ABSTRACT

Medicinal Properties of Garlic and Its Derivatives

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With more than 3,000 research papers and many ongoing investigations into its biochemical effects, garlic is one of the most studied medicinal plants. The objective of this study is to provide an understanding of the effects of garlic's organosulfur compounds and specifically, their antimicrobial, cardiovascular, and anticancer effects. It also examines the pharmacological effects and mechanisms of black garlic, a type of aged garlic derivative. By reviewing studies that explore the mechanisms of these compounds in both *in vitro* and *in vivo* investigations, the study provides evidence for garlic and its derivatives' potential as natural and safe pharmacological agents.

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MEDICINAL PROPERTIES OF GARLIC AND ITS DERIVATIVES

A Thesis Submitted to the Faculty of

Baylor University

In Partial Fulfillment of the Requirements for the

Honors Program

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Waco, Texas

May 2018

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CHAPTER ONE

Introduction

Garlic, *Allium sativum*, is part of the Liliaceae family and has a long history in folk medicine in a variety of cultures across the world. Although many of these cultures developed independently of each other, the wide availability and durability of garlic in a variety of conditions have produced similar uses of garlic in several different locations and times in history.

Garlic is native to the Tien Shan and Pamir-Alai mountains of southern central Asia. Because of its compact and durable structure, garlic is thought to have been a food source for the nomadic hunter-gatherers more than 10,000 years ago.[1] As garlic made its way around the globe, one of the earliest references to its usage comes from the ancient Egyptians, who used garlic to strengthen their slaves for manual labor, including building the pyramids.[1,2] One of the earliest records of garlic in medicine and the authoritative medical text at the time, the *Codex Ebers*, prescribed garlic as a method of treatment for abnormal growths, circulatory issues, general malaise, and parasitic infestations.[2] The Egyptian usage of garlic establishes several major themes that can be found in many of the subsequent cultures: use of garlic for maintaining and increasing strength especially in the working class, its antimicrobial properties, and its effect on circulatory diseases.

In ancient Greece, evidence for the use of garlic in both Olympic athletes' and soldiers' diets suggest that garlic may have played a role as one of the first "performance-enhancing" agents.[1–3] The father of medicine Hippocrates prescribed garlic as a way to cure pulmonary diseases, because he believed that garlic was a cleansing agent. The medical text of ancient India, *Charaka-Samhita*, also recommended garlic as a treatment option for heart disease nearly 2000 years ago. Evidence in ancient Chinese and Japanese cultures suggest that garlic was prescribed for several pulmonary and digestive ailments. Garlic was even thought to prevent heat strokes during the Middle Ages and continued to be a part of the working class diet during the Renaissance.[2] Although the science behind these ancient medical practices would not be discovered until much later, it is interesting to note that these distinct cultures used garlic for many of the same reasons and ailments.

Nowadays garlic is most commonly used as a flavoring agent in many cultures and is consumed in large quantities particularly in Korea, Japan, and Italy.[4] When administered as a supplement, garlic is most often taken raw or in an enteric-coated tablet. It is primarily used as a supplement in treating cardiovascular disease, particularly atherosclerosis. Other uses include protection against viruses such as the common cold although currently, there is no scientific proof that garlic plays a direct role in preventing or treating the common cold.[4] According to the 2012 National Health Interview Survey, around one third of American adults utilized complementary health approaches, and garlic was listed as one of the top 10 most used natural products.

However, global consumption of garlic has been declining for the past several years as some people find the pungent smell and taste of raw garlic to be unpleasant. Raw garlic has also been linked to gastrointestinal discomfort in some people, and food scientists have addressed such complaints by introducing black garlic.[5] Although black garlic's origin is unknown, it has long been a part of South Korean, Japanese, and Thai cuisine and has been introduced to other countries, such as Taiwan, 10 years ago.[6]

Recently, black garlic has been sought after by high-end chefs looking to add its sweet and sour taste and jelly-like texture to a variety of recipes.[5] Research has shown that black garlic is able to retain and even increase the bioavailability of some of fresh garlic's most beneficial compounds.[5]

With the increased use of nonvitamin, nonmineral dietary supplements and alternative medical therapies, garlic and other nutraceuticals have become the subject of extensive research regarding their potential as pharmacological agents.[7] Garlic in particular has been the subject of several of these investigations because of its wide availability and the low toxicity of its active compounds.[8] Many of these studies have focused on the organosulfur compounds in garlic, such as allicin, which exhibit several pharmacological actions that include antibiotic, antifungal, anti-cancer, pesticidal, and cardioprotective activity, as well as inhibition of platelet aggregation.[9]

In order to better understand its effect on human health, we will begin with garlic's bioactive constituents and the methods used to extract these compounds. We will then explore the bioactive molecules in black garlic that depend on different methods of processing and production and how black garlic may serve as a more preferable alternative to raw garlic in the extraction of specific compounds with pharmacological potential.

The most abundant sulfur-containing compound in garlic is alliin, which is the precursor to allicin and its organosulfur derivatives. Alliin, an alkenyl derivative of cysteine alkyl sulfoxide, is stored within the cytoplasm of an intact garlic cell.[9,10] Neither allicin, nor its derivatives, form until garlic undergoes either a mechanical or chemical breakdown. However, when an intact cell undergoes mechanical or chemical breakdown, damage to the cell's vacuole releases the enzyme alliinase, which induces the conversion of alliin to allicin. The enzyme acts as a carbon-sulfurlyase to transform alliin into a reactive intermediate called 2-propene-sulfenic acid. Subsequently, two molecules of this sulfenic acid quickly condense to form one molecule of allicin.[10] The conversion of alliin to allicin also produces pyruvate and ammonium and is illustrated in the Figure 1.1.

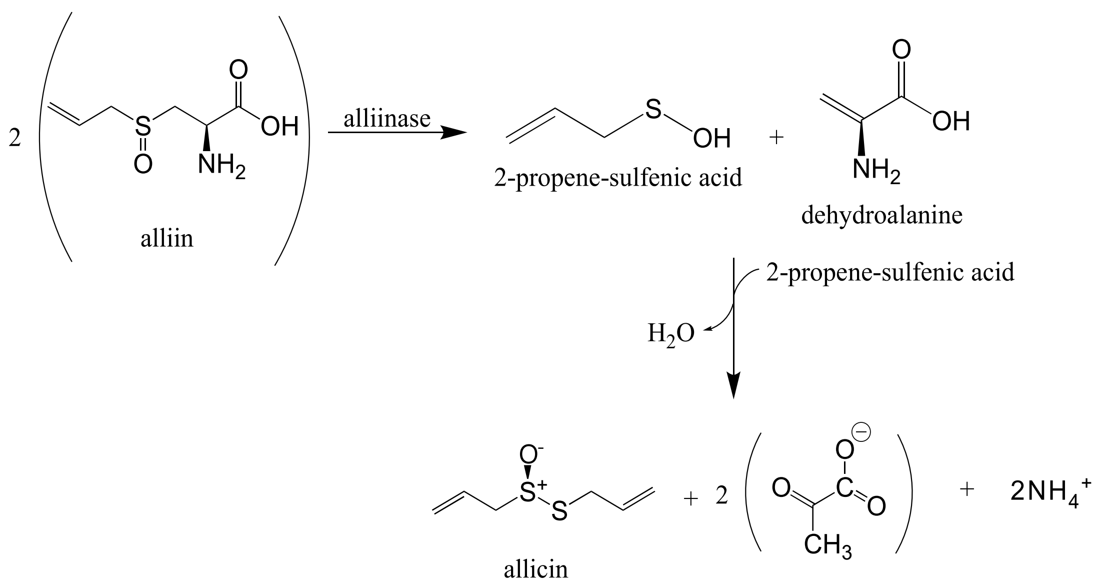


Figure 1.1 Conversion of alliin to allicin via enzymatic activity of alliinase.

The major organosulfur compounds from garlic include diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), trans-ajoene, cis-ajoene, and vinyl-dithiins. They are represented by their molecular structures in Figure 2 below. Many of these compounds exhibit multiple pharmacological actions. Although allicin displays nearly all of the pharmacological actions associated with constituents of garlic, its instability poses a significant challenge in its potential use as a pharmacological agent.[9] Therefore, the beneficial effects of garlic's organosulfur compounds are most often extracted from allicin's many derivatives.[11] Table 1.1 summarizes the structure and pharmacological actions of a few of garlic's most studied compounds.

