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Comparative Antibacterial and Synergistic Effects of Garlic and Ginger Extracts with Silver Nanoparticles against Proteus mirabilis

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Abstract

Development of antimicrobial resistance among microbes has emerged as a critical challenge in global public health, and there is an urgent need for novel antimicrobial strategies. The current study was carried out to assess the antibacterial activity of aqueous extracts of garlic Allium sativum and ginger Zingiber officinale, as well as the synergistic effect between these extracts and silver nanoparticles (AgNPs), against Proteus mirabilis. The antibacterial activity was investigated via the agar well diffusion technique, whereas MIC and MBC were determined via broth microdilution method. Synergy tests were performed using FIC index, which was supported by time-kill studies. The findings showed concentration-dependent antibacterial activities for all compounds. Silver nanoparticles revealed the highest antibacterial potential followed by garlic and ginger extracts. Combination of garlic extract with silver nanoparticles displayed synergistic interaction with FIC index of 0.42, whereas ginger combinations showed additive interactions. It can be concluded that plant extracts-nanoparticles combinations, especially those based on garlic and AgNPs, possess enhanced antibacterial potential against P. mirabilis. Nevertheless, additional research including multiple isolates and toxicity testing is required to confirm their applications.

1. Introduction

Antimicrobial resistance (AMR) has emerged as a major global health threat, significantly reducing the effectiveness of conventional antibiotics and complicating the management of infectious diseases worldwide [1,2]. Opportunistic pathogens such as Proteus mirabilis are frequently associated with urinary tract infections (UTIs) and are increasingly reported to exhibit multidrug resistance, thereby limiting therapeutic options.

Medicinal plants continue to represent a valuable source of bioactive compounds with antimicrobial properties. Allium sativum (garlic) has been extensively investigated due to its sulfur-containing compounds, particularly allicin, which disrupt bacterial cell membranes and interfere with essential metabolic processes [3]. Similarly, Zingiber officinale (ginger) contains bioactive phenolic constituents such as gingerols and shogaols, which contribute to its antimicrobial activity [4].

On the other hand, the potential use of nanotechnology has been explored for antimicrobial purposes due to its promising results. The silver nanoparticles (AgNPs) showed antibacterial properties based on different mechanisms such as damage to membranes, production of reactive oxygen species (ROS), and prevention of DNA replication [5].

Studies have indicated that the synergism between the nanoparticles and plant extracts might increase the antibacterial activity by using reduced amounts and preventing resistance development [6]. Thus, the purpose of this study is to compare the antibacterial effect of garlic and ginger extracts and to explore their synergy with the AgNPs against Proteus mirabilis.

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The results of some researches have shown that the combined use of these two components may enhance the antibacterial action through minimizing the quantity and avoiding the resistance of the bacteria to the antimicrobial agents [6]. Consequently, the objective of the present study is to analyze the antibacterial efficacy of garlic and ginger extracts as well as their synergistic action in combination with AgNPs against Proteus mirabilis.

2. Materials and Methods

2.1. Isolation and Identification of Proteus mirabilis

A total of five clinical isolate of P. mirabilis was isolated from the laboratory of Samarra General Hospital, Samarra, Iraq. The isolate was grown on nutrient agar medium at 37°C for 24 h. Identification of the bacterial strain was done by examining the colony appearance, Gram stain, and biochemical reactions such as urease test, indole test, and motility tests, according to conventional microbiological methods [7].
All isolates were confirmed as P. mirabilis and were used in subsequent antibacterial assays to ensure reproducibility and reliability of the results.

2.2. Preparation of plant

Garlic (Allium sativum) and ginger (Zingiber officinale), which were fresh, were cleaned, dried in air, and powdered. An aqueous extract was made by combining 10 g of plant powder with 100 mL of distilled water that was sterile. This mixture was stirred continuously for 24 h and was then passed through a Whatman No. 1 filter paper and refrigerated at 4°C [8,9].

2.3. Silver Nanoparticles (AgNPs)

The silver nanoparticles (AgNPs) utilized for this research work were bought from Al-Nahrein Scientific Office, Baghdad, Iraq. As per the specification details, the particles used for the experiment had an average particle size ranging between 20-40 nm and spherical in form. Prior to use, the solution containing the AgNPs was prepared using sterile distilled water and was sonicated for proper dispersion.

2.4. Preparation of test concentrations

Plant extracts stock solutions (100 mg/mL) were prepared and diluted to get the required concentrations (100, 50, 25, 12.5 mg/mL). Similarly, AgNPs were diluted with Mueller-Hinton broth at equivalent concentrations.

