## Original Research Article

# Antibacterial Activities of Extracts and Synthesized Silver Nanoparticles from *Allium Sativum* on Urinary Quinolone-Resistant *Escherichia Coli*




**ABSTRACT**

**Aims:** The study was aimed at evaluation of the antibacterial activities of crude extracts and synthesized Ag-NPs from *Allium sativum* on urinary quinolone resistant *E. coli*.

**Study design:**Cross sectional study.
**Place and Duration of Study:**Department of Microbiology, Nasarawa State University, Keffi, between November 2023 and October 2024
**Methodology:**Confirmed Samples for this study were collected from selected General Hospitals in Abuja Municipal, Nigeria. The phytochemical screening of the Allium sativum ethanolic extract (ASE) for various phytochemical constituents and detection of AgNPswas conducted using laboratory method.
**Results:**All isolates were resistant to AMP and CIP. All the *E. coli* isolated were MAR isolates. All the MAR isolates had MAR indices of ≥ 0.2. Most isolates were MDR isolates. There was also a PDR and XDR isolates among the selected tested *E coli* isolates. Phytochemical screening of aqueous, ethanolic, ethyl acetate and n-hexane leaf extracts of *Allium sativum* showed presence of only glycosides in all the extracts. Phenols and resins followed after as they were only absent in the aqueous extract of A. *sativum* while tanins, steroids, saponins and pholabatanins were present in two (2) of the four (4) extract analyzed. Antibacterial activity screening of aqueous, ethanoic, ethyl acetate and n-hexane leaf extracts of *Allium sativum* were evaluated against quinolone resistant *E. coli* showed activity by only aqueous and ethanolic extracts. The ethanolic extracts were more active. Synthesized silver nanoparticles of the active extracts have shown higher antibacterial activity against the responsive isolates than their corresponding crude extracts.
**Conclusion:**The synthesized silver nanoparticles from the active extracts exhibited greater antibacterial activity against the responsive isolates compared to their corresponding crude extracts. These findings suggest that AgNPs hold potential for use in medicine to prevent both infectious and non-infectious diseases.


*Keywords: Allium sativum, Silver nanoparticles, Escherichia coli, Biofilm, Quinolone*

## 1. INTRODUCTION

Nanotechnology involves the design, synthesis, characterization, and manipulation of materials at the nanoscale (1-100 nm) [1]. Biogenic synthesis utilizing plant-derived extracts offers an eco-friendly, cost-effective approach to nanoparticle production, and using natural bioactive compounds with inherent antimicrobial properties [1].Particles differ significantly from materials created on a larger scale from the same source material, yet the differences remain noteworthy [2, 3]. Nanotechnology implies the production, characterization, modification, and manufacturing of nanoparticles [4]. When these particles are synthesized, characterized and manipulated, and incorporated with suitable nanomaterials, their pharmacokinetic characteristics and efficacy can be improved [4, 5, 6].
Medicinal plants play a crucial role in forming capping layers that influence the size and structure of nanoparticles [7, 8]. One such plant, *Allium sativum*—commonly known as garlic—has been utilized in the synthesis of silver nanoparticles (AgNPs) [9, 10]. Renowned for its medicinal properties, garlic is widely used in cuisines across the globe to treat various ailments [11]. It is reported to exhibit anticancer, antimicrobial, antidiabetic, hypoglycemic, hypolipidemic, antiemetic, and immunomodulatory effects [11]. Phytochemical studies reveal that *Allium sativum* is rich in tannins, saponins, phenols, alkaloids, carotenoids, and flavonoids, and possesses significant antioxidant properties [12].
The present study was aimed at evaluating the antibacterial activities of crude extracts and synthesized Ag-NPs from *Allium sativum* on urinary quinolone resistant *E. coli*.

## 2. MATERIAL AND METHODS

### 2.1 Bacteria Isolates

Two hundred presumptive *E. coli* isolates [50 each from Asokoro General Hospital (AGH), Garki Hospital Abuja (GHA), Gwarimpa General Hospital (GGH), and Wuse General Hospital (WGH)] was collected. These Health facilities are located in Abuja, Nigeria. The samples were collected using NA agar slants and transported using ice pack to National Institute for Pharmaceutical Research and Development (NIPRD) for analysis.

#### 2.1.1Confirmation of *E. coli* isolates

*E. coli*was confirmed using the VITEK® 2 Compact system (bioMérieux, Marcy-l'Etoile, France). This system utilizes advanced colorimetry to analyze biochemical reactions on microbial identification cards. After inoculating the cards with an unknown organism, the system's internal optics read and compare the reactions to those in the VITEK 2 database, enabling precise organism identification. The system employs a transmittance optical method that uses various wavelengths within the visible spectrum to interpret test reactions. During incubation, reactions are monitored every 15 minutes to detect turbidity or color changes associated with metabolic activity. An integrated algorithm eliminates false readings caused by small bubbles, ensuring accuracy.

