# Green Synthesis of Silver Nanoparticles using Medicinal Plants *Berberis asiatica* and *Cassia fistula* and Evaluation of Antioxidant and Anti-bacterial Activities

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**CONFILICT OF INTEREST:** None



## ABSTRACT

The application of silver nanoparticles in various sectors including health related field is remarkably profound. Nowadays, the research of synthesizing metal nanoparticles (MNPs) using plant extracts is fascinating field as it offers the eco-friendly and cost-effective method for nanoparticle synthesis. In this study, we synthesized silver nanoparticles (AgNPs) using methanolic extract of *B.asiatica* and *C. fistula* regarding their ethnomedical importance.The synthesized AgNPs were characterized by UV-Visible spectroscopy, Fourier transform infrared spectroscopy (FTIR) and X-ray diffractometer (XRD). UV-vis spectroscopy exhibited the characteristic Surface Plasmon Peak of silver nanoparticle~420 nm.FTIR data were measured to get a preliminary idea on the functional groups responsible for the stabilization of AgNPs. XRD data confirmed the natural crystal structure with a face centered cubic of AgNPs. The antibacterial activity of biosynthesized AgNPs was assessed by testing promptly available gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* bacterial strain and antioxidant activity was calculated by DPPH assay. The overall outcomes of the studies concluded that the application of the biogenic synthesis of AgNPs of *B. asiatica*as an antioxidant and antibacterial agent is more potent showing $IC_{50}$ value 65.1±1.30 µg/mL and the highest zone of inhibition 15 mm in diameter against *S. aureus*.

**Keywords:** *Berberis asiatica, cassia fistula*, biosynthesis, silver nanocolloid, anti-bacterial potential

## 1. INTRODUCTION

Pharmaceutical industries have been using metal nanoparticles (MNPs) tremendously in the manufacturing of ointments and creams to get relief from burns and wounds associated with infections (Satyavani *et al*. 2011). Synthesizing MNPs using plant extracts is the most adequate biosynthetic method because of being eco-friendly and cost-efficient. Currently, several plant parts like leaf, fruit, stem, bark and seed extracts in different solvents are being productively applied for the synthesis of MNPs (Mittal *et al*. 2013). In the ratio of other MNPs, silver nano-particles (AgNPs) are more demanding due to their strong inhibitory activity against a number of microorganisms (Yugal Kishore Mohanta & Behera 2014). Having significant anti-microbial activity, the phyto-mediated AgNPs are widely implemented in food packaging, food and seed preservation, bio-fertilizers, cosmetics, medicines and so on (Marambio-Jones & Hoek 2010; Dipankar & Murugan 2012). The implication of AgNPs in the control of microbial and treating cancer as potential drug carrier has presently earned substantial attention (Nayak *et al.* 2016).

Several established protocols are available to synthesize AgNPs viz. chemical and photochemical reaction in reverse micelles, thermal decomposition of silver compound, radiation based, microwave-assisted process, sono-chemical process and currently becoming familiar "green chemistry technology" (Iravani *et al*. 2014). Green synthesis is more significant in the comparison of physical and chemical approaches because of being cost effective and environmentally compatible and problem free while scaling up for producing in large quantity without applying high pressure, energy, temperature and toxic chemicals (Kharissova *et al.* 2019). In the green synthesis of AgNPs, the two methods either using biological microorganisms or plant extracts based have been expended but phyto-mediated is more advantageous as it doesn't necessitate the tedious processes of preserving the cell cultures and aseptic conditions (Loo *et al*. 2018).

