

## Plant-mediated green synthesis, characterization and antibacterial efficacy of Ag-NPS using extracts of *Wrightia tinctoria* leaves for biological applications

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*Wrightia tinctoria* was one of the medicinal plants that contains enormous amount of phytoconstituents with diverse functions and the plant was said to be the safer drug widely used in treating various ailments. In this present work, silver nanoparticles were synthesized using different solvents (benzene, ethanol and aqueous) of *Wrightia tinctoria* leaves. The prepared nanoparticles are characterized by UV- Vis spectroscopy, FTIR, XRD, FESEM and HRTEM analysis. Biosynthesized silver nanoparticles of ethanol extract showed strong antibacterial activity compared to the nanoparticles synthesized from other extracts against *Staphylococcus aureus* and *Enterococcus faecalis* (gram-positive) and *Escherichia coli* and *Klebsiella pneumoniae*(gram-negative) bacterial strains by well diffusion method. In this investigation, the selected plant extract and silver nanoparticle were coated on viscose spunlaced nonwoven fabrics and subjected to qualitative and quantitative antibacterial studies.

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**Keywords:** *Wrightia tinctoria*, Silver nanoparticles, Plant extract, Antibacterial activity

### 1. Introduction

Nanotechnology is understanding, manipulating and controlling of materials ranging from 1-100 nm in diameter. The surface properties of the textile materials can be altered using nanotechnology by applying techniques like coating and finishing. The properties of the developed materials can be changed for the betterment and there are number of advancements in the technique [1]. The materials developed at nanoscale sizes possess enormous physical, chemical and biological properties because of its enhanced surface area to volume ratio [2].

Silver is used as an antibiotic since ancient times in preparing traditional and ayurvedic medicines. Silver based products and dressings are used in the treatment of burns, wounds and ulcers. It is used in treating certain chronic wounds thereby minimizing the scar formation. Silver in the form of metallic silver, silver nitrate and silver sulfadiazine is used in the treatment of wounds [3]. Silver nanoparticles (Ag-NPs) are found to have their own unique physical, chemical, biological and mechanical properties which are different from the bulk material. The properties are more affected by morphological characteristics of the nanoparticles. The large surface area to volume ratio is the reason for more interaction into the cell membranes [4]. Ag-NPs are used to treat some delayed wounds particularly in treatment of diabetic wounds. The nanoparticles heal the wound in its earlier stage itself without causing any secondary infection and with minimum scar formation [5].

Though chemical approach is the widely used method for synthesizing Ag-NPs, there is a growing environmental concern which leads to the development of ecofriendly biological method of synthesizing nanoparticles using plants, microorganisms and enzymes. Biological synthesis is proved to be competent against the physical and chemical techniques since the technique is found to be non toxic and cost effective. The nanoparticles synthesized using biological method is proved to be more antibacterial resistant against the bacterial strains. There are various factors that affect antimicrobial activity of nanoparticles such as concentration, size, chemical composition,

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shape and target microorganisms [6]. Green synthesized Ag-NPs particularly using plants are proving its efficacy in the field of medicine because of its enhanced antimicrobial, anticancer and antioxidant properties. The process is simple, safe, cost-efficient and biocompatible than the other methods and the synthesized nanoparticles are finding its applications in treatment of wounds [7].

*Wrightia tinctoria* is one of such medicinal plants that are used to treat various skin infections since bioactive compounds are present in the leaf extracts of *Wrightia tinctoria*. The plant is emerging as an alternate system in the field of medicine. The plant contains bioactive compounds like phenols and flavonoids needed for the drug development [8]. The plant is known to have diverse pharmacological activities like Anti-inflammatory activity, Anti-diabetic activity, Anti-fungal activity, Anti-viral activity, Anti-psoriatic activity, Anti-cancerous activity and Anthelmintic activity [9]. The extracts are used in the treating skin problems and the latex proteases of *Wrightia tinctoria* are found to have wound healing potential. It is suggested to heal some of the chronic wounds found in diabetic patients [10].

The present study intends to synthesize Ag-NPs from the benzene, ethanol and aqueous extracts of *Wrightia tinctoria* leaves. The phytochemical activity of the extracts was reported followed by the antibacterial activity. The selected Ag-NPs was then subjected to the characterization studies and coated on viscose nonwoven fabrics for finding out its effectiveness for biological applications particularly in developing wound dressings.

