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Research Article

Chemical Composition, Antibacterial and Antibiotic Potentiating Activity of the Essential Oil of *Allium sativum* L. Bulbils and Allicin

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Abstract

The present study aims to analyze the chemical profile, antibacterial activity, and modifying effect of the essential oil from the bulbils of *Allium sativum* L. in comparison with allicin. To this end, the plant was collected near the city of Caririaçu - CE and taken to the municipality of Juazeiro do Norte - CE for the experiments. Allicin was obtained from Sigma-Aldrich®. The bulbils were crushed and subjected to hydrodistillation using a modified Clevenger-type apparatus to obtain the essential oil. The antibacterial activity of the oil and the isolated compound was evaluated using the microdilution method against Gram-positive and Gram-negative bacteria. Finally, the modifying activity was determined in association with aminoglycoside and beta-lactam antibiotics. Regarding beta-lactams, results showed that the MIC of benzylpenicillin against *Staphylococcus aureus* was reduced from >1024 µg/mL to <1024 µg/mL. However, no activity was observed in several combinations, such as cefalotin and benzylpenicillin against *Escherichia coli*. For *Pseudomonas aeruginosa*, activity was observed in several combinations, including amikacin, with MIC reduction from 512 µg/mL to 256 µg/mL, and gentamicin, from 1024 µg/mL to 64 µg/mL. The combination of essential oil with antibiotics such as benzylpenicillin and amikacin can enhance antibacterial efficacy by altering bacterial membrane permeability. Allicin exhibited significant synergistic mechanisms when combined with aminoglycosides. Thus, further experiments with different bacterial strains are essential for the development of new therapies using garlic.

Keywords: *Allium sativum L.; Antibacterial; Allicin; Chemical profile; Modifying agent.*

1. Introduction

Throughout history, medicinal plants have played a fundamental role as sources of natural remedies and in the discovery of new treatments. The use of herbal medicines as an integral part of medical practice is becoming increasingly common in various countries, including Brazil. The utilization of medicinal plants is facilitated by the wide diversity of plant species and the affordable cost associated with therapy, which generates interest among health programs and professionals in the field [1,2].

In this context, a wide variety of active phytochemical compounds derived from these plants have been used both in the prevention and treatment of serious diseases. This natural wealth serves as the basis for herbal medicines, highlighting their complexity and distinction from synthetic drugs [3,4].

Additionally, antimicrobial properties found in plant extracts and essential oils, resulting from their secondary metabolic processes, have been empirically recognized over many centuries and recently confirmed by science. Studies on such characteristics in native plants have been conducted in various countries, including Brazil, which boasts a rich flora and a longstanding tradition in the use of medicinal plants as antibacterial or antifungal agents [5,6].

Therefore, numerous studies have been dedicated to investigating the biological properties of essential oils derived from *Allium* species, highlighting their remarkable antioxidant and antimicrobial activities. All oils exhibited antioxidant capacity, with shallot and leek standing out in this aspect. Furthermore, they demonstrated potent antibacterial activity, with special emphasis on garlic, onion, and Chinese chive. The organosulfur compounds present in these plants have shown promising therapeutic potential in combating various diseases, including cancer and cardiovascular conditions [7].

In light of this, the plant *Allium sativum* L., known as garlic, is widely recognized for its remarkable biological and medicinal properties, thanks to its rich composition of various bioactive compounds. These compounds include phenolics, essential oils, sulfur compounds, flavonoids, volatile substances, minerals, and vitamins. Such diversity of compounds grants garlic a wide range of health benefits, such as antioxidant, antimicrobial, anti-inflammatory, and cardiovascular properties [8,9].

Moreover, species of the genus *Allium* possess a complex chemical composition, characterized by the abundant presence of organosulfur compounds and polyphenols. Additionally, they are important sources of carbohydrates, essential amino acids, and vitamins, standing out especially for their significant concentration of flavonoids, known for their antioxidant activity. Studies have also demonstrated the antimicrobial efficacy of these plants against a variety of microorganisms. These properties contribute not only to the nutritional value of *Allium* species but also to their therapeutic potential [10] (Fredotović & Puizina, 2019).

