Antioxidant and antibacterial activity of silver nanoparticles synthesized by Cestrum nocturnum

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Abstract

Background: Silver nanoparticles are toxic to bacteria and have widespread application in different research areas.

Objective: The aim of this study was to synthesize silver nanoparticles using an aqueous leaf extract of Cestrum nocturnum and to test its antioxidant and antibacterial activities.

Materials and methods: The silver nanoparticles were synthesized by addition of 20 ml extract (8% w/v) with 180 ml silver nitrate solution (1 mM). The synthesis of silver nanoparticles was confirmed by UV–Vis spectrophotometer. The silver nanoparticles were characterized by X-ray diffractometer, Transmission Electron Microscope, Scanning Electron Microscope and Fourier Transform Infra-Red spectroscopy. The antioxidant property of silver nanoparticles was analyzed by the 2, 2-diphenyl-1-picrylhydrazyl, hydrogen peroxide, hydroxyl radical and superoxide radical scavenging methods. The bacteriostatic and bactericidal activity of silver nanoparticles against Escherichia coli, Enterococcus fae-calis, and Salmonella typhi was determined using bacterial growth inhibition method. The antibacterial sensitivity and Minimum Inhibitory Concentration (MIC) of silver nanoparticles was determined against the bacteria.

Results: The results confirmed that the silver nanoparticles synthesized by C. nocturnum extract were crystalline in nature, average particle size was 20 nm and were mostly spherical in shape. The antioxidant methods confirmed that the silver nanoparticles have more antioxidant activity as compared to vitamin C. The silver nanoparticles have strong antibacterial (maximum Vibrio cholerae and minimum E. faecalis) activity. The MIC value of silver nanoparticles was 16 µg/ml (Citrobacter), 4 µg/ml (E. faecalis), 16 µg/ml (S. typhi), 8 µg/ml (E. coli), 8 µg/ml (Proteus vulgaris), and 16 µg/ml (V. cholerae).

Conclusion: Green synthesized silver nanoparticles have strong antioxidant and antibacterial activity due to the presence of bioactive molecules on the surface of silver nanoparticles.

Keywords: Cestrum nocturnum, silver nanoparticles, Green synthesis, Antioxidant, Antibacterial activity.

1. Introduction

Nanoparticles can be created by physical, chemical or biological method. Biological method holds better chances as it is environment friendly and economical. Biological method consists of using micro-organisms or medicinal plants for the production of nanoparticles. Using medicinal plants is advantageous as their medicinal properties are added to the nanoparticles during synthesis. Phyto-compounds of plants provide antioxidant property and the plants which have antibacterial activity provide additional antioxidant and antibacterial property to the nanoparticles [1].

From ancient times, silver has been used as an anti-microbial agent and silver-based compounds are much cheaper than gold based [2]. Further, silver nanoparticles (AgNPs) are non-toxic to eukaryotic cells including humans, but it has high toxicity against prokaryotic cells such as micro-organisms like bacteria, viruses, and fungi [3]. The AgNPs have unique chemical, optical, electrical, magnetic and mechanical properties. These unique properties of AgNPs are of interest to researchers in investigating its applications.
in nanomedicine such as anti-plasmodial [4], anti-microbial [5],
targeted drug delivery [6], sensing and imaging [7], anti-fungal [8],
anti-platelet [9], anti-cancerous [10] and wound healing [11]. The
advancement in the synthesis of silver nanoparticles has expanded
a strong impact in many scientific areas. Due to low yield and the
use of toxic compounds physical and chemical methods are not
suitable for the synthesis of silver nanoparticles respectively.
Micro-organism based AgNPs synthesis is also not preferred
because most of the microbes are pathogenic [12]. Several works
already reported that plants extracts used for the synthesis of
AgNPs such as Memecylonedule [13], Callicarpa mairgany [14],
Terminalia chebula [15], Trachyspermum ammi and Papaver somniferum
[16]. Bauhinia variegate L. [17], Hevea brasiliensis [18], Aloe vera [19]
and tea leaf [20]. Cestrum nocturnum belong to the family Sol-
anaceae. This plant contains bioactive molecules such as alkaloids,
flavonols glycosides, steroidal saponins, phenols, fatty acids and
essential oil [21]. The extract of C. nocturnum has been used in burn
and swelling, analgesic and bactericidal activity, local anesthetic
effect, inhibitory effect on the central nervous system, cardiac
arrhythmic, tumor inhibition and antioxidant activity [22]. Due to
the above said reasons present work designed to use C. nocturnum
leaves extract for the synthesis of AgNPs from silver nitrates and test
its antioxidant property and antibacterial activity against hu-
man pathogenic bacteria such as Citrobacter, Salmonella typhi,
Enteroccus faecalis, Escherichia coli, Proteus vulgaris and Vibrio
cholerae was evaluated. Further, Bacterial Growth Inhibition (BGI)
of AgNPs against E. faecalis, E. coli and P. vulgaris were determined.

