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Green Synthesis and Antibacterial Activity of Cobalt Nanoparticle from *Calotropis gigantae*

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Abstract. Cobalt nanoparticles (CoNPs) were successfully synthesized by taking a simple green synthetic route using metal salts and flower extracts of a wild plant *Calotropis gigantea* which act as reducing as well as the stabilizing agent. The synthesized Cobalt nanoparticles (CoNPs) were characterized using various techniques such as UV-visible spectrophotometry and Fourier transform infrared spectrometry. Size characterization of the samples was made by Dynamic light-scattering (DLS) and Transmission electron microscope (TEM). The micrographs of the synthesized CoNPs showed the formation of spherical nanoparticles with an average size of 13 ± 5 nm. The CoNPs were stable at room temperature (25 °C) for six months. Zeta potential values provided an indirect measurement of the net charge on the Cobalt nanoparticles (CoNPs) surface. The antibacterial activities of cobalt nanoparticles (CoNPs) were studied under varying concentrations of CoNPs with respect to *Staphylococcus aureus* and *Escherichia coli*.
Keywords: Nanoparticles; synthesis; characterization; hydrodynamic; zeta potential; zone of inhibition.

Resumen. Las nanopartículas de cobalto (CoNPs) se sintetizaron con éxito al tomar una ruta sintética verde simple usando sales metálicas y extractos de flores de una planta silvestre *Calotropis gigantea* que actúa como agente reductor y estabilizador. Las nanopartículas de cobalto sintetizadas (CoNPs) se caracterizaron utilizando diversas técnicas, como la espectrofotometría UV-visible y la espectrometría infrarroja por transformada de Fourier. La caracterización del tamaño de las muestras se realizó mediante dispersión dinámica de luz (DLS) y microscopio electrónico de transmisión (TEM). Las micrografías de los CoNPs sintetizados mostraron la formación de nanopartículas esféricas con un tamaño promedio de 13 ± 5 nm. Los CoNPs fueron estables a temperatura ambiente (25 °C) durante seis meses. Los valores potenciales de Zeta proporcionaron una medición indirecta de la carga neta en la superficie de nanopartículas de cobalto (CoNPs). Las actividades antibacterianas de las nanopartículas de cobalto (CoNPs) se estudiaron bajo concentraciones variables de CoNPs con respecto a *Staphylococcus aureus* y *Escherichia coli*.
Palabras clave: Nanopartículas; síntesis; caracterización; hidrodinámico; potencial zeta; zona de inhibición.

Introduction

Nanotechnology is regarded as a distinct field of research in modern science and technology with multidirectional applications. Research and development in this field are growing rapidly throughout the world.

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A major output of this activity is the development of new materials on the nanometer scale, including nanoparticles. Nanoparticles exhibit completely new or improved properties compared with larger particles of the bulk materials and these novel properties are derived due to the variation in specific characteristics such as size, distribution, and morphology of the particles [1-2].

Due to the growing demand for various nanoparticles, it is necessary to develop synthesis methods that are cost-effective and environment-friendly. Green synthesized nanoparticles have been of immense interest for their unique chemical and physical properties and potential technological applications in various fields ranging from catalysis to disease diagnosis [3]. Conventional methods of NPs synthesis mostly rely on the use of synthetic chemicals and prolonged heating [4]. Replacement of toxic chemicals as a reducing and stabilizing agent is the major concern of this new approach. Reports about the successful synthesis and subsequent stability of NPs by using different biomaterials sourced from the plant are now increasing day by day [5-6]. In this respect, plant mediated green synthesis of nanoparticles is gaining importance and there is an increasing demand for "green nanotechnology" [7]. Cobalt nanoparticles (CoNPs) could be efficient nanoparticles as they possess good catalytic [8-9] and high performance permanent magnetic properties [10-11] and also possess biomedical [12] and cytotoxic activity [13]. Cobalt-based nanoparticles may be produced as cobalt oxide, organic metal compounds or biopolymers [14]. Plant mediated synthesis of CoNPs was carried out using aqueous flower extract of *Calotropis gigantea*. The *Calotropis* has several uses from the ancient time. The different parts of the plant are used in Indian traditional medicine for the treatment of painful muscular spasm, dysentery, fever, rheumatism, and asthma. The development of nanoscience and nanotechnology presents opportunities for exploring the bactericidal effect of metal nanoparticles. The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes, and is not merely due to the release of metal ions in solution [15-16]. Recent studies using metallic nanoparticles have demonstrated a broad range of antimicrobial activity against numerous pathogenic bacteria [17-19] viruses [20], pathogenic fungi [21], and eukaryotic microorganisms [22]. However, there are still no available reports in the scientific literature on antibacterial activity of CoNPs against pathogenic bacteria causing diseases. *Staphylococcus aureus* and *Escherichia coli* were widely used in bacterial experiment. *S. aureus* and *E. coli* live on the body surface of mammals and sometimes occur infection to them. Therefore, S. aureus and E. coli strains were selected for this antibacterial investigation. The current study assessed the applicability of cobalt nanoparticles (CoNPs) for their promising antibacterial activities against *S. aureus* and *E. coli*.

