

Green Synthesis and Characterization of Silver Nanoparticles using Crude Extract of *Crotalaria burhia*

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Authors' Contributions

1 Conception & Study design, Data Analysis and/or Interpretation.

2 & 4 Data Collection & Processing, Drafting of Manuscript.

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ABSTRACT

Background: Appearance of antibiotic resistance has raised the demand to find alternative therapies and modified drug delivery system of medicinal plants to treat bacterial infections.

Objective: The aim of this study is the green synthesis and characterization of silver nanoparticles by using crude extract of *Crotalaria burhia* and to evaluate their antibacterial potential.

Methods: The roots and stems of plant were used to prepare the crude extract. The phytochemical analysis of different compounds in extract was performed. 1mM AgNO₃ and different concentrations of plant extract were used for the green synthesis of silver nanoparticles. The particles size and zeta potential were measured by zeta sizer while surface morphology of silver nanoparticles was observed with Scanning Electron Microscope (SEM). The antibacterial activity of silver nanoparticles was performed by 96 well microdilution plate method.

Results: The particle size and zeta potential of optimized formulation was 92 nm and -24.8 mV. The SEM analysis showed that silver nanoparticles are irregular and spherical shape. The antibacterial activity showed that MIC value of silver nanoparticles was lower for *E. coli* than *S. aureus*.

Conclusion: Silver nanoparticles possess potent bactericidal activity against *E. coli* and moderate activity against *S. aureus*. It had been concluded that these nanoparticles can be used against multi-drug resistant bacterial infections.

Keywords: *Crotalaria burhia*; Silver nanoparticles; Antibiotics resistance; Antibacterial.

INTRODUCTION

Medicinal plants and their potential chemical constituents play a major role in the well-being of poor communities around the world [1]. Phytomedicine has a wide range of therapeutic benefits, therefore they are considered appropriate in the treatment of

diseases and has a central place in healthcare around the world [2]. Phytomedicine has lesser side effects than synthetic medicines and is of considerable significance in the treatment of transmissible diseases [3].

Crotalaria burhia is a desert plant. The Genus *Crotalaria burhia* which belongs to family Fabaceae is

commonly present in dry places of the west Afghanistan, India as well as Pakistan [4]. The novel drug delivery system is an advanced and modern system which is designed for the optimum delivery of drugs. Recent advances in drug pharmacokinetics & pharmacodynamics has led the giant pharmaceutical manufacturer to design different drug delivery system of conventional drugs, because synthesis of new drug molecule is expensive as compared to development of new drug delivery system [5]. In the 21st century, the use of small sized nanoparticles is a best approach for the delivery of active drug to cure different diseases. For this reason, the pharmacists, drug delivery scientists and researchers shift their focus on developing a novel drug delivery system for herbal medicines [6].

Silver nanoparticles (Ag Nps) are silver crystals, with a common particle size of 1 nm to 100 nm. They consist of a high percentage of silver oxide because of their large surface to bulk silver atoms [7]. The primary requirement of Ag NPs green synthesis is the solution of silver metal ion and a reduction biological agent. In most cases, reducing agents or other constituents in the cells act as a stabilizing and capping agent and there is no need to add external capping stabilizing agents [8]. The usage of nanotechnology in herbal drugs has demonstrated numerous benefits for herbal medicines which are improved solubility, bioavailability and improved pharmacological activity. This produces less toxicity and prevents chemical and physical degradation of the crude drug [9] So, the green synthesis of silver nanoparticles can be an effective and safe way for eradication of multidrug resistant bacterial infections [10] therefore the aim of this study is to green synthesize and characterize silver nanoparticles by using *Crotalaria burhia* plant extract and to evaluate antibacterial potential of silver nanoparticles.

MATERIALS AND METHODS

Collection of Plant

Crotalaria burhia plant was collected from the Cholistan desert of Bahawalpur, Pakistan. The plant was verified by Prof. Dr. Ghazala H. Rizwani (Director Research, Hamdard University, Karachi, Pakistan). The dried sample of plant was stored in pharmacognosy research list with reference number A138.

