Comparative and Antibactrial Studies of Biosynthesis Preparation of Silver Nanoparticles with and without Sodium Hydroxide as Precipitation Agent

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Abstract: This study presents a simple, rapid, cost-effective, and environmentally friendly green synthesis of silver nanoparticles (AgNPs) using *Cordia myxa* leaf extract as both the reducing and stabilizing agent. Silver nitrate was used as the precursor, and the synthesis was carried out at room temperature (25 °C) through two routes, with and without sodium hydroxide (NaOH) as a precipitation agent. The synthesized AgNPs were characterized by UV–Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), field-emission scanning electron microscopy (FE-SEM), and energy-dispersive X-ray spectroscopy (EDS). UV–Vis analysis confirmed the formation of AgNPs with characteristic surface plasmon resonance peaks at 420 nm and 441 nm for samples synthesized with and without NaOH, respectively. XRD analysis revealed the formation of face-centered cubic crystalline silver with average crystallite sizes of 17.58 nm and 18.75 nm for samples prepared with and without NaOH, respectively. FE-SEM images showed predominantly spherical nanoparticles with average particle sizes of approximately 143 nm and 99 nm, indicating greater aggregation in the presence of NaOH. EDS analysis confirmed the formation of silver nanoparticles with a higher silver content for the sample synthesized with NaOH (93%) than for the sample prepared without NaOH (67%). These results demonstrate that the addition of NaOH influences the morphology, aggregation behavior, and elemental purity of the synthesized AgNPs while preserving their crystalline structure.

1 INTRODUCTION

Nano-biotechnology Nanoscience has considered as a crucial division of nanotech. Nanomaterials is one of the main parts in the field of nanotechnology in term of create novel compounds with nanoscale size. The innovations of such size and modified nanomaterials made fast growth in the nanotechnology sector [1]. Ag, Au, Pd, and Pt which are Noble metals have been utilized in the synthesis of metallic nanoparticles and are mostly applied to the field that directly intouch to the human body [2]. Thus, there are huge interest by researcher to modify green, ecofriendly processes for manufacturing nanoparticle rather than using non-safe chemicals in the traditional processes [3]. The silver nanoparticles (Ag-NPs) have become the centre for many research studies and due to their unique optical, electrical, and biological properties, which offer potential impact in different disciplines such as catalysis reagents, electronic devices, and medicine [4]. Many studied of silver NPs synthetic methods on shape-control were done such as template-assisted methods, polyol routes, and seed-mediated methods [5]. Many different types of shapes of silver nano structures like tubes, cubes, spheres, wires, hollow spheres, rods, and plates exhibit different optical phenomena [6], [7]. The routes to prepare the Ag NPs can be chemical, biological, and physical, however choosing one suitable route will highly depends on the end-use. For example, the downside approach of chemical and physical would requires a wide space, and high temperature for a longer time to maintain the thermal stability of prepared Ag NPs [8]-[10]. In addition, obtaining Ag NPs using the green method has better

biocompatibility and are lower toxicity for the biotic systems [11], [12]. The concentration of phytoconstituents as bio-reducing agent plays a critical role to decide the nanoparticle morphology. Additionally, the factors loke pH, temperature, times, and concentration of silver salt would affect and determine the morphology [13]. The green method which is non-toxic and environmentally safe is the best for producing the nanoparticles in comparison to other methods [14], [15]. Our present work is to prepare the Ag-NPs using green method environment friendly natural *cordia myxa* leaves extract as capping agents and silver nitrate and is used as precursors in the presence and absence of sodium hydroxide as a precipitation agent.

2 EXPERIMENTAL

2.1 Materials and Reagents

Chemicals of analytical purity (BDH), Silver nitrate (AgNO3, 98%), NaOH, Deionized distilled water and leaves extract of *cordia myxa*.