### 2.2 Antibiotic Susceptibility Testing

The antimicrobial susceptibility testing of the bacterial isolates was carried out as earlier described by Clinical and Laboratory Standards Institute [13]. Briefly, three (3) pure colonies of the isolates was inoculated in to 5ml sterile 0.85% (w/v) NaCl (normal saline)and the turbidity of the bacteria suspension was adjusted to the turbidity equivalent to 0.5 McFarland standard. The McFarland standard was prepared as follows: 0.5 ml of 1.172% (w/v) $BaCl_2.2H_2O$ was added to 99.5 ml of 1% (w/v) $H_2SO_4$.

A sterile swab stick was soaked in standardized bacteria suspension and streaked on Mueller-Hinton agar plates and the antibiotic discs were aseptically placed at the center of the plates and allowed to stand for 1 h for pre-diffusion. The plates was incubated at 37ºC for 24 h. The diameter zone of inhibition in millimeter was measured and the result was interpreted in accordance with the susceptibility break point earlier described by Clinical and Laboratory Standards Institute [13].

### 2.3 Plant Material

*Allium sativum* was obtained on June, 2023 from a local market (Wuse Market) in Abuja/ Nigeria. The plant material was rinsed with sterile distilled water and dried [14].

#### 2.3.1 Preparation of *Allium sativum* Extracts (ASE)

Aqueous, methanolic, ethanolic and ethyl acetate *A. sativum* extract was prepared following a method described by [15] with modification. One hundred grams (100.0 g) dried *A. sativum* leaf will be soaked in 500 ml each of water, methanolic,

ethanolic and ethyl acetate for 24 h at room temperature under shaking after which the extracts was filtered through Whatman filter paper (No. 1) and concentrated to dryness using water bath at 45$^{o}$C.

**2.3.2Phytochemical Tests of *Allium sativum* Extracts (ASE)**

***2.3.2.1 Tannin Test***

Exactly 0.3 g of the powder sample was transferred into test tube and diluted in 30 ml of distilled water. The mixture was boiled in a water bath maintained at 100°Cor oven over a Bunsen flame for 10 min. The extract was filtered using a Whatman filter paper (No 45, 125mm). 2 drops of Ferric Chloride ($FeCl_3$) solution was added. Presence of catecholic tannins was indicated by appearance of green black color as described by Warnasih& Hasanah with modification[16].

***2.3.2.2 Steroid Test***

Exactly 0.5 g of extract was dissolved into 30 ml of distilled water, treated with 3 ml of $H_2SO_4$ followed by 2 ml of Chloroform. The mixture was allowed to settle for about 10 min. Formation of brown color in Chloroform layer indicated the presence of steroids as described by Warnasih& Hasanah with modification [16].

***2.3.2.3 Terpenoid Test***

Exactly 0.3 g of the extract was transferred into test tube and dissolved in 30 ml of distilled water, 0.5 ml of acetic anhydride, 0.5 ml of Chloroform and concentrated $H_2SO_4$ was added to the mixture.Formation of red violet marked the presence of terpenoid as described by Indumathi*et al* with modification[17].

***2.3.2.4 Saponin Test***

Exactly 0.3 g of extract was dissolved in 30 ml of distilled water. The mixture was boiled in the bath maintained at 100°C for 10 min and the extract was shaken vigorously. Froth formation indicated the presence of saponins in the leaves as described by Warnasih& Hasanah with modification [16].

***2.3.2.5 Flavonoid Test***

Exactly 0.3 g of extracts was dissolved in 30 ml of distilled water. The mixture was filtered using Whatman filter paper after 2 h. Exactly 10 ml was measured into test tube, 5 ml of 1M ammonia solution and concentrated $H_2SO_4$. Formation of yellow coloration indicated the presence of flavonoids as described by Warnasih& Hasanah with modification [16].

***2.3.2.6 Glycoside Test***

Exactly 0.3 g extract was mixed with 2 ml of glacial acetic acid. To the mixture, few drops of concentrated $H_2SO_4$ and $FeCl_3$ was added. Reddish brown color formation at junction of two layers and appearance of bluish green color in the upper layer indicated the presence of glycosides in the extracts as described by Warnasih& Hasanah with modification [16].

***2.3.2.7 Detection of Anthraquinone***

Exactly 200 mg of the extract was placed in a dry test tube and 2 ml of chloroform was added for 5 min. The extract was filtered and the filtrate was shaken with 2 ml of 10% ammonia solution. A pink violet or red color showed the presence of anthraquinone as described by Latha *et al* with modification [18].

***2.3.2.8 Detection of Phenols***

Exactly 100 mg of dry extract was dissolved in ethanol with 2 ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution. Ten percent aqueous ferric chloride solution was prepared by mixing 5 ml of ferric chloride to 45 ml of distilled water. Formation of a blue-green color indicated the presence of phenols [19].

***2.3.2.9 Detection of Resins***

Exactly 100 mg of dry extract was dissolved in ethanol and 5 ml of acetic anhydrite was added and dissolved by gentle heating. After cooling, 0.5 ml of concentrated sulphuric acid was added. Bright-purple colour produced indicated the presence of resins [19].

