



**Antimicrobial Effects of Red Pepper Seeds Nanoparticles against
Multidrug-Resistant *Klebsiella pneumoniae*
And *Staphylococcus aureus***

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Abstract

Background: Bio-nanoparticles are manufactured using the metabolites of microbes e.g. viruses, bacteria and true fungi including yeast and algae.

The objective of this research was to determine the red pepper seeds plant extract and silver nitrate nanoparticles effect as an antibacterial on *Klebsiella pneumoniae* and *Staphylococcus aureus* bacteria.

Materials and Methods: The red pepper seeds plant extract, silver nitrate nanoparticles were examined against *Klebsiella pneumoniae* and *Staphylococcus aureus* bacteria in the study. The silver nanoparticles were characterized using Ultra Violet-Visible spectroscopy, transmission electron microscopy and infrared spectroscopy.

Results : A total of 120 samples were gathered from patients with burns from three hospitals in Baghdad City, Iraq (e.g. Al-Kindy hospital, Ibn-Balady hospital and Al-Sader hospital) from April to July 2025. Revealing isolation 35 *Staphylococcus aureus* (29.16%), out of total cases. Additionally, 25 cases of *Klebsiella pneumoniae* (20.83%) was insulated out of total cases. Data showed the superiority of silver

nanoparticles in an inhibiting the *Klebsiella pneumoniae* and *Staphylococcus aureus* growth. A concentration of 256 µg/ml was used to antibacterial activity of plant seed extract and plant seed silver nanoparticles against *Staphylococcus aureus* and *Klebsiella pneumoniae*. The diameter of the inhibition zone of red pepper extract on *Klebsiella pneumoniae* was 21 mm at 256 µg/ml concentration, while the inhibition zone diameter of silver nanoparticles was 29 mm at 256 µg/ml. The diameter of zone of inhibition of red pepper extract was 16 mm at 256 µg/ml and silver nanoparticles on *Staphylococcus aureus* was 22 mm at 256 µg/ml. The *Klebsiella pneumoniae* was resistant to Colistin (100%), Amikacin and Imipenem (84%). While *Staphylococcus aureus* showed resistant to Amikacin (94.28%), Colistin (71.42%), Piperacillin (12%).

Conclusion: The red pepper extract and silver nanoparticles are an effective strategy that may contribute to control the growth and spread of microbes.

Keywords: Red pepper seeds, *Capsicum annuum* L., *K. pneumoniae*, *S. aureus*, Silver nanoparticles, Resistant, Inhibition, Antimicrobial.

Introduction

Nanotechnology has recently amplification in various fields including medicine, and pharmacy. Nanoparticles are manufactured in different ways. The advantages of this method are that it is environmentally friendly, does not require energy, cheap, and fast. Researchers have used extracts of plant parts such as roots, stems, leaves, fruits, or fruit peels and seeds to nanoparticles preparation. Also, different metals are the most used such as silver, gold, zinc oxide, copper oxide (Al-Amin & Jayasuriya, 2007; Kirdat *et al.*, 2021). These particles have different properties from the metals from which they were formed. The inappropriate utilize of antibiotics has resulted upgrowth of antibiotic-resistant microbial isolates. It is necessary to find out novel antimicrobial mechanisms with broad-spectrum efficacy. Recently, plant alternatives have been used to overcome microbial resistance to antibiotics and obtain effective treatment to enhance immunity. The capacity of plants to form secondary compounds in seeds, leaves, and roots, which have medical significance, such as alkaloids and phenols. Therefore, focus increased on the use of nanotechnology using plant extracts and studying by

evaluating the effectiveness of red pepper plant extract and silver nanoparticles prepared against some types of bacteria (Kirdat *et al.*, 2021; Akintelu *et al.*, 2021). Plants contain distinctive and effective organic compounds e.g. flavonoid, amino acids, carboxylic acids, ketones that might produce nanoparticles in the easy, safe, and secure ways (Politeo *et al.*, 2007; Zawar *et al.* 2023). Some researchers have widely used plant leaves in the field of biomanufacturing of silver nanoparticles, these particles had antifungal activity (Jebril *et al.*, 2020), silver nanoparticles (Jumah *et al.*, 2020) and gold nanoparticles (Wang, 2017).

The retirement began in a spherical shape and its drops reached in the range of 20 - 40 drops and this had positive effects in increasing the biomass of the stem and roots at a concentration of 10 - 100 mg / L. However, prompted the researcher to reach the use of iron nanoparticles in fertilization (Javed *et al.*, 2020). Hence, this study aimed to determine the red pepper seeds plant extract and silver nitrate nanoparticles as an antibacterial agent on *Klebsiella pneumoniae* and *Staphylococcus aureus* bacteria.

