ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

A Biomimetic Approach for Synthesis of Silver Nanoparticles using *Murraya paniculata* Leaf Extract with Reference to Antimicrobial Activity

Vikas Shrivastava, Pallavi Singh Chauhan and Rajesh singh Tomar*

Amity Institute of Biotechnology, Amity University Madhya Pradesh, Gwalior (Madhya Pradesh), Pin code: 474005

Abstract

Aim:

The present study aims to employ use of plants as a source of synthesizing silver nanoparticles with less hazards towards environment, easily scaled up, stable and economically viable.

Method:

The Silver nanoparticles were synthesized by using *Murraya Paniculata* leaf extract. The biosynthesized nanoparticles are then allowed to characterize by means of visible observation, UV Spectroscopy, SEM and TEM analysis. Inorganic nanoparticles is known to have potent antibacterial activity, thus in our study this property of nanoparticles were exploited to inhibit growth of clinically isolated micro-organisms i.e. *B.subtilis* and *P.vulgaris* which were characterized by various biochemical tests. The *in vitro* test was performed to calculate the zone of inhibition and MIC of the particles against isolated micro-organisms.

Result:

The result obtained clearly demonstrate the synthesis of spherical nanoparticles with size less than 100 nm. Also the biosynthesized nanoparticles showed good antibacterial activity against *B.subtilis* and *P.vulgaris.*

Conclusion:

The present study provides new insight into the synthesis of stable silver metal nanoparticles with less hazards along with exploring its antibacterial activity in the development of a potential tool in biomedics.

Key Words: Biosynthesis, *Murraya Paniculata*, silver nanoparticles, characterization, antibacterial activity, *B.subtilis, P.vulgaris.*

INTRODUCTION

In the present scenario nanotechnology is encompassing multiple applications in various fields. The area has proven to be merge of physico-chemical, biological and engineering sciences. In comparison to the bulk material the particles at nano level have high surface to volume ratio and thus known to have added advantage in various tasks such as bio-absorption, diffusivity, drug targeting, drug delivery etc.

The materials at the nano-range have specific properties, hence this field is attracting focus of researchers towards them. Due to the versatility of inorganic nanoparticles there exploration in the biomedical field is the prime concern of various laboratories. The inorganic nanoparticles have unique features like wide availability, controlled drug release etc. [1]

There are various studies available for the synthesis of nanoparticles by chemical and physical route, but the process usually exploits time and energy also they are hazardous to the environment. [2] Few studies have been recorded for the asymmetric shape of nanoparticles by physical means of synthesis. Green synthesis of nanoparticles by means of plants is considered to be as widely acceptable technology for synthesizing nanoparticles with less hazard and good stability (do not show agglomeration). [3] The green synthesis of nanoparticles is considered to be as bottom up approach and the plant extract having good phytochemical composition with antioxidant activity are basically chosen for the synthesizing purpose of nanoparticles. [4] It is a prerequisite to dissolve metal salt solution in water free of salinity and contamination, because the presence of Ca and Mg ions may enhance the agglomeration rate of the particles within the solution.[5]

The studies on green synthesis of nanoparticles demonstrated the use of plants and microbial species [6-11] for the stable nanoparticles synthesis with n numbers of its application in various fields. Thus the biological means of nanoparticle synthesis is the prime focus of researchers. Among various applications of nanoparticles the antimicrobial activity of the nanoparticles are the most promising area of the research now a day's [12-15]. This attempt is to exploit phytochemicals of plants in synthesizing inorganic nanoparticles for exploring its usability in various applications such as antibacterial activity etc.

MATERIALS AND METHODS

A. Materials

For synthesizing silver nanoparticles, *Murraya paniculata* leaves were collected from Amity University campus, Madhya Pradesh, India. The extract of the chosen plant leaf was used for the synthesis purpose of nanoparticles. Silver nitrate is taken for the preparation of metal salt solution.

B. **Methods**

Preparation of the Extract:
Fresh leaves (approx. 20 grams) of the chosen plant were taken, washed thoroughly and then dried so as to remove the water from the outer surface. The leaves were then crushed in a motor pistil to make the fine paste and then transferred into an Erlenmeyer flask with 100ml of distilled water. Then allowed to mix thoroughly by keeping it on a gel rocker for approximately 3 hours.

