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RESEARCH ARTICLE

Preparation and characterization of silver nanoparticles using *Rubia cordifolia* plant root extract and their microbial properties

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Abstract

The present study will focus on the synthesis of AgNPs by using plant materials. The AgNPs were synthesized using *Rubia cordifolia* plant root aqueous extracts. The green synthesized nanoparticles were characterized by UV/Visible Spectroscopy, Potentiometer, FTIR and Scanning Electron Microscope. The green synthesized nanoparticles have good inhibitory activity against *Vibrio alginolyticus, Pseudomonas aeroginosa, Shigella spp, Plesiomonas shigelloides* and *Vibrio parahaemolyticus.*

1. Introduction

Nanotechnology has been established recently as a new interdisciplinary science (Aswathi sreenivasan *et al*., 2012). Nanotechnology enters into all the field of sciences. Nanoparticles are very tiny particles that indicate 10^{-9} meters. Synthesis of nanoparticles was done previously by various chemical methods. Biologically synthesed nanoparticles are very safe and eco-friendly, when compared to chemically synthesized nanoparticles. Now a days many researchers have started reporting the nanoparticles synthesize using biological methods viz., medicinal plant materials (Nagaraj*et al*., 2012; Luca Marchiol., 2012), microorganisms (Ranganathan Nithya and Ramachandran Ragunathan., 2012), enzymes, polysaccharides (Virender K. Sharma*et al*., 2009) etc. The microorganisms are very harmful and they tolerate the antibiotics. So there is need for alternative and best eco-friendly nanoparticle synthesize method. The present study has been focused on the green synthesis of Ag nanoparticles using *Rubia cordifolia* plant root aqueous extract, characterization of the synthesized nanoparticles by UV-Visible spectrophotometer, FTIR, Potentiometer and Scanning Electron Microscope (SEM) and anaysing the antimicrobial effect of the synthesized nanoparticles.

2. Materials and methods:

2.1. Materials:

Medicinal plant *Rubia cordifolia* roots, $AgNO_3$, Double Distilled H_2O,Whatman No 1 filter paper.

2.2. Extraction of plant materials:

The roots of Rubia cordifolia was purchased from Ayurveda shop at Nagercoil, Kanyakumari (Dist), Tamilnadu, India. The plant materials was washed with distilled H_2O and dried at room temperature. Then this plant material was powdered. Take 5 grams of root powder, and boiled with 100ml of double distilled water at 90^oC in 30 minutes. The aqueous extracts were then filtered using Whatman No 1 filter paper.

2.3. Preparation of $AgNO_3$Solution:

$AgNO_3$were purchased Sarabhi Merck Limited, Baroda. 0.16grams of $AgNO_3$ was dissolved in 1000ml Double Distilled water and stored in a clean brown bottle with dark condition.

2.4. Synthesis of Ag NP's using plant extracts:

90ml of prepared $AgNO_3$ (25mM) solution was added to 10ml of (5%) plant extract and stirred in room temperature. The solution colour got changed colourless to dark reddish brown that indicates the formation of AgNPs.

Fig 1: Preparation of AgNPs using *Rubia cordifolia* plant root extract:

2.5. Characterization:

The synthesized AgNPs were analyzed using Potentiometer, UV-Visible Spectrophotometer, Fourier transform infrared spectroscopy and Scanning Electron Microscopy.

The potentiometer study was carried out with $AgNO_3$ solution and required volume of plant extract in a beaker and in certain intervals EMF was noted. The prepared samples were analyzed for the rate of absorption of UV and Visible region using of UV-Visible Spectrophotometer in the range of 200 to 1000nm. IR spectra were recorded using KBr pellets (1% w/w) on a Perkin-Elmer GX FT-IR spectrometer. The sizes of the AgNPs were analyzed using Scanning Electron Microscope. SEM micrographs have a large depth of field yielding a characteristic three-dimensional appearance useful for understanding the surface structure of a sample.

