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Original Article

Phytochemical Profile and Antimicrobial Activity of Aqueous Garlic Extract (Allium sativum L.) for Food Preservation

Elmer Torres[1], Grimaldo Quispe[1], Henry Javier[2], Carlos Raymundo[3], Heyul Chavez[4], Francisco Dominguez[4]

[1]*Altoandina National Autonomous University of Tarma, Huaraz Tarma, Peru.*
[2]*Technological University of Peru, Faculty of Engineering, Huancayo, Peru.*
[3]*R&D Lab. in Emerging Technologies, Peruvian University of Applied Sciences, Lima, Perú.*
[4]*School of Computer Engineering, Rey Juan Carlos University, Madrid, Spain.*

[3]*Corresponding Author : carlos.raymundo@upc.edu.pe*

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Abstract *- The contamination of seafood by common bacteria and the ecological limitations of antibacterials has prompted the investigation of the phytochemical profile and antimicrobial activity of aqueous garlic extract (Allium sativum L.) against four significant food-associated pathogens: Salmonella typhi, Micrococcus luteus, Staphylococcus aureus, and Escherichia coli. Absorbance curves were constructed, inhibition halos were measured, and minimum lethal concentrations were determined. This study aims to harness the properties of garlic and its derivatives, making them ideal for use in pharmaceutical and industrial products. The results showed greater inhibitory activity against Gram-positive bacteria compared to Gram-negative bacteria. Specifically, a concentration of 0.125 g/ml of the extract produced inhibition halos with diameters of 0.75 mm for S. typhi, 0.8 mm for M. luteus, 11 mm for S. aureus, and 8.25 mm for E. coli. Furthermore, a concentration of 1.0 g/ml of the extract exhibited bactericidal activity at 210 minutes against M. luteus, 330 minutes against S. typhi, and 270 minutes against S. aureus, but it showed no such effect against E. coli.*

Keywords *- Phytochemical, Antibacterial activity, Aqueous extract, Minimum lethal concentration, Inhibitory activity.*

1. Introduction

The main cause of food contamination in marine products is pathogenic bacteria, which usually have a low toxic effect; however, they can cause serious illnesses [1], such as salmonella, Micrococcus luteus [2], Staphylococcus aureus [3], and S. typhi [4, 5]. Globally, the therapeutic use of plants in traditional medicine has widely expanded [6,9]. Information on the phytochemicals in these plants is currently being used to develop new antibacterial materials for food coating [10, 14]. Among the various plants being investigated in the context of phytotherapy, garlic (Allium sativum) stands out as a promising candidate see Figure 1 [15]. Its properties and the quality of its derivatives have been extensively studied [16, 17]. For instance, the feed of tilapia was supplemented with garlic to test its efficacy in controlling Streptococcus iniae, which can cause up to 10%–15% of production loss [18]. In both fresh and saltwater, garlic solution increased survival rates due to its positive effect on intestinal health. In vitro assays revealed that its medicinal activity was 81.2% of moderate sensitivity, more potent than leaves of the mala and bohera plants. The expression of inflammatory cytokinin genes was significantly regulated by garlic powder treatment [19]. Due to its importance in food and health, substantial research has focused on Allium sativum [20-23]. With more than 500 species, Allium is the largest genus within Amaryllidaceae [24].

Fig. 1 Garlic (Allium sativum)

Some strains of Escherichia coli can acquire virulent genes and cause diseases in the intestines, urinary tract, and even the brain. For example, Shiga toxin-producing E. coli causes bloody diarrhea and fatal hemolytic uremic syndrome. Garlic (A. Sativum) exhibited antibacterial and antibiofilm activities against such E. coli isolates, with a minimum inhibitory concentration ranging between 30–140 ml, as measured using a colorimeter [25]. Three peptides from Laba garlic showed a zone of inhibition of 100 µm against E. coli and S. aureus, demonstrating an antimicrobial effect and inhibiting mycelial growth without any hemolytic activity.

High-Performance Liquid Chromatography (HPLC)–mass spectrophotometry revealed that garlic, added to a final concentration of 10% in chilli sauce, was effective against Bacillus cereus, yeast, and mold over 30 days and could easily replace the chemical preservatives normally used [26]. Despite increasing the pH (3.56–3.96), OSH (33.77–39.90), and viscosity, the taste of the garlic-supplemented chilli sauce was acceptable [27, 28].

Garlic extract is often used to treat lifestyle disorders. It is extracted at 60°C in water for 5 hours. For a higher phenol content, 1.69% methanol is used for 45 minutes. Although this increases the pH and the content of total soluble solids over time, the L value increases after 28 days [29].

