

# Biomedical Effects of Garlic

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Garlic is a perennial plant of the amaryllis family that produces strong-smelling pungent bulbs from a strong tall stem of 25–70 cm and can be grown in mild climates. Garlic is commonly used as a spice in cooking and in herbal medicine.

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## 1. Botanical Description

Central Asia is considered the home of garlic (*Allium sativum*), a member of the Amaryllidaceae family, even though it has been farmed for a long time worldwide. Garlic bulbs are composed of 10–20 cloves, and those who have flowers are hermaphrodites (some varieties do not produce flowers) <sup>[1]</sup>. Its leaves and cloves have been used as a spice, food additive, and in traditional medicine for a long time <sup>[2]</sup>. Garlic has two major subspecies: hardneck (produces flower stalks and results in a bulb circle of 6–11 cloves) and softneck (produces no flowers, and the bulb circle can result in 24 cloves <sup>[3][4]</sup>). Garlic's cultivars are divided into eight subtypes (rocamboule, marble purple, purple stripes, porcelain, glazed purple stripe, Asiatic, creole, and turban) for hardneck and into two subtypes (artichoke and silverskin) for softneck <sup>[4]</sup>. Alliums, such as garlic, produce a pungent odor when crushed. Interest in the potential benefits of this plant originates in antiquity (up to 5000 years ago). It is one of the earliest documented examples of plants used for health maintenance and treatment of disease <sup>[5]</sup>.

## 2. Phytochemicals

Garlic has various phytoconstituents, including alkaloids, saponins, flavonoids, tannins, phenolics, terpenoids, and organosulfides <sup>[6]</sup>. In addition, garlic is considered a good source of vitamins and minerals, including vitamin B1, B6, C, manganese, copper, phosphorus, selenium, and calcium <sup>[7]</sup>. Garlic's main phytochemicals are organosulfides (sulfur-containing compounds), including allicin, alliin, ajoenes (E-ajoene and Z-ajoene), sulfides (diallyl sulfide, diallyl disulfide, diallyl trisulfide), 2-Vinyl-4H-1,3-dithiin, and allyl methyl sulfide <sup>[8]</sup>. These organosulfides are produced in garlic cloves <sup>[6]</sup>. Allicin is the primary bioactive phytochemicals present in the aqueous extract of garlic and is also responsible for the characteristic odor of garlic <sup>[3]</sup>. Thus, enzyme alliinase converts allicin to alliin when the garlic cloves are sliced/crushed <sup>[9][10]</sup>. For this reason, several studies have shown that crushed fresh garlic can deliver most of its active phytochemical <sup>[8][11][12]</sup>. As allicin is chemically unstable, it rearranges into the stable phytochemical ajoene (E- and Z-) <sup>[13]</sup>. Allyl sulfides are most often found in garlic oil, and vinyl-4H-1,3-dithiin is mainly found in stir-fried garlic and garlic oil <sup>[14][15]</sup>.

## 3. Biomedical effects

### 3.1. Anticancer

Interestingly, phytochemicals such as garlic-derived allicin have been combined with commonly used anticancer drugs to enhance the therapeutic effect of current treatments. For example, an experiment performed by Tigu et al. showed that a combination of the anticancer drug, 5-fluorouracil with allicin, hindered colorectal (DLD-1) and lung cancer (SK-MES-1) cell migration and proliferation in vitro <sup>[16]</sup>. Petrovic et al. studied the effectiveness of intraperitoneal injections of ethanolic homemade garlic extract against an aggressive breast cancer tumor in BalB/c mice. The results showed that, after 28 days of treatment, cancer growth was delayed by 30% compared with untreated mice <sup>[17]</sup>. In another study, Tanaka et. al, led a randomized double-blinded study on 51 patients with colorectal adenomas that utilized high-aged garlic extract (2.4 mL/day) and low-aged garlic extract (0.16 mL/day) for 12 months. At least one adenoma decreased by 50% (>6 months of uptake) in the high-aged garlic extract group, while there was no decrease in the low-aged garlic extract group <sup>[18]</sup>. Finally, a recent meta-analysis of epidemiological articles using a total of 11 clinical trials and 12,558 cases concluded that garlic intake could reduce the risk of colorectal cancer <sup>[19]</sup>, coinciding with previous studies <sup>[20]</sup>, while another previous meta-

analysis limited to men showed no correlation [21]. These studies show that broader investigations with increased sample size are necessary to clarify the result discrepancies from several epidemiological studies.

