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## Review: antimicrobial properties of allicin used alone or in combination with other medications

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### Abstract

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Garlic (*Allium sativum* L.) is a well-known spice widely utilised for its medicinal properties. There is an extensive record of the many beneficial health effects of garlic which can be traced back to as early as the ancient Egyptian era. One of the most studied properties of garlic is its ability to cure certain ailments caused by infections. In the 1940s, the antimicrobial activities exhibited by garlic were first reported to be due to allicin, a volatile compound extracted from raw garlic. Since then, allicin has been widely investigated for its putative inhibitory activities against a wide range of microorganisms. Allicin has demonstrated a preference for targeting the thiol-containing proteins and/or enzymes in microorganisms. It has also demonstrated the ability to regulate several genes essential for the virulence of microorganisms. Recently, it was

reported that allicin may function better in combination with other antimicrobials compared to when used alone. When used in combination with antibiotics or antifungals, allicin enhanced the antimicrobial activities of these substances and improved the antimicrobial efficacy. Hence, it is likely that combination therapy of allicin with additional antimicrobial drug(s) could serve as a viable alternative for combating rising antimicrobial resistance. This review focuses on the antimicrobial activities exhibited by allicin alone as well as in combination with other substances. The mechanisms of action of allicin elucidated by some of the studies are also highlighted in the present review in order to provide a comprehensive overview of this versatile bioactive compound and the mechanistic evidence supporting its potential use in antimicrobial therapy.

## Introduction

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Garlic has a long-standing history of being both a spice as well as medicine. It has been described in a wide range of ancient texts pertaining to its widespread use throughout history for treatment of various ailments and diseases. The main compound possessing the antimicrobial activities in garlic, named as allicin, was not identified until 1944 by Cavallito and Bailey ([1944](#)). Moreover, allicin exhibits many bioactive properties that span across various fields of studies including antimicrobial ([Ankri and Mirelman 1999](#); [Bakri and Douglas](#)

[2005](#); Dwivedi et al. [2018](#)), anti-inflammatory (Alam et al. [2018](#); Metwally et al. [2018](#)), anti-cancer (Fujisawa et al. [2008a](#); Shang et al. [2019](#)) and immunomodulatory activities (Ankri and Mirelman [1999](#); Arellano Buendía et al. [2018](#); Foroutan-Rad et al. [2017](#)). It is one of the most researched natural compounds due to its impressive history and the ease by which to obtain it. Allicin has demonstrated antimicrobial activities against a broad range of microorganisms including *Staphylococcus aureus* (Leng et al. [2011](#)), *Escherichia coli* (Jabar and Al-Mossawi [2007](#)) and *Candida albicans* (Khodavandi et al. [2010](#)). It has even been reported to exhibit antiviral and antiparasitic properties. The mode of action exhibited by allicin has been associated with its selective targeting of thiol-containing proteins and enzymes of microorganisms, thus inhibiting and/or disrupting their essential metabolic pathways (Strehlow et al. [2016](#)). In addition, allicin also exerts its antimicrobial properties through suppressing the expression of certain genes that are important for the virulence of these microorganisms (Khodavandi et al. [2011a](#); Leng et al. [2011](#)). Interestingly, allicin has also demonstrated the ability to enhance the antimicrobial properties of other substances when used in combination with these substances. Under certain conditions, allicin has shown more promising results when used in combination with other substances in comparison to when it is used alone (Guo et al. [2010](#); Corral et al. [2014a](#); Corral et al. [2014b](#)). It has been hypothesised that allicin

renders the microorganisms vulnerable which allows other substances such as antibiotics or antifungals to act on them. Hence, it is anticipated that combination therapy of allicin and other antimicrobials could be a highly feasible alternative for tackling the problem of drug resistance in infectious diseases.

## Background overview of garlic and its medicinal properties

Garlic has been described for its medicinal properties in many ancient texts as far back as the reign of King Tutankhamun of Egypt in 1336–1327 BC (Rivlin [2001](#)). The most well-known record of its medicinal properties can be traced back to the Codex Ebers, an ancient Egyptian medicine papyrus dated around 1500 BC (Davis [2005](#)). It was said to have originated from the Middle East whereby it was transported by Sumerians in 2600–2100 BC to China and subsequently to Japan and Korea and beyond (Petrovska and Cekovska [2010](#); Oosthuizen et al. [2018](#)). However, there are also evidences indicating that it may have originated from somewhere along the West of China to Kazakhstan and Kyrgyzstan (Petrovska and Cekovska [2010](#)). Garlic and its fellow alliums have been recognised for their many medicinal properties. However, these properties and their *modus operandi* remain elusive until the recent decade.

Garlic is a popular spice and aphrodisiac (Majewski [2014](#)) that is often referred to as a natural antibiotic

(Adetumbi and Lau [1983](#)). During the pre-antibiotics era, garlic bulbs served as one of the main treatment modes for a wide range of ailments due to its broad-spectrum effects (Petrovska and Cekovska [2010](#)). It was used as a form of a nutrient supplement for Egyptian labourers, as treatment for weakness and skin infections in Indians as well as a relief for colic and sea sickness among Greeks. The first mention of garlic and its antimicrobial properties was reported in France during the plague of Marseilles in 1721, when four men resorted to consuming a mixture of macerated garlic and wine tincture known as '*vinaigre des quatre voleurs*' to protect themselves from getting the disease that was afflicting those around them (Harris et al. [2001](#)). Garlic was later utilised in World War II to treat wounded soldiers due to its antibiotic properties (Oosthuizen et al. [2018](#)). However, the interest in garlic had slightly diminished at this point as antibiotics had become readily available.

