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Original Article

# Green Synthesis and Characterization of Silver Nanoparticles from Stem Bark Extract of *Cordia dichotoma* G. Forst and Evaluation of their Antioxidant and Antibacterial activities

**Gone Swapna, Bhattiprolu Kesava Rao***

**ABSTRACT**

**Background:** There is a lack of scientific evidence for the fabrication of silver nanoparticles (AgNPs) using stem bark extract of medicinal plant *Cordia dichotoma* and its potential therapeutic applications. **Objectives:** In our present study we contemplated using *Cordia dichotoma* extract for fabricating AgNPs and to analyze their potential applications as antioxidant and biocidal agents. **Materials and Methods:** AgNPs were fabricated by bio reduction of silver nitrate solution with bark extract of *Cordia dichotoma*. The fabricated AgNPs were characterized using U.V visible, FT-IR, XRD, SEM, EDAX, particle size analyzer, and zeta potential. Further, evaluated for their potential antioxidant activity and compared with the crude extract of *Cordia dichotoma* and with standard ascorbic acid. The antibacterial activity was performed against both Gram-positive and Gram-negative bacteria. **Results:** The AgNPs were synthesized and UV spectra showed maximum absorbance at 430nm, which is the characteristic feature of AgNPs. The XRD peaks at (111), (220), and (311) indicate the existence of silver with a crystalline structure. The FT-IR spectra in the range of 1516 to 1634 $cm^{-1}$ reflected the presence of phenol groups which acted as the capping agents for synthesized AgNPs. The SEM images confirmed the spherical structure of AgNPs that formed aggregates with phytochemicals and appeared as composites. The average size of AgNPs is 39.8nm and exhibited good stability as evident from the zeta potential value of -33. **Conclusion:** Phytomediated eco-friendly synthesized and structurally characterized AgNPs can act as antioxidants and exhibited antibacterial activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) pathogenic bacteria.

**Key words:** *Cordia dichotoma,* Silver nanoparticles, Antioxidants, Antibacterials.

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## INTRODUCTION

Globally much research was focused on the usage of different organic and inorganic metals, and their alloys for the synthesis of metal nanoparticles.[1] Various elements and metals such as $CeO_2$, FeO, $SiO_2$, $TiO_2$, and ZnO are often used apart from other metal elements like Ag, Au, Fe, Cu, Pt, Pd, Ni, and Co, etc for the synthesis of nanoparticles.[2] These metal-based nanostructures have a small size and high surface area. Among all these metals and materials silver (Ag) occupies the predominant position. Silver and its usage for therapeutic applications are as old as human civilization. The fabrication of silver nanoparticles using plant extracts rich in secondary metabolites is the recently recognized and developed study for further enhancing the applications of silver. In this process, silver is reduced to silver nanoparticles and plant extracts act as a capping and reducing agent of silver nitrate into silver nanoparticles.[3] The ultra-small size, biocompatibility and nontoxic nature of normal tissues and eukaryotic cells at the nanoscale level are major advantages of nanoparticle applications, especially as therapeutic agents.[4]

The Boraginaceae family is one of the richest plant families in terms of medicinal plants with huge therapeutic and cosmetic applications. It Comprises plants that have important therapeutic and cosmetic applications.[5] The pharmacological effect and efficiency is associated with the presence of diverse phytochemicals such as alkaloids, flavonoids, naphthoquinones, phenols, terpenoids, and many more secondary metabolites. *Cordia dichotoma* belongs to the Boraginaceae family and native to India and other Asian countries. *Cordia dichotoma* is well known for its therapeutic and medicinal applications like curing diseases especially diarrhea, dyspepsia, dysentery fever, headache, and stomach ache, etc.[6]

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## MATERIALS AND METHODS

### Plant extract preparation

The *Cordia dichotoma* stem bark was collected, authenticated by the taxonomist, dried and powdered. The powder was sieved with mesh no 22 and stored until further use. From the plant stem bark powder 3 g was taken, boiled with 100mL of double distilled water to extract the Phytochemicals and secondary metabolites. The extract was filtered with Whatmann No- 1 filter paper and diluted with 300mL of double distilled water. Obtained solution was used for the synthesis of AgNPs.[7]

### Synthesis of AgNPs

The AgNPs were synthesized with the modified protocol of Parlinska-Wojtan.[8] For this purpose, 1mM silver nitrate solution and *Cordia dichotoma* plant stem bark aqueous extract were mixed in different ratios and finally, (1:10) ratio of plant extract to 1mM $AgNO_3$ was standardized. 400ml of plant extract (7.5mg/mL) was added to 4L of 1mM silver nitrate solution at room temperature and stored in a dark room for 24 hr. Further, the reaction mixture was subjected to centrifugation at 10,000 RPM at 4°C. The supernatant was discarded and the AgNPs were deposited as pellets at the bottom of the centrifuge tube. The obtained pellets were air-dried at 25°C for three days to make them fine powder.