Phytochemical screening of the plant extract:
The extract was then subjected to qualitative phytochemical screening, which was done to understand the phytochemical constituents of the plant extract chosen for study. For this some specific tests were performed to evaluate the presence of particular phytochemicals. The tests performed for checking the availability of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins.[16]

Synthesis of Nanoparticles:
0.01 M solution of AgNO3 was prepared in 100 ml of distilled water. The solution then allowed for mixing with 10 drops of plants extract on magnetic stirrer. The solution soon began to change the colour from foggy white to chocolaty brown. After one hour of proper mixing the solution is then allowed to take wavelength reading by means of UV Visible spectrophotometer. [17] The reading is then followed by centrifugation of the solution with 16000 rpm for 6-10 minutes. After centrifugation the pellets were collected and washed by means of various solvents. The samples are then allowed to dry over dry bath.

Analysis of Silver nanopaticles:
The analysis of the synthesized nanoparticles was done by means of visible observation, UV-Visible Spectroscopy, TEM, SEM etc.

Isolation and identification of the microbial flora:
The microbial flora was isolated from the clinical samples collected from J.H., Gwalior (M.P.). Two potent bacterial colonies appear in the culture plates named as culture 1 and culture 2. The identification of the micro-organisms was done by means of various bio-chemical tests performed in the laboratory.[18-19]

Antibacterial Analysis:
The antibacterial activities of the synthesized nanoparticles were evaluated by agar well diffusion method against micro-organisms isolated from clinical samples.

MIC detection:
The minimum inhibitory concentration of the nanoparticle was detected by means of well diffusion method against *B.subtilis* and *P.vulgaris.*

RESULT AND DISCUSSION

Qualitative analysis of phytochemicals: The plant extract chosen for study showed the presence of the following phyto chemicals, discussed in the table 1:

S.no.	Phytochemicals	Aqueous leaf extract of *Murraya koenigii*
1.	Steroids	+
2.	Alkaloids	+
3.	Flavonoids	-
4.	Triterpenoids	-
5.	Tannins	+
6.	Saponins	+
7.	Phenol	+
8.	Quinone	-
9.	Coumarin	+
10.	Protein	+
11.	Sugar	+
12.	Gum	+

Table 1: Phytochemical screening of *Murraya Paniculata* leaf extract

Visible analysis: Silver nitrate solution has turned dark brown or dark reddish in colour, which indicated the formation of Ag NPs (Fig. 1). This colour change is due to the property of quantum confinement which is a size dependent property of nanoparticles which affects the optical property of the nanoparticles.

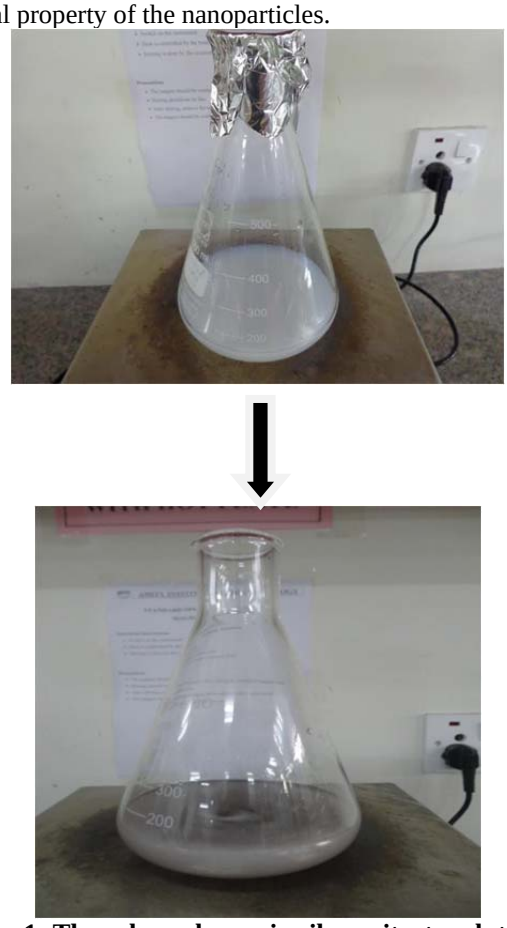
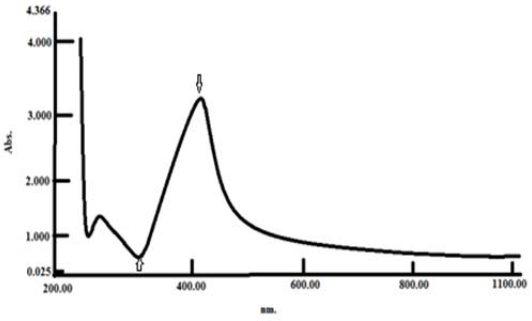


