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Investigating the Antimicrobial Properties of Garlic and Onion Against Gram Positive and Negative Bacteria, *E. coli* and *S. aureus*

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Abstract

The present investigation examines the antimicrobial potency of garlic and onion against both Gram-positive and Gram-negative bacteria, with a focus on *Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus).* The objective is to assess the viability of these natural substances as potential antibacterial alternatives amidst the escalating challenge of antibiotic resistance. Garlic, abundant in organosulfur compounds such as allicin, ajoene, and allyl sulphides, is well-documented for its bactericidal effects. Onion, which contains quercetin and sulphur compounds, also exhibits antimicrobial properties. The study aims to determines the susceptibility of the chosen antimicrobial ingredients and the minimum inhibitory concentrations (MICs) of extracts from garlic and onion when applied to both bacterial species. Findings reveal that garlic demonstrates considerable antibacterial activity against *S. aureus*, achieving a lower MIC than that observed for *E. coli*. While the antimicrobial efficacy of onion is unresolved, showing result with no significant findings against inhibiting both bacteria. The research posits that only garlic is integral to the formulation of novel antibacterial agents.

1. Introduction

In the face of a growing global health crisis marked by the rise of antibiotic-resistant bacteria, the quest for alternative antibacterial agents has never been more urgent. This technical paper delves into the antimicrobial potential of two commonly consumed vegetables: garlic and onion. Through rigorous scientific inquiry, we explore their efficacy against formidable bacterial adversaries *E. coli* and *S. aureus*, which are notorious for their roles in various infections and their increasing resistance to conventional antibiotics [1]. *S. aureus*, this Gram-positive bacterium is notorious for causing skin infections, pneumonia, and life-threatening sepsis. It has developed resistance to multiple antibiotics meanwhile *E. coli*, a Gram-negative bacterium commonly associated with urinary tract infections, gastrointestinal illnesses, and food poisoning. Like *S. aureus*, it exhibits antibiotic resistance.

Garlic and onions, both part of the allium family, offer notable health benefits. Garlic, with its high levels of vitamin B6, vitamin C, and organosulfur compound ds like allicin, has been celebrated for its ability to combat bacterial infections. Allicin, a component abundant in garlic, disrupts *S. aureus* cell membranes, inhibiting growth and promoting cell death [2]. Compounds like ajoenes and alyll sulfides contribute to garlic's antimicrobial activity. Ajoenes inhibit bacterial growth, while allyl sulfides disrupt cell membranes [3]. Garlic has been used for centuries in traditional medicine to treat infections, boost immunity, and promote overall health. Its well-documented efficacy, rich composition of organosulfur compounds, and historical relevance make garlic a compelling choice for antimicrobial research.

On the other hand, onions, rich in quercetin and sulfur compounds, are recognized for their antimicrobial properties. Quercetin is a flavonoid with antioxidant and anti-inflammatory properties. It also exhibits antimicrobial effects. Quercetin interferes with bacterial DNA replication and protein synthesis, making it a potential weapon against *E. coli* [4]. These flavourful ingredients enhance your meals and contribute to overall health. While promising, onion's antimicrobial efficacy remains an area of ongoing investigation.

While garlic exhibits significant antibacterial activity against *S. aureus*, surpassing that of *E. coli* [5][6][7], the results for onion are inconclusive, indicating a need for further research to ascertain its potential as an antibacterial agent [8].

This study aims to assess the antimicrobial potency of garlic and onion extracts against *S. aureus* and *E. coli* and determine minimum inhibitory concentrations (MICs) for these extracts. The study focuses on in vitro assessments, using bacterial strains commonly associated with infections. Limitations include variations in extract preparation methods and the need for further clinical validation.

2. Materials and Methods

2.1 Preparation of Antimicrobial Samples

Garlics and onions are readily available at nearby markets. Garlic bulbs and onions are peeled, weighed to make sure it is 100g, and cleaned before being washed. Clean garlic and onions are put into a sterile blender before being filtered through a syringe filter of 0.45 µm. The solution of the garlic and onion extract are made by adding 100 ml of distilled water to produce 100% w/v. It is kept at –20°C.