2.6. Antibacterial Activity:

The antibacterial activity of green synthesized NP's against eight bacterial isolates was evaluated by using agar well diffusion method (Ahmad and Beg, 2001). Nutrient Agar plates were inoculated with 100µl of standardized inoculums (1.5x108 CFU/ml) of each bacterium (in triplicates) and spread with sterile swabs. Wells of 8 mm size are made in the agar plates containing the bacterial Inoculums. Wells were filled with liquid solution of nanoparticles in the ratio of 25µl, 50µl, 75µl and 100µl. Standard chemical solution (silver nitrate) was used as a negative control. The plates thus prepared were left at room temperature for ten minutes for allowing the diffusion of the extract into the agar. After incubation for 24 hrs at 37^{o}C, the plates were observed. If antibacterial activity was indicated by an inhibition zone surrounding the well containing the nanoparticles. The zone of inhibition was measured and expressed in millimeters. Antibacterial activity was recorded if the zone of inhibition was greater than 8 mm (Hammer, *et al*., 1999; Mariselvam[a]*et al*., 2012). The antibacterial activity results were expressed in term of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and >18mm as very active (Junior and Zanil, 2000; Mariselvam[b]*et al*., 2012).

3. Results and Discussion:

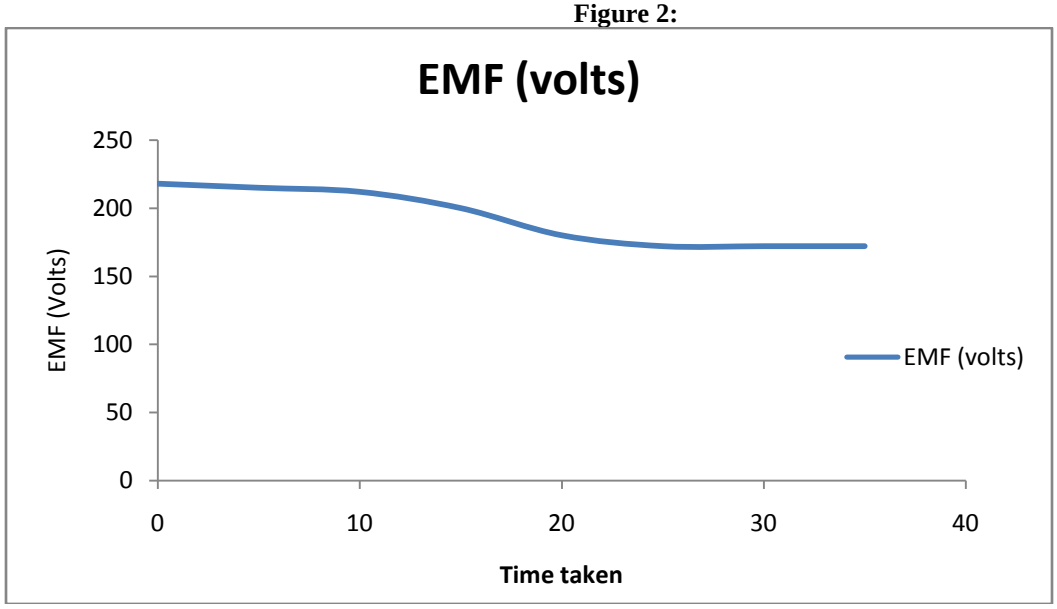
3.1. Potentiometer study:

The $AgNO_3$ solution is reduces to form the AgNPs using the plant extract. The plant extract act as a reducing agent and capping agent. The kinetics of $AgNO_3$ solution was measured by potentiometer. The time taken by Ag^+ ions to reduced from $AgNO_3$ solution was measured as the Emf (volts) rating (Table 1). The Ag^+ ions reduction time is 25 minutes because the EMF ranges were constant after 25 minutes.

Table 1: Kinetic of Ag NP's synthesized using *Rubia cordifolia* root extract:

S.No	Time taken (minutes)	EMF (volts)
1	0	218
2	5	215
3	10	212
4	15	200
5	20	180
6	25	172
7	30	172
8	35	172

Figure 2:



3.2. UV-Visible Spectrophotometer Study:

The colour of the silver nanoparticles prepared using plant root aqueous extract was dark brown. The absorption strongly depends on the particle size, dielectric medium and chemical surrounding the UV-Vis absorption spectra of the silver nanoparticles dispersed in H_2O. The absorption peak (Surface Plasmon Resonance (SPR)) is obtained in the UV range at 380 nm (Fig 3).

