



## Medicinal Benefits of Allicin in Black Garlic and Its Potential Impact on Post-Harvest Degradation: A Review

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### ABSTRACT

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Black garlic, a fermented form of garlic, is known for its medicinal benefits, including antioxidant, anti-inflammatory, anti-microbial, and anti-cancer properties. The heating process of making black garlic alters its flavor profile, appearance, and bioactive properties. The paper highlights studies that investigate the potential anti-cancer, antioxidant, anti-inflammatory, anti-microbial, and hepatoprotective properties of black garlic for further research to explore the therapeutic applications of black garlic and its bioactive compounds in various clinical settings as well as the potential impact of allicin during post-harvest degradation. Black garlic is a healthy food product made by heating the entire garlic bulb for several weeks at a temperature ranging from 60°C to 90°C and a humidity of 70% to 90%. Allicin is a sulfur compound naturally present in raw garlic responsible for its pungent odor and taste. During the fermentation process of black garlic, allicin undergoes degradation into various other sulfur compounds, contributing to the transformation of the garlic's flavor, color, and texture. While the degradation of allicin is one of the crucial steps in the development of black garlic's unique characteristics, it can also have implications for the shelf life and post-harvest quality of the product. Other than the enzymatic transformation of alliin into allicin as a key event in the post-harvest deterioration of derived garlic where the release of allicin and its subsequent breakdown products contributes to the development of its characteristic flavors and aromas, temperature, pH, and humidity, can influence the stability of allicin and its breakdown products.

**Keywords:** Allicin, black garlic, medicinal benefits, post-harvest

### Introduction

Commonly used as an ingredient in Asian cuisine, garlic (*Allium sativum* L.) from the family of Alliaceae is frequently used as traditional seasoning and as medication in contemporary medicine. However, due to its unpleasant odor and taste, some find it unacceptable to be consumed raw. Heat treatment could eliminate the disagreeable taste and odor, therefore improving palatability. In order to enhance the flavor and quality of black garlic and give it new functionality, heating treatment has been a common processing method.<sup>1</sup> Black garlic (BG) has captured the interest of the public, particularly researchers and health enthusiasts regarding its therapeutic effect as well as culinary experts on its distinctive traits. BG is a healthy food product made by heating the entire garlic bulb for several weeks at a temperature ranging from 60°C to 90°C and a humidity of 70% to 90%.<sup>2</sup> This transition significantly impacts the flavor profile of the garlic and changes its appearance, giving it a deep black color.

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The bioactive properties of garlic vary as it is heated. Since it is prone to deterioration due to its unstable sulfoxide bond at high temperatures, alliin and deoxidized alliin are broken down into allyl compounds that contain sulfur, and some of these molecules provide a pleasant aroma as they undergo thermal breakdown.<sup>3,4</sup>

For a very long time, it has been utilized in medical treatments, including in ancient Egypt, China, Korea, India, and Japan.<sup>5-8</sup> BG has crossed cultural and geographic barriers to become a global phenomenon, with roots that are deeply ingrained in ancient Asian culinary traditions. In the realms of gastronomy, it offers a remarkable fusion of flavors and textures, tantalizing the palates of garlic's potential health benefits, which have garnered significant attention and triggered extensive scientific investigations into its phytochemical composition and therapeutic properties. According to recent studies, BG and its bioactive components have a range of biological activities and pharmacological characteristics that maintain and enhance their ability to prevent various diseases. Its anti-inflammatory, anti-obesity, hypolipidemic, hepatoprotective, anti-cancer, anti-allergy, immunomodulatory, nephroprotective, cardiovascular, and neuroprotective qualities comprise the majority of these benefits.<sup>3</sup>

Allicin, a sulfur compound in raw garlic, undergoes degradation during the fermentation of BG, transforming into various sulfur compounds that define the product's unique flavor, color, and texture. This degradation is crucial in developing BG's characteristics but can impact its shelf life and post-harvest quality. The breakdown of allicin and the formation of new compounds may influence stability, aroma, and taste during storage. Heat treatment accelerates this breakdown, producing water-soluble bioactive compounds like S-allyl cysteine. The production process involves variables affecting chemical

composition, offering avenues to enhance bioactive qualities and overall product quality. Harvest timing influences alliin content, impacting bioactive potency. Additionally, post-harvest circumstances, such as storage and processing, are vital for maintaining the stability of bioactive compounds. The quality of BG is intricately linked to cultivation methods, garlic genotypes, growing conditions, and processing. While alliin degradation is not directly applicable, preserving the stability of compounds like S-allyl cysteine through proper storage practices ensures BG retains its distinctive characteristics and potential health benefits over an extended period.

## Review Methods

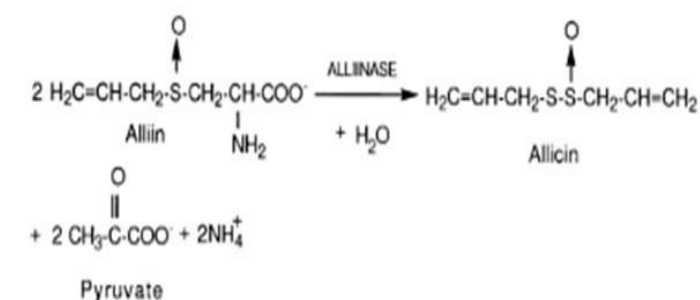
### Methods

PubMed, Scopus, ScienceDirect, and Google Scholar databases were searched for publications from 2005 to 2023. The search terms included the following: “antioxidant”, “alliin”, “harvest,” and “black garlic”. About 100 publications, including journal articles and proceedings, were reviewed, along with publications for which abstracts were accessible.

## Discussion

### Alliin and Alliin

According to Santhosha *et al.*, about 63% of water makes up 28% of fresh garlic; fructans comprise 2.3%, and organosulfur compounds, 2% of which are proteins (alliinase), 1.2% of which are free amino acids (arginine), and 1.5% of which are fiber. The unstable compounds can be oxidized and hydrolyzed because they break down easily to form alliin, which naturally builds up when garlic is stored at a low temperature. The primary sulfur-containing substances found in the bulb are allyl-cysteine sulfoxides and g-glutamyl peptides. The enzyme alliinase transforms alliin, the main allyl cysteine sulfoxide, into alliin when the bulb is processed by slicing, chopping, or squeezing.<sup>5</sup> Alliinase, the enzyme quickly converts cytotoxic cysteine sulfoxides (alliin) into alkyl alkane-thiosulfates like alliin (Fig 1).<sup>10</sup> Alliin is well known for its medicinal benefits, which include antibacterial, anti-hyperlipidemic, anti-tumor, and immunoregulatory activity (Table 2).<sup>4</sup> Garlic particular flavor and taste are attributed by the chemical alliin. BG does not have the same strong off-flavor as fresh garlic because of its lower alliin content, which was converted into antioxidant molecules such as bioactive alkaloids and flavonoid compounds as it aged. This is mentioned by Zhang *et al.* in their research, where at the time of maturity, the alliin content of BG had decreased to approximately 0.2 g/kg.<sup>4</sup> This was significantly lower than the alliin content of fresh garlic, which was approximately 3.45 g/kg. The Maillard reaction involving fructose, fructan, and other carbohydrates likely resulted from heating the garlic to lower the alliin concentration. Another possible explanation for the decreasing amount of alliin in BG is that alliin is transformed during thermal processing into S-allyl cysteine (SAC), S-allylmercapto-cysteine, arginine, and other compounds. Diallyl sulfide, diallyl disulfide, diallyl trisulfide, dithiins, and ajoene are among the compounds produced as soon as alliin and other thiosulfates are broken down (Table 1).<sup>10-13</sup>