2.2 Preparation for Bacteria Sample

Colonies of *S. aureus* and *E. coli* are easily accessible. Each test tube contains 10 mm of the Mueller Hinton Broth insert. Next, pick one colony of each bacteria using a toothpick, place it in each test tube, and shake. The bacteria growth cell was placed in the incubator and left for 16–18 hours. Subsequently, distilled water was created as the blank for the UV/Vis test and bacteria was diluted for the OD test. The OD test used *S. aureus* and *E. coli* as the blanks in UV/Vis, and the concentration of bacteria was measured at a wavelength of 625 nm, or between 0.008 and 0.12 UV/Vis Spectrophotometer [7] [8].

2.3 Preparation of Agar Media

38 g of dehydrated Mueller-Hinton agar powder should be weighed. To distribute the powder, 1 L distilled water is added and stirred. The medium is heated for one minute while stirring often. Autoclave for 15 minutes at 121°C. Once at room temperature, let it cool. To provide equal depth, pour Mueller Hinton Agar onto sterile petri plates set on a flat, horizontal surface [9][10].

2.4 Antimicrobial Susceptibility

To obtain an equal inoculum, 50 µm of *E. coli* and *S. aureus* that are already in the desired optical density are uniformly dispersed across the plate in three directions using a sterile cotton swab for the test culture. The dish is left to dry for three to five minutes. On top of the agar, 5 mm diameter disks are well. In one disk, distilled water for negative gram and co-amoxiclav for positive gram, and 30 µm of 10% solutions (v/v) of fresh garlic and fresh onion are added to the other disks. For 20 hours, the plates are incubated at 37°C. Using a ruler, the zone of inhibition is determined [9][10].

2.5 Minimum Inhibitory Concentration (MIC) Test of Material by Using Dilution Method

500 µm of Mueller Hinton Broth was poured into the microtiter plate's hole for the MIC test. The sample was subsequently mixed and diluted for the following hole after 500 µm had been added to each of the initial holes. Finally, 500 µm of the diluted bacteria was put into each microtiter plate hole. The microtiter plate was incubated for 16–18 hours. 50 µm of the MIC was streaked over the agar after the microtiter plate's bacteria had been incubated. Afterwards, the MIC agar was incubated.

3. Results and Discussion

The discussion is made to compare the efficacy of garlic, onion, and the mixture of both samples against *S. aureus* and *E. coli*.