Allicin is the main constituent responsible for the pharmacological properties of garlic. As a sulfur compound, it exhibits antioxidant activity due to its structure similar to dimethyl sulfide, known for its effectiveness in neutralizing free radicals. This results in beneficial effects on blood coagulation, prevention of atherosclerosis, regulation of cholesterol levels, and reduction of oxidative stress. In addition to its antioxidant properties, allicin also demonstrates antibacterial, antifungal, and antiparasitic activity [11-14].

With the growing global concern about antibiotic resistance and the need to find effective therapeutic alternatives, garlic (*Allium sativum* L.) has been traditionally recognized for its antimicrobial properties, being considered a natural and accessible source of bioactive compounds, which may lead to the development of safer and more sustainable therapies. By comparing these characteristics to the isolated compound (allicin), we can foster new therapeutic approaches in combating bacterial infections. In this context, the present study aims to analyze the chemical profile and antibacterial activity of the essential oil from *Allium sativum* L. bulbs in comparison with allicin.

2. Materials and Methods

2.1 Type and Location of Study

This research was an experimental study, including a pre-test to evaluate a hypothesis. The findings contributed to the refinement of established scientific techniques, applied to deepen knowledge on previously studied aspects. Variables in this study were manipulated to allow analysis of cause and effect of a particular event [15]. Experiments were conducted in the Microbiology and Bromatology laboratories at Centro Universitário Doutor Leão Sampaio-UNILEÃO, Health Campus, in Juazeiro do Norte, Ceará, Brazil.

2.2 Selection, Collection, and Identification of Plant Material

Bulbs of *Allium sativum* L. were collected in the municipality of Caririaçu, Ceará, from an organic plantation, and transported to Juazeiro do Norte, Ceará, to the Microbiology and Bromatology laboratory at Centro Universitário Doutor Leão Sampaio-UNILEÃO for this study. They were pre-selected based on growth stage and plant condition. A voucher specimen was deposited in the Dárdaro de Andrade Lima Herbarium at Universidade Regional do Cariri (URCA) for registration.

2.3 Acquisition of Products

Allicin, with purity over 98%, was purchased from Sigma-Aldrich® (Sigma, St. Louis, USA). This compound, a primary derivative of garlic (*Allium sativum*), is well-known for its antimicrobial and antioxidant properties. Choosing high-purity allicin ensures experimental reliability, attributing observed effects to the active substance under study without significant interference from impurities. Unless specified otherwise, all reagents and compounds used followed the quality standards provided by Sigma-Aldrich®, renowned for high-precision scientific research products.

Fresh plant material (bulbs) was crushed and subjected to hydrodistillation using a modified Clevenger apparatus for 2 hours. Yield was calculated based on the oil's weight relative to the material's weight before extraction. The essential oil was treated with anhydrous sodium sulfate (Na_2SO_4) and stored in amber bottles under refrigeration until analysis [16].

2.4 Antibacterial Evaluation and Minimum Inhibitory Concentration (MIC)

Antibacterial activity was assessed using the microdilution method based on CLSI [17]. Standard bacteria were used: two Gram-positive (*Staphylococcus aureus* ATCC 12624 and *Enterococcus faecalis* ATCC 4083) and two Gram-negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 15442). Multidrug-resistant strains were also tested: *Staphylococcus aureus* 10, clinical isolate of *Enterococcus faecalis*, *Escherichia coli* 06, and clinical isolate of *Pseudomonas aeruginosa*. All strains were provided by the Laboratório de Pesquisa de Produtos Naturais (LPPN) at Universidade Regional do Cariri (URCA) upon request, availability, and approval from the laboratory coordination.

Bacterial strains were activated in Brain Heart Infusion Broth (BHI 3.8%) and incubated for 24 hours. After initial cultivation, the inoculum was standardized to approximately 1 x 10^8 CFU/mL (McFarland scale turbidity). This suspension was then diluted in 10% BHI broth, and 100 µL volumes were added and homogenized in microdilution plate wells with 1024 µg/mL of the plant product. Plates were incubated at 37°C for 24 hours. Experiments were performed in triplicate.