Material & Methods

Sample Collection and Isolation Bacteria from Burn

A total of 120 samples were gathered from patients with burn from three hospitals in Baghdad City, Iraq (e.g. Al-Kindy hospital, Ibn-Balady hospital and Al-Sader hospital) during the period of April to July of 2025. All samples were taken to laboratory for culturing and identification. The phenotypic characterization of the cultivable on different media and differentiation, such as colony color, height, colony edges, and texture. Therefore, pigment formation on culture media using MacConkey Agar, Mannitol Salt Agar, Blood Agar were basis for selecting and differentiating between the isolates and diagnose if isolated bacteria.

Dry Red pepper

Dry Red pepper *Capsicum annum L.* seeds were bought from the local market- Baghdad. They were classified in the General Authority for Seed Testing and Certification, Plant Genetic Resources Department- Gene Bank. The seeds were grinding and weighed at 50g using a digital scale, sterilized and left in dry containers for storage.

Preparation Standard Hot Aqueous Seeds Extract Solutions

The standard solutions required for the experiment were prepared by adding 1 ml of the seeds extract added to 99 ml of deionized water. 0.5 ml of 100x-Triton was added as an emulsifying substance to help the seeds extract mix with the water (Alnuaimi *et al.*, 2019).

Biosynthesis of Silver Nanoparticles

The seed extract was prepared by grinding the seeds of red pepper plant using an electric grinder in a fine bowl and placing them in a clean and sterile glass container, it is about 8 grams of seed powder added to 100 ml of distilled water. Then placing in a water bath at a temperature of 60 °C for 30 minutes then filtering it and adding fresh silver nitrate solution (1 mol/L) in a ratio of 9:1 and then leaving the mixture under the sun for 10 minutes (Biradar *et al.*, 2011).

Determination of Red Pepper Seeds Silver Nanoparticles MIC

Two-fold serially diluted (1–1024 µg/mL) Red Pepper Seeds Silver Nanoparticles prepared from stock (10 mg/1 mL) in microtiter suspension plates similar to McFarland Standard 0.5 (1.5×10^8 CFU). Excluding the negative control wells. At 37 °C were incubated microplate plates for (18-20 hours) (Shariati *et al.*, 2019) .

Characterization of Silver Nanoparticles

The absorption spectrum of silver nitrate solution and plant extract was measured, added silver nitrate until the appearance of color was observed using a UV/Vis/NIR Spectrophotometer within the range of 800-300 nm and a resolution of one nanometer (Reig *et al.*, 2002). The samples were prepared for the tests using silver nanoparticles solution and centrifuging at a rate of 7800 rpm for 30 minutes and the disposing of the filtrate was precipitate and dried. Determination the shape and size of the particles, and the quantity of the dried precipitate was dissolved in ethanol. The suspension was exposed to an ultrasonic bath (BRANSON, 1510) for 30 min. Then, a drop of the suspension is put on a carbon-coated copper grid and leftover to dry completely with a Transmission Electron Microscope (TEM) (JEM-1011, JEOL, Japan, Tokyo) at an accelerating voltage of 90 kV. (James, 2025). Furthermore, the Fourier Transform Infrared Spectrometer (FT-IR)" was utilized to obtain the FT-IR of the 600–4000 cm⁻¹ Ag NPs.

Antibiotic susceptibility test for isolates

The disk diffusion method as mentioned by the Clinical Laboratory Standards Institute was used to perform antibiotic susceptibility testing CLSI 2023. Antibiotic tablet of Amikacin (AK), Cefepime (CEF), Ciprofloxacin (CF), Colistin (CO), Imipenem (IPM), Tazobactam Ticarcillin (TAZ), Ceftazidime (CAZ), Piperacillin (PC) were used for antibiotic susceptibility testing (AST). Isolates that exhibit resistance to more than three different classes of antibiotics have been certified as multi-drug resistant (MDR) (Dubey *et al.*, 2010). In this respect, *Escherichia coli* ATCC strain 25922 as control was obtained from Biology Department laboratory- Mustansiriyah University.