## **2. Materials and methods**

### **2.1. Preparation of leaf extracts**

The whole healthy and fresh leaves of *Wrightia tinctoria* were collected from the agricultural field of Erode district, Tamilnadu, India. The collected leaves were washed thoroughly with running tap water and double distilled water in order to remove all contaminants. The leaves were kept away from direct sunlight and shade dried at room temperature for 15 days to avoid degradation of the compounds. The method helps to remove moisture from the surface of the leaves and the leaves were then finely powdered. The powder was extracted using Soxhlet apparatus with benzene, ethanol and aqueous solution and the process was carried out until the solvent in the siphon tube becomes colourless. The solvent extracts were reduced in rotary evaporator at 65°C and the reduced extracts were further dried in lyophilizer and stored in refrigerator at 4°C for further analysis.

### **2.2. Phytochemical analysis**

Phytochemical screening helps to analyse the presence of secondary metabolites in the freshly prepared plant extracts (benzene, ethanol and aqueous) of *Wrightia tinctoria* by using standard methods.

#### **2.2.1. Phenolic compounds**

50 mg of each plant extract was taken and dissolved in 5 ml of distilled water to which few drops of 5% ferric chloride solution was added. The presence of phenolic compound was indicated by the formation of dark green colour.

#### **2.2.2. Alkaloids**

2 ml of each plant extract was evaporated to dryness and 5 ml of 2N hydrochloric acid was added to the residue which was heated on a boiling water bath. The mixture was then cooled and filtered. The filtrate was divided into three equal portions and treated with equal amount of Mayer's reagent, Hager's reagent and Wagner's reagent to each portion. The formation of turbidity or precipitation indicated the presence of alkaloids.

#### **2.2.3. Carbohydrates**

About 2 ml of distilled water was added to each solvent free plant extract and then few drops of Molisch's reagent were added. The presence of carbohydrates was indicated by the appearance of violet or reddish violet colour.

#### **2.2.4. Reducing sugars**

About 1 ml of water was added to extracts and then treated with 5-8 drops of Fehling's solution (A and B). The mixture was kept on a hot water bath for a period of 15 minutes. The brick red precipitate indicated the presence of reducing sugars.

#### **2.2.5. Test for glycosides**

To solvent free plant extracts, about 1 ml of glacial acetic acid and then few drops of ferric chloride solution and concentrated sulphuric acid were added. The presence of glycosides was indicated by the formation of bluish green colour.

#### **2.2.6. Test for tannins**

About 1 ml of water and 2 drops of ferric chloride solution were added to the extracts and formation of blue colour and green colour indicated the presence of gallic tannins and catecholic tannins respectively.

#### **2.2.7. Test for terpenoids**

To the plant extracts, 2 ml of chloroform was added followed by 3ml of concentrated sulphuric acid. The presence of terpenoids was indicated by the formation of reddish brown colour.

#### **2.2.8. Test for flavonoids**

About 2 ml of each extract solution was taken and treated with few drops of concentrated 2N hydrochloric acid and 0.5 g of magnesium turnings. The pink or magenta red colour formation indicated the presence of flavonoids.

#### **2.2.9. Test for saponins**

About 2ml of distilled water was added to solvent free plant extracts and shaken vigorously. The foam formation indicated the presence of saponins.

### **2.3. Green Synthesis of Ag-NPs**

10 ml of benzene, ethanol and aqueous extracts of *Wrightia tinctoria* leaves were taken separately in conical flasks and the extracts were mixed with 90 ml of 1 mM aqueous silver nitrate solution at room temperature. The mixture were incubated under dark conditions in a rotary shaking incubator and monitored periodically for colour change. The formation of Ag-NPs was confirmed by the colour change from green to yellowish brown and also scanning by UV-Visible Spectrophotometer (UV/VIS 3000+ Double Beam UV Visible Ratio- Recording Scanning Spectrophotometer from Lab India) in the range of 300 nm to 700 nm. This spectroscopy analysis was carried out to measure the bioreduction of silver ions ( $\text{Ag}^+$  ions to  $\text{Ag}^0$  ions) in the green synthesized Ag-NPs. The nanoparticles were centrifuged at 10000 rpm for 30 minutes, lyophilized and stored for further studies.

