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Facile Green Synthesis and Biological Activities of Silver Nanoparticles using *Coldenia procumbens* Linn.

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The facile green synthesis of silver nanoparticles is finding an imperative use in different fields due to their potential applications. The present study is focused on the green synthesis of silver nanoparticles from *Coldenia procumbens* Linn., in an eco-friendly way in order to reduce the toxicity from chemical methods. The zeta potential, size of the silver nanoparticles was measured and the characterization was done from UV-visible spectroscopy, Fourier transform infrared spectroscopy, X-ray diffraction and scanning electron microscopy. The disc diffusion method has used to evaluate the antimicrobial efficacy of synthesized nanoparticles with different bacterial pathogens and the antioxidant capability of silver nanoparticles was tested and has shown significant DPPH radical scavenging activity.

Keywords: Silver nanoparticles, *Coldenia procumbens* Linn., Biological activities.

INTRODUCTION

From centuries, medicinal plants have been used in Siddha, Ayurveda and Unani medicines as they are rich sources of unique constituents for treating various ailments. Recently, many such plants are getting the growing importance in different research fields. Nanotechnology is one of the upcoming areas of research in modern medicine in 21st century [1]. In general, particles with size 1 to100 nanometers are considered as nanoparticles. Nanoparticles can be synthesized by various techniques like chemical and physical methods such as chemical reduction [2], electrochemical reduction [3] and photochemical reduction [4]. The synthesis of nanoparticles by chemical methods requires short period on large quantity, but it will produce hazardous byproducts which leads to environment pollution [5]. Hence, there is a need for the development of an eco-friendly synthesis of nanoparticles in biological approaches like the synthesis of nanoparticles from microorganisms, enzymes, plants and plant extracts [6]. Among that plants are the better alternative for the synthesis of nanoparticles as they are cheap, safe to handle, most abundant and are rich sources of secondary metabolites like terpenoids, flavones, ketones, aldehydes, carboxylic acids and amides. These phytochemicals in plant extracts may act both as reducing agents and also as stabilizing agents in the synthesis of nanoparticles [7]. The nanoparticles from different metals like silver, gold, copper, zinc and platinum have been synthesizing from biological resources. Especially at nanoscale, the silver particles showed physico-chemical properties and biological activities due to its higher surface area allowing a larger amount of atoms to interact with their surroundings. The plant mediated green synthesis of silver nanoparticles is developing tremendously as a new technique to meet the demand of a current market in the medical field to develop the new drugs of less toxicity. Hence, the species from Boraginaceae [8] family *i.e.* *Coldenia procumbens* Linn., has been selected for the synthesis of AgNPs following facile green synthesis. *Coldenia procumbens* Linn. [9,10] grows like an annual herb, and a common weed in India. The plant is known to be efficacious in treating fever, haemorrhoids and scorpion stings. In the traditional system of medicine, the plant was reported to be used as anti-inflammatory [11], antimicrobial [12], analgesic [13], antidiabetic [14], CNS depressant [15], *etc*. The preliminary investigation of this plant has shown the presence of flavonoids, carbohydrates, glycosides, steroids and alkaloids [16]. The active constituents like coumestan derivative wedelolactone [17] and rare cyano-glucosides [18] were also extracted from this plant. A GC-MS analysis of volatile components of leaves of *Coldenia procumbens* Linn., has shown 20 compounds with

9,12-octadecadienoic acid, hexadecanoic acid as major compounds [19]. The facile green synthesis of silver nanoparticles from an aqueous extract of *Coldenia procumbens* Linn. is reported here for the first time which is enhancing the biological importance of this plant.

EXPERIMENTAL

Plant material: The aerial parts of *Coldenia procumbens* Linn., were collected from moist place of agricultural land at Nunna near Vijayawada, India. The voucher specimen was authenticated by Prof. V. S. Raju and kept in the Department of Botany, Kakatiya University, Warangal, India with accession number 1877. The aerial parts were collected, shade dried and the dried plant material was ground into fine powder, labeled and kept for further analysis.