Experimental

Preparation of flower extract and synthesis of CoNPs

Fresh flowers of *Calotropis gigantea* was collected during the summer month of May from Patna university campus of Patna district region, Bihar, India. These flowers were then washed several times with water to remove the dust particles. The flowers were washed with double distilled water and soaked in ethanol for 30-40 sec. It was then dried at room temperature (37 °C) in shade to remove the moisture. To prepare a flower extract, 10g of finely chopped flowers was added to 200 mL of deionized water and incubated on a hot plate at 200°C for 15 min. After cooling the extract was filtered using Whatman No.1 filter paper and the filtrate was stored at 4°C for further use. Cobaltous chloride ($CoCl_2$) was purchased from Sigma Aldrich Chemicals, St Louis, MO, USA was used for the synthesis of CoNPs.

Characterization of Cobalt Nanoparticles

The absorption spectrum of the sample was measured on a Shimadzu UV-1800 UV–vis spectrophotometer operating at the wavelength of 280-700 nm. IR spectra were recorded on Perkin–Elmer FTIR- 1710 spectrophotometer within the region of 4000–400 cm^{-1} using KBr. The size, size distribution of particles and zeta potential were measured using a Nano ZS zeta sizer system (Malvern Instruments) with a laser wavelength of 633 nm (He–Ne), a scattering angle of 173°, and a measurement temperature of 25 °C. The size of nanoparticles was confirmed through transmission electron microscopy (TEM). The TEM images of nanoparticles were obtained with a JEOL JEM-1200EX transmission electron microscope operating at 120 kV.

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Antibacterial activity

The *S. aureus* and *E. coli* cells used in the present study were obtained from the Institute of Microbial Technology, Chandigarh. The media used for the antibacterial test were Muller Hinton Agar. The bacterial cells were maintained on nutrient agar (Hi Media, India) slopes at 4 °C and sub cultured before use. The antibacterial assays were done by disc diffusion method [23] to establish the antibacterial activity of the cobalt nanoparticles (CoNPs). Agar plates were inoculated with bacterial strain under aseptic conditions and wells (diameter = 6 mm) were filled with 50 µl of the test samples and incubated at 37 °C for 24 hours. The final concentrations of CoNPs were 50, 100, and 150 µg/mL. After the incubation period, the diameter of the growth inhibition zones was measured. Inhibition of the bacterial growth was measured in mm. Tests were performed in duplicate.

Results and discussion

Green synthesis of CoNPs

Green synthesis of CoNPs was carried out with the help of floral extract of medicinal plant *Calotropis gigantea* as shown in Fig. 1. The presence of compounds like Folic acid, 5-hydroxymethyl furfural bearing –OH group and saccharides like 6-acetyl b-D-mannose provides the strong antioxidant potential to this plant that make it a potential reducing as well as stabilizing agent for the synthesis of metal NPs.

