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Investigation of Biophysical Molecular Properties by Temperature Influence on the Silver Nanoparticles Synthesized Via Green Methods Using Beta Vulgaris: Applications of Antibacterial Agents

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ABSTRACT

Introduction: Beta vulgaris has traditionally been utilized for the treatment of anemia, cancer prevention, fever management, anticoagulation, sclerosis, and bodily detoxification. Beets have been utilized for these purposes by numerous ancient tribes throughout antiquity.
Material and Methods: The major purpose of this research was to investigate the impact of betalains derived from beetroot (Beta vulgaris) on the biosynthesis of silver nanoparticles (AgNPs).
Results: The in vitro antibacterial effectiveness of Beta vulgaris L. pomace extract was evaluated at a dose of 100 mg/ml against clinical isolates and reference strains of *Escherichia coli* and *Klebsiella pneumoniae*. Well diffusion (50, 100 µl) and disc diffusion (15 µl) were other methods utilized. Fourier transform infrared spectroscopy, scanning tunneling microscopy (STM), and scanning electron microscopy (SEM) were employed to analyze the physical properties of the synthesized silver nanoparticles. Antimicrobial characteristics, silver nanoparticles, agar well-diffusion method.
Conclusion: This distinctive green synthesis technique for generating silver nanoparticles obviates the necessity for detrimental solvents and byproducts, rendering it an optimal instrument for promoting green technology in nanotechnology.

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Introduction

In the field of biomedicine, nanotechnology, nanoparticles, have several uses such as contrast agents, photothermal agents, radiosensitizers, cellular imaging, molecular diagnostics, and targeted therapy in the treatment of cancer. Their unique optical, electrical, magnetic, oxidation-resistant, structural, compositional, and morphological properties, in addition to their stability, make these materials very useful [1-8]. Silver nanoparticles, often known as AgNPs, are a nanomaterial that holds extensive significance. A study conducted by the manufacturer had previously indicated that the nanoparticles is capable of healing full-thickness excisional in vivo wounds on mice [1]. Many researchers have suggested that this information is accurate. It has been observed that dressings containing impregnated silver nanoparticles (Ag NPs) improve wound healing in both normal and diabetic rats [2]. In addition to toxicity tests on human cells and cancer cells, efficacy tests on the antibacterial silver were carried out using human keratinocytes [3]. Additionally, they have exhibited some anti-inflammatory effects in peritoneal adhesion, which was written about in a separate paper [4]. As a result, the purpose of this research was to investigate if collagen hydrogels are, in fact, an efficient method for protecting silver nanoparticles (AgNPs) from being contaminated by microorganisms. Individual keratinocytes and dermal fibroblasts from the epidermis were included in this evaluation [5]. Collagen-coated silver nanoparticles (AgNPs) were tested for their ability to inhibit the growth of many types of microorganisms, including S. aureus, S. epidermidis, E. coli, and P. aeruginosa under the microscope [6]. In today's world, a wide variety of microorganisms, including bacteria, fungi, and viruses, exhibit a high level of sensitivity to metallic nanoparticles (for example, antibacterial activity). Because of the level of progress that has been made in the creation of nanotechnology, nanoparticles made of metal and metal oxide are currently being created in order to combat harmful microorganisms or in other systems that limit the pathogenicity of these bacteria [7]. There is a greater potential for contact between microbial lipid membranes and metal nanoparticles

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due to the fact that these nanoparticles are extremely small and have a high surface area to volume ratio [8]. As a result of the fact that antibacterial metal nanoparticles are immobilized and integrated on surfaces, there is sufficient evidence to suggest that it has the potential to be utilized in a wide variety of applications, ranging from medical devices and instruments to water treatment systems, food manufacturing and distribution, and other areas [2]. The evaluation of the antibacterial efficacy of polymers that have been mixed with metal nanoparticles has been the subject of a great number of papers [9]. Another intriguing alternative is the formation of nanoparticles by the process of whole-body extraction. Consequently, our research involves the utilization of silver particles derived from beetroot extract and an examination of the properties of the molecular material in relation to bacterial activity.

Materials and Methods

Material

Sampling plants for research

The research obtained conventional beets (Beta vulgaris) from established vendors located in Baghdad. The verification process has confirmed the origin and validity of Beta vulgaris.