Preparation of plant extract: A fine powder of *Coldenia procumbens* Linn. (3 g) was taken in a 250 mL beaker and 100 mL of double distilled water was added and kept on a magnetic hot plate at 65 ºC. The stirring was continued at 500 rpm until the colour of the extract changed to dark brown colour. The plant extract was filtered with Whatman filter paper 41 twice to get clear extract and stored at 4 ºC for further use.

Synthesis of AgNPs: For the synthesis of AgNPs, 195 mL of 1 mM $AgNO_3$ solution and 5 mL of plant extract were placed in a flask and stirred vigorously at room temperature and kept for 12 h. The colour change in the solution, *i.e.* from yellowish brown to dark brown indicates the formation of AgNPs and the UV-visible spectroscopy confirms the formation of AgNPs at different time intervals. For further characterization, AgNPs were separated over continuous centrifugation at 6000 rpm for 20 min. The supernatant was discarded and the suspension was collected and dried to get black powder of silver nanoparticles.

Synthetic mechanism of AgNPs: The secondary metabolites present in the plant extract acts as capping and stabilizing agents and undergoes reduction to convert silver ions into silver nanoparticles. It is reported that the mechanism of nanoparticles formation involves mainly three stages *i.e*. reduction of ions, clustering and further nanoparticles growth depends upon the concentration of the reducing agent, pH and $AgNO_3$ [20]. The preliminary investigation of this plant has shown the presence of flavonoids, carbohydrates, glycosides, steroids and alkaloids [16]. These secondary metabolites generally involved in the reduction of Ag^+ into Ag^0.

UV-visible spectroscopy: The UV-visible spectrum of silver nanoparticles was studied on Thermo Scientific spectrophotometer. A sample solution (1 mL) was collected hourly to monitor the reduction of silver ions into silver nanoparticles, followed by dilution with 2 mL of deionized water and scanned in the range of 300-800 nm. An absorbance on UV-visible spectrophotometer at different time intervals, *i.e.* for 0, 1 and 2 h confirmed the formation of AgNPs.

FTIR spectroscopy: The FTIR spectroscopy analyzed the dried powder of silver nanoparticles synthesized from *Coldenia procumbens* Linn., through the formation of potassium bromide pellet method and the spectrum was recorded in the wavelength range of 4000-500 cm^{-1} using Thermo Nicolet Nexus 670 Spectrometer.

X-ray diffraction analysis: The crystalline structure of synthesized AgNPs was carried out by X-ray diffraction analysis. The X-ray diffractogram of synthesized AgNPs from *Coldenia procumbens* Linn. was carried out on a PW 1830 Philips instrument (Netherlands) with copper as the main source and operated at a voltage of 40 kV and a current of 30 mA with Cu radiation.

SEM analysis: The SEM analysis of synthesized AgNPs was done on field emission SEM Carl Zeiss model Merlin compact microscope using a 30 KeV electron beam. The size and shape of silver nanoparticles can be done by SEM analysis through the preparation of sample film on a carbon coated copper grid.

EDX analysis: The energy dispersive X-ray spectrum was recorded with an Oxford Instruments X-Max^N SDD (50 mm^2) system and INCA analysis software. EDX was carried out to confirm the formation of elemental silver synthesized from an aqueous extract of *Coldenia procumbens* Linn.

Zeta potential and particle size analysis: The zeta potential and particle size of bio-reduced AgNPs were measured on Horiba scientific SZ-100. The zeta potential is used to study the stability and surface characteristics of AgNPs. The dynamic light scattering technique has measured the particle size of AgNPs.

Antibacterial activity: The antibacterial potential of synthesized AgNPs was carried out using a disc diffusion method. The four bacterial cultures of *Escherichia coli*, *Staphylococcus aureus*, *Lactobacilli* and *Enterobacter* were inoculated overnight and spread over freshly prepared nutrient agar media with 6 mm diameter discs. Different concentrations of silver nanoparticles *i.e*. 20, 30, 40 and 60 µg/mL were poured onto the discs and incubated at 37 ºC for 24 to 48 h with standard drug ciprofloxacin as control. The measurement of zone of inhibition around the discs in diameter has determined the antibacterial efficacy of the synthesized AgNPs from *Coldenia procumbens* Linn.

