Antimicrobial Activity of Garlic Derivatives on Common Causative Microorganisms of the External Ear Canal and Chronic Middle Ear Infections

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Original Investigation

Abstract

Objective: Today, antibiotic resistance is increasing and evolving into an important health problem. Therefore, it is important to research on alternative therapies to antibiotics. This study aimed to investigate the inhibitory effect of four garlic derivatives on microorganisms commonly isolated in ear infections.

Methods: The antimicrobial activities of allicin, s-allyl cysteine (SAC), diallyl disulfide (DADS), and s-allyl mercaptocysteine (SAMC) were investigated on standard strains of commonly isolated microorganisms using the broth microdilution method. The test strains were selected among the microorganisms responsible for chronic suppurative otitis media and otitis externa. These microorganisms were *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Enterococcus faecium*, *Candida albicans*, and *Candida tropicalis*.

Results: Minimum inhibitory concentration (MIC) values of allicin and SAC ranged from 0.125 to 20 µg/mL for fermentative bacteria (*E. coli* and *K. pneumoniae*), 20 to 80 µg/mL for non-fermentative bacteria (*P. aeruginosa* and *A. baumannii*), 5 to 10 µg/mL for gram-positive cocci (*S. aureus* and *E. faecium*), and 40 to 80 µg/mL for yeasts (*C. albicans* and *C. tropicalis*). MIC values of DADS ranged from 40 to 80 µg/mL for fermentative bacteria, 40 to 160 µg/mL for non-fermentative bacteria, 40 to 80 µg/mL for gram-positive cocci, and 20 to 40 µg/mL for yeasts. The MICs of SAMC were >640 µg/mL for the tested bacteria and yeasts.

Conclusion: Both allicin and SAC showed antimicrobial activity against the tested microorganisms, even at low concentrations. These two derivatives may be used to treat infections in the future.

Keywords: Garlic, antimicrobial agent, chronic otitis media, otitis externa, microbiologic technique

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Introduction

In the presence of tympanic membrane perforation, ear discharge lasting longer than six weeks is defined as chronic suppurative otitis media (CSOM), and inflammation or infection of the external auditory canal is defined as otitis externa (OE) (1). Infectious agents including bacteria, fungi, and viruses are responsible for CSOM. The most common causative agents of CSOM are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterobacteriaceae*, such as *Proteus* spp., and *Klebsiella pneumoniae* (2-4). However, when disruption occurs in external auditory canal defense, new pathogenic flora can develop and cause OE.

P. aeruginosa and S. aureus are the main causes of OE. Other microorganisms such as *Proteus mirabilis, Klebsiella. pneumoniae, Escherichia coli, Streptococcus pyogenes, Staphylococcus epidermidis*, and the *Candida* species have also been isolated in samples of OE (5-7). Infections are often polymicrobial, and anaerobic bacteria can also be detected in these infections (2).

Antibiotic resistance is becoming an important health problem with increasing mortality and morbidity rates (8); therefore, it is imperative to investigate alternative therapies to presently used antibiotics. The use of plants as natural health care resources has become increasingly popular. Garlic (*Allium sativum L.)*, a member of the *Liliaceae* family, has antibacterial and antioxidant effects

(9-12). It might be a candidate molecule to interrupt antibiotic resistance and provide synergy with antimicrobial drugs (12).

Garlic has some biologically active compounds such as allicin (diallyl thiosulfinate), s-allyl cysteine (SAC), s-allyl mercaptocysteine (SAMC), and diallyl disulfide (DADS). The inhibitory effect of garlic derivatives on the common causal agents of middle and outer ear canal infections has not been studied in detail. Therefore, this study aimed to investigate the antimicrobial activities of four garlic derivatives (GDs), allicin, SAC, SAMC, and DADS, on the common causative microorganisms of CSOM and OE.

Methods

Ethics Approval

The study was initiated after receiving the approval of the local ethics committee (Date: 15 August 2017; No: 2017/0282). Informed consent was not required since the study was not performed on humans or human subjects.