The articles describing different methods applied in the green synthesis of AgNPs by using plant extracts have been published in the renewed journals. However, there is still a demand for such procedures which are environmentally friendly, commercially survival and economically sustainable to synthesize plant based AgNPs (Beyene *et al*. 2017). Regarding current trends in the synthesis of AgNPs, using plants and plant based products are being a fascinating research domain (Khandel *et al.* 2018). A variety of ethno-medicinal plants are immensely used in the production of herbal medicine and their bioactive compounds are demanded in extremes for healthcare and nutritional products as a principal ingredient. In our study, *B. asiatica* and *C. fistula* were chosen concerning their medicinal values after literature survey. Berberis genius is massively used in many medical fieldssince ancient periods for the treatment of wound healing, eye disease fever, kidney and gall balder stones, jaundice, vomiting during pregnancy, rheumatism, and several other illnesses. Several bioactive compounds found on the roots and leaf of *B. asiatica* areBerberine, Colunbamamine, Jatrrohirine, Karachine, Oxyacanthine, Oxyberberine, Palmitine, Taxilamine and Tetrahydropalmitine (Bhardwaj & Kaushik 2012). In Ayurveda, *B. asiatica*is used singly or in combination with other medicinal plants for healing a variety of ailments such as enlargement of spleen, leprosy, rheumatism, fever, morning or evening sickness and snakebite (Belwal *et al.* 2017). AgNPs synthesized from the steam bark extract of *B. asiatica* showed potent antioxidant and antimicrobial activities (Bhandari *et al.* 2000).Thus we use the leaf of *B. asiatica* for the synthesis of AgNPs. The AgNPs synthesized using India dwelling *C. fistula* has established effective results for antioxidant, antimicrobial and anticancer activities (Mohanta *et al.* 2016).No further literatures are available describing antioxidant and antibacterial activities and the green synthesis of AgNPS using Nepal inhabiting *C. fistula* medicinal plant. So, we synthesized AgNPs using *C. fistula* and reported its antioxidant and antibacterial activities for the first time.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Plant specimens were collected from Kailali and Gandaki districts of Nepal in October 2018. Table 1 contains descriptive information of the plants used in the study. Chemicals and reagents consumed in the experiment are of molecular grade. Water used during experiment was PCR-grade obtained from Milli-Q water purification. Origin Pro 18 and Graph Pad Prisma 8 software were used for data analysis.

**Table 1** Details of the medicinal plants used in the experiment

| Scientific Name | Local name | Locality | Altitude | Parts used | % yield (Methanol) |
|---|---|---|---|---|---|
| *Berbarisasiatica* | Chuthro | Low camp, Mardi Himal | 3200 m | Leaves | 15.5 |
| *Cassia fistula* | Rajbrikshya | Dhangadi, Kailali | 109 m | Leaves | 12.8 |

### 2.2 Extract Preparation

The medicinal plants were collected (Table 1) and washed thoroughly with distilled water then dried at room temperature for a month. The dried plants material was pulverized by a grinder and extracted in methanol by soxhlet extraction process. Thus obtained extract was concentrated using a rota evaporator and made completely dried keeping at room temperature. The extract was stored at -20 °C for further studies.

### 2.3 Biosynthesis of Silver Nanoparticle

A mixture was prepared by adding 100 mg of plant extract to 100 mL of 1mM silver nitrate solution. The color of the mixture was changed from brown to yellow after incubating at the room temperature for a day. The reduction of Ag+ was examined by scanning spectrophotometer and sample absorbance was taken in the range of 350-700 nm at minimum scanning rate. Distilled water (RO) taken from Milli-Q was used as the blank reference in the

baseline correction of the spectrometer. After 24 hours, the prepared AgNPs was centrifuged at 9000 rpm for 20 min at 25°C (Ahmed *et al*. 2016). The supernatant liquid was discarded and obtained pellet was washed with distilled water. The purified AgNPs were dried in a hot air oven at 25-26 °C and stored in an eppendorf tube at 4°C until further requirement.

## 2.4 Characterization of AgNPs

### 2.4.1 *UV-Vis spectroscopy*

Green synthesized AgNPs solution in deionized water was monitored by using Agilent technologies, Cary-60 UV-Vis spectrophotometer. The absorption spectra of the nanoparticles were measured between 300 to 700 nm in a quartz cuvette taking water as reference for the base line correction (Elamawi *et al*. 2018).

### 2.4.2 *Fourier Transform-Infrared (FTIR) spectroscopy*

The FTIR spectrum of biosynthesized AgNPs was recorded in FTIR spectrometer to determine the presence of different functional groups found in the sample. Initially, the background screening of potassium bromide (KBr) was done. Then, a KBr pellet was made with AgNPs and scanned again. The spectra showed different frequencies and hence the frequencies were analyzed using infrared and Raman spectroscopy table. All measurements were carried out in the range of 400–4000 $cm^{-1}$ at a resolution of 4 $cm^{-1}$ by using IRTracer-100 FTIR spectrophotometer, Shimadzu to identify the present functional groups on the synthesized AgNPs.