2.5. Antibacterial Activity Assay

The antibacterial activity of garlic extract, ginger extract, silver nanoparticles, and their combinations was determined using the Agar Well Diffusion Test as per the protocols specified by CLSI [10]. The test was conducted using the Mueller-Hinton Agar medium, while the bacterial suspension of Proteus mirabilis was prepared at 0.5 McFarland standard concentration (1 x 10^8 CFU/mL).

Inoculation of bacterial suspension was done using sterile cotton swabs evenly over the surface of the agar medium. Wells with a diameter of 6 mm were created on the agar medium using a sterile cork borer. After that, 50 µL of test solution was poured into the wells. Distilled water was taken as the negative control while ciprofloxacin (5 µg/disc) was used as the positive control to compare the antibacterial activity of the tested agents.

After culturing the media plates at room temperature for 1 hour, they were further cultured at 37 °C for 24 hours. The measurements of inhibition zones were conducted using digital calipers in millimeters. The assessment of the antibacterial activity was made according to the diameters of the inhibition zones as reported earlier [11] using modern methods of assessment of AgNPs antimicrobial activity [12].

2.6. Determination of MIC and MBCt

MIC and MBC of the compound under investigation were studied by broth dilution method in 96-well sterile microtiter plates based on the guidelines established by CLSI [10]. MIC of the compound is defined as the minimum concentration at which there is no visible bacterial growth, whereas MBC of the compound is defined as the minimum concentration at which the bacteria are killed. The method has been widely used in recent studies dealing with nanoparticles having antimicrobial activities [3].

2.7. Determination of Synergistic Activity (FIC Index)

The synergistic activity of combinations was evaluated using the fractional inhibitory concentration (FIC) index:

FIC = (MIC_A in combination / MIC_A alone) + (MIC_B in combination / MIC_B alone)

The interaction was interpreted as follows:

- ≤ 0.5: Synergistic
- 0.5–1: Additive
- 1–4: Indifferent
- 4: Antagonistic

2.8. Statistical analysis

Each experiment was performed in triplicate, and data are presented as means ± SD. Statistical analysis was carried out using one-way ANOVA, followed by Tukey's post hoc test ($p < 0.05$).

All assays, such as Agar well diffusion, MIC, MBC, and FIC, were done in triplicate (n = 3), and their outcomes were reported as mean ± SD. For statistical analysis, one-way ANOVA followed by Tukey's post hoc test was employed, and $p < 0.05$ was regarded as statistically significant.

3. Results and Discussion

3.1. Results

The antibacterial activity of garlic extract, ginger extract, and silver nanoparticles AgNPs was concentration-dependent against *P. mirabilis* as shown in Table 1 and Figure 1. The MIC and MBC values for garlic extract are lower than ginger extract, while AgNPs have the highest antibacterial activity. The positive control (ciprofloxacin 5 µg/disc) exhibited a significant inhibition zone against *P. mirabilis*, confirming the susceptibility of the tested isolates and validating the experimental conditions. (Table 1 and Figure 1 and Figure 2).

Table 1. Zone of inhibition (mm) of tested agents against P. mirabilis at different concentrations Values are expressed as mean ± SD of triplicate experiments (n = 3).

Concentration (mg/mL)	Garlic (Mean ± SD)	Ginger (Mean ± SD)	AgNPs (Mean ± SD)	Garlic + AgNPs (Mean ± SD)	Ginger + AgNPs (Mean ± SD)
25	11.2 ± 0.6	8.4 ± 0.5	14.6 ± 0.7	18.9 ± 0.8	16.7 ± 0.7
50	14.8 ± 0.7	11.6 ± 0.6	18.3 ± 0.8	23.7 ± 0.9	21.0 ± 0.8
75	18.6 ± 0.8	14.9 ± 0.7	22.5 ± 0.9	27.9 ± 1.0	25.4 ± 0.9
100	22.4 ± 0.9	18.1 ± 0.8	26.8 ± 1.1	31.6 ± 1.2	29.3 ± 1.1