### Identification of allicin from garlic (*Allium sativum* L.)

Garlic began to garner interest once again when Cavallito and Bailey ([1944](#)) identified the main compound responsible for the antimicrobial properties of garlic as allicin, a thiosulfinate that comprises more than 70% (*w/w*) of thiosulfinates extracted from fresh garlic (Rybak et al. [2004](#); Salehi et al. [2019](#)). Since then, garlic along with its chief bioactive component allicin has been associated with a wide range of antimicrobial

properties as well as beneficial effects towards the cardiovascular and immune system (Harris et al. [2001](#); Oosthuizen et al. [2018](#)). Allicin has been identified as a potential alternative antimicrobial agent (Hunter et al. [2005](#)) to overcome the problem of rising antimicrobial resistance (AMR) as several researchers proposed that it is 1000-fold easier for microorganisms to develop resistance against antibiotics than against allicin (Gupta and Viswanthan [1995](#); Ankri and Mirelman [1999](#)). It has been speculated that most bacteria are unable to develop resistance towards allicin as this compound employs a mode of action that differs from that of antibiotics (Jabar and Al-Mossawi [2007](#)), whereby it targets primitive functions essential for life that leaves no room for mutation or metabolic adaptation (Fujisawa et al. [2009](#)).

Aside from its antimicrobial properties, allicin has also been reported to exert a wide range of properties such as antiplatelet, antithrombosis, antioxidant, anti-inflammatory, immunomodulatory and neuroprotective activities, with one of its most notable features being anticancer activity (Fujisawa et al. [2008a](#); Shang et al. [2019](#)). Garlic contains a wide range of bioactive compounds that contribute to its anticancer properties, particularly its allylsulphide derivatives (Bayan et al. [2014](#)) such as allicin, diallyl sulphide (DAS), diallyl disulphide (DADS), diallyl trisulphide (DATS), E/Z-ajoene, S-allyl-cysteine (SAC) and S-allyl-cysteine sulfoxide (alliin) (Yoo et al. [2014](#);

Kodera et al. [2017](#); Shang et al. [2019](#)). Previous studies have reported the association between the consumption of garlic and the decrease in prevalence of certain types of cancers such as stomach, colorectal, oesophageal and prostate cancers. However, the findings in these studies could not always be replicated, and this has been attributed to host factors such as genetics and body composition as well as external factors such as the preparation of the garlic supplements. A summary of the epidemiological studies of garlic on various cancers deduced that the effects are more prominent in cancers of the gastrointestinal tract (Nicastro et al. [2015](#)). Besides that, allicin has exhibited the ability to interrupt cholesterol biosynthesis through inhibition of squalene monooxygenase enzyme activity, thus indicating its potential in areas such as antithrombosis (Gupta and Porter [2001](#)).

Allicin (diallyl thiosulphinate)

Chemistry and biological properties of allicin

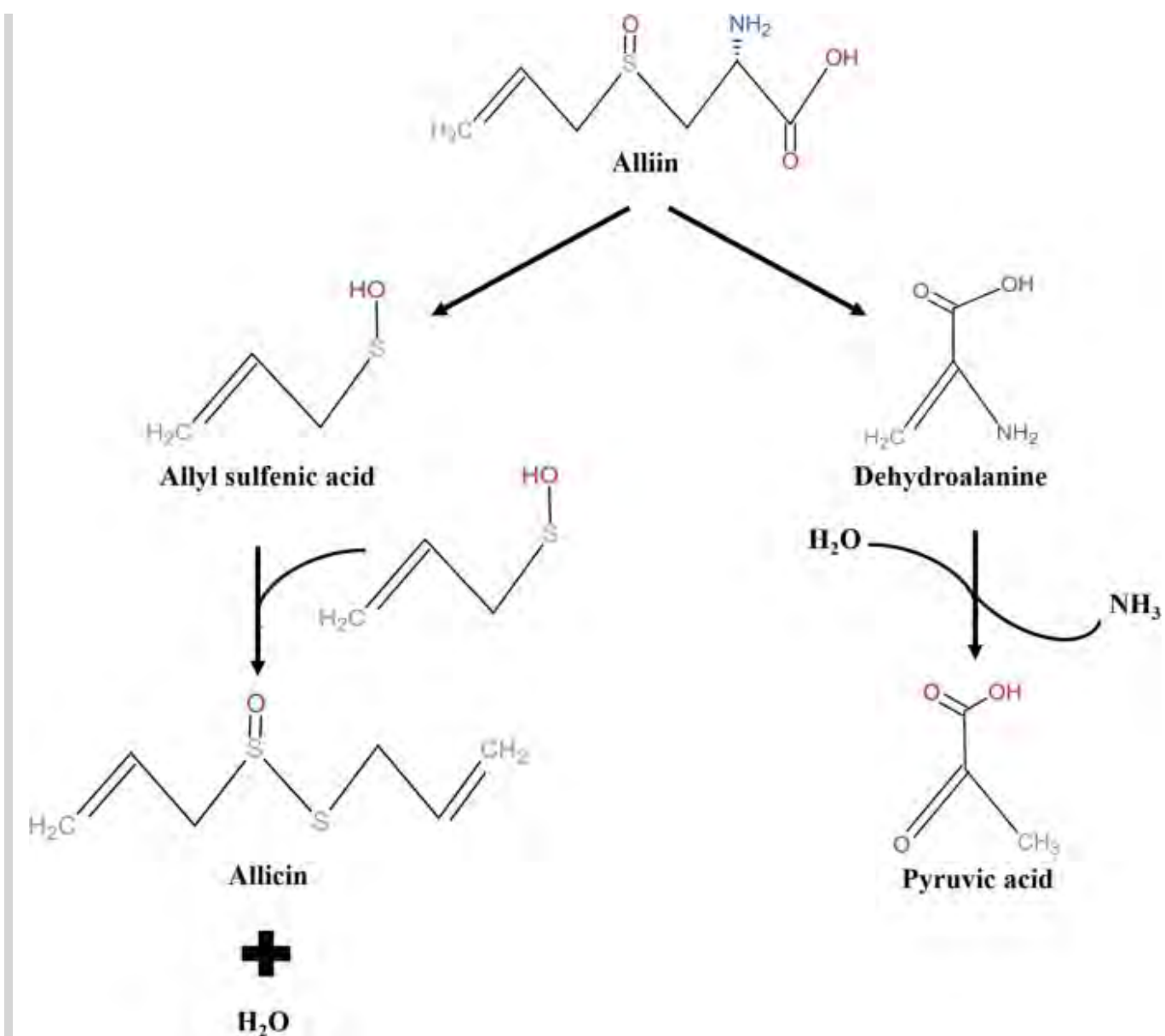
Allicin is the sulphur-containing, bioactive compound derived from freshly chopped garlic when the phosphopyridoxal enzyme known as alliinase catalyses the conversion of the non-proteinogenic amino acid, alliin (allyl cysteine sulphoxide), to form allyl sulphenic acid (Bayan et al. [2014](#)). Two allyl sulphenic acid molecules will then condense spontaneously to form allicin through the removal of water (Salehi et al. [2019](#)). This reaction is represented in Fig. [1](#). Allicin is the

