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## Biological properties and therapeutic applications of garlic and its components

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Garlic is one of the most widely employed condiments in cooking. It has also been used since ancient times in traditional plant-based medicine, largely based on its organosulfur compounds. The objective of this study was to provide updated information on the biological and therapeutic garlic properties. Garlic has been found to possess important biological properties with high therapeutic potential, which is influenced by the mode of its utilization, preparation, and extraction. It has been attributed with antioxidant, anti-inflammatory, and immunomodulatory capacities. Garlic, in particular its organosulfur compounds, can maintain immune system homeostasis through positive effects on immune cells, especially by regulating cytokine proliferation and expression. This may underlie their usefulness in the treatment of infectious and tumor processes. These compounds can also offer vascular benefits by regulating lipid metabolism or by exerting antihypertensive and antiaggregant effects. However, further clinical trials are warranted to confirm the therapeutic potential of garlic and its derivatives.

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## Introduction

Garlic (*Allium sativum*) has been widely employed in cooking and medicine since ancient times.<sup>1</sup> It continues to serve as a therapeutic resource in traditional healing, which uses plants with medicinal properties to treat diseases.<sup>2–4</sup> Garlic contains phenolic compounds, saponins, polysaccharides, and organosulfur compounds, among other components.<sup>5</sup> Its organosulfur compounds are responsible for its most important biological and pharmacological properties and are extracted and isolated for therapeutic purposes.<sup>6,7</sup> Compounds derived from garlic have been attributed with anti-inflammatory, antioxidant, and antimicrobial activities with beneficial effects against cardiovascular diseases, cancers, and immune system disorders.<sup>2,4,7</sup>

Garlic can serve as a poultice in solid powder form but is more frequently prepared as a liquid in water, oil, or alcohol solvents. The mode of preparation influences its composition and its biological effects.<sup>4,8</sup> However, the separation and

isolation of the organosulfur components of garlic is challenging due to their physicochemical properties, and variability in their proportions among garlic preparations hampers reproduction and validation of the effects observed in different studies.<sup>6</sup> Also, despite the fact that garlic supplementation has been classified as a safe product, some non-serious gastrointestinal side effects have been reported.<sup>9,10</sup>

The aim of this study was to provide an update of scientific evidence on the biological properties of garlic and its chemical components and their relevance for the management of health and disease.

## Characteristics and composition of garlic

Garlic contains more than 200 chemical compounds with multiple properties. It is 65% water, 28% carbohydrates, 2.3% organosulfur compounds, 2% proteins, 1.2% free amino-acids, and 1.5% fiber. It also contains fat-soluble vitamins (vitamin A, vitamin K, and vitamin E), water-soluble vitamins (vitamin C, B-complex vitamins: B1, B2, B3, B6, and B8), and minerals (Ca, Fe, Mg, P, K, Na, and Zn). Organosulfur compounds give garlic its characteristic taste and odor as well as its pharmacological properties.<sup>11</sup>

Moreover, garlic has both non-volatile and volatile organosulfur compounds. Non-volatile precursor compounds of sulfur in garlic include  $\gamma$ -glutamyl-S-allyl-L-cysteine and its sulfide, known as alliin. Alliin is only present in intact garlic, being transformed into other compounds when the bulb is macerated or crushed. In contrast, volatile organosulfur com-

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pounds are generated during the processing of garlic products, being divided into three subgroups according to their chemical nature (Fig. 1): (a) thiosulfinate, which result from the conversion of sulfoxides by enzymatic reaction when raw garlic is processed. (b) Volatile organosulfurs such as allicin (diallyl thiosulfinate), one of the most biologically active compounds, which is absent in intact garlic but produced by the reaction of alliin with the enzyme alliinase when the bulb is ground, crushed, or damaged. Allicin is highly unstable and instantly decomposes into diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), and/or sulfur dioxide ( $\text{SO}_2$ ), among other compounds. This subgroup also includes methyl allyl disulfide and methyl allyl trisulfide. (c) Water-soluble organosulfur compounds, generated during the aqueous or alcoholic extraction of garlic through the decomposition of  $\gamma$ -glutamyl-S-allyl-L-cysteine into S-allyl-L-cysteine (SAC).

In summary, intact bulbs contain SAC and  $\gamma$ -glutamyl-S-allyl-L-cysteine sulfoxide, vapor-distilled oils contain volatile sulfide compounds (DADS, DATS, DAS), dry garlic powder is rich in alliin and DADS, and garlic macerates are rich in organosulfur compounds, ditiins, and *E,Z*-ajoenes. Finally, mashed and ethanol-soaked aged garlic extract (AGE) contains SAC and S-allyl-mercaptocysteine (SAMC) and has shown more biological properties in comparison to the aforementioned preparations.<sup>12–15</sup> Multiple factors influence the bioactivity of garlic components, such as extraction, preparation, temperature or storage. In this context, it is reported that longer cooking time and increased temperature may reduce the proportion of phenolic compounds, such as hesperetin and 3/4-hydroxybenzoic acid, and flavonoid.<sup>16,17</sup> It should be noted that absorption, metabolism and excretion are the main indices of bioavailability of an ingested compound. Allicin and allicin-derived compounds are rapidly metabolized to allyl methyl sulfide, which is the main metabolite of allicin and an active metabolite (Lawson and Wang, 2001).<sup>18</sup> However, information on the bioavailability of organosulfur compounds is

limited. Thus, Miękus *et al.*<sup>19</sup> suggested the need to conduct a series of clinical trials in a significant number of volunteers to optimize doses and understand the factors that determine bioavailability.