Positive control (Ciprofloxacin, 5 µg/disc): 34.5 ± 1.0 mm

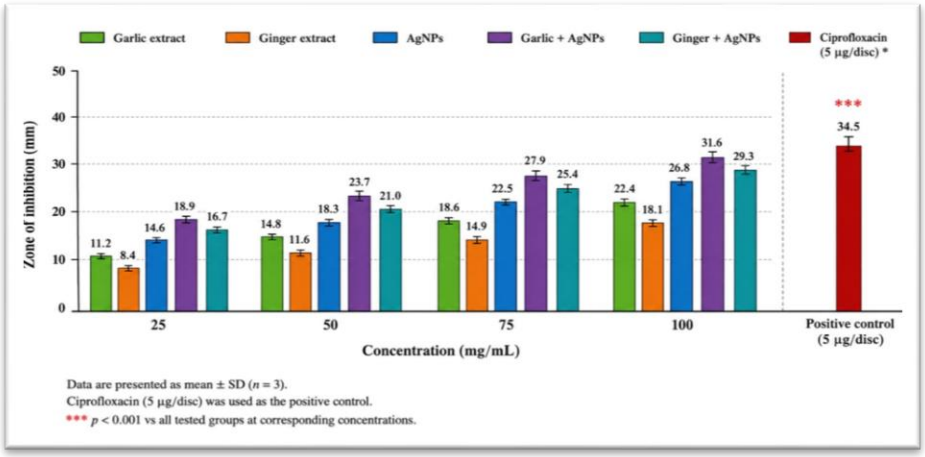

Figure 1. Antibacterial activity of garlic extract, ginger extract, silver nanoparticles (AgNPs), their combinations, and the positive control (ciprofloxacin) against P. mirabilis at different concentrations.

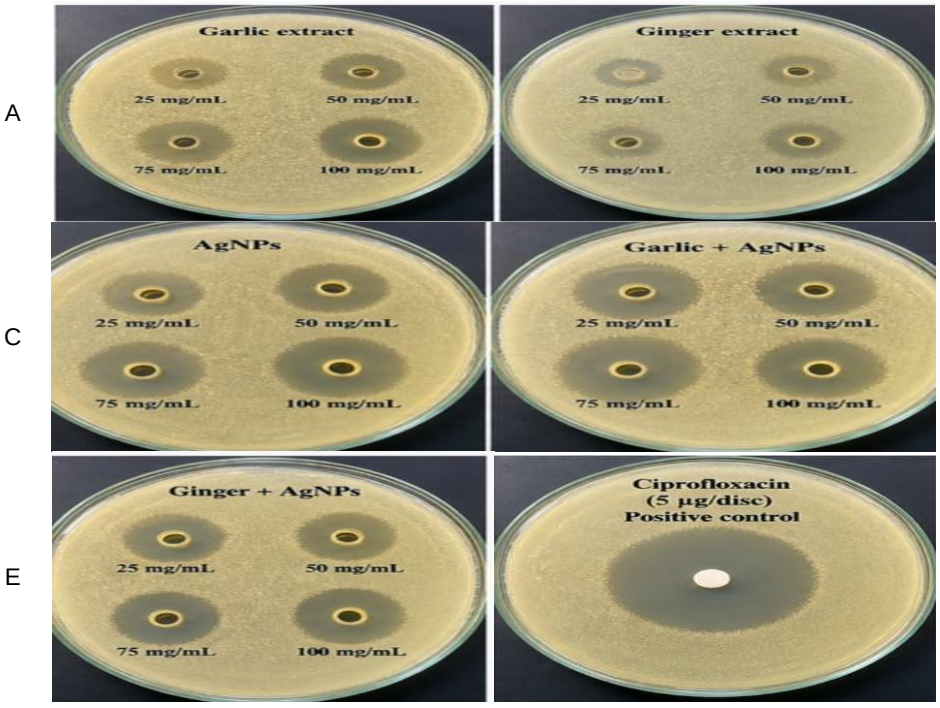

Figure 2. (A,B,C,D,E,D): Antibacterial activity of garlic extract, ginger extract, silver nanoparticles (AgNPs), their combinations, and the positive control (ciprofloxacin) against P. mirabilis at different concentrations.

Statistically significant differences in antibacterial properties were observed for all groups at the respective concentrations ($p<0.05$). The mixture of garlic extract and AgNPs demonstrated a significantly larger inhibition zone than the use of the agents separately.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the agents for their efficacy against P. mirabilis are presented in Table 2. Among all agents under evaluation, it was found that the MIC and MBC values for silver nanoparticles (AgNPs) were lowest compared to garlic and ginger extracts. Comparative representation of MIC and MBC values of all agents is presented in Figure 3, indicating their effectiveness, particularly for the efficacy of AgNPs.