Antimicrobial activities of hot aqueous extraction and silver nanoparticles

Antimicrobial Sensitivity test was used to detect the antimicrobial activities of nutrient agar (NA) plates swabbed with 12 h old sterile broth (cotton swab) cultured with bacteria. Zones of inhibition diameters were produced on every plate utilizing a sterilized cork drill, then by utilizing a sterilized syringe, into borehole and left to soak at ambient temperatures for two hours. All plates were left for 24 hours, then incubated at 37 °C (Arokiyaraj *et al.*, 2015).

The antibacterial activity of plant seed extract and plant seed silver nanoparticles against *Staphylococcus aureus* and *Klebsiella pneumoniae* was determined at concentrations of 256 µg/ml, according to (Kunwar *et al.*, 2021).

RESULTS

Isolation and Identification of Bacteria

This study revealed that 35 *Staphylococcus aureus* were isolated out of total cases. Additionally, 25 cases of *Klebsiella pneumoniae* was isolated out of total cases.

Characterization of silver nanoparticles

The results of this study of the visible and ultraviolet spectrum analysis showed the absorption spectrum of red pepper nanoparticles in high-purity water. It is showed between the high-intensity absorption peak around 415 nm and a low-intensity peak around 700 nm. It is termed

the spectral plasmonic region (SPR), which observed for red pepper nanoparticles that synthesized by red pepper nanoparticles (Figure 1).

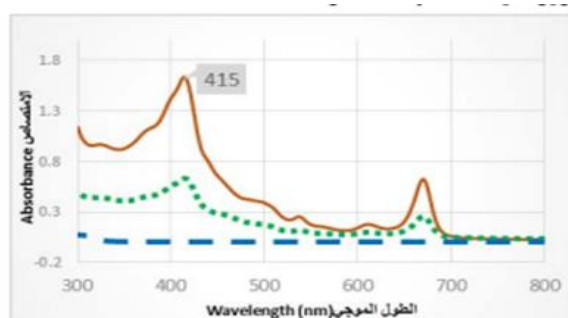


Figure 1: UV–visible spectra analysis of red pepper nanoparticles peak at a wavelength of 415 nm

Transmission electron microscopy (TEM) results showed the distribution of red pepper AgNPs and silver core diameter captured the size of particles within the range of 14-45 nanometers (Figure 2).

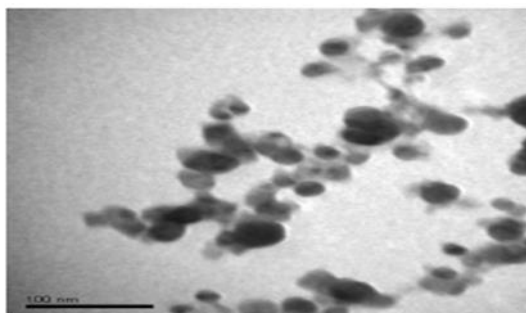


Figure 2: TEM images of red pepper nanoparticles

Fourier transform infrared (FTIR) shows the infrared spectroscopy of the red pepper extract by broad peak at 3290 cm^{-1} represents. The vibration frequency of the C-H stretching bonds is measured the functional group of alkynes. At the peak at 2824 cm^{-1} indicates N-H stretching bonds of the moiety amine salt. The board peak at 2752 cm^{-1} represents the stretching vibration of the frequency of the O-H stretching bond. The broad stretch at 1621 cm^{-1} represents the vibration that stretches the frequency of the C=O stretching bond of the functional group conjugated alkenes. The broad stretch at $1462, 1367\text{ cm}^{-1}$ represents the

bending vibration of the regularity of C-H bending bond of the functional group alkenes. The broad stretch at 1241 cm⁻¹ depicts the stretching vibration of the frequency C-N stretching bond of the functional group amine (Figure 3).