## Biological activity of garlic

The different biological mechanisms of garlic are summarized in Table 1.

### Antioxidant activity

The oxidation of DNA, proteins, and lipids by reactive oxygen species (ROS) plays a key role in aging and in the development of cancers and cardiovascular, inflammatory, neurodegenerative, and age-related diseases.<sup>20,21</sup>

The antioxidant potential of garlic largely derives from its saponins, flavonoids, organosulfur compounds, and phenolic compounds. This potential is higher in aqueous extracts (*e.g.*, AGE) than in fresh garlic or other preparations, likely due to the presence of water-soluble organosulfur compounds with antioxidant capacity (*e.g.*, SAC and SAMC). Furthermore, a synergistic antioxidant effect has been observed between these compounds and flavonoids, saponins, and certain micro- and macro-nutrients also present in aqueous garlic extracts.<sup>11,14</sup> Phytochemicals in these extracts can exert a double action by favoring the expression of antioxidant enzymes (*e.g.*, superoxide dismutase [SOD] and catalase) and increasing cell glutathione (GSH) levels.<sup>22,23</sup> In a recent meta-analysis of randomized controlled trials, serum SOD levels and total antioxidant capacity were found to be increased by supplementation with 80–4000 mg day<sup>−1</sup> of garlic for 2–24 weeks, with a reduction in malondialdehyde production.<sup>24</sup> An *in vitro* study found that ajoene, a chemically stable by product of garlic, can activate nuclear factor 2 (Nrf2), regulating the expression of glutamate-cysteine ligase (GCL), raising levels of GSH, a potent antioxidant, and increasing the expression of other genes that encode cysteine-metabolizing enzymes.<sup>25</sup> Nrf2 controls the expression of numerous genes involved in the defense against xenobiotic agents and oxidative stress and is considered a key regulator of oxidative stress and the inflammatory response. For instance, it induces the gene expression of heme oxygenase-1 (HO-1), an enzyme that catalyzes degradation of the heme group, generating carbon monoxide (CO), biliverdin, and iron. This enzyme is activated by oxidative stress or by the presence of cytokines or other mediators during inflammatory processes.<sup>25</sup> In a study of human endothelial cells, AGE was found to reduce oxidative stress in a dose-dependent manner by activating the Nrf2 pathway through an increase in the expression of GCL and HO-1.<sup>26</sup> Oral administration of AGE (100–200 mg kg<sup>−1</sup>) to male albino rats with indomethacin-induced gastric inflammation had a gastroprotective effect that was attributed to its anti-inflammatory and antioxidant properties.<sup>27</sup> In this regard, it was reported in an *in vitro* study that allicin attenuates oxidative stress responses induced by lipopolysaccharides (LPS) of Gram-negative bacteria *via* the

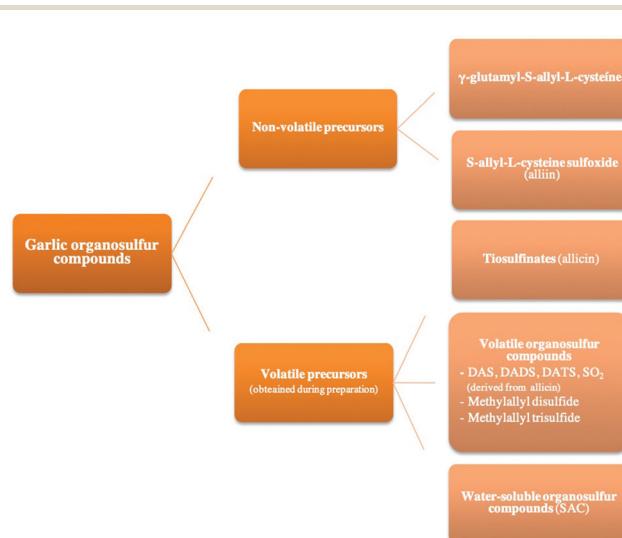


Fig. 1 Organosulfur compounds of garlic.



