



Green Synthesized Cobalt Nanoparticles using *Asparagus racemosus* root Extract & Evaluation of Antibacterial activity

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Abstract : In the present study, cobalt nanoparticles were synthesized by an ecofriendly and cost effective method using *Asparagus racemosus* root extract and characterized using various techniques such as UV-visible spectrophotometry, Fourier transform infrared spectrometry and Scanning electron microscopy coupled with Energy dispersive micro analysis. The spectroscopic methods confirmed the formation of cobalt nanoparticles and the microscopic technique confirmed the shape and size of the cobalt nanoparticles as spherical with an average particle size of 48nm. Antibacterial activity of the synthesized nanoparticles was measured by disc diffusion method. The cobalt nanoparticles showed effective antibacterial activity against human pathogenic bacteria *S. Dysenteriae* & *E. faecalis* when compared with antibiotic Ciprofloxacin.

Keywords : Cobalt nanoparticles, antibacterial activity, *S. dysenteriae* & *E. faecalis*, antibiotic Ciprofloxacin.

Introduction:

Nanoparticles are the nano-sized particles [1,2] which have found various applications in the fields of medicine [3,4,5,6], biology [7,8,9,10], catalysis [11,12,13] etc. The nanoparticles can be synthesized by physical, chemical or biological methods. Cobalt nanoparticles (Co-NPs) can be synthesized by various approaches like ultrasonic spray pyrolysis, DC magnetron sputtering [14], thermal decomposition [15], electrochemical [16] and Liquid-Phase Reduction [17] process and also by biological methods such as microbial synthesis [18] of nanoparticles.

Recently, many studies have proven that the plant extracts act as a potential precursor for the synthesis of the nanomaterials in non-hazardous ways. The plants are used successfully in the synthesis of several greener nanoparticles such as cobalt, copper, silver, gold, palladium, platinum, zinc oxide and magnetite.

Plant mediated biological synthesis of nanoparticles is gaining importance due to its simplicity, cost effective and eco-friendliness [19,20]. Co-NPs could be efficient nanoparticles as they possess good catalytic [21,22] and high performance permanent magnetic properties [23,24] and also possess biomedical [25] and cytotoxic [26] activity.

Asparagus racemosus is a plant used in traditional Indian medicine (Ayurveda). The root is used to make medicine. Don't confuse *asparagus racemosus* with *Asparagus officinalis*, which is the type of asparagus that is commonly eaten as a vegetable.

People use *Asparagus Racemosus* for upset stomach (dyspepsia), constipation, stomach spasms, and stomach ulcers. It is also used for fluid retention, pain, anxiety, cancer, diarrhea, bronchitis, tuberculosis, dementia, and diabetes. Some people use it to ease alcohol withdrawal. Women use *asparagus racemosus* for premenstrual syndrome (PMS) and uterine bleeding; and to start breast milk production. *Asparagus racemosus* is also used to increase sexual desire (as an aphrodisiac). Hence, in view of its medicinal importance we have selected *Asparagus Racemosus* extract for the biosynthesis of Cobalt nanoparticles in the present study.

2. Material and Methods:

2.1 Plant Collection and preparation of root extract and 10mM Cobalt acetate solution:

Asparagus racemosus roots were collected from the local market, Andhra Pradesh, India (Fig 1A). The roots were rinsed profusely with distilled water followed by organic free water to remove the dust and other contaminants, then dried at room temperature (35°C) in shade to remove the moisture. To prepare a root extract, 25gms of roots were added to 150ml of organic free water and incubated on hot plate at 80°C for 30min. After cooling, the extract was filtered using whatman No.1 filter paper and the filtrate was stored at 4°C for further use. To prepare 10mM cobalt solution, 0.3gms of cobalt acetate (AR grade) was mixed in 100ml of organic free water and stored in a bottle.

2.2 Synthesis of Cobalt Nanoparticles using the *Asparagus racemosus* roots Extract:

The aqueous root extract of *Asparagus racemosus* and 10 mM Cobalt acetate solution were mixed in the ratio of 1:5 and incubated on hot plate at 60°C for 90 min until change in colour was observed.

