Original Article

Effect of Silver Nanoparticles (Ag NPs) Prepared Using Clove Extract Against *Escherichia coli*

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Abstract

Background: *Escherichia coli* bacteria is widespread and cause health problems for humans and animals alike. Scientific studies have noted in recent years the increasing resistance of this bacteria to available antibiotic, which necessitated the search for alternatives to traditional antibiotics that were effective and safe at the same time. **Objective:** The impact of clove-derived silver nanoparticles on *E. coli* that was clinically isolated from patients in Iraq will be covered in this study. **Materials and Methods:** In this study, 11 samples were collected from Iraqi patients in different Baghdad hospitals suffering of UTIs, and chemical tests were performed on *E. coli* O157:H7, biofilm formation and their sensitivity to antibiotics were tested. The clove plant extract was prepared by green methods, and silver nanoparticles were prepared from the clove extract by biosynthesis method. The effectiveness of the nanomaterials was also tested in the laboratory. **Results:** After testing the clove extract, the results indicated its activity against *E. coli*, and the Minimum inhibitory concentration (MIC) was determined to be 18.75 and 9.37 as sub-MIC. Also, the effectiveness of silver nanoparticles prepared from cloves was also tested, and 3.75 was the MIC and 1.87 as sub-MIC. This study showed the effectiveness of clove extract and silver particles prepared from it against *E. coli*, which requires more studies to determine that it is clinically safe and effective *in vivo*. **Conclusions:** Silver nanoparticles that have been produced have antioxidant and superoxide scavenging properties. Ag NPs' functional groups on the surface are what cause this activity to happen. Furthermore, because of their tiny in size, Ag NPs exhibit potent antibacterial action against *E. coli* bacteria.

Keywords: Clove extract, *Escherichia coli*, Green method, *in vivo*, silver nanoparticles

Introduction

Numerous illnesses in the human body are brought on by *Escherichia coli*, including otitis media, UTIs, wound infections, and bacteremia.[1]The *E. coli* pathogen isolates have particular characteristics that enable them to colonize and remain on infection sites.[2,3] The *Escherichia* genus, the most prevalent facultative anaerobe that survives in the human large intestine is *E. coli*. Despite the majority of *E. coli* strains alive inoffensively in the colon, numerous pathogenic strains can cause intestinal also, extraintestinal complications.[4,5] Human infecting strains of extraintestinal pathogenic *E. coli* (ExPEC) are phylogenetically connected to one another and share a large number of virulence genes.[6,7] One of the largest issues that society is currently experiencing is the antibiotic resistance of the *E. coli*. Resistance in bacteria has expanded and developed as a result of the repetitive, and indiscriminate, use of antibiotics in medicine. Antimicrobial resistance to *E. coli* can be either naturally occurring or acquired.[8-10]

The emergence of resistant bacterial strains is a result of the increased exposure to antibiotics during the treatment of bacterial diseases. Studies on the use of natural botanicals as substitute antibacterial agents in topical preparation as well as formulation for systemic use have increased recently.[11,12]

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Since ancient times, people have utilized clove (*Syzygium aromaticum L.*) as a food preservative or for medical purposes. The antifungal, antibacterial, antiviral, and characteristics of clove were only a few of their many medical uses.[13,14] Significant levels of volatile oil, which is used to medicines, are present in cloves. Additionally, cloves are an important source of phenolic chemicals such flavonoids, hydroxycinamic acids, hydroxybenzoic acids, and hydroxyphenyl propenes. The primary bioactive component of clove is eugenol.[15]

The food and drug administration has recently approved nano-medicines for use in humans. Nanoparticles have produced some fascinating results in microorganisms as a result of their precise targeting, biocompatibility, bioavailability, and multifunctional capabilities.[16] Previous studies suggested that clove extract could be used to create silver nanoparticles. The antibacterial properties of silver nanoparticles (Ag NPs) are thought to be caused by their significant surface oxidative activity, which may be harmful to the structures and functions of microbial cells. Ag NPs can therefore cause cytotoxicity, genotoxicity, and even cell death.[17-19]

Material and Methods

Collection of samples

Eleven UTI patients had midstream urine samples taken in the morning in a sterile screw-cap jar. A calibrated loopful of the urine samples was then added to the culture media (MacConkey, Blood, and EMB agar), and the culture was carried out aerobically at 37°C for 18 h. Additionally, in Baghdad, after being clinically isolated from patients from Iraq, *E. coli* O157:H7 was discovered utilizing the Vitek 2 technique.