Fig. 1. Flowers of *Calotropis gigantea.*

The aqueous flower extract of *Calotropis gigantea* and different concentrations (1 mM, 2 mM, 5 mM, 10 mM, 15 mM, and 20 mM) of cobaltous chloride solution was mixed thoroughly in the ratio of 1:3. The content was left at room temperature for 24 h. After 24 hours, a distinct change in the color of the experimental samples was observed. The color of experimental samples turned light brown. Change in color in the experimental samples clearly indicated the formation of cobalt nanoparticles. All the six experimental samples were centrifuged at 5000 rpm for 45 minutes at 4˚C, following which the pellet was re-dispersed in sterile distilled water (5 mL) to get rid of any uncoordinated biological molecules. The process of centrifugation and re-dispersion in sterile distilled water was repeated to ensure purification of nanoparticles. At the bottom of the centrifuge tube very small amount of green precipitate with pH 10 was observed. The precipitate was then resuspended using the small amount of distilled water and the particles remained in the suspension with a very high concentration.

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UV/VIS analysis

The formation of metal nanoparticles by reduction of the aqueous metal ions during exposure of *Calotropis gigantea* extract was easily followed by UV/Vis spectroscopy. The sharp exciton absorption is positioned at 319 nm for all six samples are shown in Fig. 2, the sharpness of peak suggested that formed CoNPs were stable while the higher intensity of peak suggests that the yield of CoNPs is high.

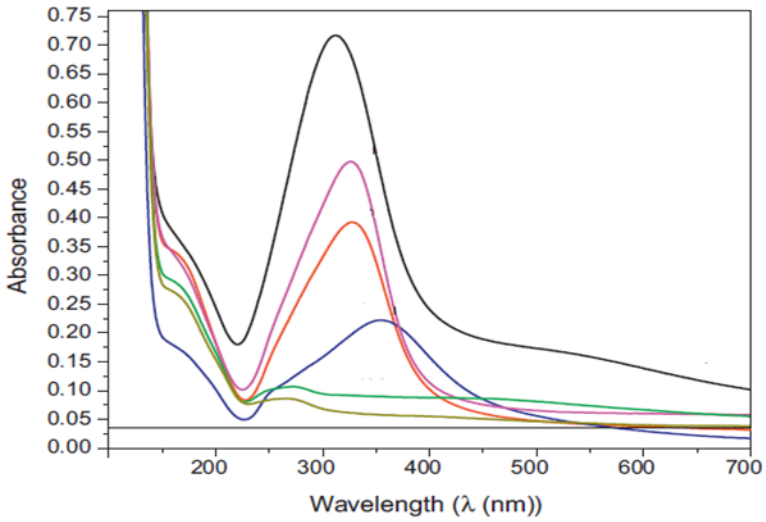

Fig. 2. UV visible spectra for synthesized cobalt nanoparticles.

There are no additional peaks in this region that indicated that pure CoNPs were formed [24]. The position of absorption bands for CoNPs depends on several factors such as temperature, synthetic method, size and shape of NPs. Intensity and sharpness of the peak suggest that formed CoNPs were well dispersed and stable with no aggregation and high yield. The light brown color could be due to the excitation of surface plasmon vibrations, typical of the cobalt nanoparticles [25].

FTIR analysis

FTIR analyses were carried out to identify the functional groups responsible for capping and stabilizing of the bioreduced CoNPs synthesized using plant extract. The indication of the synthesis of CoNPs was corroborated with the appearance of new groups in synthesized CoNPs. It is evident from the spectra of flower extract has a variety of functional groups (Fig. 3). Broad band at 3350 cm^{-1} was due to the hydroxyl functional group. The bands observed at around 2950 cm^{-1} and 2825 cm^{-1} are for CH_3 and CH_2 groups respectively of the hydrocarbons. Strong absorption bands at around 1120 cm^{-1} due to C-O stretching of the ethers.