Antibacterial activity was detected using the colorimetric method with 25 µL of sodium resazurin (0.01%) after the incubation period. The minimum inhibitory concentration (MIC) was determined as the lowest product concentration capable of inhibiting bacterial growth [18].

2.5 Evaluation of Modifying Activity

The modulation test was conducted in the presence and absence of the natural compound through microdilution in triplicate. To evaluate modulatory activity, the MIC of the ethanolic extract and essential oil was tested against aminoglycoside antibiotics (gentamicin and amikacin) and beta-lactams (benzylpenicillin and Cefalotin) in combination with bacterial strains *Staphylococcus aureus* 10 (multidrug-resistant), clinical isolate of *Enterococcus faecalis* (multidrug-resistant), clinical isolate of *Pseudomonas aeruginosa* (multidrug-resistant), and *Escherichia coli* 06 (multidrug-resistant).

The amount of essential oil or ethanolic extract was calculated at sub-inhibitory concentration (MIC/8). Bacterial inocula in 10% BHI were distributed in microplates, followed by microdilution of 100 µL antibiotic solutions (1024 µg/mL). Serial dilutions were performed to obtain antibiotic concentrations ranging from 512 to 0.5 µg/mL.

The test included a positive control containing only antibiotics and microorganisms. Microdilution plates were incubated at 37°C for 24 hours, and readings were taken using sodium resazurin as previously described [19].

2.6 Statistical Analyses

Results were evaluated using linear regression models, analyzed by two-way ANOVA followed by Bonferroni's test, using GraphPad Prism 7.0 software. Results with $p<0.05$ were considered statistically significant.

3. Results and Discussion

3.1 Chemical Profile Survey

In a study conducted by [20], the chemical composition analysis of garlic essential oil revealed five major components, corresponding to the largest peaks in the chromatogram: allyl disulfide (30.24%), allicin (27.82%), methyl-allyl disulfide (8.51%), alliin (5.78%), and allyl sulfone (4.19%).

The chemical composition of the bulb of some garlic species identified a more complex chemical profile. The main compound found was allicin (50.9%), followed by other sulfur compounds such as diallyl disulfide (27.9%), ethyl diallyl trisulfide (3.1%), 2,2-bis(ethylthio)propane (2.0%), (Z)-allyl propyl sulfide (1.4%), and methyl allicin (1.3%), as described by [21].

Allium species have a chemical composition rich in organosulfur compounds, polyphenols, carbohydrates, essential amino acids, and vitamins. Additionally, they are significant sources of flavonoids, notable for their antioxidant action, and have demonstrated antimicrobial efficacy against various microorganisms, as stated by [10] Fredotović and Puizina (2019). However, several studies, such as [22], confirm that the primary antimicrobial activity of garlic is attributed to allicin; however, the extraction method can directly influence the bioactive compounds present.

Several compounds present in garlic have unique characteristics, such as diallyl disulfide, an organophosphorus compound with antioxidant and anticancer capabilities, which disrupts cellular processes involved in tumor progression, such as the epithelial-mesenchymal transition, as described by [23].

The study conducted by [24] identified the main components of garlic as diallyl trisulfide, diallyl disulfide, and allyl methyl trisulfide. Hydrodistillation produced similar compositions, with high levels of diallyl trisulfide. In contrast, steam distillation resulted in higher concentrations of diallyl sulfide and diallyl disulfide but reduced yields of diallyl trisulfide. This phenomenon highlights the importance of the extraction method in the efficacy of bioactive compounds, directly influencing their use in therapeutic treatments.

It was reported in a study by [25] that allicin caused a global increase in the oxidation degree of the *Pseudomonas fluorescens* proteome after a 15-minute treatment. They also demonstrated that allicin inhibited DNA gyrase activity in vitro. This underscores the importance of allicin as an oxidizing agent and suggests potential tolerance mechanisms in bacterial strains. These studies highlight the therapeutic value of allicin and the complexity of its action mechanisms in controlling bacterial infections.

Through inverted inhibition zone tests conducted by [26], it was demonstrated that allicin is capable of inhibiting fungal growth through the gaseous phase of the solution. The results revealed an average inhibition zone of 1965 mm^2 for *Rhizopus stolonifer* and 2405 mm^2 for *Mucor racemosus*. Additionally, in agar diffusion tests, allicin solutions presented an inhibition zone greater than 800 mm^2, confirming their antifungal capability.

