Updates on the antimicrobial properties of garlic (*Allium sativum*) biomolecules in the treatment of human infectious diseases

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Abstract

Garlic (*Allium sativum*) is extensively consumed as a seasoning and can be classified as a medicinal food due to its anti-inflammatory and antimicrobial properties. This study summarizes the garlic biomolecules and presents an update on garlic's main antimicrobial properties, correlating them with its phytochemical composition. This review article is based on a search for articles in the SCOPUS, PubMed, and SciELO databases, using ((anti-infective agents) OR (antimicrobial)) AND (garlic) descriptors. Garlic is a good option for association with traditional medicines and emerges as a potential source of phytochemicals. It has applications *in natura*, in aqueous and alcoholic extracts, and as essential oils. The macrostructure of garlic is divided into roots, bulb, stem, clove and leaves. Phenolic compounds and flavonoids, which are important constituents in its antimicrobial character, are found in the leaves. Allicin, garlic's main bioactive component, is mostly located in the stem and leaves. The effects of garlic influence the metabolism, the stabilization of the plasma membrane, the adhesion of microorganisms and the formation of biofilms by bacteria, mainly *Escherichia coli* and the fungus *Candida albicans*. This study concludes that *Allium sativum* is an option in the development of potential antimicrobial phytochemicals and an excellent contributor to the strengthening of the immune system.

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Introduction

Antimicrobials – including antibiotics, antivirals, antifungals and antiparasitics – are medicines used in the prevention and treatment of infectious diseases in humans, animals and plants. Antimicrobial resistance (AMR) occurs when bacteria, viruses, fungi and parasites no longer respond to antimicrobial drugs. As a result of drug resistance, antibiotics and other antimicrobial drugs become ineffective and infections become difficult or impossible to treat, thus increasing the risk of the spread of diseases, serious illness, disability and death. AMR is a natural process that occurs over time through genetic changes in pathogens. Its emergence and diffusion are accelerated by human activity, especially by the misuse and overuse of anti-

microbials to treat, prevent or control infections in humans, animals and plants. Antimicrobial resistance (AMR) is a major global threat to public health and development. Bacterial AMR was directly responsible for an estimated 1.27 million global deaths in 2019 and contributed to 4.95 million deaths.[1]

AMR puts many of the gains of modern medicine at risk. It makes infections more difficult to treat and significantly increases the risks associated with other medical procedures and treatments. In addition to death and disability, AMR has significant economic costs. The World Bank estimates that AMR could result in US$1 trillion in additional healthcare costs by 2050 and losses of US$1 trillion to US$3.4 trillion in gross domestic product (GDP) per year by 2030[2]. Priorities for addressing AMR in human health include strategic innovation and the research and development of new medicines. In this regard, compounds extracted from plants show promise. Garlic has potential as an antimicrobial agent, and the dissemination of knowledge and research on this plant should be encouraged.

The use of plants to treat illnesses and promote overall well-being dates back thousands of years. Species such as *Zingiber officinale* (ginger), *Mystirica frangrans* (nutmeg) and *Allium sativum* (garlic) gained prominence due to their antimicrobial activity and are currently being studied as potential medicines to combat microorganisms.[3,4,5] However, it should be noted that the use of these species goes back to antiquity, as exemplified by *Allium sativum*, which was used by many people to treat diseases. Since that time, garlic has been credited with fungicidal and bactericidal properties, in addition to being considered useful in the treatment of heart disease, headaches, diabetes and even cancer.[6,7,8]

The *Allium sativum* species belongs to the Amaryllidaceae family, has Asian origins and is produced all over the world. It is composed of a subglobose bulb, divided between 620 bulbils (garlic cloves) surrounded and held together by protective leaves. The bulbils are attached to a stem, which has some fibrous roots on its lower surface. Each bulbil has an ovoid shape, is asymmetrical and has a whitish, pink, or violet color. In cross-section, the bulbil is formed by an outer part, called the scarious prophyll, which surrounds a reserve cataphyll, a fleshy structure, in which the main assets with vegetable medicinal properties are found.[3,9]

The search for alternative antimicrobial medicines, such as garlic, has gained importance in the current context, in light of the growth in bacterial resistance and the difficulty in combating pathogens that cause common infections by use of traditional antibiotics. This phenomenon is related to the misuse and abuse of these medications. In this sense, research into new antimicrobials can help in the search for effective treatment options against bacterial resistant infections.[10,11] Therefore, the main objective of this study is to provide an update on the antimicrobial properties of the species *Allium sativum*, through the compilation and critical analysis of available sources, in order to understand how this vegetable can influence the treatment of infectious diseases. Finally, we also seek to understand the varied chemical components that can be extracted from the different structures of the plant.

