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Formulation of silver nanoparticles using methanolic extract of stem of plant *Desmodium gangeticum*, their characterization and antibacterial and anti-oxidant evaluation

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Abstract
Objectives: The primary objective of this study is to present an evidence-based perspective of silver nanoparticles green synthesis and evaluation of their antibacterial and antioxidant activity.

Methods: Silver nanoparticles are formulated by using methanolic extract of stem of plant *Desmodium gangeticum* and their characterization was performed. Further they were analysed for their anti-bacterial and antioxidant activity by using disc diffusion method for antibacterial evaluation and DPPH(2,2-Diphenyl 1-picryl hydrazyl) scavenging assay for antioxidant evaluation.

Results and discussion: Different concentrations of silver nitrate solution were prepared and reacted with extract solution in different ratios. Silver nanoparticles are formulated by using *Desmodium gangeticum* plant extract by reduction of Ag^+ to Ag^0 from silver nitrate solution. The light yellow colour was observed that changes to dark brown which indicates the formation of nanoparticles. Results from UV-vis (ultra violet-visible spectroscopy) analysis, zeta potential analysis, FTIR (Fourier Transform Infrared) spectroscopy and scanning electron microscopy were obtained good and satisfactory. The results obtained from the antibacterial and anti-oxidant assay showed that the antibacterial and anti-oxidant activity of formulated silver nanoparticles was more than that of the plant extract.

Conclusion: The obtained results suggested that the formulated silver nanoparticles of methanolic extract of stem of plant *Desmodium gangeticum* possess efficient anti-bacterial and antioxidant activity.

Keywords: *Desmodium gangeticum*, disc diffusion, zeta potential, Fourier Transform Infrared, scanning electron microscopy.

1. Introduction
In this era, nanotechnology is one of the most interesting area which is used to describe the creation and utilization of materials with structural features between those of atoms and bulk materials with at least one dimension in the nano range [1-2]. In this study, we have formulated silver nanoparticles with the help of *Desmodium gangeticum* extract by reduction of Ag^+ to Ag^0 from silver nitrate solution. Silver nanoparticles are particles of silver, i.e. silver particles of between 1 nm and 100 nm in size [3]. The medical properties of silver have been known for over 2,000 years. Since the nineteenth century, silver-based compounds have been used in many antimicrobial applications. It is a well-known fact that silver ions and silver-based compounds are highly toxic to micro-organisms which include sixteen major species of bacteria [4-5]. This aspect of silver makes it an excellent choice for multiple roles in the medical field.

2. Materials and Methods
Silver nitrate was purchased from A.B. enterprises Mumbai, Methanol was purchased from S.D. Fine Chemical Ltd. Mumbai, DPPH was purchased from Sisco Research Laboratories pvt. Ltd. Mumbai. All other chemicals used were of analytical grade.
Equipments used were purchased from Remi, Mumbai, India.

2.1 Preparation of methanolic extract of stem of plant *Desmodium gangeticum*
The plant was authenticated at CSIR National Institute of Science Communication and

Information Resources, New Delhi. The plant was firstly washed, cleaned and maintained in the department. 25 g of fresh stem of the plant was sliced and air-dried at room temperature. The sliced, air-dried plant was milled into fine powder in a warring commercial blender. The powdered plant material was extracted by using 200 ml methanol as solvent for 72 hours in soxhlet apparatus, then the extract was filtered and distilled on a water bath, finally giving 32.8% yield of dark green, resinous crude methanolic stem extract of plant *Desmodium gangeticum*.

2.2 Formulation of silver nanoparticles using the obtained extract

Analytical grade silver nitrate($AgN0_3$) was prepared in various concentration and was used for the experiment. Different concentrations of silver nitrate solution (0.1 M, 0.01 M, 0.001 M) were prepared and interacted with the plant extract in different mixing ratios (1:1, 1:2, 2:1) for different time periods at different temperature conditions in a rotary shaker at different rpm. Immediately after the addition plant extract to $AgNO_3$ aqueous solution, a light yellowish color was observed which changed to dark brown color. This change of color indicates that the formation of AgNP has taken place [6-7].