**Table 1** Key findings of the biological activity of garlic

Biological activity	Mechanism of action	References
Antioxidant activity	<p>Garlic phytochemicals could promote the expression of antioxidant enzymes, such as SOD or catalase, and increase cellular GSH levels. Supplementation with 80–4000 mg day<sup>-1</sup> of garlic for 2–24 weeks increases serum SOD levels, increasing total antioxidant capacity through a reduction of malondialdehyde.</p> <p>Ajoene exerts its antioxidant function through the activation of Nrf2, which regulates GCL expression and increases GSH levels, among other mechanisms of action. Likewise, AGEs could reduce the oxidative stress of endothelial cells by activating the Nrf2 pathway, through an increase in the expression of GCL and HO-1.</p> <p>Allicin attenuates oxidative stress responses caused by different factors through the Nrf2 pathway and regulation of apoptosis.</p> <p>AGEs play an antioxidant role against damage to DNA structures caused by ultraviolet radiation, performing antimutagenic, anticarcinogenic and reparative activities on damaged structures.</p>	<p>Liu <i>et al.</i> 1992 Borlinghaus <i>et al.</i> 2014 Askari <i>et al.</i> 2021</p> <p>Kay <i>et al.</i> 2010 Hiramatsu <i>et al.</i> 2016 Zhang <i>et al.</i> 2017 Liu <i>et al.</i> 2015</p> <p>Corzo-Martínez <i>et al.</i> 2007 Khanum <i>et al.</i> 2004</p>
Anti-inflammatory activity	<p>A reduction in adipocyte IL-6 and MCP-1 secretion, as well as up-regulation of the expression of genes involved in inflammatory response has been documented after alliin treatment.</p> <p>Treatment with garlic extracts on mononuclear cells showed inhibition of IL-17 secretion. IL-14 secretion was not affected after treatment with garlic extract on mononuclear cells.</p> <p>AGE reduces the production of IL-12, TNF-<math>\alpha</math> and IL-6.</p> <p>Garlic and its components may modulate the inflammatory response induced by T helper cells.</p> <p>Garlic components have the potential to inhibit NF-<math>\kappa</math>B, thereby reducing the inflammatory response mediated by TNF-<math>\alpha</math>, IL-1, IL-6, MCP-1 and IL-12, among others.</p>	<p>Quintero-Fabián <i>et al.</i> 2013</p> <p>Moutia <i>et al.</i> 2016</p> <p>Hodge, Hodge, and Han 2002 Larypoor <i>et al.</i> 2013 Liu <i>et al.</i> 2009</p> <p>Rodrigues &amp; Percival 2019</p>
Antimicrobial activity	<p>Garlic extracts have been shown to have antibiofilm and antibacterial effects against <i>Bacillus cereus</i>, <i>Staphylococcus aureus</i>, <i>Micrococcus</i> spp., <i>Escherichia coli</i>, <i>Klebsiella</i> spp., <i>Proteus</i> spp., <i>Aspergillus versicolor</i>, <i>Penicillium citrinum</i>, and <i>Penicillium expansum</i>.</p> <p>Anti-fungal activity of garlic against <i>Penicillium funiculosum</i> and <i>Candida albicans</i> has been demonstrated.</p> <p>Garlic has been shown to have antiviral activity through inhibition of retrotranscription and viral DNA synthesis capacity.</p> <p>Garlic and its organosulphur and flavonoid components exert antiviral action against SARS-CoV-2 infection via inhibition of the 6LU7 protein of the active region of the pathogen.</p> <p>Garlic has therapeutic action in COVID-19 disease by strengthening immune system cells and decreasing the secretion of proinflammatory cytokine and leptin.</p> <p>Garlic extract inhibits the penetration and proliferation of the influenza A (H1N1) virus. Ajoene obtained from garlic inhibits the NA protein of the influenza A (H1N1) virus, which is responsible for the onset of viral infection.</p> <p>Garlic-derived DATS treatment applied to human lung epithelial cells reduced viral load, increased viral gene expression, and decreased proinflammatory cytokine expression.</p> <p>Garlic compounds such as glutamyl cysteine, DATS, allin or allicin possess HIV-1 inhibitory activity.</p>	<p>Marefati <i>et al.</i> 2020 Fratianni <i>et al.</i> 2016 Nakamoto <i>et al.</i> 2020</p> <p>Li <i>et al.</i> 2014 Li <i>et al.</i> 2016</p> <p>Rouf <i>et al.</i> 2020</p> <p>Pandey <i>et al.</i> 2019 Sen <i>et al.</i> 2020 Thuy <i>et al.</i> 2020</p> <p>Donma &amp; Donma 2020</p> <p>Mehrbod <i>et al.</i> 2009 Sahoo <i>et al.</i> 2016</p> <p>Ming <i>et al.</i> 2021</p> <p>Gökalp <i>et al.</i> 2018</p>
Immuno-modulatory activity	<p>The consumption of garlic and its derivatives has been shown to exert immunomodulatory activity depending on the dose administered.</p> <p>Garlic's protein fraction and AGE have been shown to stimulate lymphocytes, natural killer cells and macrophages.</p>	<p>Kuttan 2000 Charron <i>et al.</i> 2015 Arreola <i>et al.</i> 2015 Colić <i>et al.</i> 2002</p> <p>Ebrahimi <i>et al.</i> 2013 Moutia, Habti, &amp; Badou 2018 Rodrigues &amp; Percival 2019 Percival 2016</p>

SOD: superoxide dismutase; GSH: glutathione; Nrf2: nuclear factor erythroid 2-related factor 2; GCL: glutamate-cysteine ligase; AGE: aged garlic extract; HO-1: heme oxygenase-1; IL: interleukin; MCP-1: monocyte chemotactic protein; TNF- $\alpha$ : tumor necrosis factor  $\alpha$ ; NF- $\kappa$ B: nuclear kappa B transcription factor; NA: neuraminidase; DATS: diallyl trisulfide; HIV-1: human immunodeficiency virus type 1.