Antioxidant activity: DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable radical and the free radical scavenging activity of an aqueous plant extract and the synthesized AgNPs was measured using this DPPH radical in hydrogen donating or radical-scavenging ability. The solution of DPPH, *i.e.* 0.1 mM in methanol was prepared and 1.0 mL of this solution was added to 3 mL of AgNPs solution in water at different concentrations (25-200 µg/mL). The solution was incubated for 30 min and after 30 min, the absorbance was measured at 517 nm. Ascorbic acid was used as a standard. The capability of an aqueous plant extract and the synthesized AgNPs to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenged (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

where, $A_{control}$ is the absorbance of the control reaction; A_{test}/std is the absorbance in the presence of extracts.

RESULTS AND DISCUSSION

A solution of $AgNO_3$ (1 mM) was added to the plant extract of *Coldenia procumbens* Linn. in a 250 mL conical flask and kept over 24 h as the reaction time at room temperature. The UV-visible spectroscopy monitored the reduction of silver ions into AgNPs in the presence of an aqueous extract of *Coldenia procumbens* Linn. Initially the colour of AgNPs in aqueous solution is yellowish brown due to surface plasmon

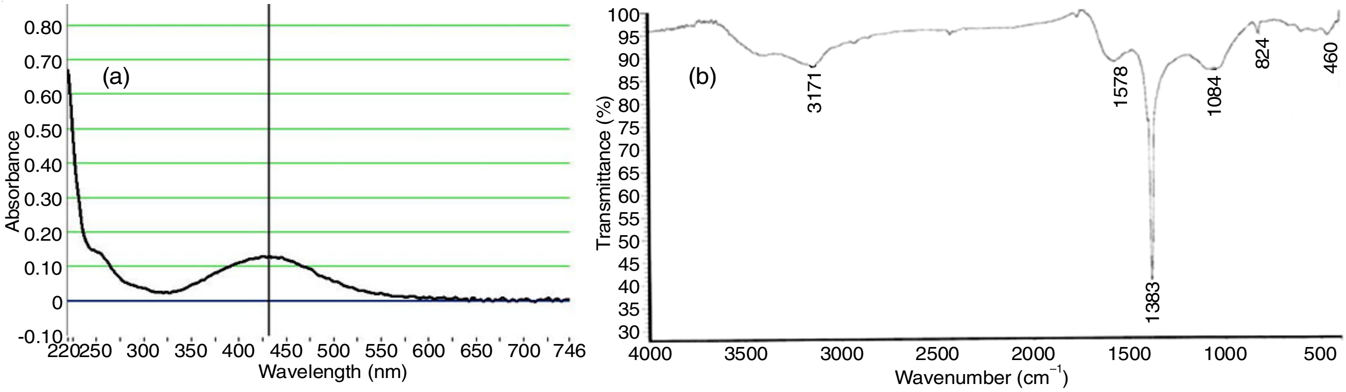

Fig. 1. (a) UV-Visible spectrum of AgNPs, (b) FTIR spectrum of AgNPs

vibrations [21]. As the reaction time increases, the AgNPs colour changes to reddish brown that indicates the formation of AgNPs. Further, the formation of AgNPs was confirmed by observing a distinct maximum absorption at 432 nm in UV-visible spectrum (Fig 1a). As the time increases, the absorption of AgNPs was also found to be increased. The incubation time is directly related to the size, shape and the synthesis rate of AgNPs [22].

The FTIR studies were carried out to identify the active biomolecules responsible for capping and stabilization of AgNPs. The powder of synthesized AgNPs was characterized in the range of 4000-400 cm^{-1} using a KBr pellet method and the FTIR spectrum of AgNPs synthesized from *C. procumbens* Linn. is shown in Fig. 1b. The peak at 3171 cm^{-1} showed the strong broad band corresponds to N-H stretch of amide group, the peak at 1578 cm^{-1} corresponds to C=C stretching. The band at 1383 cm^{-1} can be attributed to -C-O stretching mode corresponds to the presence of carbonyl groups. The absorption band at 1084 cm^{-1} corresponded to the presence of fatty acids and the peaks at 824 and 460 cm^{-1} corresponds to the other functional groups in the AgNPs [23].