Materials and methods

A search was made for original articles on the PubMed, Scopus and Scielo platforms, following PRISMA guidelines (Reporting for Systematic Review and Meta-Analysis Protocols)[12] to guide the research. Two different reviewers, using pre-established standards, independently read articles that presented studies on the antimicrobial properties of garlic and reached a consensus on which ones would be selected and which ones excluded. The descriptors were identi-

fied using the MeSH terms (Medical Subject Headings) thesaurus and an advanced search was carried out using Boolean operators. Both platforms used a combination of descriptors: "Garlic AND Anti-infective agents" and "Garlic AND Antimicrobial".

Table 1. Search strategy for the literature review and number of results found in the databases

	PubMed	Scopus	Scielo
Identification (total)	67	306	67
(Garlic) AND (Anti-infective agent)	33	161	47
(Garlic) AND (Antimicrobial)	34	144	17

End date of search: 31/Jan/2024

Source: The authors (2024).

First, the titles and abstracts of the studies were read. Then, the texts selected in the first stage were read and a final selection of articles for review was made. The inclusion and exclusion criteria for the articles were discussed and agreed among the researchers.

Studies were excluded according to the following criteria: i) showing the properties of garlic, but not its antimicrobial properties; ii) showing plants' antimicrobial properties, but not those of garlic; iii) lack of an original study (reviews, editorials, comments, letters); iv) articles in languages other than Portuguese, English or Spanish. The following inclusion criteria were used: i) original articles (clinical trials, randomized clinical trials, controlled clinical trials and case reports); ii) demonstrated association of antimicrobial properties and garlic; iii) articles that analyzed the antimicrobial properties of garlic in humans, animals and *in vitro*; iv) articles in Portuguese, English or Spanish; and v) articles on garlic on its own were included in this research.

Table 2. Flow diagram of survey results, based on preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement

IDENTIFICATION	ARTICLES IDENTIFIED PubMed (67) Scielo (67) Scopus (306)	TOTAL 440
SCREENING	**440 articles**	**337 articles excluded.** 58 were duplicates. Garlic not associated with antimicrobial properties Lack of originality
ELEGIBILITY	**103 article titles or summaries read**	**57 articles excluded** 6 articles -> Garlic associated with antibiotics 5 articles -> Garlic associated with other therapies 20 articles -> Garlic in food 20 articles -> Garlic against oral pathogens Others
INCLUDED	**46 articles**	17

Source: The authors (2024).

After application of the criteria, 103 articles were selected. These were tabulated and classified as follows: garlic by itself (46 articles); garlic with antibiotics (6 articles); garlic with other substances (5 articles); garlic in food (20 articles); garlic against oral pathogens (20 articles); others (6 articles). Only articles solely about garlic are reviewed in this research.

Results

Initially, 440 articles were selected, but only 46 met the pre-established criteria and were eligible to take part in this review. The selected articles were reviewed, and some topics on *Allium sativum* (garlic) were found to be frequently addressed. These can be divided into the following categories: phytochemical composition of the extract; antimicrobial properties and sensitive pathogens; efficacy of garlic extract when compared to those of other plants; efficacy of garlic extract when compared to broad-spectrum antibiotics used in medical practice (Table 3).