Nrf2 pathway.<sup>28</sup> It was also observed that allicin has a protective effect *in vitro* against traumatic or ischemic neuronal damage by regulating apoptosis and oxidative stress pathways.<sup>29</sup>

The antioxidant properties of garlic also act by reducing free radicals. Thus, Corzo-Martínez *et al.* reported that AGE can counter ultraviolet radiation-induced DNA damage.<sup>30</sup> Antimutagenic activity has been shown by certain sulfur com-



ponents (e.g., DAS) through their action on DNA repair mechanisms, avoiding the onset of carcinogenesis.<sup>31</sup>

Locatelli *et al.*<sup>6</sup> highlighted the antioxidant capacity of allicin, ajoenes, and vinylidithiins (2-VD) in garlic, reporting that allicin can eliminate 1,1-diphenyl-2-picrylhydrazyl radical (DPPH<sup>•</sup>) and 2,20-azino-bis-(3-ethylbenzo thiazoline-6-sulfonic acid) diammonium salt (ABTS) and reduce iron levels.

### Anti-inflammatory activity

The anti-inflammatory activity of garlic extracts is well documented. Quintero-Fabián *et al.* reported that treatment of LPS-stimulated 3 T3-L1 adipocytes with alliin reduces the gene expression and protein synthesis of interleukin-6 (IL-6) and monocyte chemotactic protein (MCP-1) and upregulates genes related to the immune response.<sup>32</sup> Other authors found that treatment with garlic extract inhibited the expression of IL-17 by peripheral blood mononuclear cells without affecting their expression of IL-4.<sup>33</sup>

In another *in vitro* study, Hodge *et al.* reported that AGE reduces the production of IL-12 and other proinflammatory cytokines and increases IL-10, which inhibits the production of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and IL-6.<sup>34</sup> Inhibition of Th1 proinflammatory cytokines by allicin was observed by Larypoor *et al.*<sup>35</sup> However, a study in rats found that garlic oil had dual effects on the balance of T helper cells (Th1-Th2) according to the dose, with low doses obtaining a Th1 response whereas high doses a Th2 response.<sup>36</sup>

Nuclear kappa B transcription factor (NF- $\kappa$ B) is a key regulator of the expression of genes involved in inflammation, including the enzymes nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). NF- $\kappa$ B overactivation is associated with various chronic inflammatory diseases, and suppression of the NF- $\kappa$ B pathway by anti-inflammatory drugs favors their prevention and treatment.<sup>26</sup> The NF- $\kappa$ B transcription factor can be inhibited by caffeic acid, SAC, and DATS, among other compounds derived from garlic, thereby inhibiting multiple genes that encode cytokines related to the proinflammatory response (e.g., TNF- $\alpha$ , IL-1, IL-6, MCP-1, and IL-12).<sup>14</sup> In another investigation, a garlic protein of 14 kDa isolated from AGE significantly inhibited the production of NO, prostaglandin E (PGE), TNF- $\alpha$ , and IL-1 $\beta$  by LPS-activated macrophages in a dose-dependent manner with no toxic effects. As observed in other studies, the anti-inflammatory effect was largely mediated by the inhibition of iNOS and COX-2 expressions through inactivation of the NF- $\kappa$ B pathway.<sup>37</sup>

### Antimicrobial activity

Ample evidence has also been published on the antimicrobial and antifungal properties of garlic and some of its components.<sup>38</sup> Fresh garlic extract was found to inhibit the growth of *Bacillus cereus*, *Staphylococcus aureus*, *Micrococcus* spp., *Escherichia coli*, *Klebsiella* spp., and *Proteus* spp., using 1% chloramphenicol as reference antibiotic.<sup>39</sup> In addition, Fratianni *et al.* observed that "Rosato" and "Caposele" garlic varieties exert antimicrobial action against *Aspergillus versicolor*, *Penicillium citrinum*, and *Penicillium expansum*.<sup>40</sup> The sulfur-

ous compounds of garlic can impair biofilm formation by acting as quorum-sensing inhibitors, hampering early bacterial adhesion and the secretion of extracellular polymeric substances and virulence factors.<sup>36</sup> There is evidence that garlic has antifungal activity against *Penicillium funiculosum* and *Candida albicans*.<sup>41,42</sup> It has also been demonstrated anti-viral activity against multiple pathogens. It acts by blocking the entry of viruses into cells, inhibiting viral polymerase-RNA, reverse transcriptase, DNA synthesis, and the transcription of immediate-early gene 1 (IEG1) and downregulating the extracellular signal-regulated kinase (ERK)/mitogen activated protein kinase (MAPK) signaling pathway.<sup>43</sup> Various researchers have explored the therapeutic potential of garlic against SARS-CoV-2 infection disease. Some authors have observed that garlic and its bioactive compounds may act against this disease by inhibiting target protein 6LU7 of SARS-CoV-2.<sup>44-46</sup> It has also been proposed that garlic may mitigate the effects of COVID-19 on the immune system by strengthening immune system cells and inhibiting the secretion of proinflammatory cytokines and leptin.<sup>47</sup> Moreover, some authors point out that garlic and its components may also have antiviral activity against influenza A (H1N1) and avian influenza (H9N2) viruses.<sup>48-50</sup> Similarly, garlic compounds such as glutamyl cysteine, DATS, allin and allicin have been shown to inhibit the activity of human immunodeficiency virus type 1 (HIV-1).<sup>51</sup> However, numerous studies indicate that supplementation with garlic or its derivatives could exert an undesirable interaction with prescribed antiretroviral drugs against HIV disease.<sup>52,53</sup>