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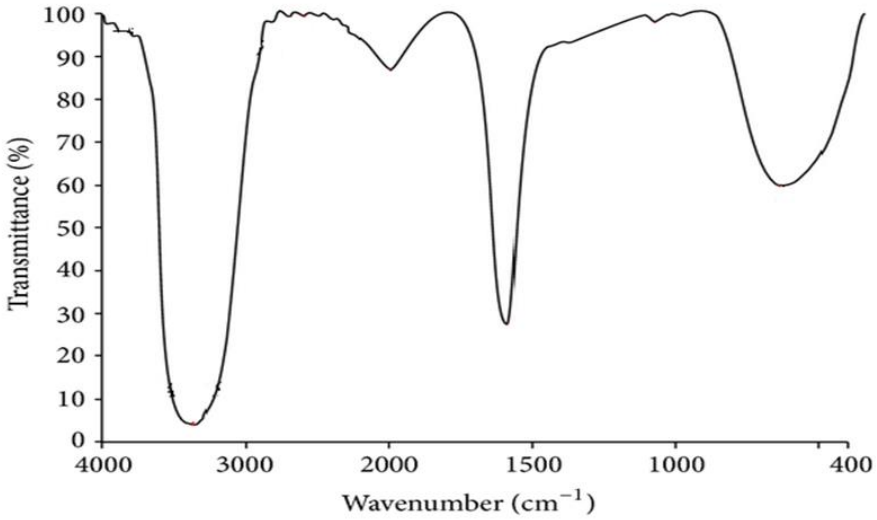

Fig. 3. FTIR spectra of cobalt nanoparticles.

FTIR spectrum of CoNPs also showed strong bands for halogens at 1020 cm^{-1}, due to C-F stretching and there was also some extent of carbonyls -C=O peak that was observed at 1640 cm^{-1}. FTIR peaks that were corresponding to alcohols, geminal methyls, and ether linkages indicate the presence of flavones and terpenoids [26] responsible for the stabilization of the CoNPs synthesized by the *Calotropis gigantea* flower extract.

TEM analysis

The particle size, shape, and distribution were characterized using the TEM. The TEM image and size distribution of CoNPs are shown in Fig. 4 and Fig. 5. The morphology of cobalt nanoparticles is spherical with a uniform size and is almost homogeneous as is evident from Fig. 4. It is easy for the CoNPs to aggregate due to the small dimensions and high surface energy of the particles. From the TEM image the particle size histogram (Fig. 5) was determined which further probed the size distribution of the CoNPs. The diameter sizes of the CoNPs were found to be in the range of 8 to 30 nm approximately with a narrow size distribution. The average particle size is found to be 13 ± 5 nm. The results are in agreement with those previously reported for the green synthesis of CoNPs [27].

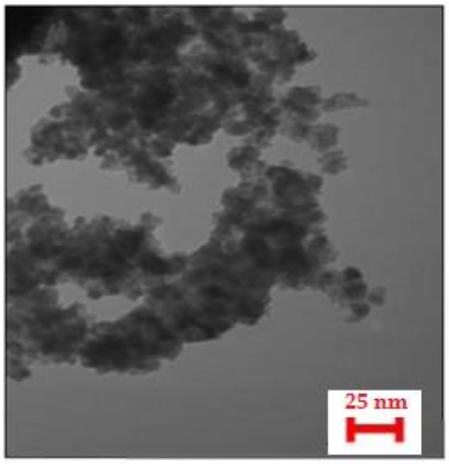

Fig. 4. TEM image of the CoNPs.

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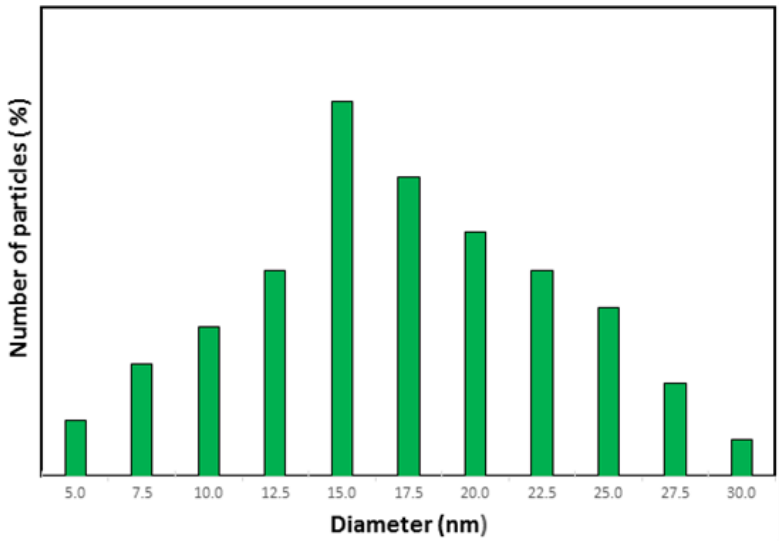

Fig. 5. Particle size distribution of CoNps.