The X-ray diffraction study (XRD) is used for the phase identification of AgNPs and the characterization of crystal structure of AgNPs can be studied with this technique. The XRD pattern of synthesized AgNPs at different diffraction peaks are shown in Fig. 2. The peaks at 2θ 38.20º, 44.30º, 64.40º and 77.60º representing (111), (200), (220) and (311) of Bragg's reflections with the face centered cubic (FCC) crystalline structure of AgNPs which are in good agreement with the reported values previously [24]. Few intense XRD peaks at 27.80º, 32.50º, 46.10º, 54.90º and 81.60º (2θ) were also observed which correspond to AgCl face centered cubic structure [25]. Generally, the broader peaks in XRD of AgNPs related to the size of particle. The broader peaks signify the smaller particle size of AgNPs.

The SEM images of synthesized AgNPs are shown in Fig. 3 with different magnification scale. The formation of AgNPs and their morphological dimension can be studied with SEM by using SEM grid. A very small quantity of the sample was dropped on the carbon coated copper grid and thin films of the sample were prepared, then the film on SEM grid was dried under a lamp for 5 min. The shape of the majority of AgNPs was proved to be spherical as shown in Fig. 3. The size of some of selected AgNPs in the sample was found in the range of 40-

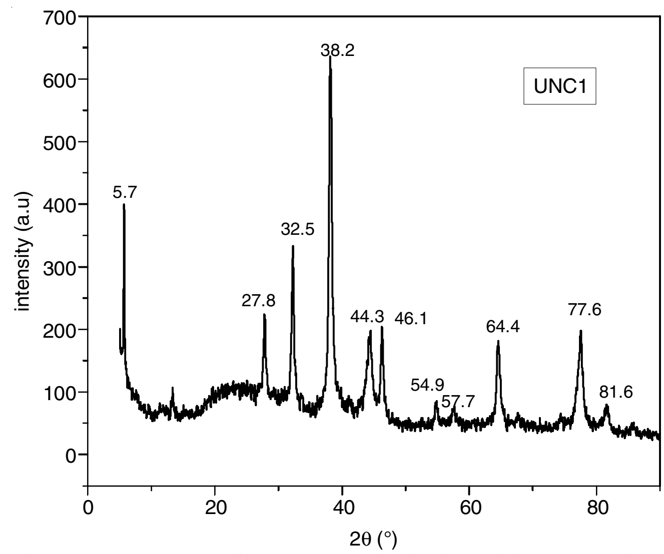

Fig. 2. XRD spectrum of AgNPs synthesized using *Coldenia procumbens* Linn. extract

108 nm having polydispersions. Aggregation of smaller particles with the plant extract can lead to the formation of larger silver particle size in the powder of AgNPs and this aggregation can be avoided by sonication methods.

The elemental composition of synthesized AgNPs can be achieved by employing EDX analysis. The weight and an atomic percentage of elements in synthesized AgNPs are shown in Fig 4. It has been observed from the results that the strong peak is for silver (Ag) particles and weak peaks for carbon (C), oxygen (O). They may have resulted from the bio-organic molecules from plant extract bounded to the surface of AgNPs [26]. The carbon peak may be due to the carbon grid that was used for analysis. The quantitative results from EDX confirmed the silver metal with 95 weight % and 69.79 atomic %.

From Fig. 5, silver nanoparticles synthesized from *Coldenia procumbens* Linn. has shown sharp peak at -27.8 mV indicates the negative Zeta potential of the surface of AgNPs and considered that they have incipient stability in an aqueous medium. Generally, zeta potential of synthesized nanoparticles should be higher than +30V or lower than -30V [27].

The average size of the AgNPs and their polydispersity index (PDI) were recorded on Horiba particle size analyzer. The size of silver nanoparticles was found to be 8.2 nm (Fig. 6). The average particle diameter was 4843.1 nm and the polydispersity index was 0.428.