Phytochemical profiling is used to determine the content of anthraquinones, anthrones, chromones, coumarins, pyrans and pyrones, alkaloids, flavonoids, carbohydrates and related compounds, amino acids, and lipids in plant extracts. These compounds possess various activities, such as antioxidant, antimicrobial, antimalarial, antiviral, hepatoprotective, anticancer, and wound healing activities [30, 31].

Phytochemical profiling of aculeata leaves (Ora-pro-nobis) was performed to determine their activity against K. pneumoniae compared with ampicillin, showing inhibitory effects without hepatotoxicity [32].

Phytochemicals in ergot table olives have also been profiled [33]. The phenotypic structures of allelochemical compounds from okra (Abelmoschus spp.) and their resistance against Aphis gossypii Glover, A. caillei, and A. esculentus can be used to improve crop productivity.

Bioactive compounds responsible for aloe vera's use in nutritional supplements and cosmetics have also been identified [31, 34]. Main studies on bacteria and their corresponding phytochemical profiles are described in Table 1.

Methods for phytochemical profiling include oxidative hemolysis and inhibition assay, inhibition of thiobarbituric acid reactive substances production, reduction of 2,2-diphenyl-1-picrylhydrazyl radical, reduction of 2,2-azino-bis cation (3-ethylbenzothiazolin-6-sulfonate), and hydroxyl radical removal assay [32]. These methods are mostly performed in vitro to simulate gastrointestinal digestion, and commonly found chemicals include secoiridoids, with isomers of oleuropein and comselogoside, and elenolic acid, whose antioxidant potential was determined by HPLC [33].

The foliar extracts of Fouquieria splendens, examined by HPLC with diode-array detection, ultraviolet-visible spectrophotometry, and attenuated total reflection Fourier transform infrared spectroscopy, showed significant variations in their phenolic compounds under different conditions [36, 37]. Plants with a higher phenol content demonstrate better antioxidant activity [38].

The antibacterial properties of plants have been widely studied. Ottonia anisum exhibits activity against larvae of Aedes aegypti and interferes in the pupal and adult stages at a concentration of 200 µg/ml. Its environmental safety makes it a better pesticide than synthetic compounds, and it is also an economical antiviral against rainforest viruses such as dengue, Zika, yellow fever, and chikungunya. The pure extract was spectrophotometrically examined to determine the amount of deuterated dimethyl sulfoxide [39].

The phytochemicals megareveratrol and naringenin counteract the effects of E. coli in apple cider over 14 days of storage at 4ºC, making them viable as natural antimicrobial additives due to their sensory characteristics [40].

The leaves of caamembeca (Polygala spectabilis - EFC) and figo (Ficus carica - EFF) possess potent antimicrobial activity against Clostridium perfringens, E. coli, P. aeruginosa, and S. aureus. Trichoderma inoculated into potato dextrose agar in liquid media controls cocoa pod rot, reducing Phytophthora palmivora incidence from 40.61% to 38.02% at 12 WAA [41].

Table 1. Bacteria studied and source

Bacteria studied	Source
S. aureus, E. coli, C. albicans	[35]
S. aureus, B. subtilis, E. coli, S. typhimurium, B. turengensis, P. aeruginosa, K pneumoniae, R. oryzae, A. niger, M. luteus, and E. faecalis	[34]
E. Coli	[25]
Candida, Trichophyton, Cryptococcus, Aspergillus, Trichosporon and Rhodotorula	[24]
lumbricoides, Ascaridia galli.	[23]
S. aureus MSSA, *P. aeruginosa* and *K. pneumoniae* ESBL, *E. faecalis, E. coli, S. aureus* MRSA and *E. coli* MBL	[20]
Oreochromis niloticus and the control of *Streptococcus* infection	[19]

Despite extensive research on garlic's antimicrobial properties, there remains a significant gap in understanding its potential as a natural preservative in various food matrices. This study aims to fill this gap by exploring the phytochemical profile and antimicrobial efficacy of aqueous garlic extract (Allium sativum L.) specifically for food preservation.

Compared to previous studies, this research focuses on the practical application of garlic extract in inhibiting common foodborne pathogens and extending the shelf life of perishable food products. By leveraging advanced phytochemical profiling techniques and comprehensive antimicrobial assays, this study provides novel insights into the functional benefits of garlic in food preservation, highlighting its potential as a natural, safe, and effective alternative to synthetic preservatives.