**Figure 1:** Conversion of alliin into alliin in BG

### Medicinal Benefits of Alliin

BG has lower amounts of alliin, a substance with anti-inflammatory and antioxidant qualities, than fresh garlic. Still, during the fermentation process, other chemicals, like S-allyl cysteine (SAC), are significantly enhanced. (+) S-(2-propenyl)-L-cysteine sulfoxide (alliin) is the precursor substance of allyl 2-propenethiosulfinate (alliin), also known as diallyl thiosulfate.<sup>1</sup> Sulfur compounds, including SAC, have been found to have antioxidant properties and are regarded as effective scavengers of free radicals.<sup>21-24</sup> Due to their extensive bioactivities, these chemicals have been linked to potential health benefits, such as cardiovascular benefits (lipid management, blood pressure reduction, platelet inhibition, and decreased fibrinolytic activity) and immune system support (antineoplastic and immunostimulant agent).

Besides, alliin, an organosulfur molecule derived from garlic, has been shown in numerous studies to enhance antioxidant activity, induce cell cycle arrest, and encourage tumor cell death. Karnjanapratum *et al.* also support this matter by stating these compounds have a close relationship with the pharmacological and biological characteristics of anti-tumor, anti-cancer, anti-allergic, and hypolipidemic properties in BG due to the heat treatment.<sup>25</sup> Their antioxidant, anti-cancer, anti-inflammatory, anti-microbial, and hepatoprotective properties make them promising subjects of scientific inquiry and hold significant promise in the development of natural remedies and therapeutic agents. They should be explored deeply in various clinical settings, from adjunctive treatments in cardiovascular diseases to cancer prevention and treatment, as their potential role in managing infections and modulating the immune system offers novel approaches to healthcare.

### Anti-cancer

Particularly BG, garlic compounds have been demonstrated to affect a growing array of molecular pathways associated with carcinogenesis, including DNA adduct formation, free radical scavenging, mutagenesis, cell proliferation, differentiation, and angiogenesis. A number of studies published in the past several years have demonstrated the antiproliferative properties of various chemicals produced from BG. One of the components in BG, called organosulfur compounds (OSC), could inhibit the proliferation of cancer cells in a variety of anatomical regions and modify several important components of cellular signal transduction pathways associated with the apoptotic process. One potent ingredient called diallyl sulfide has been shown to both speed up the metabolism of testosterone and prevent oxidative stress brought on by testosterone.

A study conducted by Purev *et al.* showed that BG possessed anti-cancer properties that resulted in the cell viability of breast, gastric, lung, and liver cancer cells.<sup>24</sup> In all cancer cell lines, the 70% and 90% BG extract were found to have a great amount of anti-cancer activity, demonstrating that human cancer, including lung carcinoma epithelial cells line (A549), breast cancer cell line (MCF-7), gastric adenocarcinoma hyper diploid cell line (AGS), and liver cancer cell line (HepG2) were highly susceptible to the dose-dependent effects of the BG. For AGS human gastric cancer cells, one of the most common human malignant tumors, BG decreases MMP-9 (matrix metalloproteinase-9) and MMP-2 activity, as well as cell motility and invasiveness.<sup>25</sup> Park *et al.* mentioned that BG may have therapeutic relevance in the treatment of leukemia, as the hypothesis proposed that it inhibits the development of leukemic cells by causing caspase-dependent apoptosis via both intrinsic and extrinsic pathways.<sup>26</sup> Other than that, Wang *et al.* studied the level of apoptosis in BG *in vitro* and *in vivo*. After being exposed to BG for 24 hours, the morphology of the cells treated with BG shifted from a more elongated fibroblast-like morphology to a round, smaller, and packed form of epithelial cells.<sup>27</sup> This observation suggests that the cells started to inhibit morphological features of apoptosis *in vitro*, which explains why the effects of BG are dose-dependent. Based on the observation of spleen proliferation, the data showed that the tumor growth rate was significantly reduced in mice treated with BG.

Another recent study by Howard *et al.*, Pinto *et al.*, and Sigounas *et al.* mentioned that one of the BG compounds, S-allylmercapto-cysteine (SAMC), has been shown to have anti-cancer effects on bladder, ovarian, liver, and prostate cancers.<sup>28,30,31</sup> It was demonstrated by altering the expression of prostate biomarkers and using testosterone to boost the expression of E-cadherin, proving that SAMC had protective effects against prostate cancer cells. Additionally, SAMC can limit bladder cancer cell survival, invasion, and migration by inactivating the inhibitor of the differentiation-1 pathway and increase mitogen-activated protein kinases (MAPK) inhibitor-induced apoptosis by activating the transforming growth factor-beta (TGF $\beta$ ) signaling pathway.<sup>32,33</sup>

#### Antioxidant

Alliin, a potent sulfur-containing compound found in fresh garlic, exhibits notable antioxidant properties contributing to its potential health benefits. The antioxidant activity of alliin stems from its ability to neutralize free radicals, which are reactive molecules associated with oxidative stress and cellular damage. Alliin's unique chemical structure allows it to act as a scavenger for free radicals, preventing them from causing harm to cellular components. This antioxidant capacity is particularly crucial in mitigating oxidative stress, a process implicated in developing various chronic diseases, including cardiovascular conditions and certain types of cancer. Alliin's antioxidant properties also extend to its ability to enhance the body's natural defense mechanisms, supporting overall immune function. While alliin is known for its characteristic pungency and anti-microbial properties, its role as a powerful antioxidant underscores its potential contribution to maintaining cellular health and preventing oxidative damage in the body.