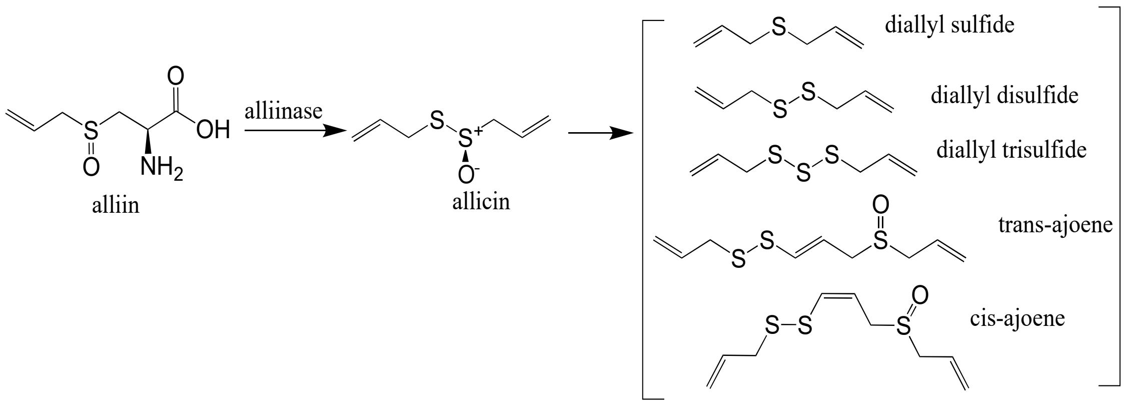
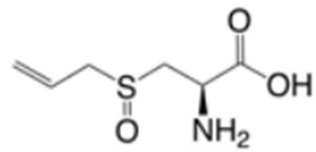
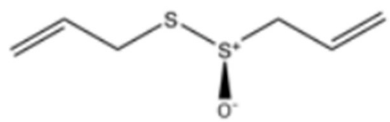
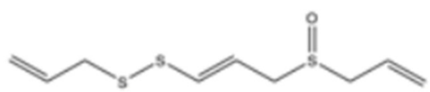
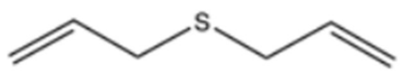
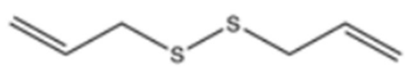
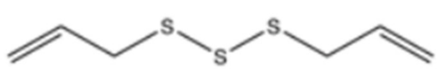


Figure 1.2 Molecular structures of alliin, allicin, and some of its organosulfur derivatives.

Along with its many health benefits, allicin is also responsible for the characteristic smell and taste of freshly prepared garlic.[12] Extraction methods have a critical influence on the organosulfur compounds' actions as certain properties are only exhibited through specific extraction methods. For example, the aqueous and ethanolic extracts of garlic had significantly different levels of inhibitory effect on the growth of *Escherichia coli* and *Salmonella typhimurium*: whereas the ethanolic extract exhibited considerable inhibitory effect, the aqueous extract had little or no inhibition.[8] Commercially available preparations of garlic such as garlic powder tablets, oil of steamed distilled garlic, aged garlic extract, and ether-extracted oil of garlic all contain different constituents due to differences in extraction methods.[13]

Table 1.1 Active compounds of garlic and their pharmacological effects.[9]

Compound	Molecular structure	Antimicrobial	Cardioprotective	Anticancer	Antifungal
Alliin			+		
Allicin		+	+	+	+
Ajoene		+	+	+	+
Diallyl-sulfide (DAS)			+	+	
Diallyl-disulfide (DADS)		+	+	+	
Diallyl-trisulfide (DATS)			+	+	

Similar to the observation that different extraction methods yield different organosulfur compounds in fresh garlic, variations in the production of black garlic lead to changes in its content. The heating process used in producing black garlic causes a Maillard reaction that gives it its distinct black color and produces antioxidant effects as evidenced by experiments using *in vitro* electron-donating ability assay and trolox equivalent antioxidant capacity assay.[5] Fresh garlic is placed in a temperature and humidity controlled room without any additives or other treatments, but the duration and the temperature to which garlic is exposed varies with each manufacturer, with higher temperatures requiring a shorter processing period.[5] A qualitative comparison concluded that black garlic processed at 70°C has the best flavor and homogeneity of its black color.[14]

Analysis of black garlic produced at varying temperatures determined that levels of moisture, amino acid nitrogen, and allicin decreased with increasing temperature, while reducing sugar, 5-hydroxymethylfurfural, total phenols, total acid contents, and browning increased.[14] The absence of fresh garlic's pungent smell in black garlic can be attributed to the gradual reduction of allicin in black garlic as it undergoes the heating process. In addition, 70°C provides optimal levels of reducing sugar and 5-hydroxy-methylfurfural, which are important intermediates in the Maillard reaction.[6] Compounds with known activity against diseases, such as polyphenol, flavonoids, and some intermediates of Maillard reaction that show antioxidant properties, increase in black garlic and contribute to its significantly higher biological activity.[6] Table 1.2 has been adapted from a review by Ryu and Kang and compares the contents of black garlic and fresh garlic.

Table 1.2 Comparison of the phytochemical components of aged black garlic (ABG) and fresh raw garlic (FRG).[5]

Components	Content in ABG	Content in FRG	Changes in ABG compared to FRG
Total sugar (%)	1.6	0.2	↑
	28.2±1.2	4.2±0.1	↑
Allicin (mg/100g)	-	362±1	↓
	20	345	↓
Thiosulfate (mmol/100g)	9.12±0.05	0.65±0.03	↑
	0.3	10.5±0.4	↓
	0.8	0.1	↑
S-allyl cystein (SAC) (mg/100g)	8.5±0.1	2	↑
	19.4	2.4	↑
	9.8±0.2	2.2	↑
	11.4±0.9	2.3	↑
Protein (%)	1.0±0.1	0.7	↑
	9.1	8.4	↑

With more than 3,000 research papers and many ongoing investigations into its biochemical effects, garlic is one of the most studied medicinal plants.[9] The objective of this study is to provide an understanding of the effects of garlic's organosulfur compounds and specifically, their antimicrobial, cardiovascular, and anticancer effects. It also examines the pharmacological effects and mechanisms of black garlic, a type of aged garlic derivative. By reviewing studies that explore the mechanisms of these compounds in both *in vitro* and *in vivo* investigations, the study provides evidence for garlic and its derivatives' potential as natural and safe pharmacological agents.

CHAPTER TWO

Antimicrobial Properties

Infectious diseases remain one of the leading causes of death worldwide, accounting for 15 million deaths in 2015 and estimated to still cause 13 million deaths in 2050. With the epidemiological shift the number one cause of death around the world has changed to ischemic heart disease and stroke, both of which are non-communicable diseases, but infectious diseases remain the leading causes of morbidity and mortality in low-income regions. Graph (b) in Figure 2.1 shows the top 10 infectious disease in 2010, including respiratory infections as the leading cause of death by infectious diseases. In fact, infectious diseases was the leading cause of death in low-income countries in 2010.

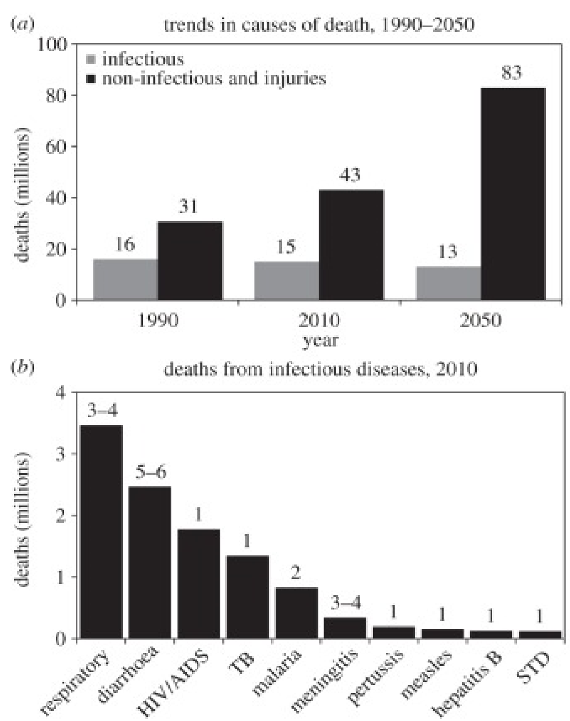


Figure 2.1 (a) Estimated deaths in 1900, 2010, 2050 from infectious vs. non-infectious diseases. (b) Top 10 causes of death from infectious diseases in 2010.[15]