most abundant thiosulfinate found in garlic (Cai et al. [2008](#)). It is reported that approximately 3.4 to 4.6 mg of allicin can be extracted from every gram of fresh garlic (Rybak et al. [2004](#)). However, the concentrations of alliin and alliinase vary with different garlic species (Wu et al. [2014](#)). Allicin is not found in raw uncut garlic as alliin and alliinase are kept in separate microcompartments in garlic plant cells as a defence mechanism against invading microorganisms (Ellmore and Feldberg [1994](#); Weiner et al. [2009](#)). Damage of the thin membrane separating these microcompartments through crushing of garlic bulbs results in exposure of the substrate, alliin, to the enzyme, alliinase, and leads to production of allicin. Its actions are said to be rapid and localised to preserve the alliin in other parts of the garlic from future attacks by invasive microorganisms (Ankri and Mirelman [1999](#); Jabar and Al-Mossawi [2007](#)). The effects of allicin are also detrimental to the host plant and hence its tissues cannot be exposed to allicin for too long (Leontiev et al. [2018](#)). Alliinase, the main enzyme crucial for the formation of allicin is irreversibly deactivated at acidic pH which explains the lack of allicin in the human body upon consumption.

**Fig. 1**







Depiction of the biosynthesis of allicin from alliin. Alliin is converted to allyl sulphenic acid and dehydroalanine by the enzyme, alliinase. Two molecules of allyl sulphenic acid will then condense spontaneously to result in a molecule of allicin. (Adapted with permission from Gruhlke et al. [2016](#))

Allicin is a volatile molecule that is poorly miscible in water and is responsible for the typical odour as well as taste of freshly crushed garlic (Colin-Gonzalez and Santamaria [2017](#)). It has been described as a colourless and oily substance by

Cavallito and Bailey (reviewed by Petrovska and Cekovska [2010](#)). Allicin is unstable at room temperature and rapidly degrades at temperatures higher than 80 °C with concomitant decreasing antimicrobial properties (Curtis et al. [2004](#); Fujisawa et al. [2008a](#); Leontiev et al. [2018](#)). According to Chen et al. ([2017](#)), two of the main bioactive components derived from allicin elucidated through high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) were as 3-vinyl-1,2-dithiacyclohex-5-ene and 2-vinyl-4H-1,3-dithiin.

Due to its reactive nature and depending on the environmental and/or processing conditions (Harris et al. [2001](#); Majewski [2014](#)), allicin will react with thiol groups from other proteins or from itself to form bioactive compounds such as ajoenes, vinyl dithiins and sulphides (Jacob and Anwar [2008](#)) identified as DAS, DADS and DATS. It is noteworthy to remember that ajoenes and vinyl dithiins are produced as a result of the decomposition of allicin in the presence of solvents that are less polar than water (Rybak et al. [2004](#)). On the other hand, the preparation of allicin at –70 °C in dilute aqueous solutions has been observed to be stable over a 2-year period and has been speculated to remain stable beyond 2 years (Koch and Lawson [1996](#); Borlinghaus et al. [2014](#)).

Factors that can impact the stability of the bioactive compounds in garlic include heat, storage time, pH,

temperature and processing (Nicastro et al. [2015](#)). Harris et al. ([2001](#)) mentioned the importance of proper storage conditions of garlic extracts as they observed a reduction in the antibacterial effects exerted by allicin when it was stored at room temperature in comparison to when it was stored at between 0 and 4 °C. A similar finding was noted by Curtis et al. ([2004](#)) and Fujisawa et al. ([2008a](#)). Previous studies reported an increase in alliin content in garlic stored over time due to the conversion of  $\gamma$ -glutamyl-cysteine to S-allylcysteine, a precursor of alliin. Similarly, S-allylcysteine was observed to be stable for up to a year under ambient temperatures (Lawson and Gardner [2005](#); Nicastro et al. [2015](#)). Fujisawa and co-authors (Fujisawa et al. [2008b](#)) noted that temperature and pH could also contribute to the metabolism of allicin into secondary compounds. This was demonstrated through the addition of vinegar to garlic formulation which lowered the pH and increased the half-life of allicin (Kamruzzaman et al. [2014](#); Wang et al. [2014](#)). It was also observed by Nicastro et al. ([2015](#)) who noted that the stability of allyl thiosulfinates of freshly crushed garlic was minimally affected 3 days after addition into condiments such as yogurt and creamy horseradish. These findings have implications for the processing of garlic in supplements and as food additives.