Alliin, an organosulfur molecule derived from BG, has been shown in numerous studies to enhance antioxidant activity in addition to causing cell cycle arrest and inducing death in tumor cells. One of the primary benefits of alliin and its derivatives in BG is their potent antioxidant activity. A comparison of raw and BG development reveals a possible increase in the total polyphenol content, antioxidant capacity, and total polyphenol index. The process of preparing BG involves heat treatment, causing the unstable compounds to be converted into stable soluble compounds with improved antioxidant properties. According to earlier research by Lee *et al.* on BG, the chemical S-allyl-cysteine, which is generated from alliin, and polyphenol levels have increased, referring to the result from the amount of polyphenols increased between 3-fold and 6-fold in BG cloves, concluding the increased of antioxidant potential.<sup>34</sup> Another study by Kim *et al.* reported that compared to fresh garlic, the total phenolic and flavonoid content of BG was much higher.<sup>35</sup> In particular, the BG extract's polyphenol content increased almost seven times when compared to the fresh garlic extract's, which enhanced the antioxidant activity. Zhang *et al.* also concluded that during the thermal treatment, the antioxidant activities of BG, such as 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH), hydroxyl radical scavenging (HO) and ferric reducing power activities (FRAP) activity, gradually increased.<sup>1</sup> After three days of heat treatment, the DPPH radical scavenging ability rose quickly from 30 to 76%, then grew again to 95% in the next three days, and finally reached a maximum after six days. While the level of HO scavenging activity in fresh garlic was lower than in BG (3.1%), it grew steadily to 30.8% as heating times increased from 0 to 10 days. As for the FRAP value, the reducing power of the fresh garlic samples was 0.08 mmol/L, whereas the BG sample grew gradually to 0.21 mmol/L after ten days of thermal processing.

#### Anti-inflammatory

Alliin and its derivatives' interactions with the body's inflammatory processes play a key role in the relationship between BG's alliin and any potential anti-inflammatory qualities. Although the body uses inflammation as a complex biological response and one of its defense systems, excessive or chronic inflammation can cause several health problems, including chronic illnesses. Many inflammatory diseases, such as atherosclerosis, rheumatoid arthritis, cancer, and allergies, have been linked to the pathogenesis of excessive reactive oxygen

species (ROS) generation, which results in oxidative stress and protein oxidation. Polymorphonuclear neutrophils (PMNs), which are engaged in the host defense response, are among the cells that produce ROS. By oxidizing critical cell signaling proteins like tyrosine phosphatases, ROS causes endothelial dysfunction. Several studies have shown that elevated ROS levels cause inflammation and worsen illness. Antioxidant phytochemicals have been created to treat inflammatory diseases since these compounds are thought to have anti-inflammatory properties.<sup>36-39</sup>

Colorimetric techniques using various substrates are typically utilized to ascertain the anti-inflammatory activities. The evaluation and comparison of various BG properties with fresh garlic included measures such as superoxide dismutase (SOD) activity, ferrous iron chelating (FIC) capacity, ferricyanide reducing power, free radical, and nitrite scavenging activity.<sup>40-43</sup> Among the substances exhibiting anti-inflammatory properties in BG are 5-hydroxymethylfurfural, 2-linoleoylglycerol, and pyruvate. Research by Jeong *et al.* demonstrated that pyruvate can decrease the nitric oxide (NO) that lipopolysaccharide (LPS)-induced RAW264.7 cells produce, release prostaglandin E2 (PGE2), also known as dinoprostone and inhibit the activities of cyclooxygenase-2 inhibitors (COX-2 inhibitor) and 5-lipoxygenase, pro-inflammatory cytokines, and leukotrienes.<sup>40</sup> This concludes that elevated sugar and decreased alliin concentrations after BG digestion are the causes of the low anti-inflammatory effects in LPS-activated RAW264.7 cells. Other than that, 2-linoleoylglycerol derived from BG suppresses the levels of pro-inflammatory cytokines, nitric oxide (NO), and PGE2 in LPS-induced RAW264.7 cells via inhibiting the signaling pathways of mitogen-activated protein kinases.<sup>44</sup> The primary source of tumor necrosis factor-alpha (TNF- $\alpha$ ) is macrophages, but it can also be produced by glial cells, Kupffer cells, T and B lymphocytes, and keratinocytes. TNF- $\alpha$  receptors with high affinity are present in a number of tissues, including the liver, muscles, intestines, and lungs. BG also reduces TNF- $\alpha$ -induced a nuclear factor pathway (NF- $\kappa$ B), a prototypical pro-inflammatory signaling pathway in human umbilical vein endothelial cells (HUVECs), phorbol 12-myristate-13-acetate-induced production in COX-2 and PGE2 through the NF- $\kappa$ B inactivation, and NO and pro-inflammatory cytokines in LPS-induced RAW264.7 cells and in septicemia mice.<sup>45-47</sup>

#### Anti-microbial

The antibacterial properties of BG are attributed mostly to alliin and other sulfur-containing chemicals. Many gram-positive, gram-negative, and acid-fast bacteria, such as *Proteus* species, *Vibrio*, *Staphylococcus*, and *Salmonella*, can be effectively combated by garlic.<sup>48</sup> According to Abubakar, it was found that the aqueous extract of BG can be used in conjunction with conventional antibiotics to combat the agents of nosocomial infections, which are highly common in hospitals.<sup>49</sup> This means that the gram-positive strain of *Staphylococcus aureus* was more vulnerable to the toxic effects of BG than its gram-negative counterparts. The alliin in BG may shield the stomach from the formation of *Helicobacter pylori*, a bacterium that has been directly connected to gastrointestinal cancer. Alliin breaks down further to produce ajoene. Ajoene plays a role in the anticoagulant effects of garlic. It may be the component that prevents clogged blood arteries, which can lead to atherosclerosis. It was discovered by Tian *et al.* that pure alliin exhibited antibacterial efficacy against enterotoxigenic strains of *Escherichia coli* that were resistant to multiple drugs.<sup>58</sup> Although BG's efficacy was lower than that of fresh garlic extracts, it demonstrated antibacterial activity against therapeutically significant pathogens such *Candida albicans*, enterohemorrhagic *Escherichia coli* O157:H7, MRSA (methicillin-resistant *Staphylococcus aureus*), and *Pseudomonas aeruginosa*. Broad antifungal selectivity has been shown for alliin (diallylthiosulfate), produced from the alliin by the garlic enzyme alliinase. Ankri and Mirelman demonstrated that alliin from garlic has antifungal activity, namely against *Candida albicans*, and that alliin and antibody-alliinase conjugates are effective against mouse pulmonary aspergillosis as conducted by Appel *et al.* *in vivo* study.<sup>47,48</sup> An additional *in vitro* investigation demonstrated alliin's inherent antifungal properties and how well it works in combination with

azoles to cure candidiasis.<sup>50</sup> Research examining Amphotericin B's (AmB) efficacy against *Candida albicans* has shown that allicin significantly enhances AmB's efficacy against *Saccharomyces cerevisiae*, *Candida albicans*, and *Aspergillus fumigatus* *in vitro* and *in vivo*.