2. Materials and Methods

2.1. Materials

The laboratory equipment used in this study were as follows: electronic shaker (KS 250 Basic Capacity 500 rev/min), compound microscope (Leica 5000), drying hood, racks, test tubes, flasks, pipettes, electric cooker (HELIOS), incubator (Memmert and JP Selecta model 207), kitchen equipment (Bain Marie) from 0ºC–100ºC, vacuum pump (Groschopp), refrigerator (Coldex Model R162), weighing balance (DIAL GRAM, of 310 g capacity), micropipettes of 10–100 µl capacity, type, seed homogenizer, and electric cooker. The chemical reagents used were as follows: 96% alcohol, sterile water, NaCl, detergent, 0.1 N hydrochloric acid, 0.1 N sodium hydroxide, silica gel, ninhydrin reagent, ferric chloride, magnesium tape, iodine alcohol, Dragendorff's reagent, nitric acid, and Benedict's reagent.

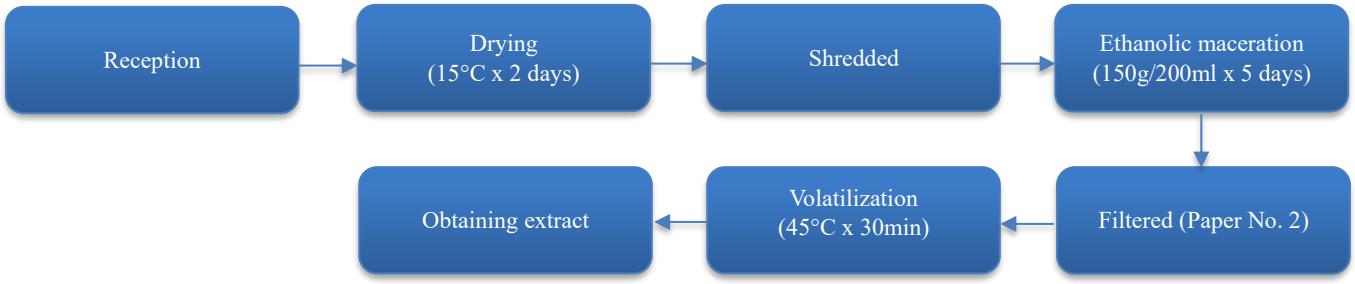

Fig. 2 Obtaining the aqueous extract

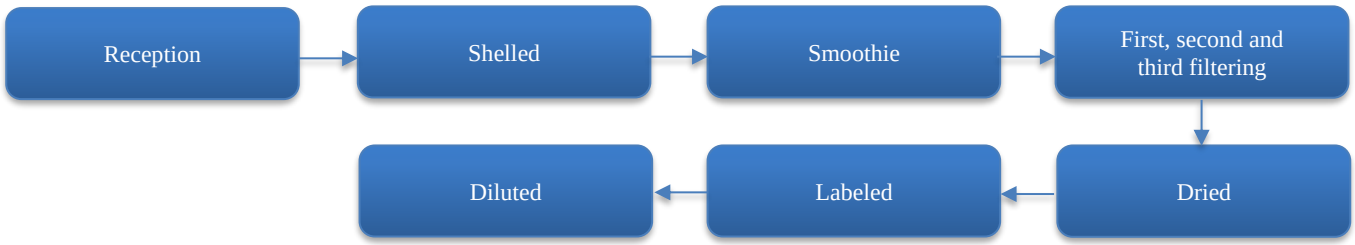

Fig. 3 Obtaining the ethanolic extract

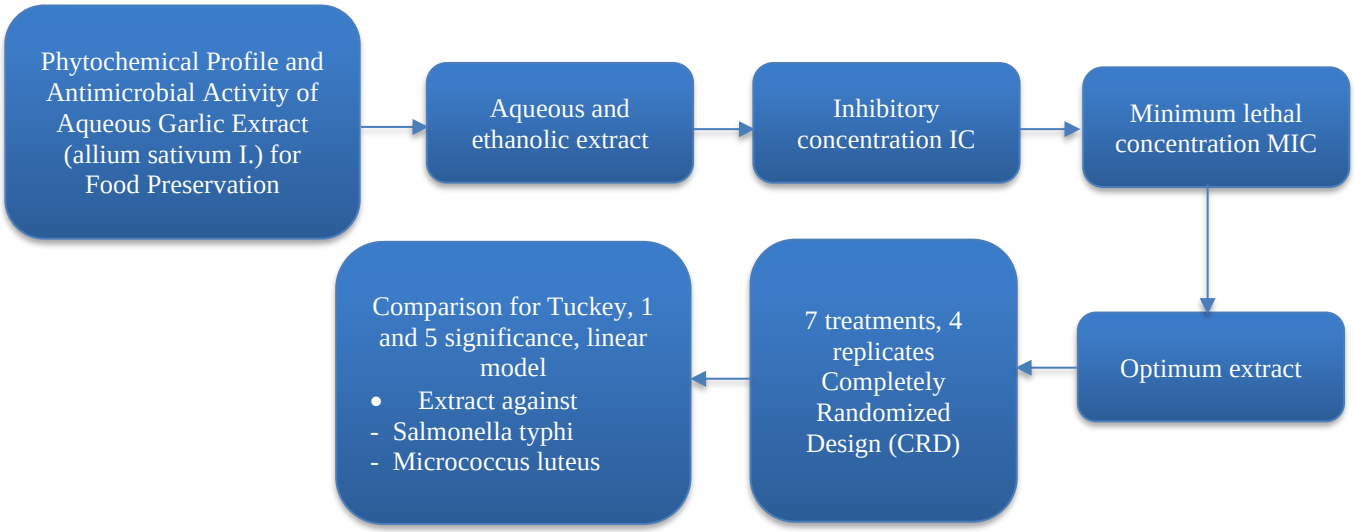

Fig. 4 Phytochemical profile and antimicrobial activity methods

2.2. Methods

The first step in obtaining an aqueous garlic extract involves harvesting fresh garlic bulbs of the Serrano variety from the Tarma province in Peru. After manual shelling, the garlic is liquefied with the addition of 10% water relative to the bulb's weight. The aqueous extraction was carried out using 46.67% w/v biodistilled water, with the largest particles being removed during the initial filtration. The extract then underwent a second filtration through No. 2 filter paper and a third filtration through a 0.5 µm membrane.

The resulting antimicrobial liquid was dried with silica gel, labeled, and diluted in distilled water to the following concentrations: 1 gram per milliliter, 0.5 grams per milliliter, 0.25 grams per milliliter, and 0.125 grams per milliliter [42-44] (see Figure 2). To obtain an ethanol extract, garlic of the serrano variety was dried for 2 days at 15°C with natural ventilation, crushed in a mortar, added to alcohol at 150 g/200 ml for days with constant agitation, filtered through a No. 2 filter paper, and incubated in a water bath at 45°C for 30 minutes to volatilize the organic solvent see Figure 3.