### 2.4 Synthesis of Silver Nanoparticles (AgNPs)

Ten milliliters of the *A. sativum* extract were added to 90 ml AgNO3 solution (0.1 M). The mixture was stirred continuously for 15 minutesusing a magnetic stirrer. In order to avoid silver's natural oxidationprocess, the solution was stored in the dark [20].

### 2.5 Characterization of ASE and AgNPs

*Allium sativum* extracts and Silver Nanoparticles were characterized using UV-visible spectra analysis, a UV-vis spectrophotometer (UV-3000 PC, UK) was used totake consistent readings at a wavelength range of 365-540 nm. A spectrometer was used in order to carry out the Fouriertransform infrared (FTIR) analysis, Spectra were collected using atransmission mode that ranged from 4000-440 cm1 [20].

All analyses were performed in theDepartment of Molecular Biology, Niger-Delta University, Wilberforce Island, Bayelsa State, Nigeria.

### 2.6 Determination Antibacterial activity of ASE, and ASE-AgNPs

The extract of *A. sativum* and AgNPs were tested for their ability to inhibit the growth of bacteria through the useof the agar well diffusion method [21]. Mueller Hinton agar was madeand used according to the manufacturer's instructions. An aliquot of 0.1ml of overnight bacterial culture (adjusted to 0.5 McFarland turbiditylevel) was streaked entirely on the Mueller Hinton agar using a sterileswab stick. A sterile 5 mm cork borer was used to insert five wells inthe culture medium. Using a sterile micropipette, Various amounts of the crude extracts (50 mg, 20 mg, 12.5 mg, 6.25 mg and 3.125 mg); and Ag-NPs (50 µg, 25 µg, 12.5 µg, 6.25µg and 3.125 mg/ml) were dispensed into each well of the MHA streaked with the test organism and incubated at 37°C for 24 h. The diameter of the inhibition zone was measured using meter rule.

### 2.7 Statistical Analysis

Statistical analysis was performed on all results using Graph Pad Prism version 9 for Windows, (GraphPad Software, San Diego, CA, USA).

## 3. RESULTS AND DISCUSSION

### 3.1 Confirmation of *Escherichia coli*

The phenotypic characteristics of test urinary *E. coli* isolates from patients in selected hospitals are summarized in Table 1. The isolates exhibited typical *E. coli* characteristics including pink colonies on MacConkey agar, metallic green sheen on EMB agar, and Gram-negative rod morphology. The biochemical profile (indole+, MR+, VP-, citrate-, ONPG+) further confirmed their identification as *E. coli*.

**Table 1.Cultural, Morphological and Biochemical characteristics of Test *Escherichia coli* isolated from patients with suspected urinary tract infections in selected general hospitals in Abuja Municipal, Nigeria**

| **Cultural characteristics** | **Morphological characteristics** | | **Biochemical Characteristics** | | | | | | | | | | | | **Inference** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gram reaction | Morphology | IND | MR | VP | CT | TDA | ONPG | LYS | ORN | UR | NT | $H_2S$ | MAL | |
| Pinkish colonies on MCA and Greenish metallic sheen on EMB agar | - | Rod | + | + | - | - | - | + | + | + | - | + | - | - | *E. coli* |

*+ = Positive, - = negative, IND = Indole; MR = Methyl red;Vp = Voges-Proskauer, CT = Citrate, LYS = Lysine, ORN = Ornithine; ONPG = Ortho-Nitrophenyl-β-galactosidase, UR = Urease, NT = Nitrate, $H_2S$ = Hydrogen Sulphide, Mal = Malonate, TDA = Phenylalanine deaminase*

### 3.2 Antimicrobial Susceptibility Profile of the selected Test *Escherichia coli* isolates

The antimicrobial susceptibility profiles of the selected tested *E. coli* isolates from urine of the patients in the selected general hospitals are as shown in Table 2. All isolates were resistant to AMP, S, and CIP. All the *E. coli* isolated were MAR isolates. All the MAR isolates had MAR indices of ≥ 0.2. Most isolates were MDR isolates. There was also a PDR and XDR isolates among the selected tested *E coli* isolates.

**Table 2. Antimicrobial Resistance Profile of Selected Test Quinolone resistant *Escherichia coli* isolated from urine of Patients attending selected hospitals in Abuja, Nigeria**

| Isolate | Source | Antimicrobial Resistance Class | Antimicrobial Resistance Phenotype |
|---|---|---|---|
| **EC1** | GHA | MDR | S,FOX,CN,CIP,AMP,OFX,NA |
| **EC2** | WGH | MDR | S,CTX,CAZ,FOX,CIP,AMP,NA |
| **EC3** | WGH | MDR | S,SXT,CTX,CAZ,CIP,AMP,OFX,NA |
| **EC4** | AGH | MDR | S,SXT,CTX,CN,CIP,AMP,OFX,NA |
| **EC5** | AGH | MDR | S,SXT,CTX,CAZ,FOX,CIP,AMP |
| **EC6** | GHA | MDR | AMC,S,SXT,FOX,CN,CIP,AMP,OFX |
| **EC7** | GHA | MDR | AMC,S,SXT,CTX,CAZ,FOX,CIP,AMP |
| **EC8** | GHA | MDR | S,SXT,CTX,CAZ,FOX,CN,CIP,AMP |
| **EC9** | WGH | XDR | AMC,S,SXT,CTX,CAZ,FOX,IPM,CIP,AMP |
| **EC10** | GHA | PDR | AMC,S,SXT,CTX,CAZ,FOX,CN,IPM,CIP,AMP |