Types of bacteria

Al-Karkh University of Science's Department of Microbiology generously provided us with strains of *Escherichia coli* and *Staphylococcus aureus* bacteria. At 4 ^{0}C, strains were kept on nutrient agar slants.

Plant extraction of the beetroot

The experimental task concept was categorized into two stages: the initial preparation involved the use of (Beta vulgaris) S1 aqueous plant extract combined with atmospheric steamed distilled water at room temperature, while the subsequent heating phase was designated as S2 stages. Beetroots underwent a cleaning process using potable water to ensure they are free from impurities. Proceed by fragmenting the beetroot into small pieces and allowing them to desiccate in the air for several days. The dried beetroot was subsequently processed using a mixer grinder. Ten grams of beetroot powder were dissolved in 100 milliliters of distilled water in a single batch. One sample was left at room temperature, while the other was subjected to boiling. The mixture was subsequently allowed to stand undisturbed for 5 minutes before being filtered using filter paper. The plant extract was subsequently subjected to centrifugation at 4000 rpm for 15 minutes and stored in the refrigerator at 4°C until further analysis. A stock solution of silver nitrate (AgNO3) was prepared at a concentration of -10 mM by dissolving 1.7 g of AgNO3 in 100 ml of water. A stock solution of 100 mM was prepared for each metal solution that was formulated. The synthetic process was conducted through dilution with beetroot extract, maintaining a constant volume of 2 ml of beetroot extract while varying the concentrations of silver nitrate solution at 1, 1.5, 2, 2.5, 3, and 5 mM in deionized water. The mixture was combined for thirty minutes using a magnetic stirrer.

Nano-Silver Synthesis via Living Organisms

Beetroot significantly changed the color of these solutions, turning them from a vibrant ruby red to a brown hue. The interaction between silver ions (Ag ions) and the subsequent creation of silver nanoparticles (AgNPs) is responsible for this change. After being separated from the extract by centrifugation, the sediment was dried in a controlled environment at 25°C. To characterize, classify, and optimize the produced silver nanoparticles (AgNPs) and silver nanoparticle samples in line with the main goal of this investigation.

Synthetic green silver nanoparticle characterization

Additionally, various techniques were utilized, including X-ray diffraction (XRD) with the Empyrean from Malvern Panalytical, pattern analysis, energy dispersive X-ray (EDX) using Zeuss, Fourier-transform infrared spectroscopy (FTIR) from Jasco Inc., UV-Visible spectroscopy by Malvern Panalytical, and atomic force microscopy (AFM), to improve the effectiveness of the synthesized silver nanoparticles (AgNPs). The antibacterial activity of the AgNPs was assessed in liquid nutrient media and on agar plates. Propagation of Escherichia coli cultures: The cultivation process utilized a feeding medium consisting of 1 g yeast extract, 1 g beef extract, and 0.5 g sodium chloride in 100 ml distilled water, aimed at promoting the colony formation of E. coli. The bacteria were cultured with a lawn spread on the plate surface at specific concentrations as outlined below: (K) 10^{-3} (O); (V) 10^{-4}; (□QWU) 10^{-5} The inoculum was produced by cultivating several cryopreserved Escherichia coli strains in a nutrient-rich medium overnight. Silver nanoparticles (NPs) were prepared by diluting them in water at the designated concentration. In this experimental setup, E. coli cultures were inoculated at a concentration of 10^6 cells/ml and incubated at 37° C with a rotation speed of 200 rpm. All prepared flasks were retained for experimentation, during which the growth behavior of bacterial cells was examined after the injection of varying amounts (0.5 mM, 10 mM, and 15 mM) of silver nanoparticles. Silver nanoparticles (AgNPs) at concentrations of 0 mM, 0.2 mM, 0.3 mM, and 0.6 mM were introduced into separate flasks to investigate the cellular development patterns under consistent experimental conditions across different doses. Each flask had a total volume of 50 milliliters with a concentration of 3 mg/ml. This method entails the application of a specific amount of the microorganism onto the most robust media available. Following this, the antimicrobial filter paper discs are carefully placed on the agar surface. Petri plates are then placed in the incubator under ideal conditions to facilitate growth. The antimicrobial agent is administered to an agar plate, resulting in discs that indicate the zones of inhibition to control bacterial growth.