#### Hepatoprotective

Hepatoprotective activities refer to the capacity of specific compounds, frequently obtained from natural sources, to shield the liver from harm and enhance its general well-being and functionality, which include metabolizing nutrients, detoxifying harmful substances, and maintaining various physiological processes. Besides, hepatoprotective agents aim to support and enhance these functions, particularly in the face of challenges such as oxidative stress, inflammation, and exposure to toxins. Numerous substances exhibiting hepatoprotective qualities have been discovered in BG, distinguished by their anti-inflammatory, detoxifying, and antioxidant qualities. According to Kim *et al.*, the increase in hepatic activities of aspartate aminotransferase, alanine, lactate dehydrogenase, and aminotransferase brought on by alcohol was considerably attenuated by BG.<sup>61</sup> BG treatment significantly decreased the quantity of thiobarbituric acid-reactive chemicals in the liver, heart, and plasma. Antioxidant enzyme activity, including glutathione reductase, glutathione peroxidase, and catalase, as well as the quantity of glutathione in the liver, were significantly elevated. These compounds also could neutralize the damaging effects of free radicals, lessen hepatic tissue inflammation, and encourage the renewal and repair of damaged cells. These kinds of actions are essential for avoiding or treating liver disorders and illnesses.

Chronic diseases related to the liver are often associated with prolonged exposure to risk factors such as excessive alcohol consumption, viral infections (e.g., hepatitis B and C), non-alcoholic fatty liver disease (NAFLD), and certain medications. The liver's continuous exposure to these stressors can lead to chronic inflammation and oxidative damage, eventually resulting in conditions like liver fibrosis, cirrhosis, or hepatocellular carcinoma. Besides, Shin *et al.* hypothesized that BG can provide protection against liver damage brought on by a variety of stimuli.<sup>51</sup> In rats, aspartate transaminase (AST) and alanine transaminase (ALT), indicators of hepatocellular injury, were elevated when exposed to carbon tetrachloride; however, with the presence of D-galactosamine in BG, this outcome is possibly inhibited. This proves that BG therapy inhibited hepatocellular damage. BG can potentially prevent hepatocyte necrosis, which may lessen hepatic damage in chronic liver illnesses. According to a different study by Tran *et al.* BG reduced dyslipidaemia and the rise of ALT and AST levels brought on by chronic carbon tetrachloride (CCl<sub>4</sub>) intoxication.<sup>60</sup> Lee *et al.* demonstrated that BG possesses the hepatoprotective ability to prevent tert-Butyl hydroperoxide (tBHP)-induced effects on rat clone-9 hepatocytes' oxidative stress, inflammation, lipid peroxidation, and cell death as the result showed significant reduction of tBHP induced cell death of rat clone nine hepatocytes (P<0.05).<sup>61</sup>

Therefore, natural compounds with hepatoprotective properties include S-allyl cysteine, found in BG, and various polyphenols, flavonoids, and other bioactive molecules in fruits, vegetables, and herbs. These compounds exhibit hepatoprotective effects and contribute to overall health, potentially reducing the risk of developing chronic diseases.<sup>49,50</sup>

#### Stability of Allicin

Other than the enzymatic transformation of alliin into allicin as a key event in the post-harvest deterioration of derived garlic where the release of allicin and its subsequent breakdown products contributes to the development of its characteristic flavors and aromas, temperature, pH, and humidity, can influence the stability of allicin and its breakdown products.

#### Temperature

The fermentation process of BG involves exposing fresh garlic bulbs to controlled conditions of heat and humidity for an extended period.

This process transforms alliin into various bioactive compounds since it is popularly known as an unstable component, including S-allyl cysteine. Higher temperatures can accelerate the degradation of certain sulfur-containing compounds in BG or may lead to chemical reactions that result in the breakdown or alteration of the compounds formed during fermentation, potentially affecting the flavor and nutritional profile of BG. Excessive heat may contribute to the loss of volatile compounds responsible for the characteristic aroma. Since the environment that allicin is in has an impact on its stability, it is easily transformed into different compounds after it is produced and is subjected to unfavorable temperatures or solutions.<sup>62</sup> Research stated that chemical and biological experiments showed that allicin extracts are unstable and rapidly deteriorate at high temperatures.<sup>63,64</sup> According to Wang *et al.* in a study of allicin stability, the assessed concentrations of 9.03, 6.02, and 3.01 mg/L at different storage temperatures (-20, 4, 25, 40, 55, 70, and 80°C), allicin was unstable for at least 20 days at 4°C and 40 days at -20°C with no obvious degradation, but it degraded rapidly at temperatures above 40°C and much more swiftly at temperatures over 70°C. At a temperature over 40°C for 120 hours (5 days), allicin entirely decomposed.<sup>65</sup>

#### pH

The pH stability of allicin during the post-harvest process is a critical factor that influences its preservation and bioactivity. Allicin, a sulfur-containing compound found in raw garlic, is known for its pungent odor and taste. However, during post-harvest processing, particularly in the fermentation of BG, allicin undergoes degradation into various sulfur compounds, transforming flavor and other characteristics. The stability of allicin is influenced by the pH conditions during this process. Allicin is known to be more stable in neutral environments and less stable in acidic and alkaline conditions. All three concentrations of 9.03, 6.02, and 3.01 mg/L of allicin demonstrate by Wang *et al.* a consistent trend in terms of stability in various pH solutions.<sup>65</sup> Allicin stability in an aqueous extract was highest in solutions with a pH of 5-7, where no apparent degradation occurred over a period of five days. In solutions with a pH of more than 11 or less than 1.5, the stability drastically decreased, and in those cases, complete breakdown was observed in two hours. Besides, Freeman and Kodera concluded that allicin was highly sensitive to pH because of its great sensitivity to low pH, which supported a previous theory that allicin finds it difficult to stay intact in the stomach, where pH is typically around 2.<sup>66</sup>

In summary, while the pH stability of allicin is more unstable during post-harvest, the overall pH conditions during the fermentation process and subsequent storage of BG can influence the stability of the sulfur-containing compounds formed during this transformation. Therefore, the pH levels in the post-harvest environment play a crucial role in determining the rate of allicin breakdown and the formation of new compounds, as well as controlling the quality and properties of the final product. It is particularly relevant during the fermentation of BG, as maintaining optimal pH levels contributes to the stability of the transformed sulfur compounds, including S-allyl cysteine.