Silver nanoparticle Ag NPs synthesis using clove essential oil in a green method

With some changes from Ojha *et al*.[20] silver nanoparticles from green synthesis were created utilizing clove ethanolic extracts. About 5 mL of clove extract were sprayed individually dropwise into 95 mL of 10 mM silver nitrate $AgNO_3$ solution under ultrasonic conditions using an ultrasonic power of 100 W and a frequency of 42 kHz. The solution was created by dissolving 1.69 g of silver nitrate into 1 L of deionized water. The solutions were sonicated for 20 min, agitated for 30 min at 25°C at 800 rpm, and then stored there for 1 day in opaque bottles. The reaction mixture was cleaned after 1 day by centrifuging it for 10 min at 10,000 rpm to obtain clear supernatant. The final colloid samples were stored in opaque flasks at 4°C. The color of the solutions shifted from clear yellowish to dark greenish brown during the course of 5 days. This alteration in color indicates the formation of silver nanoparticles produced through green synthesis.

The prepared nanoparticles' characterization

The two types of produced nanoparticles mentioned were characterized using various tools, as demonstrated below:

The scanning electron microscopy SEM

A little drop of each type of nanoparticle was put to a copper grid that had been coated with carbon, and it was allowed to dry for 5 min under a mercury lamp. After that, measurements were made using a constant voltage and magnifications of 5000, 10,000, 20,000, and 50,000 [Figure 1].[21,22]

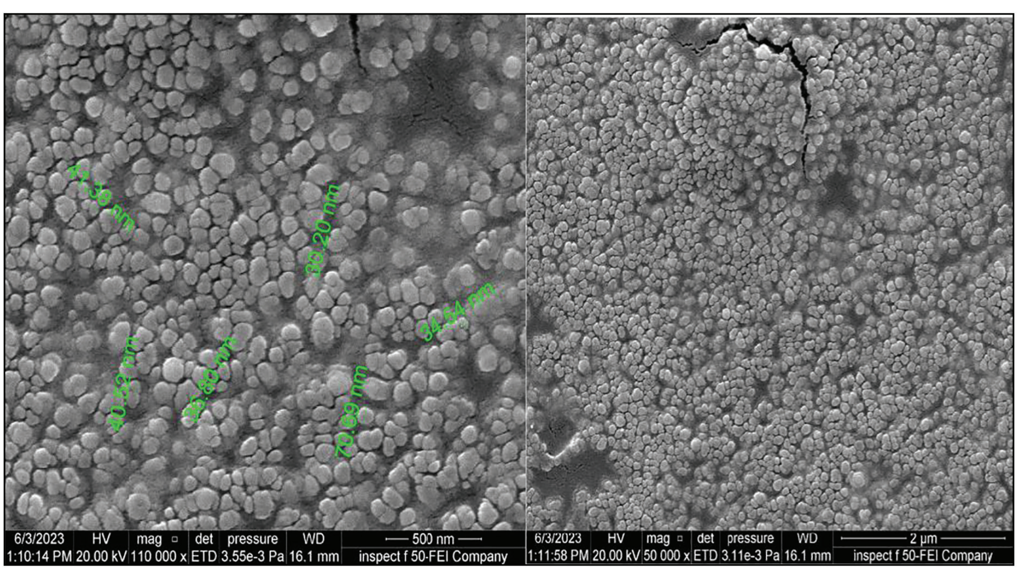

Figure 1: Atomic force microscopy (AFM) for Ag NPs

The atomic force microscopy atomic force microscopy (AFM)

A thin film of the sample from each type of nanoparticle was made by pouring 100 µL of the sample onto a glass slide. This film was then allowed to dry for 5 min. The slides were then scanned using the AFM.[23,24]

X-ray diffractometer XRD

On a glass slide, a thin water-suspended film of each type of nanoparticle was created and left to dry. *X*-ray deflection (XRD) design was recorded by, employing X-ray diffractometer at 2θ/*θ* scanning mode (operational voltage 40 kV and current 30 mA, Cu K (a) radiation (λ = 1.540).[20] Data were recorded for the 2*θ* range of 10° to 80° with a step of 0.0200°. The XRD pattern result was interpreted using the Joint Committee on Powder Diffraction Standards' standard reference (JCPDS card number 04-0783) for the characterization of Ag NPs.[25] Using the Debye–Scherrer equation, the prepared samples' particle size was calculated as follows:

$D = 0.9\lambda/\beta\cos\theta$: Where D is the crystal size, λ: is the wavelength of x-ray, θ: is the diffraction angle (Braggs angle) in radians and β: is the full width at half maximum of the peak in radians.[26,27]

The Zeta potential analyzer

The stability of the created nanoparticles was assessed in terms of zeta potential using a graphing program and a zeta potential analyzer that can work between −160 mV and +160 mV [Figure 2].[28,29]

Silver nanoparticle FTIR (Fourier transform infrared) analysis

Infrared spectroscopy using Fourier transforms Using an FTIR spectrophotometer, the produced formulations' transmittance was measured, Biosynthesis silver NPs was sent for FTIR, at Laboratories of the Ministry of Science and Technology in Baghdad.[30]

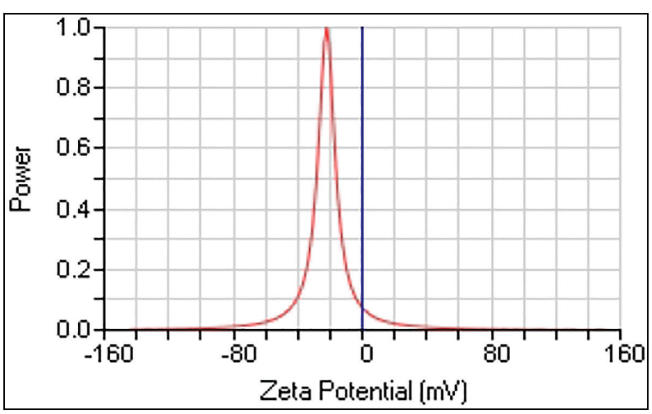

Figure 2: Result of Zeta potential analyzer for Ag NPs

Estimation of the silver ion content of silver nanoparticles made using green synthesis

Using an atomic absorption spectrophotometer, the concentration of silver ions for each of the produced green synthesis silver nanoparticles cloves that were specified in was assessed after stabilizing the color shift of the green silver nanoparticle solutions.[31]

Minimum inhibitory concentration (MIC) analysis of clove extract and silver NPs

The micro-titer plate method was used to visually evaluate the MIC after the incubation durations. Clove extract, Ag NPs, and silver nanoparticles were diluted twice in succession (1024, 512, 256, 128, 64, 32, 16, 8, 4, and 2 µg/mL) were made using 100 µL of brain heart infusion (BHI) as the diluent. With the exception of the positive control wells, which contain BHI and 10 µL of bacterial culture without clove extract and Ag NPs, and the negative control wells, which contain just 200 µL of BHI, A 10 µL bacterial suspension of *E. coli* matching to the McFarland standard no. 0.5 (1.5×10^8 CFU/mL) was added to all wells from 2 to 11 after 100 µL of high concentration clove extract or nano-silver were removed and applied to the well.[2] Micro-titer plates were incubated at 37°C for 18–20 h. Each well received 30 µL of Resazurin dye, which was then incubated for 2 h to look for any color changes. The MIC of the clove extract and Ag NPs can be determined by looking at the color. According to Ohikhena *et al.*,[32] the sub-MIC Concentrations were identified visually in broth micro dilutions as the lowest concentrations at which the resazurin dye broth assay's color changed from blue to pink [Figure 3].

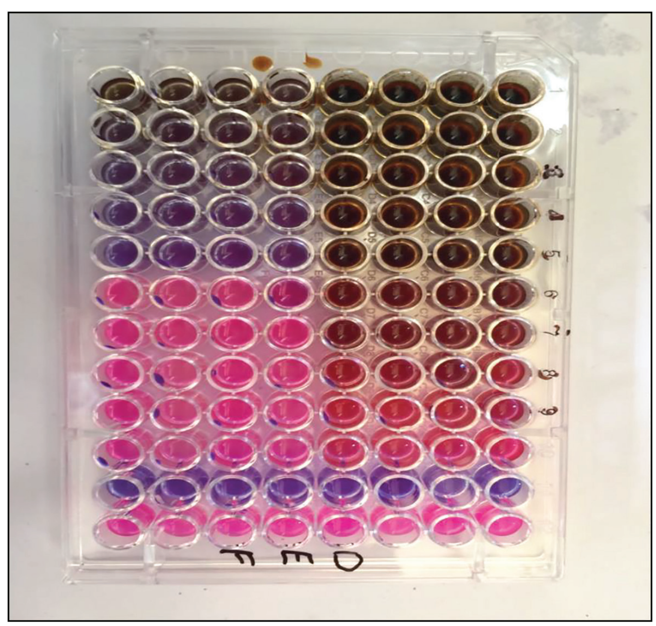

Figure 3: The MIC, sub-MIC and extra sub-MIC of Ag NPs and clove extract to inhibited *E. coli* biofilm

Antioxidant activity

By 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, the results were confirmed by compered between Ag NPs and vitamin C (Ascorbic acid) has the antioxidant activity.[33]

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 87 (including the number and the date in May 11, 2022) to get this approval.