Results

The absorption of plasmon by the silver nanoparticles in the sample of interest was indicated by a distinct peak at 409 nm to 437 nm observed in the UV-visible spectra study performed during the biogenesis of silver nanoparticles using betalain. The most basic principle of biosensing is the generation of a surface plasmon resonance (S. P. R.) absorption band through the stimulation of these unbound electrons in metallic nanoparticles [10]. The collective oscillation is a result of the metal nanoparticles' electrons oscillating in tandem with one another and in harmony with the waves of light. Surface plasmon oscillations of silver nanoparticles are clearly correlated with the data peaks. Figure 1, The finished sample's absorption spectra.

A careful observation about the comparison of figure (2a and 2b) showed in subtle variation occur on strength as well as position of absorbance peak that may be associate to development of nanoparticles. The shifts in peak position observed at 3384.17 cm^{-1} and 2854.70 cm^{-1} could result from disruption of hydrogen bonding, which plays a key role in the reduction process of nanoparticles as less emphasized peaks were also recorded at 1089.88 cm^{-1} and 1049.55 cm^{-1} [11-13]. Physical characteristics the surface morphology of the biosynthesized silver nanoparticles was revealed solely by the scanning electron microscopy. The AgNPs produced using the green approach may be observed in Figures 3a and 3b, which demonstrate the results at both room temperature and boiling point. Figure 3c shows an example of an energy-dispersive x-ray (EDX) spectrum that was used to determine the sample's constituent makeup. In this intermediate area presented in a variety spectrum showed there is discrete maximum as represent of Ag from 2 kV to 4 kV. The magnitudes of these peaks are directly related to Ag characteristic line. Quantitative analysis revealed that 67.93% of the analyzed samples had silver levels. Other peaks of the EDX spectrum oxide: oxygen 14.90%, chlorine 15.56%, and calcium 1.78% as shown in the figure 3c.

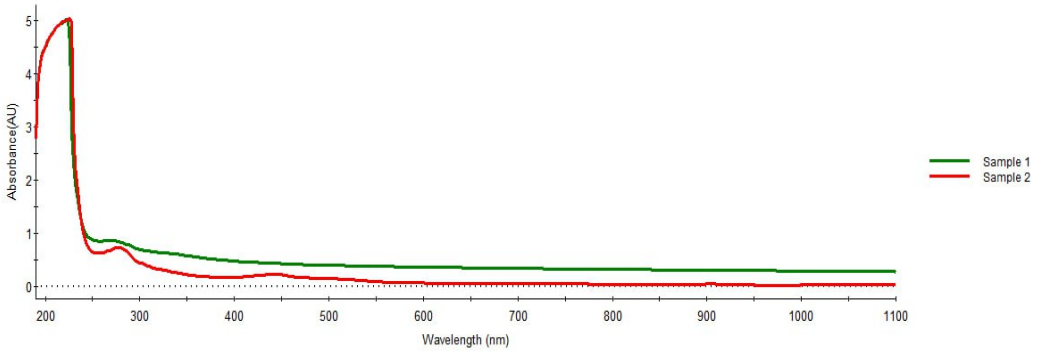

Figure 1. UV-visible spectrum analysis of the biogenesis process of silver nanoparticles