*EC= Escherichia coli; AMP= Ampicillin; AMC= Amoxicillin/Clavulanic acid; S= Streptomycin; CN= Gentamicin; SXT= Cotrimoxazole; CAZ= Ceftazidime; CTX= Cefotaxime; FOX= Cefoxitin; CIP= Ciprofloxacin; IPM= Imipenem; AGH= Asokoro General Hospital; GHA= Garki Hospital Abuja; GGH= Gwarimpa General Hospital; WGH= Wuse General Hospital; MDR= Multidrug resistance(non-susceptible to ≥1 agent in ≥3 antimicrobial categories); XDR= extensive drug resistance (non-susceptible to ≥1 agent in all but ≤2 antimicrobial categories); PDR= pan drug resistanceresistance (non-susceptible to all antimicrobial listed); NMDR= non-multidrug resistance*[22].

### 3.3 Phytochemical Constituents of the Extracts of *Allium Sativum*

The phytochemical constituents of the aqueous, ethanolic, hexane and ethyl acetate extracts of *A. sativum* are presented in Table 3. All the extracts were found to contain glycosides. Phenols and resins followed after as they were only absent in the aqueous extract of A. *sativum* while tanins, steroids, saponins and pholabatanins were present in two (2) of the four (4) extract analyzed.

**Table 3. Phytochemical Constituents of Crude Extracts of *Allium sativum***

| Phytochemical Constituents | Extracts | | | |
|---|---|---|---|---|
| | Aqueous | Ethanolic | Ethyl acetate | n-Hexane |
| Tanins | + | - | + | - |
| Steroids | + | + | - | - |
| Saponins | + | + | - | - |
| Flavonoids | + | + | + | - |
| Glycosides | + | + | + | + |
| Anthraquinone | - | - | + | + |
| Phenols | - | + | + | + |
| Resins | - | + | + | + |

*+ means present, - means absent*

### 3.4Antibacterial Activities of *Allium sativum* Extracts and Synthesized Silver Nanoparticles on the isolates

The antibacterial activities of the aqueous and ethanolic extracts, and their corresponding synthesized silver nanoparticles evaluated as inhibition zone diameter (IZD) (in millimeter) against the Test and Control isolates are as presented respectively in Table 4 and Table 5. Both the crude extracts and Ag-NPs were active against the isolates, but the ethanolic were more active.

For the aqueous extract (Table 4), the IZDs ranged from 0-23 mm (crude)0-33 mm (Ag-NPs) at the various amounts tested. The antibacterial activity was concentration-dependent. The crude was inactive against isolates EC4 and EC9 at all the amount of extract tested; and EC2 and EC6 were more responsive to the action of the extract. The Ag-NPs was generally more active than the crude; and more isolates (EC1, EC2, EC3 and EC8)were more responsive to the action of the Ag-NPs. Previously inactive EC4 and EC9 were also responsive at the highest amount (50 mg) tested.

For the ethanolic extract (Table 5), the IZDs ranged from 0-27 mm (crude) and 0-34 mm (Ag-NPs) at the various amounts tested. The antibacterial activity was also concentration-dependent. The extract was also inactive against isolates EC4 and EC9 at all the amount of extract tested; and EC2, EC6 and EC8 were more responsive to the action of the crude. The Ag-NPs was also generally more active than the crude; and more isolates (EC1, EC2, EC3, EC6 and EC8) were more responsive to the action of the Ag-NPs. Previously inactive EC4 and EC9 were also responsive at the highest amount (50 mg) tested.

**Table 4. Antibacterial Activities of *Allium sativum* Aqueous Extracts and Synthesized Silver Nanoparticles (Ag-NPs) on Quinolone Resistant *Escherichia coli***

| Isolates | Inhibitory Zone Diameter (mm) at various amounts of Aqueous Extract and Ag-NPs | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Aqueous Extract (mg) | | | | | Ag-NPs/Aqueous Extract (µg) | | | | |
| | 3.125 | 6.25 | 12.5 | 20 | 50 | 3.125 | 6.25 | 12.5 | 20 | 50 |
| **EC1** | 0 | 0 | 8 | 9 | 13 | 0 | 7 | 10 | 13 | 15 |
| **EC2** | 0 | 5 | 7 | 10 | 12 | 0 | 7 | 9 | 11 | 14 |
| **EC3** | 0 | 0 | 0 | 4 | 7 | 0 | 0 | 5 | 8 | 11 |
| **EC4** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| **EC5** | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 3 | 7 |
| **EC6** | 0 | 0 | 8 | 10 | 10 | 0 | 0 | 8 | 12 | 17 |
| **EC7** | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 12 |
| **EC8** | 0 | 0 | 4 | 5 | 6 | 0 | 0 | 7 | 10 | 13 |
| **EC9** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| **EC10** | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 9 |
| **EC ATCC 25922** | 0 | 5 | 10 | 15 | 23 | 8 | 15 | 20 | 25 | 33 |