2.2 Instruments and Apparatus

The data for all resulted samples were obtained at the college of Science/Tehran University-Iran. For scanning angles, diffractometer was used running at 30 mA and 40 kV (XPERT-Pro), temperature 20 to 80°, the XRD pattern was obtained by irradiation the samples with a Siemens D500. The surface morphology investigation were conducted using the Scanning Electron Microscope model ZEISS: Sigma VP. The FT-IR spectra for obtained silver nanoparticles were determined at University of Diyala /College of Education for Pure Science using Spectrophotometer Schimadzu/Japan with the range of 4000 –500 cm^{-1}.

2.2.1 Preparation of Aqueous Cordia Myxa Leaves Extract

The leaves of the *cordia myxa* plant were collected and washed with distilled water, dried, then crashed into powder. The fine powder of *cordia myxa* leaves (5.0 g) were put in distilled water (100 mL) and boiled at 80 °C for 30 minutes. Then, the resulted extract was filtered through using (Whatman filter paper 1) and was left in a refrigerator at 4 °C for later use. (Fig. 1).

2.2.2 Green Synthesis of Silver Nanoparticles Using Cordia Myxa Leaves Extract

Method 1. The salt solution was prepared by dissolving $AgNO_3$ (2.0 g) in deionized water (100 mL). The solution of extract was added gradually to the solution under stirring at 30 °C, then the mixture was warmed to 80 °C for 2 hours. The mixture was filtered, the obtained precipitate was washed with deionized water and EtOH to obtain silver nanoparticles Ag-NPs (Fig. 2).

Method 2. The salt solution was prepared by dissolving a 2.0 g ($AgNO_3$) in 100 mL deionized water. The solution of extract was added stepwise to the solution under stirring at 30 °C, then the mixture was warmed to 80 °C, followed by addition of sodium hydroxide solution (0.1 M) dropwise to the mixture (brine and leaves extract), to obtain the pH at approximately 14, the mixture was left at constant stirring. After 2 hours the mixture was filtered, and the precipitate was collected and washed with deionized water and ethanol to obtain a pH of approximately 7 to obtain silver nanoparticles Ag-NPs (Fig. 2).

3 RESULTS AND DISCUSSION

3.1 Characterization of synthesized silver nanoparticles

Two different synthetic routs with and without precipitation agent were carried out to study the synthesis of silver nanoparticles, and were analyzed using FTIR, XRD, FESEM, EDX and UV spectrphotometry.

3.2 UV-Vis Analysis

Surface plasmon resonance is a unique feature for metal nanoparticles, which can be utilized to determine the size and the distribution of the nanoparticles in the optical spectroscopy. The impure nanoparticle solution that obtained was purified by the centrifugation at 10000 rpm for 45 minutes, then followed by re-dispersion of the pellet in distilled water. Reduction of silver ions into Ag-NPs by treating with plant extracts with and without NaOH solution were observed by altering the colour, which is the common method as indicator for the formation

of Ag-NPs (Fig. 3) [16]. The absorption peak of Ag-NPs was appeared at 420 nm (2.473 ABS) and 441 nm (0.842 ABS) for with and without NaOH as precipitation agent, respectively. The difference between the intensity of the peak for both method is because of the effect of electrons surface palsmon resonance in the reaction mixture which was the reason of brown colour [17].

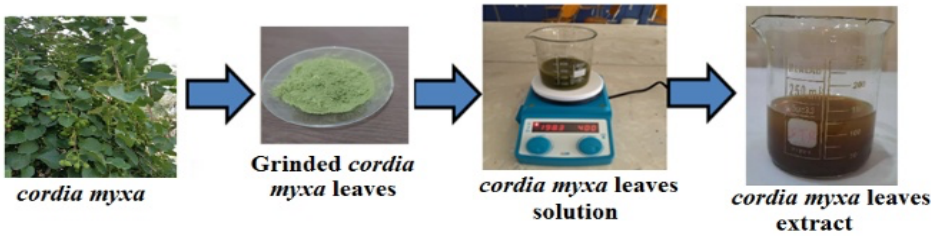

Figure 1: Preparation of *cordia myxa* leaves extract.