Fig. 1: The colour change in silver nitrate solution treated with *Plant* leaf extract

UV analysis: The UV absorption peak of silver nanoparticles range from 400 nm – 450 nm, UV-Vis spectra shows the peaks approximately at 410.00 nm (Fig. 2), clearly indicating the formation of Ag NPs by the plants extracts.



Measurent properties
Wavelength Range (nm): 220.00 to 1100.00
Scan Speed: Medium
Scnning Interval: 0.5
Auto Sampling Interval: Enabled

S.no	p/v	Wavelength	Abs.	Description
1.	⇩	412.00	3.135	
2.	⇧	336.50	0.286	

Fig. 2: The UV–Visible absorption spectra recorded from the silver nanoparticles solution after 2.5-3 hour of reaction.

SEM analysis: SEM analysis shows that the plant leaf extract have tremendous capability to synthesize silver nanoparticles which were roughly spherical in shape and are below 100 nm (Fig. 3).

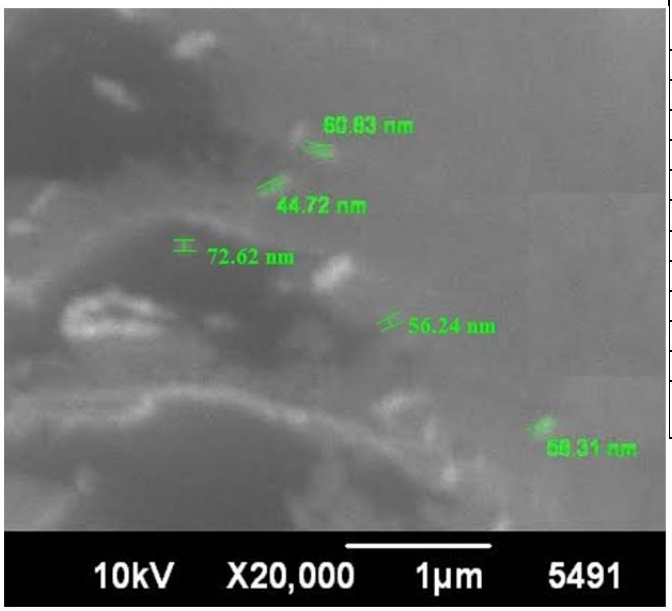


Fig.3: The SEM analysis of synthesized silver nanoparticles.

TEM analysis: Shape and size distribution of the synthesized Ag nanoparticles were characterized by transmission electron microscopic (TEM) study. The shape appeared to be slightly spherical and size of the NP is below 100nm.