### Characterization of fabricated AgNPs-*Cordia dichotoma* composite

The fabricated, fine powdered AgNPs- *Cordia dichotoma* composite was subjected to further physical and chemical characterization using various spectroscopic and microscopic techniques as follows.

### UV-Visible spectroscopy

The reduction of silver ions was observed by the periodical analysis of the 5 ml of the AgNPs reaction mass sample by the U.V (Ultra Violet) spectrum (Model: Thermo scientific) in the range of 200-800nm to detect the surface Plasmon resonance peak of AgNPs. The spectra were recorded at different time intervals of every 4 hr during the reaction incubation time.[9]

### Fourier Transform Infra Red (FT-IR) spectroscopy

The fabricated AgNPs were subjected to FT-IR spectral analysis using BRUKER spectrometer to identify the functional groups associated with the bio reduction and capping agents present on the synthesized composite.[10]

### XRD analysis

The diffraction pattern of fabricated nanoparticles was measured on the X-ray diffractometer. (PW 1830, Philips) for identification of silver element.[11]

### SEM with EDAX

The Scanning Electron Microscopy (SEM) analysis was carried out on the Field emission scanning electron microscope (FESEM) Carl Zeiss model Merlin compact microscope using a 30Kev electron beam. The AgNPs sample was coated on the carbon (silica) coated film or copper grid. The film was allowed to dry after coating it with AgNps under a mercury lamp for 5 min and then SEM images were captured at different magnifications.

Energy-dispersive X-ray Spectroscopy (EDAX) was recorded by using an Oxford instrument X-Max$^N$SDD (50mm$^2$) system and Integrated Calibration and Application tool (INCA) analysis software which is coupled with the SEM instrument. The SEM coupled with EDAX was used to identify the morphology and elemental composition of the fabricated AgNPs.[12]

### Zeta potential

The stability of fabricated AgNPs was measured by analyzing the zeta potential.[13]

### Particle size analysis

The size of the fabricated AgNPs was measured on Horiba scientific SZ- 100 instrument.[14]

### Biological activities of AgNPs

The fabricated AgNPs were investigated for potential antioxidant and antibacterial activities.

### Antioxidant activity

Antioxidant assay for the synthesized AgNPs was evaluated based on the DPPH assay method. The modified method Brand-Williams was used for the analysis.[15] The antioxidant activity was measured for *Cordia dichotoma* plant extract, fabricated AgNPs and compared with the standard ascorbic acid. For this purpose, 5mg of plant extract and AgNPs are dissolved in 1mL of HPLC water respectively and from this stock solution, aliquots of plant extract and AgNPs are prepared in 25, 50, 100, 150, 200 µg/mL concentrations. 3mL of the extract solution was added to the 1mL of 0.1mM solution of DPPH in methanol. The absorbance was calibrated after 30min at 517nm. DPPH in methanol was used as a negative control. The percentage of scavenging activity was measured by the following equation.[16]

$$\text{DPPH Scavenged (\%)} = [(A_{control} - A_{test})/ A_{control})] \times 100.$$

### Antibacterial activity of AgNPs

The synthesized AgNPs were assessed for their antibacterial activity by using the disc diffusion method. The antibacterial activity was evaluated against two Gram-positive bacteria *Staphylococcus aureus*, and *Lactobacillus* bacteria. The Gram-negative bacteria used for evaluation include *Escherichia coli* and *Enterobacter*. Streptomycin was used as the standard for comparison. 50µL of 24 hr old bacterial cultures were spread over the surface of nutrient agar plate using an aseptic swab. Sterile antimicrobial susceptibility discs were used and they were loaded with 20, 30, 40and 50 µL of fabricated AgNPs solution of 1mM concentration. The AgNPs solution-loaded discs were placed on the agar plate and incubated at 37°C for 24 hr. After the incubation period, the antibacterial activity of AgNPs was assessed by measuring the zone of inhibition using a calibrated scale.[17]