### 2.4.3 *X-ray diffraction (XRD)*

The pellets of the phyto-mediated AgNPs was prepared in autoclaved Milli-Q water at 10,000 rpm for 15 minutes. Thus prepared pellets were oven dried at 50 °C to analyze the particle size by XRD. The nature of AgNPs was studied by using Bruker D2 Phaser Diffractometer, which was observed at 30 kV voltage and 10 mA current with a monochromatic Cu Kα radiation source(λ = 0.15418 nm) having 2θ angles ranging from 20° to 80°.

## 2.5 Antioxidant Activity

### 2.5.1 *Free radical scavenging ability on 2, 2-diphenyl-2-picrylhydrazyl (DPPH)*

Ascorbic acid in the concentration of 10, 20, 60, 80, 100, 120 and 150 µg/mL was prepared as reference and sample solutions of crude plant extract and AgNPs of the concentration 2000, 1500, 1000, 500, 250, 125 and 62.5 µg/mL were prepared in DMSO. DPPH solution of 0.01mM was made in absolute methanol.100 µL of 0.1mM DPPH solution was added to 100 µL of each concentration of the sample and ascorbic acid solution. The mixtures were prepared in 96 well plates and were kept in dark for 30 minutes. Similarly, 200 µL of 0.1 mM DPPH solution was used as control. Thirty minutes later, the absorbance was taken at 517 nm. The capability to scavenge the DPPH radical was calculated by using the following equation (Nagaich *et al.* 2016):

$$\text{Percentage Scavenging} = \times 100$$

$A_o$= Absorbance of DPPH solution and $A_t$= Absorbance of test or reference sample

The percentage scavenging was plotted against concentration and regression equation to obtain $IC_{50}$ (concentration required to inhibit DPPH radical formation by 50%) values of the samples.

## 2.6 Antibacterial Activity

### 2.6.1 *Bacterial strains*

Antimicrobial activity of the crude plant extract as well as biosynthesized AgNPs was evaluated by calculating zone of inhibition against both gram-positive *S. aureus (ATCC 25923)* and gram-negative *E. coli (ATCC 25922)*bacterial strains.

### 2.6.2 *Inoculation*

A pure colony of each bacterial strain was inoculated in the 5 mL nutrient broth till the growth was equivalent with MacFarland standard (0.5%) as recommended by WHO. The inoculated culture bottles were kept in the incubator at 37 °C for 3-4 hrs. The turbidity of the sub-cultured bacterial suspension was adjusted at freshly prepared 0.5 McFarland standards. These bacterial inoculums were used for swabbing on the MHA plates to test the antibacterial potential of the AgNPs.

### 2.6.3 *Agar well diffusion method*

The well diffusion method using Muller Hinton agar (MHA) medium was applied to calculate the antibacterial activity of both the crude extract and synthesized AgNPs samples. The required number of wells was built in MHA using cork borer (6 mm diameter). Inoculums containing $10^6$ CFU/mL of bacteria were spread with a sterilized cotton swab on the MHA medium plates. 20 µL each extract of the concentration 100, 50, 25 and 12.5mg/mL was added in the wells and same volume of extraction solvent i.e methanol and DMSO was used as negative control. 1mg/mL of streptomycin was taken as positive control. Similarly, 20 µL of AgNPs prepared from different aqueous plant extract was filled in the well where aqueous plant extract was taken as negative control and 1mg/mL of

streptomycin was taken as positive control. Plates were left till the extract diffused into the medium, covered the plate and incubated at 37 °C for 24 h. After overnight incubation, the diameter of the zone of inhibition (ZOI) in mm were measured (Regmi *et al.* 2019).The samples showing ZOI greater or equal to 8 mm in diameter were considered that the samples were effective as antibacterial agent.