Preparation of Crude Extract

Stem and roots of *Crotalaria burhia* plant were taken and shade-dried. The whole dried-plant was ground to fine powder by using grinder. Powdered material of plant was added into hydroethanolic (30/70 v/v) solvent for 3 days. The plant and solvent mixture was agitated daily to dissolve phytochemicals in solvent. After 3 days, the mixture was filtered on muslin cloth and Whatman filter paper. The solvent of filtrate was evaporated on rotary evaporator at 35°C and a thick gummy extract was obtained which was stored at 4°C in refrigerator [11].

Green Synthesis of Silver Nanoparticles

40 ml of 1Mm silver nitrate solution was taken and different concentrations of plant extract were added in it (Table 1). The reaction mixture was placed on magnetic stirrer for 6 hours with constant magnetic stirring at 400 rpm. The color of solution changed from orange to dark brown which indicated the synthesis of silver nanoparticles. The synthesis of silver nanoparticles was further confirmed with UV analysis at 400 to 700 nm wavelength. The liquid sample of silver nanoparticles was centrifuged at 12000 rpm for 30 minutes. The pellets of Ag NPs were washed with water and lyophilized on lyophilizer. After lyophilization, the solid sample of nanoparticles was stored at 4°C for further characterization and antibacterial assay [11].

Table 1. Different Concentrations of Plant Extract. Green Synthesis of Silver Nanoparticles.

Formulation No.	Silver nitrate 1mM (ml)	Plant extract (mg/ml)
1.	40	5
2.	40	10
3.	40	15
4.	40	20
5.	40	25

The silver nano particles were powdered and characterized by visual observation, UV visible spectroscopy, FTIR spectroscopy, zeta sizer and Scanning Electron Microscopy (SEM). UV visible spectrophotometry was performed to analyze the reduction of silver into silver ion which refers to completion of reaction. FTIR spectroscopy (Bruker

Tensor 27 series) at wavelength range of 500 to 4000 cm^{-1} . The transmittance spectra were analyzed on OPUS software running on Windows PC. The zeta sizer (Malvern instruments, UK) was used to analyze the size and zeta potential of silver nanoparticles. SEM analysis was performed at Central Research Lab, Lahore College for Women University (LCWU), Lahore. In SEM analysis, the surface morphology and shape of silver nanoparticles was analyzed. high-resolution images were captured at 25 kV [12].

Anti-Oxidant Activity

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Method

10 μl drug solution and 90 μl of 100 μM methanolic solution of DPPH was taken in 96 well microplate (Figure 1) and incubated for half hour at 37°C. After incubating the samples were analyzed at 517nm wavelength with the help of microplate reader. The absorbance value was measured thrice and compared with the standard [13].

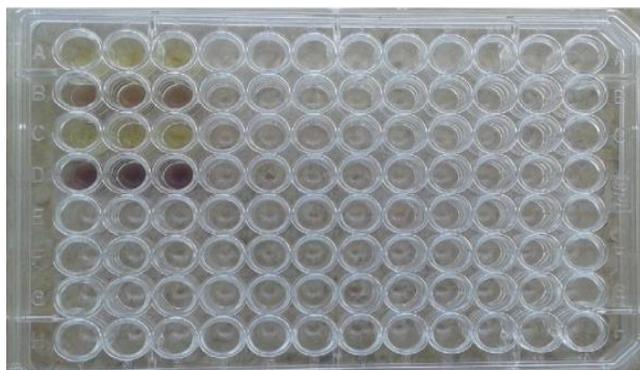


Figure 1. DPPH assay by 96 microwell plates method.

IC⁵⁰ value was measured by Ez-fit-5 Perrella Sientic Inc. USA. The final inhibitory percentage was calculated by following mathematical equation

Scavenging potential (%) = $\frac{\text{Abs. of Control} - \text{Abs. of test}}{\text{Abs. of control}} \times 100$

Absorbance of test = Radical effect of test compound

Abs. of control = Total radical effect without any inhibitor.

Antibacterial Activity

The antibacterial activity of silver nanoparticles was performed against two bacterial strains *E. coli* (Gram negative) and *S. aureus* (Gram positive). The 96 well microdilution plate method with broth medium was used to determine the minimum inhibitory concentration (MIC). The bacterial strains (5×10^5 CFU/ml concentration) were exposed with different

dilutions (different concentrations) of silver nanoparticles, plant extract and silver nitrate. The 96 well plates were incubated at 37°C for 24 hours in aerobic condition. The concentration which restrict the visual growth of bacteria was considered as MIC. The standard for antibacterial activity was Ciprofloxacin [14].