Table 3. Subject, objective, methods and highlights extracted from the articles included in this study

Subject	Aim	Method	Highlights
Phytochemical composition[13-19]	Analysis of the phytochemical composition of *Allium sativum* in different regions of the world. Trials were carried out to identify and quantify the phenolic and organosulfur compounds, vitamins, minerals, and volatile and non-volatile substances present in garlic; in addition, the main active compounds in garlic were identified with regard to their antimicrobial and antioxidant properties, as well as the influence of processing methods on such properties.	The garlic was harvested in different parts of the world (e.g. Australia, Nigeria and Brazil). The plant was dried at room temperature or in the sun, crushed using a pestle or other instrument and then transformed into powder. From this, aqueous or alcoholic extracts were obtained, using techniques such as ultrasound, Soxhlet, Headspace SPME and maceration. The extracts were filtered for antimicrobial analysis. Techniques of diffusion in agar, microdilution in well or disk were then used. Finally, the minimum and maximum inhibitory concentration was determined for each microorganism tested and any growths were analyzed. The inhibition zones for each pathogen were measured. Three different methods were tested to observe the respective impact of these methods on the chemical composition of the garlic. Three different methods were tested: shade drying, freeze drying and microwave drying. In addition, the impact of roasting garlic on its biochemical properties was also evaluated.	Garlic has several bioactive compounds with antimicrobial properties. The most important component is allicin; however, several studies have emphasized the importance of y-glutamyl-S-allyl-cysteine, γ-glutamyl-phenylalanine and E- and Z-ajoenes. Ajoenes gained prominence in the study against pathogens involved in Tinea and showed good results, being a possible ally to terbinafine, the drug currently used against this condition. Thirty organosulfur compounds and 51 non-volatile compounds were also identified in garlic, including dipeptides, flavonoids and phenolic acids. Many studies still need to be carried out on substances that are unknown or have not been the subject of extensive study. In addition, with regard to drying methods, studies found that freeze drying, and microwave drying were the best ways to preserve the composition of garlic. The type of drying that had the least impact on antimicrobial potential was freeze drying. Finally, with regard to the roasting process, the results concluded that roasted garlic had better bioactive properties than garlic *in natura*.
Antimicrobial properties and sensitive pathogens[5,7,20-36]	Evaluation of garlic and its antimicrobial properties, with emphasis on determining which microorganisms are sensitive to the plant and at what concentration.	Pathogens collected from different sites (skin, mouth, vagina, prostheses, among others) were isolated and placed in specific culture media (Blood agar, Nutrient agar, Sabouraud agar) depending on the microorganism under evaluation. Subsequently, aqueous garlic extract, garlic essential oil or garlic *in natura* (depending on the study) were added in different concentrations using the microdilution technique in wells, agar diffusion or disc diffusion and determining the maximum and minimum inhibitory concentration. The plates were subsequently checked for the presence of any bacterial growth.	The studies found that various pathogens were sensitive to garlic extract, which effect was related to the concentration of the extract. For this reason, the garlic extracts were tested at different concentrations to assess their antimicrobial capacity. At a concentration of 25mg/mL, none of the pathogens evaluated - *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* - were sensitive. At a concentration of 50mg/mL only *E. coli* was sensitive, showing an inhibition zone of 5cm. At a concentration of 75mg/mL, a zone of inhibition measuring 8cm was observed for *S. aureus* using the aqueous extract (EA) and 10cm using the alcoholic extract (EAL), a zone of inhibition for *E. coli* of 9cm (EA) and 10cm (EAL) and a zone of inhibition for *K. pneumoniae* of 8cm (EA) and 7cm (EAL). In this test, all the pathogens proved to be sensitive, but some studies have questioned the sensitivity of *E. coli*, which has proven to be resistant in other experiments. Furthermore, garlic has shown antifungal potential against *Candida* spp. and *Sporothrix schenckii*. Studies have also tested garlic's ability

Source: The authors (2024).

Table 3. Subject, objective, methods and highlights extracted from the articles included in this study (cont.)