#### Humidity

One of the major issues during the fermentation process is a substantial loss of color, appearance, flavor, taste, and chemical components. Once the fermentation process is complete, the BG bulbs must be stored to maintain quality. Storage conditions play a significant role in preserving the flavor and health-promoting compounds of BG. BG is sensitive to environmental factors, including moisture. Another element that may affect the stability of the bioactive substances in BG is moisture. Excess moisture can contribute to the growth of mold or undesirable microorganisms, leading to spoilage. Heat treatment causes the moisture content to drop, extending the garlic's shelf life.<sup>67</sup> Moist conditions may also trigger chemical reactions that alter the composition of compounds in BG, potentially affecting its quality. At approximately 40% moisture content, it appears that BG could be somewhat drier and have less elasticity. BG gets too difficult to consume when the moisture content drops below 35%.<sup>4</sup> Heat and storage duration are both considered to be the circumstances that are specifically present during the manufacture of

BG from fresh garlic, which are the primary factors that affect the breakdown of ascorbic acid.

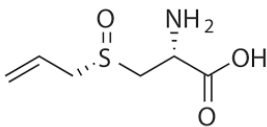
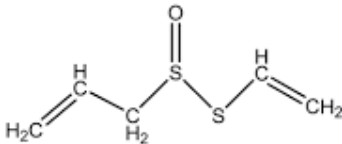
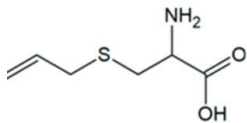
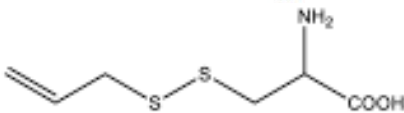
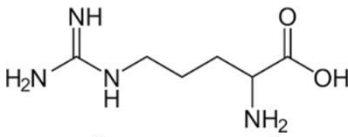
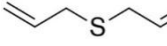
#### Role of allicin in post-harvest deterioration

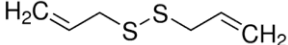
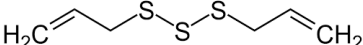
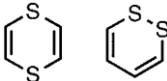
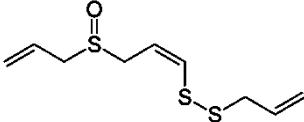
Alliin is a sulfur compound naturally present in raw garlic and is responsible for its pungent odor and taste. During the fermentation process of BG, alliin undergoes degradation into various other sulfur compounds, contributing to the transformation of the garlic flavor, color, and texture. While the degradation of alliin is one of the crucial steps in the development of BG's unique characteristics, it can also have implications for the product's shelf life and post-harvest quality. The breakdown of alliin and the formation of new compounds may impact the garlic's stability, aroma, and taste during storage. High molecular weight polysaccharides were broken down more quickly by heat treatment into low molecular weight oligosaccharides and monosaccharides, which in turn created water-soluble bioactive compounds, including polyphenol, tetrahydro-b-carbolines, alkaloids, and substances that resembled flavonoids.<sup>13,66</sup> The production process involves a number of variables that impact the chemical composition of BG; as a result, it may be a helpful way to improve not just the bioactive qualities but also the finished product quality. According to Bloem *et al.* during the growing season, there is a rising tendency in the translocation of alliin from leaves, where its production occurs, to bulbs.<sup>67</sup> Although alliin accumulates in leaves during the early stages of growth, alliin and its precursors are transferred from leaves to bulbs when bulb formation begins, and the plant gets closer to the harvesting stage. Therefore, late harvesting might be employed as a method to increase the alliin content, which would then boost the bioactive potency of the finished product.

Conversely, Horníková *et al.* conducted a study exploring the total sulfur-containing amino acid content in various garlic genotypes and their sulfoxide content changes during storage.<sup>68</sup> They observed a significant increase in the content of S-alk(en)ylcysteine sulfoxides.

This increase was attributed to the conversion of c-glutamyl dipeptides into sulfoxides rather than being linked to water loss. Although isoalliin is a minor constituent of garlic, it holds substantial technological importance, as it serves as a trigger and precursor for the development of unwanted blue or blue-green discoloration in many commercial garlic products. Consequently, it is essential to process garlic promptly after harvesting to prevent this discoloration. Since these amino acids function as precursors for a variety of physiologically active chemicals, there are circumstances in which larger amounts of free S-alk(en)cysteine sulfoxides are advantageous, notwithstanding these potentially negative impacts on the food and pharmaceutical industries.<sup>68</sup> Therefore, the quality of BG, as reflected by its chemical composition and the content of bioactive compounds, is intricately linked to post-harvest circumstances. Emphasis should be placed on the goal of attaining optimal quality by carefully considering cultivation methods, selecting suitable garlic genotypes, and ensuring favorable growing conditions. Furthermore, the processing phase requires meticulous attention since the organosulfur compounds responsible for BG's beneficial properties are notably unstable and susceptible to the various treatments applied during processing. In summary, while alliin degradation is not directly applicable to BG, the post-fermentation stability of compounds like S-allyl cysteine can be influenced by storage conditions. Proper storage practices are essential to prevent undesirable changes in BG flavor, aroma, and nutritional content, ensuring that it maintains its distinctive characteristics for an extended period. To preserve the quality of BG, it is typically recommended to store it in a cool, dry place in order to avoid direct exposure to sunlight and prevent moisture buildup so that the stability of the bioactive compounds formed during fermentation is maintained and preserved. This will ensure the shelf life of BG is extended while retaining its unique flavor and potential health benefits.

**Table 1:** Molecular structure of BG compound

Compound	Molecular structure	Function	References
Alliin		Act as a precursor to allicin	14
Allicin		Scavenge free radicals and inhibition of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase.	15,16
S-allyl cysteine (SAC)		Capture ROS, including O <sub>2</sub> <sup>-</sup> and H <sub>2</sub> O	16–18
S-allylmercapto-cysteine		Help neutralize free radicals in the body, which are unstable molecules that can cause cellular damage and contribute to various chronic diseases.	18
Arginine		An amino acid that helps the body build protein	19
Diallyl sulphide		Induce apoptosis and inhibit cells in the G2/M phase to suppress the growth of cells.	19

Diallyl disulphide		Able to suppress chemically induced carcinogenesis in rodents as well as regulate carcinogenesis in a variety of organs, including the colon	19
Diallyl trisulfide		Increase the levels of brain-derived neurotrophic factor (BDNF) and nuclear factor erythroid 2-related factor 2 (Nrf2) to reduce neuropathic pain	20
Dithiins		Phytochemicals help to keep blood pressure, cholesterol, and triglyceride levels down, as well as improve overall wellness.	21
Ajoene		Hydroxyl radical scavenging activity, the inhibitory effect of nitric oxide formation	22