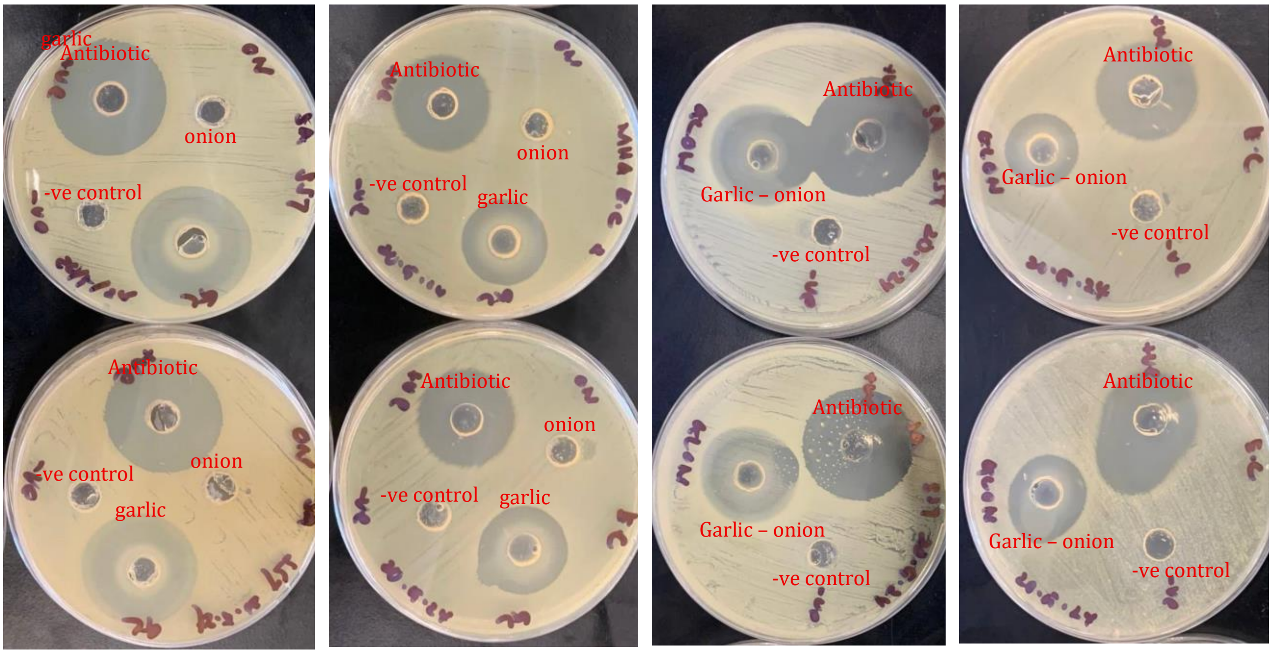
3.1 Susceptibility Test

Susceptibility testing is a method used to determine a microbe's vulnerability to various antimicrobial materials, which helps identify effective treatments for a specific organism. In this study, we conducted susceptibility tests to evaluate whether extracts from garlic and onion could inhibit bacterial growth. Disks impregnated with garlic and onion extracts were placed on agar plates. These plates had been inoculated with *S. aureus* and *E. coli*.

Fig. 1 illustrates the results of the susceptibility tests on four agar plates, labelled (a) through (d), which display the inhibition zones produced by the tested materials against different microbes. Specifically, plates (a) and (c) were inoculated with *S. aureus*, while plates (b) and (d) were inoculated with *E. coli*.

Plates (a) and (b) show the susceptibility of these different microbes to single antimicrobial materials, namely garlic and onion, as indicated by the labels in Fig. 1. In contrast, Fig. 1(c) and (d) demonstrate the susceptibility of the same microbes to a mixture of garlic and onion extracts. The experiments were controlled using both a negative control and a standard antibiotic to validate the results.

Antibiotics demonstrated a significant zone of inhibition, confirming the bacteria's susceptibility to a known antimicrobial. The negative control, labelled "-ve control," showed no zone of inhibition, indicating that the carrier medium alone has no antimicrobial effect. The visible zones of inhibition around the garlic and onion samples on each plate demonstrate their antimicrobial properties against both *S. aureus* and *E. coli*.



(a) (b) (c) (d)

Fig.1 *The inhibition area produced by the antimicrobial samples*

The susceptibility testing visually demonstrated the antimicrobial properties of garlic and onion extracts. Fig. 1 shows plates inoculated with *S. aureus* (a, c) and *E. coli* (b, d). The controls worked as expected where the antibiotic disc consistently produced a large zone and clear of inhibition area where both *E. coli* and *S. aureus* did not grow, confirming the bacteria's susceptibility. The negative control (sterile water) showed no inhibition, proving that the disc material itself had no effect.

When tested individually, garlic extract was effective against both *S. aureus* and *E. coli*, as shown by the clear zones of inhibition in Fig. 1(a) and Fig. 1(b). However, onion extract was only effective against *S. aureus*. It produced a clear zone in Fig. 1(a) but failed to inhibit *E. coli* growth in Fig. 1(b). When the two extracts were combined, the mixture successfully inhibited both *S. aureus* and *E. coli* as in Fig. 1(c) and Fig. 1(d). The inhibitory effect of the mixture seems to be primarily due to the dominant properties of the garlic extract, as the zones of inhibition were comparable to those of garlic alone. This suggests the effect is additive, where the combined effect is equal to the sum of the individual effects, rather than synergistic, which would produce a greater effect than the sum of the parts.

In summary, garlic extract is a promising broad-spectrum natural antimicrobial, while onion extract's activity appears more selective. Further research is needed to quantify these effects and identify the specific compounds responsible for the observed inhibition.

The results demonstrate the antibacterial effects of garlic and a garlic-onion mixture on *S. aureus* and *E. coli*. For *S. aureus*, garlic shows a strong antibacterial effect with an inhibition zone of 4 mm, while the garlic-onion mixture displays a moderate effect with an inhibition zone of 2 mm. Conversely, garlic exhibits no antibacterial activity against *E. coli*, as indicated by an inhibition zone of 0 mm. However, the mixture of garlic and onion shows some effectiveness, with a 2 mm inhibition zone.