Subject	Aim	Method	Highlights
Antimicrobial properties and sensitive pathogens[5,7,20-36]			to inhibit fungi that cause Tinea. Ointments containing the extract were used and the results were promising, but more studies are needed on this topic. The anti-*Giardia* capacity of garlic extracts was also evaluated, and garlic was shown to be a potential medicine for use against this protozoan, since allicin has the capacity to inhibit the pathogen.
Comparison with other antibiotics[34-44]	Comparison of the antimicrobial effects of *Allium sativum* with those of traditional medicines already used to treat infections. Antibiotic and antifungal drugs were analyzed against a variety of pathogens common in clinical practice. The studies varied in the way the plant was analyzed, with the majority using the aqueous extract of the plant. The degrees of inhibition, response time and doses required for each therapy were compared, with most studies seeking to find the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC) for garlic and the drugs according to each pathogen analyzed.	Most of the studies analyzed the minimum concentrations using the agar diffusion method. Samples of bacteria and fungi were isolated from various places, such as vaginal lesions, the nasopharynx of infected people, beef, urine, among others. After isolation, each pathogen was cultivated in the appropriate medium for its growth, respecting the incubation time required for the analysis to be carried out. Meanwhile, garlic was prepared for addition to the microorganisms' culture medium. Most of the studies used the plant's aqueous extract, made by mixing a certain amount of the plant in grams with a few mL of distilled water. Another group of studies used fresh garlic, cut into discs and applied directly to the pathogens. In the case of the isolated analysis of certain garlic compounds, the extraction processes used materials such as methanol. Subsequently, the garlic samples and traditional medicines were added to the microorganism isolates by diffusion methods in Petri dishes or by microdilution in test tubes. Finally, the macroscopic aspects of the result were observed, such as the appearance of turbidity and the measurement of the diameter of the inhibition halos. Microscopic characteristics were analyzed using electron microscopy, spectrophotometry and colorimetry methods. Most of the analysis used a simultaneous negative control.	Most studies have shown that garlic has similar or even greater antimicrobial potential than some traditional medications. In studies comparing the effects of the plant with the efficacy of fluconazole on *Candida* spp. isolates, the combination of two garlic extracts proved to be more effective than the drug in inhibiting the growth of the pathogen. In another study, resistant strains of some microorganisms, such as *Methicilin Resistant Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*, also proved resistant to various concentrations of garlic extracts. The plant's main mechanisms of action include destabilizing the plasma membrane and interfering with the metabolism of pathogens. Much of the research suggests that the plant has the potential to be combined with traditional medicines to enhance the treatment of infections in the community and in health centers. However, it is important to emphasize that most of the studies have been carried out using *in vitro* tests and more *in vivo* tests need to be conducted in order to draw more solid conclusions about the efficacy and safety of using garlic.
Comparison with other plants[28,39,45-53]	Comparison of the antimicrobial effects of *Allium sativum* with other plant varieties and other natural products.	The main compounds of each plant or natural product were extracted. Different methods were used in the varieties of studies analyzed. Garlic compounds were extracted under pressure and with the addition of an extractant, such as distilled water, methanol or physiological NaCl solution. Their antimicrobial properties were analyzed using the diffusion method, with subsequent calculation of the zone of inhibition around the site where the extract was applied. The efficacy of extracts obtained from garlic was compared with those of other varieties, such as ginger, chili, lemon and honey. Each study selected a specific pathogen, for example, *Streptococcus mutans, Escherichia coli*, among others.	The comparison of the antimicrobial activities of garlic species with those of other plants, showed that *Allium sativum* was much more effective. In comparative studies that also analyzed lemon, honey and ginger, garlic showed a potent anti-infective activity against a greater number of pathogens and faster action in improving symptoms and remission of the disease. In other studies, which also evaluated the properties of some onion species, garlic showed efficacy against almost all pathogens, while the other plants showed little or no effect. It should be noted that a large part of garlic's antimicrobial character is derived from the presence of allicin and phenolic compounds in its composition. Finally, all the studies highlight the need for further analysis to consolidate and innovate knowledge about the use of garlic and other plants in the treatment of infections.

Source: The authors (2024).

Discussion

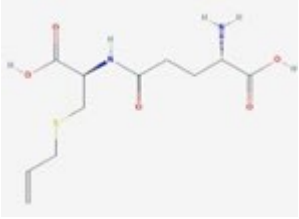
Antimicrobial resistance is a public health problem, the solution of which depends on the search for therapeutic alternatives for treatment of resistant microorganisms. Among the promising alternatives, pharmacological repositioning and research on the development of new drugs stand out. Research into the pharmacological potential of plants is one possible avenue of action and has been gaining prominence in recent years. Since garlic is a food with pharmacological properties that are already well-known to many civilizations, scientific experimentation to evaluate therapeutic evidence is essential. This study aims to gather and discuss the results of research on the antimicrobial potential of garlic to facilitate new studies based on the existing scientific evidence.