The averages of the inhibition halos obtained after the effect of the aqueous extract against S. typhi and M. luteus were compared using Tukey's test at a significance level of 1 and 5 in a linear model. Figure 4 describes each process in more detail. The phytochemical profile was assessed using in vitro tests performed in the Universidad Nacional Autónoma Altoandina de Tarma laboratory. Secondary metabolites in the aqueous extract of garlic were identified by spectrophotometry, and minerals were quantified by atomic absorption.

Alkaloids were determined by reacting 1% HCl with Dragendorff's reagent, saponins by persistent foaming, tannins using 1% ferric chloride to precipitate and color the sample, total proteins by the xanthoprotein reaction, starch-type polysaccharides with drops of Lugol, reducing-type sugars using Benedict's reagent, and amino acids using ninhydrin reagent. Dragendorff's reagent was prepared by first dissolving basic bismuth nitrate in a solution of tartaric acid and water, then combining it with a potassium iodide solution.

Specifically, 1.7 g of basic bismuth nitrate was dissolved in a solution of 20 g of tartaric acid in 80 ml of water and mixed with 16 g of potassium iodide in 40 ml of water. This mixture was equalized in volume and refrigerated at 4°C. Finally, 5 ml of this solution, at room temperature (25°C) and standard atmospheric pressure (1 atmosphere), was dissolved in a solution of 10 g of tartaric acid in 50 ml of water (see Figure 5). To determine the inhibitory concentration of aqueous garlic extract (A. sativum L.), first, Trypticase soy agar (TSA) was poured into 12 Petri dishes (15 ml each).

After 24 hours, Culture Medium Semisolid Agar containing Trypticase soy broth + 0.6% of granulated agar was poured onto the TSA (15 ml each) at 40ºC. Wells, 6 mm in diameter, were pierced, and the aqueous garlic extract was inoculated at varying concentrations: 1 gram per milliliter, 0.5 grams per milliliter, 0.25 grams per milliliter, and 0.125 grams per milliliter (40 µl each).

The plates were incubated for 24 hours at 35°C, after which the inhibition halos were measured in millimeters see Figure 6. To determine the minimum lethal concentration of aqueous garlic extract, 1 g of garlic extract was dissolved in 1 mL of distilled water, 100 µl of pathogenic microorganisms were added, and the suspension was incubated at 35°C for 24 hours. The colony-forming units per milliliter of inoculum were measured (see Figure 7). To prepare the TSA culture medium, the required ingredients were weighed and placed in an Erlenmeyer flask, except for the granulated agar, which was added hot. Distilled water was then added as needed.