These findings suggest that garlic's antibacterial properties are potent against *S. aureus* but ineffective against *E. coli*. The effectiveness against *S. aureus* may be attributed to the organosulfur compounds in garlic, which are known to have diverse antibacterial properties, including bactericidal action, inhibition of biofilm formation, and interference with quorum sensing (bacterial communication) [3].

A notable and interesting observation is the different responses of the two bacterial strains to the treatments. While garlic alone was effective against *S. aureus* but not *E. coli*, the garlic-onion mixture had a moderate inhibitory effect on *S. aureus* and some inhibitory action against *E. coli*. This difference highlights that the antibacterial efficacy of these treatments varies significantly between the two bacterial strains. This could be due to differences in cell wall composition or metabolic pathways that make one bacterium more susceptible to certain compounds than the other.

3.2 Minimum Inhibitory Concentration (MIC)

MIC is the minimum inhibitory concentration, measured in milligrams per millilitre (µg/mL) of an antibacterial property (garlic, onions, and a mixture of garlic and onions), that totally inhibits the test strain of an organism from growing visibly [11]. The average was determined by calculating the number of colonies that appeared on the plate**.**

Using viable plate counts to determine the minimum inhibitory concentration (MIC) against *S. aureus* and *E. coli*, bacterial cells are spread and antimicrobial samples from a liquid culture are placed onto an agar plate. After incubation, the number of colonies that developed was counted. The results of MIC for *S. aureus* were shown in Fig. 2 and those for *E. coli* in Fig. 3.

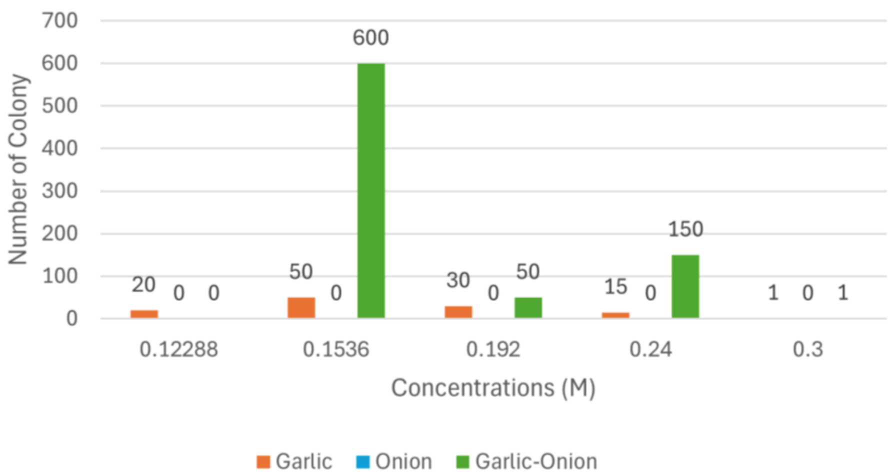


Fig. 2 *Minimum inhibitory concentration (MIC) S. aureus for garlic, onion and garlic-onion*

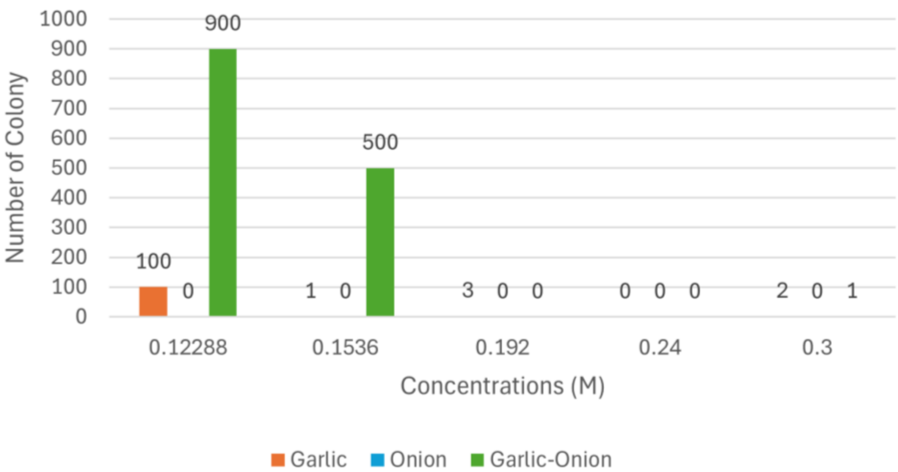


Fig. 3 *Minimum inhibitory concentration (MIC) E. coli for garlic, onion and garlic-onion*