## 3. RESULTS AND DISCUSSION

### 3.1 Plant Materials

Plants having high medicinal values used locally as a medicine for the remedy of the spectrum of illness were selected for the study. Bioactive compounds possessing pharmacological activity were extracted by using methanol as solvent through soxhlet extractor. The yield of *B. asiatica* was obtained in 15.5 % while *C. fistula* yield was 12.8 %. Climatic conditions, parts of the plant used, extraction time, temperature and extraction procedure play apivotal role in the isolation of bioactive compounds

### 3.2 UV-vis Spectra Analysis of AgNPs

An UV–visible spectrum is a well-known technique to prove the formation of metal nanoparticles provided surface plasmon resonance exists for the metal nanoparticles. Having free electors on metal nanoparticle produces the band of surface plasmon resonance (SPR) because of the high energy electronic vibration in the resonance. Changing color from yellow to brown informed the synthesis of AgNPs. The spectrum displays the vital role of $AgNO_3$ along with the presence of plant constituents in the formation of AgNPs. The AgNPs of plants *B. asiatica* and *C. fistula* extracts showed the UV absorption peak at the range of 400-450 nm. *B. asiatica*and *C. fistula* exhibited the peak at 450 nm and 445 nm respectively in the UV-Vis spectroscopy (Fig.1). The spectra demonstrated a gradual decrease of the absorbance from the wavelength from 450 to 380 nm.The occurrence of the silver SPR band in the range of 400-450 nm indicates the formation of AgNPs (El-Shanshoury *et al*. 2011;Umoren *et al*. 2014; Vijay Kumar *et al*. 2014).

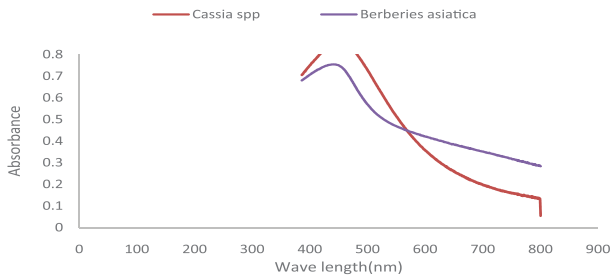


**Fig. 1.** UV-Vis spectra showing surface Plasmon resonace of biosynthesized AgNPs of the plants.

### 3.3 FTIR Spectroscopic Analysis

The AgNPs in powder form was used for the analysis of FTIR to verify the presence of functional groups which are essential for the reduction of AgNPs from the plant samples. FTIR analysis of the AgNPs was performed in the range of 4000-500 $cm^{-1}$. Upon performing FTIR, it was observed that the AgNPs showed the peak at the range of 3400-3700$cm^{-1}$due to the presence hydroxyl group (-OH) and at the range of 1690-1630 $cm^{-1}$shows the presence of carbonyl group (-C=O), which were mainly responsible for bio-reduction of silver nitrate and synthesis of its nanoparticles. The absorption spectrum of *B. asiatica*synthesized AgNPs (Fig. 2) recordedthe major peak at 3442 $cm^{-1}$ due to O-H stretching vibration in alcohol and phenol and the peak at 1633 $cm^{-1}$suggests the presence of carbony groups. The AgNPs of *C. fistula* showed the dominant peaks at 3441 $cm^{-1}$due to O-H stretching vibration in alcohol and phenol and at 1635 $cm^{-1}$due to the presence of carboxy groups.The Fig. 2 and Fig. 3 indicate the presence of many fundamental groups involved in conversion of silver ions to silver nanoparticles.