*EC= Escherichia coli; Ag-NPs= Silver Nano-Particles*

**Table 5.Antibacterial Activity of *Allium sativum* Ethanolic Extractsand Silver Nano-Particles (Ag-NPs) on Quinolone Resistant *Escherichia coli***

| Isolates | Inhibitory Zone Diameter (mm) at various amounts of Ethanolic Extract and Ag-NPs | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ethanolic Extract (mg) | | | | | Ag-NPs/Ethanolic Extract (µg) | | | | |
| | 3.125 | 6.25 | 12.5 | 20 | 50 | 3.125 | 6.25 | 12.5 | 20 | 50 |
| **EC1** | 0 | 0 | 8 | 11 | 14 | 0 | 8 | 11 | 14 | 16 |
| **EC2** | 0 | 11 | 17 | 21 | 25 | 0 | 7 | 9 | 13 | 16 |
| **EC3** | 0 | 0 | 0 | 8 | 14 | 0 | 0 | 5 | 10 | 12 |
| **EC4** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| **EC5** | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 4 | 9 |
| **EC6** | 0 | 0 | 9 | 11 | 12 | 0 | 0 | 9 | 14 | 18 |
| **EC7** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **EC8** | 0 | 0 | 6 | 8 | 9 | 0 | 0 | 7 | 10 | 13 |
| **EC9** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| **EC10** | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 9 |
| **EC ATCC 25922** | 0 | 8 | 10 | 19 | 27 | 10 | 17 | 21 | 27 | 34 |

*EC= Escherichia coli; Ag-NPs= Silver Nano-Particles*

*Allium sativum* is said to have high antibacterial properties on a wide spectrum of gram-positive and gram-negative bacteria [23]. Allicin has been found to be the active ingredient in *A. sativum* and it works as an antimicrobial agent by inhibiting DNA and protein synthesis moderately and inhibiting RNA synthesis completely as a primary target [24, 25]. *Allium sativum* is also rich in anionic components such as nitrates, chlorides and sulfates and other water soluble components found in plants and these components may have antimicrobial properties [24]. Research by Olusanmi and Amadi [26] showed the presence of flavonoids, which was similar in this present study where flavonoid was present in the aqueous and ethanolic extract of *A. sativum* but absent in the N hexane and ethyl acetate extracts. This study also showed the presence of glycosides in all the extracts of *A. sativum*and steroids in the aqueous and ethanolic extracts of *A. sativum* which is also similar to the study of Akintobi*et al*. [27] which showed the presence of glycosides and steroids in *A. sativum*. Tannins can inhibit the reverse transcriptase enzyme and DNA topoisomerase so that bacterial cells cannot be formed [28]. Similarly, steroids can interfere with the lipid membrane function, which causes leakage of the liposomes so that the cells are brittle and lysis [29]. A research by Reiter *et al* shows that the main component responsible for inhibiting and killing bacteria is allicin [30].The solvent used in preparation of the extracts played a major role in the inhibitory effect of the extract [31].

*A. sativum* was shown to have antimicrobial activity against *E. coli* [24, 32] which dully supports this study. Though few studies have shown that *E. coli* can be resistant to garlic extracts [33].

Both *A. sativum* and ciprofloxacin (CIP) showed similar levels of antimicrobial activity against*E. coli*. This was attributed to similarity in the mechanism of action of *A. sativum* and CIP. Allicin, the active ingredient of *A. sativum*, acts by partially inhibiting DNA and protein synthesis and also totally inhibiting RNA synthesis as a primary target [34]. Similarly, CIP inhibits bacterial DNA gyrase, and thus interferes with DNA transcription and other activities involving DNA [35]. There was also some similarity in the modes of action of garlic and AMP, as both inhibit cell wall synthesis [36].There was a significant difference ($p < 0.01$) in effectivity between *A. sativum* and ciprofloxacin, which could have arisen as a result of genetic differences in the sensitivities of the isolates to the antimicrobial agents and differences in the modes of action of the antibiotics. The cell wall structural nature of gram-negative enteric bacteria may be responsible for the observed susceptibility in this study. The cell membrane of *E. coli* has been reported to contain 20% lipid [37]. The polysaccharides and the lipid contents of the cell wall affect the permeability of allicin and other garlic constituents, and thus the observed susceptibility to garlic [38].