The mixture was homogenized with a glass rod and heated on a tripod with an asbestos grid until the ingredients dissolved. After cooling to 50ºC–55ºC, the pH was adjusted to 7.2–7.4 using 0.1 N HCl or NaOH. The medium was then distributed into glass bottles, filled halfway, and capped with aluminum foil and straw. The bottles were sterilized in an autoclave at 121ºC and 15 pounds of pressure for 15 minutes. After cooling to 50ºC–55ºC, 15 ml of the medium was poured into each plate under aseptic conditions. To confirm sterility, the plates were incubated at 35ºC for 24 hours and appropriately labeled.

For inoculating the microorganisms, the liquid-to-liquid method was employed in test tubes, ensuring the internal walls were not touched. The tubes were incubated at 37ºC for 24 hours and labeled with the group number, strain name, and date.

To assess the garlic extract, seven treatments, each of the aqueous and ethanolic extracts and four replicates of each treatment were used. The interaction between the factors under investigation was determined using Tukey's test, with a significance level of 1% and 5%.

The linear model obeys the following formula:

$$Y_{ij} = \mu + T_i + E_{ij} \qquad (1)$$

The antibiotic activity of the extract against some pathogenic food-associated bacteria was qualitatively assessed using the agar diffusion method.

Briefly, a standard solution of the test microorganism was inoculated at various concentrations along with the extract. After an appropriate incubation period, a distinct inhibition zone could be observed. In this study, 90 µl of the garlic extract was impregnated onto filter paper disks.