## Conclusion

Through its antioxidant, anti-inflammatory, anti-microbial, immunoregulator, and hypolipidemic properties, BG has demonstrated a variety of therapeutic benefits in multiple studies, including anti-diabetic, anti-cancer, anti-obesity, immunomodulatory, cardioprotective, hepatoprotective, and neuroprotective effects. In conclusion, allicin, a key sulfur compound in raw garlic, undergoes a significant transformation during the fermentation process of BG, contributing to its unique flavor, color, and texture. This transformation, while essential for creating the distinctive characteristics of BG, can also impact its shelf life and post-harvest quality. Heat treatment during processing can further alter the chemical composition, yielding water-soluble bioactive compounds. The cultivation practices, genotype selection, and growing conditions play a critical role in determining the quality of garlic, both pre-and post-harvest. Additionally, the pH and humidity can influence the alliin content and, consequently, the bioactive potency of the final product. Thus, ensuring the quality of BG requires a holistic approach, considering all these factors in cultivation, processing, and post-harvest handling.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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**Table 2:** Anti-cancer, antioxidant, anti-inflammatory, anti-microbial and hepatoprotective properties of BG

Properties	Method	Procedure	Result	Reference
Anti-cancer	MTT assay	In RPMI 1640, $5 \times 10^6$ cells/mL of lymphocytes were seeded in 96-well plates along with samples and mitogens like LPS (B lymphocyte mitogen, 1 $\mu$ g/mL) and Con A (T lymphocyte mitogen, 3 $\mu$ g/mL). The cells were then cultivated for 44 hours. Each culture well was then filled with 5 mg/mL MTT solution.	The 70% and 90% BG extracts were shown to have significant anti-cancer activity in all cancer cell lines, showing that the BG's dose-dependent effects were extremely susceptible to the human cancer A549, MCF-7, AGS, and HepG2 cells.	24
		U937 cells were subjected to different BG sample concentrations for a duration of 24 hours or 10 $\mu$ g/ml for the specified durations.	BG has a cytotoxic effect on U937 cells, as evidenced by the time- and concentration-dependent declines in the viability of BG-treated cells.	26
		In 96-well plates, cells were seeded, and 24 hours later, SAMC was added. Following SAMC therapy, cell viability was assessed every 24 hours for four days. Prior to testing, cells were cultured for five hours at 37°C with the addition of 5 mg/ml MTT solution.	Similar levels of cell proliferation suppression across all three cell lines indicate that SAMC has a comparable impact on androgen-dependent and independent prostate cancer cells.	29
Caspase activity assay		The lysis buffer was used to lyse the harvested cell pellets, and the supernatants were then collected. Equal amounts of protein were incubated with reaction buffer and colorimetric substrate, acetyl (Ac)-Ile-Glu-Thr-Asp (IETD) p-nitroaniline (pNA) for caspase-8, Ac-Leu-Glu-His-Asp (LEHD)-pNA for caspase-9, and Ac-Asp-Glu-Val-Asp (DEVD)-pNA for caspase-3, respectively, at 37°C for 2 h in the dark.	The cleaved levels of caspase-9, -8, and -3 were elevated by BG in a concentration-dependent manner. In cells treated with BG, there was also a notable concentration-dependent increase in cleaved poly (ADP-ribose) polymerase (PARP), a substrate of caspase 3.	26
Matrix metalloproteinases (MMPs) gelatine zymographic analysis		50 $\mu$ g of soluble and precipitated stomach tissue proteins were separated electrophoretically on gels, which were then incubated for 1 h at room temperature in renaturing buffer and 16 h at 37°C in the developing buffer. The developing solution was mixed with a particular protease inhibitor to halt the reaction.	BG decreases MMP-9 (matrix metalloproteinase-9) and MMP-2 activity, as well as cell motility and invasiveness. An increase in transepithelial electrical resistance served as evidence that the inhibitory effects of ABG on cell motility and invasiveness were linked to a tighter tight junction (TJ).	25
Apoptosis of SGC-7901 cells.		SGC-7901 cells were cultivated and subjected to varying BG doses in a medium containing	24 hours after exposure, the BG-treated cells' morphology altered from that of elongated	27

		10% fetal bovine serum at 37°C in an incubator that was humidified and included 5% CO <sub>2</sub> .	fibroblast-like cells to that of spherical, smaller, and packed epithelial cells, suggesting that SGC-7901 cells started to show signs of apoptosis.	
	Colony forming assay	After 24 h, 300 cells per well were treated concurrently with medication and vehicle control. Colonies with more than 50 cells were counted after seven days.	The addition of SAMC to the docetaxel-treated cells resulted in a dose-dependent decrease in PC3 cells' capacity to form colonies, indicating an additional inhibitory action of SAMC on docetaxel. When compared to single agent treatment, SAMC enhanced the efficacy of docetaxel on colony formation inhibition by 9–50%.	29
	HRPC CWR22R nude mice mode	The mice underwent scrotal castration and were subsequently implanted in the flank after 48 h. The 100 µL of tumor suspension contained a 1:1:1 ratio of the mechanically minced tumor, RPMI, and unilateral Matrige, which was inserted through a tunneled 23G needle while the mice were under general anesthesia.	Docetaxel with SAMC together had a 53% greater potency than docetaxel alone.	29
	Flow cytometric analysis	Following the simultaneous addition of SAMC, docetaxel, and SAMC + docetaxel to adherent cells in fresh RPMI, adherent and floating cells were produced for study after a 24-hour period.	Docetaxel-induced G2/M phase cell cycle arrest and apoptotic induction were facilitated by SAMC.	29
	Western blot	For SDS-PAGE, cell lysate protein from adherent and floating cells was loaded onto 10-12% polyacrylamide gels and then placed onto a polyvinylidene difluoride membrane. Membranes were blocked overnight in 10% non-fat milk and incubated for 1 hr at room temperature with primary antibodies: Actin, Bcl-2, Bax, Cleaved Caspase 3, PARP.	SAMC encouraged the induction of apoptosis and G2/M phase cell cycle arrest caused by docetaxel.	29
Antioxidant	1,1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging activity	0.5 mL of the sample was combined with 2.5 mL of the 0.2 mM DPPH ethanol solution.	After three days of heat treatment, the DPPH radical scavenging ability rose quickly from 30 to 76%, then to 95% in the next three days, and finally reached its peak after six days.	1
		80 mL of a 0.02 mM 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) methanol solution was	The DPPH radical scavenging activity of the 70% ethanol extracts was higher than that of the	24