According to [27], allicin, derived from garlic, is known for its antioxidant and antimicrobial properties. It can reduce oxidative stress by modulating enzymes such as NOX, SOD, and CAT. Furthermore, it has shown therapeutic potential in neuroinflammation and neurodegenerative diseases like Alzheimer's and Parkinson's, as well as aiding in psychoneurological conditions such as ASD and ADHD. Its chemical structure is represented in Figure 1.

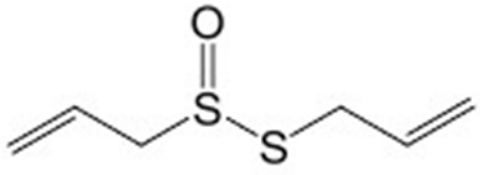

Figure 1. *Chemical structure of allicin.*

These properties make allicin a multifunctional molecule with a significant impact on applications across various fields, confirming its relevance as a target for studies. The presence of sulfur compounds, such as allicin, diallyl disulfide, and diallyl trisulfide, highlights its therapeutic potential in areas such as microbiology, oncology, and inflammatory and neurodegenerative diseases.

3.2 Antibacterial Activity

The results obtained from the essential oil of the bulb of *Allium sativum* (OEBAS) and allicin indicated a minimum inhibitory concentration (MIC) of 1024 µg/mL, suggesting low antibacterial activity for both ATCC bacterial strains and multidrug-resistant strains. OEBAS contains various compounds with antibacterial activity.

Allicin reacts with thiol (-SH) groups in proteins and enzymes in the bacterial membrane. This reaction leads to the oxidation of these groups, compromising the integrity of the bacterial cell membrane and increasing cell permeability. Consequently, homeostasis is disrupted, and function is lost, which may lead to cell death, as suggested by [28] and [29]. However, the allicin concentration may have influenced the observed results.

According to [30], tests identified an MIC of 15.62 mg/mL for *Pseudomonas aeruginosa*, corroborating the findings. Some compounds, such as diallyl disulfide, methyl allyl trisulfide, and diallyl trisulfide, were found in abundance in the essential oil.

A study conducted by [31] indicated the absence of bacteriostatic activity of garlic essential oil against *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739), and *Pseudomonas aeruginosa* (ATCC 9027), demonstrating its inefficacy in inhibiting bacterial growth, which aligns with the obtained results.

[32] identified high resistance of a *P. aeruginosa* strain to allicin vapor, with no detectable MIC, demonstrating the various resistance mechanisms of the tested strain, even in contact with compounds with high antibacterial activity. Similarly, [33] stated that isolated allicin does not exhibit strong antibacterial activity, identifying an MIC >512 µg/mL against *Staphylococcus spp.* and *P. aeruginosa*. This suggests that allicin's action against strains with resistance mechanisms may lead to its low efficacy in eliminating pathogens, justifying the results obtained.

According to [34], the MIC found for *Staphylococcus aureus* strains using garlic extract was 400 µg/mL. A study using different extraction methodologies for garlic components identified variations in biological activities, including antibacterial activity, which decreased due to differences in component concentrations, as stated by [35] and [36]. Thus, the high MIC may be related to the extraction method of the essential oil and allicin.

Bacterial resistance mechanisms, such as efflux pumps, may be directly related to the low detected antibacterial activity. Once intracellular concentrations of toxic compounds reach high levels, efflux pump expression occurs, expelling the material into the extracellular environment, as described by [37].

Studies conducted by [38], identified higher antibacterial activity in pure allicin compared to garlic extract—up to twice as effective as isolated allicin. This suggests that the chemical composition of the extract and its interaction with allicin enhance antibacterial activity. The presence of synergistic and antagonistic compounds may have influenced the obtained results.

According to [29], a possible mechanism affecting protein synthesis involves cysteine residues reacting with allicin, leading to disulfide exchange. This interaction may affect amino acid chains, influencing antibacterial action and inducing protein defects.