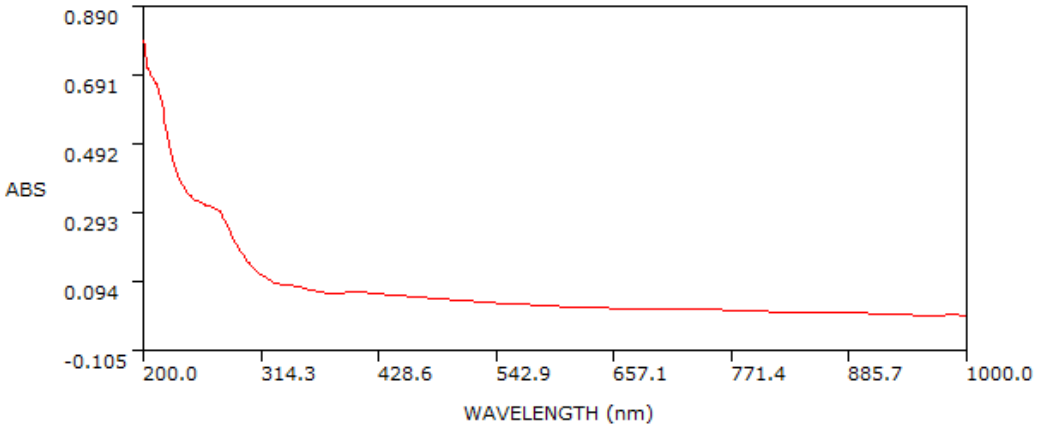
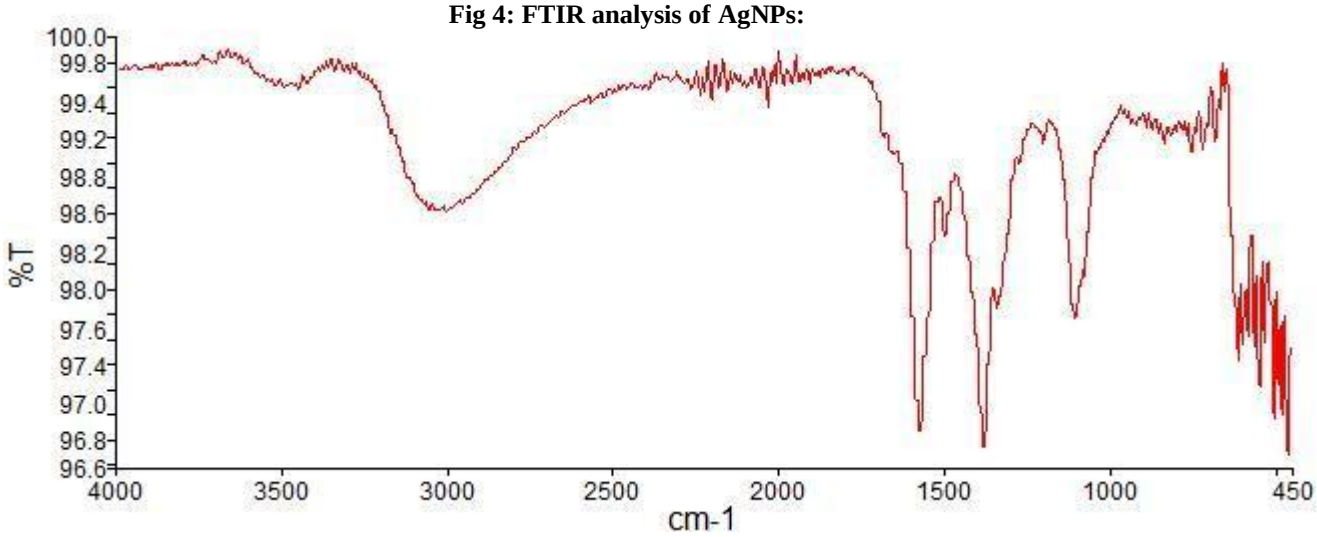


Fig 3: UV-Spec analysis of AgNPs:

3.3. FTIR:

The figure 4 shows FTIR analysis of the green synthesized AgNPs. The figure shows that the strong bands at 1125cm-1, 1345cm-1 and 1773cm-1.

Fig 4: FTIR analysis of AgNPs:



3.4. SEM:

Figure 5 shows SEM images of Ag nanoparticle's surface morphology. The obtained SEM images, confirms that the formed silver nanoparticles were about the 1µm and 0.5µm and these silver nanoparticles are comparitively smaller in size.