### **2.4. Antibacterial activity of plant extracts and Ag-NPs**

Agar well diffusion method was used to study the antibacterial activity of plant extracts and Ag-NPs synthesized using benzene, ethanol and aqueous extracts of *Wrightia tinctoria* leaves on Mueller-Hinton agar plates (HiMedia Laboratories Pvt. Ltd., Mumbai, India). Inhibition against two gram-positive bacterial cultures named *Staphylococcus aureus* and *Enterococcus faecalis* and two gram-negative bacterial cultures named *Escherichia coli* and *Klebsiella pneumoniae* were measured. Nutrient agar solution was poured into the petriplates and the agar plates were prepared by swabbing the plates with overnight grown bacterial cultures using sterile cotton swab. The procedure was carried out by varying the concentration of plant extract and silver nanoparticles. Wells of 5mm diameter were punctured and various concentrations (25µl-100µl) of each plant extract and silver nanoparticle were loaded into the respective wells on the agar plates and were incubated for 24 hours. The standard antibiotic found to be resistant from antibiotic susceptibility testing was selected and used as a negative control. The Mueller-Hinton agar plates containing the

plant extracts, Ag-NPs and standard negative control (antibiotic) were then incubated at 37°C for 24 hours and the zone of inhibition was measured.

## **2.5. Characterization of synthesized Ag-NPs**

The following characterization studies were performed on the selected synthesized Ag-NPs based on the above studies.

### **2.5.1. X-ray diffraction analysis (XRD)**

The X-ray diffraction patterns of the nanoparticles was recorded in the 2θ range (10°-80°) using powder X-ray diffractometer (PANalytical X'Pert-PRO Powder X-Ray Diffractometer) at the wavelength of 1.5406. The diffracted intensities were recorded by continuous scanning at 40kV and 30mA. Particle size calculations were performed using the diffraction patterns of Ag-NPs according to the line width of the maximum intensity reflection peak.

### **2.5.2. Fourier transform infra-red spectroscopy analysis (FTIR)**

The spectra of the synthesized Ag-NPs were recorded using FTIR spectrometer (IR Prestige 21, Shimadzu) to detect the functional groups responsible for stabilization and reduction processes. The FTIR spectra were analyzed in the range of 500-4000 cm<sup>-1</sup> operating at a resolution of 1cm<sup>-1</sup>.

### **2.5.3. Field emission scanning electron microscopy (FESEM)**

The morphological analysis of the synthesized nanoparticles was done using field emission scanning electron microscope (SIGMA HV-Carl Zeiss with Bruker Quantax 200-Z10 EDS Detector) to study the size and shape of nanoparticles. The images were recorded in various magnifications, operating at 8.00 kV – 10.00 kV.

### **2.5.4. High resolution transmission electron microscopy (HRTEM)**

It is used to analyze the particle size and shape of the green synthesized nanoparticles. The images were recorded in various magnifications using FEI-Tecnai G2 20 S-TWIN High Resolution Transmission Electron Microscope, operating at an accelerating voltage of 200 kV. The microscope is equipped with Energy- Dispersive X-Ray Spectroscopy (EDS) for performing the chemical analysis of the nanoparticles.

## **2.6. Coating on fabric**

The fabric used for the study was viscose spunlace nonwoven procured from The South India Textile Research Association (SITRA), Coimbatore. The fabric sample was then coated with 5% of selected plant extract and synthesized silver nanoparticles of *Wrightia tinctoria* leaves. The application of coating was done by using exhaust method.

### **2.6.1. Qualitative antibacterial evaluation on the coated fabrics (Parallel streak method)**

In parallel streak method, nutrient agar plate was inoculated with streaks of test bacteria and the coated textile samples were placed immediately over these streaks. The method involves finding out the effect of antibacterial agent against the bacterial cultures. The nutrient agar plate containing the coated fabric samples were incubated for 24 hours at 37°C. The bacterial growth was examined around the edges and underneath the fabrics. In the present study, both the plant extract and silver nanoparticle coated fabrics were subjected to qualitative antibacterial evaluation (Parallel streak method- AATCC 147-2016) against the bacterial cultures *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniae* ATCC 4352. Both bacteriostatic activity and growth was observed in the coated textile samples.

### **2.6.2. Quantitative antibacterial evaluation on the coated fabrics (Percentage reduction test)**

The antibacterial activity of the coated fabric samples were tested according to percentage reduction test (AATCC 100-2019) against the bacterial cultures *Staphylococcus aureus* ATCC

6538 and *Klebsiella pneumoniae* ATCC 4352. 0.1 ml sample were spread on to the sterile nutrient agar plates and the dilution medium used was Dey- Engley Neutralizing broth. The test samples (4.8 cm in diameter) were incubated at 37°C for 24 hrs. After incubation, the percentage in bacterial reduction was calculated on both plant extract and silver nanoparticle coated fabric samples.