Preparation of Garlic Derivative Solutions

GDs were dissolved in suitable solutions: Allicin was in liquid form in a concentration of 50 mg/mL. SAC in sterile distilled water, SAMC in water containing 30 mM HCL, and DADS in methanol. Then, stock solutions (1280 µg/mL) of each derivative were prepared in Mueller-Hinton broth media (Mueller-Hinton broth media; Merck, Darmstadt, Germany). These dilutions were used immediately. The antimicrobial activity of methanol, 30 mM HCL, and dimethyl sulfoxide (DMSO) in stock garlic solutions and their serial dilutions between 1/2 and 1/256 were also tested.

Preparation of Control Antibacterial and Antifungal Solutions

Ciprofloxacin and fluconazole were used as controls to verify the experimental conditions in antibacterial and antifungal activity tests, respectively. These antibiotics were also used to evaluate the antimicrobial activities of the GDs. Commercial powders of ciprofloxacin (Ciprofloxacin; Sigma, St. Louis, MO, USA) and fluconazole (Fluconazole; Sigma-Aldrich, St. Louis, MO, USA) were dissolved in water, and 1,280 µg/mL stock solutions of ciprofloxacin and fluconazole were prepared.

Test Strains

Antimicrobial activities of the GDs were tested on standard strains of *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecium* ATCC BAA2127, *Candida albicans* ATCC 102316, and *Candida tropicalis* ATCC 13803. The test strains were selected among the microorganisms responsible for CSOM and OE.

Determination of Antimicrobial Activities Against Bacteria

The minimum inhibitory concentration (MIC) of each GD was determined using the broth microdilution method recommended by the Clinical Laboratory Standard Institute (CLSI) (13). Serial dilutions (0.125–640 µg/mL) of the GDs were prepared in Mueller-Hinton broth media. Bacterial strains grown on Mueller-Hinton agar plates were suspended in Mueller-Hinton broth. The cell densities were adjusted to 0.5 McFarland standards (1–1.5 × 10^8 cells/mL for bacteria) and were diluted to 1/100. Working inoculums (0.1 mL) of each bacterial strain were added into the microplate wells containing the previously prepared serial dilutions of the GDs and ciprofloxacin. The inoculated plates were sealed with lids and incubated at 35°C for 24 hrs. Both sterile medium and growth control wells were included in each experiment. The MICs were defined as the lowest concentration of the GD, which prevented visible growth of the test strain. Each test was performed in triplicate.

A loop-full of medium from wells with no visible bacterial growth was spread onto the Mueller-Hinton agar (Mueller-Hinton agar; Merck, Darmstadt, Germany) to determine the minimum bactericidal concentration (MBC). MBCs were determined after overnight incubation. MBC was considered as the lowest concentration yielding less than four colonies (13).

Determination of Antimicrobial Activities Against Yeast

The antifungal activities of the GDs were determined using the broth microdilution method recommended by the CLSI (13). First, 1280 µg/mL of each GD solution was prepared in Roswell Park Memorial Institute (RPMI)-1640 media (RPMI 1640 media; Sigma, St. Louis, MO, USA) buffered with MOPS [3-(N-morpholino) propanesulfonic acid]. Then, serial dilutions (0.125-640.0 µg/mL) were prepared in RPMI-1640 Media (RPMI-1640 Media; Sigma, St. Louis, MO, USA). Test yeast strains were suspended in RPMI media with a density of 1–5 × 10^6 cells/mL for yeasts. Working inoculums (0.1 mL) of each *Candida* species were inoculated into the appropriate microtiter wells. The microtiter plates were covered with lids and incubated at 30°C for 24 to 48 hrs. Appropriate controls were used as described above. The lowest concentration of a GD that inhibited ≥50% growth of the tested strains compared to the growth in the control well was defined as the MIC. Each test was performed in triplicate. The minimum fungicidal concentration (MFC) of each GD, which was the lowest concentration yielding less than four colonies on Sabouraud dextrose agar (Sabouraud dextrose agar; Merck, Darmstadt, Germany), was determined using the methods described previously (14).

Statistical test was not used in this study. In the current study we aimed to screen the antimicrobial activities of four GDs and to compare their MIC values with ciprofloxacin and fluconazole to determine whether they had any potential antimicrobial effect or not. In this experiment, since we did not obtain quantitative results obtained from many different clinical isolates, we were unable to compare our results with a statistical method.