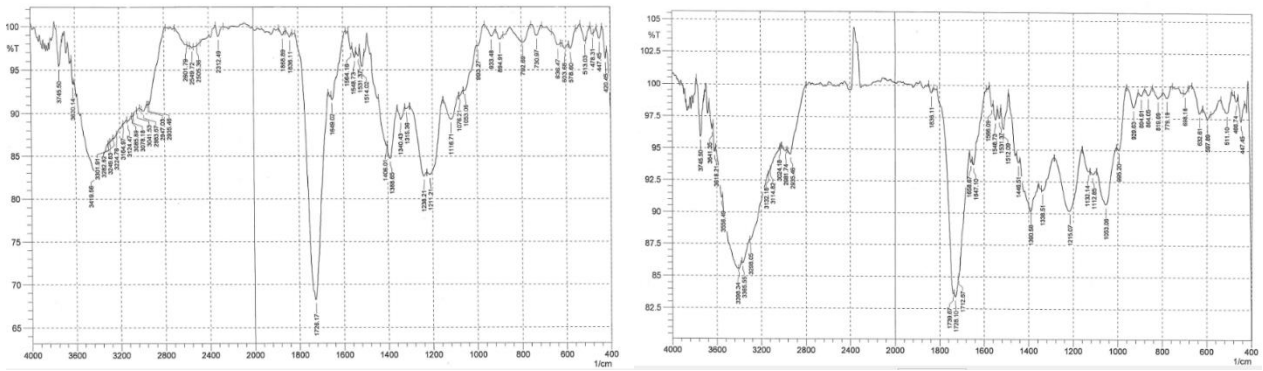

Figure 2. FTIR spectrum of the aqueous extract of Ag nanoparticles (Ag NP).

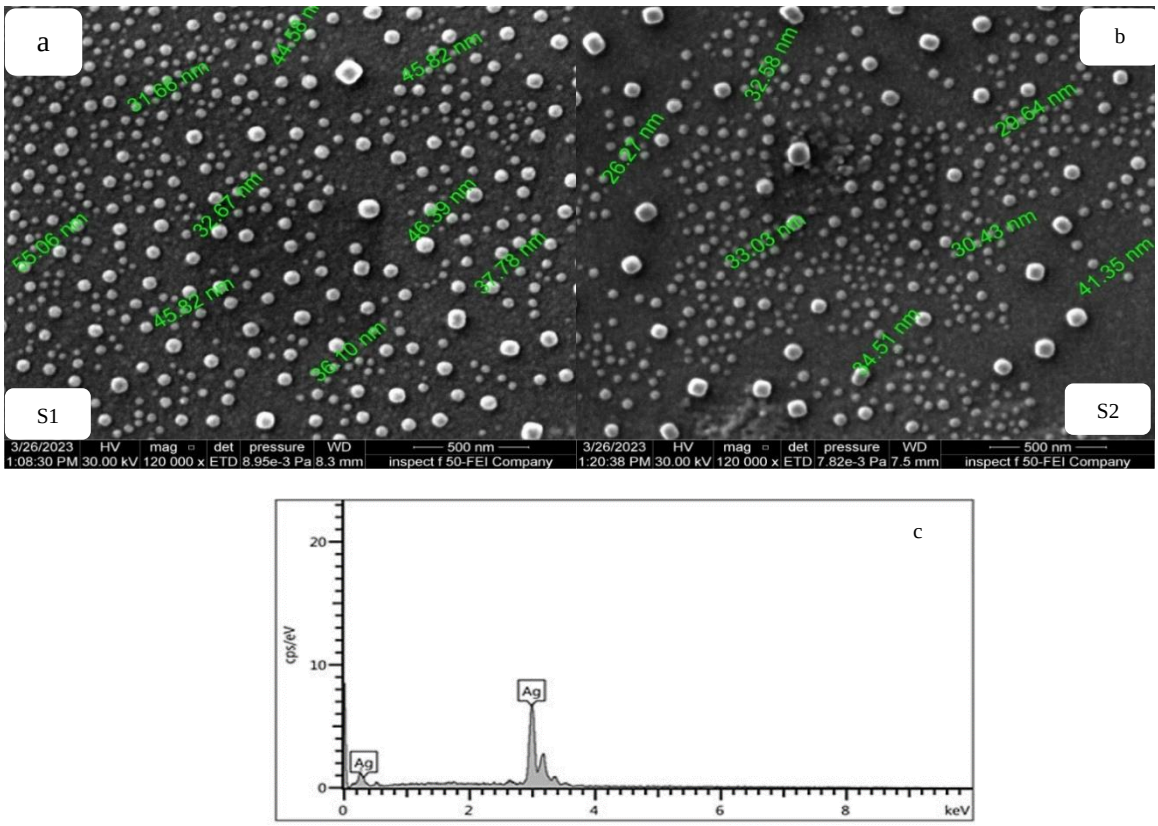

Figure 3. Show the morphology of the silver nanoparticles by SEM (a &b) & EDX spectrum of the sample c

These nanoparticles were characterized by scanning electron microscopy (SEM) and found that, the images displayed a poly-dispersed spherical morphology with high degree of dispersion and aggregation. The preparation of the medium in which Ag nanoparticles were synthesized was performed and indirectly heated for 4 h, making most of the nanoparticle's aggregate. Figure 4 depicts atomic force microscopy (AFM) image analysis of silver nanoparticles (Ag NP's) prepared by a green chemistry method.