### Immunomodulatory activity

Garlic can contribute to maintaining immune system homeostasis through its immunomodulatory action on different cell populations, alongside its antiapoptotic, antiparasitic, and anticarcinogenic effects.<sup>13</sup>

Animal experiments showed that garlic consumption can increase the number of immune cells and the total number of bone marrow cells.<sup>54</sup> A study in humans found that expression of the gene encoding the NFAM1 protein is increased by the addition of 5 g of raw mashed garlic to one meal every day. NFAM1 is a type I membrane receptor that activates cytokine gene promoters (e.g., IL-13 and TNF- $\alpha$ ) and is involved in B cell development and signaling.<sup>55</sup>

Various garlic derivatives have been found to have a dual effect, exerting either a stimulatory or inhibitory effect on monocytes and lymphocytes according to the dose.<sup>13,56,57</sup> For instance, concanavalin A-stimulated lymphocyte proliferation is inhibited by high doses of garlic extract but increased by low doses.<sup>48</sup> The same dual effect has been observed with DATS.<sup>57</sup>

The protein fraction of garlic has demonstrated stimulatory effects on lymphocytes, natural killer (NK) cells, and macrophages. Specifically, it was found to increase the mitogenic activity of human peripheral blood lymphocytes and CD8 $^{+}$  T cells, producing an increase in the delayed hypersensitivity reaction and promoting an effective cellular response.<sup>58</sup>

AGE has also exhibited this immunomodulatory property, activating and increasing the population of NK cells.



Supplementation with AGE in mice increases the activation of NK and cytotoxic T cells in mice and improves the immune response in humans by augmenting the proliferation of NK and T $\gamma$ δ cells.<sup>11,13,14,59</sup>

## Therapeutic activity against diseases

Given its antioxidant, immunomodulatory, and anti-inflammatory properties, researchers have explored the usefulness of garlic in the treatment of various diseases (Table 2).

### Infectious processes

Various authors have studied the action of garlic and its components against infectious processes. Thus, the severity and duration of respiratory tract infections and their symptoms were reduced after the administration of allicin *versus* placebo.<sup>60</sup> In a mouse study, garlic oil and DADS were found to have protective effects against respiratory tract inflammation caused by cigarette smoke, reducing the inflammatory cell count and the production of IL-1 $\beta$ , IL-6, and TNF- $\alpha$ .<sup>61</sup> Similarly, experimental studies in adults and children showed that daily supplementation with allicin or long-releasing garlic capsules for 12 or 20 weeks respectively, significantly reduced common cold virus infections in adults and acute respiratory infections in children, with no side effects as a result of the administration.<sup>62,63</sup>

In a mouse model of induced polymicrobial sepsis, the administration of methyl sucrose 3-formyl-4-methylpentanoate, isolated from garlic, reduced peritoneal fluid colony-forming units (CFUs) produced by the stimulation of ROS generation, diminished lymphocyte apoptosis in the spleen, and inhibited the production of proinflammatory cytokines (e.g., TNF- $\alpha$ , IL-1 $\beta$ , and IL-6).<sup>64</sup>

Lee *et al.* evaluated the growth and immune response of laying chickens fed with a mixture of phytogenic extracts (garlic and cilantro) and probiotics.<sup>65</sup> They reported an increase in the proliferation of lamina propria leukocytes and in their cytotoxic activity against tumor cells, also observing an improvement in the egg production but not growth of the animals. The inhibition of LPS-induced TNF- $\alpha$  and IFN- $\gamma$  secretion and the reduction in fecal *E. coli* count were similar between chickens fed with this mixture and those treated with antibiotics.

### Tumoural processes

Garlic has demonstrated anticarcinogenic properties against colorectal, breast, liver, and gastric cancers, among others.<sup>66–69</sup> In 2020, Zhang *et al.* reported that garlic had therapeutic potential not only to prevent but also to treat cancer, proposing possible mechanisms of action.<sup>70</sup> The aforementioned beneficial effects of garlic on the immune system would also contribute to controlling tumor processes.<sup>11</sup> *In vitro* and *in vivo* studies have demonstrated the capacity of garlic to inhibit cell division, block carcinogen activation, reduce microbiological growth, scavenge ROS, induce superoxide dismutase, increase proinflammatory cytokine release, and reduce cell prolifer-

ation.<sup>71</sup> These effects have been associated with specific compounds of garlic, especially DADS, DATS, SAC, and SAMC.<sup>72</sup> Treatment with garlic extract attenuated cell migration and induced death in rat sarcoma cells, and pre-exposure to the extract completely inhibited the growth of implanted tumor cells.<sup>73</sup> Likewise, treatment with protein fractions extracted from garlic reduced the tumor size in mice with implanted breast tumor cells, increasing T cell activation and the intra-tumor infiltration of lymphocytes at all studied doses.<sup>56</sup>