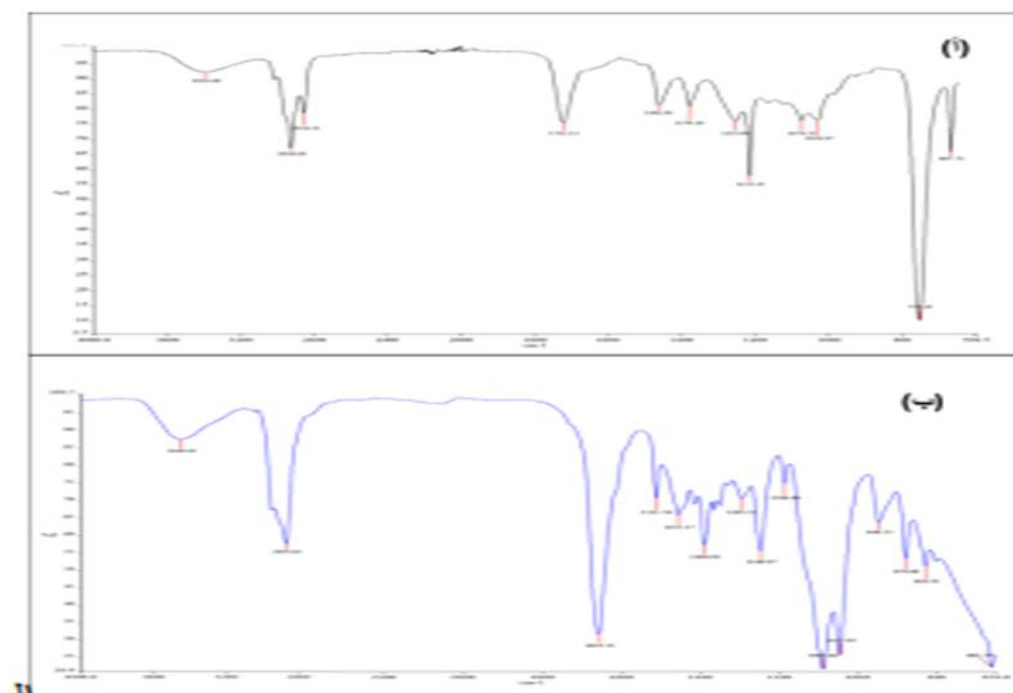


Figure 3: Infrared spectrum analysis of the Red Pepper plant extract
Antibiotic Susceptibility Test for Isolates

The results of this study showed that *Staphylococcus aureus* were resistant to Amikacin (94.28%), Piperacillin (88.57%), Cefepime and Tazobactam/Ticarcillin (85.71%), Ceftazidime (62.85%), Colistin (71.42%), Ciprofloxacin (54.28%), Meropenem (40%). Also, Imipenem (28.57%). *Klebsiella pneumoniae* was resistant to Colistin (100%), Amikacin and Imipenem (84%), Cefepime and Tazobactam/Ticarcillin (72%), Ciprofloxacin (40%), Ceftazidime (32%) and Piperacillin (12%). *K. pneumoniae* was resistant to Colistin and Tobramycin (100%), Amikacin and Imipenem (87.5%), Cefepime, Gentamicin and Tazobactam/Ticarcillin (62.5%), Ciprofloxacin (37.5%), Ceftazidime

(25%) and Piperacillin (12.5%), while no resistance to Meropenem was found (Table 1).

Antibacterial activity of red pepper extract and red pepper nanoparticles

The results of This study proved antibacterial activity of plant seed extract and plant seed silver nanoparticles against *Staphylococcus aureus* and *Klebsiella pneumoniae* at concentrations of 256 µg/ml significantly higher than other concentrations . The diameter of the inhibition zone of red pepper extract on *Klebsiella pneumoniae* was 21 mm (A) at 256 µg/ml , while the inhibition zone diameter of silver nanoparticles was 29 mm (A1) at 256 µg/ml. The diameter of zone of inhibition of red pepper extract was 16 mm (B1) at 256 µg/ml and silver nanoparticles on *Staphylococcus aureus* was 22 mm (B) at 256 µg/ml (Figure 4).



Figure 4: Inhibition zones of

- A- Hot aqueous extract of red pepper seeds
- A1 silver nanoparticles against *Klebsiella pneumoniae*
- B silver nanoparticles against *Staphylococcus aureus*
- B1- Hot aqueous extract of red pepper seeds

Discussion

Many studies have indicated the using plant extracts of red pepper nanoparticles because they contain many secondary metabolites that play a major and effective role of nanoparticles (Petla *et al.*, 2011; Reda *et al.*, 2019). Previous studies indicated the ability of the isolated compound pearl from henna leaf extract to reduce and produce gold and silver nanoparticles due to its content of secondary carbonyl and hydroxyl groups. Also, proteins and some amino acids present in the soybean leaf extract Max Glycine reduce palladium ions to palladium nanoparticles (AL-Kazaz, 2014).