RESULTS

Phytochemical Analysis

On phytochemical analysis of hydro-alcoholic extract of plant high amount of carbohydrates was found. The mild amount of saponins, phenols, tannins, proteins and quinines was found in extract. The mild quantity of fats and oils and high quantity of flavonoids was also found in *Crotalaria burhia* extract. Mild to moderate quantity of alkaloids and glycosides was also present. While high quantity of terpenes was present in this crude extract (Table 2).

Table 2. Phytochemical Analysis of *Crotalaria burhia*.

Sr No.	Chemical compound	<i>Crotalaria burhia</i> extract
1	Tannins	+
2	Alkaloids	++
3	Glycosides	++
4	Saponins	+
5	Terpenes	++
6	Fats and oils	++
7	Proteins	+
8	Phenols	+
9	Carbohydrates	+++
10	Quinones	+
11	Flavonoids	+++

+ indicate mild, ++ indicate moderate and +++ indicate high amount of compounds in the crude extract. While, - indicate the absence of compound in the crude extract.

Visual Observation

The concentration of silver nitrate was kept constant and different concentration of plant extracts were added into the AgNO_3 solution. The color of solution changed from light faint to yellowish brown and colloidal brown at the end. This color change pattern indicates the formation of silver nanoparticles.

UV Visible Spectroscopy

To confirm the synthesis of nanoparticles UV visible spectroscopy was performed. The maximum absorbance band of silver nanoparticles was seen at 450 nm which indicate the green synthesis of silver nanoparticles.

FTIR Spectroscopy

The FTIR-spectrum of silver nanoparticles showed all major peaks of plant extract. The OH-group peak was seen at 3385.04 cm^{-1} and C=O group's peak was seen at 1660.09 cm^{-1} . The NH group peak was observed at 1596.20 cm^{-1} (Figure 2).

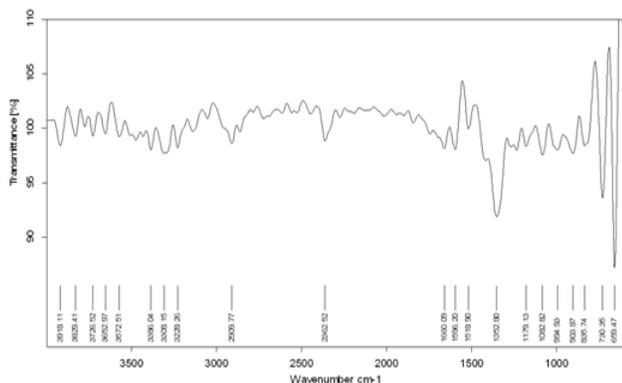


Figure 2. FTIR spectrum showing the different functional groups of silver nanoparticles.

Particle Size and Zeta Potential

The different formulation yields different sized silver nanoparticles. The optimum formulation was formulation 1 (Table 3) whose particle size was 92nm (Figure 3) and zeta potential was -24.8 mV (Figure 4).

Table 3. Average Particle Sizes of Different Formulations.

Formulation	Average Particle Size (nm)
1	92
2	98
3	101
4	105
5	113

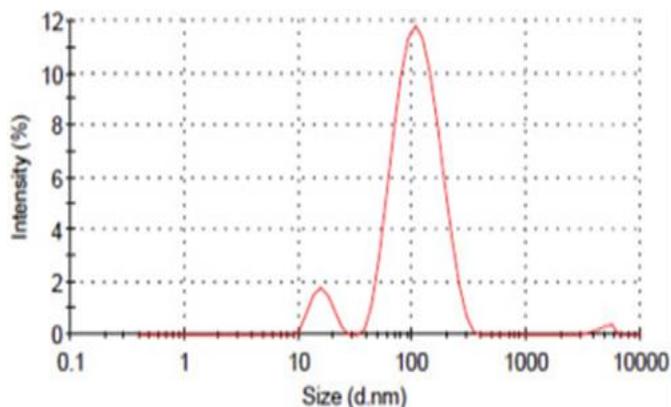


Figure 3. Graph showing variable particle sizes of silver nanoparticles of *Crotalaria burhia*.