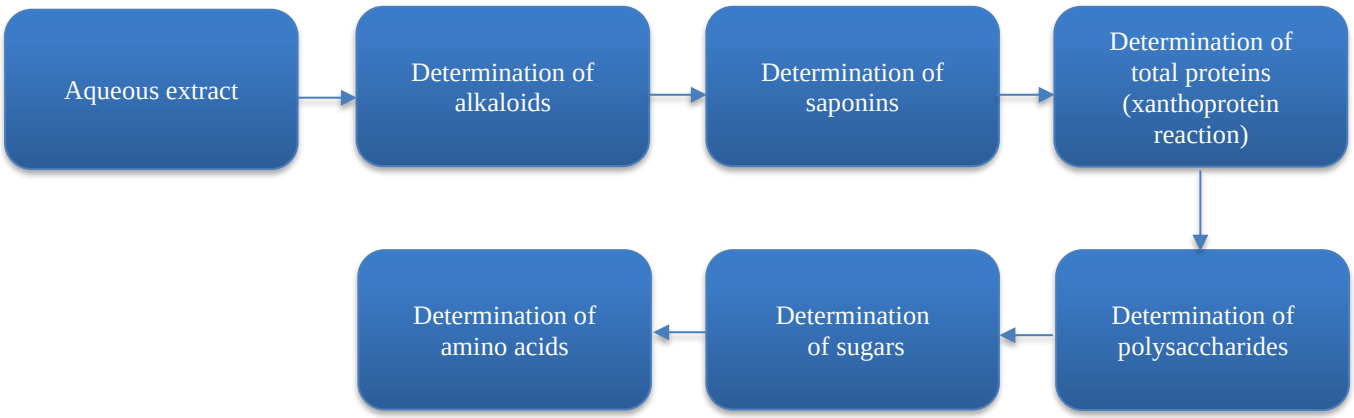

Fig. 5 Phytochemical march

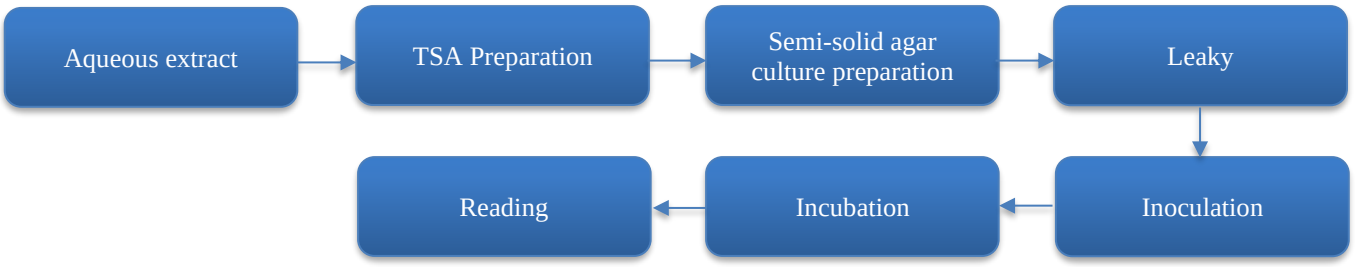

Fig. 6 Inhibitory concentration measurement

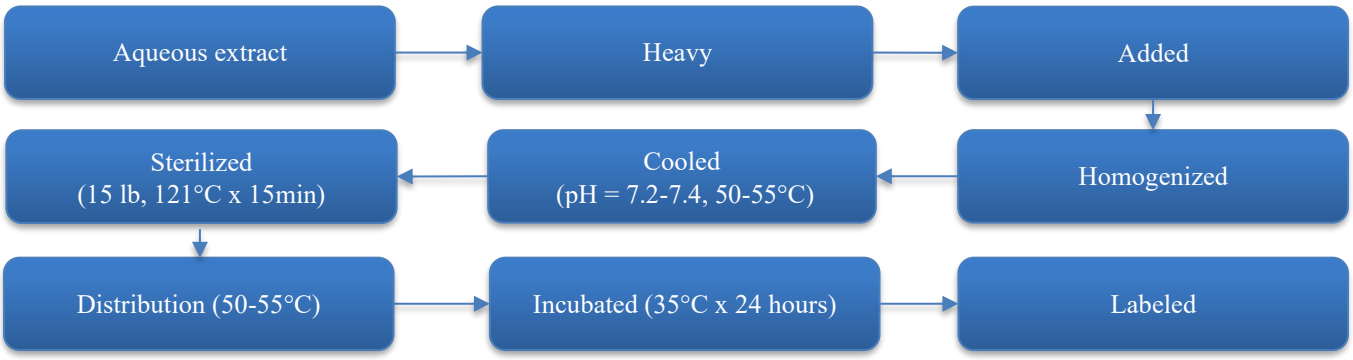

Fig. 7 Determination of minimum lethal concentration

Four concentrations of the extract—1 gram per milliliter, 0.5 grams per milliliter, 0.25 grams per milliliter, and 0.125 grams per milliliter—were used against S. typhi, M. luteus, S. aureus, and E. coli, with four repetitions to determine the inhibitory concentration. The concentration that produced the best inhibition halos was used to establish the minimum lethal concentration at seven different times, with four repetitions each.

For the experimental design to determine the aqueous extract of garlic (Allium sativum L.) against Salmonella typhi and Micrococcus luteus, using a completely randomized design in the determination of the Inhibitory Concentration and the Minimum Lethal Concentration, the means were compared using Tukey's test, with a significance level of 1% and 5%, with the assistance of Statgraphics software.

3. Results and Discussion

The characteristics of the serrano garlic bulbs, such as protein, pH and acidity, are described in Table 2.

Table 2. Characteristics bulb and aqueous garlic

Product	Characteristic	Quantity
Garlic bulb	Protein	8.10%
	pH	6.96
	Acidity	0.08%
Aqueous garlic	pH	7.15
	Acidity	0.12%