## RESULTS

### UV-Visible spectroscopy

The nanoparticles were fabricated by reducing silver nitrate into AgNPs by using *Cordia dichotoma* stem bark aqueous extract as a bio reductant and capping agent. The synthesis was initially confirmed by observing colour change from light yellow to red color and further confirmed by UV-Visible spectroscopy. A characteristic broad peak at 430nm was observed. The result of UV-Visible spectroscopy was represented in Figure 1. The UV analysis was performed to identify the Surface Plasmon Resonance characteristic feature of fabricated AgNPs. An increase in the absorbance was observed with an increase in time and evaluated up to 24 hr. There was no significant increase in the absorbance observed after 20 hr.[18]

### FT-IR

The FT-IR spectrum demonstrated the presence of 1516, 1634 $cm^{-1}$ 1650, 2347, 2825, and 3200 to 3900 $cm^{-1}$ peaks. These peaks are

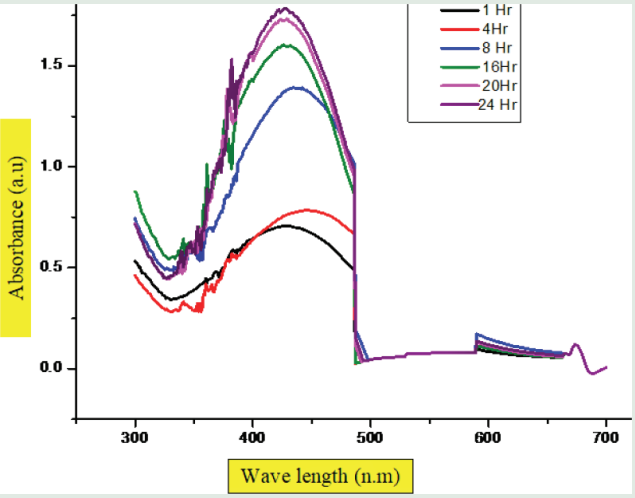


**Figure 1:** U.V visible spectrum of AgNPs at different time intervals.

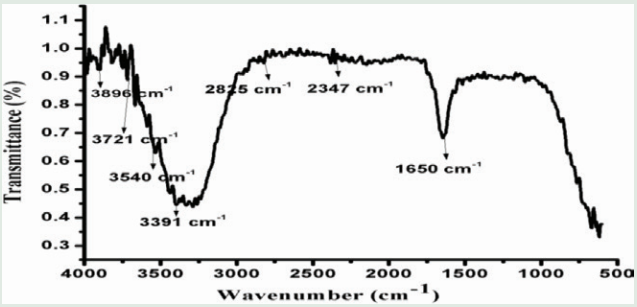


**Figure 2:** FT-IR spectra of the synthesized AgNPs.

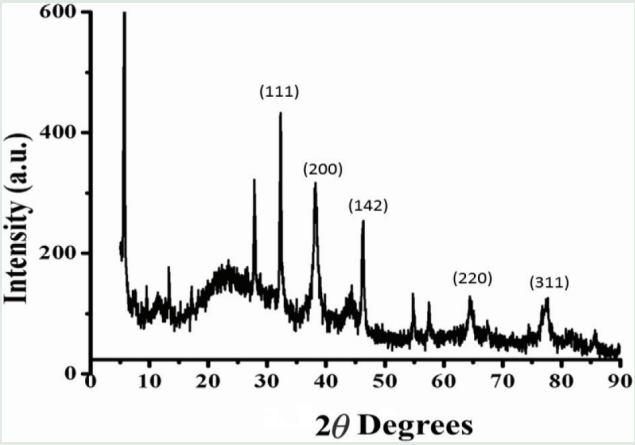


**Figure 3:** XRD of the synthesized AgNPs.

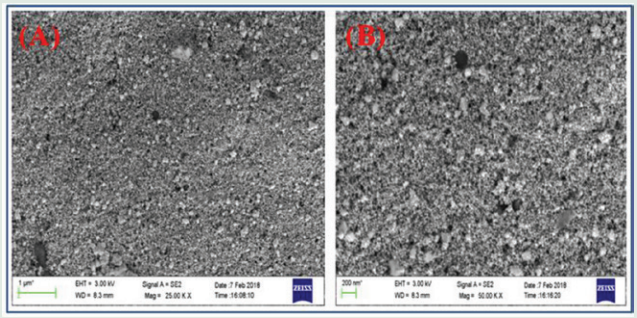


**Figure 4:** Corresponds to the SEM images of the synthesized AgNPS. Image (A) corresponds to the AGNPS-Plant extract composite at lower magnification. Image (B) corresponds to the SEM image at further higher magnification.