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of tested agents against P. mirabilis

Agent	MIC (mg/mL)	MBC (mg/mL)
Garlic extract	25	50
Ginger extract	50	75
AgNPs	12.5	25

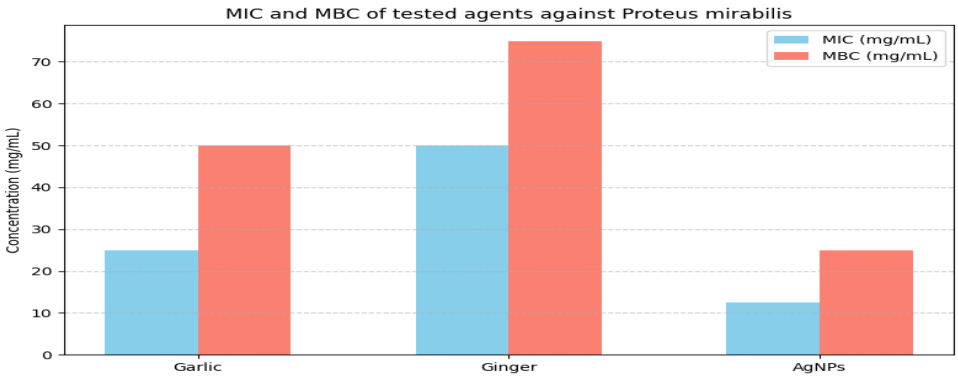

Figure 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of garlic extract, ginger extract, and silver nanoparticles against P. mirabilis.

According to the analysis of the FIC index, the mixture of garlic and AgNPs (0.42) and garlic, ginger, and AgNPs (0.39) had synergistic activity. On the other hand, ginger and AgNPs (0.75) and garlic and ginger (0.88) had additive activity but not synergistic (Table 3).

Table 3. Fractional Inhibitory Concentration (FIC) indices for combinations

Combination	FIC index	Interpretation
Garlic + AgNPs	0.42	Synergistic
Ginger + AgNPs	0.75	Additive
Garlic + Ginger	0.88	Additive
Garlic + Ginger + AgNPs	0.39	Synergistic

This was confirmed using a time-kill test, showing that the synergistic combinations were effective in reducing bacterial load after 24 hours as compared to the use of the compounds as monotherapies ($p < 0.05$).

As far as concentration is concerned, the antibacterial properties of garlic extract, ginger extract, and silver nanoparticles (AgNPs) against *P. mirabilis* have been observed to escalate gradually. Garlic extract has been observed to possess a higher inhibitory potential than ginger extract at all concentrations of the extracts used, whereas AgNPs possess the highest antibacterial properties.

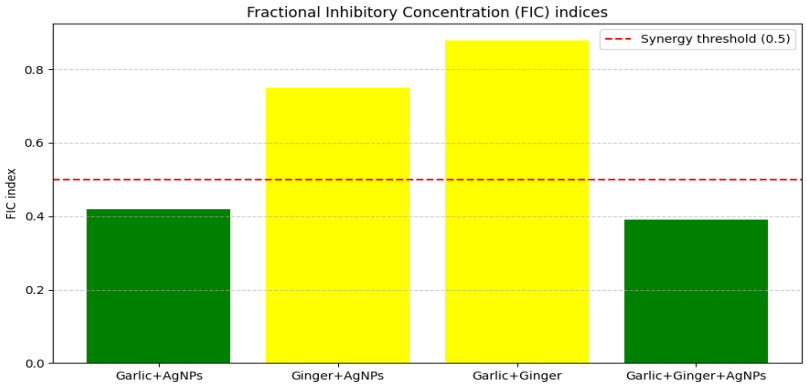

Figure 4. Fractional Inhibitory Concentration (FIC) indices for different combinations of garlic extract, ginger extract, and silver nanoparticles against Proteus mirabilis**.**

3.2. Discussion

In the current study, garlic extract was found to have higher antibacterial activity against *Proteus mirabilis* compared to ginger extract, whereas silver nanoparticles had the highest inhibitory effect compared to other compounds. This is supported by previous findings that garlic extract possesses potent antibacterial activity, which can be ascribed to compounds such as allicin and other sulfur compounds that can interfere with bacterial cell membrane structure, inhibit enzymes, and disrupt essential bacterial metabolism [14]. On the other hand, ginger extract showed lower antibacterial activity compared to garlic extract, which is supported by previous findings and recent literature that showed that ginger extract possesses moderate antimicrobial activity, mainly against Gram-negative bacteria [15].

It is worthy of note that the combination of garlic extract and AgNPs demonstrated marked synergistic antibacterial activity against *P. mirabilis*, as confirmed by the fractional inhibitory concentration (FIC) index of 0.42. This implies an enhanced mechanism of action for the killing of bacteria by the combination of bioactive phytochemicals and nanomaterials. This type of synergistic effect between plant extracts and metal nanoparticles has also been reported in some recently published literature, where enhanced antibacterial activity was attributed to the action of nanoparticles and the enhanced intracellular accumulation of plat-derived compounds [6,12].