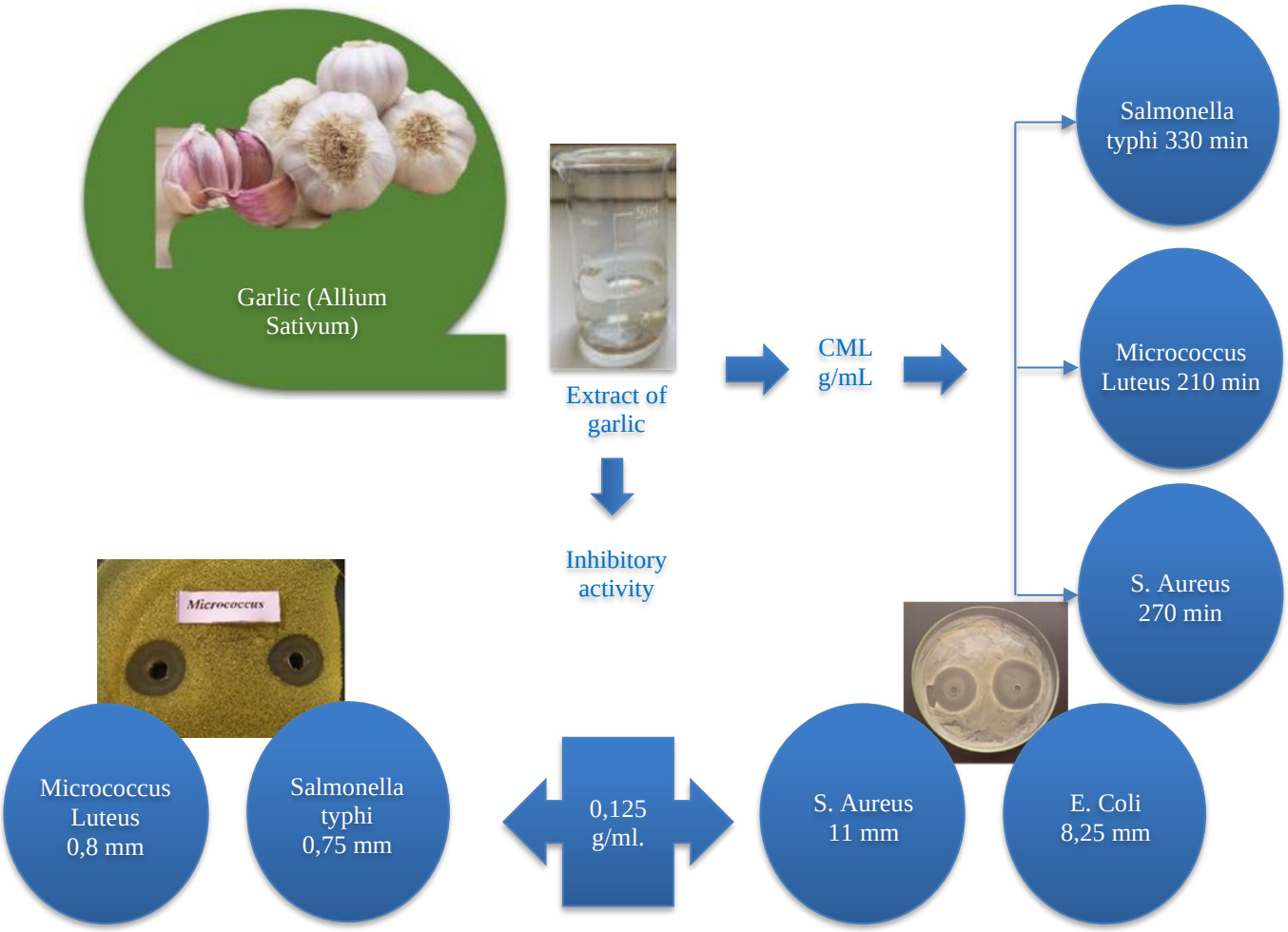

Fig. 8 Inhibitory concentration against pathogens

Table 3 shows the data obtained from phytochemical profiling. A precipitate was formed when the alkaloids in the extract reacted with Dragendorff's reagent. Steroidal saponins were detected since the foam formation lasted 30 minutes. Similarly, the absence of tannins was noted. In the test for amino acids, a blue coloring was observed, indicating the presence of alliin (s-allyl-L-cysternasulfoxide).

In fact, when the garlic is crushed, it releases the enzyme allizinase, which converts alliin into 2-propenesulfonic acid, which is also transformed into allicin (C6H10OS2) [45]. Figures 8, 9, and 10 show the inhibitory concentration of the aqueous garlic extract against S. typhi, M. luteus, S. aureus, and E. coli. We found that 1 g/ml of aqueous garlic extract exhibited statistically significant inhibitory activity, but the doses of 0.5 and 0.25 g/ml were very close in their activities also, so either of them can be used. Further, 1 g/ml of extract produced an inhibition halo of 45 mm against E. coli, while 0.5 g/ml produced a halo of 19 mm [28]. The dose of 1 g/ml of aqueous garlic extract produced inhibition halos with a diameter of 34 mm, indicating that at this concentration, bacterial growth is inhibited [25], [28].

For Gram-positive bacteria, 1 g/ml of aqueous garlic extract is an optimal inhibitory concentration, producing halos with an average diameter of 34.35 mm. The action of the extract would begin by inhibiting cell wall synthesis, which in turn causes cell wall injury, cell membrane inhibition, inhibition of protein synthesis and nucleic acids, ultimately inhibiting bacterial growth (see Figure 10).

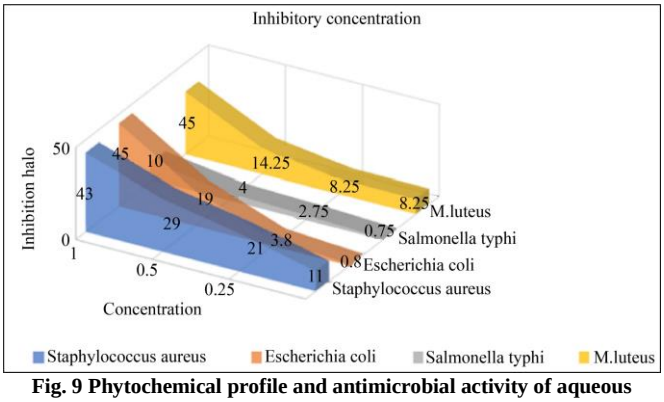

Fig. 9 Phytochemical profile and antimicrobial activity of aqueous garlic extract (Allium sativum L.) for food preservation