characteristic features of functional groups having phenolic compounds and flavonoids. For instance, the strong intense peaks at 1650represent the (C=C) groups from aromatic rings. 2347 represents (C≡O), aldehydic C-H stretch at 2825 cm, the peaks in the range of 3200 to 3900 were due to the OH stretching in alcohols and phenols.[19] The FT-IR spectrum is represented in Figure 2.

## XRD analysis

The XRD pattern was measured and analyzed from a diffraction angle range of 0° to 80°. The XRD spectrum was represented in Figure 3. The XRD diffraction peaks can be indexed to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) reflections of face-centred cubic structure of metallic silver which appropriately matched the standard diffraction data of silver reported by the joint committee on powder diffraction standards (JCPDS) file no: 04-0783. The smaller size of AgNPs in the sample resulted in the line widening of peaks. The other peaks at 2θ = 27.19°, 32.24°, 54.69°, 57.36°, and 76.58° have resulted from the secondary metabolites of AgNPs.[20]

## SEM analysis

The SEM images of AgNPs-*Cordia dichotoma* composite revealed that they are in spherical shape and agglomerated. SEM micrographs for synthesized silver nanoparticles were recorded at 100μM and 200μM, It was evident from SEM analysis is that the AgNPs are spherical closely arranged and agglomerated.[21] The secondary metabolites acted as reducing and capping agents. The SEM images at low and high magnification were represented in Figure 4.

## EDAX

The EDAX spectrum revealed the presence of elemental silver with a percentage of 80%. Apart from silver, the EDAX spectrum also revealed the presence of other elements like carbon, oxygen, sodium, and nitrogen at different concentrations which are from the phytoconstituents. The EDAX spectrum was represented in Figure 5.

## Zeta potential

Zeta potential technique measures the long-term stability of colloidal AgNPs. The metal nanoparticles with a large positive or negative zeta potential, repel each other and do not agglomerate. Agglomeration occurs in nanoparticles that have less positive and less negative charges because of the absence of repulsive forces. From the zeta potential analysis, the fabricated AgNPs exhibited a value of -33, which indicated the good stability of AgNPs. The zeta potential spectrum was represented in Figure 6.

## Particle size analysis

The particle size of the fabricated AgNPs showed an average particle size of 39.8nm. The AgNPs exhibited relatively narrow particle size

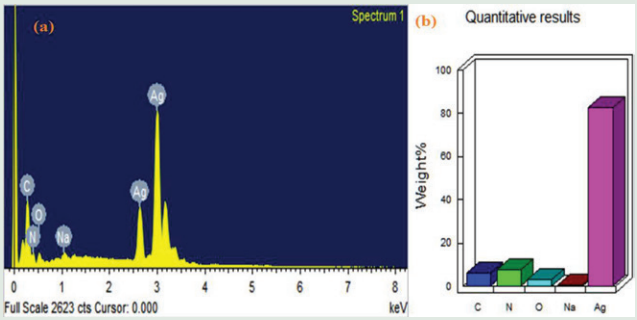


**Figure 5:** EDAX spectrum of the synthesized AgNPs. Image (a) corresponds to the EDAX spectrum with different elements. Image (b) corresponds to the bar graph representation of various elements as represented in weight%.