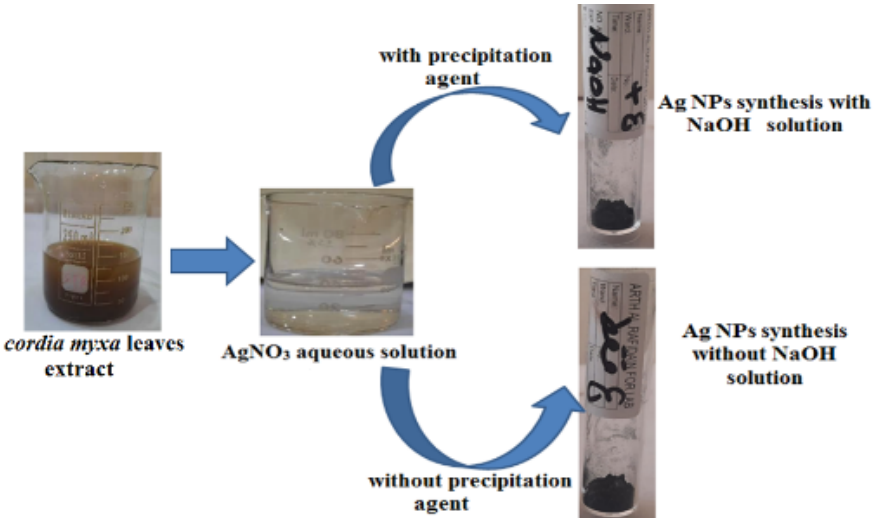

Figure 2: Preparation of silver nanoparticles by *cordia myxa* leaves extract as a reduction factor and with/without NaOH as precipitation agent.

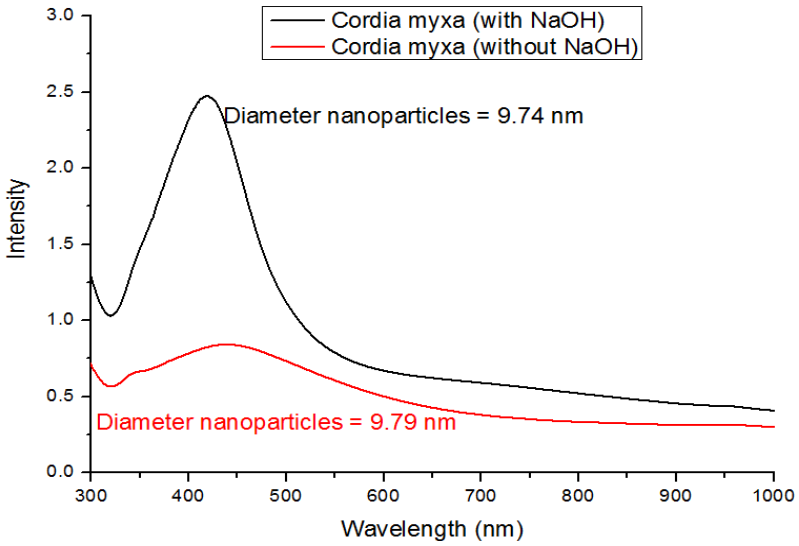

Figure 3: Ultraviolet-visible spectra of silver nanoparticles synthesized by *cordia myxa* leaves extract with/without NaOH.