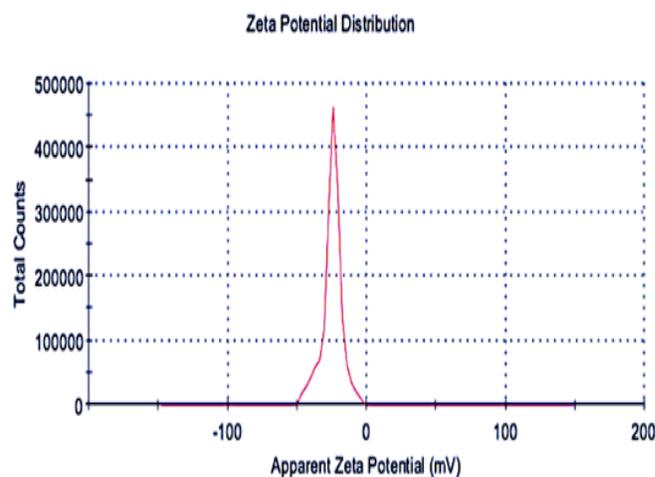


Figure 4. Graph indicating Zeta Potential distribution of silver nanoparticles.

Scanning Electron Microscopy (SEM)

The high-resolution SEM image of optimized formulation 1 was analyzed to study the outer shape of Ag NPs. The irregular, clumpy and some spherical type silver nanoparticles were observed in SEM image. Some particle seems big and some seems small in size (Figure 5).

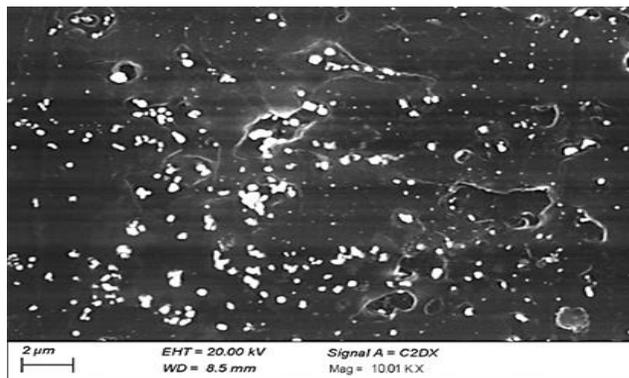


Figure 5. SEM image of variable sizes of silver nanoparticles of *Crotalaria burhia*.

Antioxidant Activity

Diphenyl-1-Picrylhydrazyl (DPPH) Method

Antioxidant effect of *Crotalaria burhia* plant extract was measured by DPPH free radical scavenging assay method. Vitamin C (Ascorbic acid) was used as standard in this method. The results showed that it possess antioxidant potential (Table 4). The %age inhibitory effect of *Crotalaria burhia* extract was 80.16 ± 0.21 while that of standard was 92.15 ± 0.17 (Figure 6).

Table 4. Scavenging Potential of *Crotalaria burhia* Plant Extract.

Scavenging potential of <i>Crotalaria burhia</i> plant extract		
Concentration (mg/ml)	Scavenging potential (%)	IC ₅₀ (mg/ml)
5.00	80.16	1.79
2.50	65.24	
1.25	40.23	
0.75	25.33	

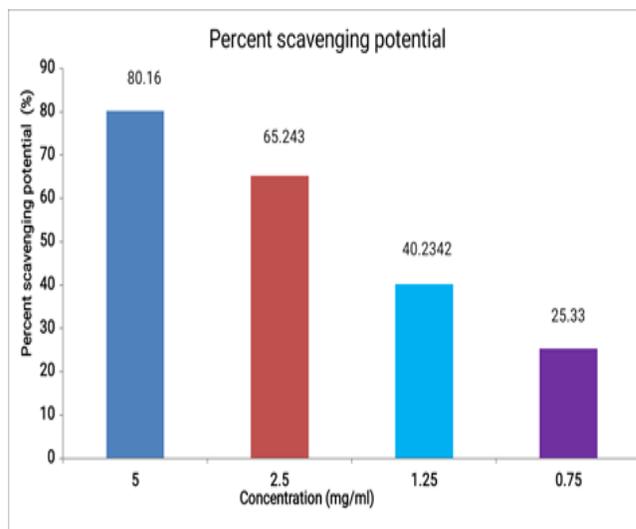


Figure 6. Percentage scavenging potential of *Crotalaria burhia* plant extract

Antibacterial Activity

The minimum inhibitory concentration of optimized formulation of standard was 2.1 mg/ml against *E. coli* and 2.4 mg/ml against *S. aureus* while the MIC value of silver nanoparticles was 2.5 mg/ml and 3.1 mg/ml against *E. coli* and *S. aureus* respectively (Table 5).