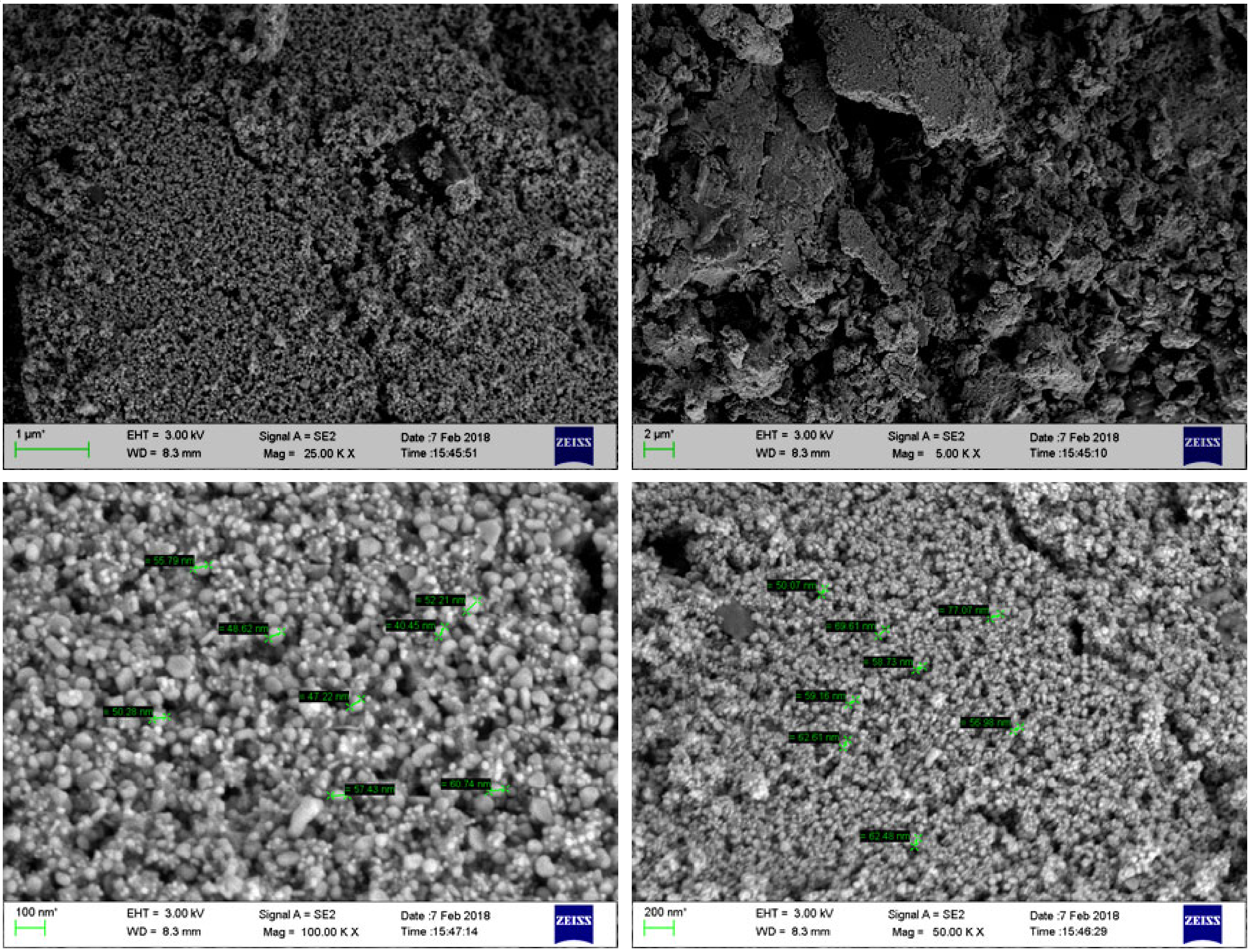

Fig. 3. Scanning electron microscopy images of AgNPs synthesized using *Coldenia procumbens* Linn. extract