### 3. Results and discussion

#### 3.1. Phytochemical analysis

Active phytochemical components such as phenolic compounds, alkaloids, carbohydrates, reducing sugar, glycosides, tannins, terpenoids, flavonoids and saponins were present in extracts of *Wrightia tinctoria* leaves and these were responsible for the antimicrobial, antioxidant and antitumor properties needed for the therapeutic treatment [11], it was given in Table 1. Presence of all these phytochemical components was observed in the ethanol extract. More chromogenic reaction found in the ethanol extract may be the reason for the presence of all active phytochemicals [12] whereas benzene extracts reported the absence of alkaloids, glycosides, tannins and saponins. Also the screening revealed the absence of phenols and flavonoids in the aqueous extracts of *Wrightia tinctoria* leaves.

Table 1. Phytochemical analysis of *W.tinctoria* leaf extracts.

Phytochemicals	Benzene extract	Ethanol extract	Aqueous extract
Phenol	+	+	-
Alkaloids	-	+	+
Carbohydrates	+	+	+
Reducing sugars	+	+	+
Glycosides	-	+	+
Tannins	-	+	+
Terpenoids	+	+	+
Flavonoids	+	+	-
Saponins	-	+	+

+ presence, - absence

#### 3.2. UV-Vis spectroscopy analysis

The Ag-NPs synthesized using benzene, ethanol and aqueous extracts of *Wrightia tinctoria* leaves were analysed by UV-Vis spectroscopy. The positioning of surface plasmon resonance (SPR) confirmed the formation of Ag-NPs. The spectra showed the activity of silver nitrate and the role of reducing agent in the nanoparticle formation [13]. The previous studies reported that the absorption spectra of Ag-NPs range from 400-450 nm and the SPR band of the present study showed the spectra range of 420-436nm, indicating the formation of Ag-NPs. The narrow peaks indicated the synthesis of nanoparticles in smaller size and the broadening of the peaks indicated that the particles were polydispersed [14]. The synthesized nanoparticles from benzene, ethanol and aqueous extracts of *Wrightia tinctoria* leaves displayed a strong peak centered around 436 nm, 425 nm and 420 nm, respectively and is shown in Fig.1.

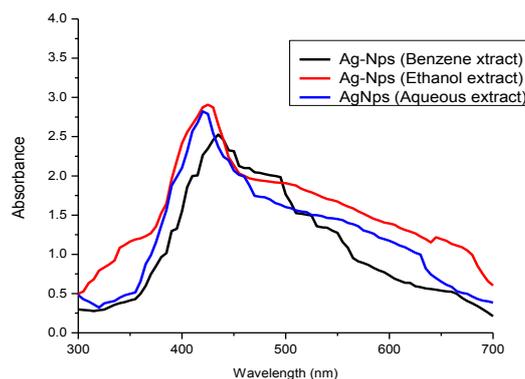


Fig.1. UV-Vis absorption of Ag-NPs synthesized from different solvent extracts of *W.tinctoria* leaves.

### 3.3. Antibacterial activity plant extracts and Ag-NPs

The antibacterial activity of the extracts and synthesized Ag-NPs was carried out against *Staphylococcus aureus* and *Enterococcus faecalis* (gram- positive) and *Escherichia coli* and *Klebsiella pneumoniae* (gram- negative) by well diffusion method, shown in Figs 2 and 3. Also Table 2 and 3 shows zone of inhibition of the plant extracts and synthesized silver nanoparticles measured at four different concentrations (25 $\mu$ l, 50  $\mu$ l, 75  $\mu$ l & 100 $\mu$ l) respectively. The synthesized Ag-NPs from benzene, ethanol and aqueous extracts showed better activity than the plant extracts against all bacterial strains since silver was found to exhibit strong toxicity against bacterial strains [15]. The antibacterial activity gets increased while increasing the concentration in both plant extracts and silver nanoparticles [16]. Both the plant extracts and synthesized Ag-NPs have stronger antibacterial activity than the standard antibiotics (Methicillin and Penicillin) that was used as negative control for the study [17]. Biosynthesized Ag-NPs of ethanol extract showed maximum inhibition compared to the nanoparticles synthesized from other extracts against all bacterial strains [18]. Further, all plant extracts were observed to have minimum antibacterial activity against all gram positive bacterial strains compared to gram negative bacterial strains [16] and this may be due to the thin cell wall found in the gram negative bacteria [19]. Earlier it was reported that, Ag-NPs exhibited high antibacterial activity against gram positive bacterial strains compared to gram negative [13] and the same was inferred in the present work. Also the antibacterial activity was found effective in the small sized nanoparticles compared to large sized nanoparticles because of its high surface area for bacterial interaction [20].