When the AgNP solution is made, it contains several big clusters of nanoparticles that aggregate, as can be seen in figure 4. Additionally, this image emphasizes a clearly identifiable morphological feature. Surface island-like formations are a distinguishing feature of Ag nanoparticle thin films. This X-ray diffractogram shows the aqueous solution with Ag nanoparticles, which were made from chemically extracted beetroot peel (Figure 5).

Table 1 presents the identifying parameters of Ag nanoparticles, which have been indexed by the JCPDS 04-0783 structures.

The Bragg reflections index (111), (220), and (311) obtained from the as-synthesized AgNPs indicate that they possess a face-centered cubic (FCC) structure, confirming their crystalline nature. Nevertheless, it is worth noting that the peaks exhibit significant broadening, suggesting that the composition of the substance consists of silver crystallites of minute dimensions. The size of nanoparticles has a significant influence on the observed X-ray diffraction (XRD) patterns, leading to several distinctive properties. These characteristics encompass atypical positions, magnitudes, and dimensions of peaks.

Antibacterial screening

The purpose of this research was to determine whether silver nanoparticles (Ag NPs) made from beetroot extract utilizing a green chemistry approach had any antibacterial effects. Beetroot extract and silver nanoparticles were used to test for Klebsiella pneumoniae and Escherichia coli. In the investigation, both reference cultures and wild isolates were used. A larger concentration or volume of beetroot extract was required due to the low efficacy of Beta vulgaris L. extracts, as shown in earlier research. As a result, other approaches were used in this study, such as the disc diffusion method that uses discs with limited capacity and the agar-well diffusion method that uses holes with different sizes and volumes. The results of the antibacterial efficacy of silver nanoparticles (Ag NPs) that were made using beetroot extract employing green chemistry principles are shown in table (2). Researchers looked at how these nanoparticles affected the bacteria Klebsiella pneumoniae and Escherichia coli. The degree of susceptibility was highest for Escherichia coli among all the tested microorganisms. Indicators of the extract's bactericidal action, such as halo zones, proved this to be the case [13].