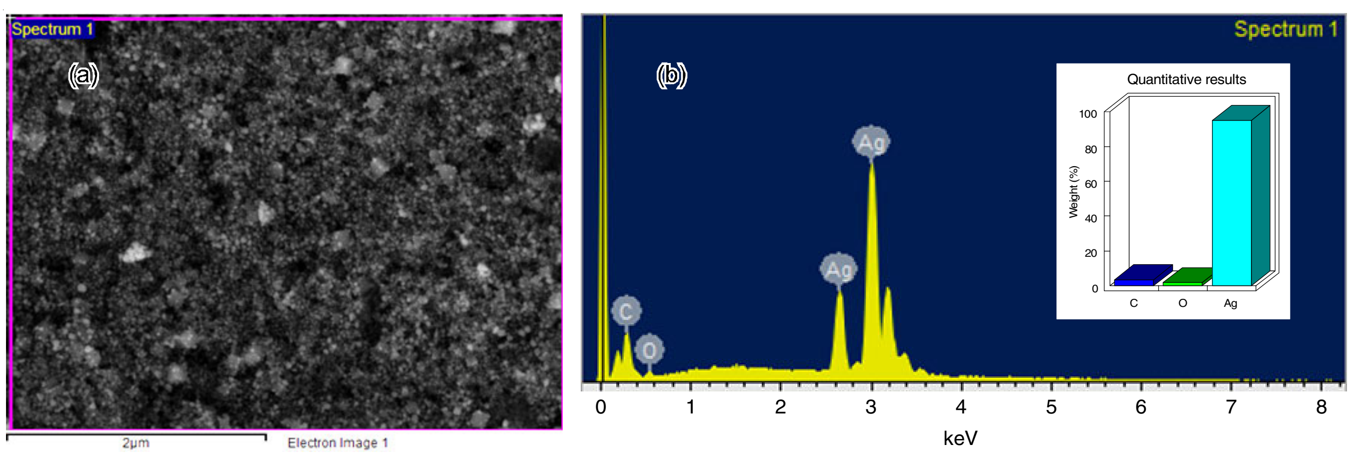

Fig. 4. (a) EDAX images of AgNPs synthesized using *Coldenia procumbens* Linn., extract, (b) elemental composition of AgNPs with quantitative results

Antibacterial activity: The antibacterial potential of AgNPs synthesized from an aqueous extract of *C. procumbens* Linn., was effectively accessed against *E. coli*, *S. aureus*, *Lactobacilli* and *Enterobacter* and compared with the standard antibiotic drug ciprofloxacin. The disc diffusion method was used for testing the antibacterial property [28]. The AgNPs synthesized from *Coldenia procumbens* Linn. has shown prominent inhibition against *Staphylococcus aureus* and *Lactobacilli*, but not inhibited the growth of *E. coli* and *Enterobacter* (Table-1). The different mechanisms of antimicrobial action of AgNPs have been proposed in different ways. It is believed that the positively charged silver ions attached to the negatively charged bacterial cell wall which leads to denaturation of protein and finally cell death by interacting with sulfur- and phosphorus-

TABLE-1
ZONE OF INHIBITION OF *E. coli*, *Staphylococcus aureus*, *Lacto bacilli* AND *Enterobacter* BY AgNPs SYNTHESIZED FROM *Coldenia procumbens* Linn.

Name of the bacteria	Zone of inhibition by ciprofloxacin (mm)	Zone of inhibition by AgNPs in diameter (mm) at different concentrations			
		20 µg/mL	30 µg/mL	40 µg/mL	60 µg/mL
E. coli	42	–	–	–	–
Staphylococcus aureus	28	12	12	16	15
Lacto bacilli	37	15	13	15	12
Enterobacter	32	–	–	–	–

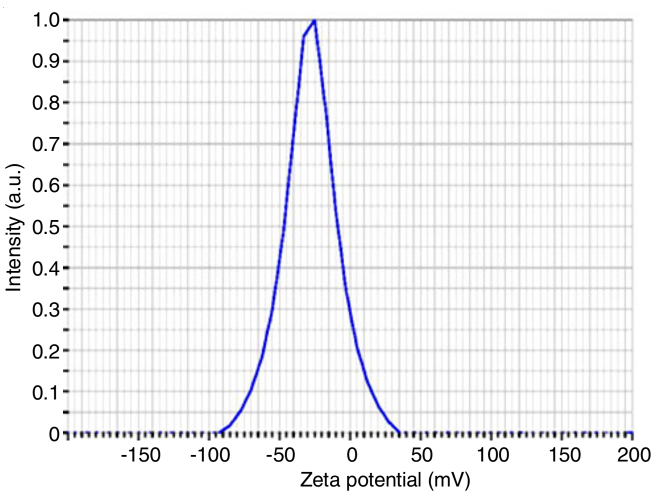

Fig. 5. Zeta potential of AgNPs synthesized using *Coldenia procumbens* Linn. extract