Table 3. Phytochemical profile

Phytochemical test	Analysis method	Coloration of the aqueous extract	Coloration of the ethanolic extract
Alkaloids	Dragendorff reagent	(+) White precipitate form	(+) Brown precipitate form
Saponins	Foam Test	(+) Foams longer than 30 minutes	(+) Foams longer than 30 minutes
Tannins		(-) White color without precipitate	(-) Transparent color, no precipitate
Total proteins		(+) Orange	(+) Orange
Polysaccharides	Starch Type	(-) No presence of Polysaccharides (Starch Type), brown coloration	(-) No presence of Polysaccharides (Starch Type), brown coloration
Sugars	Reducer type	(+) Red	(+) Ethanolic extract has red color
Amino acids	Ninhydrin reagent	(+) Blue	(-) No blue coloration became transparent color

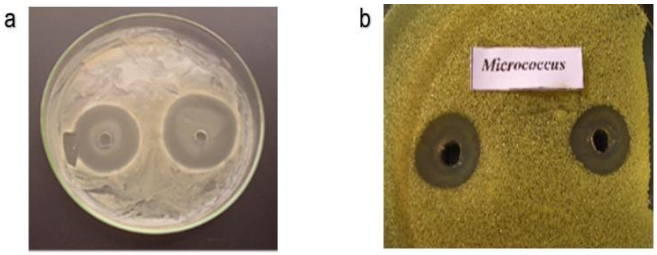

Fig. 10 Inhibitory concentration against the following: (a) Salmonella typhi; (b) Micrococcus luteus

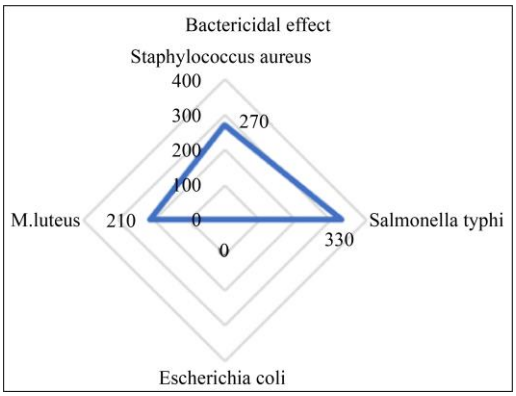

Fig. 11 Bactericidal effect on 4 pathogens

Table 4. Statistical difference in effects

	Between Strains	Between Concentrations
Chi-Square	6,473	5,882
gl	3	3
Six. asymptotic	0,091	0,118

The inhibitory effects of ethanolic extracts have been studied in S. typhi and E. coli, with inhibition zone diameters of 25.66 mm and 24.33 mm recorded, respectively [46]. The activity of the garlic extract exceeds these values. In addition, the garlic extract showed similar potency as the methanolic extract of *Rydingia michauxii* [47], slightly improving the inhibition diameter values of 11.5 mm or less than 10 mm against M. luteus [34, 35]. The aqueous garlic extract showed no bactericidal effect against E. coli, while 1 g/ml of the extract induced a bactericidal effect on S. typhi over 330 minutes of exposure (see Figure 11). The extract first inhibits cell wall synthesis, which lyses the bacterial cell and inhibits the cell membrane and translation of the genetic material, thereby blocking nucleic acid synthesis [45]. This result was highly significant, as determined by the Kruskal–Wallis test (α = 95%). The study has demonstrated antibacterial effects against Gram-positive bacteria that occur more frequently in food and differ from those already studied: Bacillus cereus MTCC 1272, S. aureus subsp. aureus MTCC 96, and Listeria monocytogenes MTCC 657 [48]. The bactericidal effect exhibited by 1.0 g/ml of the extract was 3.5 h for M. luteus and 5.5 h for S. typhi [37]. Garlic peptides have antimicrobial activity similar to that of megaresveratrol and naringenin, but the different garlic by-products have different effects. However, the effects of the median inhibition concentrations do not differ significantly (Table 4). Garlic extracts have effective antibiotic activity against recognized pathogens that are commonly present in food and constantly infect humans, as stated by Pasteur in the "Germ Theory" as seen in [49, 50].

4. Conclusion

Phytochemical analysis of aqueous garlic extract revealed the presence of alkaloids, saponins, tannins, total proteins, polysaccharides, sugars, and amino acids. Aqueous extract of garlic and its derivatives are ideal for the design of pharmacological and industrial eco-friendly bactericidal products. A concentration of aqueous garlic extracts of 0.125 g/ml produced inhibition halos of 0.75 mm against S. typhi, 0.8 mm against M. luteus, 11 mm against S. aureus, and 8.25 mm against E. coli. The bactericidal effect exerted by 1.0 g/mL of the extract was 210 min for M. luteus, 330 min for S. typhi, 270 min for S. aureus, and no effect on E. coli. Garlic extracts have effective antibiotic activity against foodborne pathogens that constantly infect humans. The study shows a higher inhibitory activity against Gram-positive bacteria compared with Gram-negative bacteria.

Data Availability Statement

All data, models, and code generated or used during the study appear in the submitted article.

Acknowledgments

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