Table 2. Antibacterial activity of plant extracts and synthesized Ag-NPs against gram-positive bacterial strains.

Solvents	Zone of inhibition, mm															
	<i>Staphylococcus aureus</i>								<i>Enterococcus faecalis</i>							
	Plant extract ( $\mu$ l/ml)				Ag-NPs ( $\mu$ l/ml)				Plant extract ( $\mu$ l/ml)				Ag-NPs ( $\mu$ l/ml)			
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
<b>Benzene</b>	7.0	7.6	8.0	-	8.0	10.0	12.0	13.5	-	-	6.0	6.0	10.0	13.0	14.0	14.5
<b>Ethanol</b>	9.0	10.0	10.5	11.0	11.0	10.0	12.0	11.5	7.0	7.5	8.0	8.0	10.0	13.5	14.5	16.0
<b>Aqueous</b>	8.0	8.0	10.0	10.5	-	-	-	6.0	-	-	6.5	7.0	7.0	11.0	11.5	12.0

Table 3. Antibacterial activity of plant extracts and synthesized Ag-NPs against gram-negative bacterial strains.

Solvents	Zone of inhibition, mm															
	<i>Escherichia coli</i>								<i>Klebsiella pneumoniae</i>							
	Plant extract (µl/ml)				Ag-NPs (µl/ml)				Plant extract (µl/ml)				Ag-NPs (µl/ml)			
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
<b>Benzene</b>	-	-	6.0	6.5	6.5	10.0	12.0	13.0	-	7.0	7.2	7.5	10.0	11.0	11.5	11.6
<b>Ethanol</b>	9.0	9.5	10.0	11.0	9.5	10.0	12.5	14.0	-	6.0	6.5	6.5	9.0	13.0	13.5	14.0
<b>Aqueous</b>	-	7.0	7.0	7.5	-	-	7.5	8.0	6.0	6.0	6.0	6.5	7.0	8.0	10.0	11.5