2. Materials and methods

All the chemicals used were of analytic grades. Silver nitrate
(Sigma Aldrich, 99%), DPPH (SRL, 99%), Methanol (Molychem, 99%),
Vitamin C (Himedia, 99–100%), Hydrogen peroxide (Fischer Scien-
tific, 30%), NADH (SRL, 98%), PMS (SRL, 99%), NBT (SRL, 99%)
deoxyribose (SRL, 98%), FeSO4-EDTA (Sigma Aldrich, 99%), tri-
chloroacetic acid (Fischer Scientific, 98%), TBA (Himedia, 98%) and
NaOH (Fischer Scientific, 97%) were purchased. This study was
performed in collaboration of the Department of Biochemistry and
Department of Microbiology, Institute of Medical Sciences, Banaras
Hindu University, Varanasi, India.

2.1. Preparation of C. nocturnum extract

C. nocturnum leaves were procured from the botanical garden of
Banaras Hindu University, Varanasi, UP, India. The leaves were
dried and powdered using a grinder. The aqueous extract (8% w/v) of C.
nocturnum was prepared using 500 ml Erlenmeyer flask containing
8 g powder and 100 ml deionized water and heated at 70 °C using a
hot plate for 2 h. The extract was obtained by centrifuge the
mixture at 3000 rpm for 5 min followed the filtration using
Buchner funnel and Whatman no. 1 filter paper. These filtrates were
stored in the refrigerator for further use [23].

2.2. Synthesis of silver nanoparticles

20 ml extract was added to the 180 ml silver nitrate solution
(1 mM) in 250 ml volumetric flask and stirred twice times for 5 min
using a magnetic stirrer at room temperature. The change in color
of the solution after 1 week indicated the reduction of silver nitrate
into AgNPs. Then 2 ml aliquots were taken every 5 h till 24 h and
absorbance (λ, 200–600 nm) was analyzed using UV–Vis spec-
photometer (Systronics, AU-2701). After the completion of the
reaction, the solution was centrifuged at 5000 rpm for 15 min and
the pellet was collected. Then pellets were washed three times
using the 5 ml deionized water and centrifuged at 5000 rpm for
15 min. Further, the pellets were dried in hot air oven at 80 °C for 5 h [23].

2.3. Characterization of silver nanoparticles

The AgNPs were powdered and characterized by X-Ray
Diffraction (XRD), Transmission Electron Microscopy (TEM), Energy
Dispersive Spectrophotometer (EDS), Scanning Electron Micro-
scopy (SEM) and Fourier Transform Infra-Red spectroscopy (FTIR).
XRD (Bruker Advanced D8, Eco) was performed for the phase
identification of AgNPs. TEM (FEI, TECNAL G2 20 TWIN) was carried
out to find the structure and size distribution of silver nanoparticles
and EDS was performed to check whether the nanoparticles were
made of elemental silver or not and SEM (JEOL-MODEL 6390) was
used to analyze the shape and size of AgNPs. FTIR (Varian Excalibur
3000, Palo Alto, CA) was used to check the capping agents on the
surface of AgNPs.

2.4. Antioxidant activity

2.4.1. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method

The method was performed according to the Bhakya et al. (2016)
with slight modification [24]. The free radical scavenging activity
of AgNPs and standard vitamin C was determined using the stable
radical DPPH. 1 ml of different concentrations (10, 20, 30, 40, 50, 75
and 100 μg/ml) of AgNPs was mixed with 1 ml freshly prepared
DPPH (1 mM in methanol) solution and vortexed thoroughly. Then
the solution was incubated at room temperature in the dark for
30 min. The absorbance was recorded at 517 nm using UV–Vis
spectrophotometer (Systronics, AU-2701). DPPH (all the reagent
except the sample) was used as a control and methanol was used as
a blank solution. The free radical scavenging activity was expressed
as the percentage of inhibition which was determined using the
following formula [24],

\[
\% \text{ of scavenging} = \frac{P_c - P_s}{P_c} \times 100
\]  

(1)

where \(P_c\) is the absorbance of control and \(P_s\) is the absorption of
AgNPs/vitamin C.