Results

The diagnosis of the bacteria was confirmed by culturing them on the media (MacConkey, Blood, and EMB agar). *Escherichia coli* O157:H7 appears metal bright colonies on the EMB agar medium, and the diagnosis was confirmed through Vitek 2 technique.

Table 1: The MIC and sub-MIC of clove extract on *Escherichia coli*

Clove extract		
Isolates no.	**MIC value**	**Sub MIC**
E. coli No. 1	18.75	9.37
E. coli No. 2	18.75	9.37
E. coli No. 3	18.75	9.37
E. coli No. 4	18.75	9.37

Table 2: The MIC and sub-MIC of silver nanoparticles on *Escherichia coli*

Silver nanoparticles		
Isolates no.	**MIC value**	**Sub MIC**
E. coli No. 1	3.75	1.87
E. coli No. 2	3.75	1.87
E. coli No. 3	3.75	1.87
E. coli No. 4	3.75	1.87

In the present investigation, biofilm-forming bacteria and antibiotic-resistant *E. coli* O157:H7 were inhibited by clove plant extract. The clove extract's MIC and sub-MIC against four different isolates of the aforementioned bacteria are shown in Table 1.

On the other hand, four isolates of antibiotic-resistant, biofilm-forming *E. coli*, isolated from patients in Iraq, were evaluated in this investigation to determine the MIC and sub-MIC of silver nanoparticles [Table 2].

Also, in the current study, after the effect of clove extract and Ag NPs on inhibiting antibiotic-resistant *E. coli* O157:H7 was studied, the effect of clove extract and Ag NPs on biofilms of *E. coli* was studied, as it was shown that these substances have an inhibitory effect on the production of The biofilm was measured by measuring the optical density, as it was lower than that of bacteria without a control factor. As for the concentration sub-MIC, the effectiveness of these inhibitory substances was higher than the concentration of the MIC. This may be due to the fact that the diluted concentration of MIC is able to reach the largest number of bacteria and increase the inhibitory effectiveness, as in Table 3.

Discussion

Research into the antibacterial properties of clove extract has advanced significantly. This study looked at how clove extract affects antibiotic-resistant *E. coli*. Due to it having some antibacterial properties, clove extracts are used in herbal medicine and many food products as flavor enhancers. A previous studies conducted by Packyanathan and Prakasam[34] and Faleh *et al.*[35] indicated results similar to the results of this study regarding the effect of cloves against *E. coli*. The mechanisms of action of the extracts are unknown, however they contain aliphatic alcohols, thymol, terpenes, eugenol, flavones, and glycosides of phenolic monoterpenoids in addition to other antimicrobial substances. These substances have the ability to exhibit a variety of antibacterial effects against both bacteria and fungi, whether working alone or in combination. Clove oils display antibacterial activity against *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa*, as well as against *Streptococcus pyogenes*, *Salmonella*, *Bacteroides*, and *Candida albicans* at various dilutions of the extracts. These findings are in line with

Table 3: The MIC, sub-MIC and extra sub-MIC of Ag NPs and clove extract to inhibited *Escherichia coli* biofilm

Sample	*E. coli* isolate no. 11	*E. coli* isolate no. 32	*E. coli* isolate no. 8	*E. coli* isolate no. 12
G –ve control	0.15	0.15	0.15	0.15
G +ve control	0.32	0.34	0.66	0.32
MIC Ag NPs	0.006	0.008	0	0.1
Sub MIC Ag NPs	0.012	0.01	0.1	0.01
Extra MIC clove extract mg/dL	0.0	0.0	0.0	0.0
Extra sub-MIC clove extract mg/dL	0.0	0.0	0.0	0.0

those of other studies.[36] The MIC and sub-MIC assay can demonstrate a substance's antibacterial activity and serve as a reliable gauge of the dosage required to stop the spread of germs. Clove extract was found to be antibacterial when its MIC was evaluated against test organisms *E. coli*.[37]