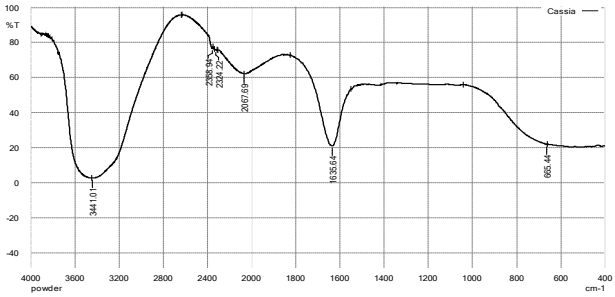


**Fig. 2.** FTIR spectrum of the AgNPs synthesized from *B. asiatica (chutro)*

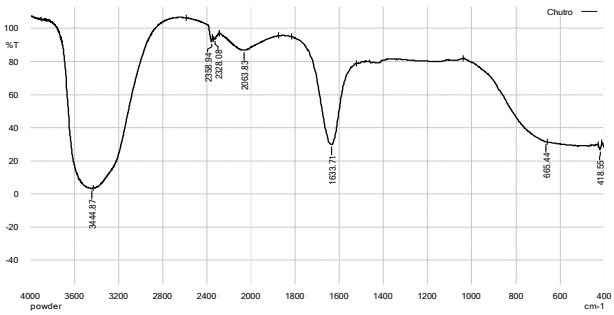


**Fig.3.** FTIR spectrum of the AgNPs synthesized from *C.fistula*

### 3.4 XRD Analysis

The crystallize size and structure of AgNPs was studied by using XRD. AgNPs of *B. asiatica* (Fig. 4) observed diffraction peak in XRD at $2\theta$ = 32.1, 38.3, 44.5, 64.5 and 77.5 and *C. fistula* (Fig. 5) demonstrated the dominant

peaks at 29.1, 38.1, 43.7, 64.3 and 77.3. These found XRD peaks confirmed that biosynthesized AgNPs nanocrystal and crystalline in sturcture where the peaks corresponded with standard. The peaks seen in XRD can be attributed to the planes (122), (111), (200), (220) and (311) facet of silver crystal respectively (Roy *et al.* 2015). From the XRD patterns, peak intensity, position and full width at half maximum (FWHM) were analyzed using Debye–Scherrer's formula to determine the crystallite size. Some peaks in the spectra couldn't assign due to the presence of some bioorganic compounds/protein(s) in the extracts and crystallizes on the surface of the silver metal (Philip 2011). The AgNPs particle in crystalline structure was calculated applying Debye–Scherrer's equation (Ajitha *et al*. 2014):

$D = 0.9 \lambda / \beta \cos\theta$ where, $\lambda$ = wavelength of the x-ray

$\beta$ = broadening of the diffraction line measured as half of its maximum intensity in radians and

$\theta$ = Bragg's diffraction angle

The crystalline size of the AgNPs was estimated from the line width of the (111) XRD peak of the both samples. The AgNPs particle size of *B. asiatica* and *C. fistula* was found 13 and 15 nm respectively.

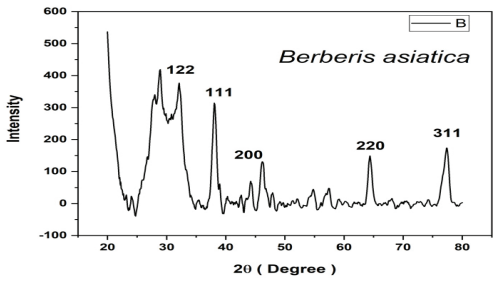


**Fig. 4.** XRD pattern of the AgNPs synthesized from *B. asiatica.*

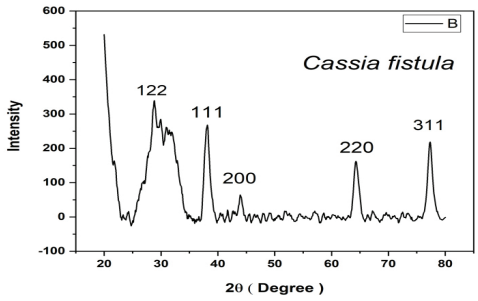


**Fig. 5.** XRD pattern of the AgNPs synthesized from *C. fistula.*

## 3.5 Antioxidant Activity of AgNPs

The biogenic synthesis of AgNPs using *B. asiatica* exhibited significant antioxidant potential having $IC_{50}$65.1±1.30µg/mL but crude methanolic extract showed130.4±0.12µg/mL. Similarly, the AgNPs of *C. fistula* had antioxidant activity of $IC_{50}$100.2±0.82**µg**/mL but respective crude extract possessed significantly higher $IC_{50}$ (Table 2). Many papers have been published reporting potential antioxidant activity of green synthesized AgNPs from *P. pinnate* (Priya *et al.* 2016) and *E. suberosa* (Yugal K. Mohanta *et al.* 2017). The results concluded that the implication of AgNPS as a natural antioxidant is more effective in the comparison of its crude extract for health protection from different oxidative stress linked with degenerative diseases. AgNPs can be used in animal models and also in clinical trial after evaluating their antioxidant activity.