2.4.2. Hydrogen peroxide scavenging activity

This method was performed according to the Keshari et al., 2016
[25], 0.1 ml AgNPs (25–250 μg/ml) in phosphate buffer (50 mM,
\(pH = 7.4\)) was mixed with 0.3 ml phosphate buffer (50 mM,
\(pH = 7.4\)) and 0.6 ml hydrogen peroxide solution (2 mM H2O2 in
phosphate buffer, 50 mM, \(pH = 7.4\)). The mixture was vortexed and
after 10 min the observance was recorded at 230 nm using UV–Vis
spectrophotometer (Systronics, AU-2701). Vitamin C was used as
standard while phosphate buffer (50 mM, \(pH = 7.4\)) was used as
blank. The percentage of hydrogen peroxide scavenging activity
was calculated using Formula 2: [25]

\[
\text{Scavenging} (\%) = \frac{O_c - O_s}{O_c} \times 100
\]  

(2)

where \(O_c\) represents the absorbance of control (all the reagent
except the test sample) and \(O_s\) absorbance of AgNPs/Vitamin C.

2.4.3. Hydroxyl radical (OH·) scavenging activity

The OH· radicals scavenging activity was determined according
to the Keshari et al., 2016 [25], 0.075 ml AgNPs (25–250 μg/ml in
methanol), 0.45 ml sodium phosphate buffer (200 mM, \(pH = 7.0\),
0.15 ml deoxyribose (10 mM), 0.15 ml FeSO4–EDTA (10 mM),
0.15 ml H2O2 (10 mM) and 0.525 ml deionized water were mixed.
The mixture was kept in the digital incubator for 4 h. The reaction was stopped by the addition of 0.75 ml trichloroacetic acid (2.8%) and 0.75 ml TBA (1% in 50 mM NaOH). Then the solution was kept in boiling water bath for 10 min and cooled using tap water. The absorbance of the solution was determined at 520 nm. Methanol was used as blank while vitamin C was used as a standard. The percentage of hydroxyl radical scavenging activity was calculated using Formula 3: \[ \text{Scavenging (\%)} = \frac{H_c - H_s}{H_c} \times 100 \] (3)

whereas \( H_c \) is the absorbance of control (all the reagent except the test sample) and \( H_s \), absorbance of AgNPs/vitamin C.

2.4.4. Superoxide (\( O_2^- \)) radical scavenging activity
This activity of AgNPs was determined according to Keshari et al., 2017 [25]. Superoxide radicals are generated by the oxidation of NADH in Nicotinamide adenine dinucleotide (NADH) – phenazine methosulphate (PMS) system and analyzed by the reduction of Nitro blue tetrazolium (NBT). 0.2 ml AgNPs (100–500 \( \mu \)g/ml in methanol), 1 ml Tris–HCl buffer (16 mM, pH = 8), 1 ml NBT (50 \( \mu \)M), 1 ml NADH (78 \( \mu \)M) and 1 ml PMS (10 \( \mu \)M) were mixed and kept for 5 min at 25 °C. The absorbance was recorded at 560 nm using UV–Vis spectrophotometer (Systronics, AU-2701). Vitamin C was used as standard and prepared same as AgNPs. The inhibition percentage of superoxide generation was calculated using Formula 4: \[ \text{Inhibition (\%)} = \frac{Sc - Ss}{Sc} \times 100 \] (4)

whereas \( Sc \) is the absorbance of control (all the reagent except the test sample) while \( Ss \) is the absorption of AgNPs/vitamin C.

2.5. Antibacterial activity

2.5.1. Analysis of anti-microbial sensitivity
The antibacterial activity of AgNPs, extracts and silver nitrates was determined against the \textit{Citrobacter}, \textit{S. typhi}, \textit{E. faecalis}, \textit{E. coli}, \textit{P. vulgaris} and \textit{V. cholerae}. Pure cultures of bacteria were sub-cultured on agar-solidified Luria broth (LB) medium. The bacteria were swabbed onto the agar plates using a swab. Then extracts, AgNPs and AgNO\(_3\) were dropped on sterile discs while deionized water was used as a control. Then the plates were incubated overnight in the incubator at 37 °C. The inhibition zone around dropping was measured using an ordinary scale [26].