2.3 Characterization

2.3.1 UV-Visible spectroscopy

Preliminary characterization of the metallic nanoparticles was carried out using UV-Visible spectroscopy (ultraviolet-visible spectroscopy). The reduction of silver ions to the nanoparticle form was monitored by measuring the UV-Visible spectra of the solutions after diluting the sample with Millipore water 20 times [8]. The spectra were recorded on UV-Visible double beam spectrophotometer from 200 to 600 nm.

2.3.2 Fourier transform infrared spectroscopy

The lyophilized powders of the silver nanoparticles were subjected to FTIR spectroscopy measurements. The measurements were carried out on a Perkin-Elmer Spectrum-One instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets [9].

2.3.3 Zeta potential analysis

The zeta potential of the synthesized nanoparticles was determined by means of zeta potential analyzer. The measurement of zetapotential is based on the direction and velocity of particles under the influence of known electric field [10-11].

2.3.4 Scanning electron microscopy

The formulated silver nanoparticles are characterized by SEM for determining their morphology i.e. shape and size. SEM can produce very high-resolution images of a sample surface, revealing details less than 1 nm in size [12].

2.4 Antibacterial assay

Disc diffusion method [13]

The stock cultures of bacteria were rejuvenated in broth media by inoculation and grown for 18 hours at 37 °C temperature. The above media was poured into agar plate in which wells are drilled. The drilled wells are poured with the silver nanoparticles in different concentrations of 1 µg, 5 µg, 10 µg, 20 µg. Separate wells of Amoxicillin (for gram +ve bacteria) and Streptomycin (for gram –ve bacteria) [25 µg each] are used in the test as a control for comparison purpose. The agar plates are incubated for 24 hours at the temperature 37 °C and the zone of inhibition is noted.

2.5 Anti-oxidant assay

DPPH scavenging assay [8, 13, 14]

Different concentrations of the nanoparticles were mixed with 2 mL of 100 µl M DPPH solutions. The samples were vortexes and allowed to scavenge DPPH in dark for 30 min. The absorbance of the supernatants after centrifugation at 3000 rpm for 15 minutes was measured at 517 nm in UV-vis spectrophotometer. In all the cases, measurements were done in triplicates. The scavenging percentage was calculated using the formula:

$$\text{DPPH scavenging} = \frac{(AC\text{-}As)}{AC} \times 100$$

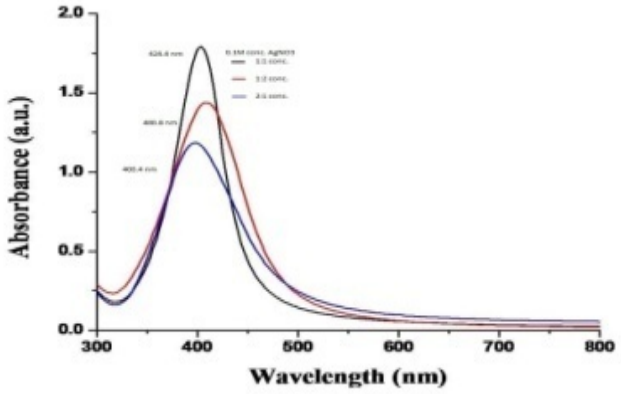
Where AC and AS are absorption of blank DPPH and DPPH subjected to interact with the sample at 517 nm, respectively.

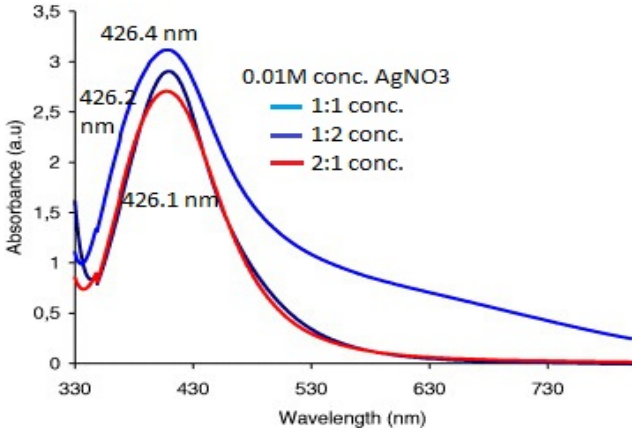
3. Results and Discussion

The methanolic stem extract of plant *Desmodium gangeticum* obtained was resinous and dark green in colour and the yield obtained was 32.8%. The change in colour from light yellowish to dark brown indicated the formation of silver nanoparticles, which was due to the reduction of Ag^{+}.