Results

Antibacterial (MIC and MBC) and antifungal (MIC and MFC) activities of the GDs tested are shown in Tables 1 and 2, respectively. MIC values of allicin and SAC ranged from 0.125 to 20 µg/mL for fermentative bacteria (*E. coli* and *K. pneumo-*

Table 1. Antibacterial activity (MIC and MBC) of the allicin, S-allylcysteine, S-allylmercaptocysteine, and diallyl disulfide

	S-Allylcysteine		Allicin		S-Allylmercaptocysteine		Diallyl disulfide		Control antibiotic (Ciprofloxacin)
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC
E. coli ATCC 25922	0.125	0.250	0.125	0.250	>640	>640	80	160	0.125
K. pneumoniae ATCC 700603	20	40	20	40	>640	>640	40	80	0.250
P. aeruginosa ATCC 27853	80	160	80	160	>640	>640	160	320	1
A. baumannii ATCC 19606	20	40	20	40	>640	>640	40	80	0.03
S. aureus ATCC 29213	10	20	10	20	>640	>640	80	160	0.06
E. faecium ATCC BAA2127	5	20	5	20	>640	>640	40	160	0.06

MIC: minimum inhibitory concentrations (μg/mL); MBC: minimum bactericidal concentration (μg/mL)

Table 2. Antifungal activity (MIC and MFC) of the allicin, S-allylcysteine, S-allylmercaptocysteine, and diallyl disulfide

	S-Allylcysteine		Allicin		S-Allylmercaptocysteine		Diallyl disulfide		Control antifungal (fluconazole)
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC
C. albicans ATCC 102316	80	160	80	160	>640	>640	20	40	0.5
C. tropicalis ATCC 13803	40	80	40	80	>640	>640	40	80	0.5

MIC: minimum inhibitory concentrations (μg/mL); MFC: minimum fungicidal concentration (μg/mL)

niae), 20 to 80 μg/mL for non-fermentative bacteria (*P. aeruginosa* and *A. baumannii*), 5 to 10 μg/mL for gram-positive cocci (*S. aureus* and *E. faeceum*), and 40 to 80 μg/mL for yeasts (*C. albicans* and *C. tropicalis*). MIC values of DADS ranged from 40 to 80 μg/mL for fermentative bacteria, 40 to 160 μg/mL for non-fermentative bacteria, 40 to 80 μg/mL for gram-positive cocci, and 20 to 40 μg/mL for yeasts. The MICs of SAMC were > 640 μg/mL for the tested bacteria and yeasts.

No antimicrobial activities were detected at the tested concentrations of methanol, HCL and DMSO. The obtained MIC values of ciprofloxacin for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, *S. aureus*, and *E. faecium* were 0.125, 0.250, 1.0, 0.03, 0.06, 0.06 μg/mL, respectively. The MIC values of fluconazole for C. albicans and C. tropicalis were 0.5 μg/mL (Table 1, 2). These values were in the ranges reported by the CLSI (13, 14). These results confirmed the suitability of our experimental conditions.

Discussion

Otitis media (OM) is a significant public health problem. In developing countries, 21,000 people die due to OM complications every year, and the prevalence of hearing loss caused by OM is reported as 30 per 10,000 individuals worldwide (range: 0.7-95) (9). Chronic suppurative otitis media can especially result in hearing loss. The treatment of ear infections is based on topical and systemic antibiotherapy and surgery (1).

Recently, the efficacy of the antimicrobials has become limited due to resistance and biofilm formation. Overuse of antibiotic prescriptions is a major burden on the healthcare economy, and antibiotic resistance is problematic in many countries (14). The annual antibiotic treatment costs reportedly increase by 14.1% when antibiotic resistance increases by 5% in acute otitis media (15). In Turkey, according a published article in 2018, the total annual treatment costs for acute bacterial rhinosinusitis and OM were US$101,499,040 and US$57,191,330, respectively (16).