The protein fraction of garlic has been found to modulate NK cell activity in mice, while AGE can increase the number and activity of NK cells in healthy individuals and in patients with advanced cancers.<sup>11,14,74,75</sup>

Cytokines also play an important role in antitumor immunity. Supplementation with allicins of Ehrlich ascites carcinoma (EAC) cells treated with tamoxifen (antiestrogenic drug for breast cancer) increased the expression of cytotoxic damage markers and TNF- $\alpha$  levels.<sup>76</sup> In another study, AGE significantly increased the apoptosis of acute lymphoblastic leukemia cells without altering the activation of T cells or the production of IFN- $\gamma$ , IL-2, and TNF- $\alpha$ , whereas T cell proliferation and cytokine production were reduced by treatment with cytostatic agents.<sup>77</sup> In a mouse study, treatment with AGE produced a cytokine release pattern that favored Th1 cell action and antitumor immunity.<sup>35</sup>

Other human randomized clinical trials have evaluated the effectiveness of garlic against disorders resulting from infection with the bacterium *Helicobacter pylori*. It was found that combined vitamin and garlic supplementation treatment (extract and oil) for 7.3 years reduced the prevalence of precancerous gastric lesions and reduced the risk of mortality from gastric cancer after a follow-up of 22 years.<sup>78,79</sup> Indeed, this is in accordance with the findings of Ishikawa *et al.*, who observed an improvement in NK cell activity after 6 months of AGE supplementation in patients with advanced pancreatic, liver and colon cancer.<sup>80</sup> Similarly, other authors demonstrated that daily supplementation with high-dose AGE (2.4 mL) could inhibit the progression of colorectal adenoma.<sup>81</sup> None of these studies found significant adverse effects as a consequence of supplementary therapy.

### Activity at vascular level

Garlic has been attributed with beneficial effects on the cardiovascular system by improving lipid profile, endothelial function, and blood pressure.<sup>7,82</sup>

A meta-analysis published by Silagy and Neil in 1994 confirmed that garlic and its derivatives can reduce levels of triglycerides and cholesterol.<sup>83</sup> Allicin can also suppress cholesterol biosynthesis by inhibiting the key enzymes squalene-monooxygenase and acetyl-CoA synthetase.<sup>84,85</sup> In addition, (1*R*,3*S*)-1-methyl-1,2,3,4-tetrahydro- $\beta$ -carboline-3-carboxylic acid obtained from garlic was found to prevent adiposity by regulating the expression of genes involved in the lipid metabolism of adipocytes, downregulating lipogenic genes and upregulating lipolytic genes.<sup>86</sup> Likewise, experimental studies are in agreement with these findings, observing garlic supplementation to