### 3.2. Antioxidant

Garlic's phytochemicals also promote an antioxidant effect. The antioxidant properties of garlic might be associated with two of its main phytochemicals, alliin, and allicin. Bhatt and Patel et al. prepared 900 mg of cooked versus raw garlic and incubated these samples with gastric enzymes. These results showed that cooked garlic lost 90% of phenolic content, leading to less antioxidant activity due to heat (evaporation of active compound) than raw garlic [22]. Lei et al. demonstrated that the scavenging activity of black fermented garlic ethanolic extract is concentration-dependent. This study also showed that this garlic extract increased the mean longevity of flies (*Drosophila melanogaster*) compared to controls [23]. In a more translational scenario, a randomized, double-blind clinical trial on seventy women with rheumatoid arthritis evaluated the effects of garlic in pain mitigation. Patients received 1000 mg of garlic for a total of 8 weeks. Results showed that pain after activities decreased in the garlic group compared to the placebo. This effect from garlic was attributed to a decrease in oxidative stress, a common feature in this disease [24]. However, there are mixed results in the literature about the oxidative stress reduction mechanism.

### 3.3. Antimicrobial

#### 3.3.1. Antiviral

Several studies have shown the antiviral effect of garlic. Pre-clinical studies elucidated that garlic and its organosulfides phytochemicals have great activity against several human and animal viruses by inhibiting viral RNA polymerase, reverse transcriptase, and downregulation of the extracellular-signal-regulated kinase/mitogen-activated protein kinase signaling pathway [25]. The variety of viruses attacked by garlic are adenovirus [26]; SARS-CoV-1 [27]; dengue [28]; herpes simplex [29] influenza A, B, and H1N1 [30][31]; hepatitis [32]; HIV [33]; and rotavirus [34]. Furthermore, in a very recent study, essential garlic oil was found to be acting on the angiotensin-converting enzyme 2 (ACE2) and largely on the main protease of SARS-CoV-2 (PDB6LU7). This activity is crucial to diminish the impact of the host receptor of SARS-CoV-2, and this study proposes that garlic oil active compounds can be used as a COVID-19 preventive treatment [35].

#### 3.3.2. Antibacterial

The antibacterial effect of garlic was analyzed in vitro using fresh garlic juice in agar plates against *E. coli*, *P.mirabilis*, *K.pneumoniae*, *S.aereus*, and *P.aeruginosa*. The results showed a dose-dependent inhibition in all bacterial strains exposed to a garlic concentration higher than 10% [36]. In another study, two different aqueous garlic extracts (from *Allium sativum* and *Allium tuberosum*) were tested in rats infected with one penicillin-sensitive (ATCC 25923) and one methicillin-resistant (ATCC 33592) *S. aureus*. The two species of garlic were administered orally at 100 and 400 mg/kg) every 6 hours for 24 hrs. Results showed that both garlic extracts could reduce the infection but not against the resistant strain [37]. Several in vitro studies demonstrated the antibacterial effect of fresh garlic extract on *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *P. aeruginosa*, and *S. aureus* [36], and also against multidrug-resistant *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *Serratia marcescens*, and methicillin-resistant *S. aureus* [38]. In a clinical trial that involved 15 patients with *Helicobacter pylori*, the results showed that the urease breath test to detect *H. pylori* was lower in patients who took 3 g of garlic cloves twice a day, demonstrating its antimicrobial effect [39].

#### 3.3.3. Antifungal

Various studies have discussed the antifungal effect of garlic. Li et al. showed that garlic oil had an inhibitory effect against *Candida albicans* at a concentration of 0.35 µg/mL [40]. Aala et al. performed an experiment that evaluated the structural characteristic of *Trichophyton rubrum* in response to garlic and allicin aqueous extracts. The results showed that the allicin extract was more effective in impeding the growth of fungal cells by changing fungi morphology [41]. Another in vitro study indicated that 0.125 and 0.0313 % of garlic oil had a strong antifungal activity by penetrating hyphae cells and destroying their organelles against *Penicillium funiculosum* [42].

### 3.4. Anti-Inflammatory

The anti-inflammatory effect of garlic was studied by several research groups. Overall, the studies agreed on the antioxidant and anti-inflammatory properties of garlic. However, the results for the mechanisms activated/inhibited by the phytonutrients themselves and their phytochemicals are diverse. We understand that this could be possible due to different garlic preparations and also by the "double-edged sword" of nitric oxide. For example, in an in vitro study, Lee and coworkers showed garlic's anti-inflammatory activity at µM concentrations. They demonstrated that garlic's organosulfides Z- and E- ajoene and analogs inhibited nitric oxide/prostaglandins and nitric oxide

synthase/cyclooxygenase, the phosphorylation of p38 mitogen-activated protein kinases, and, also, the expression of the following pro-inflammatory cytokines: tumor necrosis factor- $\alpha$ , interleukin-1 $\beta$ , and -6 in a lipopolysaccharide-induced macrophage cell line [43]. In a double-blind clinical trial study, anti-inflammatory effects in 40 peritoneal dialysis patients were investigated by administering a garlic extract twice daily for 8 weeks. The results demonstrated that garlic diminished inflammatory markers in end-stage renal disease patients, specifically interleukin-6, C-reactive protein, and erythrocyte sedimentation rate in the treated group [44]. On the other hand, a previous in vivo study concluded that garlic inhibits platelet aggregation by activating nitric oxide (NO) synthase and the production of NO [45].