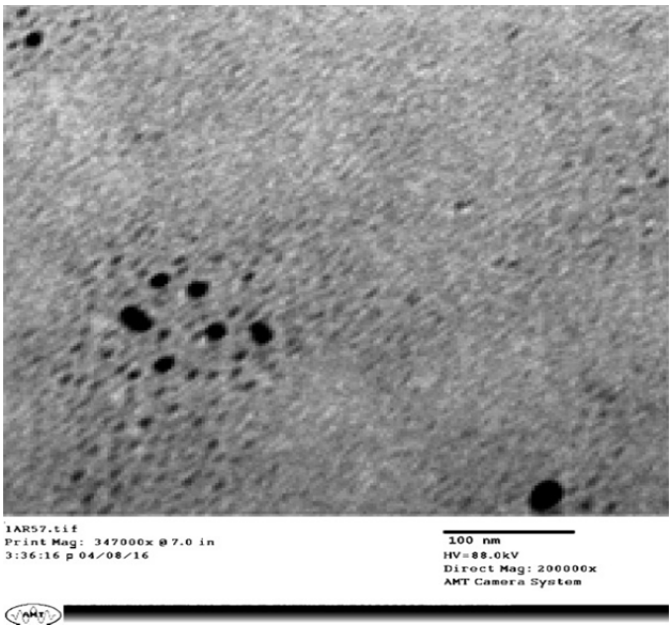


Fig. 4: The TEM analysis of synthesized silver nanoparticles

Identification of the microbial flora: The identification of the two potent colonies isolated from the clinical isolated were done by means of morphology analysis, gram's staining and various biochemical tests, and the results obtained are given here in Table 2:

Tests	Result	
	Culture 1	Culture 2
Gram staining	Gram positive Bacilli	Gram negative bacillus
Glucose fermentation	-	+
Mannitol fermentation	+	-
Methyl red	+	+
Motility	-	+
H2S production	-	+
Indole production	-	+
Lactose fermentation	-	-
Citrate utilization	-	-
Catalase test	+	+
Voges proskeaur	+	-
Sucose fermentation	+	+
Simmons citrate agar test	+	-

Table 2: Identification of the microorganism by biochemical tests

The result thus obtained by biochemical estimation clearly demonstrates the presence of *B.subtilis* in culture 1 and *P.vulgaris* in culture 2.

Antibacterial activity evaluation by agar well diffusion method: The antibacterial activity of the synthesized nanoparticles was evaluated against *B.subtilis and P.vulgaris*(Fig. 5). Antibacterial potential of AgNO3 and plant extract were also tested. Also one well is kept as control in which the suspension medium of the particles was taken i.e. distilled water.

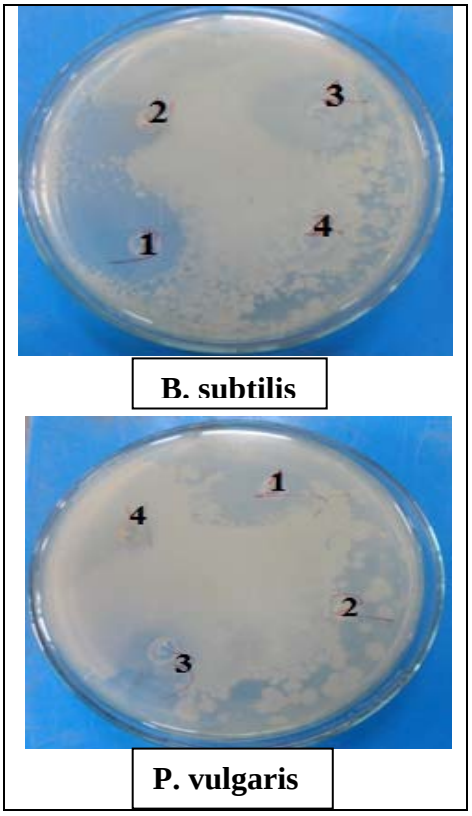


Fig. 5: Showing antibacterial activity evaluation by agar well diffusion method

The exact mechanism behind the antibacterial activity of metal nanoparticles is still unknown. , but the possible mechanism may be the bonding between nanoparticles and sulphur in proteins having thiol group in the cell, which ultimately affect the ion transport mechanism and so on. [20]

Zone of Inhibition: The antibacterial activity was evaluated by measuring zone of inhibition (Table 3) and the results are tabulated here as:

Sample Culture	Zone of Inhibition (in mm)			
	Ag NP's	AgNO3	Plant extract	Control
B.subtilis	17	12	- (little activity is seen with no clear zone)	nil
P.vulgaris.	18	10	Nil	nil

Table 3: The evaluation of antibacterial activity of synthesized silver nanoparticles, AgNO3, and chosen plant extract against *B.subtilis and P.vulgaris.* Here 1 is Ag NP's, 2 is AgNO3, 3 is plant extract and 4 is kept control.

