 5 may have more potent activity against bacterial pathogens.

Antimicrobial activity of compounds without sulfur atom derived from garlic. Matsuura *et al* isolated new furostanols termed proto-eruboside-B and satiboside-B from a crude glycoside fraction of garlic. They also found that these saponins transform into spinostanol form by endogenous β -glucosidase during processing period (42,43). Notably, spinostanol from eruboside-B inhibits the growth of *Candida albicans*, whereas furostanol from proto-eruboside-B, does not (44). Koderá *et al* isolated a phenolic antimicrobial compound, 3-hydroxy-5-methoxy-6-methyl-2-n-pentyl-4*H*-pyran-4-one, termed allixin (45). This compound was phytoalexin; however, the antimicrobial activity was very low.

3. Effects of sulfur compounds on biofilm formation and quorum sensing

Bacteria have a barrier system, biofilm formation, which inhibits the entry of disinfectants, antibiotics and host

immune molecules into the bacterial cells and is a major cause of the drug-resistance of bacteria (44). In addition, QS molecules, such as AHL regulate biofilm formation, intercellular communication, bacterial population and other processes (3,4,11,46). The inhibition of biofilm formation and QS has been studied in various scientific and technological fields. Certain natural products have been reported to provide effective resources for the inhibition of biofilm formation. Rasmussen *et al* performed screening to identify QS inhibitors (QSIs) by using a novel genetic system and found that toluene extract of garlic inhibited biofilm formation (13). This result suggests that hydrophobic compounds extracted from garlic might have activity as a QSI (47). Allicin prevents biofilm formation by inhibiting early bacterial adhesion and EPS secretion (42,48,49). In addition, allicin inhibits the secretion of virulence factors by regulating QS (50). Ajoene regulates biofilm formation by blocking the QS-induced production of virulence factors (51,52). DAS₂ inhibits the biofilm formation of *Pseudomonas* spp by inhibiting the production of virulence factors via the regulation of QS at the concentration of 0.16-1.28 mg/ml with no effects on microbial growth (44). In addition, DAS₂ inhibits the formation of biofilm by suppressing the expression of key QS-related genes (44). Moreover, in the *S. aureus* QS system, a peptidic compound having a thioester group acts as an autoinducer (53). It is expected that DAS₂ may also inhibit QS through the reaction with the thioester group of the autoinducer. These hydrophobic compounds may contribute to the reduction of undesirable impacts of microorganisms on humans and they can be expected to suppress the development of drug resistance due to biofilm formation of bacteria.

4. Conclusion

Various hydrophobic compounds derived from garlic and its preparations have broad-spectrum antimicrobial activities. In particular, allicin and its derivatives have been studied extensively as antimicrobial active ingredients and have shown the inhibitory activity of biofilm formation by inhibiting QS. However, these compounds are unstable and could not be used against systemic infections. Therefore, the development of allicin derivatives with sufficient stability may lead to the development of superior compounds with greater antimicrobial activity and more potent inhibitory activity against biofilm formation for the treatment of drug-resistant bacteria.

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Authors' contributions

MN and YK conceived this review. MN, KK and JIS analyzed the relevant literature. MN wrote the first draft of the manuscript and produced the figures. KK, JIS and YK critically revised the manuscript. All authors have reviewed and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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