### 3.5. Immunomodulatory

As previously described here, garlic induces multiple different functions, including antioxidant, anti-microbial, and anti-inflammatory properties leading to an improvement in the immune response. The immune response induced by the garlic phytochemical allicin was studied in female BALB/c mice. Results showed that allicin treatment reduced parasitaemias and enhanced pro-inflammatory mediators during malaria infection in a dose-dependent manner [46]. In addition, Bruck et al. studied the immune response of allicin in induced liver damage BALB/c male mouse. Results showed that allicin-treated mice showed decreased levels of the pro-inflammatory tumor necrosis factor- $\alpha$ , aminotransferases, and improved hepatic necroinflammation [47]. A randomized, double-blind clinical trial studied the immune and inflammatory effects of 3.6-g aged garlic extract administered daily in 51 obese adults for 6 weeks. Results showed that patients who took the extract supplementation had less pro-inflammatory cytokines, such as interleukin-6 and tumor necrosis factor- $\alpha$  [48]. In a separate study, the immune effect of aged garlic extract supplementation was analyzed in a randomized, double-blind trial with 120 healthy participant adults to examine the proliferation of immune cells and the severity of symptoms during cold and flu season. Results showed that the garlic extract induced increased levels of NK cells and  $\gamma\delta$ -T cells, and reduced the severity of symptoms, days, and incidence [49]. This immune response of garlic is due to the scavenging of oxidizing agents, thereby preventing the formation of pro-inflammatory messengers, such as COX and LOX. In addition, one of the main mechanisms observed is through immunomodulation of inflammatory cytokines and direct stimulation of immune cells [50].

### 3.6. Predicted gastrointestinal (GI) absorption

The physicochemical properties for the main five most bioactive phytochemicals in garlic (aliin, allicin, diallylsulfide, z-ajoene and 2-vinyl-4H-1,3-dithiin) were calculated based on the combination of Lipinski's, Ghose's, and Veber's rules (L-Ro5, GF, VR), summarized as follow: molecular weight (160-500 Da); hydrogen bond donors  $\leq 5$ ; hydrogen bond acceptors  $\leq 10$ ; molar refractivity (40-130); lipophilicity (-0.4–5.6); rotatable bonds  $\leq 10$ ; polar surface area  $< 140$ ; total number of atoms (20-70) [51, 52, 53, 54]. These are described as an approximation for the pharmacokinetics of a molecule in the body. From garlic's phytochemicals, 40% (aliin and z-ajoene) comply with all of the "drug-likeness" rules. The 60% (allicin, diallylsulfide, and 2-vinyl-4H-1,3-dithiin) violate the total number of atoms ( $< 20$ ), polar surface area ( $< 40$ ), and MW  $< 160$  Da. Accordingly, diallylsulfide is predicted to have the lowest GI, followed by 2-Vinyl-4H-1,3-dithiin and then, allicin.

## 4. Contraindications

There is limited data about the safety of garlic supplements [55]. Hoshino et al. administered 40 mg of different garlic preparations to adult dogs, and his results showed significant damage caused to gastric mucosa by raw garlic powder and gastric redness caused by boiled garlic powder. Interestingly, no adverse effect was caused by the ingestion of raw garlic extract [56]. In 2014, the first case of pneumonia caused by fermented black garlic was discovered in a 77-year-old female patient who came into the hospital with shortness of breath and cough after taking black garlic. The patient showed health improvement when she stopped taking black garlic [57]. In addition, the first case of drug-induced liver injury by the mild periportal cholestatic reaction was reported in a 43-year-old patient who suffered from hepatopulmonary syndrome following a liver transplant by taking a high dose of *Allium sativum* as treatment. The patient's liver enzymes returned to normal after discontinuation of the treatment [58]. According to the National Institutes of Health, garlic supplements may increase the risk of bleeding. As we mentioned before, garlic displays strong antioxidant properties [24] that could lead to the inhibition of platelet adhesion and aggregation [58]. Through these mechanisms, garlic intake might increase the risk of bleeding when combined with other anticoagulants. However, this property would help patients with cardiovascular diseases by the strong garlic antihypertensive action.

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