2.5.2. Analysis of minimum inhibitory concentration (MIC)
The MIC of different compounds (AgNPs and AgNO\(_3\)) was determined using broth microdilution method. 0.5 McFarland’s bacterial suspension was poured into each microtiter plates. Then different concentration (0, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, and 256 \( \mu \)g/ml) of AgNPs and AgNO\(_3\) was used against the selected bacteria. The bacterial strains were incubated with AgNPs by two folds serial dilution and kept at 37 °C for 24 h [27].

2.5.3. Analysis of bacterial growth inhibition
The role of AgNPs on bacterial growth was determined in LB media. Few bacterial cells (\textit{E. coli}, \textit{S. typhi} and \textit{E. faecalis}) were inoculated in the LB media and kept at 37 °C, 250 rpm for overnight. Then culture was mixed with fresh LB media to maintain the OD 0.1 at 600 nm (OD, 0.1 represents \( 10^8 \) cells/ml). Then equal amounts (50 \( \mu l \)) of each concentration of AgNPs (0–100 \( \mu \)g/ml) and fresh bacterial culture was mixed in a microtiter plate. The absorbance was recorded at 600 nm using an Elisa reader (Thermo scientific, Multiskan Ex) every hour for 6 h [27].

3. Results

3.1. Formation of silver nanoparticles
The formation of nanoparticles started after the mixing the extract with the silver nitrate solution. The visible color changes was recorded at 600 nm using an Elisa reader (Thermo scientific, Multiskan Ex) every hour for 6 h [27].
(light yellow to dark brown) of the solution and spectral analysis confirmed the formation of silver nanoparticles. The absorption band of AgNPs was observed at 442 nm due to the Localized Surface Plasmon resonance (LSPR) (Fig. 1A and B).

3.2. X-ray diffractometry

The peaks at the $2\theta$ values 38.06, 44.23 and 67.43 represented the planes at 111, 200 and 220 respectively. These planes confirmed the AgNPs synthesized by extract was crystalline in nature (Fig. 2).

3.3. Transmission electron microscopy

TEM results confirmed the synthesized AgNPs were spherical in shape (Fig. 5A) and the size of AgNPs ranged from 5 to 45 nm. The average mean size of AgNPs was 20 nm (Fig. 5C). The diffraction pattern confirmed the formation of metallic AgNPs (Fig. 4B). EDS spectrometer analysis confirmed the presence of silver signals. The silver signal confirmed the presence of elemental silver (peak, 3 KeV) due to localized surface plasmon resonance (Fig. 4D).

3.4. Scanning electron microscopy

The SEM results confirmed that the shape of AgNPs was spherical and variable in size. The size of AgNPs was ranged from 15 to 28 nm (Fig. 4).

3.5. Fourier Transform Infra-Red spectroscopy

FTIR data of AgNPs provided different absorption peaks which assigned the different functional group of phyto compounds. The absorption peaks at 3477, 3348, 2917, 2346, 2208, 2003 and 1656 cm$^{-1}$ were assigned the O-H stretch of phenolic compounds, N-H stretch of primary and secondary amines and amides, C-H stretch of methyl groups, H-C=O stretch of aldehydes, C=N stretch of nitriles, C=C stretch of alkenes, C=O stretch of carbonyl groups of flavonoids and tannins respectively (Fig. 3).

3.6. Antioxidant activity

3.6.1. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method

The results confirmed the AgNPs and vitamin C has the antioxidant activity. The AgNPs has 29.55% antioxidant activity while vitamin C has 24.28% antioxidant activity. The results confirmed the
3.6.2. Hydrogen peroxide scavenging activity
The results confirmed the AgNPs has 45.41% hydrogen peroxide scavenging activity while the standard vitamin C has 65.63% hydrogen peroxide scavenging activity. This result proved the vitamin C has strong hydrogen peroxide scavenging activity as compared with AgNPs (Fig. 6).

3.6.3. Hydroxyl radicals scavenging activity
The results proved the AgNPs has 20% hydroxyl radical scavenging activity and vitamin C has 9.47% hydrogen peroxide scavenging activity. The results showed the AgNPs has strong hydrogen peroxide scavenging activity as compared with standard vitamin C (Fig. 6).

3.6.4. Superoxide scavenging activity
The results confirmed the AgNPs has 8% superoxide scavenging activity while standard vitamin C has 32% superoxide scavenging activity. The results indicated the vitamin C has greater superoxide scavenging activity when compared with AgNPs (Fig. 6).

3.7. Anti-microbial activity of silver nanoparticles

3.7.1. Screening of anti-microbial activity
The results confirmed that the antibacterial activity was present in AgNPs, AgNO₃ and no antibacterial activity was observed in the extract. The zone of inhibition confirmed the AgNPs has greater antibacterial activity as compared with standard vitamin C (Fig. 6).