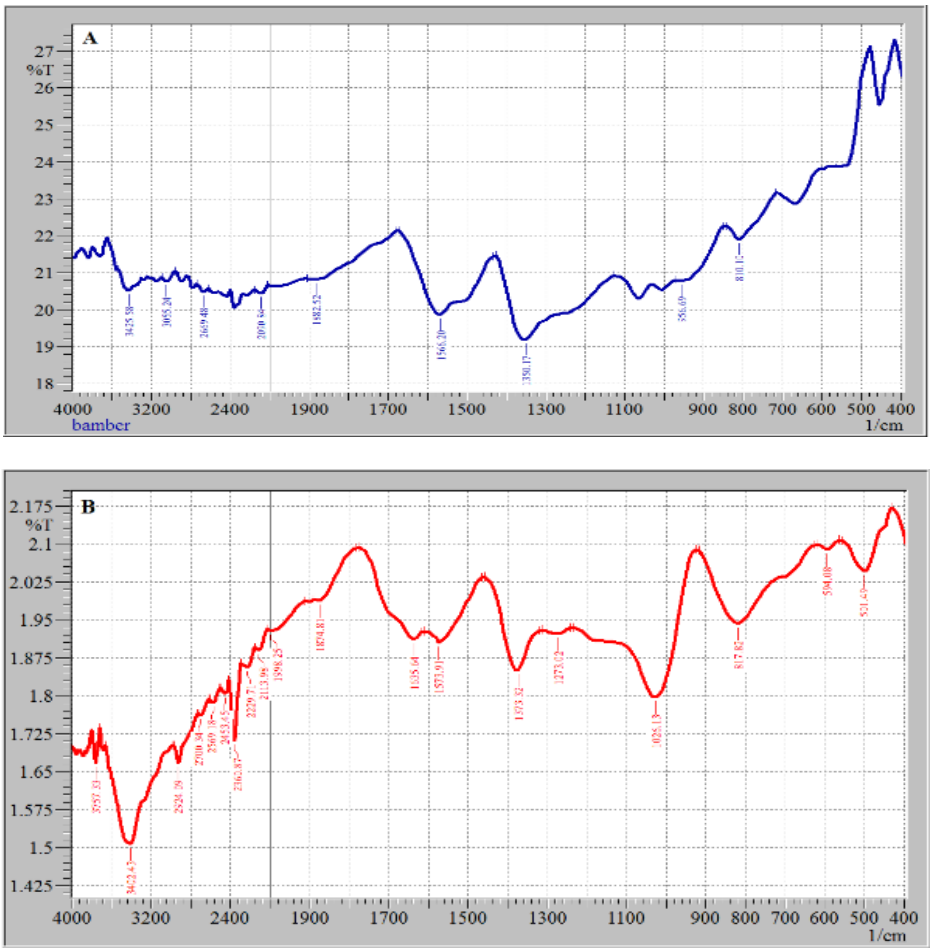

Figure 4: FT-IR analysis of Ag NPs using cordia myxa leaves extract: a) with NaOH, b) without NaOH.

Silver nanoparticles diameter was calculate by Haiss equation [18]:

$$d = \ln(\lambda_{SPR} - \lambda_0)/L_1/L_2.$$

Where: d refer to the particle diameters of the Ag-NPs, λ_{SPR} represent the wavelength at maximum absorption, λ_0 refer to wavelength at the minimum absorption and L_1 and L_2 are parameters equal to 6.53 and 0.0216, respectively. Ag-NPs diameter was found about 9.75 nm and 9.80 nm for Ag-NPs with and without NaOH solution, respectively.

3.2.1 FT-IR analysis

FT-IR spectrophotometer was used to recognize the possible biomolecules for the reduction of the Ag^+ ions and capping of bioreduced Ag- NPs. Figure 4 a and b, represents the FT-IR spectrum of synthesized Ag-NPs using *Cordia myxa* leaves extract with sodium hydroxide solution. The band at 1026 cm^{-1} can appointed for C–O, the band at 1635 cm^{-1} might related to chelated carbonyl group or OH of carboxylic group in the sennosides [19]-[21]. The C=C group of aromatic ring appeared at 1511 and 1450 cm^{-1}. The stretching C–H band appeared at 2924 cm^{-1}. The bands at 3435 and 3425 cm^{-1} can be assigned to OH group. The FT-IR data indicates that the biological molecules could possibly be involve for both synthesis and stabilization of Ag-NPs, thus, the FT-IR study reveals the multi-functionality of the aqueous extract of *Cordia myxa* where reduction and stabilization occur simultaneously [22].