Contemporary studies have confirmed the importance of the medicinal properties of this plant in the context of infectious diseases, due to its antibacterial, antifungal and antiviral characteristics. Much of this function is known to be derived from the organosulfur compounds, flavonoids and terpenoids present in garlic. [8,45] Recent research analyzed the inhibitory potential of the vegetable on the growth of microorganisms that cause recurrent infections in the community and in hospital contexts. Several studies have already demonstrated the action of *Allium sativum* in controlling *Escherichia coli*, *Candida albicans* and *Streptococcus* spp.[14,15,16] Furthermore, an ability to inhibit the growth of viruses such as *Coxsackievirus* spp. and herpes simplex virus types 1 and 2 was also observed.[47]

The phytochemical composition of the extract made from garlic cloves was found to be rich in bioactive compounds that enhance its medicinal and nutritional properties. The main active compounds in garlic are organosulfur constituents, such as allicin, which have different mechanisms of action related to fighting infections. They are responsible for many of garlic's health benefits, including antioxidant and antibacterial activity. In this regard, these components can prevent the formation of biofilm by bacteria, destabilize the plasma membrane of microorganisms and interfere with the synthesis of DNA and RNA, in addition to other processes that act on the different forms of growth and survival of pathogens.[3,4]

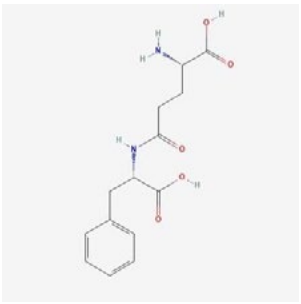
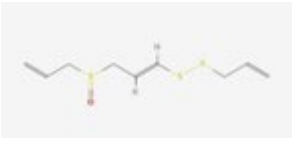
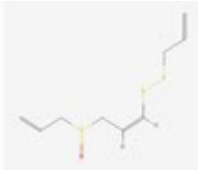
According to Torres *et al.*[15] and Barbu *et al.*,[14] other compounds with antibacterial function are y--glutamyl-S-allyl-cysteine, γ-glutamyl-phenylalanine and E- and Z-ajoenes. Garlic also contains phenolic compounds, such as flavonoids and phenolic acids. Other important substances include vitamins, especially vitamin C, and minerals, such as selenium and magnesium.

Table 4. List and structure of some compounds isolated from Allium Sativum

Compounds	Molecular formula	Structure
Allicin	C6H10OS2	
Gama-glutamyl-S allycisteine	C11H8N2O5S	

Source: https://pubchem.ncbi.nlm.nih.gov/

Table 4. List and structure of some compounds isolated from Allium Sativum (cont.)

Compounds	Molecular formula	Structure
Gama-glutamyl-phenylalanine	C14H18N2O5	
E-ajoene	C9H14OS3	
Z-ajoene	C9H14OS3	

Source: https://pubchem.ncbi.nlm.nih.gov/

In line with this finding, another study[45] detailed the phytochemical composition of five parts of the food that are usually wasted – the root, garlic clove skin, garlic peel, flower stalk and leaf – and quantified the phenolic compounds and flavonoids present in them and their antioxidant capacity. The article concluded that the garlic leaf had an even higher content of phenolic compounds and flavonoids than the garlic clove and the other parts, as well as having an effective antioxidant and antitumor power. Allicin was found in significantly lower quantities in the flower stalk and leaves than in the garlic clove.

Research has been conducted to test the antimicrobial capacity of the aqueous/alcoholic extract of garlic against gram-positive and gram-negative bacteria and fungi. The main pathogens tested were *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida* spp.[14,35,45]

Vargas *et al.*[48] analyzed garlic extracts with different concentrations of allicin and concluded that a garlic extract containing 3mL of allicin was able to completely inhibit the growth of the bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* and was unable to inhibit the growth of *Escherichia coli*, with this pathogen being more resistant to the antimicrobial compound studied at this concentration.

The other concentrations studied were unable to prevent bacterial multiplication. From a similar perspective, Abidullah *et al.*[49] also tested the efficacy of the aqueous and alcoholic extract of garlic against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* at concentrations of 100mg/mL and observed the formation of an inhibition zone of 12mm (*S. aureus*), 7mm (*K. pneumoniae*) and 10mm (*E. coli*). It was therefore concluded that the extract was effective against these micro-organisms and that, at higher concentrations, *E. coli*

is sensitive to garlic. Finally, the antifungal potential of garlic, which was proven in a clinical trial[23] carried out with women diagnosed with candidiasis, is also worth highlighting. The women were given garlic tablets to treat the fungus, and the results showed that garlic could be a possible alternative to fluconazole, currently the gold standard drug for treatment of this condition. However, further studies are needed.