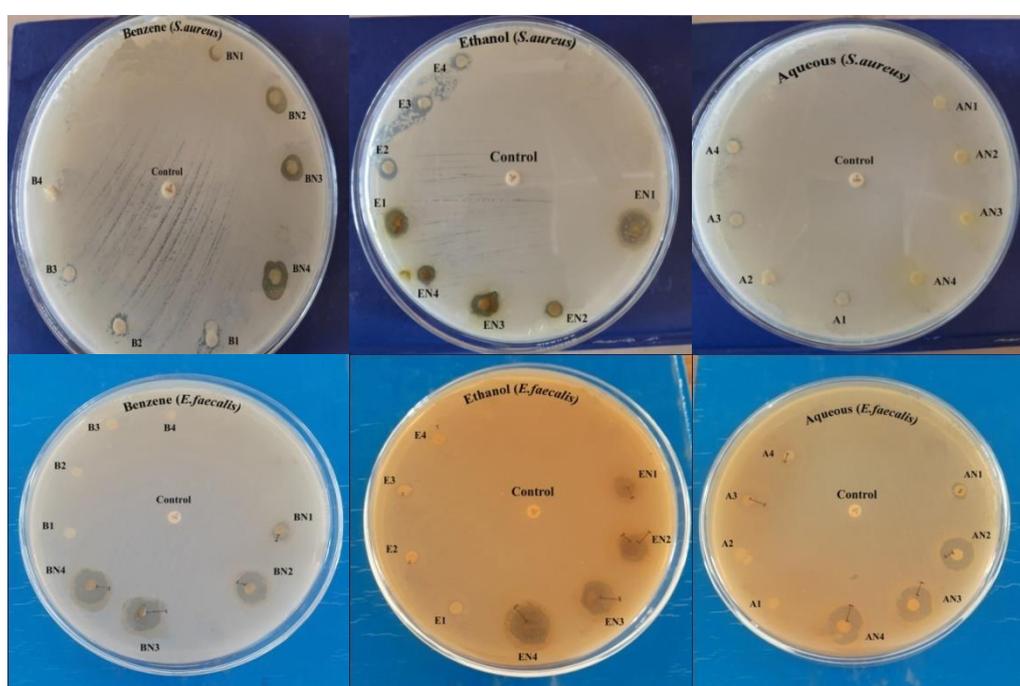


Fig.2. Antibacterial activity of plant extracts and synthesized Ag-NPs against gram-positive bacterial strains (a) *Staphylococcus aureus* (b) *Enterococcus faecalis*.

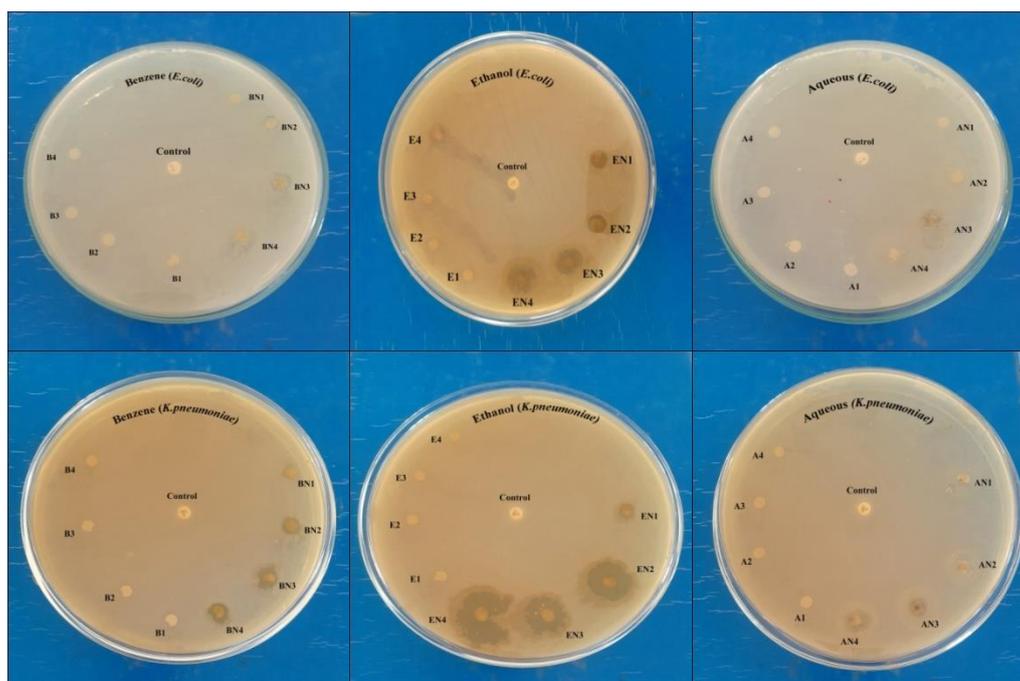


Fig.3. Antibacterial activity of plant extracts and synthesized Ag-NPs against gram-negative bacterial strains(a) *Escherichia coli*(b) *Klebsiella pneumoniae*

### 3.4. X-ray diffraction analysis (XRD)

The X-ray diffraction (XRD) pattern of Ag-NPs synthesized using ethanol extract of *Wrightia tinctoria* was shown in the Fig.4. The average crystallite size was calculated using Debye- Scherrer's equation based on the values of major peaks observed in the XRD patterns [21]. The synthesized Ag-NPs showed diffraction peaks at about  $38.14^\circ$ ,  $45.50^\circ$ ,  $64.73^\circ$  and  $77.50^\circ$  that correspond to (111), (200), (220) and (311) crystalline planes, respectively and the average crystallite size was found to be 29.27 nm. The presence of sharp peaks clearly indicated the crystallinity of the nanoparticles in the Ag-NPs. The other sharp peaks observed were due to the crystallization of bioorganic phase present on the surface of the nanoparticles. The pattern observed may be indexed to the face-centered cubic structure of silver [22] and was found well matched with the standard powder diffraction card of JCPDS (File no. 04 – 0783) [23]. The above result matched well with earlier reports of Ag-NPs synthesized using different leaf extracts of the plants [18].