3.2.2 XRD analysis

The silver nanoparticles Ag-NPs that papered with NaOH and without NaOH was synthesized by eco-friendly method, and powder XRD was used to identify the particles of Ag and to characterize the information of structure for products of both methods. For XRD pattern of silver nanoparticles Ag-NPs with NaOH and without NaOH see Figures 5 and 6.

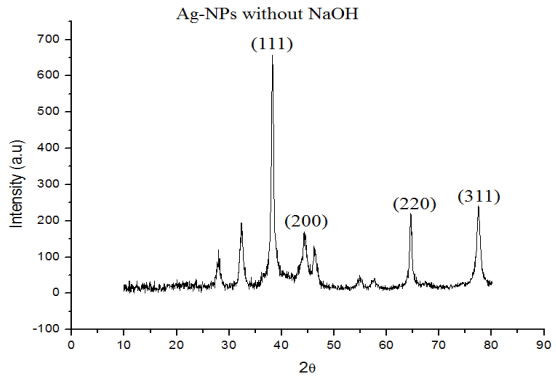

Figure 5: XRD pattern of cordia myxa leaves extract synthesized silver nanoparticles without NaOH.

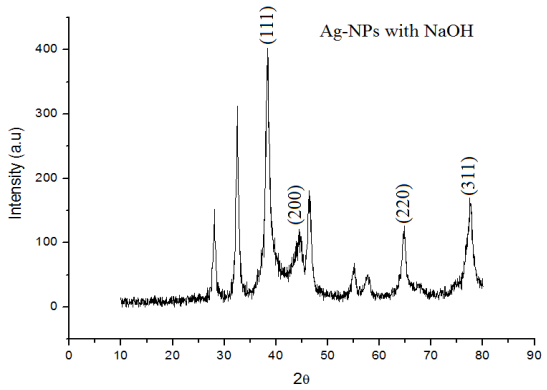

Figure 6: XRD pattern of cordia myxa leaves extract synthesized silver nanoparticles with NaOH.

The pattern of Ag-NPs with NaOH significantly exhibit the main peaks at (2θ) 38.3858° corresponds to the (111), 44.5271° to (200), 64.7496° to (220) and 77.6113 to (311) planes. In comparison of JCPDS no: 89- 3722 [23], the typical pattern of green-synthesis of Ag-NPs is possessing a cubic structure with average crystalline size 17.58 nm, and was estimated using Debye–Scherrer's equation [24] and lattice constant about 4.070964 °A. While, the pattern of Ag-NPs without NaOH gave main peaks at (2θ) 38.2424° corresponding to the (111), 44.3754° (200),, 64.5878 (220) and 77.5156° (311) planes, with cubic structure, average crystalline size 18.75 nm and lattice constant about 4.081258 °A. On the other side, three unassigned peaks appeared at (28.0672°, 32.4729°, 46.3993°) and (27.9915°, 32.3722°, 46.322°) for Ag-NPs with NaOH and without NaOH respectively, such weaker than those for silver nanoparticles. This might be because of the precipitated bio-organic impurities on the Ag-NPs surface and appearances of these peaks are due to the the leaf extracts since it contains phyto-chemical compounds [25]. These unpredicted peaks have high intensity in the case of Ag-NPs with NaOH sample compare as Ag-NPs without NaOH.

3.2.3 FE-SEM image

The FE-SEM images obviously revealed the spherical morphology and different sizes of obtained samples see Figures 7 and 8. The FE-SEM image (Fig. 7) shows that Ag-NPs without NaOH was spread out over the surface to maintain the relative uniformity with average size diameter about 99 nm and less agglomerated. The aggregation might be due to the encapsulation of biomolecules with Ag-NPs. The process for the preparation of *cordia myxa* leaves extract mediated Ag-NPs was repeated with NaOH as precipitation agent and the surface morphology of the particles was found to be different with average size diameter 143 nm (Fig. 8). Thus, FE-SEM analysis depicts the potentiality of sodium hydroxide solution to Increases the aggregation and average size diameter of Ag-NPs [26].