		mixed with 20 mL of the sample solution.	90% ethanol extracts, and the 70% BGE extract exhibited the highest scavenging activity of any extract tested.	
		Both the BG extract and the ascorbic acid standard solution were serially diluted at seven different concentrations (400 to 6.25 µg/mL). Equal volumes of freshly made 0.004% (w/v) DPPH methanolic solution were combined with 1 mL of each concentration.	After comparing the aqueous extract's concentration to standard ascorbic acid at 400 µg mL <sup>-1</sup> (72.57%), the percent inhibition of DPPH scavenging was found to be 65.51%	53
Ferric reducing power activities (FRAP)		The FRAP solution was heated to 37°C, and 0.3 mL of the sample was combined with 2.7 mL of the heated FRAP solution.	After 10 days of thermal processing, the BG samples' reducing power steadily increased to 0.21 mmol/L.	1
		2.5 mL each of 200 mM sodium phosphate buffer (pH 6.6) and 1% potassium ferricyanide were combined.	The reducing power was higher for BG and 70% ethanol extract.	24
		To 2.5 mL of phosphate buffer (0.2 M, pH = 6.6) and 1% potassium ferricyanide. [K <sub>3</sub> Fe(CN) <sub>6</sub> ]. 1 ml of each sample concentration was added. After adding 2.5 mL of 10% trichloroacetic acid (TCA) to the reaction mixture, the mixture was centrifuged for 10 min at 3000 rpm.	Higher reducing powers were shown by the aqueous extract and standard ascorbic acid, which increased in a concentration-dependent linear pattern.	53
Hydroxyl Radical scavenging (HO RSA)		10 mmol/L of phosphate-buffered saline (pH 7.4), 1 mL of safranin T solution (40 µg/mL), 1 mL of H <sub>2</sub> O <sub>2</sub> (3%), and 1 mL of EDTA-Na <sub>2</sub> -Fe (II) (0.15 mol/L) were mixed with 0.2 mL of sample	As heating duration increased from 0 to 10 days, BG's capacity to scavenge HO rose steadily to 30.8%.	1
Total and oxidized glutathione (GSH + GSSG)		GSH was conjugated with 2-vinylpyridine and extracted from the mixture in order to measure the GSSG level.	Allicin exposure causes endothelial cells to produce more cellular glutathione. After 28 hours of treatment, allicin increased the glutathione level up to eight times at a dose of 10–20 µM in a concentration- and time-dependent way.	54
Trolox equivalent antioxidant capacity (TEAC) assay		After one week of adaption, three-week-old mice were fed with 5% freeze-dried garlic or aged BG for 7 weeks.	TEAC values of garlic and aged BG were 13.3 ± 0.5 and 59.2 ± 0.8 mol/g wet weight, respectively. <i>In vitro</i> and <i>in vivo</i> , BG exhibits higher antioxidant activity than garlic.	34

	Total polyphenol content (TPC)	A 1/10 dilution of Folin-reagent Ciocalteu's in water, containing 5.0 mL, was placed into separate tubes with 1.0 mL of the diluted sample. Afterward, 4.0 mL of a 7.5% w/v sodium carbonate solution was added.	When compared to fresh garlic, the TPC in BG rose by a factor of 4–10.	35
		9 mL of distilled water was mixed with 1 mL of the sample (100 µg/mL) or standard gallic acid solution (6.25 to 200 µg/mL), and then 1 mL of Folin-reagent Ciocalteu's was added (diluted 10 times). 5 min later, 10 mL of 7% Na <sub>2</sub> CO <sub>3</sub> and 25 mL of distilled water were added.	A significant quantity of phenolic acid (306.77 ± 1.53 mg GAE/g dry extract) was observed in the extract, suggesting that the polyphenolic metabolites obtained from BG have a strong antioxidant effect.	53
	Total flavonoid content (TFC)	The sample was mixed with 20 mL of 80% methanol, allowed to extract for 2 h at room temperature, and centrifuged for 15 min at 18,000 rpm. 80% methanol was used to make an extract that filled a 100 mL container. After removing 0.5 mL of it, 0.5 mL of a 2% ethanolic AlCl <sub>3</sub> solution was added.	The TFC in BG was approximately 1.1–1.5 times higher than that of fresh garlic.	35
		After adding 1 mL of BG extract (100 µg/mL) and 5 mL of distilled water, 5% NaNO <sub>2</sub> (0.3 mL) was mixed and preserved for 5 min. Afterward, the reaction mixture was combined with 0.6 mL of 10% AlCl <sub>3</sub> and 2 mL of 1 M NaOH, and it was left to incubate for 5 min at room temperature.	The extract demonstrated a notable concentration of flavonoids (252.22 ± 1.09 mg QE/g dry extract), demonstrating the potent antioxidant potential of BG in the aqueous extract.	53
Anti-inflammatory	Lipopolysaccharide-induced inflammatory response model	AGE-1 and AGE-2, cell viability, as well as nitric oxide, prostaglandin E <sub>2</sub> , and pro-inflammatory cytokine [interleukin-6 (IL-6), TNF-α, and IL-1β] levels were measured. Reverse transcription polymerase chain reaction and western blotting were used to determine the mRNA and protein expression levels of cyclooxygenase-2 and inducible nitric oxide synthase.	Prostaglandin E <sub>2</sub> and nitric oxide levels dropped significantly as AGE-1 concentration increased (IC <sub>50</sub> = 1.41 µg/mL and 29.6 µg/mL, respectively), whereas AGE-2 levels did not. AGE-1 treatment also reduced the expression of cyclooxygenase-2 and inducible nitric oxide synthase mRNA and protein, and inducible nitric oxide synthase secretion in a dose-dependent manner. These results suggest that BG may have a significant impact on inflammatory factors and may be a useful anti-inflammatory therapeutic agent.	44
	Hypotonicity-induced	Using a sterile washing buffer, the	Compared to normal aspirin (92.23%), the	53

	hemolysis assay	precipitated RBC portion was centrifuged three times for five minutes at 2500 rpm (0.9% NaCl). A 10 mM phosphate buffer solution was used to create 10% (v/v) RBC. Aspirin was used as the standard, and the samples were serially diluted to create aliquots of 5 different concentrations (ranging from 25 to 400 µg/mL) in phosphate buffer solution. Next, a 2 mL Eppendorf tube was filled with 600 µL of isotonic solution, 600 µL of sample or standard, and 600 µL of RBC suspension.	aqueous extract's 400 µg/mL <sup>-1</sup> concentration resulted in a 71.70% suppression of hemolysis. With an IC <sub>50</sub> of 147.59 ± 2.98 µg/mL <sup>-1</sup> , the extract showed a significant inhibitory effect, while aspirin had an IC <sub>50</sub> of 59.08 ± 3.02 µg/mL <sup>-1</sup> .		
	15-Lipoxygenase (15-LOX) inhibition assay	0.2M borate buffer was used to reconstitute 975 µL of 15-LOX solution (3000 U/mL) at pH 9.0. was combined with 25 µL of one of the following concentrations: blank (0.1 M PBS solution), standard (Quercetin), or sample extract (50 to 400 µg/mL). To start the enzymatic reaction, each tube was mixed with 517.5 µM linoleic acid substrate solution (reconstituted with 0.2 M borate buffer at pH 9.0).	As the aqueous extract was concentrated to 400 µg/mL <sup>-1</sup> , the maximum percentage of inhibition was 72.46% when compared to conventional quercetin (96.76%). The aqueous extract demonstrated substantial inhibitory activity with an IC <sub>50</sub> of 250.05 ± 8.48 µg/mL <sup>-1</sup> , whereas Quercetin possessed an IC <sub>50</sub> of 19.62 ± 6.52 µg/mL <sup>-1</sup> .	53	
Anti-microbial	Antibacterial assays	20 g of BG were dissolved in 10 ml of solvent to reconstitute them. Each of the 50 mg/ml crude extracts was pipetted into 500 µl holes bored into the agar. The negative controls were 500 µl of each pure solvent, while the positive control was 500 µl of an antibiotic solution containing 50 mg/ml of metronidazole. After 1 h of drying on a level bench, the plates were incubated for 18 h at 37°C.	<i>S. aureus</i> (NSA1) was most susceptible to the active principles present in garlic, closely followed by <i>S. pneumoniae</i> (NSP1)	49	
	Kirby-Bauer method	diffusion	Blood Agar Plate (LAD) media is used for <i>Streptococcus pneumoniae</i> media, and Muller Hinton Agar (MHA) media is used for the bacteria <i>Klebsiella pneumoniae</i> . Amoxicillin was the positive control, and DMSO was the negative control. At 37°C, the agar medium was incubated for 24 to 48 h.	When compared to the ethanol extract of a single garlic clove, the BG extract showed a greater inhibitory zone average value on the development of <i>S. pneumoniae</i> and <i>K. pneumoniae</i> .	55
			The media plate was divided into four	The antibacterial activity of single BG is higher	56