Table 5. MIC Values of Standard, Silver Nanoparticles, AgNO₃ and *Crotalaria burhia* Plant Extract.

Bacterial strain	Standard Ciprofloxacin (mg/ml)	Silver nanoparticles (mg/ml)	Control (mg/ml)	
			AgNO ₃	<i>C. burhia</i> extract
<i>E. coli</i>	2.1	2.5	4.3	6.9
<i>S. aureus</i>	2.4	3.1	5.4	7.2

The MIC value of silver nanoparticles was a little bit higher than standard (Ciprofloxacin).

DISCUSSION

Crotalaria burhia is a medicinal plant which is rich in glycosides, flavonoid, alkaloid, antioxidants and carbohydrates. Previous studies reported that *Crotalaria burhia* have diverse pharmacological potential such as anticancer, analgesic, anti-inflammatory anti-oxidant and anti-microbial activities [15]. The green synthesis of Ag NPs (silver nanoparticles) includes the use of bacteria, fungi, algae, yeast and/or plant extracts for the reduction of silver [16]. Silver nanoparticles have potent antibacterial activity [17]. The bactericidal activity of Ag NPs without causing toxicity to human cells, can make them a proper substitution for antibiotics [10].

The aim of this research study is the green synthesis and characterization of silver nanoparticles by using crude extract of *Crotalaria burhia* plant extract and to evaluate the antibacterial potential of silver nanoparticles. Abdel-Aziz and his colleagues also confirmed the antioxidant and antibacterial activity of silver nano particles of *Chenopodium morale* leaf [18]. Antibacterial activity of silver Nano particles of many plants have been confirmed like *Cestrum nocturnum* [19], *Rhazya stricta* [20], *Ferula gumosa*, *Ferula latisecta*, *Teucrium polium* and *Trachomitum venetum* leaves and stems extract [21], *Zea mays* [22] and *Malus domestica* [23] etc.

In the 21st century, the use of small sized nanoparticles is a best approach for the delivery of active drug to cure different diseases, For this reason,

the pharmacists, drug delivery scientists and researchers shift their focus on developing a novel drug delivery system for herbal medicines [6].

In our study different formulations of our plant extract yielded different sized silver nanoparticles. It all depends on the concentration of plant extract. High concentration of plant extract causes agglomeration of silver nanoparticles which cause big particles size [24]. The small size of silver nanoparticles increases the antibacterial potential of silver. The small size allows them to permeate into the bacterial membrane. The small sized silver nanoparticles have large surface to volume ratio area so they can interact with bacteria easily and can provide potent bactericidal activity [25]. Thus, in our study the optimum formulation of silver nanoparticles whose particle size was 92nm and zeta potential was -24.8 mV showed high antibacterial activity against both bacteria *E. coli* and *S. aureus*. It was observed that MIC for *E. coli* was low as compared to *S. aureus*. As reported in previous studies, the AgNPs have no significant antibacterial activity against *S. aureus* because it has thick peptidoglycan layer in its cell wall. This thick layer of peptidoglycan cause difficulty in absorption of silver nanoparticles into bacterial cytoplasm [17]. So, *E. coli* is less susceptible to *C. burhia* plant extract and its silver nanoparticles.

CONCLUSION

This study showed that these silver nanoparticles have potent antibacterial activity against *E. coli* and moderate antibacterial activity against *S. aureus*. Hence, it has been concluded that these nanoparticles can be used against multi drug resistant bacteria. In the future, the antitumor effect of these silver nanoparticles can be assessed by performing cytotoxic studies on different cancer cell lines.

Conflict of interest

The authors declare that there are no conflicts of interest.

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