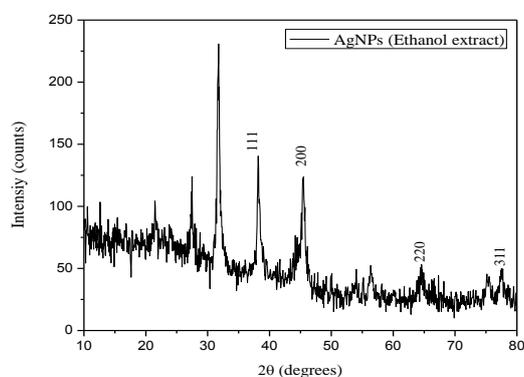


Fig.4. XRD pattern of Ag-Nps synthesized from ethanol extract of *W.tinctoria* leaves

### 3.5 Fourier transform infra-red spectroscopy analysis (FTIR)

Fig.5 reveals absorption peaks of the synthesized Ag-NPs at around 3360.90, 2916.73, 2848.98, 1732.55, 1455.39, 1377.94, 1243.13, 1167.81, 1087.22, 879.58, 718.44 and 655.89  $\text{cm}^{-1}$ . The peaks were shifted to higher wave number after reacting with silver nitrate. The peak observed at 3360.90  $\text{cm}^{-1}$  is associated with strong and broad O-H stretching of alcohol. The peak at 2916.73  $\text{cm}^{-1}$  and 2848.98  $\text{cm}^{-1}$  represent C-H stretching of alkanes. The peak at 1732.55  $\text{cm}^{-1}$  indicates C=O stretching of aldehyde. The peak at 1455.39  $\text{cm}^{-1}$  represents C-H bond of alkenes. The peak at 1377.94  $\text{cm}^{-1}$  corresponds to O-H bending of phenol. The peak obtained at 1243.13  $\text{cm}^{-1}$ , 1167.81  $\text{cm}^{-1}$  and 1087.22  $\text{cm}^{-1}$  denote C-N stretching of amines. The absorption peak at 879.58  $\text{cm}^{-1}$  represents C-H bending out of the plane. The peak at 718.44  $\text{cm}^{-1}$  was attributed to the C=C bending of alkanes. The peak observed at 655.89  $\text{cm}^{-1}$  refers to C-Br stretching of alkyl halide. The Ag-NPs were found to be stable because of the phytoconstituents and other functional groups detected and the reason for the stability may be the protein that covers the nanoparticles thus acting as capping, reducing and stabilizing agents preventing from agglomeration and also accounting for the reduction of silver nanoparticles [24].

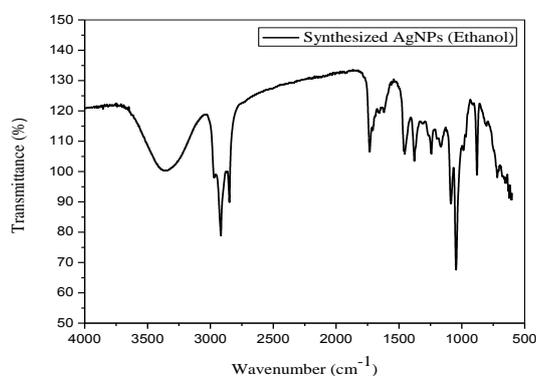


Fig.5. FTIR spectrum of Ag-NPs synthesized from ethanol extract of *W.tinctoria* leaves.

### 3.6. Field emission scanning electron microscopy (FESEM)

The surface morphology of the Ag-NPs synthesized from ethanol extract showed several large irregular shaped nanoparticles due to the aggregation of smaller ones. The average size of the nanoparticles was found to be 28nm and the overall particle size of the nanoparticles range between 10 -36 nm and most of the nanoparticles were found spherical in shape as shown in the Fig.6. Similar results were found in the Ag-NPs synthesized from different plant extracts by many researchers [25,26].

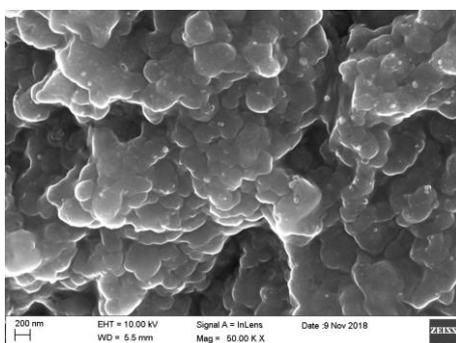


Fig.6.FESEM image of synthesized Ag-Nps.