Graph (a) in Figure 2.1 illustrates the slow rate of decline in infectious disease mortality and the rising burden of non-communicable diseases, such as cardiovascular diseases and cancers. Consequently, low- and middle-income countries face a "double burden" of infectious and non-infectious diseases.[15]

Part of the difficulty in developing effective treatment against pathogenic microorganisms is the emergence of microorganisms that are resistant to current antibiotic therapy.[16] In the last decade, much research has been dedicated to finding natural alternatives or adjuvant to existing antibacterial therapies in an effort to develop treatments with high efficacy against antibiotic resistant microorganisms but which have few adverse effects.[16] According to the World Health Organization, of the 1,400 known species of microbial pathogens, approximately 20 species are responsible for nearly two-third of all infectious diseases around the world.[15]

Table 2.1 List of species against which *Allium sativum* exhibits antimicrobial effects.[18]

Bacteria inhibited	Fungi inhibited
B. cereus	*A. flavus*
B. subtilis	*A. niger*
Clostridium botulinum	*Aspergillus parasiticus*
Enterobacter aerogenes	*C. albicans*
E. coli	*Cryptococcus*
E. coli O157	*Endomyces fibuliger*
Y. enterocolitica	*P. commune*
L. monocytogenes	*Penicillium cyclopium*
S. enteritidis	*P. roqueforti*
Salmonella typhi	*Rhodotorula*
S. typhimurium	*S. cerevisiae*
S. aureus	*Torulopsis*
Shigella	*Trichosporon*
Staphylococcus epidermidis	

Louis Pasteur was one of the first people to describe the antimicrobial properties of garlic in 1858,[16] and since then allicin and its organosulfur derivatives have been identified as the species largely responsible for the effect.[8,9] Allicin inhibits a broad spectrum of targets, including bacteria, fungi, oomycetes, and protozoa,[17] and Table 2.1 lists some of the susceptible bacterial and fungal species.

Although the exact mode of action through which allicin confers antimicrobial effects upon garlic is unknown, allicin's classification as a naturally occurring thiosulfinate means that it is highly reactive towards thiols and participates in extensive redox reaction mechanisms.[17,18] As illustrated in Figure 1.1, allicin is produced via an enzymatic conversion of a comparatively redox-inactive sulfoxide, namely alliin.[18] Thiosulfinates are characterized not only by their high reactivity but also selectivity towards thiols, with one molecule of thiosulfinate exhibiting the capacity to consume up to four thiol equivalents. Despite their selectivity for thiols, however, thiosulfinates target any cysteine residue in any protein they encounter.[18] Allicin is thought to disrupt bacterial cell function and stability by forming allyl-disulfide species with free amino acid L-cysteine, which plays a crucial role in many biochemical and biosynthetic pathways.[16] For example, cysteine is a precursor for methionine, thiamine, biotin, lipoic acid, coenzyme A and coenzyme M. In addition, cysteine is involved in the biogenesis of [Fe –S] clusters, found in the catalytic site of several enzymes that aid protein folding and assembly via formation of disulfide bonds. Cysteine derivatives, such as thioredoxin and glutathione, help protect cells against oxidative stress.[19] Therefore, while mammalian cells with low levels of glutathione are able to evade such aggressive oxidation reactions, many microbes, bacteria, and fungi are subject to destruction by compounds such as allicin and exhibit little chance of developing resistance.[18]

Helicobacter pylori: an Introduction

Allicin's antimicrobial effects have led to investigations into the potential use of allicin as a pharmacological agent. Extensive research studied the effects of allicin and its potential in eradicating *Helicobacter pylori*, the bacteria responsible for the most common gastrointestinal diseases in humans.[16] Despite the advancements in treatment made in recent years, *H. pylori* remains one of the most prevalent chronic bacterial infections in humans, affecting an estimated 50% of the world population.[20,21] *H. pylori* infects the mucosal layer of the gastrointestinal system and is associated with diseases such as chronic gastritis, peptic ulcer disease, gastric carcinoma, refractory iron deficiency anemia, and vitamin B12 deficiency.[21]

Helicobacter pylori Studies

In 2001, a study examined the *in vivo* efficacy of garlic in treating *H. pylori* infection in 210 patients. Of the six different trial groups, only two groups received allicin either alone or as a supplement to a standard treatment of lansaprasol 30 mg, clarithromycin 500 mg, and amoxicillin 1 g twice a day. Of the 30 patients in the group treated with standard antibiotics and allicin supplement, 27 (90%) patients showed eradication of infection, and 7 (23%) patients from the group treated with allicin alone showed infection eradication.[22]

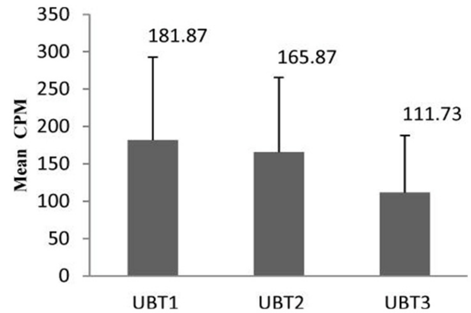


Figure 2.2 Comparison of the mean count per minute (CPM) from urease breath tests (UBT1, 2, and 3) that measure urease activity level of *H. pylori*.[23]

In a more recent study, 15 patients were given a urease breath test (UBT), a standard method of detection for *H. pylori* infections. One group received no treatment and was considered the control group, while a second group was prescribed 3g of garlic cloves each day for 3 days. Both groups were given a UBT after 3 days, and the UBT results showed a significant difference before and after garlic consumption. Figure 2.2 shows the results of UBTs administered each day during the 3-day treatment course for the experimental group.

Possible Modes of Action against Helicobacter pylori

Although the exact mechanism is still under investigation, allicin most likely inhibits *H. pylori* proliferation in the stomach by interfering with enzymes and proteins that contain thiol groups.[23] In particular, allicin's reactivity and selectivity towards cysteine allow it to target toll-like receptor 4 (TLR4) on the cell surface to inhibit the activity of B cells, which increase the expression of inflammatory cytokines and induce the characteristic immune responses in cancer and inflammation.[23] Cañizares et al. reported similar findings with acetonic garlic extracts that exhibited bacteriostatic properties against *H. pylori* in an *in vitro* study, suggesting the synergistic effects of garlic in treating *H. pylori* with extracts from a relatively cheap and easy processing method.[24]

Diallyl trisulfide, also known as allitridi, is an organosulfur derivative of allicin that also exhibits antimicrobial activity against *H. pylori*.[25] According to experiments conducted by Liu et al., allitridi administered at its minimum inhibitory concentration (MIC) of 1 μg/mL downregulated two important virulence proteins found in *H. pylori*: cytotoxin-associated gene A (CagA) and neutrophil-activating protein A (NapA).[25] CagA-positive *H. pylori* infections are associated with increased levels of gastric inflammation and gastric adenocarcinoma.[26] NapA has been known to participate in the recruitment and activation of neutrophils and monocytes that produce reactive oxygen species.[25] Therefore, allitridi's ability to downregulate CagA and NapA suggests the potential use of garlic in treating *H. pylori* infections by targeting the bacteria's virulence factors.

Biofilm-Forming Species: an Introduction

Biofilm-forming species have led to another issue in treating microbial infections, especially in chronic diseases. Biofilm is an aggregated community of surface-associated bacterial cells that often demonstrates extremely high resistance to antibiotics and immune responses in comparison to planktonic cells. An extracellular polysaccharide matrix allows biofilm-embedded bacteria to evade host immune responses and antibiotic treatments.[27–29] The presence of pathogenic microbial colonies is associated with various chronic diseases, such as cystic fibrosis and endocarditis, and the spread of slime-associated nosocomial infections found on implanted medical devices.[27] *Proteus mirabilis* in particular has been determined to be the leading cause of recurrent urinary tract infections (UTIs) in patients with indwelling catheters or structural abnormalities in the urinary tract.[28] *Pseudomonas aeruginosa* has been associated with nosocomial and wound infections, along with genetically inherited cystic fibrosis. *P. aeruginosa* is thought to form biofilms via quorum sensing, a system that uses *N*-acyl-homoserine lactone to coordinate gene expression.[29]