2.3 Characterization of Cobalt Nanoparticles:

2.3.1 UV-vis spectrophotometry:

UV-visible spectrophotometer was employed for the spectrometric analysis of bio-synthesized cobalt nanoparticles. The reduction of cobalt was measured periodically at 200–700 nm. A spectrum of nanoparticles was plotted with wave length on x-axis and absorbance on y-axis.

2.3.2 Fourier transform infrared (FTIR) Spectroscopy:

For removing the biochemical compounds or uncapping ligands of the nanoparticles, the 500 mL residual solution of reaction mixture was centrifuged at 10,000 rpm for 30 min and the precipitate was re-suspended in 10 mL organic free water. The centrifugation and re-suspension processes were repeated for 5 times. The purified suspension was dried in an oven at 60°C to obtain the stable powder and analyzed by Fourier transform infrared spectrum (FTIR), Perkin Elmer-RX1 spectrophotometer.

2.3.3 Scanning electron microscopy–energy dispersive X-ray (SEM–EDX) microanalysis:

Scanning electron microscope (SEM) analysis was carried out using scanning electron microscope machine compatible with EDX machine. The reaction mixture was centrifuged at 10,000 rpm for 30 min and the pellet was re-dispersed in 10 mL ethanol and washed 3 times with Millipore water to obtain the pellet. The pellet was re-suspended in Millipore water, ultrasonicated and thin films of sample were prepared on carbon coated copper grid and analysed for size and shape determination. The particle size and shape of nanoparticles can be analysed by using image magnification software compatible with SEM and helps in determining the presence and formation of Cobalt nanoparticles. The Electron Dispersive X-ray Microanalysis which confirm the presence of elemental cobalt signal.

2.3.4 Transmission electron microscopic examination (TEM)

Transmission electron microscopic examination was done to know the morphology of cobalt nanoparticles, using high-resolution analytical transmission electron microscope (Phillips, Netherland Model: Technai20). In this examination, we used centrifuged powder of the solution of cobalt nanoparticles. For TEM analysis, the specimen was suspended in distilled water, dispersed ultrasonically to separate individual particles, and one or two drop of the suspension deposited onto holey-carbon coated copper grids and dried under Infrared lamp.

2.3.5 Antibacterial activity

The antibacterial assays were done on human pathogenic strains like *S. dysenteriae* and *E. faecalis* by disc diffusion method. Luria Bertani (LB) broth/agar medium was used to cultivate bacterial strains. Fresh overnight inoculum (100 μ L) of each culture was spread on to Luria Bertani agar plates. Sterile Whatman No.1 paper discs of 5mm diameter containing 10 μ L of *Asparagus racemosus* root extract (5 μ g), 10 μ L of Co-NPs (1mg/mL), 10 μ L of Cobalt solution (10mM) and 10 μ L of Ciprofloxacin(1mg/mL) were placed in each plate in serial order. After overnight incubation at 37 $^{\circ}$ C, zone of inhibition was measured (diameter in mm). The bactericidal activity is evaluated by the size of clear zone and greater the zone of inhibition greater the bactericidal activity.

3. Results and Discussion:

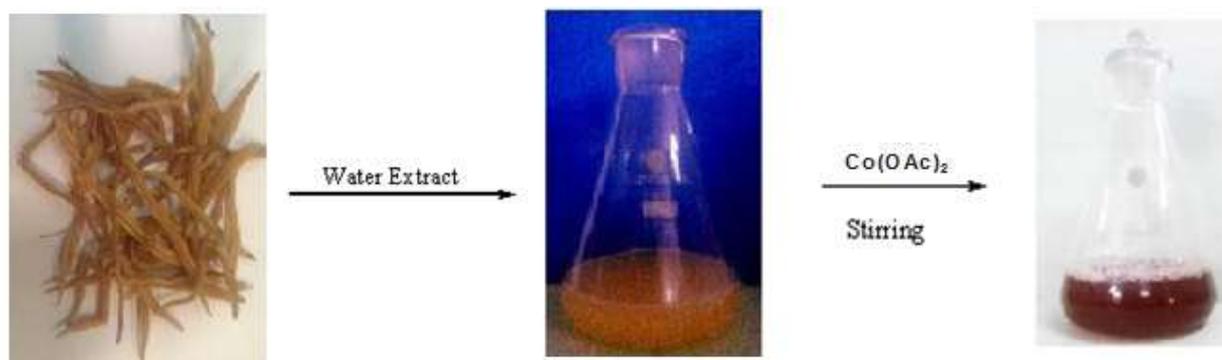
3.1 Synthesis of Cobalt Nanoparticles using *Asparagus racemosus* root extract:

This study deals with the synthesis, characterization and exploring biomedical applications of cobalt nanoparticles, synthesized by using root extract of *Asparagus racemosus*. The synthesized Co-NPs were reddish brown in color. The color of the extract changed from light yellow to reddish brown after addition of Cobalt acetate and on incubation for 90min at 60 $^{\circ}$ C. The colouration was due to the excitation of the surface Plasmon vibration of the Co-NPs. Change in colour after the reduction of cobalt ions to cobalt nanoparticles is shown in (Fig.1).

3.2 Characterization of Cobalt Nanoparticles:

3.1.1 By color change

The sequential color change indicates the formation of Co-NPs by our plant materials. This is the primary test for the checking of formation of Co-NPs.



Color was changed from yellow to light brown and After 18-24hrs color was changed into dark brown (Fig. 1)

The color reduction of $\text{Co}(\text{OAc})_2$ into Nanoparticles was visibly evident from the color change. Stem powder was added into a cobalt acetate solution. Within few minutes the appearance of brown color was observed and it indicates the formation of Co-NPs. The color was changed from yellow to light brown (Fig. 1). After 18-24hrs color was changed into dark brown (Fig. 1). This color change indicates the formation of Co-NPs.

UV-visible and TEM studies:

Figure 2 shows the UV-vis spectra of Cobalt colloid obtained. The surface Plasmon resonance (SPR) band is broad indicating poly-dispersed nanoparticles. A smooth and narrow absorption band at 438 nm is observed which is characteristic of mono-dispersed spherical nanoparticles. UV-visible spectroscopy is one of the most widely used techniques for structural characterization of Cobalt nanoparticles. The surface plasmon resonance (SPR) band (λ max) around 438 nm broadened and slightly moved to the long wavelength region, indicating the presence and formation of Cobalt nanoparticles. The optical absorption spectra of metal nanoparticles are dominated by surface Plasmon resonances (SPR), which shift to longer wavelengths with increasing particle size. The position and shape of plasmon absorption of cobalt nanoclusters are strongly dependent on the particle size, dielectric medium, and surface-adsorbed species. The surface plasmon absorption of cobalt nanoparticles have the short wavelength band in the visible region around 438 nm is due to the transverse electronic oscillation.

The TEM images obtained for colloid is shown in figure-3. It is clear from the TEM images in figure 3 that the particle size, nearly spherical particles of average size 48 nm is obtained. The TEM image confirms the particles are spherical in shape.

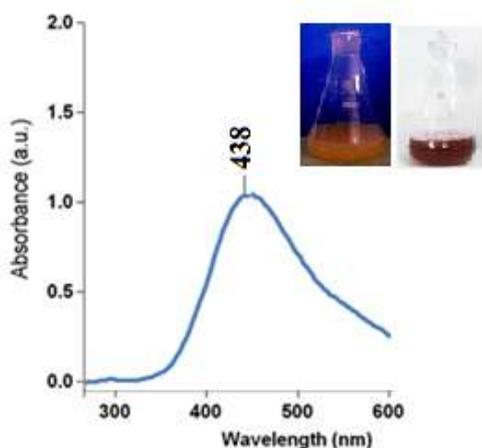


Figure 2: (a) UV-visual absorption spectra of cobalt nanoparticles after 24 h of reaction. (b) Picture of flask containing the solution of aqueous *Asparagus racemosus* root extract filtrate with of cobaltacetate in Erlenmeyer flask, before reaction (flask 1) and after 24 h of reaction (flask 2).