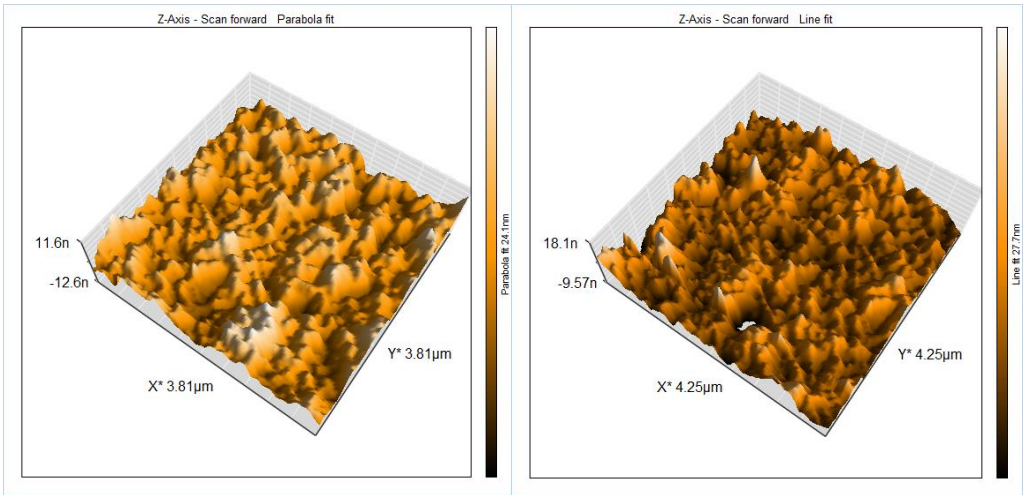

Figure 4. AFM images of Ag NP's aqueous solution extracted from beetroot plant

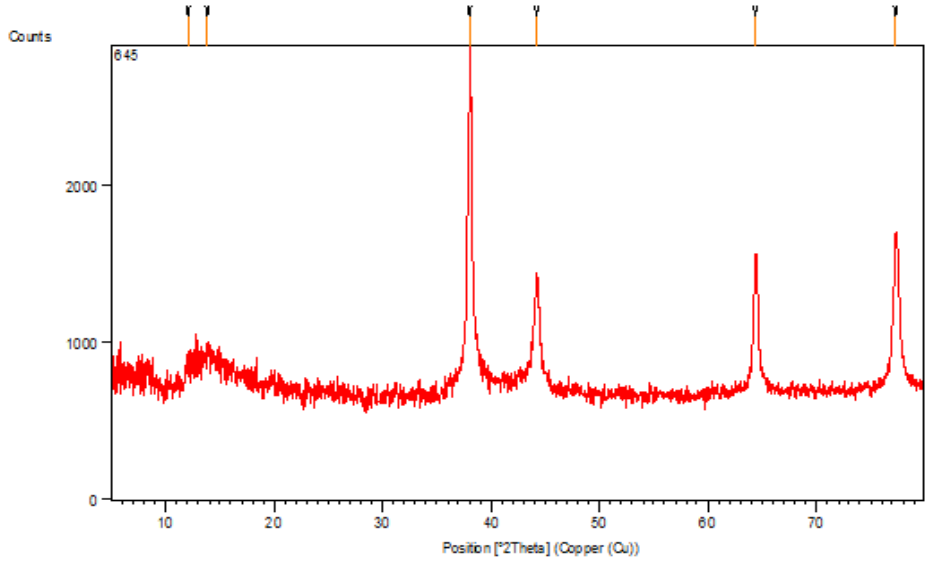

Figure 5. XRD pattern of Ag NP's aqueous solution extracted from beetroot plant

Table 1. The identification parameters of silver nanoparticles (Ag NP's) derived from the X-ray diffraction (XRD) findings.

2θ (Deg.)	FWHM (Deg.)	dhkl Exp.(Å)	G.S (nm)	dhkl Std.(Å)	hkl	Card No.
38.0330	0.7800	2.3640	10.8	2.3651	(111)	JCPDS Silver: 04-0783
44.2730	0.6240	2.0442	13.7	2.0642	(200)	JCPDS Silver: 04-0784
64.4750	0.7540	1.4441	12.5	1.4621	(220)	JCPDS Silver: 04-0785
77.3970	0.7520	1.2320	13.5	1.2331	(311)	JCPDS Silver: 04-0786

Table 2. Antibacterial activity of Ag NP's and beetroot extract against Escherichia coli and Klebsiella pneumonia

Sample	*Escherichia coli*		*Klebsiella pneumonia*	
	Inhibition zone (mm)		Inhibition zone (mm)	
1	19	23	20	15
2	17	12	15	8

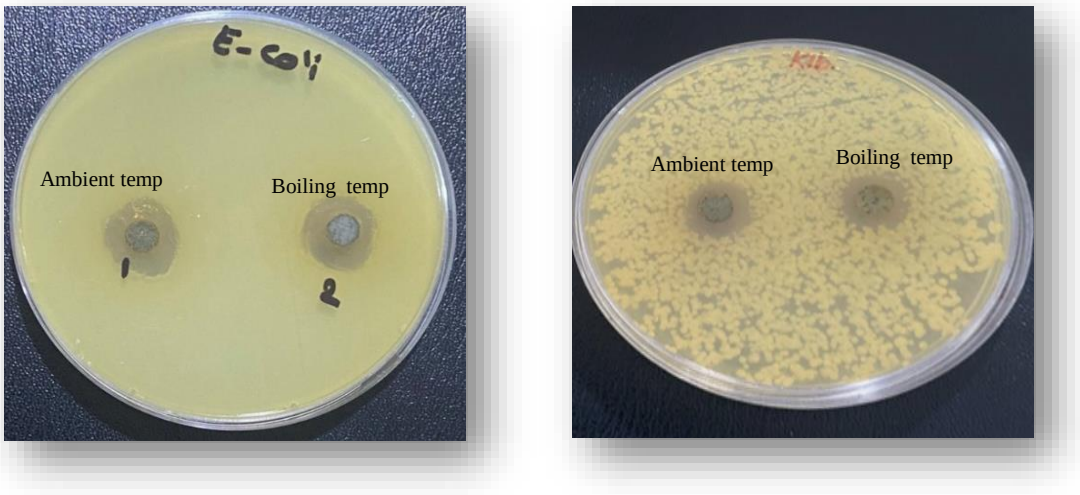

Figure 6. Antibacterial activity of AgNPs beetroot extract on *E. coli & Klebsiella*