Biofilm-Forming Species Studies

In 2012, Jakobsen et al. studied the effects of ajoene on inhibiting the biofilm formation of *P. aeruginosa*. The study discovered that the concentration of rhamnolipid (heat-stable hemolysin), a virulence factor controlled by quorum sensing, was inversely correlated to the concentration of ajoene. Rhamnolipid destroys polymorphonuclear leukocytes via lytic necrosis and is responsible for the tissue damage and evasion of host immune response in biofilm-forming bacteria. A concentration of 20 μg/mL of ajoene was enough to reduce the rhamnolipid content to one-third of that for the untreated culture, and at 80 μg/mL, rhamnolipid could not be detected.[29]

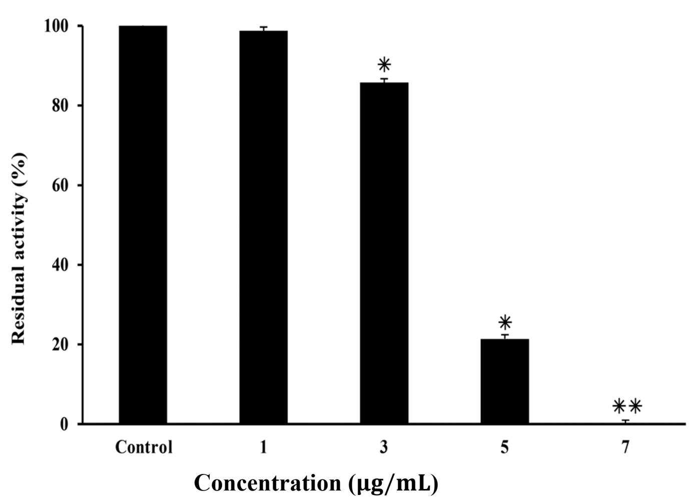


Figure 2.3 Inhibition of urease activity in bacterial cell lysate using allicin.[28]

In a more recent study, Ranjbar-Omid et al. demonstrated allicin's ability *in vitro* to inhibit biofilm formation and urease activity of *Proteus mirabilis*. Urease is an important target for treating *P. mirabilis*, because it catalyzes the hydrolysis of urea to ammonia and carbon dioxide. The alkaline properties of ammonia cause damage to host tissues by affecting enzyme activities that may result in the formation of bladder and/or kidney stones as a result of a weakened ability of the immune system in an elevated pH environment.[28,30] Derivatives of ammonia, such as ammonium hydroxide and monochloramine, are toxic to mammalian cells. Monochloramine, in particular, is known to induce DNA damage.[30] As demonstrated in Figure 2.3, allicin inhibited urease activity in a concentration dependent manner with IC50 achieved with 4.5 μg/mL of allicin and complete inhibition with 7 μg/mL. In addition, allicin also reduced the formation of biofilm in a concentration dependent manner. Concentrations of 16 and 32 μg/mL of allicin showed maximum reduction in biofilm development by around 29% and 34%, respectively, in a standard strain of *P. mirabilis*. Allicin showed similar results in clinical strains isolated from patients with UTIs.[28]

Another study examined the antimicrobial effects and the effects against the formation of biofilm of methanol and ethanol extracts of garlic against *S. aureus, B. cereus, S. pneumoniae, P. aeruginosa, E. coli,* and *Klebsiella penumoniae.* Although the garlic extracts only demonstrated antimicrobial effects against *B. cereus* in solid medium performed via disk diffusion analysis, both extracts exhibited antimicrobial effects against all six pathogenic microbes in broth medium. In addition to antimicrobial activity, inhibition and destruction of biofilm was observed in varying degrees, suggesting that the efficacy of garlic's inhibitory effect on biofilm formation depends on the type of solvent, concentration of extract, and type of bacteria.[27] This is illustrated in Figure 2.4.

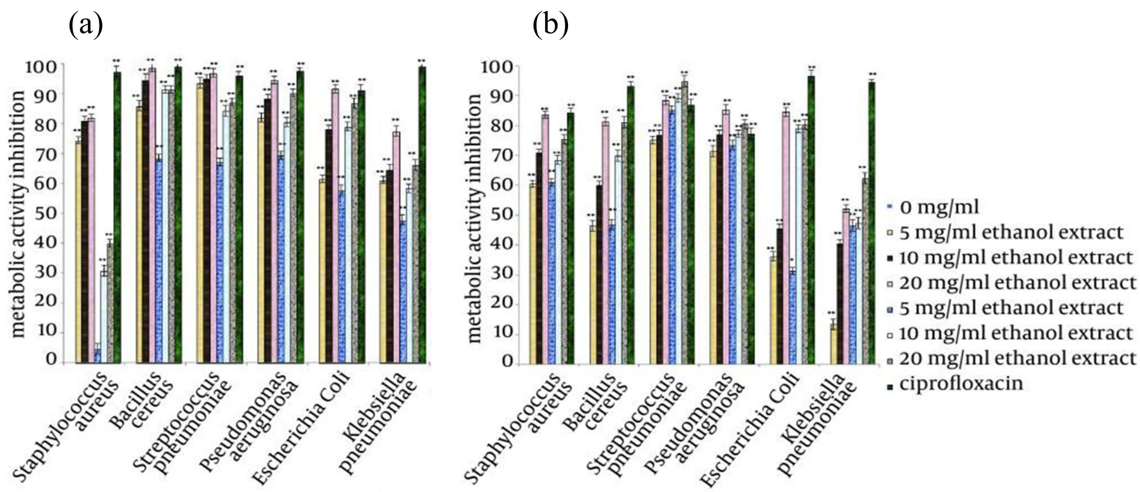


Figure 2.4 (a) Reduction of biofilm formation for the test bacteria treated with different concentrations of *Allium sativum* for 24 hours[27]; (b) Disruption of biofilm formation for the test bacteria treated with different concentrations of *Allium sativum* for 24 hours.[27]

Possible Modes of Action against Biofilm-Forming Species

Although the study by Mohsensipour et al. did not investigate the exact mechanism by which garlic demonstrates antimicrobial and biofilm inhibitory effects, it can be proposed that there are several different inhibitory mechanisms by which garlic can work against the various mechanisms of biofilm formation and development in the six different bacterial species used.[27] Allicin in particular is known to inhibit pneumolysin O (PLY) and streptolysin O (SLO), which are virulence factors present in bacterial species such as *P. mirabilis*, by binding to cysteinyl residues in the binding sites.[31,32] In addition to allicin's high affinity towards thiol groups, its low molecular weight and simple structure allow the organosulfur compound to enter the active sites of thiol-containing enzymes and toxins to inhibit biofilm formation and confer antimicrobial effects.[28]

Antibiotic Resistance: an Introduction

Increasing antimicrobial drug resistance in disease-causing pathogens has raised serious concerns and posed significant difficulties in the prevention and treatment of infectious diseases.[16] Acquisition and transmission of antimicrobial resistance among common species of infectious organism such as *S. aureus* are of major concern in hospital settings, where nosocomial infections commonly occur.[33] Antibiotic resistance contributes to the "double burden" of diseases for low to middle-income populations, because according to a multinational survey by the World Health Organization, respondents from lower income countries were more likely to have taken antibiotics in the month preceding the survey.[34] Causes of the antibiotic resistance crisis include overuse, inappropriate prescriptions, extensive agricultural use, and reduced production of new antibiotic treatments.[35] Resistant strains of *S. aureus* and *Enterococcus* species pose the largest threat amongst the Gram-positive pathogens with methicillin-resistant *S. aureus* (MRSA) killing more Americans per year than HIV/AIDS, Parkinson's disease, emphysema, and homicide combined. Among Gram-negative pathogens, the emergence of multidrug resistant (MDR) bacilli, *Enterobacteriaceae* (mostly *Klebsiella pneumoniae*), *Pseudomonas aeruginosa*, and *Acinetobacter* have been found in healthcare settings. Extended-spectrum beta-lactamase-producing *E. coli* and *Neisseria gonorrhoeae* are emerging even outside of hospital settings.[35]

Antibiotic Resistance Studies

In his study, Abubakar demonstrated that crude garlic extracts at a concentration of 100 mg/mL successfully inhibited the growth of bacteria such as *S. aureus*, *E. coli*, *S. pneumoniae* and *P. aeruginosa* with the gram-positive *S. aureus* demonstrating the greatest susceptibility.[33]

Table 2.2 Inhibition of resistant strains of *S. aureus* and *E. coli* due to streptomycin, sterile garlic extract, and combinations of streptomycin and garlic extract.[36]

Drug	Dose	Zone of inhibition (mm ± S.E.M.)	
		S. aureus ATCC BAA 1026	*E. coli* ATCC 10536
Streptomycin[a] (control)	10 mg/ml	7 ± 0.2887	8 ± 0.2887
Sterile garlic extract[a]	50%	14 ± 0.5775**	14.5 ± 0.2887**
Streptomycin[b]	10 mg/ml	15 ± 0.2887**	16 ± 0.2887**
Streptomycin[c]	10 mg/ml	22 ± 0.2887**	24 ± 0.5774**
Streptomycin[d]	10 mg/ml	26 ± 0.2887**	28 ± 0.2887**

[a]Solvent: sterile distilled water. [b]Solvent: 50% sterile garlic extract. [c]Solvent: 75% sterile garlic extract. [d]Solvent: 100% sterile garlic extract. ***P* < 0.01 as compared with control according to one-way ANOVA post-test.