**Table 2**. Antioxidant activity ($IC_{50}$) of AgNPs and crude extract of the medicinal plants.

| S.N. | Plant species | $IC_{50}$ MeOH extract (µg/mL) | $IC_{50}$ AgNPs (µg/mL) |
|---|---|---|---|
| 1. | *B. asiatica* | 130.4±0.12 | 65.1±1.30 |
| 2. | *C. fistula* | 170.5±1.12 | 100.2±0.82 |

## 3.6 Antibacterial Activity of AgNPs

Plant based AgNPs are used extensively in the field of medicine, health and environment. We examined the antibacterial potential of AgNPs by calculating the zone of inhibition against both gram-positive and gram-negative bacteria. The details are shown in Fig. 6 and Table 3.The maximum ZOI exhibited by the AgNPs of *B. asiatica* against*S. aureus was* 15 mm depicted in Fig. 6(A**)** and the minimum inhibition less than 8 mm was performed by the AgNPs of *R. australe against K. pneumonia* shown in Fig. 6(C). The findings indicated that the biosynthesized AgNPs is more effective in the comparison to the crude extract because AgNPs contains large surface area that facilitates ameliorating contact with bacterial cell wall (Ibrahim 2015). Many similar results were published previously explaining inhibition of AgNPs against *E. coli* using *Menthapiperita* (Mubarak Ali *et al*. 2011) and *Acalyphaindica* (Krishnaraj *et al.* 2010).

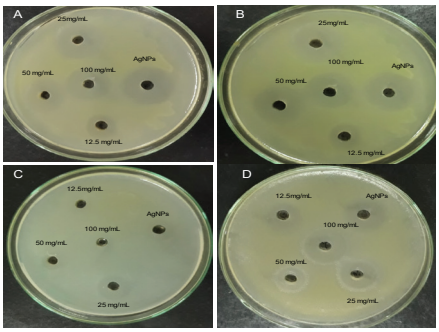


**Fig. 6.** Antimicrobial activity of different concentration of crude extract and AgNPs of *B. asiatica*

(A) *S. aureus*, (B) *E. coli* and *C. fistula* (C) *S. aureus* and (D) *E. coli*.

The mechanism behind the antimicrobial activity of AgNPs is established as the electrostatic attraction between the positive charges on the $Ag^+$ ion and the negative charges developed on the bacterial cell wall due to phospholipidsbilayers (Raut *et al.* 2010). The working process of AgNPs against microorganisms is really intriguing. In the first step, AgNPs attaches on the cell wall and membrane then penetrate inside the cells and start damaging inner structures of the microorganisms. In the third step, nanoparticles stimulate cellular toxicity and oxidative stress by producing reactive oxygen species. Finally, error is occurred in the signal transduction pathway and organism remains no longer alive (Tian *et al*. 2017).

**Table 3.** Zone of Inhibition (mm) of plant extracts and nanoparticles in gram positive (*S. aureus*) and gram negative (*E. coli*) bacteria.

| Plants | Concentration (12.5 mg/mL) | Zone of inhibition (mm) | |
|---|---|---|---|
| | | *S. aureus* | *E. coli* |
| *B. asiatica* | Crude | 9 | 7 |
| | AgNPs | 15 | 14 |
| *C. fistula* | Crude | 6 | 8 |
| | AgNPs | 7 | 12 |

## 4. CONCLUSION

The green synthesis of AgNPs were performed by using methanolic extract of medicinal plants *B. Asiatic and C. fistula*. The synthesized AgNPs were characterized by UV-Vis spectrophotometer demonstrating wavelength at 400-450 nm. FTIR data supported the presence of functional groups by appearing the peak at the range 3400-3700 $cm^{-1}$ of the hydroxyl group (O-H) and the peak at 1690-1630 $cm^{-1}$ of the carbonyl group (C=O). XRD analysis predicted that biosynthesized AgNPs nanocrystal and crystalline in sturcture were the sample peaks corresponded with standard. AgNPs prepared from *B. asiatica* showed more potent antioxiant activity ($IC_{50}$65.1±1.3**µg**/mL) than the others. AgNPs of *B. asiatica* demonstrated more effective antibacterial property against *S. aureus* (15 mm) among all others. Therefore, biosynthesized AgNPs is more valuable than their corresponding crude extracts. These green synthesized AgNPs would be applied in different medical sectors in near future.

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