According to [39] (Reza, Sutton, and Rahman, 2019) and [40], *Pseudomonas aeruginosa* possesses multiple resistance mechanisms, dynamically altering its resistance profile in response to environmental pressures, such as antibiotic exposure or host immune responses. Additionally, its outer membrane is naturally impenetrable to many antibiotics, preventing their entry into cells. This range of mechanisms is directly related to the observed results.

The detected MIC may reflect allicin’s high instability. As described by [41], its thiol-rich chemical structure makes it highly susceptible to thermal and oxidative degradation, leading to rapid conversion into other compounds, such as diallyl disulfide and ajoene, which may reduce its effects against bacteria with resistance mechanisms.

The interaction between different components of the oil may result in antagonistic or synergistic effects that influence final potency. In this complex matrix, a higher concentration may be required to achieve an effective MIC against the tested bacteria.

3.3 Modifying Activity

The results obtained from microdilution tests are presented in the graphs. The essential oil from the bulbs of *Allium sativum* (OEBAS) in combination with aminoglycosides corresponds to figure 2 and 3, while OEBAS in action with beta-lactams corresponds to figure 4 and 5.

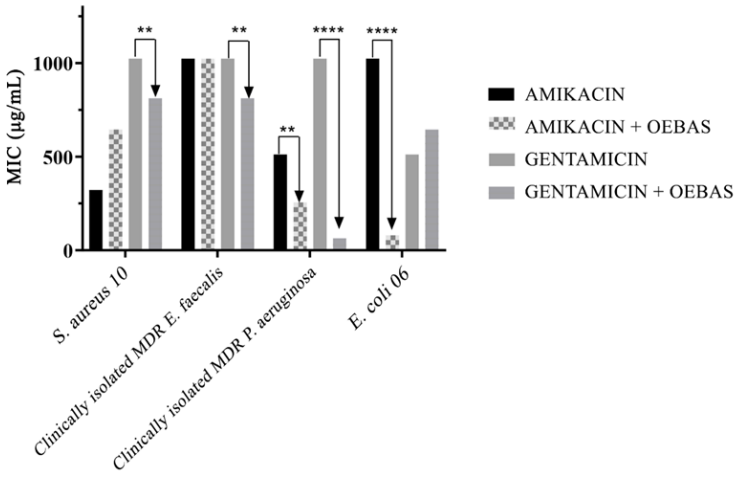

Fig. 2. *Result of the modifying potential of OEBAS on the antibiotic activity of aminoglycosides against strains of Staphylococcus aureus 10 (Multidrug-resistant), Enterococcus faecalis clinical isolate (Multidrug-resistant), Pseudomonas aeruginosa clinical isolate (Multidrug-resistant), Escherichia coli 06 (Multidrug-resistant). Two-way ANOVA followed by Bonferroni post-test, using GraphPad Prism 7.0 software. ****p <0.0001.*

The results obtained showed that the MIC was 512 µg/mL (Amikacin) to >256 µg/mL (Amikacin + OEBAS) for *Staphylococcus aureus*. For Gentamicin, the MIC was 1024 µg/mL to <1024 µg/mL in combination with OEBAS. For *Enterococcus faecalis*, the MIC was 1024 µg/mL for both Amikacin and Amikacin + OEBAS, showing no modifying activity. Gentamicin had an MIC of 1024 µg/mL, which decreased to <1024 µg/mL with OEBAS.

For *Escherichia coli*, the MIC was <1024 µg/mL (Amikacin) to 64 µg/mL (Amikacin + OEBAS), while for Gentamicin, the MIC shifted from 512 µg/mL to >512 µg/mL in combination with OEBAS. For *Pseudomonas aeruginosa*, the MIC was 512 µg/mL (Amikacin) to 256 µg/mL (Amikacin + OEBAS). For Gentamicin, the MIC changed from 1024 µg/mL to 64 µg/mL.

Aminoglycosides, such as Amikacin and Gentamicin, act by binding to the 30S subunit of the bacterial ribosome, blocking mRNA translation and generating defective proteins, leading to bacterial death. Additionally, they can inhibit translation initiation and generate reactive oxygen species, as described in studies by [42] and [43]. These properties explain their bactericidal efficacy but also their vulnerability to resistance due to enzymatic modifications, ribosomal target alterations, and changes in cell permeability.