Discussion

The results clearly demonstrate that the highest wavelength-absorbance value was observed in the visible region for the surface plasmon resonance (SPR) of metallic silver. This finding provides confirmation of the structural characteristics of the silver nanoparticles (NPs). Figure 2 displays the Fourier Transform Infrared (FTIR) spectrum of the aqueous extract of Ag nanoparticles (Ag NP).

Figure 2 demonstrates that there might be a -OH functional group present due to the large absorption frequency at 3322.38 cm^{-1}. Alkanes have a peak at 2973.32 cm-1 wavenumber, which is correlated with the stretching of C-C-H bonds. A frequency of 1379.81 cm^{-1} was determined to be the C=O stretching frequency in betalain. Separate bands at 1085.99 cm^{-1} and 1044.55 cm^{-1} were observed, matching to the primary vibrational stretch frequency associated with the alcohol group and the symmetric and asymmetric stretching of the C-O-C bond, respectively. The peak at 880.52 cm^{-1} (stretching frequency) confirms the presence of C-COOH. The examination of the nanoparticles produced by Fourier Transform Infrared (FTIR) is shown in Figure 2 b. C-N-H stretching vibration of carboxylate nanoparticles may explain the trend of a decreased signal from P-F at 881.12 cm^{-1}. Optimal conditions for the AgNPs spectra revealed a distinct peak at 3412 cm^{-1}, which may be explained by the vibrational stretching bands of the OH groups. Furthermore, stretching of C-H bonds in alkanes is correlated with a maximum at 2926 cm^{-1}. The major vibrational mode associated with the stretching of the amide bond's carbonyl group is responsible for the adsorption peak at 1634 cm^{-1}. C-N bond stretch or OH bond vibration is attributed to the peak at 1384 cm^{-1}. A vibrational mode owing to the C-OH bond is shown by the presence of a peak at 1051 cm^{-1} in the spectrum. Particular absorbance peaks in the Fourier transform infrared (FTIR) spectra of the extract and AgNPs are characterized by functional groups. Illustration 2. Hence, the extract's functional groups are crucial for stabilizing produced nanoparticles and reducing silver ions. Findings and analysis. Figure 6 illustrates However; it should not be assumed that the plant is completely inert only because it does not have any antibacterial properties or only has little activity against certain bacteria. To be more specific, there could not be enough of the active ingredients to properly inhibit cell multiplication. Substantial doses are required to prove inactivity. Another consideration is the potential addition of other components when larger amounts of extract are used. It is possible that these components will have antagonistic effects or will diminish the beneficial effects of the bioactive substances. Although Klebsiella pneumonia was one of the Gram-positive bacteria tested, the effects of applying different concentrations of Beta vulgaris L. extract and Ag NPs were not statistically significant.

Conclusion

The research hypothesized that there would be a notable antibacterial effect of beet root extract against Klebsiella pneumonia and Escherichia coli. Researchers found that silver nanoparticles (Ag NPs) have powerful antibacterial effects, which bodes well for the development of new antimicrobial drugs to combat bacterial infections. The fact that they are harmless to the environment is just one of the many appealing aspects of them. A multi-method analytical technique was used to characterize the nanoparticles. We used UV-VIS absorbance spectra spectroscopy to confirm that the Ag nanoparticles produced via bio-reduction exhibited surface plasmon resonance. The presence of diffraction peaks at specific locations in the X-ray diffraction (XRD) pattern is indicative of nanoparticle crystallography. Physical characteristics the generated nanoparticles had a spherical shape. This study's biosynthetic technique to producing Ag nanoparticles (Ag NPs) has various benefits over chemical synthesis methods. Among its many benefits are the fact that it is non-toxic to nature, biodegradable, and extremely radio-

safe. Furthermore, biologically produced silver nanoparticles showed potent antibacterial activity against a wide variety of therapeutically important pathogenic microbes.

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