Palaksha, Ahmed, and Das also noted similar results in studying the efficacy of garlic on streptomycin-resistant strains of gram-positive *S. aureus* and gram-negative *E. coli*.[36] Observation of clear zones of inhibition in plates inoculated with both streptomycin and garlic extracts suggested that inhibition of microbial growth was enhanced by the presence of garlic extracts.[36]

Cutler and Wilson studied the effects of allicin on MRSA in particular and suggested the possibility of the organosulfur compound as an alternative to topical agents used to treat and decrease the spread of MRSA. The MIC value for the majority (88%) of the 30 clinical isolates was 16 µg/mL, with all strains inhibited at 32 µg/mL.[37] Another study evaluated the bactericidal effect of allicin *in vitro* against 20 clinical isolates of *S. aureus,* 20 of *S. epidermis*, and 31 of *P. aeruginosa.* Although allicin did not exhibit significant antibacterial activity alone, it increased the efficacy of three conventional antibiotics, cefazolin, oxacillin and cefoperazone used in treating the three different bacterial species.[38] Such findings suggest potential use of garlic and its derivatives in synergy with conventional antibiotics to produce more effective antimicrobial activity, especially in infections that exhibit drug resistance.[8]

Possible Modes of Action against Antibiotic Resistant Species

Similar to the way in which garlic extracts inhibit *H. pylori* infections and the formation of biofilm, it is suspected that garlic also suppresses the growth of antibiotic resistant bacteria via organosulfur compounds such as allicin that inhibit a number of thiol-containing bacterial enzymes.[16] Allicin is also thought to have a variety of targets within the bacterial cell, thereby contributing to its diverse antimicrobial effects including inhibition of acetyl CoA formation, DNA and protein synthesis, and RNA polymerase.[37]

Black Garlic and Antimicrobial Activity

Because much of the allicin in fresh garlic is lost in the process of producing black garlic, the latter demonstrates significantly less antimicrobial activity. Therefore, this property is found almost exclusively in fresh garlic and serves as a limitation on the use of black garlic as an antimicrobial agent.

CHAPTER THREE

Cardiovascular Effects

Cardiovascular Disorders: an Introduction

The recognition of the critical role of diet in the management and prevention of cardiovascular diseases has led to an increased interest in alternative medical therapies, including garlic and its cardioprotective effects.[13,39–41] Cardiovascular disease is the leading cause of death around the world, accounting for approximately 31% of deaths globally and 23.4% of deaths in the U.S. in 2015.[42] More than 130 million (45.1%) U.S. adults are expected to have at least one form of cardiovascular disease by 2035, increasing the financial burden to $1.1 trillion.[43] Risk factors for cardiovascular diseases are often lifestyle-related and include unhealthy diet, tobacco use, excessive alcohol consumption, lack of physical activity, high blood pressure, high blood lipids, and obesity.[39] With the growth of the aging population, there has been an increase in the total number of cardiovascular diseases.[44]

Hypertension: an Introduction

Hypertension, also known as raised or high blood pressure, refers to a systolic blood pressure (SBP) of 140 mmHg or higher, or a diastolic pressure (DBP) of 90 mmHg or higher, or both.[40] High blood pressure is a major risk factor for cardiovascular diseases and contributes to the development of stroke, kidney failure, and premature disability and mortality. Of the 17 million deaths that occur from cardiovascular diseases each year, complications of hypertension account for 9.4 million deaths.[45] The number of people diagnosed with hypertension globally has been on the rise since 2000 and is expected to continue increasing into 2025, indicating an increasingly aging population that will suffer from one or more cardiovascular diseases in adulthood. Blood pressure is a heritable trait and genetics account for approximately 30% of the variance in blood pressure. However, excess intake of salt, calories, and alcohol, along with lack of physical activity, also contribute to the development of hypertension.[46]

Hypertension Studies

A study on induced hypertension in rats and the effects of garlic demonstrated a dose-dependent reduction in SBP. The male Wistar rats in the experimental group underwent a surgical procedure to place a silver clip on the right renal artery, which increased the blood pressure. The control group underwent the same procedure, including anesthesia and flank incision, but without the placement of the silver clip. Both groups were given intravenous infusion of aqueous garlic extract. Bolus infusions of garlic extracts were also used.

As Tables 3.1 and 3.2 show, bolus infusion of 20 mg/kg in hypertensive rats decreased SBP from 180$\pm$3 to 150$\pm$3 mmHg and DBP from 150$\pm$3 to 110$\pm$6 mmHg. The same dose decreased SBP from 90$\pm$4 to 70$\pm$5 mmHg and DBP from 72$\pm$5 to 50$\pm$4 mmHg in normotensive rats.[47] Another study using dogs demonstrated significant reduction in blood pressure for several hours after intragastric administration of garlic powder.[48]

Table 3.1 (a) Effect of bolus infusion of garlic on blood pressure and heart rate of hypotensive rats (b) Effect of bolus infusion of garlic on blood pressure and heart rate of normotensive rats.[48]

(a)

	Systolic (mmHg)	Diastolic (mmHg)	Pulse pressure (mmHg)	MAP (mmHg)	Heart rate (beats/min)
Pre- infusion	180 ±3	150 ±3	30 ±3	160 ±3	406 ±6
After infusion (20mg/ml)	150 ±5*	110 ±6*	40 ±5#	123 ±5*	250 ±5*
% reduction or increase	16.7	26.7	33.3#	23.1	38.4

MAP= Mean Arterial Pressure, * = P<0.05 compared with pre-infusion of extract, # = p<0.05 compared with pre-infusion of extract, n = 6.

(b)

	Systolic (mmHg)	Diastolic (mmHg)	Pulse pressure (mmHg)	MAP (mmHg)	Heart rate (beats/min)
Pre-infusion	90 ±4	72 ±5	18 ±5	78 ±5	378 ±5
After infusion (20mg/ml)	70 ±5*	50 ±4*	20 ±5#	56 ±5*	207 ±5*
% reduction or increase*	22.2	30.6	11.1	28.2	45.2

* = p<0.05 compared with pre-infusion of extract, # = P<0.05 compared with pre-infusion of extract, n = 6.

A meta-analysis by Silagy and Neil identified eight studies on the effects of dried garlic powder in humans. The analysis included a total of 415 subjects and three studies that were conducted specifically on hypertensive individuals. Seven out of eight studies compared the effect of garlic on hypertension to that of a placebo, and three of these studies demonstrated a significant reduction in SBP. Although four of the studies showed a reduction in DBP, the overall effect was greater for SBP than DBP.[49] Meta-analysis of 20 trials with 970 participants also suggested that garlic supplements significantly lower SBP by a mean difference of 5.1±2.2 mmHg and DBP by a mean difference of 2.6±1.6 mmHg. A review of the hypotensive effect of garlic on hypertensive participants in particular also revealed an average decrease of 8.6±2.2 mmHg and 6.1±1.3 mmHg for SBP and DBP, respectively.[50]

Possible Modes of Hypotensive Action

Several mechanisms explaining the hypotensive effects of garlic have been proposed, but further research is needed in order to gain a clearer understanding of how garlic's metabolites work to lower blood pressure.[40,47] According to the results from the study by Nwokocha et al., it is clear that garlic reduces blood pressure in a dose dependent manner by a mechanism not involving acetylcholine.[47] Heart rate and contractility is affected by β_1 adrenergic receptor stimulation.[51] Nwokocha et al. treated mice receiving garlic infusions with atropine, an anticholinergic agent, and observed no significant difference in the effect of garlic. This result indicates that the cholinergic system has no influence on the hypotensive action of garlic.[47]