The enhanced antibacterial effect of the combination of garlic extract and AgNPs might be attributed to the ability of AgNPs to increase the permeability of the bacterial cell membrane, thereby allowing the penetration of the antibacterial components of garlic into the bacterial cell.

Synergism that exists between the garlic extract and AgNPs can be said to arise from the increased permeability of the bacterial cell membranes caused by the presence of nanoparticles, thus enabling the entry of bioactive compounds like allicin. Nevertheless, additive effect that occurs in the case of ginger extract and AgNPs conforms to what has previously been reported concerning moderate enhancement effects of ginger extract combinations[5].

Although the results demonstrate promising antibacterial activity, this study was limited to a single clinical isolate. Therefore, further investigations involving multiple isolates and in vivo models are required to validate the therapeutic potential of these combinations.

The observed synergistic bactericidal effects could have potential clinical applications, as combination therapies could potentially reduce the doses of antimicrobial agents and still prevent the development of antimicrobial resistance.

4. Conclusions

In this current study, it was found that garlic and ginger aqueous extracts exert antibacterial activities on P.mirabilis, with garlic being more effective compared to ginger. The silver nanoparticles (AgNPs) showed strong antibacterial and bactericidal actions out of all the other test samples. The combination of garlic extract and AgNPs showed a synergistic antibacterial effect as proven through FIC index determination. This synergistic effect is because AgNPs were able to breach the bacterial cell membrane, allowing the bioactive compound (allicin) of garlic extract to penetrate the bacteria. Nevertheless, the results generated from the above experiments are limited to in vitro studies using only one isolate of bacteria. Future studies should involve different bacteria isolates, toxicity assessments, and even animal testing for further evidence.

Conflicts of Interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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التأثيرات المضادة للبكتيريا والتآزرية المقارنة لمستخلصي الثوم والزنجبيل مع جسيمات الفضة النانوية ضد بكتيريا ***Proteus mirabilis***

الملخص

أصبح تطور مقاومة المضادات الحيوية بين الأحياء المجهرية الممرضة يمثل تحديًا رئيسيًا في الصحة العامة العالمية، مما أدى إلى الحاجة الملحّة لتطوير استراتيجيات بديلة مضادة للميكروبات. هدفت هذه الدراسة إلى تقييم الفعالية المضادة للبكتيريا للمستخلصات المائية للثوم *Allium sativum* والزنجبيل *Zingiber officinale*، بالإضافة إلى دراسة التأثير التآزري لهذه المستخلصات عند دمجها مع جسيمات الفضة النانوية (AgNPs) ضد بكتيريا *Proteus mirabilis*.

تم تقييم النشاط المضاد للبكتيريا باستخدام طريقة الانتشار بالحفر على الآغار، في حين تم تحديد التركيز المثبط الأدنى (MIC) والتركيز القاتل الأدنى للبكتيريا (MBC)باستخدام طريقة التخفيف المجهري في المرق. كما تم تقييم التأثيرات التآزرية باستخدام مؤشر التركيز المثبط الجزئي(FIC) . وأظهرت جسيمات الفضة النانوية أعلى فعالية مضادة للبكتيريا، تلتها مستخلصات الثوم ثم الزنجبيل. كما أظهر مزيج مستخلص الثوم مع جسيمات الفضة النانوية تأثيرًا تآزريًا بمؤشر FIC بلغ 0.42، في حين أظهرت التركيبات المعتمدة على الزنجبيل تأثيرات إضافية. بالإضافة إلى ذلك، أكد اختبار القتل الزمني زيادة الفعالية القاتلة للبكتيريا في التركيبات التآزرية.تستنتج الدراسة أن تراكيب المستخلصات النباتية مع الجسيمات النانوية، وخاصة مزيج الثوم مع جسيمات الفضة النانوية، أظهرت فعالية مضادة للبكتيريا بصورة محسنة ضد بكتيريا *P. mirabilis* تحت الظروف المختبرية. ومع ذلك، فإن هناك حاجة إلى المزيد من الدراسات التي تشمل عزلات سريرية متعددة، وتقييم السمية، والدراسات الحية (In vivo) للتحقق من إمكانية تطبيقها العلاجية مستقبلًا.

الكلمات المفتاحية: الثوم، الزنجبيل، جسيمات الفضة النانوية، التأثير التآزري، بكتيريا *Proteus mirabilis*، النشاط المضاد للبكتيريا.