DLS analysis

Dynamic light scattering (DLS) that utilizes time variation of scattered light from suspended particles under Brownian motion to obtain their hydrodynamic size distribution is the most popular technology in sizing nanoparticles [28]. All measurements reported in this paper were made at the temperature of 25°C. Three repeat measurements on each sample were made to check the repeatability of the results obtained. The particle size distribution (PSD) of synthesized cobalt nanoparticles of different concentrations i.e. 1 mM, 2 mM, 5 mM, 10 mM, 15 mM, and 20 mM have the average size of 608.7 d.nm, 669.0 d.nm, 403.5 d,nm, 670.0 d.nm, 385.0 d.nm and 595.5 d.nm respectively. After comparing the above six results it can be concluded that the concentrations with 2 mM, 5 mM, 10 mM, 15 mM and 20 mM gave uniform distribution of particles but the concentration with 1mM (Fig. 6) does not obey this principle. Among them, the sample with a concentration of 15 mM (Fig. 7) is very appropriate since it gives the lowest average size of nanoparticles.

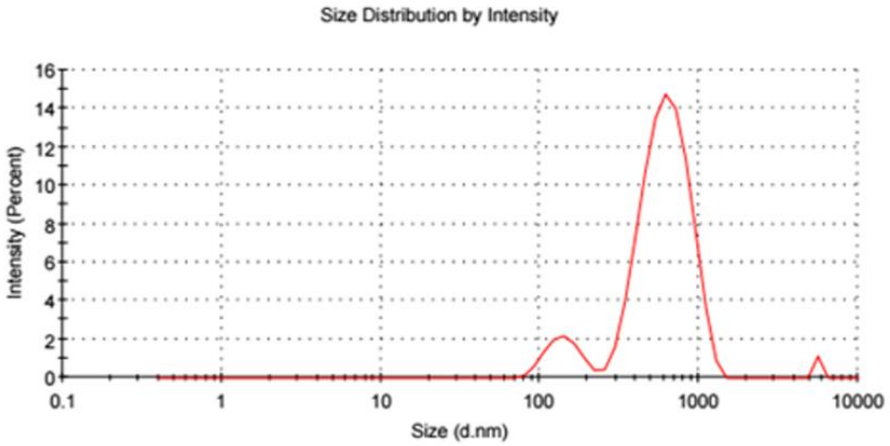

Fig. 6. DLS for 1 mM cobalt nanoparticles.

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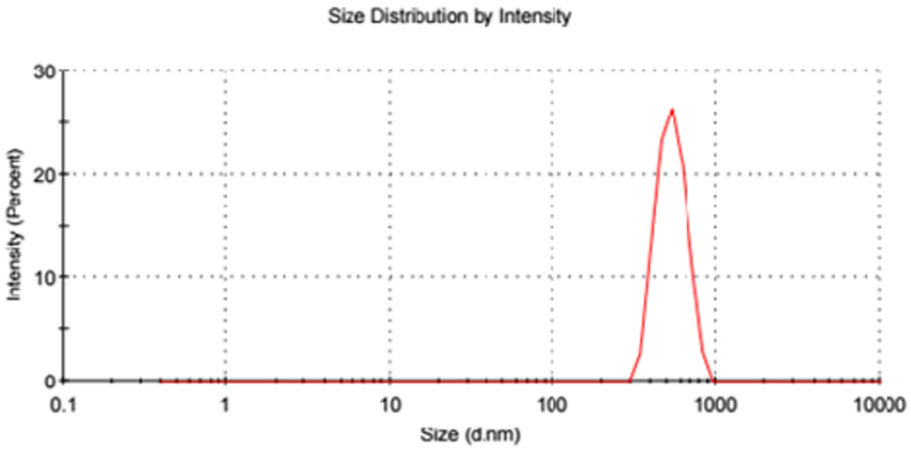

Fig. 7. DLS for 20mM cobalt nanoparticles.