		sections: part 1 and part 2 were filled with dish samples of garlic or BG extract; part 3 was the antibiotic ciprofloxacin as a positive control; and part 4 was dimethyl sulfoxide as a negative control (DMSO). The agar medium was incubated for 24-48 hours at 37°C.	against <i>Streptococcus pyogenes</i> , as evidenced by the statistically significant difference between the antibacterial activity of garlic and BG ethanol extract on the growth of <i>Streptococcus mutants</i> and <i>Enterococcus faecalis</i> with P values of 0.001 and 0.03 respectively (P < 0.05).	
	<i>In vitro</i> anti-MRSA activity (Methicillin-resistant <i>Staphylococcus aureus</i> )	Mice were infected by injecting 200 µL of MRSA-PBS solution, which contained 107 cfu, via the tail vein. Garlic extract at 100% and 50%, diallyl sulfide (DAS) at 10% and 5%, and diallyl disulfide (DADS) at 1% and 0.5% were utilized.	Garlic extract and two diallyl sulfides taken orally dramatically reduced MRSA viability in plasma, liver, kidney, and spleen (P < 0.05). The levels of fibronectin and interleukin-6 in the plasma of MRSA-infected mice were dramatically elevated (P < 0.05) by MRSA infection; on the other hand, the levels of these substances were significantly decreased (P < 0.05) upon oral administration of garlic extract and two diallyl sulfides (DAS).	58
	<i>in vitro</i> growth of <i>Helicobacter pylori</i> (Hp)	The sample was rolled onto a slide for Gram staining after being streaked over a Pylori-agar plate. For 7 days, the plate was incubated at 37°C with microaerophilia.	The substances exhibit a synergistic effect on the suppression of Hp <i>in vitro</i> growth.	57
Hepatoprotective	CCl <sub>4</sub> or D-galactosamine-induced liver injury model	For seven days, SD rats were given an oral pre-treatment consisting of either saline as the carrier or the relevant dosages of ABG (100 or 200 mg/kg). To cause liver damage, either D-galactosamine (400 mg/kg in saline) or CCl <sub>4</sub> (20% in olive oil, 2 ml/kg) was administered orally to 16-hour-fasted SD rats.	Rats given ABG treatment prevented the concentration-dependent rise in liver weight caused by CCl <sub>4</sub> . Additionally, CCl <sub>4</sub> treatment markedly increased the level of AST and ALT, a marker of hepatocellular damage, which was dose-dependently decreased by BG administration. When D-galactosamine was given to SD rats, their liver weight significantly increased, which was in line with the findings of the CCl <sub>4</sub> -induced liver damage model.	51
		After intraperitoneal injection of CCl <sub>4</sub> to induce AHI, the mice in the control, CCl <sub>4</sub> , silymarin, and BG groups were given distilled water, silymarin, and various fraction extracts of BG orally.	The hepatoprotective effect of BG's n-butanol layer extract (BA) and water layer extract (WS) was demonstrated by the reduction of hepatic malondialdehyde (MDA), alanine aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) levels.	58

	60 adult male Wistar rats were divided into three groups: Group 1 received 0.9% isotonic saline, Group 2 received 1.9mg/kg CCl <sub>4</sub> mixed with olive oil, and Group 3 received 100mg/kg BG + CCl <sub>4</sub> . The rats were killed after four weeks, and the liver tissue was embedded in paraffin after being fixed in 10% formaldehyde. Tissue sections with a thickness of 5μ were stained with hematoxylin and eosin and evaluated using light microscopy.	There was a significant difference (p=0.001) in the liver's biochemical and histological results between the BG+ CCl <sub>4</sub> and control groups. When comparing CCl <sub>4</sub> groups to controls and BG+ CCl <sub>4</sub> groups, there was a substantial alteration in liver enzymes and histology (p=0.001).	59
	Four groups of mice were created at random: extract control, CCl <sub>4</sub> intoxication, control, and administrated CCl <sub>4</sub> and extract group. In order to develop chronic liver injury mode, mice were administered a dose of 1 ml/kg body weight of CCl <sub>4</sub> orally twice a week for 28 days. Additionally, for 30 days, mice were cotreated with CCl <sub>4</sub> and 200 mg/kg body weight of single bulb BG extract via gastric gauge.	BG ameliorated dyslipidemia and the elevation of ALT and AST levels induced by chronic CCl <sub>4</sub> intoxication	60
High-fat diet-induced liver steatosis and injury model-induced hepatic injury	A control diet was given to the mice, while the other mice were given a diet heavy in fat. In five days, a week for 4 weeks., patients received 100, 200, or 400 mg/kg of BG.	The effects of BG therapy on the rise in body weight caused by HFD were significant. Aspartate transaminase (AST) and alanine transaminase (ALT), two indicators of hepatocellular injury, were up-regulated by HFD, and their plasma levels decreased considerably with BG treatment.	51
Ethanol-induced oxidative liver damage model	Rats were divided into three groups: a saline (WT) group, an ethanol (ET) group (15 mL=kg of body weight 20% [wt=vol] ethanol), and an ethanol + ABG (ET +ABG) group (ethanol + 100 mg=kg of body weight ABG).	Administering BG resulted in decreased liver weights, a drop in the epididymal and total fat pad (P<.05), a reduction in the evident fatty alterations surrounding the portal triad, and a decrease in the accumulation of fat in the liver. Alkaline phosphatase, lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase all showed a marked reduction in their hepatic activity when exposed to BG.	61

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