Through a series of experimenting with different extraction methods, the ethyl alcohol method was identified as the ideal method to isolate allicin

(Harris et al. [2001](#)). The differences in efficiency of various extraction methods had been attributed to the hydrophobic nature of allicin. Meanwhile, the loss of activity of allicin in water and 100% ethanol when compared to other solvents has been associated with the chemical instability of allicin (Fujisawa et al. [2008a](#)).

### Cell toxicity

Allicin molecules have the ability to easily pass through the cell membrane to reach cellular compartments and react with free thiol groups due to their hydrophobic nature (Fujisawa et al. [2008a](#); Salehi et al. [2019](#)). As all living cells contain thiol groups, allicin may function as a dose-dependent biocide with the ability to kill eukaryotic cells (Gruhlke et al. [2016](#)). A concentration exceeding 60 mg/L has been identified as the toxic dose for mammalian cells (Shadkchan et al. [2004](#)). Similarly, previous studies conducted on mice indicated that high dosages of allicin at 60 mg/kg and 120 mg/kg body weight via intravenous injection and subcutaneous administration, respectively, are lethal (Koch and Lawson [1996](#)). Findings from previous studies indicate that the dose required for allicin to exert its antimicrobial effects on pathogens such as *Aspergillus* spp. at 8 to 32 mg/L in vitro (Shadkchan et al. [2004](#)), and *Plasmodium* at 5 to 8 mg/kg in infected mice (Coppi et al. [2006](#)), was much lower than the toxic dose against animals. Besides that, animal cells are observed to be more resilient to allicin as they have

a redundancy in protease function that are not common among microorganisms and single-cell protozoans (Coppi et al. [2006](#)). Most of the host proteins in animal cells are found intracellularly. Due to their higher concentrations of glutathione, mammalian cells have been described to be relatively protected from the similar fate of microorganisms in the presence of allicin (Ankri et al. [1997](#); Rabinkov et al. [1998](#); Anufrieva et al. [2015](#)). The low levels of glutathione in many microbial pathogens in comparison to their eukaryotic hosts (Smirnova and Oktyabrsky [2005](#)) could contribute to the sensitivity of these microorganisms to allicin. Glutathione is a tripeptide that is made up of glutamic acid, cysteine and glycine. It serves as one of the important elements that control the cellular redox potential in the body. A change in the GSH:GSSG balance which triggers the oxidation of GSH and leads to an increase in glutathione dimers (GSSG) would promote the glutathiolation of protein surface free cysteine. Thus, this shields the cysteine groups from overoxidation during oxidative stress (Borlinghaus et al. [2014](#)).

Cysteine and glutathione (GSH) are said to hinder the thiolation activity exerted by allicin (Bayan et al. [2014](#)) through their sulphhydryl groups (Jonkers et al. [1999b](#)). The importance of the sulphhydryl group to the inhibitory actions of garlic is demonstrated in the study by Gupta and Porter ([2001](#)) as they reported that thiol-containing

compounds such as glutathione were able to prevent and reverse the inhibition exerted by garlic. The role of glutathione was apparent in a comparison study conducted by Gruhlke and his co-authors (Gruhlke et al. [2016](#)) in which they reported that cell lines with lower initial glutathione content such as the human umbilical vein endothelial cells (HUVEC) were more sensitive to treatment by allicin than the other cell lines tested, which leads to GSH oxidation and subsequently cell death. This observation was supported by Fujisawa et al. ([2009](#)) who demonstrated that the antibacterial properties of allicin towards a strain of MRSA and *E. coli* were inhibited through the addition of cysteine, glutathione or coenzyme A. The authors described the inhibition as a result of the S-allyl moiety originating from allicin binding with the –SH group of these compounds, and thus preventing them from binding with the thiol groups of the targeted bacteria (Fujisawa et al. [2009](#)).

### Antimicrobial properties of allicin

Allicin has been described as the main substance responsible for the antimicrobial activities associated with garlic. Indeed, when allicin is not present or when alliinase is inhibited, the antimicrobial activities of garlic are lost (Farbman et al. [1993](#); Jonkers et al. [1999a](#)). The inhibitory effects exhibited by allicin are comparable, if not stronger than some conventional antibiotics such as penicillin, tetracycline (Majewski [2014](#)) and kanamycin (Curtis et al. [2004](#); Fujisawa et al.

[2009](#)). Unlike the commonly utilized antibiotics, the inhibitory effects of allicin have been described to target a broader spectrum of microorganisms (Borlinghaus et al. [2014](#)) encompassing bacteria, yeasts, fungi and even parasites.