Gram-positive and Gram-negative pathogens exhibit various resistance mechanisms, such as efflux pumps, expression of genes that modify antibiotic-binding proteins, and the presence of beta-lactamase enzymes that inhibit antibiotic effects, as stated by [44], justifying the lack of inhibitory activity of antibiotics against multidrug-resistant strains.

Some mechanisms present in *Escherichia coli*, such as antibiotic-modifying enzymes, outer membrane porin remodeling, enhanced efflux pump activity, and alteration of antibiotic target sites, justifying the high MIC found, considering its ability to reduce the antibacterial effect of substances, as described by [45].

The evaluation of modifying activity conducted by [46] using fresh garlic extract identified a synergistic activity of Gentamicin against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*, indicating that some compounds in the extract may alter the behavior of resistant strains to certain antibiotics, with some of these compounds also present in the oil.

According to [47], garlic was able to inhibit the bacterial growth of *Pseudomonas aeruginosa* strains in contact with macrolides and aminoglycosides, with inhibition occurring at concentrations below 128 µg/mL. The results obtained are consistent with what is described in the literature.

The study by [48] observed that allicin significantly reduces the minimum inhibitory concentration (MIC) of antibiotics against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, with MIC reductions of up to 50% for some combinations, but antagonism with β-lactams under specific conditions.

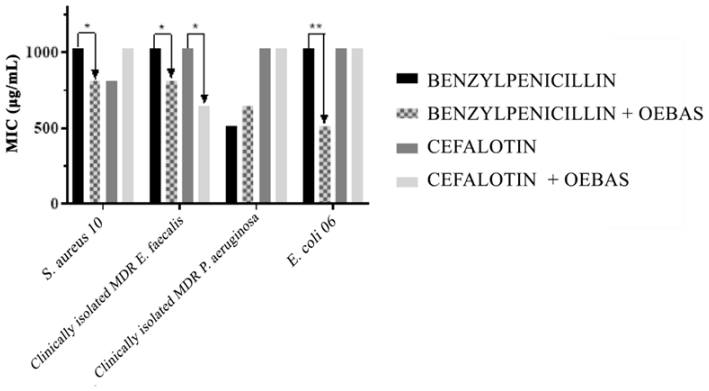

***Figure 3**. Result of the modifying potential of OEBAS on the antibiotic activity of beta-lactams against strains of Staphylococcus aureus 10 (Multidrug-resistant), Enterococcus faecalis clinical isolate (Multidrug-resistant), Pseudomonas aeruginosa clinical isolate (Multidrug-resistant), Escherichia coli 06 (Multidrug-resistant). Two-way ANOVA followed by Bonferroni post-test, using GraphPad Prism 7.0 software. ****p<0.0001.*

The results obtained for beta-lactams were as follows: an MIC of 1024 µg/mL for *Escherichia coli* (Cefalotin / Cefalotin + OEBAS) and an MIC of 1024 µg/mL for *Pseudomonas aeruginosa* (Cefalotin / Cefalotin + OEBAS). A modifying activity was observed for *Staphylococcus aureus*, with an MIC of >1024 µg/mL (Benzylpenicillin) decreasing to <1024 µg/mL (Benzylpenicillin + OEBAS).

MIC values of <1024 µg/mL (Cefalotin) increased to >1024 µg/mL (Cefalotin + OEBAS) for *Staphylococcus aureus*, while for *Escherichia coli*, the MIC of >1024 µg/mL (Benzylpenicillin) decreased to 512 µg/mL (Benzylpenicillin + OEBAS). For *Pseudomonas aeruginosa*, the MIC of 512 µg/mL (Benzylpenicillin) increased to >512 µg/mL (Benzylpenicillin + OEBAS). For *Enterococcus faecalis*, the MIC changed from >1024 µg/mL to <1024 µg/mL for the beta-lactams tested.

Staphylococcus aureus is resistant due to its ability to reduce cell membrane permeability, making it more difficult for antibiotics to enter, in addition to producing enzymes such as β-lactamase, which degrade β-lactam antibiotics. Another resistance strategy is the modification of penicillin-binding proteins (PBPs), as seen in MRSA, which makes it resistant to methicillin, as described by [4], justifying the results found.