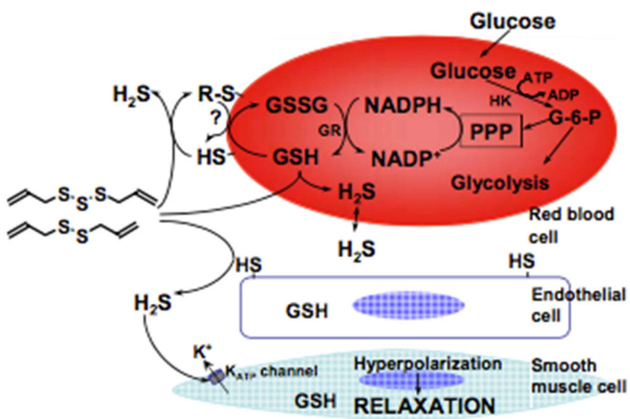


Figure 3.1 Proposed model of garlic-induced H_2S effect in the vascular system. Diallyl-disulfide (DADS) and diallyl-trisulfide (DATS) react with the thiol groups on the surface of red blood cell to produce H_2S, which leads to vasorelaxation via vascular smooth muscle cell ATP-linked hyperpolarization.[53]

Some of the possible mechanisms that have been proposed are vasorelaxation through H_2S production, inhibition of angiotensin-converting enzyme *in vitro* via gamma-glutamylcysteines, endothelium-derived relaxing and constricting factors, and beta-adrenoceptor blocking action.[47] Vasorelaxation through H_2S production occurs via human red blood cells that convert garlic's organic polysulfides into H_2S, an endogenous vascular cell signaling molecule that functions to protect the cardiovascular system. Effects of H_2S on cell signaling and cardioprotection are thought to be similar to those of

CO and NO, including decrease in blood pressure, cardioprotection against ischemic reperfusion damage, and oxygen-dependent vasorelaxation.[52]

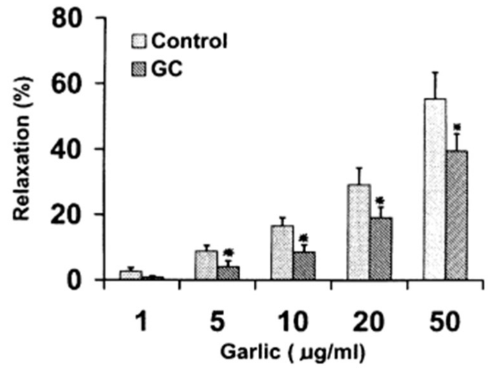


Figure 3.2 Reduced ability of garlic to cause vasorelaxation (expressed as percent inhibition of contraction produced by phenylephrine) as a result of blocking ATP mediated K^+ channels using glybenclamide.[54]

Ashraf et al. proposed that the hypotensive effect of garlic is derived from garlic's ability to induce vasorelaxation via endothelium-mediated mechanisms. According to the study, garlic stimulates the release of endothelium-derived hyperpolarizing factors and also inhibits cyclooxygenase generated contracting factors in order to cause vasorelaxation. Glybenclamide, an ATP mediated K^+ channel blocker, was used to elucidate the possible mechanism through which garlic caused blood pressure lowering. As shown in Figure 3.2, blocking ATP mediated K^+ channels prohibited the release of endothelium-derived hyperpolarizing factors and thus significantly lowered the efficacy of garlic.[53]

Atherosclerosis: an Introduction

Atherosclerosis, like many other heart disorders, is a complex, multifactorial disease. It is defined as the accumulation of lipids and fibrous elements in the subendothelial area of the arterial wall. Early lesions of atherosclerosis involve lipid-laden macrophage build up in the subendothelial layer that may progress to fibrous lesions with lipid-rich necrotic debris and smooth muscle cells. These lipid-laden cells contribute to the inflammatory responses in the arterial wall. Atherosclerosis is the leading cause of heart disease and stroke, along with other fatal conditions such as calcification, rupture, and hemorrhage.[54,55]

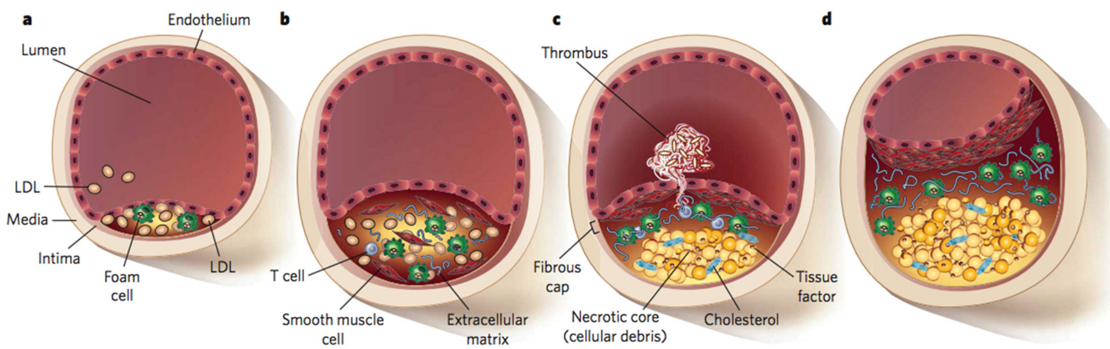


Figure 3.3 Initiation and progression of atherosclerosis. (a) Early lesion formation within the subendothelial layer; (b) Progression of early lesion into a lesion that is vulnerable to rupture; (c) Exposure of thrombogenic material; (d) If the lesion does not rupture, the buildup of plaque can encroach on the lumen and result in obstructive disease.[56]

A multitude of both genetic and environmental factors are known to contribute to its etiology, and hypercholesterolemia remains one of the major risk factors and a focus of much of the research regarding atherosclerosis and understanding the mechanistic development of the disease.[54] A 13-year study of 2,974 patients diagnosed with hypercholesterolemia determined that administration of lipid-lowering drugs significantly lowered the presence of plaques, which is used as a marker of atherosclerosis.[57] Low-density lipoprotein (LDL) cholesterol in particular is associated with the progression of atherosclerosis, while high-density lipoprotein (HDL) cholesterol concentrations are negatively associated.[54]

Atherosclerosis Studies

Garlic exhibits antiatherosclerotic, specifically cholesterol lowering, effects and even shows the ability to cause regression of already-present atherosclerosis in both *in vitro* and *in vivo* studies.[39,41] A 16-week study of the effects of raw and frozen garlic extracts on hypercholesterolemic rats demonstrated a decrease in plasma total cholesterol and, more significantly, a decrease in LDL in comparison to the control group.[58] In another study, 12 weeks of oral administration of allicin in mice produced a decrease in hepatic cholesterol storage and lipid accumulation in hepatic cells. Both of these changes were reflected in an overall decrease in body weight over the 12 week course and suggested allicin's role in alleviating liver stress related to high cholesterol content and the development of fatty liver associated with atherosclerosis.[59] Coronary artery calcification (CAC) serves as a model marker of coronary atherosclerosic burden, and daily garlic intake has revealed an attenuation of CAC progression in asymptomatic and intermediate-risk patients with coronary artery disease.[39]

Due to the multifactorial etiology of cardiovascular diseases, garlic's efficacy in the presence of a variety of drugs must be tested. For example, garlic is often used with antihypertensive drugs to decrease cardiovascular complications. However, conventional hypertensive drugs, such as propranolol (PRO) and hydrochlorothiazide (HYD), are reported to increase serum lipid levels, while captopril (CAP) shows a hypolipidemic effect. Asdaq et al. conducted a study examining the hypolipidemic effect of garlic in patients given PRO, HYD, or CAP. Under the influence of PRO and HYD, the efficacy of garlic's hypolipidemic effect decreased. However, co-administration with CAP suggested an augmentation in garlic's efficacy, pointing to a synergistic effect that could potentially be used to target a multitude of causes for cardiovascular disease.[41]

Banerjee and Maulik's meta-analysis revealed more than 46 studies since 1975 involving lipid-lowering effects of garlic in humans. The majority of these studies used garlic powder for 4 to 16 weeks in hyperlipidemic patients. While most demonstrated that garlic lowered the serum cholesterol and serum triglyceride levels, only one-third of the studies specifically measured lipoproteins and reported significant decreases in LDL ranging from 11 to 26%.[40] However, the extent of garlic's efficacy varies from study to study, and the hypocholesterolemic effect of garlic needs further research.