Previous studies highlighted that the concentration of allicin, temperature and cultivation conditions of garlic may affect the antimicrobial properties of allicin (Adler and Beuchat [2002](#)). The inhibitory activity demonstrated by allicin had been attributed to the inhibition of RNA synthesis as previously noted against *S. typhimurium* (Feldberg et al. [1988](#)). Kyung ([2012](#)) reported that allicin inhibits the growth of microorganisms through the natural reaction that occurs between its –S(O)–S-group and the –SH group of the cellular proteins in the microorganisms to form mixed disulphides. The reactive nature of allicin is contributed by the oxygen atom in thiosulfinates which exerts an electron-withdrawing effect to create an electrophilic sulphur centre that can readily react with thiol groups (Leontiev et al. [2018](#); Salehi et al. [2019](#)). The disulphide bonds that form between the sulphhydryl groups of proteins and allicin have been described to play an essential role as the addition of  $\beta$ -mercaptoethanol, which functions to break disulphide bonds, has been observed to inhibit the synergistic effects of allicin when in combination with cysteine (Jonkers et al. [1999b](#)). The rapid reaction that takes place between allicin and the microbial thiol-containing proteins and

enzymes would interfere with essential microbial metabolism (Strehlow et al. [2016](#)) and bacterial nutrition (Bakri and Douglas [2005](#)). Similarly, allicin has been reported to inhibit acetyl-CoA synthases in yeasts (Focke et al. [1990](#); Wallock-Richards et al. [2014](#)).

### Antibacterial properties

Cavallito and Bailey ([1944](#)), the first researchers to describe allicin, noted its inhibitory activities towards various bacteria including *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Streptococcus viridans*, *Bacillus subtilis*, *Bacillus typhosus*, *Bacillus paratyphosus*, *Morganella morganii*, *Bacillus enteriditis*, *Salmonella typhimurium*, *Shigella dysenteriae* and *Vibrio cholerae*. They reported the inhibitory effect exerted by allicin as bacteriostatic (as all the test pathogens could resume growth once cultured in fresh media), but nonetheless, potent. Similarly, Hughes and Lawson ([1991](#)) reported inhibitory effects of allicin against *S. aureus* and *E. coli*.

Moreover, other researchers have also reported the ability of allicin to inhibit the growth of a broad spectrum of bacteria such as *E. coli*, *Salmonella*, *Listeria monocytogenes* (Adler and Beuchat [2002](#)), *Pseudomonas* (Karuppiah and Rajaram [2012](#)) and *Klebsiella* (Bayan et al. [2013](#)).

Allicin has shown the ability to inhibit the growth of oral bacteria such as *Porphyromonas gingivalis* and *Streptococcus mutans*, with an immediate



effect on the former, whereby the authors surmised to be due to the thinner cell wall of *P. gingivalis* (Bakri and Douglas [2005](#)). On the contrary, Fujisawa et al. ([2009](#)) noted that allicin was more effective against the Gram-positive pathogen, MRSA in comparison to *E. coli*. Jabar and Al-Mossawi ([2007](#)) reported a similar finding whereby allicin extracted via the aqueous extraction method was able to inhibit the growth of several drug-resistant strains of *S. aureus*, *E. coli*, *K. aerogenes* and *S. enterica goldcoast* with higher inhibition against the first pathogen. Recently, Getti and Poole ([2019](#)) reported that allicin acted on the peptidoglycan layer of *S. aureus* by reacting with the thiol group of the enzymes involved in the synthesis of peptidoglycan as observed through MALDI-TOF mass spectrophotometry.

A higher protein content in the cell walls has been reported to interfere with the antibacterial effects exhibited by allicin, thus explaining the differences in the inhibitory activity demonstrated towards different microorganisms (Fujisawa et al. [2009](#)). Images obtained from scanning electron microscopy (SEM) indicate that allicin may alter the permeability of the bacterial membrane, thus destroying the cell structural integrity and eventually leading to cell death (Chen et al. [2017](#)). However, it is observed that the proteins do not completely diminish the inhibitory effects exerted by allicin.

Besides that, allicin has been shown by Wallock-Richards et al. (2014) to have the ability to exert antibacterial activities towards *Burkholderia cepacia* complex, which is a phytopathogen as well as a human pathogen that commonly causes infection in cystic fibrosis (CF) patients. The proteomics analysis result in this work is in agreement with previous reports on the mode of action employed by allicin as it observably modified the catalytic cysteine residue of *B. cepacia*'s thiol-dependent peroxiredoxin (BCP), resulting in bacterial growth inhibition and ultimately cell death. Similarly, allicin has been described to indiscriminately exert the same antimicrobial activities towards the probiotic *Bifidobacterium* (Booyens et al. 2013), hence suggesting the need for caution while taking probiotic supplements together with garlic.

A study conducted by Jonkers and co-authors (Jonkers et al. 1999a) demonstrated that garlic could inhibit the growth of five clinical isolates of *Helicobacter pylori*. On the contrary, Graham et al. (1999) and Aydin et al. (2000) reported that garlic oil, which contains 70 to 75% of allicin, did not exert any inhibitory effects towards *H. pylori* upon consumption by patients. A follow-up study by Koçkar et al. (2001) comparing the effects of allicin, ascorbic acid and beta carotene respectively towards *H. pylori*-positive individuals led the authors to speculate the possibility of utilizing allicin as a means of treatment. However, allicin

was most effective at a higher dosage of 4200 mg/day with an eradication rate of 27/30 (90%) in comparison to 1200 mg/day which had an eradication rate of 7/30 (23%) (Marchese et al. [2016](#)). It is noteworthy to remember that allicin has been reported to form other intermediates that may not possess antibacterial properties prior to its entry into the stomach due to its reactive nature (Amagase [2006](#)). Recently, allicin has been reported to inhibit the growth of several drug-sensitive and drug-resistant strains of *Mycobacterium tuberculosis* both in vitro and in vivo without any adverse effects on the host (Dwivedi et al. [2018](#)). It was observed that allicin not only inhibited *M. tuberculosis* but also boosted the host immune system. Similarly, the authors indicated the possibility of utilising allicin as an adjunct with antibiotics commonly used to treat tuberculosis (TB) although the outcome of this remains uncertain.