Zeta potential measurement

Zeta potential is a very important parameter to determine the surface charge of the nanoparticles. Its value is closely related to nanoparticles stability and particle surface morphology. Zeta potential values provide an indirect measurement of the net charge on the nanoparticle (NP) surface. The Zeta potential measurements of cobalt nanoparticles synthesized with different concentrations of 1 mM, 2 mM, 5 mM, 10 mM, 15 mM, and 20 mM are -17.9 mV, -19.2 mV, -13.8 mV, -24.1 mV, -27.0 mV, and -25.8 mV respectively. The general guidelines available through literature classifying nanoparticles with zeta potential values of ±0-10 mV, ±10-20 mV, ±20-30 mV, and > ± 30 mV as highly unstable, relatively stable, moderately stable and highly stable, respectively are common [29]. From the analysis, it can be concluded that concentration 10 mM, 15 mM, and 20 mM are moderately stable Fig. 8 and Fig. 9.

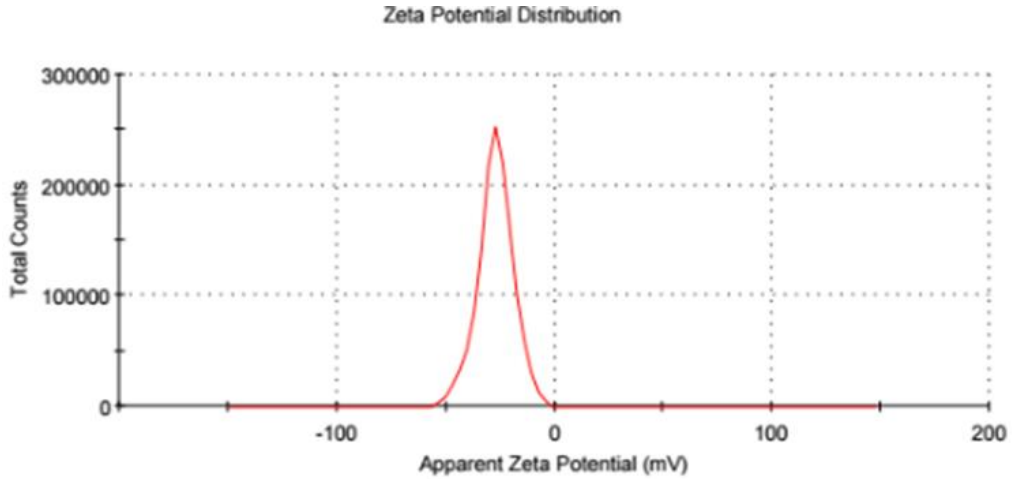

Fig. 8. Zeta potential measurements for CoNPs synthesized witn 15mM $CoCl_2$.

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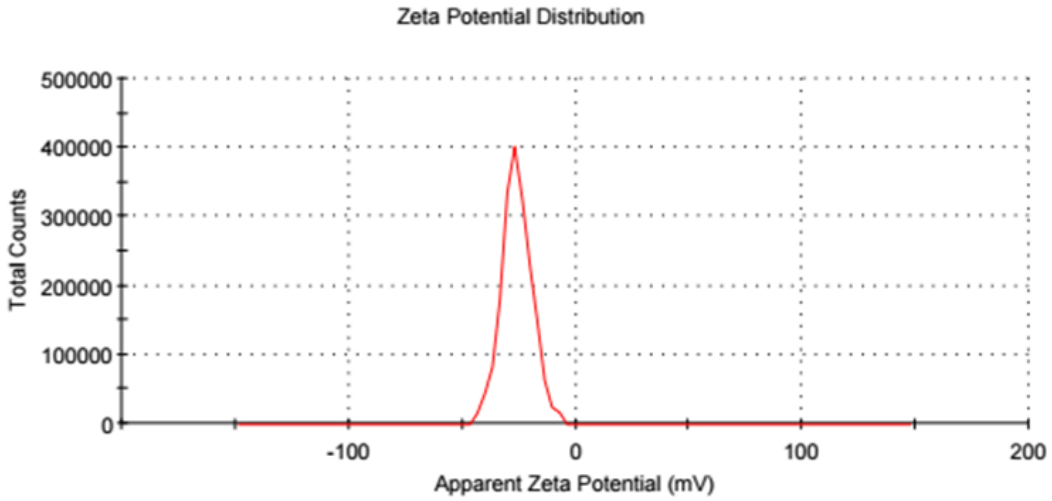

Fig. 9. Zeta potential measurements for CoNPs synthesized with 20mM $CoCl_2$.