3.1 Characterization

3.1.1 UV-vis spectral analysis





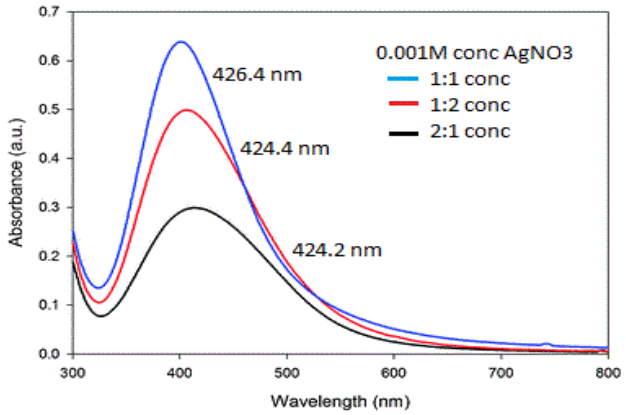


Fig 1: Graphs showing UV-vis absorption spectra of silver nanoparticles at different ratios

The graphs obtained shows the wavelength of silver nanoparticles between 400 nm to 426 nm.

3.1.2 Fourier transform infrared spectral analysis

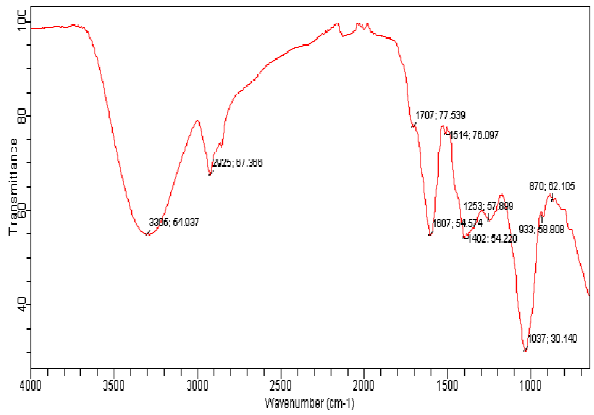


Fig 2: FTIR graphical representation of formulated silver nanoparticles

The FTIR spectrum of formulated silver nanoparticles showed the bands in ranges of alcohol, alkane, carbonyl, aromatic, alkyl halide and alkene groups. It indicated that silver nanoparticles are surrounded by phenols, alkanes, carbonyls, alkyl halides and alkene.