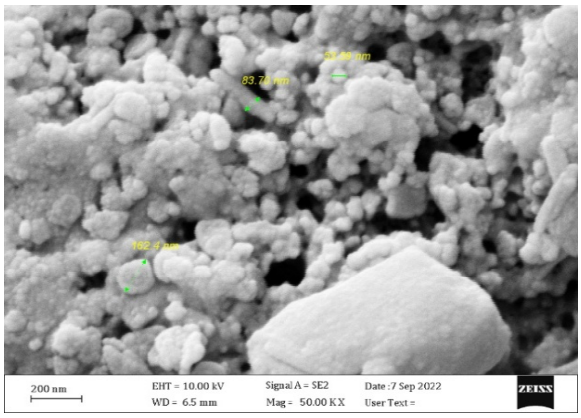

Figure 7: FE-SEM image of bio-prepared Ag-NPs in aqueous extract of *cordia myxa* without NaOH.

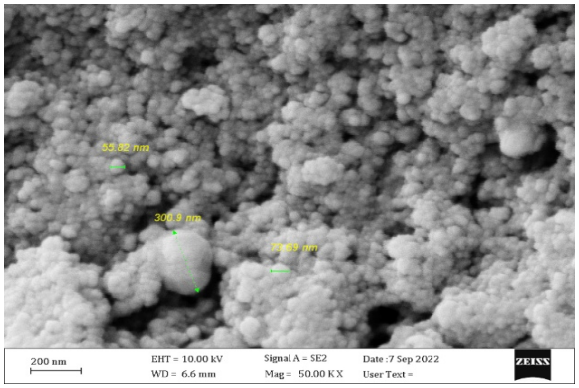

Figure 8: FE-SEM image of bio-prepared Ag-NPs in aqueous extract of *cordia myxa* with NaOH.

3.2.4 EDS Analysis

Figure 9 and 10 illustrates the EDS analysis, which was obtained for the prepared silver nanoparticles, Figure 9 represent Ag-NPs without NaOH, shows a weak signal at 3.1 keV energy level for silver with (Ag = 67%); other weak signals for Au, C, Cl and O were observed as well. The at 3.1 keV of major emission energy indicates the correct identifying of Ag. In other hand, the Ag-NPs with NaOH sample gave a strong signal at 3.1 keV energy level for Ag with (Ag = 93%) in comparison to Ag-NPs without NaOH, other weak signals for Au, C, Cl and O were observed as well, see (Fig. 10).

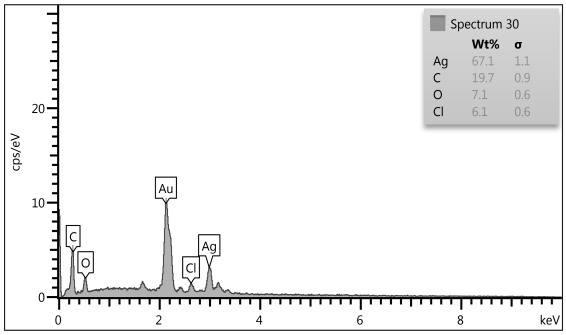

Figure 9: EDS analysis of Ag NPs synthesis using *cordia myxa* leaves extract without NaOH.

3.2.5 The Antibacterial Activities

The biological activity of synthesized compounds that obtained using plant extracts were studied using disk diffusion method, against four pathogenic identified bacterial isolates in Baqubah Teaching Hospital and Al-Amine laboratories, Diyala/ Baqubah, two G^{+ve} bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and two G^{-ve} bacteria (*Escherichia coli* and *Klebsiella pneumonia*). Biological activity as antibacterial agents of some synthesized compounds were tested against some identified and isolated bacteria, against two-gram positive isolates (Gr^{+ve}) (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and two gram-negative bacterial isolates (G^{-ve}) (*Klebsilla pneumonia* and *Escherichia coli*), these bacteria were selected because of their importance in the medical drug and antibiotic. The results in Tables 1 to 4 exhibit the result of the antibacterial activity for the synthesized compounds in using two concentration (50, 100) mg/mL and gave moderate to good effects as the antibacterial agents compare to standard antibiotic (Tetracycline, Piperacillin).