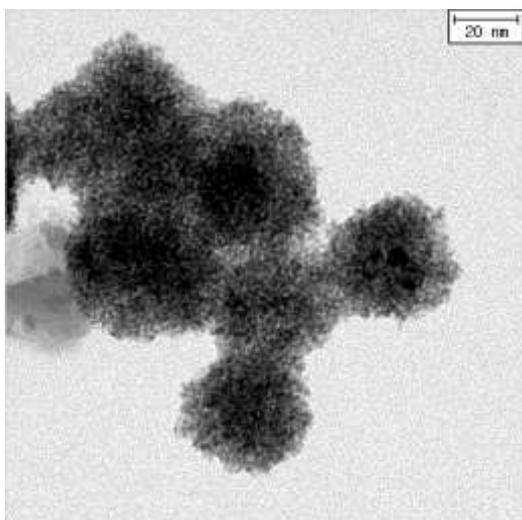


Figure 3: Transmission electron micrographs of the cobalt nanoparticles used in this work. (a) The bar marker represents 20 nm.

FTIR and XRD studies:

Figure-4 shows the XRD pattern of Co nanoparticles obtained using *Asparagus racemosus*. The diffraction peaks appeared at 27.81, 32.11, 39.10, 46.11, 64.4 and 77.20. The average crystallite size according to Scherrer equation calculated using the highest peak of the 46.11 is found to be 48.36 nm, nearly in agreement with the particle size obtained from TEM image.

FTIR measurement was carried out to identify the possible biomolecules responsible for capping and efficient stabilization of Co nanoparticles synthesized using *Asparagus racemosus*. Figure-5 shows the FTIR spectrum of Co nanoparticles obtained in this study. In the IR spectrum of *Asparagus racemosus* capped Co nanoparticles, the spectrum showed absorptions at 3442.90 (OH), the band observed at 1597 arise C=O of -COOH, respectively. The band observed at 1357 cm^{-1} is due to C-O stretching mode. The very strong band at 1072 cm^{-1} arises from C-O-C symmetric stretching and C-O-H bending vibrations of protein in the *Asparagus racemosus*.

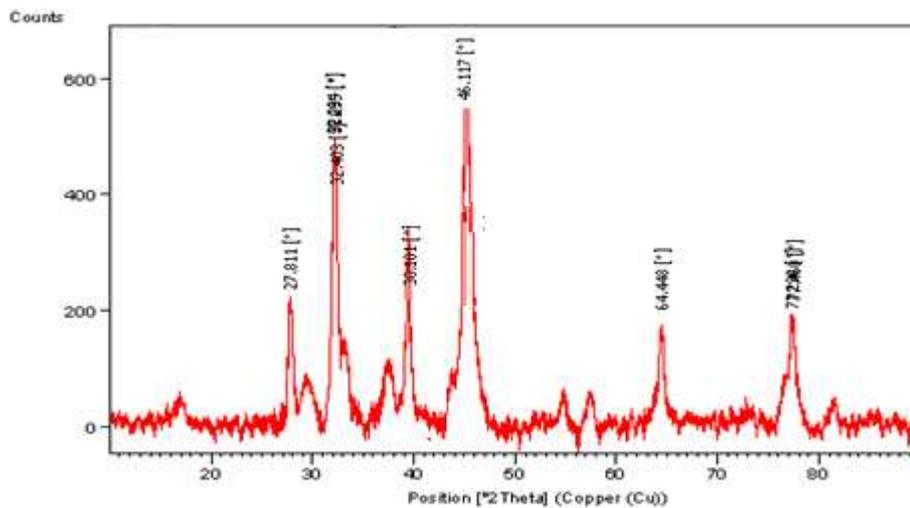


Figure 4: X-ray diffraction pattern of Co-NPs at room temperature synthesized by *Asparagus racemosus* root extract with $\text{Co}(\text{OAc})_2$ solution.