In this study, water, ethanol, nhexane and ethyl acetate were utilized to prepare the extracts with various concentrations. N hexane and ethyl acetate solvent did not have antibacterial activity on the isolates. Water and ethanol had antibacterial activity on the isolates. From the study, it was observed that ethanol showed larger inhibitory zones for the isolates tested [24]. Rafińska*et al*. [39] revealed the active compounds from *A. sativum* had poor solubility in water hence having lower antibacterial activity than ethanol. The results from the present study exposed that the compounds present in *A. sativum*, extracted by ethanol had antibacterial activity. These findings demonstrated the good solubility of the active compounds in ethanol as the organic solvent.

A color change from light pale to brown was observed on mixing of *A. sativum* extracts with 3 mM silver nitrate solution. The visible color change indicates the formation of silver nanoparticles. This may be as a result of $AgNO_3$ reduction and stimulation of surface Plasmon resonance. No precipitation was observed. The color change was stable even after completion of the reaction. The UV-vis spectroscopy is one of the most widely used simple and sensitive technique for the analysis of nanoparticle synthesis. The color exhibited by the samples is due to the excitation of electrons of the transition metals which affects the absorbance in the ultraviolet region. The silver nanoparticles synthesized by *A. sativum* extracts were confirmed by UV-vis spectrophotometer. The weak absorption peak at shorter wave lengths due to the presence of several organic compounds which are known to interact with silver ions.

The biologically synthesized silver nanoparticles using medicinal plants were found to be highly active against *E. coli.* The Ag-NPs showed high antibacterial activity against all the *E. coli* isolates. The ionic silver strongly interacts with thiol group of vital enzymes and inactivate the enzyme activity in the bacterial [40]. Studies have shown that DNA loses its replication ability once the bacteria have been treated with silver ions [41, 42]. Deepachitra*et al*. [43] mentioned that the pathogenic effect of nanoparticles can be attributed to their stability in the medium as a colloid, which modulates the phosphotyrosine profile of the pathogen proteins and arrests its growth. The growth of the microorganisms was inhibited by the synthesized Ag-NPs showed variation in the inhibition of growth of microorganisms may be due to the presence of peptidoglycan, which is a complex structure and after contains teichoic acids or lipoteichoic acids which have a strong negative charge. This charge may contribute to the sequestration of free silver ions. Thus gram positive bacteria may

allow less silver to reach the cytoplasmic membrane than the gram negative bacteria [43]. The Ag-NPs synthesized from plant species are toxic to multi-drug resistant microorganisms [44].

## 4. CONCLUSION

This study revealed that phytochemical screening of aqueous, ethanoic, ethyl acetate and n-hexane leaf extracts of *Allium sativum*indicated the presence of only glycosides in all the extracts.Also, antibacterial activity screening of aqueous, ethanoic, ethyl acetate and n-hexane leaf extracts of *Allium sativum* evaluated against quinolone resistant *E. coli,* showed activity by only aqueous and ethanoic extracts.Furthermore, the synthesized silver nanoparticles of the active extracts showed higher antibacterial activity against the responsive isolates than their corresponding crude extracts. From the results, AgNPs can be employed in the field of medicine to prevent infectiousand non-infectious diseases.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images

## ETHICAL APPROVAL

Appropriate ethical committee approval was obtained prior to start of the research and is available for review.

## REFERENCES


1Lal, S. S., & Nayak, P. L. (2012). Green synthesis of gold nanoparticles using various extract of plants and spices. *Int J Sci InnovDiscov*, *2*(3), 325-350.

2. Jwad, K. H., Saleh, T. H., & Abd-Alhamza, B. (2019). Preparation of Aluminum oxide nanoparticles by laser ablation and a study of their applications as antibacterial and wounds healing agent. *Nano Biomed. Eng*, *11*(3), 313-9..

3. Maeah, R. K., Hasoon, B. A. A. H., Abdalwahab, A. I., Al-Azawi, K. F. F., &Hameedi, W. B. A. (2020). Biosynthesis of silver nanoparticles using Hibiscus sabdariffa and their biological application. *EurAsian Journal of Biosciences*, *14*(2), 3377-3383.

4. Jawad, K. H. (2023). Laser ablation mediated ZnO nanoparticles inhibit growth and biofilm forming potential of urinary tract bacterium Proteus mirabilis. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, *14*(1), 015002.

5. Okafor, F., Janen, A., Kukhtareva, T., Edwards, V., & Curley, M. (2013). Green synthesis of silver nanoparticles, their characterization, application and antibacterial activity. *International journal of environmental research and public health*, *10*(10), 5221-5238.

6. Al-Saadi, H. K., Awad, H. A., Saltan, Z. S., Hasoon, B. A., Abdulwahab, A. I., Al-Azawi, K. F., & Al-Rubaii, B. A. (2023). Antioxidant and Antibacterial Activities of Allium sativum Ethanol Extract and Silver Nanoparticles. *Tropical Journal of Natural Product Research*, *7*(6).

7. Rauwel, P., Küünal, S., Ferdov, S., &Rauwel, E. (2015). A review on the green synthesis of silver nanoparticles and their morphologies studied via TEM. *Advances in Materials Science and Engineering*, *2015*(1), 682749.