3.1.3 Zeta potential analysis

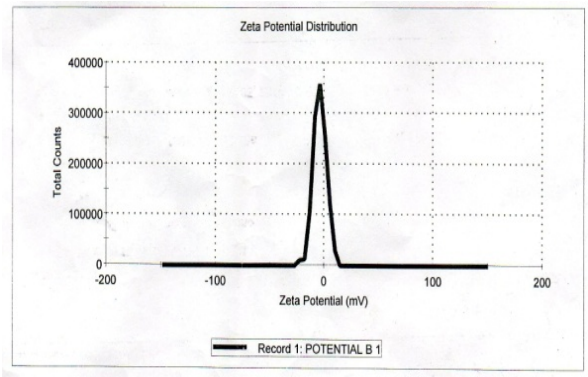


Fig 3: showing graphical representation of zeta potential analysis

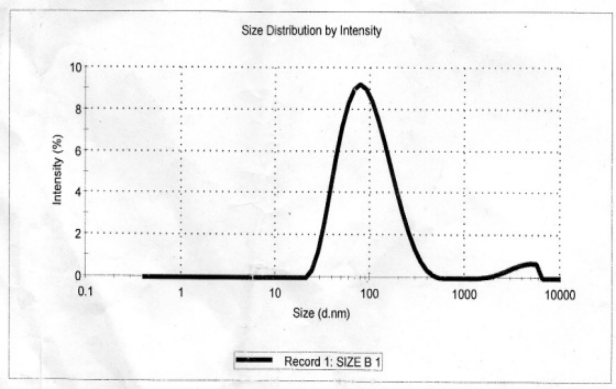


Fig 4: showing graphical representation of size distribution analysis

- Zeta potential -3.95 mV
- Z-average 77.59 d. nm

3.1.4 Scanning electron microscopy

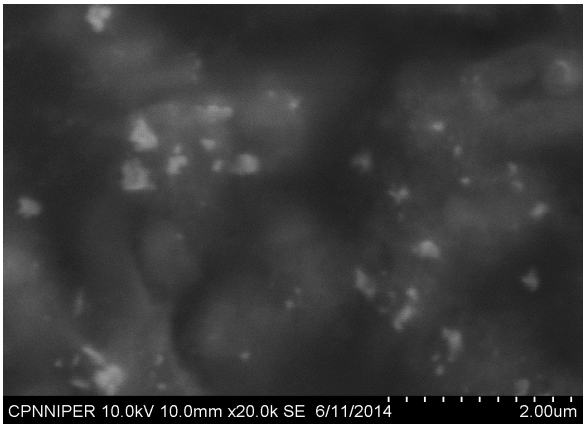


Fig 5.

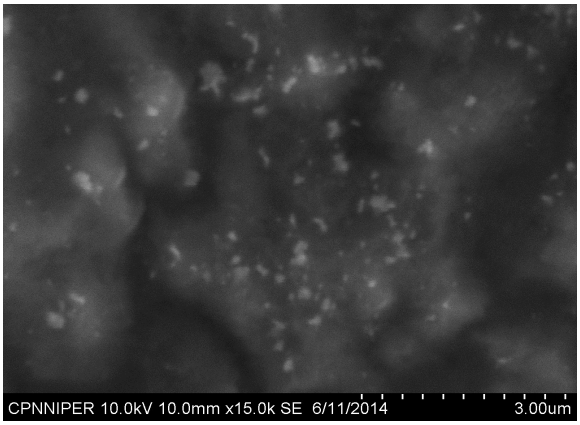


Fig 6.

SEM images showing silver nanoparticles at 2.00 µm (Fig no. 5) and 3.00 µm (Fig no. 6)

3.2 Antibacterial assay

The following figure shows a clear inhibition zone with silver nanoparticles whereas the standard antibiotic Amoxicillin and Streptomycin shows smaller zone of inhibition as compared to the silver nanoparticles treated discs.