The resistance of *S. aureus* isolates for Ciprofloxacin was confirmed by previous studies (Hawraa, 2014; Mohammed & Maki 2017). Amikacin was 81%, Tobramycin (76.47%) and Ciprofloxacin (52.94%), which is close to the result of this study. The Gentamicin was (50%) close to the study, where previous report found that Cefepime was (75%)(Jassim, 2013). The resistance of *K. pneumoniae* isolates for Amikacin and Gentamicin were (66.6%) and (83.3%) respectively, this is consistent with previous studies by (AL-Samaraey, 2021; AL-Taai, 2016), and Ciprofloxacin (Al-Mathkhury & Assal, 2012), because the β -lactam family that inhibit the synthesis of the bacterial cell wall. Whereas, *Staphylococcus aureus* showed high resistance to these antibiotics because these bacteria secreted the beta-lactamase enzyme in addition to changing the binding sites (Alshammari & Al-Skhattat 2015). According (Reygaert, 2013). This indicates that the plasmid is the source of the prescription for resistance to these antibiotics. It can be said that the use of red pepper extract and silver nanoparticles prepared from it, is an effective strategy that may contribute to controlling the growth and spread of microbes (AL-Oqaili, 2018).

Previous reports declared that silver nanoparticles synthesized utilizing the alcoholic extract of *Capsicum frutescens* and monodisperse particles 20-25nm showed surface plasmon resonance band at 385-435nm (Yousif *et al.*, 2019). The role of phenol and aliphatic compounds in the stabilization of silver nanoparticles (Dubey *et al.*, 2010). It has a high proportion of Polyphenolic compounds, vitamin C, vitamin A, and Beta-carotene are present. The turnout of these components in the red pepper

extract solution work together to detoxify making them more powerful (Shankar *et al.*, 2017; Howard *et al.*, 2000). Silver nanoparticles utilizing the aqueous extract of *Capsicum*, created The spherical shape of silver nanoparticles was typical with a 20-40 nm size range. The AgNPs were effective against *Klebsiella pneumoniae* and *Staphylococcus aureus*, which proved their antibacterial action. In medical and textile products, silver nanoparticles are widely used as broad-spectrum antimicrobial agents (Mohammed *et al.*, 2017).

Secondary metabolites found in medicinal plants, such as alkaloids, flavonoids, tannins, and terpenoids, contribute to their rich source of antimicrobial agents (Bhandari *et al.*, 2016; Mole, and Waterman, 1987). This study's findings are in part compatible with previous investigations of antimicrobial activity on *Capsicum annuum* (Berber *et al.*, 2013). Red pepper nanoparticles were used in the study, which demonstrated excellent action against gram-positive and gram-negative organisms. Silver's ability to kill bacterial strains and other microorganisms present in medical and industrial processes has been long-recognized (Dorantes *et al.*, 2000). It can be said that the use of red pepper extract and silver nanoparticles prepared from it is an effective strategy that may contribute to controlling the growth and spread of microbes (Meredith, (2011).

Fourier transform infrared (FTIR) analysis is one of the important techniques for rapid and efficient identification of encase compounds. Surface properties of red pepper nanoparticles showing transmittance as a function of wave number investigated by FTIR (AL-Hadidi *et al.*, 2014). FTIR examination of the infrared spectrum allows us to understand the macro particles and nanoparticles of red pepper Comparing the FTIR spectrum analysis of the red pepper extract before and after adding silver nitrate and forming silver nanoparticles that might increase the broad stretching bond of the functional group primary amine (AL-Hadidi *et al.*, 2014; Patkowska *et al.*, 2025).

Conclusion

Controlling the growth and spread of microbes may be possible with the use of red pepper extract and silver nanoparticles, which are effective strategies. Using the red pepper AgNPs and silver core diameters, the size of particles was characterized by a range of 14-45 nanometers. The red

pepper extract's infrared transform has the highest broad peak at 3290 cm⁻¹.

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Table (1): Antibiotic Sensitivity test results of Studied bacteria

Antibiotic Disc (cod)	<i>Staphylococcus aureus</i> / Resistant No.	<i>Staphylococcus aureus</i> Sensitive No.	<i>Klebsiella pneumoniae</i> / Resistant NO.	<i>Klebsiella pneumoniae</i> Sensitive No.
Amikacin (AK)	94.28% (33)	5.71% (2)	84% (21)	16% (4)
Cefepim (CEF)	85.71% (31)	11.42% (4)	72% (18)	28% (7)
Ciprofloxacin (CF)	54.28% (19)	45.71% (16)	40 % (10)	60% (15)
Colistin (C)	71.42% (25)	28.57% (10)	100% (25)	0% (0)
Imipenem (IPM)	28.57% (10)	71.42% (25)	84% (21)	16% (4)
Tazobactam/Ticarcillin (TAZ)	85.71% (31)	11.42% (4)	72% (18)	28% (7)
Ceftazidime (CAZ)	62.85% (22)	37.14% (13)	32% (8)	68% (17)
Piperacillin (PC)	85.57%(31)	11.42% (4)	12%(3)	88% (22)