The obtained negative zeta potential for the present nanoparticles indicates that negative charge was developed over the surface of CoNPs.

Antibacterial activity

The minimum inhibitory concentration (MIC) was applied to determine the lowest concentration that inhibits the growth of the microorganism completely. CoNPs synthesized with *Calotropis gigantea* leaf extract showed a strong inhibitory action against *Staphylococcus aureus* and *Escherichia coli*. The antibacterial activity of cobalt nanoparticles (CoNPs) at three different concentrations viz: 50 µg/mL (C1), 100 µg/mL (C2) and 150 µg/mL (C3) along with positive (Ab) and negative control (con) against *S. aureus* is shown in Fig. 10.

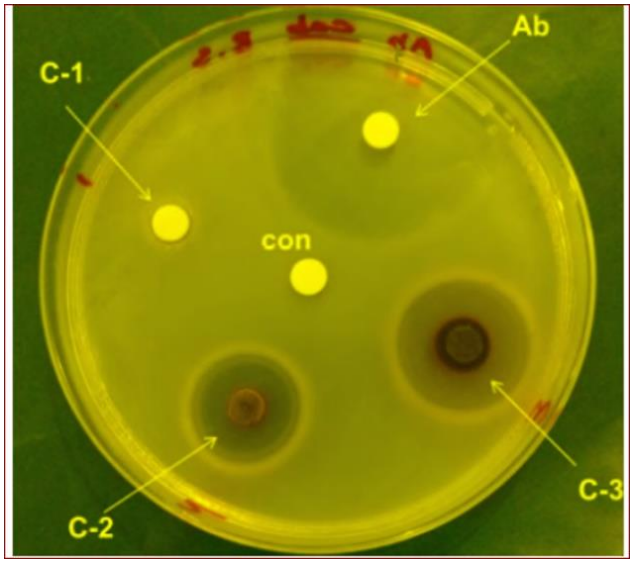

Fig. 10. Antibacterial activity of CoNPs against *S. aureus*.

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The MIC of CoNPs against *S. aureus* and *E. coli* was 100 μg/mL. When 100 and 150 μg/mL was used, inhibition in growth was observed; however, when 50 μg/mL CoNPs was applied, growth was only marginally inhibited. Interestingly, CoNPs showed a significant growth inhibition compared with the control and have shown better antibacterial effectiveness at higher concentration. The identical antibacterial activity was also shown by the other bacterial strains *E. coli*. Agar diffusion test was performed for qualitative measurement of the bactericidal effect of the cobalt nanoparticles. The diameters of the zone of inhibition (ZOI) were determined and these are presented in Table 1. The absence of growth around the CoNPs is an indirect measure of the ability of the nanoparticles to inhibit the growth.

Table 1. Zone of inhibition against bacteria strains by different concentrations of CoNPs.

Treatment	**Zone of inhibition (mm)**	
	Staphylococcus aureus	***Escherichia coli***
C1	3.1	3.7
C2	7.4	7.3
C3	11.7	11.1
Ab	18.2	20.5
con	0.0	0.0

The inhibitory action of C3 against all the bacterial strains is higher than other samples. This could be attributed to its small size and the accompanied higher surface area of the nanoparticles. The increase in surface area increases the number of atoms on the surface, which leads to an increase in the biological activity.

Conclusion

This study reports the reliable and eco-friendly processes for the synthesis of metallic nanoparticles. This green approach was simple and cost-effective for the preparation of stable cobalt nanoparticles by reduction of cobalt nitrate solution with a reduction method using flower extracts of a wild plant *Calotropis gigantea* as the reducing agent. Synthesis of metallic nanoparticles using green resources is a challenging alternative to chemical synthesis. UV–vis, FT-IR, TEM, zeta potential and DLS were used for studying structural characteristics which certified the formation of CoNPs. Synthesized cobalt nanoparticles have good ability to inhibit the microbial growth thus can be used as a reliable antibacterial agent.

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