In general, garlic has greater antimicrobial potential than other vegetables when comparisons are made. Farbman *et al.*[50] evaluated the in vitro activity of garlic and onion species against a variety of bacteria and concluded that garlic is more effective, emphasizing the importance of components such as allicin. Onions did not exhibit any inhibition of the variety of pathogens tested. In addition, when studying the antimicrobial properties of a range of foods used in Indian cuisine (garlic, chilies, ginger, onions), Indu *et al.*[5] concluded that garlic was effective against all the bacteria investigated, such as *E. coli* and *L. monocytogenes*. The other vegetables, on the other hand, proved to be less or not at all effective. These conclusions demonstrate the need to devote more resources and studies to the properties of garlic, since it is one of the vegetables with the greatest antimicrobial potential known today.

Finally, it is worth noting that many authors have also evaluated the antimicrobial properties of the plant in comparison with traditional medicines. Ebrahimy *et al.*[44] compared the effects of garlic capsules in the treatment of *Candida* spp. vaginitis with the commonly used drug fluconazole, finding no significant differences in the response to treatment following the two interventions. Indu *et al.*[5] compared the activity of the plant with the performance of traditional antibiotics, such as ciprofloxacin and chloramphenicol, and the plant showed excellent antimicrobial activity and could be an alternative treatment in light of the increasing resistance and development of new strains of pathogens. Another study evaluated the antimicrobial potential of allicin against *T. rubrum*, the fungus that causes dermatophytosis, comparing treatment with ketoconazole. The study concluded that the concentration of allicin required to have the same effect as ketoconazole is higher, but that one substance is almost as effective as the other, and called for more in vivo experiments to advance knowledge of these healing properties of garlic.[24] These conclusions confirm the plant's potential to become an alternative or an ally to traditional treatments, thus helping to expand therapeutic approaches to community illnesses.

Conclusion

The studies analyzed show that *Allium sativum* is a plant with great antimicrobial potential, and appears to be a good option for association with traditional medicines or, possibly, replacement of them in the treatment of important conditions, such as infections by *Eschericia Coli*, *Staphylococcous aureus*, *Klebisiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida* spp. This anti-infective property is derived from a variety of plant components, especially allicin and phenolic compounds. The studies evaluated the effects of garlic prepared in different ways, such as *in natura*, in aqueous and alcoholic extracts, as essential oils and in other forms.

One of the issues analyzed during the search for studies was the macrostructure of garlic, its divisions, and their possible relation with its properties. In this sense, the plant is divided into roots, bulb, stem, clove and leaves. The bulb is formed by the garlic clove, a fleshy structure, which is covered by a film. The largest number of phenolic compounds and flavonoids, which are important constituents in the antimicrobial character of the vegetable, were

found in the leaf. Meanwhile, allicin, the main bioactive component of the plant, is mostly located in the stem and leaves.

Among several conclusions, we can highlight the effects of garlic on the metabolism, in the destabilization of the plasma membrane of pathogens and in the adhesion of microorganisms, in addition to its influence on the formation of biofilms by bacteria. In this context, the action of the vegetable against the bacteria *Escherichia coli* and the fungus *Candida albicans*, which are relevant microorganisms in the treatment of patients in intensive care units, was deemed to be effective.

Based on all the knowledge acquired and considering the effectiveness of garlic, as well as the safety and the growth of microbial resistance to traditional medicines, one can conclude that *Allium sativum* presents a possible option in the treatment of patients and an excellent contributor to the strengthening of the human immune system. The vegetable appears to be an accessible and promising alternative and requires further studies to develop effective and reliable treatments for the best-known pathogens. The present study is an instrument to consolidate this knowledge and to promote new research on this subject.

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Declaration of interest statement

No potential conflict of interest was reported by the authors.

Data availability statement

The datasets generated used and analyzed during the current study are available from the corresponding author upon reasonable request.

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