8. Muthukumaran, U., Govindarajan, M., Rajeswary, M., & Hoti, S. (2015). Synthesis and characterization of silver nanoparticles using Gmelina asiatica leaf extract against filariasis, dengue, and malaria vector mosquitoes. *Parasitology research*, *114*, 1817-1827.

9. Mikaili, P., Maadirad, S., Moloudizargari, M., Aghajanshakeri, S., &Sarahroodi, S. (2013). Therapeutic uses and pharmacological properties of garlic, shallot, and their biologically active compounds. *Iranian journal of basic medical sciences*, *16*(10), 1031.

10. Abdul-HamzaHasoonALzubedy, B., MohammedA, RaghadkwaterMaeahALkazaly, M. A. A., Kareem, Z. A., Al-Sadie, H. A. K. H., &Hadebsadoon, A. (2016). Biological effect of Lawsoniainermis plant. *Al-Mustansiriyah Journal of Science*, *27*(4).

11. Abdulwahab, A. I., Hasoon, B. A., Maeah, R. K., & Al-azaw, K. F. (2021). Preparation of Mentha Crispata Extract and Detection of its Biological Application. *Sys Rev Pharm*, *12*(1), 1151-1155.

12. Hussein, N., Maeah, R., Sharba, Z., Aasoon, B., Sulaiman, G., Taha, A., & Jwad, K. (2019). Cytotoxic, antioxidant and antibacterial activities of crude extract of Syzygium aromaticum plant. *Plant Arch*, *19*, 350-355.

13. EUCAST, T. (2020). European committee on antimicrobial susceptibility testing. *Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 10.0*.
14. Al-Saadi, H. K., Awad, H. A., Saltan, Z. S., Hasoon, B. A., Abdulwahab, A. I., Al-Azawi, K. F., & Al-Rubaii, B. A. (2023). Antioxidant and Antibacterial Activities of Allium sativum Ethanol Extract and Silver Nanoparticles. *Tropical Journal of Natural Product Research*, *7*(6).
15. Mohana, D. C., & Raveesha, K. A. (2007). Anti-fungal evaluation of some plant extracts against some plant pathogenic field and storage fungi. *Journal of Agricultural Technology*, *4*(1), 119-137.
16. Warnasih, S., & Hasanah, U. (2019). Phytochemical characterization and tannin stability test from kluwek (PangiumeduleReinw). *Journal of Science Innovare*, *1*(2), 44-49.
17. Indumathi, C., Durgadevi, G., Nithyavani, S., & Gayathri, P. K. (2014). Estimation of terpenoid content and its antimicrobial property in Enicostemmalitorrale. *Int J ChemTech Res*, *6*(9), 4264-4267.
18. Chaitanya Latha, B., Ahalya, S., Divya Naidu, P., Mounica, K., & Ravi Kumar, A. (2017). Phytochemical evaluation of Andrographis paniculata, Cassia angustifolia and Eclipta alba. *Indian Journal of Research in Pharmacy and Biotechnology*, *5*(2), 160-163.
19. Alhajali, O., & Ali-nizam, A. (2021). Phytochemical screening and antibacterial activity of Pistacia atlantica and Pinus canariensis extracts. *Journal of the Turkish Chemical Society Section A: Chemistry*, *8*(2), 403-418.
20. Siddiqui, T., Zia, M. K., Muaz, M., Ahsan, H., & Khan, F. H. (2023). Synthesis and Characterization of Silver Nanoparticles (AgNPs) using Chemico-physical Methods. *Indonesian Journal of Chemical Analysis (IJCA)*, *6*(2), 124-132.
21. Mariadoss, A. V. A., Ramachandran, V., Shalini, V., Agilan, B., Franklin, J. H., Sanjay, K., ... & Ernest, D. (2019). Green synthesis, characterization and antibacterial activity of silver nanoparticles by Malus domestica and its cytotoxic effect on (MCF-7) cell line. *Microbial pathogenesis*, *135*, 103609.
22. Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., ... & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection*, *18*(3), 268-281.
23. Moghadam, F. J., Navidifar, T., & Amin, M. (2014). Antibacterial activity of garlic (Allium sativum L.) on multi-drug resistant. *Int J Enteric Pathog*, *2*(2), e16749.
24. Shobana, S., Vidhya, V. G., & Ramya, M. (2009). Antibacterial activity of garlic varieties (ophioscordon and sativum) on enteric pathogens. *Current Research Journal of Biological Sciences*, *1*(3), 123-126.
25. Rahman, S., Parvez, A. K., Islam, R., & Khan, M. H. (2011). Antibacterial activity of natural spices on multiple drug resistant Escherichia coli isolated from drinking water, Bangladesh. *Annals of clinical microbiology and antimicrobials*, *10*, 1-4.
26. Olusanmi, M. J., & Amadi, J. E. (2010). Studies on the antimicrobial properties and phytochemical screening of garlic (Allium sativum) extracts. *Ethnobotanical leaflets*, *2009*(9), 10.
27. Akintobi, O. A., Onoh, C. C., Ogele, J. O., Idowu, A. A., Ojo, O. V., &Okonko, I. O. (2013). Antimicrobial activity of Zingiber officinale (ginger) extract against some selected pathogenic bacteria. *Nature and science*, *11*(1), 7-15.
28. Simaremare, E. S., Gunawan, E., Yarangga, I., Satya, M. D., &Yabansabra, Y. R. (2020, July). Antibacterial and toxicity activities itchy leaves (Laportea decumana, Roxb. Wedd) extract. In *Journal of Physics: Conference Series* (Vol. 1503, No. 1, p. 012041). IOP Publishing.
29. Crowley, J., Withana, M., & Deplazes, E. (2022). The interaction of steroids with phospholipid bilayers and membranes. *Biophysical Reviews*, *14*(1), 163-179.
30. Reiter, J., Hübbers, A. M., Albrecht, F., Leichert, L. I. O., & Slusarenko, A. J. (2020). Allicin, a natural antimicrobial defence substance from garlic, inhibits DNA gyrase activity in bacteria. *International Journal of Medical Microbiology*, *310*(1), 151359.
31. Ekwenye, U. N., &Elegalam, N. N. (2005). Antibacterial activity of ginger (Zingiber officinale) roscoe and garlic (Allium sativum) extracts on Escherichia coli and Salmonella typhi.
32. Mohsenipour, Z., &Hassanshahian, M. (2015). The effects of Allium sativum extracts on biofilm formation and activities of six pathogenic bacteria. *Jundishapur journal of microbiology*, *8*(8).
33. Esimone, C. O., Okoye, F. B. C., Odimegwu, D. C., Nworu, C. S., Oleghe, P. O., &Ejogha, P. W. (2010). In vitro antimicrobial evaluation of lozenges containing extract of garlic and ginger. *International Journal of Health Research*, *3*(2), 105-110.
34. Bhatwalkar, S. B., Mondal, R., Krishna, S. B. N., Adam, J. K., Govender, P., & Anupam, R. (2021). Antibacterial properties of organosulfur compounds of garlic (Allium sativum). *Frontiers in microbiology*, *12*, 613077.
35. Shariati, A., Arshadi, M., Khosrojerdi, M. A., Abedinzadeh, M., Ganjalishahi, M., Maleki, A.,&Khoshnood, S. (2022). The resistance mechanisms of bacteria against ciprofloxacin and new approaches for enhancing the efficacy of this antibiotic. *Frontiers in public health*, *10*, 1025633.
36. Eja, M. E., Asikong, B. E., Abriba, C., Arikpo, G. E., Anwan, E. E., & Enyi-Idoh, K. H. (2007). A comparative assessment of the antimicrobial effects of garlic (Allium sativum) and antibiotics on diarrheagenic organisms. *Southeast Asian journal of tropical medicine and public health*, *38*(2), 343.