3.7.2. Minimum inhibitory concentration (MIC)
The results proved the MIC value of AgNPs against selected bacteria was varied and this variability depends upon the bacterial strains. The MIC values of AgNPs against bacteria were 16 μg/ml (Citrobacter), 4 μg/ml (E. faecalis), 16 μg/ml (S. typhi), 16 μg/ml (E. coli), 8 μg/ml (P. vulgaris) and 8 μg/ml (V. cholerae) (Fig. 8).

3.7.3. Bacterial growth inhibition (BGI)
The BGI results confirmed the AgNPs has bacterial growth inhibition property. When the variable concentration of AgNPs (0–100 μg/ml) was used against the E. coli, E. faecalis and S. typhi bacteria, the slope of bacterial growth was decreased (Fig. 9).

4. Discussion
The present work explains the synthesis of AgNPs using C. nocturnum leaves extract. When the extract was added to the silver
nitrate solution the color of the solution was changed from light yellow to dark brown color. The dark brown color of solution confirmed the reduction of silver nitrates into the silver nanoparticles. Further, the formation of silver nanoparticles was confirmed by the UV–Vis spectrophotometer (λ, 300–700 nm). The absorbance band at 442 nm was recorded due to localized surface plasmon resonance (LSPR) and confirmed the formation of silver nanoparticles (Fig. 1). However, lack of LSPR suggests the formation of ultrasmall silver nanoparticles or the silver cluster, which contains a small number of atoms [28]. The synthesis of AgNPs by green route becomes popular because of no use of toxic chemicals, cheap, eco-friendly and suitable for pharmaceutical and biomedical applications [15]. Recently, several kinds of literature proved that bacteria have been resistant to antibiotics and alternative antibiotics required [29]. The XRD results confirmed the formation of crystalline silver nanoparticles (Fig. 2). The SEM results proved that the variable size silver nanoparticles were synthesized (15–28 nm) (Fig. 4). The TEM results confirmed silver nanoparticles was spherical and few are oval in shape, and silver nanoparticles are variable in size and maximum 20 nm size silver nanoparticles were synthesized. The EDS results confirmed the presence of elemental silver and EDS signal between the 3 KeV confirmed the elemental silver [14] (Fig. 5). The bioactive compounds of the extract have been responsible for the reduction and formation of silver nanoparticles [30]. FTIR results confirmed the various functional group presents on the surface of bioactive compounds. This functional group is responsible for the capping of silver nanoparticles and stable in nanosize (Fig. 3). The DPPH, Hydrogen peroxide, hydroxyl radicals, superoxide scavenging methods confirmed the silver nanoparticles has antioxidant, hydrogen peroxide, hydroxyl radicals and superoxide scavenging activities (Fig. 6). These properties of silver nanoparticles occur due to the presence of functional groups on the surface of silver nanoparticles. The results of antibacterial sensitivity confirmed the silver nanoparticles and silver nitrates have antibacterial activity. The AgNPs have the maximum zone of inhibition against the *Vibrio cholerae* (41 mm) and...
minimum against E. faecalis (15 mm) bacteria (Fig. 7, Table 1). The MIC results proved the MIC value of AgNPs against the Citrobacter, E. faecalis, S. typhi, E. coli, P. vulgaris and V. cholerae bacteria were 16, 4, 16, 16, 8, 8 and 16 µg/ml respectively (Fig. 8). Furthermore, the BGI result confirmed the bacteriostatic and bactericidal activity of AgNPs against the E. faecalis, E. coli and P. vulgaris bacteria and AgNPs behaves dual behavior as bacteriostatic in lower concentration and bactericidal at higher concentration.

5. Conclusion

Present work describes the formation of silver nanoparticles with the help of C. nocturnum extract. The leaves of C. nocturnum have bioactive compounds which responsible for the reduction and capping of silver nitrates into silver nanoparticles. The capping agent provides stability to the AgNPs. The synthesized AgNPs has antioxidant, hydrogen peroxide, hydroxyl radicals, and superoxide scavenging activity. This activity occurs due to the presence of functional groups on the surface of AgNPs. Moreover, AgNPs have strong antibacterial activity against the selected bacteria due to the small size and presence of capping agents. These silver nanoparticles might be used as antibiotics in future due to non-toxic, cheap, eco-friendly and highly effective against the bacteria.

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Conflict of interest

None.

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