Garlic's antimicrobial properties are demonstrated by the significant decrease in *S. aureus* colonies, with only one colony found at the highest tested amount. Onion alone was highly effective, as no colonies were seen at any tested amount. The combination of garlic and onion produced inconsistent outcomes. Strong inhibition was seen with only one colony at the highest amount tested, while Too Much To Count (TMTC) colonies were observed at a specific intermediate amount, indicating a potential interaction effect that decreased effectiveness at this level. Moderate inhibition was noted with 30 and 15 colonies at various intermediate amounts, showing that increasing amounts generally lead to stronger inhibition of *S. aureus*. Interestingly, the garlic-onion combination showed no colonies at a certain intermediate amount but showed an increase to one colony at the next higher amount, possibly due to improper mixing and transfer to the plate.

As the amount of garlic increases for *E. coli*, the number of colonies often falls, suggesting strong antibacterial capabilities. No colonies were seen at higher amounts, indicating significant inhibition. However, there was significant variation in the data, with 2 colonies at the highest amount tested and 100 colonies at a specific lower amount. Onion alone was highly effective, as no colonies were found at any tested amount. The results for the garlic-onion combination were inconsistent, with TMTC colonies at one intermediate amount, indicating decreased effectiveness, and TMTC colonies at another intermediate amount, suggesting moderate inhibition. Strong inhibition was evident at higher amounts, with no colonies observed [12]. However, colonies appeared again at the highest amount, which may be due to errors during mixing and transferring of the mixture.

The most significant finding is at a certain amount for *S. aureus*, where the garlic-onion combination is highly effective, with almost no colonies. For *E. coli*, the key discovery is at another specific amount, where the combination of garlic and onion significantly inhibits bacterial growth, with almost no colonies. In contrast, neither garlic nor onion alone demonstrated substantial inhibition at any amount. Both garlic and onion alone showed some inhibition but were less effective than the combination.

In summary, while all three substances inhibit bacterial growth, the garlic-onion combination stands out as particularly effective for both bacteria. This indicates that *S. aureus* and *E. coli* are susceptible to the antibacterial effects of both garlic and the garlic-onion combination. Onion may require a stronger dosage than tested to inhibit both bacteria, as research has shown that onion possesses antimicrobial properties, albeit not as abundantly as garlic.

4. Conclusion

The experiment demonstrates that garlic possesses strong antibacterial qualities against *S. aureus* and *E. coli*, with significant inhibition of *S. aureus* as evidenced by the observation of only two colonies at the highest tested amount. At lower amounts, garlic produced fewer colonies. The combination of garlic and onion yielded inconsistent results, indicating complex interactions affecting their combined effectiveness. This combination showed moderate to high colony counts for *S. aureus* at intermediate amounts but significant inhibition at the lowest and highest amounts. Against *E. coli*, garlic alone was less effective, showing significant fluctuations in colony counts, while the garlic-onion combination demonstrated solid inhibition at higher amounts but was less effective at lower amounts.

The Nephelometer susceptibility test revealed that garlic had a high antibacterial effect on its alone, with an inhibition area of 4 mm for both bacteria; the garlic-onion combination, on the other hand, exhibited moderate inhibition, with an area of 2 mm. This test indicated no inhibition in onions alone. These results imply that garlic is less consistently effective against *E. coli* even while it possesses strong antibacterial qualities against *S. aureus* [13]. The inconsistent outcomes of the garlic-onion mixture show how these two compounds' interactions require more research. In summary, the investigation validates the antibacterial properties of both garlic and onion. Garlic exhibits concentration-dependent efficacy, while onion's effectiveness is undetected with the concentrations prepared. However, the efficacy of the combination fluctuates, indicating the need for more investigation to fully comprehend the underlying interactions.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***study conception and design:*** *Nor Nadria Abdul Nadir Nurijil;* ***data collection:*** *Yang Nurin Qistina Yang Oriyana Mazri, Nurul Athirah Yuszaarul;* ***analysis and***

interpretation of results: *Nor Nadria Abdul Nadir Nurijil;* ***draft manuscript preparation:*** *Nor Nadria Abdul Nadir Nurijil. All authors reviewed the results and approved the final version of the manuscript.*

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