Tests conducted by [33] using beta-lactams in combination with allicin demonstrated a high synergistic capacity against *Staphylococcus aureus*, which is consistent with the results obtained.

Pseudomonas aeruginosa is known for its efflux pumps (such as MexAB-OprM), which expel antimicrobials from the cell. This bacterium also possesses enzymes that degrade antibiotics, such as β-lactamases. Additionally, modifications in its membrane porins limit the entry of drugs, including β-lactams and aminoglycosides, as described by [40] and [49]), supporting the findings.

The lack of activity observed for *Escherichia coli* against Cefalotin may be related to its resistance mechanisms and the absence of modifying activity against these mechanisms. Some β-lactamase-producing strains can degrade the antibiotic before it reaches its target, as described by [50] Bajaj, Singh, & Virdi (2016).

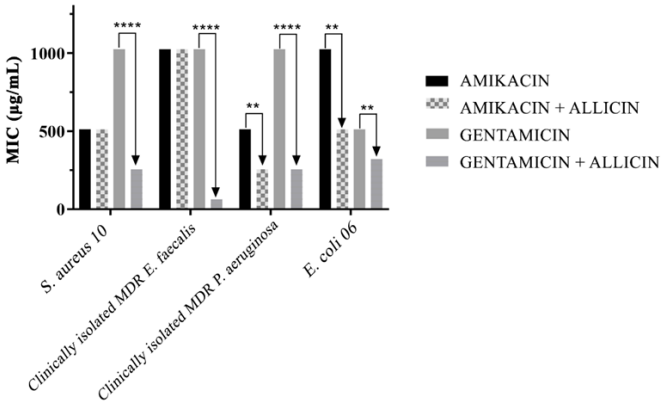

Figure 4. *Result of the modifying potential of allicin on the antibiotic activity of Cefalotin against strains of Staphylococcus aureus 10 (Multidrug-resistant), Enterococcus faecalis clinical isolate (Multidrug-resistant), Pseudomonas aeruginosa clinical isolate (Multidrug-resistant), Escherichia coli 06 (Multidrug-resistant). Two-way ANOVA followed by Bonferroni post-test, using GraphPad Prism 7.0 software. ****p<0.0001.*

The results obtained were an MIC of 1024 µg/mL (Gentamicin) reduced to 256 µg/mL (Allicin + Gentamicin) and an MIC of 512 µg/mL (Amikacin / Amikacin + Allicin) for *S. aureus*. The MIC for *Escherichia coli* was >512 µg/mL (Amikacin) reduced to 512 µg/mL (Allicin + Gentamicin), and 512 µg/mL (Gentamicin) reduced to <512 µg/mL (Allicin + Gentamicin). For *Pseudomonas aeruginosa*, the MIC of 512 µg/mL (Amikacin) decreased to 256 µg/mL, and the MIC of 1024 µg/mL (Gentamicin) dropped to 256 µg/mL (Allicin + Gentamicin). The MIC for *Enterococcus faecalis* was 1024 µg/mL (Amikacin) reduced to 64 µg/mL (Allicin + Gentamicin) and 1024 µg/mL (Gentamicin / Allicin + Gentamicin).