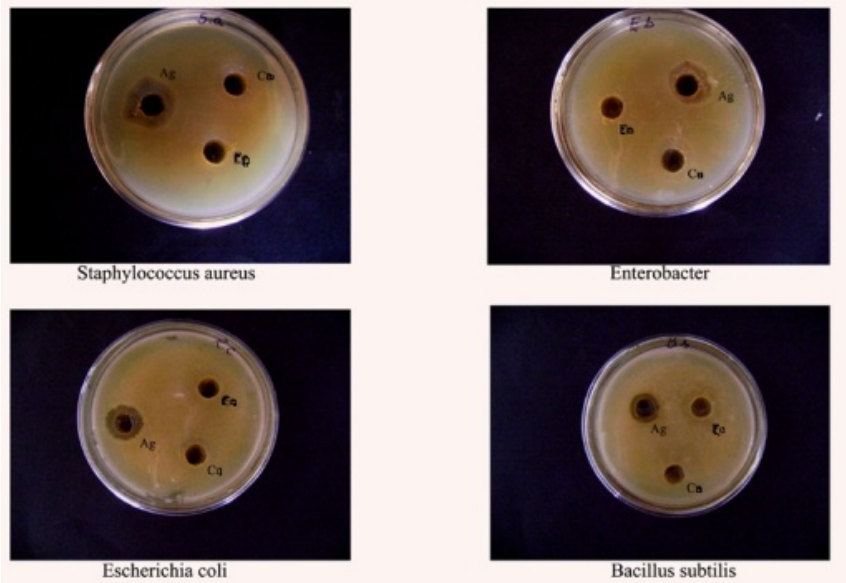


Fig 7: Images of antibacterial activity of different concentrations of silver nanoparticles (1 µg, 5 µg, 10 µg) on *Staphylococcus aureus, Enterobacter*, *E. coli* and *Bacillus subtilis* (Au-silver nanoparticles, Co-standard)

Table 2: Zone of inhibition of antibacterial assay of silver nanoparticles

Bioactive agent		Zone of inhibition (Diameter, cm)			
	Concentration	*E. coli*	*Bacillus subtilis*	*Staphylococcus aureus*	*Enterobacter*
Ag nanoparticles	1 µg	3.2	2.5	3.2	3.1
	5 µg	4.2	3.3	3.4	3.4
	10 µg	4.3	3.8	4.2	4.2
	20 µg	4.5	4.1	4.3	4.3
Amoxicillin	25 µg	0.8	Nil	0.8	3.8
Streptomycin	25 µg	1.5	0.9	1.5	2.6

3.3 Anti-oxidant assay

The antioxidant activity of formulated silver nanoparticles was estimated by comparing the percentage inhibition of formation of DPPH radicals with that of ascorbic acid. Silver nanoparticles showed moderate antioxidant activity when compared to ascorbic acid. The DPPH radical scavenging activity of silver nanoparticles increased with increase in concentration. The colour changes from purple to yellow after reduction, which can be quantified by its decrease absorbance at wavelength 517 nm. These results revealed that the silver nanoparticles are free radical inhibitor or scavenger acting possibly as primary antioxidants.

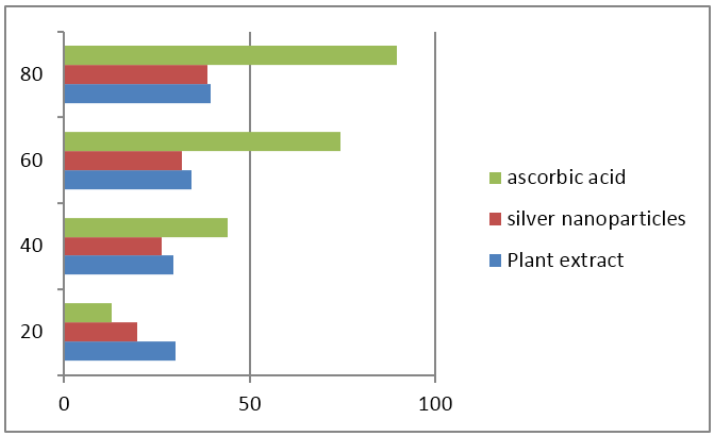


Fig 8: Graphical representation of anti-oxidant activity (%) of silver nanoparticles

Table 3: DPPH scavenging activity of the silver nanoparticles (DPPH radical scavenging activity [%])

Concentrations µg/ml	Plant extract	IC_{50} µg/ml	Silver nanoparticles	IC_{50} µg/ml	Ascorbic acid	IC_{50} µg/ml
20	26.96 ± 0.09		19.75 ± 0.15		12.67 ± 0.06	
40	29.56 ± 0.12	Not applicable	26.26 ± 0.07	Not applicable	44.16 ± 0.21	45.74
60	34.37 ± 0.15		31.72 ± 0.09		74.42 ± 0.12	
80	39.33 ± 0.15		38.54 ± 0.12		89.51 ± 0.09	

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Little achievements often require long, tortuous effort and bitter experiences including some sacrifices. And this is only possible when the almighty GOD keeps his handful of blessings on the head of anybody. I would like to submit everything beneath the feet of GOD. I acknowledge my parents for their blessings, encouragement and support which lead me to the path of success in my life. I would like to acknowledge my brother for his help and support in completing my project work.

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