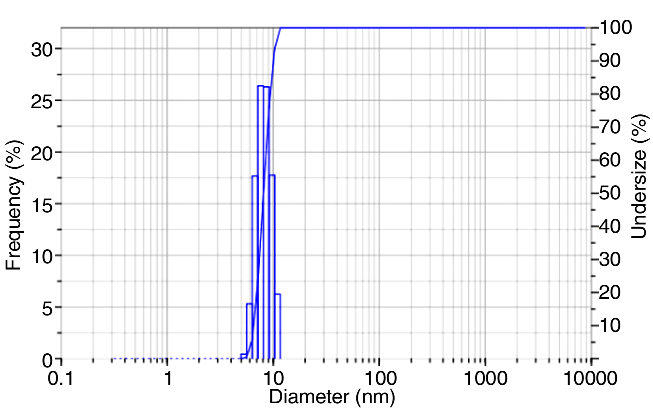

Fig. 6. Particle size distribution of AgNPs synthesized using *C. procumbens* Linn. extract

containing compounds such as DNA [29]. The power functions such as permeability and respiration will be disturbed when the AgNPs attached to the surface of cell membrane.

DPPH radical scavenging activity: The scavenging activity of synthesized AgNPs is shown in Fig 7. The absorbance of DPPH radical is usually done at 517 nm with purple colour, but upon reduction with the antioxidants the DPPH free radical becomes paired with hydrogen and forms DPPHH. As a consequence, the absorbance of DPPH will decrease with the colour change of yellow [30]. The absorbance of DPPH was measured spectrophotometrically to determine the radical scavenging ability of synthesized AgNPs from *Coldenia procumbens* Linn. The results have shown that the percentage of radical scavenging activity increased by increasing the concentration of AgNPs in a dose dependant manner and the absorbance of DPPH radical in AgNPs was decreased as AgNPs scavenged the DPPH radical.

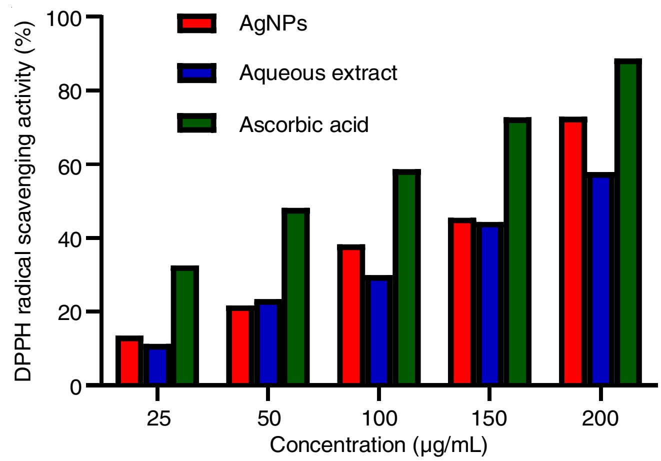

Fig. 7. DPPH radical scavenging activity of AgNPs synthesized from *Coldenia procumbens* Linn. extract

The reducing power of AgNPs was increased with the increasing concentration of AgNPs and maximum reduction was at 200 µg/mL. Hence, the silver nanoparticles synthesized from *Coldenia procumbens* Linn., have shown significant antioxidant activity. Ganesan *et al.* [31] have reported the antioxidant activity of the methanolic extract of *Coldenia procumbens* Linn., but the synthesized AgNPs from this plant is more advantageous as it has shown antioxidant activity in nanoscale.

Conclusion

The AgNPs from *Coldenia procumbens* Linn. were successfully synthesized in a facile and eco-friendly way and various techniques like UV, FTIR, XRD and SEM have used to determine the size and shape of AgNPs. The antibacterial efficacy of AgNPs was also evaluated against Gram-positive and Gram-negative bacteria by disc diffusion method. The AgNPs have scavenged DPPH radical and possesses antioxidant activity which helps in developing new methodologies in biomedical and pharmaceutical fields. The secondary metabolites from the plant extract of *Coldenia procumbens* Linn., acts as reducing agents in converting silver ions into AgNPs by an eco-friendly synthesis.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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