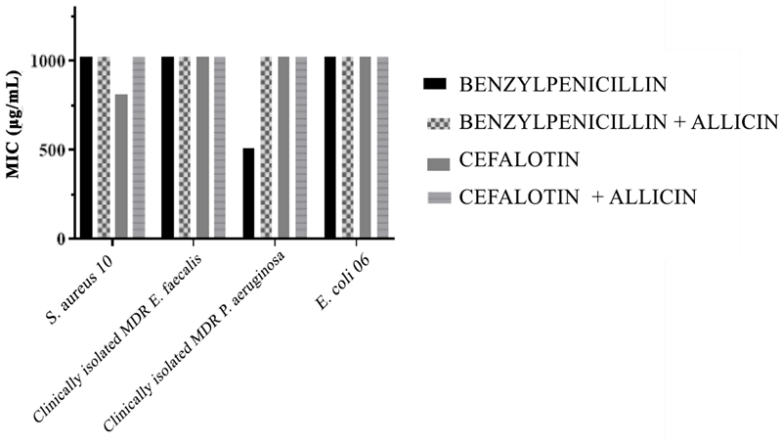

Figure 5. *Result of the modifying potential of OEBAS on the antibiotic activity of benzylpenicillin against strains of Staphylococcus aureus 10 (Multidrug-resistant), Enterococcus faecalis clinical isolate (Multidrug-resistant), Pseudomonas aeruginosa clinical isolate (Multidrug-resistant), Escherichia coli 06 (Multidrug-resistant). Two-way ANOVA followed by Bonferroni post-test, using GraphPad Prism 7.0 software. ****p<0.0001.*

The results obtained were an MIC of <1024 µg/mL (Cefalotin) reduced to <512 µg/mL (Cefalotin + Allicin) for *Staphylococcus aureus*, while for *Pseudomonas aeruginosa*, the MIC of 512 µg/mL (Benzylpenicillin) increased to >1024 µg/mL (Benzylpenicillin + Allicin), showing an antagonistic effect in both tests.

In some cases, there was no activity: *Staphylococcus aureus* (Benzylpenicillin), *Escherichia coli* (Cefalotin and Benzylpenicillin), *Pseudomonas aeruginosa* (Cefalotin), and *Enterococcus faecalis* (Cefalotin and Benzylpenicillin).

According to results obtained by [46], fresh garlic extract exhibited a modifying effect on *Pseudomonas aeruginosa* against gentamicin, improving its activity and making drug-resistant bacteria treatable with the clinically recommended dose. This may explain the results obtained for aminoglycosides.

According to [51], allicin can alter homeostasis due to its chemical composition, triggering a strong oxidative stress response. In some cases, this homeostasis alteration can affect antibiotic entry or even reduce its effect due to lack of internalization, justifying the observed absence of synergistic activity and the antagonism found.

Studies conducted by [41] showed that allicin reduces the minimum inhibitory concentration (MIC) of antibiotics such as gentamicin and amikacin against resistant bacteria. However, in some cases, antagonism may occur depending on the compound used, explaining the results found.

Gram-negative bacteria possess various mechanisms that block antibiotics, and this intrinsic resistance can lead to a lack of activity even when substances are used to mediate chemical reactions, as described by [52].

According to studies by [53] allicin is a compound capable of altering membrane stability due to its lipophilic nature. This alteration can directly influence antibiotic entry, thereby impacting its effectiveness.

Allicin was unable to interact synergistically with beta-lactams, possibly due to the absence of other compounds mediating the reactions or a lack of synergy between the mechanisms of action of beta-lactams and allicin, which explains the antagonism. The concentration used in the preparation of the product may have also influenced resistance in some strains.

4. Conclusion

The conclusion regarding the effects of garlic essential oils and antibiotics, such as benzylpenicillin and amikacin, highlights a potential enhancement of antibacterial efficacy. This interaction is likely due to the ability of sulfur compounds and their interactions with allicin present in garlic oil to disrupt the bacterial cell membrane, increasing permeability and facilitating the entry of antibiotics into the cells. This enhanced permeability may reduce the required antibiotic concentration to achieve the desired effect, improving efficacy against bacteria with robust resistance mechanisms. However, results indicate that allicin is a significant compound in some cases, demonstrating high synergistic activity with specific antibiotics.

Nevertheless, contradictory results compared to literature may be explained by experimental variables or the specific nature of the resistance in the studied strains. For example, certain factors, such as inadequate concentrations of essential oil compounds or variations in the chemical composition of the oil (which may differ depending on its origin and extraction process), can impact the effectiveness of the combination.

Additionally, the presence of adaptive mechanisms in bacterial strains, such as changes in efflux pumps or membrane fluidity, may reduce the efficacy of the combination. These mechanisms explain the discrepancy between experimental results and literature, which often consider ideal conditions or less resistant bacterial strains. Moreover, further experiments with different resistant strains are essential, as each strain possesses unique resistance mechanisms that may interact differently with the essential oil compounds. Additionally, testing different concentrations is necessary to obtain reliable results.

Conflict of Interest

The authors declare no conflict of interest.

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