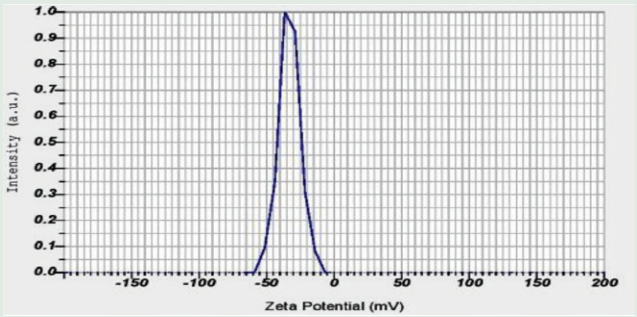


**Figure 6:** Zeta Potential of the synthesized AgNPs.

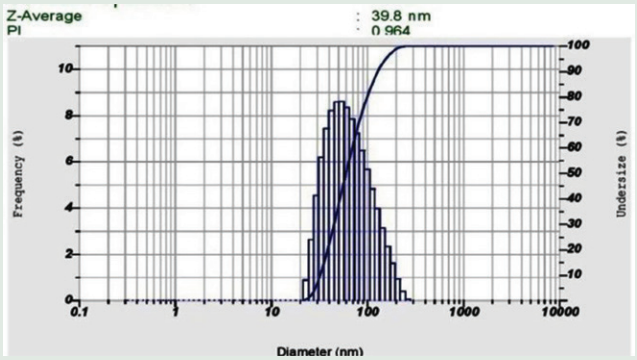


**Figure 7:** Particle size analysis spectra of the synthesized AgNPs.

distribution (z-average = 39.9 nm). Nanoparticle size distribution is related to the PDI value. PDI is measured as 0.964. The results of particle size analysis were represented in Figure 7. The PDI value of 0.96 is considered as highly polydisperse system. The most prominent, efficient, and effective nanoparticles were determined to be in the range of 1-100 nm size. Such a novel size gives these particles the characteristics of both bulk materials and molecular structures. The smaller the size, the increase in surface area with altered and enhanced physical and chemical properties while compared to their counterparts which are at a large size.

## Antioxidant activity

The antioxidant activity of fabricated AgNps and *Cordia dichotoma* stem bark aqueous extract, along with the standard ascorbic acid was represented in Table 1 and Figure 8. From these results, we can confer that the percentage of DPPH scavenged activity of AgNPs is greater than *Cordia dichotoma* stem bark aqueous extract alone, and the activity is close to the standard ascorbic acid. The present study of antioxidant activity for *Cordia dichotoma* plant stem bark mediated silver nanoparticles is the first-ever report.

The $IC_{50}$ values of the *C. dichotoma*, AgNPs of *C. dichotoma* and ascorbic acid are 96.30, 78.9 and 70.1 **µ**g/mL respectively, and the value of AgNPs are less than the plant extract itself and close to the standard ascorbic acid. The $IC_{50}$ values were calculated by linear regression analysis of dose response curve plotting between % of absorbance and different concentration of extracts.[22]

## Antibacterial activity

The antibacterial activity was measured using the disc diffusion method. After 24 hr of incubation, the zone of inhibition in mm was measured. The antibacterial activity results images were represented in Figure 9 along with the images of standard streptomycin. The activity of AgNPs against *Staphylococcus aureus* was high (16.1± 0.21) at 50 µL concentrations when compared to other strains. The results as represented from Table 2 suggested that AgNPs showed an almost similar effect on both Gram-positive and Gram-negative bacteria conferring that silver nanoparticles can act as broad-spectrum antibiotics.

**Table 1: DPPH scavenged percentage of *Cordia dichotoma* bark, *Cordia dichotoma* AgNPs and standard Ascorbic acid.**

| Concentrations ( µg/mL) | *Cordia dichotoma* Aqueous stem bark extract DPPH scavenged (%) | *Cordia dichotoma* AgNPs DPPH scavenged (%) | Ascorbic acid DPPH scavenged (%) |
|---|---|---|---|
| 25 | 27.8 ± 0.43 | 30.4±0.08 | 32.5±0.16 |
| 50 | 44.2 ± 0.53 | 46.4±0.12 | 48.24±0.11 |
| 100 | 55.9 ±0.16 | 58.6±0.24 | 60.2.±0.12 |
| 150 | 62.2±0.20 | 68.5±0.08 | 72.8±0.16 |
| 200 | 74.5± 0.16 | 81.4±0.16 | 88.7±0.12 |