Natural products with low side effects and high therapeutic effect can be used as alternatives to antibiotic resistance in the treatment of infections (17). Garlic is a widely used herbal remedy; it has many health benefits, including antimicrobial activity, and few side effects (18, 19). Garlic otic solution containing extracts of mullein flowers, garlic, yarrow, calendula flowers, and vitamin E, has been found to be effective as oral amoxicillin and topical anesthetics (20, 21). Another study found garlic to be as effective as ketoconazole against fungi (22). The in vitro antimicrobial activity of garlic oil and four diallyl sulfides was investigated against *P. aeruginosa* and *K. pneumoniae*. It was found that the most effective compound was diallyl monosulfide followed by DADS, diallyl trisulfide (DAT), and diallyl tetrasulfide (DATS) (23).

In our study, the MIC values of DADS ranged from 40 to 80 μg/mL for fermentative bacteria, 40 to 160 μg/mL for non-fermentative bacteria, and 40 to 80 μg/mL for gram-positive cocci. The MIC values against yeast ranged from 20 to 40 μg/mL. Although this compound exhibited both microbiostatic and microbicidal activities on the tested microorganisms, this effect was far below the antimicrobial activity of ciprofloxacin and fluconazole, suggesting that this compound should not be considered as an alternative to commercial antimicrobials.

S-allyl cysteine is a water-soluble organosulfur compound. Animal studies have shown that blood concentration and pharmacokinetic parameters are strongly associated with orally admin-

istered SAC doses (24). It has an antioxidant capacity as well as cardioprotective, anti-apoptotic, and anti-inflammatory effects (25). However, there is no available data on antimicrobial activity against infectious agents.

Allicin is one of the most biologically active compounds of garlic, showing antimicrobial activities against gram-positive and gram-negative bacteria and yeast. However, it is unstable at room temperature (26). An et al. (27) showed that allicin prominently increased the effect of Amphotericin-B against *C. albicans* in vitro and in vivo, although allicin did not show a fungicidal effect. On the other hand, allicin-inspired pyridyl disulfides displayed synergy with vancomycin against vancomycin-resistant *Staphylococcus aureus* (28).

Our study found that allicin and SAC were the most effective compounds; they exhibited the lowest MIC values among the tested compounds. Their MIC values against *E. coli* (0.125 µg/mL) were equal to that of ciprofloxacin. *E. faecium* was the second most susceptible bacterium inhibited with a concentration of 5 µg/mL. These compounds showed less inhibitory effect on *S. aureus* (MIC = 10 µg/mL), *K. pneumoniae* (MIC = 20 µg/mL), *A. baumannii* (MIC = 20 µg/mL), *P. aeruginosa* (MIC = 80 µg/mL), and yeast (MIC ranged from 40 to 80 µg/mL). The MIC values of these GDs indicate that it may be possible to allow their clinical use; however, further in vitro and in vivo studies are necessary to determine their toxic concentrations in humans.

SAMC is a water-soluble GD that can inhibit tumor genesis and attenuate aminoglycoside-induced hearing loss (20, 29). However, to our knowledge, the possible antimicrobial activity of SAMC has not been studied to date. In the current study, it was found that SAMC may have antimicrobial activity even though it was the lowest compared to the other GDs. The MIC values of SAMC were found to be >640 µg/mL for bacteria and yeasts. The MIC values for the other GDs were found between 5 and 0.125 for S-allylcysteine and allicin, 40 and 80 for diallyl disulfide, and 0.03 and 0.125 for ciprofloxacin as control.

Conclusion

Although allicin and SAC showed antimicrobial activity in vitro, they are not as effective as ciprofloxacin and fluconazole against the tested bacteria and yeast. In the future, these two derivatives might be used to treat infections; however, further studies are needed to test possible interactions in the tested compounds and between these derivatives and commercial antibiotics. Additional studies are also needed to show the antimicrobial activities of these compounds against the clinical isolates, to determine toxicity levels on human cells, and to standardize their usage doses for patients. In addition, further studies on combinations of GDs with antibiotics will provide valuable information about the clinical values of these natural antimicrobials.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of İstanbul Medeniyet University, Göztepe Training and Research Hospital (Date: 15 August 2017; No: 2017/0282).

Informed Consent: Informed consent was not required since the study was not performed on humans or human subjects.

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Conflict of Interest: The authors have no conflicts of interest to declare.

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