Possible Modes of Antiatherosclerotic Action

A potential mode of action for garlic's antiatheroclerotic properties is the inactivation of the cyclooxygenase pathway. In atherosclerosis, inflammation of the endothelium exacerbates the vascular damage by enhancing cyclooxygenase-dependent free radical production leading to the inactivation of NO and other endogenous endothelium-derived relaxing factors. By inhibiting the cyclooxygenase pathway, garlic can prevent further damage to the endothelial wall.[53] An *in vitro* study of garlic's ability to slow atherogenesis demonstrated that garlic consumption suppressed the oxidation of LDL. In doing so, garlic was able to attenuate the effects of oxidized LDL. These include vascular dysfunction caused by exerting direct cytotoxicity toward endothelial cells and enhancing the proliferation of various cell types that are indicative of atherosclerosis progression.[60]

Platelet Aggregation: an Introduction

Elevated platelet aggregation is the precursor to a multitude of cardiovascular diseases, including coronary artery disease, atherosclerosis, and venous thromboembolism.[13,39,40] Injured vessels expose collagen, laminin, and von Willebrand factor, which cause platelets to adhere and aggregate in order to trigger inflammation and thrombosis at the site of injury.[39,40] One of the most adverse consequences of platelet aggregation is when it is accompanied by atherosclerosis. The combination of vascular diseases may eventually cause total blockage of blood flow, resulting in myocardial infarction and thromboembolic diseases, such as pulmonary embolism or stroke.[40]

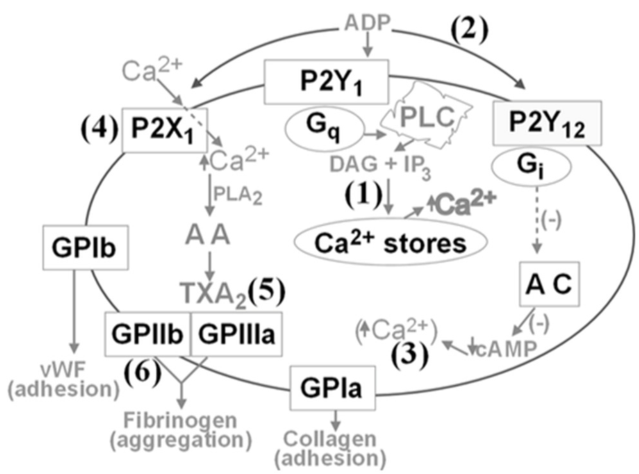


Figure 3.4 Major pathways of platelet activation and aggregation. Activation of platelets begins with the binding of ADP to the $P2Y_1$, $P2Y_{12}$, and $P2X_1$ receptors. (1) Binding to the $P2Y_1$ receptor results in the subsequent release of Ca^{2+}; (2) Binding to the $P2Y_{12}$ receptor inhibits adenylyl cyclase (AC), which decreases cAMP levels and (3) increases intracellular Ca^{2+} concentration; (4) Binding to the $P2X_1$ receptor also increases Ca^{2+} levels and leads to shape change and aggregation of platelets.[13]

Platelet Aggregation Studies

Several *in vitro* studies have examined the effects of garlic extracts in inhibiting platelet aggregation. Aqueous extract of garlic has been shown to prevent platelet aggregation and activation induced by ADP, epinephrine, collagen, and arachidonate in a dose-dependent manner.[40] Many other animal model studies demonstrated similar results and concluded that raw garlic extracts were superior in their ability to inhibit platelet aggregation than boiled garlic extracts.[13,40]

Possible Modes of Anti-Platelet Aggregate Action

Garlic extracts target multiple steps in the platelet activation and aggregation pathway in order to exert an anti-platelet aggregating effect. Previous studies have suggested the suppression of calcium mobilization as a potential mode of action.[13,40] Resting platelets are discoid in shape and require activation in order to undergo a shape change for aggregation. The binding of ADP to the $P2X_1$ purinergic receptor found on the plasma membrane of inactivated platelets results in the influx of calcium. Consequently, platelets become activated and undergo a shape change to become spherical with extending pseudopods for adhesion. Although the exact mechanism is unknown, allicin and ajoene have been shown to inhibit platelet aggregation by interfering with the calcium ionophore and thus lowering the cytosolic calcium concentration.[13]

Another study likened garlic's mechanism of anti-platelet aggregation to that of clopidogrel, a prodrug used widely for its platelet aggregation inhibition.[61,62] The active metabolite of clopidogrel, a thienopyridine derivative, irreversibly binds to the $P2Y_{12}$ receptor found on the plasma membrane of platelets and thus prevents the binding of ADP to the receptor.[62] As seen in Figure 3.4, the binding of ADP to the $P2Y_{12}$ receptor is one of the many necessary steps in platelet activation.

Black garlic and cardiovascular diseases

More so than fresh garlic extracts, aged black garlic extracts have been studied extensively in regards to cardiovascular health due to their increased antioxidant activity. Elevated concentrations of organosulfur compounds such as S-allyl-cysteine (SAC) and diallyl-disulfide (DADS) have been reported to act as potent inhibitors of cholesterol synthesis.[40] An *in vitro* study of aged black garlic extracts enriched with polyphenols and S-allylcysteine demonstrated vasodilating effects and increased release of NO. In addition, the enriched aged black garlic extracts induced the relaxation of coronary arteries before and after ischemic reperfusion and prevented the ischemic reperfusion induced decrease in myocardial contractility.[63]

Aged garlic extract supplemented with B vitamins, folic acid, and L-arginine (AGE+S) demonstrated similar results. After a year of consuming AGE+S capsules containing 250mg of aged garlic extract daily, the 33 patients in the experimental group showed significantly lower CAC progression and total cholesterol and LDL decrease in comparison to those of the control group.[64] Budoff's findings in a placebo-controlled, double-blind, randomized study of 19 high-risk patients specifically examined black garlic's ability to slow the progression of CAC in conjunction with statin therapy and aspirin. The one-year study yielded an overall reduction in progression of CAC in the group that received black garlic extracts in addition to the baseline statin therapy. The control group showed a mean CAC progression rate of approximately 22.2% per year, while the experimental group showed a reduced CAC progression rate of approximately 7.5% per year.[65]

A study by Ha et al., demonstrated a potential mechanism through which black garlic exhibits its antiatherosclerotic effects. By measuring the expression of the hepatic sterol regulatory element binding protein-1c (SREBP-1c), a key transcription factor of lipid metabolism, Ha et al. were able to observe the level of fat synthesis in the liver of rats on high fat diets. The results from rats that received high fat diet with 0.5% (5 g/ kg diet) and 1.5% (15 g/ kg diet) black garlic extract demonstrated decreased activity of biomarkers of atherosclerosis such as HMG-CoA reductase and Acyl-CoA cholesterol acyltransferase (ACAT), which regulate cholesterol synthesis and catalyze esterification of cholesterol, respectively.[66,67]

Another study examined the cardiovascular health of its subjects by monitoring the C-reactive protein (CRP), a nonspecific marker of inflammation and mild predictor of cardiovascular diseases. Administration of aged garlic extract and Coenzyme Q10, an electron carrier in aerobic respiration used to prevent oxidation of LDL, over a 12-month period yielded lower CRP levels and slower progression of CAC.[68] The improved cardiovascular health of those who received aged garlic extract in comparison to that of the control group indicates aged garlic's ability to work in synergy with pre-existing therapies for cardiovascular diseases to amplify the effects of treatment options.[68,69]

Aged garlic extracts also exhibit anti-platelet aggregating effects, and studies have suggested that the metal chelation properties of aged garlic extracts may be responsible. As with fresh garlic extracts, aged garlic extracts are thought to interfere with the calcium influx necessary for the platelet shape change and subsequent aggregation in the activation pathway.[13] A study demonstrated that aged garlic extract suppressed calcium mobilization via its metal chelating property and did not exhibit the same effects when diethyl ether extract of aged garlic was used.[70] Complexing metal ions, such as calcium, not only affects the platelet activation and aggregation pathway, but also provides cardioprotection against a major cause of atherosclerosis: LDL oxidation.