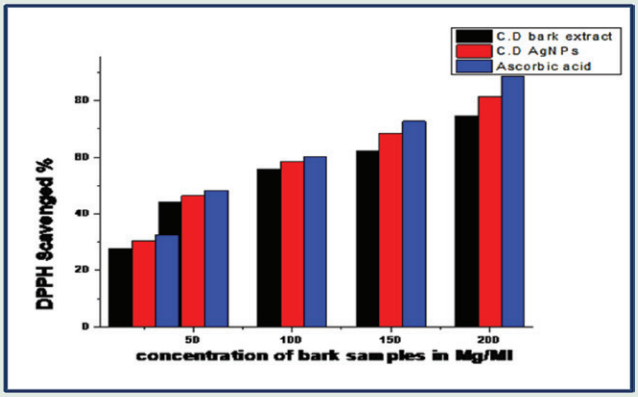


**Figure 8:** Corroborates the graphical representation of the antioxidant activity of the synthesized AgNPs.

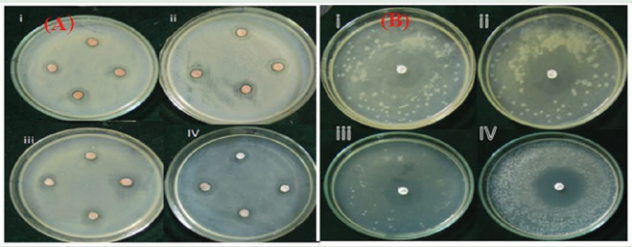


**Figure 9:** Image section A corresponds to the antibacterial activity of AgNPs. i. *Escherichia coli*, ii. *Enterobacter* sp, iii. *Staphylococcus aureus*, iv. *Lactobacillus*. Images from B correspond to the antibacterial activity against standard streptomycin. Imag- i. *E. coli*, ii. *Enterobacter*, iii. *Staphylococcus aureus*, iv. *Lactobacillus*.

**Table 2: Zone of inhibition values (mm) of AgNPs at variable concentrations.**

| Concentration of AgNPs (µL/disc) | Zone of Inhibition of test Organisms (m.m) | | | |
|---|---|---|---|---|
| | *Escheria coli* | *Staphylococcus aureus* | *Lactobacillus* | *Enterobacter* |
| 20 | 11.93±0.41 | 12.93±0.12 | 13.86± 0.49 | 12.96± 0.16 |
| 30 | 12.96±0.16 | 13.25± 0.12 | 14.13± 0.20 | 13.06± 0.12 |
| 40 | 13.93±0.41 | 14.2± 0.21 | 15.06± 0.20 | 14.06± 0.20 |
| 50 | 15.93±0.12 | 16.1± 0.21 | 16.06± 0.16 | 15.96± 0.20 |
| streptomycin | 24.5± 0.40 | 21.93± 0.33 | 22.06± 0.20 | 26.83± 0.28 |

## DISCUSSION

Globally, much attention was focused on green synthesized metal nanoparticles because of their ease, one-pot, eco-friendly, and economically feasible methodologies of synthesis and characterization. Based on the current research and available experimental data from the literature, the medicinal plant *Cordia dichotoma* is the richest source of secondary metabolites with potential therapeutic applications. Phytochemicals exhibit greater reduction and stabilization of metallic chemicals like silver nitrate which can be reduced to silver nanoparticles. Gomathi S *et al*., 2017 used *Eugenia jambolana* leaf extract for the fabrication of AgNPs in the presence of plant secondary metabolites like alkaloids, flavonoids, saponins, and sugar compounds.[23] The phenols present in the *Cordia dichotoma* reduced the silver nitrate to the AgNPs. The methodology followed in this study is a rapid, economically feasible, and most efficient approach for the bio reduction and aggregating AgNPs with therapeutic applications. Many of the researchers used a similar bio reduction method for the synthesis of metallic nanoparticles using plant extracts. The change in color of the reaction mixture was observed after 30 min due to the formation of silver nanoparticles. The broad peak observed in U.V Visible spectroscopy in the range of 410nm to 490nm is the characteristic feature of silver nanoparticles. The broad peak in U.V Visible spectroscopy is due to Surface Plasmon Resonance. Brause *et al*., represented that optical absorption spectra of metallic nanoparticles are extensively dominated by Surface Plasmon Resonance and the obtained peak can be correlated with the particle size. With the increase in particle size, the absorbance peak of AgNPs in aqueous medium shifts to longer wavelengths.[24] According to Nikita Dalal *et al*., The relation between size and wavelength is linear.[25] The FT-IR spectra exhibited the presence of diverse functional groups suggesting and confirming the formation of aggregates and composites of synthesized AgNPs with plant secondary metabolites especially phenols. Reddy NJ synthesized silver nanoparticles using the aqueous extract of *Piper longum* fruit extract and reported that the polyphenols present as secondary metabolites stabilized the silver nanoparticles.[26] The XRD analysis revealed that they are crystalline in nature with face centered cubic structure (FCC). A similar result of crystalline nature was reported by Moldovan *et al*. They synthesized spherical AgNPs from *Sambucus nigra* fruit extract.[27] SEM analysis revealed that they are spherical and compactly arranged. Plant extract directs the size and shape of silver nanoparticles, Logaranjan *et al*., 2016, prepared the AgNPs using aloe vera, and observed that that aloe vera gel played a prominent role in controlling the size and shape of the AgNPs,[28] by using the plant extract of *Fraxinus excelsior*, Praveen M *et al*., synthesized similar type of spherical shaped silver nanoparticles.[29] The synthesised silver nanoparticles of *C.dichotoma* also have effective antibacterial activity against Gram-positive bacteria like *Staphylococcus aureus*, *lactobacillus*, and against the Gram-negative bacteria *Escherichia coli* and *Enterobacter* strains. The variation in the antibacterial activity of silver nanoparticles is due to the variation in the degree of susceptibility for the cell walls of the tested organisms due to their structural variations. The possible mechanism for the antibacterial activity of AgNPs is by binding to the bacterial cell membrane and penetrates the cell, causes cell death. The other mode of action by AgNPs includes synergistic action of silver and AgNPs binds to the sulfur-containing proteins of the cell wall to increase the cell wall permeability of antibiotics.