### Antifungal properties

Allicin has demonstrated inhibitory activities against different fungi ranging from yeasts to filamentous fungi. It is an attractive alternative to conventional antifungals as its derivatives have also been reported to stimulate cell immunity and are less harmful to the host (Davis [2005](#)). As observed with bacteria, the sulphide molecules of allicin and its derivatives interact with the sulphur groups of other proteins and/or amino acids in fungi to hinder important functions. The many studies on

allicin and its mode of action against fungal species have uncovered a variety of mechanisms. Allicin can impede biofilm growth of *Candida albicans* and disrupt the fungal cell membrane as observed through SEM studies (Khodavandi et al. [2011a](#)). In another study, transmission electron microscopy (TEM) results demonstrated that allicin could cause damage to *Trichophyton rubrum* cells through the disorganisation and dissolution of the cytoplasmic content as well as the detachment of the cell membrane from the cell wall (Aala et al. [2013](#)). Meanwhile, based on findings from allicin-treated *Saccharomyces cerevisiae*, Gruhlke et al. ([2010](#)) concluded that allicin serves as a redox toxin that disrupts the redox environment of yeast cells, leading to induction of apoptosis. The authors also stated that allicin at higher doses causes the yeast cells to become excessively oxidised and undergo necrosis.

Hughes and Lawson ([1991](#)) demonstrated that allicin was effective in inhibiting the growth of reference strains of *A. fumigatus*, *C. stellatoidea*, several species of *Trichophyton* sp. and *Microsporum* sp. Similarly, Sajali and co-authors (Sajali et al. [2013](#)) observed anti-hyphal activities of allicin against *A. fumigatus* ATCC 36607 at 3.2 µg/mL and perturbation to the cell surface morphology as well as hyphal morphogenesis under SEM. More recently, allicin has been reported to inhibit the growth of several plant infecting pathogenic fungi such as *Erwinia carotovora*,

*Xanthomonas campestris* pv. *Malvacearum*,  
*Fusarium proliferatum*, *Alternaria brassicicola*  
and *Magnaporthe grisea*.

In terms of animal models of fungal infections, allicin has been shown to improve the survival rates of mice infected with *A. fumigatus*, *A. niger* and *A. terreus*, by reducing the fungal load (Shadkchan et al. [2004](#)). Khodavandi et al. ([2011b](#)) also reported that allicin was able to increase the mean survival time of mice infected with *C. albicans* from 8.5 to 16 days. Due to its short half-life (50 min), allicin probably reduced the germination of spores and growth of hyphae in vivo (Borhan-Mojabi et al. [2012](#)) through the combined effect with other allicin derivatives that were released from its degradation (Shadkchan et al. [2004](#); Khodavandi et al. [2011b](#)).

### Antiparasitic properties

Allicin has been reported to exhibit antiparasitic properties against several types of parasites. It was observed to interfere with the growth of *Plasmodium berghei* and *Plasmodium yoelii* sporozoites by inhibiting their cysteine proteases, which play a crucial role in infecting mammalian cells. It was also observed that allicin was able to hinder the growth of *Plasmodium sporozoites* at its pre-erythrocytic stage, thus preventing its development into malaria (Coppi et al. [2006](#)). A study conducted on rodent malaria model demonstrated that allicin could enhance the host

innate and adaptive immunity by increasing the number of macrophages, cytokines and CD4<sup>+</sup> T cells as well as promoting the maturation of dendritic cells (Feng et al. [2012](#)).

Besides that, allicin has been reported to exert anti-Leishmanial activity both in vitro at 50 µM and in vivo in BALB/c mice by reducing the size of the lesions (Metwally et al. [2016](#)). Allicin has also exhibited the ability to reduce the *Leishmania infantum* load in hamsters in a dose- and time-dependent manner but at higher efficacy when used in combination with amphotericin B (Corral et al. [2014b](#)). In their follow-up study, Corral et al. succeeded in identifying the mechanism of action of allicin for inducing cell necrosis as the disruption of Ca<sup>2+</sup> homeostasis and oxidative stress (Corral et al. [2016](#)). In addition, a recent study comparing various thioallyl compounds extracted from garlic exhibited the superiority of allicin in inhibiting the growth of *Giardia duodenalis* trophozoites (Argüello-García et al. [2018](#)). The authors associated the inhibitory action of allicin with its ability to interfere with the trophozoite cell integrity and its thiol-disulphide exchange capacity.

### Antiviral properties

Allicin has been reported to exert inhibitory effects against several types of viruses including influenza B, human cytomegalovirus, herpes simplex virus Type 1 and 2, parainfluenza virus Type 3, vesicular stomatitis virus, vaccinia virus and human

rhinovirus Type 2 (Ankri and Mirelman [1999](#)). A clinical study involving 146 volunteers demonstrated that individuals who were prescribed allicin supplements were less likely to succumb to flu and had a faster recovery rate (Josling [2010](#)). However, follow-up studies involving a larger sample size of individuals have been recommended (Lissiman et al. [2009](#)). Allitridin, a derivative of allicin has shown the ability to inhibit human cytomegalovirus in mice by preventing Treg expansion and modulating the host immune system to clear the virus (Li et al. [2013](#)). Besides that, it was reported that allicin could reduce the inflammation and oxidative stress in human cells infected with dengue virus (Hall et al. [2017](#)).