As Figure 3.5 indicates, the oxidation of LDL in endothelial or vascular smooth muscle cells requires active metal ions, such as copper.[71] Aged garlic extracts have been shown to chelate copper ions and thus inhibit LDL oxidation *in vitro*.[72]

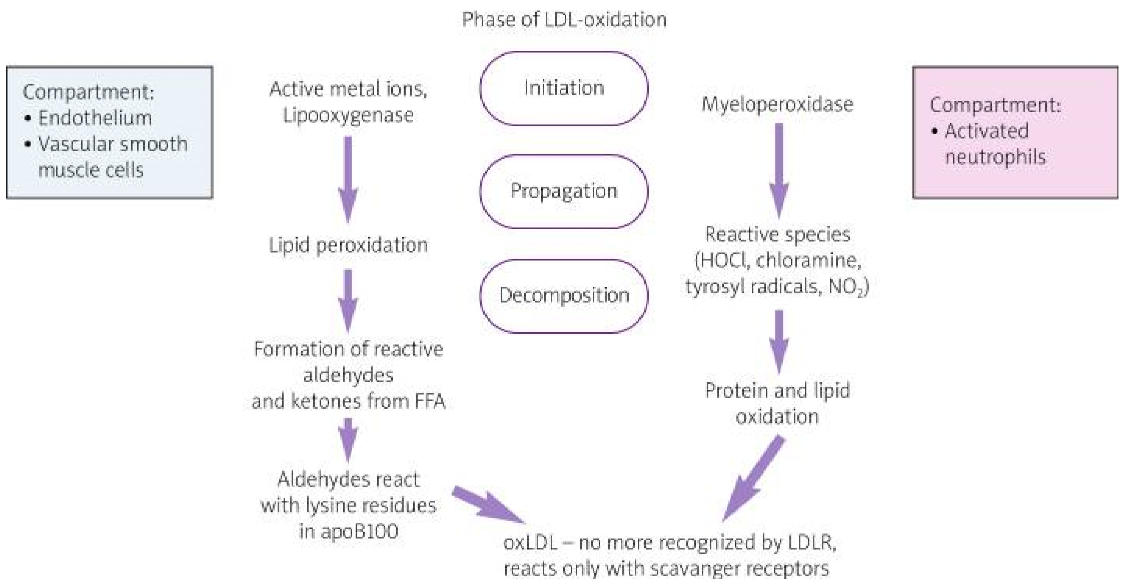


Figure 3.5 Various mechanisms of LDL oxidation.[71]

CHAPTER FOUR

Anticancer Properties and Conclusion

Cancers: an Introduction

Cancer constitutes an overwhelming disease burden not only in the United States but also globally. Recent statistical analyses have shown that the burden of cancer is falling increasingly on middle- and low-income countries.[73,74] The *World Cancer Report 2014* published by the World Health Organization's International Agency for Research on Cancer estimated a global incidence of 14 million new cancer cases in 2014 and expected the figure to rise to 19.3 million by 2025.[74] Cancer statistics in the United States show similar patterns with nearly 1.7 million new cancer cases made and more than 600,000 cancer-related deaths in 2017.[75] Primary prevention of cancer has been proposed as the most effective means of combating the alarming rate of cancer prevalence. The present knowledge of risk factors, such as smoking, infection, and excessive alcohol consumption, suggests that nearly half of all cancers could be prevented.[73,74] New and creative strategies need to be implemented to increase healthy and cancer-preventing behavior with an emphasis on middle- and low-income populations.[75]

Epidemiological studies have revealed a connection between the consumption of garlic and reduced incidence of prostate, colorectal, laryngeal, and gastric cancers. As one of the oldest and most studied medicinal plants, garlic exhibits significant potential as an alternative or supplement to existing chemotherapies.[76,77]

Cancer Studies

One of the first epidemiological studies performed in China compared two population groups for garlic consumption and rates of stomach cancer. The study revealed that a group from Cangshan county consumed nearly 20 g of garlic per day and had one of the lowest stomach cancer death rates (3/100,000), while another group from Qixia county had a stomach cancer death rate more than three times that of the Cangshan county group. Researchers postulated that the increased cancer rate could be related to the near lack of garlic consumption in Qixia county since garlic was thought to reduce nitrate reduction to nitrite and subsequently nitrosoamines, which are potent carcinogens.[76]

As Fleischauer and Arab's meta-analysis of epidemiological studies of garlic and cancer reveal, the inconsistencies among the studies proposes an issue with determining the exact method of preparation and minimum inhibitory concentration of garlic against cancer cells. Data suggest that garlic has shown greatest potency and preventative effect against gastric and colon cancers.[78]

Possible Modes of Anticancer Action

Several *in vitro* studies have been performed in an effort to narrow down the exact molecule of anticancer action. Bat-Chen et al. examined allicin's effect on colon cancer cells and determined that allicin exerts its effect by inducing apoptosis via the activation of nuclear erythroid-related factor-2 (Nrf2). Nrf2 is a transcription factor associated with genes that encode antioxidant proteins and detoxifying enzymes, and when Nrf2 is activated, the oxidative stress on cancer cells eventually leads to cell death. In addition, allicin treatment was also related to cytochrome c release from the mitochondria, which signals apoptosis.[77]

Some of garlic's organosulfur compounds, diallyl-sulfide, diallyl-disulfide, and diallyl-trisulfide, inhibited the migration and invasion of colon cancer *in vitro*. All three compounds downregulated many of the molecules involved in metastasis of cancer cells, specifically matrix metalloproteinase-2 (MMP-2), which has been found in invasive tumor cell lines and is thought to play a role in tissue remodeling. Increased level of MMP-2 is associated with the ability of cancer cells to successfully invade distant sites in the body by degradation of the extracellular matrix.[79] Diallyl-disulfide also exhibited antiproliferative action in colon cancer cells by inducing histone acetylation and subsequent G2/M cell cycle arrest in tumor cells.[80]

Black Garlic and Cancer

Black garlic also exhibits anticancer effects, mostly in gastric and colon cancer. Aged black garlic extract was able to suppress development of inoculated tumor cells in a tumor-bearing mouse model, and induce apoptosis in gastric cancer cells *in vitro*. Research suggested that aged black garlic's antioxidant properties may play a role in its prophylactic and therapeutic effects. The process of preparing aged black garlic involves the conversion of several of fresh garlic's organosulfur compounds into water-soluble organosulfur compounds, such as S-allyl cysteine (SAC) and S-allylmercapto-L-cysteine (SAMC). SAC and SAMC exhibit high radical scavenging activity and remove reactive oxygen species, which are capable of changing multiple cellular processes in carcinogenesis.[81]

Aged black garlic also employs many of the same modes of anticancer action as fresh garlic extracts. In an *in vitro* study, aged black garlic extracts induced apoptosis in colon cancer cells by inhibiting the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) intracellular signaling pathway. The PI3K/Akt pathway is often activated in cancer cells and is involved in many of the carcinogenic pathways such as tumor cell proliferation and migration. Aged black garlic upregulates phosphatase and tensin homolog (PTEN), which represses the PI3K/Akt intracellular signaling pathway and eventually induces apoptosis by inhibiting cell growth.[82] Figure 4.1 illustrates the dose-dependent manner in which aged black garlic extracts increased the concentration of PTEN.

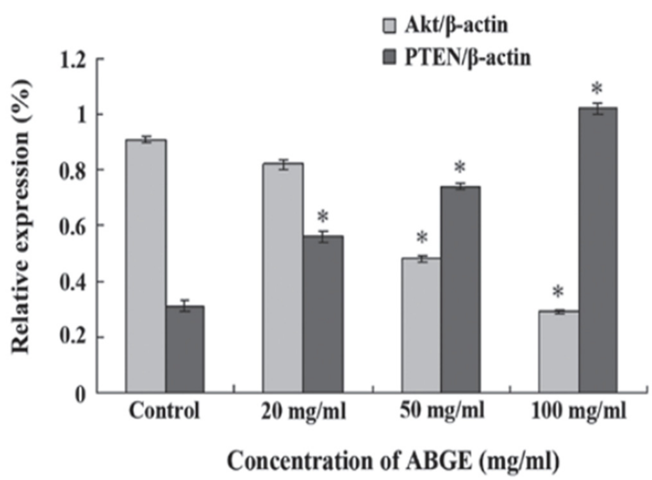


Figure 4.1 Effect of aged black garlic extract (ABGE) on Akt protein and PTEN protein expression in colon cancer cells treated with 20 mg/mL, 50 mg/mL, and 100 mg/mL of ABGE.[82]

Conclusion

Based on the evidence presented in this study, it is evident that garlic and its derivatives possess a number of pharmacological properties. Although only three properties—antimicrobial, cardioprotective, and anticancer—were explored in this paper, the wealth of scientific literature on garlic's beneficial effects points to garlic's immense potential as a pharmacological agent. The organosulfur compounds present in garlic have already been identified as the molecules responsible for many of the effects, but the specific modes of action are still in need of clarification. Based on this analysis, further research is required in order to fully understand the mechanisms of garlic and its derivatives in the body before they are introduced as safe and effective pharmacological agents.

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