The AgNPs also exhibits excellent antioxidant properties when compared with the plant extract and the $IC_{50}$ values are close to the standard ascorbic acid. The lower $IC_{50}$ values of AgNPs when compared to the plant extract itself are evident that they are effective free radical scavengers. According to jun *et al*., the $IC_{50}$ values in the range of 50-100 µg/mL is considered as active free radical activity.[30] The hydroxyl group-containing molecules are responsible for the antioxidant activity. The apigenin, hydroxyl group containing molecule was already reported from the plant. Antioxidant activity of the green synthesized AgNPs were reported in Chenopodium Murale leaf extract[31] Priya *et al*., 2016 has studied the antioxidant activity of silver and gold nanoparticles from the bark of Plumbago zeylanica and concluded that the AgNPs showed better antioxidant activity than the plant extract itself.[32]

Based on our literature survey, we can conclude that the study of antibacterial and antioxidant activity of AgNPs fabricated using the aqueous extract of stem bark of *Cordia dichotoma* is reported for the first time.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**U.V:** Ultra violet spectroscopy; **SPR:** Surface Plasmon resonance; **FT-IR:** Fourier Transform Infra-Red spectroscopy; **XRD:** X-ray powder diffraction; **SEM:** Scanning Electron Microscopy; **EDAX:** Energy-dispersive X-ray Spectroscopy; **PDI:** Poly dispersity Index; **ZOI:** Zone of inhibition; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl, **$IC_{50}$:** Half Maximal Inhibitory Concentration.

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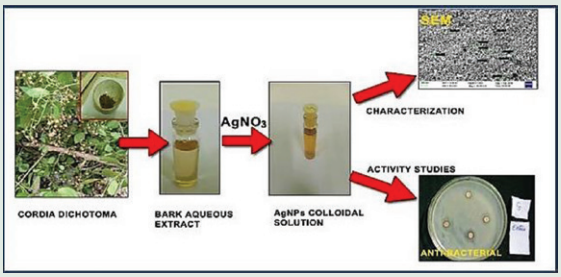
## GRAPHICAL ABSTRACT





## SUMMARY

Silver at the nanoscale level provides excellent, efficient altered physical and chemical properties and which can be further enhanced when the fabrication on synthesis method is through plant extract-based bio reduction. In the present study, AgNPs were synthesised by using stem bark aqueous extract of the *Cordia dichotoma*. The synthesized AgNPS were characterized by various spectroscopic and microscopic techniques such as U.V-Visible, FT-IR, SEM, EDAX, Particle analyzer, and Zeta potential. From the results, it can be inferred and concluded that the synthesized AgNPs have all the structural and physical characteristic properties to be considered as nanoparticles with potential therapeutic applications viz., antioxidant and antibacterial activity against both Gram-positive and Gram-negative pathogenic bacteria. As natural or synthetic antioxidants have limitations of degradation during delivery and poor bioavailability, the AgNPs synthesized using *Cordia dichotoma* can be best explored to use as natural antioxidants. However, bio-efficacy studies are needed to be performed in animals.