**Table 2** Summary of the therapeutical activity of garlic

Therapeutic applications	Mechanism of action	Ref.
Infectious processes	The administration of allicin and garlic oil has been shown to have beneficial effects against respiratory tract infections. Oral allicin supplementation treatment may prevent common cold virus infection. Daily administration of 300 mg extended-release garlic capsules for 20 weeks reduced the morbidity of acute respiratory infections in children aged 10–12 years. Treatment with garlic extract in a sepsis mouse model showed favourable results against the infectious process.	Yarnell (2018) <sup>60</sup> Ko <i>et al.</i> (2018) <sup>61</sup> Josling (2001) <sup>63</sup> Andrianova <i>et al.</i> (2003) <sup>62</sup> Lee <i>et al.</i> (2015) <sup>64</sup>
Tumoural processes	Anti-tumour properties have been found in garlic, mainly in some compounds such as DADS, DATS, SAC, and SAMC.  Treatments with garlic extract and garlic protein fractions reduced the tumour activity of rat sarcoma cells and breast tumour cells. The protein fraction of garlic and AGE was shown to modulate the activity of natural killer cells in both healthy populations and individuals with advanced cancer pathologies.	Miraghajani <i>et al.</i> (2018) <sup>72</sup> Liu <i>et al.</i> (2019) <sup>67</sup> Zhang <i>et al.</i> (2020) <sup>70</sup> Zhou <i>et al.</i> (2020) <sup>68</sup> Hu <i>et al.</i> (2002) <sup>73</sup> Ebrahimi <i>et al.</i> (2013) <sup>58</sup> Moutia, Habti and Badou (2018) <sup>11</sup> Rodrigues and Percival (2019) <sup>14</sup> Ishikawa <i>et al.</i> (2006) <sup>74</sup> Fallah-Rostami <i>et al.</i> (2013) <sup>75</sup> Suddek (2014) <sup>76</sup> Hodge <i>et al.</i> (2008) <sup>77</sup> Larypoor <i>et al.</i> (2013) <sup>35</sup> You <i>et al.</i> (2006) <sup>78</sup> Li <i>et al.</i> (2019) <sup>79</sup>
Activity at vascular level	Garlic and its components showed a beneficial effect in the treatment of tumours by modulating cytokine novels, such as TNF- $\alpha$ , IFN- $\gamma$ , IL-2, as well as the activity exerted by T-cells. Vitamin supplementation in combination with garlic for 7.3 years showed to reduce the prevalence of precancerous gastric lesions. Oral vitamin and garlic supplementation treatment administered for 7.3 years was found to reduce the risk of mortality of gastric cancer after a follow-up of more than 22 years. Daily supplementation treatment with AGE (500 mg) for 6 months in patients with advanced cancer of the digestive system (pancreatic, colon and liver cancer) showed an improvement of NK cell activity. A 12-month supplemental treatment with high-dose AGE (2.4 mL day <sup>-1</sup> ) suppressed the number and size of colorectal adenomas. Garlic and its constituents may reduce disorders related to the cardiovascular system by reducing triglyceride and cholesterol levels.	Ishikawa <i>et al.</i> (2006) <sup>80</sup> Tanaka <i>et al.</i> (2006) <sup>81</sup> Gupta and Porter (2001) <sup>84</sup> Silagy and Neil (1994) <sup>83</sup> Ried (2016) <sup>98</sup> Baek <i>et al.</i> (2019) <sup>86</sup> Sangouni <i>et al.</i> (2020) <sup>87</sup>  Zadhoush <i>et al.</i> (2021) <sup>88</sup> Adler and Holub (1997) <sup>89</sup>  Kannar <i>et al.</i> (2001) <sup>90</sup> Zeb <i>et al.</i> (2018) <sup>91</sup>  Jung <i>et al.</i> (2014) <sup>92</sup>
Prebiotic agent	The components of garlic may regulate lipid metabolism in adipocytes. Oral garlic supplementation (400 mg per day) for 12 weeks was shown to significantly reduce blood concentrations of transaminases and cholesterol in patients with non-alcoholic fatty liver disease. Daily supplementation of 800 mg of garlic in patients with polycystic ovary syndrome reduces plasma levels of total cholesterol, LDL, but not HDL. Supplementation daily with 900 mg of AGE in men with moderate hypercholesterolemia for 12 weeks significantly reduced serum triglyceride and LDL levels, as well as lowered systolic and diastolic blood pressure. Supplementation treatment of 9.6 mg allicin for 12 weeks in patients with hypercholesterolemia reduced total, LDL and HDL cholesterol levels. Garlic supplementation (2 g day <sup>-1</sup> ) in patients with hyperlipidemia significantly reduced plasma levels of total and LDL cholesterol, and increased HDL after 60 days. Similarly, this supplementation therapy reduced systolic blood pressure values, but not diastolic blood pressure. Oral black AGE supplementation treatment in patients with mild hypercholesterolemia (6 g day <sup>-1</sup> ) for 12 weeks did not show a reduction in serum LDL and triglyceride levels, although it did significantly increase HDL levels. Thiosulfonates act as potent inhibitors of platelet aggregation. Components of garlic have been shown to have antihypertensive properties, with similar results to drugs commonly used to treat hypertension.	Zeb <i>et al.</i> (2018) <sup>91</sup>  Briggs <i>et al.</i> (2000) <sup>93</sup> Dubey <i>et al.</i> (2017) <sup>95</sup> Chaupis-Meza <i>et al.</i> (2014) <sup>96</sup> Gao <i>et al.</i> (2020) <sup>97</sup> Zhang <i>et al.</i> (2017) <sup>28</sup>  Zhang <i>et al.</i> (2013) <sup>101</sup> Ried (2020) <sup>102</sup>  Askari <i>et al.</i> (2021) <sup>24</sup> Kaur <i>et al.</i> (2016) <sup>103</sup> Thomson <i>et al.</i> (2016) <sup>104</sup> Wang <i>et al.</i> (2017) <sup>105</sup> Sangouni <i>et al.</i> (2021) <sup>106</sup> Yang <i>et al.</i> (2016) <sup>107</sup>
Therapeutic endocrine and metabolic effects	Treatments with garlic extracts have demonstrated an improvement in glucose, fructosamine and glycosylated hemoglobin levels, and a weight loss in diabetic rats.  In humans, garlic has been demonstrated to improve components of the metabolic syndrome such as increasing high-density cholesterol and reducing waist circumference, weight, arterial blood pressure and insulin resistance.	DADS: diallyl disulfide; DATS: diallyl trisulfide; SAC: S-allyl-L-cysteine; SAMC: S-allyl-mercaptopcysteine; AGE: aged garlic extract; LDL: low-density lipoprotein; HDL: high-density lipoprotein.