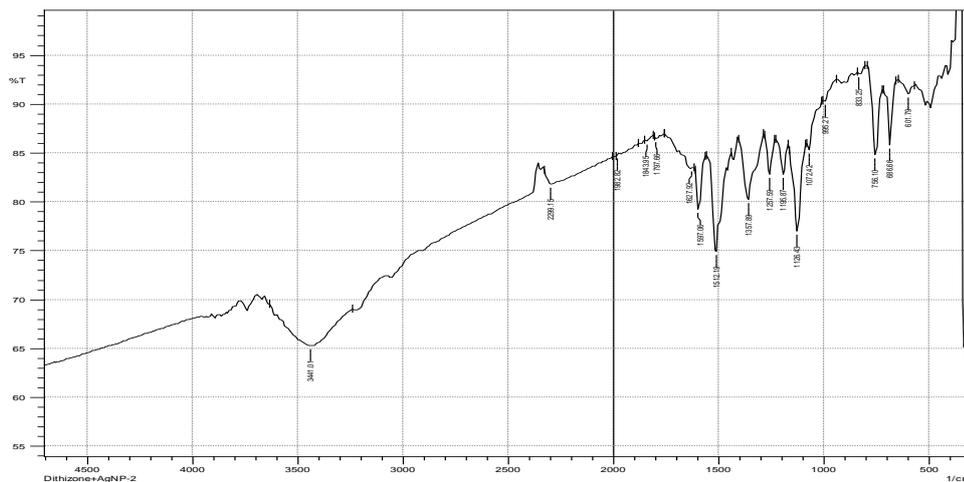


Figure 5: FT-IR spectra of *Asparagus racemosus* mediated cobalt nanoparticles.

SEM & EDX Studies:

The SEM images obtained for colloid is shown in figure 6. It is clear from the SEM images that the particles are nearly crystalline.

The Scherrer rings, characteristic of fccCobalt is clearly observed, showing that the structure seen in the SEM image are nano crystalline in nature. It is observed that the cobalt nanoparticles are scattered over the surface and no aggregates are noticed under SEM. The difference in size is possibly due to the fact that the nanoparticles are being formed at different times.

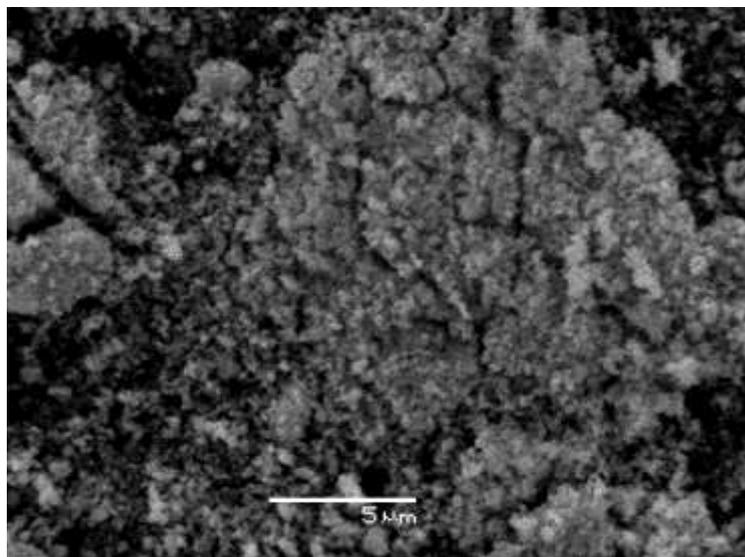


Figure: SEM images of Cobalt nanoparticles using this Method.

3.3 Antibacterial activity of Cobalt nanoparticles by Disc diffusion method:

Antibacterial activity of synthesized cobalt nanoparticles against Gram negative organisms (*S. dysenteriae* and *E. faecalis*) was observed and zone of inhibition was measured. The results indicated that cobalt nanoparticles synthesized from *E. faecalis* root extract showed effective antibacterial activity on Pathogenic bacteria.

Table: 1 showing the antibacterial activity by zone of inhibition against human pathogens.

Description	Zone of inhibition(mm)*	
	<i>S. dysenteriae</i>	<i>E. faecalis</i>
Co Solution	07	06
Co-Np's	14	13
Ciprofloxacin	15	15

[†]50μg of compound (1μg/μl concentrated), * 6mm is the well size

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