### 3.7. High Resolution Transmission Electron Microscopy (HRTEM)

The nanoparticles synthesized using ethanol extract were found dispersed, almost spherical in shape with few hexagonal nanoparticles. The size of the particles range from 10 – 40 nm with mean particle size of 25 nm. The patterns of SAED spots have been indexed according to (111), (200), (220) and (311) planes and is shown in Fig.7. These represented Face Centred Cubic (FCC) structure of silver and crystalline nature of the synthesized nanoparticles [27]. Energy-dispersive X-ray analysis (EDX) was carried out to confirm the presence of elemental silver and is presented in Fig.8. The study revealed strong signal in the silver region and confirmed the formation of Ag-NPs. Further the synthesized Ag-NPs showed optical absorption peak approximately at 3 keV. Other elemental signals recorded in the study were due to the presence of metabolites present in the extracts of *Wrightia tinctoria* leaves [28].

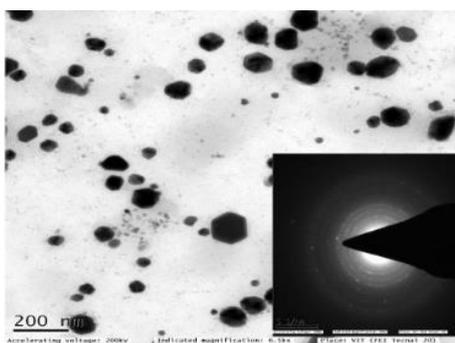


Fig.7.HRTEM image of Ag-NPs with SAED spots.

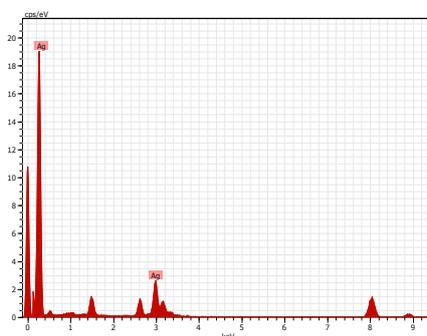


Fig. 8. Energy- dispersive X-ray (EDX) spectrum of Ag-Nps.

### 3.8. Qualitative antibacterial evaluation on coated fabric samples

The qualitative antibacterial evaluation on the ethanol plant extract and silver nanoparticle coated fabric samples was assessed by parallel streak method AATCC 147-2016. It was found that the zone of inhibition by parallel streak method of silver nanoparticle coated fabric sample was 0.2 mm against *S.aureus* and no zone was reported against *K.pneumoniae*. It was clear that the ethanol plant extract coated fabric samples doesn't exhibited any zone against both the test organisms. Also there was no bacterial growth observed underneath and along the edges of all the coated fabric samples against the bacterial strains except the ethanol plant extract coated fabric sample against *S.aureus*. The results clearly showed that bactericidal activity of the silver nanoparticle coated fabrics was found to be higher than the ethanol plant extract coated fabrics and similar results was shown by [29].

### 3.9. Quantitative antibacterial evaluation on coated fabric samples

The quantitative antibacterial evaluation on the ethanol plant extract and silver nanoparticle coated fabric samples was assessed by percentage reduction test AATCC 100-2019. It

was evident that plant extract coated fabric showed 99.99% reduction against *S. aureus* and 93.50% reduction against *K.pneumoniae* whereas the silver nanoparticle coated fabric showed 94.03% reduction against *S. aureus* and 99.99% reduction against *K.pneumoniae* and this proved the potent antibacterial property of the plant leaves in both the stages.

#### 4. Conclusion

The study revealed the effective biological reduction of AgNO<sub>3</sub> into Ag-NPs using different solvent extracts of *Wrightia tinctoria* leaves. The biomolecules present in the plants act as both capping and reducing agents and were found to possess medicinal values helping in the reduction and stabilization process. The antibacterial activity was found to be effective in the ethanol extract and Ag-NP synthesized using ethanol extract when compared to other solvent extracts and nanoparticles against the bacterial strains.

The present work proved the remarkable efficiency of the synthesized Ag-NPs to be used for biomedical applications particularly in antimicrobial textiles, healing of wounds and ulcers because of the found remarkable antibacterial activity and the nanosize of the particles that helps to easily penetrate into the cell wall or membrane and this results in ending the multiplication process thereby destructing the cell.

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