DADS: diallyl disulfide; DATS: diallyl trisulfide; SAC: S-allyl-L-cysteine; SAMC: S-allyl-mercaptopcysteine; AGE: aged garlic extract; LDL: low-density lipoprotein; HDL: high-density lipoprotein.



be beneficial in reducing plasma levels of total cholesterol and LDL in patients with nonalcoholic fatty liver disease,<sup>87</sup> polycystic ovary syndrome,<sup>88</sup> and hyperlipidemia.<sup>89–91</sup> In contrast, Jung *et al.* observed that daily supplementation of 6 g of black AGE in patients with hypercholesterolemia did not decrease LDL, although they did report an increase in HDL.<sup>92</sup> No adverse effects were reported in any of the patients.

In relation to cardiovascular disorders, thiosulfinate such as allicin have been reported to act as potent inhibitors of platelet aggregation, thereby contributing to the prevention of cardiac and/or cerebral ischemia.<sup>93</sup> Allicin derived from garlic has also demonstrated antihypertensive properties,<sup>94,95</sup> attributed to the action of red blood cells on these polysulfides, which leads to the release of H<sub>2</sub>S.<sup>94</sup> H<sub>2</sub>S is a powerful signaling molecule that relaxes smooth muscle cells surrounding blood vessels and allows their expansion, thereby reducing blood pressures. Chaupis-Meza *et al.* found that macerated garlic extract had a dose-dependent hypotensive effect in an *in vivo* rat model, observing a dose-dependent hypotensive effect comparable to that of captopril, widely prescribed for this purpose.<sup>96</sup> Dubey *et al.* were not able to identify the underlying mechanism.<sup>95</sup> However, Gao *et al.*, who verified the anti-hypertensive effects of garlic and its hydrolysates, proposed that they may act by inhibiting the activity of angiotensin-converting enzyme (ACE), reducing the formation of ACE-II, and protecting bradykinin activity.<sup>97</sup> A meta-analysis of human studies confirmed that garlic contributes to reducing the blood pressure of hypertensive individuals and improving their cholesterol levels.<sup>98</sup> However, its effect on normotensive individuals has not been established.

Garlic also has a protective effect at endothelial level. Thus, allicin increases the proliferation *in vitro* of human umbilical vein endothelial cells.<sup>28</sup> In this regard, the proliferative capacity of this cell population is diminished in response to LPS and the cells release lactate-dehydrogenase, a marker of cytotoxicity. The presence of allicin has been found to decrease LPS-induced apoptosis, suppress ROS overproduction, reduce lipid peroxidation, and decrease endogenous antioxidant enzymatic activities in endothelial cells.<sup>99</sup> These protective effects have been attributed to the inhibition of mitochondrial dysfunction through a reduction in potential mitochondrial membrane collapse, cytochrome c synthesis, and mitochondrial ATP release.<sup>28</sup> It has been reported that allicin not only inhibits the LPS-induced inflammatory response but also dose-dependently increases the expression of liver X receptors  $\alpha$  (LXR $\alpha$ ), and the nuclear receptors LXR  $\alpha$  and  $\beta$  play a crucial role in regulating carbohydrate and lipid metabolism.<sup>28</sup> It was also observed that allicin can activate Nrf2 transcription factor, which regulates defenses against oxidative stress and inflammation (see above).<sup>28,100</sup> In conclusion, allicin is able to attenuate the LPS-induced inflammatory response at blood vessel level.

#### Other effects

Besides the aforementioned benefits, various studies have demonstrated the therapeutic potential of garlic as a prebiotic agent capable of acting on and increasing the diversity of

intestinal microbiota. Thus, garlic fructane was found to stimulate the proliferation of *Bifidobacteria*,<sup>101</sup> and a meta-analysis by Ried *et al.* concluded that supplementation with AGE for three months significantly augments populations of *Lactobacillus* and *Clostridia*.<sup>102</sup> Garlic has also shown therapeutic effects at endocrinol and metabolic level. For instance, treatment of type I and II diabetic rats with garlic and certain garlic extracts (*e.g.*, AGE) improved their weight and their levels of glucose, fructosamine, and glycosylated hemoglobin, thereby reducing the damage to pancreatic cells.<sup>24,103–105</sup> Garlic has also been reported to improve components of the metabolic syndrome in humans by increasing high-density cholesterol and reducing waist circumference, weight, arterial pressure, and insulin resistance.<sup>106,107</sup>

## Conclusions

Garlic and its multiple derivatives possess biological activities with high therapeutic potential against infectious, immunological, vascular, and tumor processes. They can be prepared in raw, macerated, or powder form or as an aqueous, oily, or alcoholic extract. Little clinical advantage has been taken of this natural resource, and further research is warranted in humans to support its therapeutic application.

## Author contributions

Formulation of research question: L. M. R. and C. R.; conception and design of study: L. M. R., R. I. M., C. R. and V. J. C. R.; literature search and data collection: L. M. R., E. G. R. and C. R.; data analysis: L. M. R., E. G. R., C. R., E. L. B., R. I. M. and V. J. C. R.; writing—original draft preparation, L. M. R., C. R., R. I. M. and V. J. C. R.; writing—review and editing: L. M. R., E. G. R., C. R., E. L. B., R. I. M. and V. J. C. R.; supervision: R. I. M. and C. R. All authors have read and agreed to the published version of the manuscript.

## Conflicts of interest

There are no conflicts to declare.

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