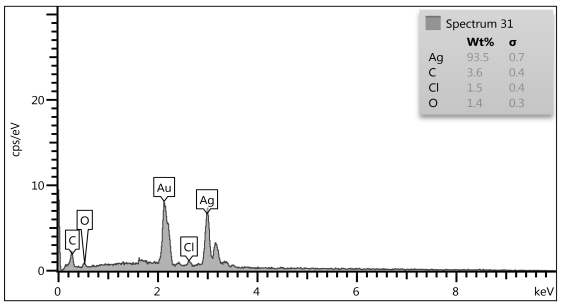

Figure 10: EDS analysis of Ag NPs synthesis using *cordia myxa* leaves extract with NaOH.

Table 1: Result for synthesized compound without NaOH against Gr+ve.

Ag-NPs / Materials	S. aureus			S. epidermidis		
	100mm	50mm	T	100 mm	50 mm	T
Alhagi graecorum	12mm	11mm	10mm	13 mm	14 mm	12 mm
Cordia Maysa	13mm	12mm	11mm	14 mm	11 mm	13 mm
Ceratonia silique	15mm	14mm	12mm	15 mm	15 mm	12 mm

Table 2: Result for synthesized compound with NaOH against Gr+ve.

Ag-NPs / Materials	S. aureus			S. epidermidis		
	100mm	50mm	T	100 mm	50mm	T
Alhagi graecorum	11mm	11mm	11 mm	14 mm	13mm	10mm
Cordia Maysa	14mm	12mm	12 mm	16 mm	12mm	15mm
Ceratonia silique	14mm	13mm	13 mm	14 mm	15mm	12 mm

Table 3: Result for synthesized compound without NaOH against Gr-ve.

Ag-NPs / Materials	E. coli			K. pneumonia		
	100 mm	50 mm	PRL	100 mm	50 mm	T
Alhagi graecorum	R	R	0	11 mm	11 mm	0
Cordia Maysa	R	R	0	13 mm	12 mm	0
Ceratonia silique	R	R	0	13 mm	13 mm	0

Table 4: Result for synthesized compound with NaOH against Gr-ve.

Ag-NPs / Materials	E. coli			K. pneumonia		
	100 mm	50 mm	PRL	100 mm	50 mm	T
Alhagi graecorum	R	R	0	12 mm	11 mm	0
Cordia Maysa	R	R	0	13 mm	12 mm	0
Ceratonia silique	11 mm	R	0	16 mm	14 mm	0

4 CONCLUSIONS

Silver nanoparticles were successfully synthesized by a simple and environmentally friendly green method using *Cordia myxa* leaf extract as both the reducing and stabilizing agent. The synthesis was carried out through two routes, with and without NaOH as a precipitation agent. Characterization by XRD confirmed the formation of face-centered cubic crystalline AgNPs with average crystallite sizes of 17.58 nm and 18.75 nm for samples synthesized with and without NaOH, respectively. UV–Vis spectroscopy showed characteristic surface plasmon resonance peaks at 420 nm and 441 nm, confirming the successful formation of AgNPs. FE-SEM analysis revealed predominantly spherical nanoparticles with average particle sizes of approximately 143 nm for the NaOH-assisted sample and 99 nm for the sample prepared without NaOH, indicating that NaOH promoted particle aggregation. EDS analysis showed a higher silver content in the NaOH-assisted sample (93%) compared with the sample synthesized without NaOH (67%). Overall, the results indicate that the use of NaOH mainly affects the morphology, aggregation behavior, and elemental composition of the synthesized silver nanoparticles, while both synthesis routes successfully produce crystalline AgNPs through a simple, rapid, and sustainable green synthesis process.

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