Mousavi and co-workers (Mousavi et al. [2018](#)) highlighted the potential of utilising allicin as an alternative treatment for genital warts caused by the human papilloma virus (HPV) among males as it is convenient and does not cause any side effects. Moreover, allicin could reduce the penetration and proliferation of Influenza A in vitro (Mehrbood et al. [2009](#)). While allicin has exhibited antiviral properties, certain types of viruses have been recorded to afflict garlic plants such as those from the *Potyvirus*, *Carlavirus* and *Allexivirus* genera resulting in reduced size and weight (Da Silva et al. [2019](#)). However, hitherto little is known about the pathogenesis of viral infections in garlic plants.

As observed with the extensive research conducted

on bacteria, fungi and parasites, it is speculated that the antiviral properties exhibited by allicin could be due to thiolation in combination with other mechanisms of action. The exact mechanisms underlying its antiviral properties remain elusive for now.

### Allicin and its anti-virulence properties

Aside from the inhibition of microbial growth, allicin has also been reported to possess anti-virulence properties (Leng et al. [2011](#)). Virulence factors such as endotoxins, exotoxins and siderophores are essential for microorganisms to invade their host, cause disease and evade detection by the host's immune system (Peterson [1996](#)). Gonzalez-Fandos et al. ([1994](#)) described allicin as being capable of inhibiting the formation of staphylococcal enterotoxins A, B and C1 at less than 1% (w/v) concentration. This reduction in enterotoxins production has been directly associated with the reduction in *S. aureus* colonies as it is evident that toxin production is correlated with abundance of colonies. In *C. albicans*, a major human opportunistic fungal pathogen, allicin, has been observed to inhibit and reduce the biofilm-forming ability, which is an important virulence factor, through the suppression of the cell wall-related gene *HWP1* (Khodavandi et al. [2011a](#)). Similarly, allicin also exhibited potent anti-biofilm activity against *S. epidermidis* ATCC 35984 at a concentration of 3.13 µg/mL compared to levofloxacin and vancomycin at 25 µg/mL and 50 µg/mL,



respectively by suppressing the genes associated with biofilm formation such as *SAPs* and *IcaA* (Wu et al. [2014](#)).

Meanwhile, Arzanlou and his co-authors (Arzanlou and Bohlooli [2010](#); Arzanlou et al. [2011](#)) demonstrated that allicin binds to the cysteine residues of pneumolysin O (PLY) and streptolysin O (SLO) of Group A streptococci (GAS) to inhibit the haemolytic activities associated with this group. Allicin was also reported to reduce the  $\alpha$ -toxin production by methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) by suppressing the transcription level of the *agrA* gene, which is responsible for the expression of certain secreted proteins (Leng et al. [2011](#)). Additionally, allicin has also been associated with the inhibition of the streptococcal pyrogenic exotoxin B production by GAS through suppression of SpeB which serves as a cysteine protease that is required for SpeBz maturation and SpeBm proteolytic activity (Arzanlou [2016](#)).

Besides that, allicin was observed to block the quorum-sensing (QS) abilities necessary for *P. aeruginosa* to establish their biofilms and prevent the activation of polymorphonuclear leukocytes (PMN), leaving them vulnerable to clearance by the host immune system (Bjarnsholt et al. [2005](#); Nazzaro et al. [2013](#)). It was noted that allicin was able to inhibit the biofilm production by *P. aeruginosa* through inhibiting early cell adhesion

and reducing the exopolysaccharide (EPS) secretion as well as downregulating the QS of this species by either preventing the adhesion of the QS molecules to their appropriate receptors or by inhibiting the expression of the QS genes (Lin et al. [2013](#)). It has been suggested that allicin would serve as an ideal therapeutic agent for blocking QS abilities and minimising the effects exerted by pathogens such as those observed in CF patients. However, further studies are necessary to determine the appropriate dose for human consumption (Bjarnsholt et al. [2005](#)).

### Antimicrobial properties of allicin in combination with other compounds

The increasing cases of antimicrobial resistance highlight the necessity of finding alternative therapeutic strategies in order to either replace or enhance the drugs that are currently available. Due to its broad-spectrum properties, allicin has been viewed as a versatile therapeutic agent for multiple ailments including infectious diseases. Previous studies utilizing allicin in combination with antimicrobials had shown that allicin can reduce the antibiotic dosage required to achieve the desired antimicrobial effects. This has the advantage of reducing the adverse effects associated with the administration of certain antimicrobials, such as AmB which has been linked to dose-dependent renal toxicity (Papich [2016](#)). It has also been suggested to reduce the cost of utilizing other means of drug delivery such as lipid carriers (Corral

et al. [2014a](#), [2014b](#)).

Table [1](#) summarises some of the previous studies performed to elucidate the antimicrobial properties of allicin alone and allicin in combination with other compounds. It is notable that the combined effects of allicin with commercialised antimicrobials were reported as early as two decades ago. The combination of allicin with other compounds was observed to decrease the MIC of antimicrobials required to inhibit the growth of microorganisms by 2-fold as noted with the fungi, *T. rubrum*, *T. mentagrophytes*, *T. verrucosum*, *M. canis*, and *Epidermophyton floccosum*, and by as much as 128-fold in the case of *S. aureus*. It was also observed to reduce the fungal and bacterial load in animal models, thereby lowering the mortality associated with some of the test pathogens as noted with *Pasteurella multocida* and *Leishmania infantum*. In some cases, the combination of allicin with antimicrobial agents was described to be more efficacious in clearing the microorganisms compared to monotherapy. Allicin alone has been reported to inhibit the growth of some drug resistant strains. Resistant strains such as MRSA succumbed to the treatment of antibiotics that it reportedly demonstrated resistance to, when subjected to treatment in conjunction with allicin. Some studies had also investigated the effects of combination of allicin with nanoparticles and nanofibers, and in general, the findings revealed an enhanced antimicrobial effect of allicin when

combined with the nanomaterials (Table 1).