AK=Amikacin, CEF=Cefepime, CF=Ciprofloxacin, CO=Colistin, IPM=Imipenem, TAZ =TazobactamTicarcillin, CAZ=Ceftazidime, PC=Piperacillin

Table (2): Inhibition zones of hot aqueous extract of red pepper and red pepper nanoparticles

Type of Bacteria	The inhibition zone diameter (mm)	
	Red pepper seeds extraction	Red pepper silver nanoparticles
<i>Staphylococcus aureus</i>	16 mm (B)	22 mm (B1)
<i>Klebsiella pneumoniae</i>	21mm (A)	29mm (A1)

التأثيرات المضادة للميكروبات للجسيمات النانوية لبذور الفلفل الأحمر ضد بكتيريا *Klebsiella pneumoniae* المقاومة للأدوية المتعددة

والمكورات العنقودية الذهبية

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مستخلص البحث:

الخلفية: تُصنع الجسيمات النانوية الحيوية باستخدام نواتج أيض الكائنات الدقيقة، مثل الفيروسات والبكتيريا والفطريات الحقيقية، بما في ذلك الخميرة والطحالب.

هدف هذا البحث هو تحديد تأثير مستخلص بذور الفلفل الأحمر وجسيمات نترات الفضة النانوية كمضادات للبكتيريا على بكتيريا *كليبسيلا الرئوية* والمكورات العنقودية الذهبية.

المواد والطرائق: تم في هذه الدراسة فحص مستخلص بذور الفلفل الأحمر وجسيمات نترات الفضة النانوية ضد بكتيريا *كليبسيلا الرئوية* والمكورات العنقودية الذهبية. وتم توصيف جسيمات الفضة النانوية باستخدام مطيافية الأشعة فوق البنفسجية والمرئية، والمجهر الإلكتروني النافذ، ومطيافية الأشعة تحت الحمراء. النتائج:

جُمعت 120 عينة من مرضى الحروق في ثلاثة مستشفيات بمدينة بغداد، العراق (مستشفى الكندي، ومستشفى ابن بلدي، ومستشفى الصدر) خلال الفترة من أبريل إلى يوليو 2025. وكشفت النتائج عن عزل 35 حالة من بكتيريا المكورات العنقودية الذهبية (29.16%) من إجمالي الحالات. بالإضافة إلى ذلك، عُرِزَت 25 حالة من بكتيريا *الكليبسيلا الرئوية* (20.83%) من إجمالي الحالات. وأظهرت البيانات تفوق جزيئات الفضة النانوية في تثبيط نمو بكتيريا *الكليبسيلا الرئوية* والمكورات العنقودية الذهبية. استُخدم تركيز 256 ميكروغرام/مل لاختبار النشاط المضاد للبكتيريا لمستخلص بذور النبات وجزيئات الفضة النانوية المستخلصة من بذور النبات ضد بكتيريا المكورات العنقودية الذهبية و*الكليبسيلا الرئوية*. بلغ قطر منطقة التثبيط لمستخلص الفلفل الأحمر على بكتيريا *كليبسيلا الرئوية* 21 مم عند تركيز 256 ميكروغرام/مل، بينما بلغ قطر منطقة التثبيط لجزيئات الفضة النانوية 29 مم عند نفس التركيز. أما على بكتيريا المكورات العنقودية الذهبية، فقد بلغ قطر منطقة التثبيط لمستخلص الفلفل الأحمر 16 مم عند نفس التركيز، بينما بلغ قطر منطقة التثبيط لجزيئات الفضة النانوية 22 مم عند نفس التركيز. أظهرت بكتيريا *كليبسيلا الرئوية* مقاومة كاملة للكوليستين (100%)، والأميكاسين، والإيميبينيم (84%). في حين أظهرت بكتيريا المكورات العنقودية الذهبية مقاومة للأميكاسين (94.28%)، والكوليستين (71.42%)، والبيبراسيلين (12%).

الخلاصة: يُعد مستخلص الفلفل الأحمر وجزيئات الفضة النانوية استراتيجية فعالة قد تُسهم في السيطرة على نمو وانتشار الميكروبات.

الكلمات المفتاحية: بذور الفلفل الأحمر، *S. aureus*، *K. pneumoniae*، *Capsicum annuum* L.، جزيئات الفضة النانوية، مقاومة، تثبيط، مضاد للميكروبات.