MIC of silver nanoparticles against *B.subtilis and P.vulgaris*: The minimum inhibitory concentration of any components is its minimum amount required to inhibit the growth of micro-organism(Table 4).

Sample Culture	MIC of silver nanoparticles (in mm)				
	NP (0.1%)	NP (0.3%)	NP (0.5%)	NP (0.7%)	NP (0.9%)
Culture 1	14	15	17	16	16
Culture 2	17	18	18	19	18

Table 4: Showing MIC of silver nanoparticles against *B.subtilis and P.vulgaris*

CONCLUSION

Green synthesis of silver nanoparticles by the help of green plants showed great capability to synthesis Ag NPs. AgNPs synthesised from leaf extract are more stable which could be due to the presence of capping and stabilizing materials within the plant extract. The UV absorption peak at 410 nm clearly indicates the synthesis of AgNPs. The SEM studies were helpful at deciphering their morphology and size. TEM analysis reveals the shape which appeared to be slightly spherical and size of the NP is below 100 nm. The biosynthesized nanoparticles showed significant antibacterial activity against clinical isolates. The study may be consider as insight for development of new drug fourmulations.

REFERENCES

1. Wang S, Mamedova N, Kotov NA, Chen W, Studer J. Nano Lett 2002; 2: 817-822.
2. Xu ZP, Zeng QH, Lu GQ, Yu AB. Chem Engin Sci 2006; 61: 1027-1040.
3. Praphulla Rao1*, Chandraprasad M S1, Lakshmi YN2, Jahnavi Rao1, Aishwarya P1, Srinidhi Shetty1. International Journal of Multidisciplinary and Current Research. 2014; 2: 2321-3124.
4. Balaprasad Ankamwar, Chinmay Damle, Absar Ahmad, MuraliSastry (2005). Journal_of_Nanoscience_and_Nanotechnology. 2005; 5(10):1665-71
5. Delay M, Dolt T, Woellhaf A, Sembritzki R, Frimmel F H. J Chromatogr A. 2011; 1218(27): 4206-12.
6. Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, et al. Nano Lett 2001; 1: 515-519.
7. Ahmad A, Mukherjee P, Senapati P, Mandal D, Islam Khan M, Kumar R. Colloids Surf B 2003; 28: 313-318.
8. Sadowski Z, Maliszewska IH, Grochowalska B, Polowczyk I, Ozlecki T. Mater Sci-Poland 2008; 26: 419-424.
9. He SY, Guo ZR, Zhang Y, Zhang S, Wang J, Gu N. Mater Lett 2007; 61: 3984-3987.
10. Pugazhenthiran N, Anandan S, Kathiravan G, Kannaian N, Prakash U, Crawford S, et al. J Nanopart Res 2009; 11: 1811-1815.
11. Minaeian S, Shahverdi AR, Nohi AS, Shahverdi HR. J Sci IAU 2008; 17: 66-70
12. Verma VC, Kharwar RN, Gange AC. Nanomedicine 2010; 5: 33-40.
13. Tomar R.S., Chauhan P.S. and Shrivastava V. World journal of pharmaceutical research, 2014; 4(1): 595-620.
14. Kumar J, Shrivastava V, Thakur S. Advanced Science Focus, 2013; 1(4).
15. Sharma N, Kumar J, Thakur S, Sharma S, Shrivastava V .Drug Invention Today, 2013; 5(1): 50-54.
16. Krishnaiah D, Devi T, Bono A, Sarbatly R. J. Medicin. Plant Res 2009; 3(2):067-072.
17. Tomar R.S., Shrivastava. International journal of multidisciplinary educational research. 2014; 2(5): 2277-7881.
18. Shrivastava V, Chauhan P.S. and Tomar R.S. World Journal Of Pharmacy And Pharmaceutical Sciences.2015; 4(5): 1929-1943.
19. Chauhan P.S., Shrivastava V, and Tomar R.S. International Journal of Pharma & Bio Sciences. 2016; 7(2): 184-195.
20. Klueh U, Wagner V, Kelly S, Johnson A, and Bryer J D. J Biomed Mater Res 2000; 53: 621-631.