**Table 1 Antimicrobial properties of allicin in combination with other antimicrobials**

Jonkers et al. (1999b) had proposed that the synergistic antibacterial activity of allicin and vancomycin against vancomycin-resistant enterococci (VRE) was associated with the ability of allicin to modify the sulphhydryl group of important proteins such as enzymes produced by the transposon *TN1546*, which encodes for vancomycin resistance and hence enabling vancomycin to exert its effect on VRE, supporting the findings by Wills (1956).

The natural antimicrobial properties of allicin such as its ability to inhibit the adherence of bacterial cells as observed with *S. epidermidis* and reduce the persistence of biofilms (Pérez-Giraldo et al. 2003) as well as expression of virulence determinants has been suggested to aid in the action of antibiotics (Abouelfetouh and Moussa 2012). Allicin has also been reported to enhance the action of antibiotics by altering the integrity and structure of microbial cells through reducing its lipid content and thus easing the entry of the antibiotic molecules (Iwalokun et al. 2004). Similarly, allicin can penetrate the membrane of target cells to exert its antimicrobial properties (Miron et al. 2000) as well

as to render the targeted cells vulnerable to the action of other antimicrobial compounds.

Besides that, allicin enhances the action of membrane-active antimicrobials such as AmB through its ability to form transient pores in artificial and biological membranes (Miron et al. [2000](#); Gruhlke et al. [2015](#); Leontiev et al. [2018](#)). An et al. ([2009](#)) hypothesised that the synergistic relationship observed between allicin and AmB could partly be due to oxidative stress brought about by allicin as previously reported by Ogita et al. ([2006](#)). Allicin binds to glutathione, which plays an important role in the GSH:GSSG coupling to maintain the redox reaction in cells, thus causing oxidative stress to the cells. On another note, some studies have observed that allicin did not exhibit significant antimicrobial activities when tested alone in comparison to when combined with other compounds (Guo et al. [2010](#); Corral et al. [2014a](#)). Hence, further study is warranted to illustrate the synergistic effects and mechanism of action of allicin in combination with other antimicrobials.

### Innovations in utilising/administering allicin in medicine

The efficacy of the antimicrobial activity associated with allicin has been observed to work in other forms as well. Cutler et al. ([2009](#)) reported potent antimicrobial activity exhibited by an allicin aqueous extract and a novel allicin topical gel towards Lancefield Group B streptococci. An attempt to enhance the antimicrobial properties of

cellulose fabrics for medical purposes, by covalent and non-covalent coating with allicin, demonstrated antimicrobial properties towards *S. aureus* (Jafary et al. [2014](#)). Due to its reactive and unstable nature, Anufrieva et al. ([2015](#)) has proposed a type of pro-drug (in which the compounds are metabolised within the body) by utilising the microbial enzyme, methionine  $\gamma$ -lyase (MGL), to catalyse the reaction of alliin and methionine sulphoxide to produce thiosulfinates such as allicin in situ. Meanwhile, Strehlow et al. ([2016](#)) reported that spray-dried alliin and alliinase would react in water to form allicin that inhibited the growth of *E. coli* which would be ideal for targeting pulmonary-associated pathogens. Additionally, Reiter et al. ([2017](#)) demonstrated that vaporised allicin was able to inhibit the growth of several strains of *P. aeruginosa*, *S. pneumoniae*, *S. pyogenes*, *S. dysgalactiae equisilimlis*, *S. aureus*, *K. pneumoniae* and *A. baumannii*. As a result of its volatile characteristics, allicin has been reported to be able to target pathogens in the lungs (Leontiev et al. [2018](#)). On another note, Soumya and co-authors (Soumya et al. [2018](#)) reported that functionalising allicin with locust bean gum nanoparticles enhanced its effects and increased its potential to be utilised for atherosclerotic treatment.

## Conclusion

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Allicin has demonstrated remarkable potential as an alternative or adjunct to conventional antibiotics

for combating antimicrobial resistance due to its ability to exert antimicrobial activities with minimal adverse effects on the host. It also shows great promise in enhancing the effects of other antimicrobial substances when used in combination with these antimicrobials. The currently available data indicates that these allicin and drug combinations not only reduced the dose required of the compounds but also minimised the undesired side effects. The information gathered from previous literature summarised in this review highlights not only the vast body of evidence supporting the use of allicin for treatment of infections but also the knowledge gaps that still exist in this field particularly in terms of its mode of action as well as its bioavailability and pharmacokinetics. Additionally, there appears to be some discrepancies in different reports on allicin's efficacy against certain microorganisms such as *H. pylori*, a fact which is probably due to the different sources of allicin used as well as the methodologies adopted. For future studies, it is therefore crucial to establish a standardised allicin extraction method that is universally adopted and to conduct more work in this field in order to better understand the underlying mechanisms for allicin's antimicrobial property as well as its safety profile for drug formulation purposes.

## Data availability

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Data sharing not applicable to this article as no

datasets were generated or analysed during the current study.

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### Ethics declarations

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#### Conflict of interest

The authors declare that they have no conflict of interest.

#### Research involving human participants and/or animals

This article does not contain any studies with human participants or animals performed by any of the authors.

#### Informed Consent

For this type of review study which does not involve any human participants, informed consent is not required.

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