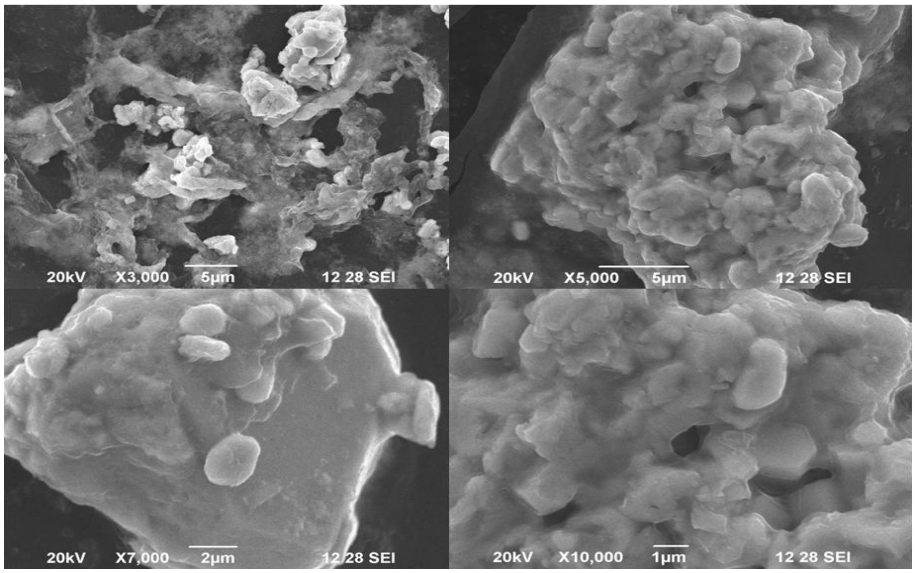


Fig 5: SEM analysis of Silver NP's:

3.5. Antimicrobial activity:

The green synthesized Silver nanoparticles using *Rubia cordifolia* plant root extract was highly inhibiting the bacterial pathogens like *Vibrio alginolyticus,Pseudomonas aeroginosa, Shigella spp, Plesiomonas shigelloides* and *Vibrio parahaemolyticus.* They had highest antimicrobial effect against *Pseudomonas aeroginosa* and *Plesiomonas shigelloides* (Table 2).

Table 2: Antimicrobial activity of green synthesized Silver NPs using *Rubia cordifolia*:

S.No	Microorganisms	Zone of Inhibition (mm)				
		25µl	50µl	75µl	100µl	S
1	*Klebsiella pneumoniae*	0	0	0	0	0
2	*Vibrio parahaemolyticus*	0	0	13	16	11
3	*Plesiomonas shigelloides*	11	13	14	16	16
4	*Shigella spp*	12	12	13	14	12
5	*E.coli*	0	0	0	0	9
6	*Streptococcus aureus*	0	0	0	0	0
7	*Pseudomonas aeruginosa*	8	9	10	10	9
8	*Vibrio alginolyticus*	9	10	11	12	11

Figure 6:

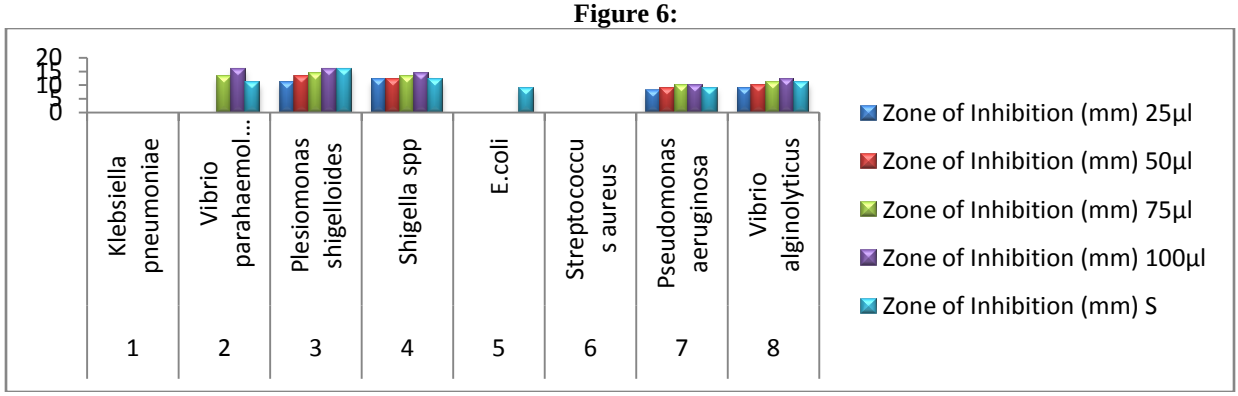


Fig 7: Antimicrobial activity of Silver NP's:

The activity of AgNPs against selected Gram negative and Gram positive microorganisms may be due to the diffusion of nanoparticles from the media to the bacterial cell wall of those microorganisms.

4. Acknowledgment

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