On the other hand, in previous studies, the growth, porousness, and morphology of the bacterial cells after action of silver nanoparticles (Ag NPs) were examined in order to better understand the antibacterial activity and mode of action of Ag NPs on *E. coli*. Previous studies found that 10 μg/mL Ag NPs could fully stop 10^7 cfu/mL *E. coli* cells from growing in liquid Mueller–Hinton agar media.[38] Also in another study conducted in 2022 by Goda *et al.*,[39] they reached summary results that Ag NPs can eliminate gram-negative and positive bacteria in cases of UTIs, noting that their effect is greater on gram-negative bacteria at lower concentrations than silver nanoparticles. Also, in a previous study by Yuan *et al.*,[40] the MICs of Ag NPs against *P. aeruginosa* and *S. aureus* to be 1-2 μg/mL. The findings from the previous study indicate that Ag NPs have antibacterial effects dependent on dose and time of bacterial exposure.

Due to its advantageous characteristics, Ag nanoparticles is well known as an antibacterial agent, and multiple investigations have revealed that Ag NPs exhibit potent antimicrobial activity. Inhibiting bacterial cell division causes this process, which ultimately kills the bacteria by dissolving their membrane structure. The results here show the possibility of a bacterial apoptosis-like response as a second bactericidal effect based on the previous report's hypothesis that Ag NPs might have a broader mode of action including membrane destruction.[41] A previous examination revealed that the ability of Ag NPs to restrict bacterial growth was confirmed by the test results for suppressing bacterial growth. When *E. coli* bacteria were exposed to a range of Ag NP concentrations (0–100 g/mL), the tendency for bacterial growth decreased, as the current study reached results similar to what was reported in this previous study.[42] Also, The results of the recent study revealed that by Elsayed *et al.*,[43] Ag NPs were effective against a wide range of bacteria including *E. coli*, with MICs ranging at 3.15. This result for the MIC of silver nanoparticles was comparable to that reported in this study (3.75) for all *E. coli* isolates studied.

The size, dispersion, shape, and release of very minute amounts of their cations on the substrates on which they are working are all factors that affect the biocidal effects of Ag NPs. Silver is the element that is, most frequently employed to create nanomaterials due to its strong antibacterial properties. Silver nanoparticles' ability to suppress bacteria is known in part. Ag NPs initially gather on the bacterial membrane's outer surface, then they penetrate the cell, altering its permeability and doing significant harm.[44] According to earlier research, Gram +ve bacteria have a more robust defensive mechanism than *E. coli*. Being a Gram-positive bacterium, it contains a coating of peptidoglycan in the cell wall and lacks the nuclear region, which is where DNA molecules are dispersed randomly, in the middle of the cell. It has also been observed that silver nanoparticles have increased biocidal activity against *E. coli* thanks to this outer layer of the cell wall's ability to stop silver ions from entering the cytoplasm.[45] This effectiveness was linked to variations in cell wall structure between Gram-negative and Gram-positive bacteria. This notion is supported by earlier research, which shows that G+ve bacteria have higher resistance to harmful environments and a wide range of disinfectants than Gram-negative bacteria. Furthermore, it has been hypothesized that Gram-negative bacteria are more vulnerable to environmental changes because their cell membrane structure is more complicated.[45] The physicochemical microenvironments found within biofilms contribute significantly to the organization of the microbial community. Silver nano material antibacterial action on bacterial biofilms may thus differ dramatically from their antibacterial effect on other bacterial cells. Unfortunately, studies into the behavior and reactivity of silver NPs in intricate systems like biofilms is still in its infancy.[46]

On the other hand, Clove oil has a strong antimicrobial effect against pathogenic bacteria. Numerous secondary metabolites present in clove oil have the ability to prevent or limit the growth of germs when used in the food sector.[47] There has been a lot of advancement in the study of clove oil antimicrobial properties. Therefore, earlier research looked into clove oil's ability to suppress the growth of *E. coli* biofilms. The prepared clove oil can break up *E. coli* biofilms and has good storage stability. Clove oil affects biofilms by preventing bacterial metabolic pathways in the membrane and the release of extracellular polymer it is essential to biofilm-forming, which causes *E. coli* bacteria to lose their capacity to build biofilm, according to the findings of the previous study to investigate the mechanism.[48]

Conclusions

Silver nanoparticles that have been produced have antioxidant and superoxide scavenging properties. Ag NPs' functional groups on the surface are what cause this activity to happen. Furthermore, because of their tiny in size, Ag NPs exhibit potent antibacterial action against *E. coli* bacteria.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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