37. Rowlett, V. W., Mallampalli, V. K., Karlstaedt, A., Dowhan, W., Taegtmeyer, H., Margolin, W., &Vitrac, H. (2017). Impact of membrane phospholipid alterations in Escherichia coli on cellular function and bacterial stress adaptation. *Journal of bacteriology*, *199*(13), 10-1128.
38. Sivam, G. P., Lampe, J. W., Ulness, B., Swanzy, S. R., & Potter, J. D. (1997). Helicobacter pylori—in vitro susceptibility to garlic (Allium sativum) extract.
39. Rafińska, K., Pomastowski, P., Rudnicka, J., Krakowska, A., Maruśka, A., Narkute, M., &Buszewski, B. (2019). Effect of solvent and extraction technique on composition and biological activity of Lepidium sativum extracts. *Food chemistry*, *289*, 16-25.
40. Oves, M., Khan, M. S., Zaidi, A., Ahmed, A. S., Ahmed, F., Ahmad, E., ... & Azam, A. (2013). Antibacterial and cytotoxic efficacy of extracellular silver nanoparticles biofabricated from chromium reducing novel OS4 strain of Stenotrophomonas maltophilia. *PloS one*, *8*(3), e59140.
41. Liao, C., Li, Y., & Tjong, S. C. (2019). Bactericidal and cytotoxic properties of silver nanoparticles. *International journal of molecular sciences*, *20*(2), 449.
42. Feng, Q. L., Wu, J., Chen, G. Q., Cui, F. Z., Kim, T. N., & Kim, J. O. (2000). A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. *Journal of biomedical materials research*, *52*(4), 662-668.
43. Deepachitra, R., Lakshmi, R. P., Sivaranjani, K., Chandra, J. H., & Sastry, T. P. (2015). Nanoparticles embedded biomaterials in wound treatment: a review. *J Chem Pharm Sci*, *8*(2), 322-329.
44. Prasad, R., & Swamy, V. S. (2013). Antibacterial activity of silver nanoparticles synthesized by bark extract of Syzygiumcumini. *Journal of Nanoparticles*, *2013*(1), 431218.