



Green Synthesis, Characterization and Antimicrobial Properties of Silver Nanowires by Aqueous Leaf Extract of *Piper betle*

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ABSTRACT

A simple, cost effective and eco-friendly green synthesis of silver nanowires using the leaf extract of *Piper betle* as a reducing agent has been investigated. The resulting silver nano wires were characterized using UV-Visible spectrophotometer, X-Ray Diffraction (XRD), Scanning Electron Microscope (SEM) and Fourier Transformations Infra Red (FTIR) spectroscopic analysis. XRD and SEM studies confirmed the formation of metallic silver nanowires with diameters in the range of 40-60 nm. FTIR analysis reveals that biomolecules with carbonyl, hydroxyl and amine functional groups have the potential for metal ion reduction and for capping the newly formed nanowires. Further the synthesized silver nanowires were tested against some common human pathogens and exhibited a moderate antibacterial activity against the tested bacterial pathogens but not in fungal pathogens.

Keywords: Antibacterial activity, Green synthesis, *Piper betle*, Silver nanowires.

INTRODUCTION

The most important and challenging aspect of modern nanotechnology is focused on the morphology controlled fabrication of nano structures because of their potential applications in optical, electronic and mechanical nano devices. Metal nanowires, as one-dimensional (1D) nanostructures, have been extensively studied on their optical properties and on their use as conductive fillers to enhance the performance of the adhesives.^{1,2} Silver nanowires have been attracting more and more attention because of their intriguing electrical, thermal and optical properties. Silver has the highest electrical conductivity among all metals, by virtue of which silver nanowires are considered as the most promising candidates in flexible electronics. Hence the mass production of silver nanowires is necessary and of great significance. Metal nanowires can be synthesized by conventional chemical and physical methods.³⁻⁵ The majority of the current chemical synthetic processes are regarded as having a relatively high environmental cost. There is increasing pressure to develop clean, nontoxic and environmentally benign synthetic technologies. Recently biosynthetic methods employing plant extracts have been emerged as environmentally sustainable alternatives to chemical synthetic procedures.⁶⁻⁸ However, the biosynthesis of silver nanowires using plant extracts has been rarely reported. The availability of nanowires in large quantity would be of great importance in microelectronics, optoelectronics, nanoscale electronic devices and other fields. In this study, we report on the simple biological synthesis of networked silver nanowires by the reaction of aqueous silver nitrate solution with an extract of *piper betle* leaves at room temperature. *Piper betle* has known medicinal properties forming an important component of ayurvedic medicine and has been used since ages to treat several ailments like

inflammation, headache, constipation and respiratory problems.

MATERIALS AND METHODS

Preparation of *piper betle* leaf extract

Fresh leaves of *piper betle* were collected from the local market and washed thrice in distilled water and dried on paper toweling. 20 gram of samples were cut into fine pieces and boiled at 100°C with 100ml of sterile distilled water for about 15 minutes. The crude extract was filtered through a Whatman filter paper (No. 40) to prepare the aqueous leaf extract. 1mM aqueous solution of Silver nitrate (AgNO₃, AR grade) was prepared and used in the synthesis of silver nanowires. 10 ml of the aqueous *piper betle* leaf extract was added to 190 ml of 1 mM aqueous AgNO₃ solution in a beaker and kept at room temperature for 48 hours for reduction.

Characterization of silver nanowires synthesized

UV-Visible Spectrum analysis

The UV-Vis spectrum for the reaction solution of silver nanowires was measured with UV-Vis Spectrophotometer (Model: HR Ocean Optics 4000).

XRD analysis

The XRD measurement was carried out for the identification of the crystallinity of silver nanowires using an XPert Pro X-ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with Cu K α radiation in a 2 θ configuration.



SEM analysis

The morphology of the synthesized silver nanowires was characterized by the Scanning Electron Microscope (FEI Quanta 250).

FTIR analysis

FTIR spectrum of the synthesized silver nanowires was recorded with a Shimadzu spectrometer (Model FTIR-8400S).

Antimicrobial evaluation

The antimicrobial activity was assayed by agar well diffusion method using 20 ml each of sterile Nutrient Agar (NA) (Hi-Media) and Potato-Dextrose Agar (PDA) (Hi-Media) for testing the bacterial and filamentous fungal activity respectively.⁹

RESULTS

UV-Vis spectrum analysis of the synthesized silver nanowires

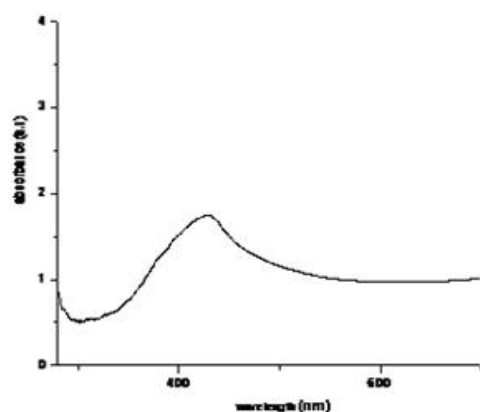


Fig. 1. UV-Vis spectrum of synthesized silver nanowires

Figure 1 shows the appearance of a single but strong surface plasmon resonance band absorption peak centered at 420 nm which indicates the formation of silver nanoparticles.¹⁰ Plasmon bands are broad with an absorption tail in the longer wavelength. The cause of the infrared absorption is the stretching vibration within molecule and could be due to the presence of nitrogen, hydrogen, carbon and oxygen bonds.¹¹ Typical nanowires exhibit aspect ratios (length to width ratio) of 1000 or more. As such they are often referred to as one dimensional (1-D) materials. Generally, one dimensional nanostructures exhibit two plasmon absorption peaks with energies characteristic of the longitudinal and the transverse axes of these particles.⁶ In this case the two peaks cannot be observed due to the overlap of the longitudinal absorption of the nanowires with different aspect ratios of relevant wavelength.

XRD spectrum analysis of synthesized silver nanowires

The silver nanowires thus obtained were purified by repeated centrifugation at 5000 rpm for 10 minutes followed by re-dispersion in 10 ml of de-ionized water. Fig. 2 shows the XRD pattern of silver nanowires. The

peaks at $2\theta = 38.16^\circ, 44.54^\circ, 64.58^\circ$ and 77.61° correspond to the (111), (200), (220) and (311) planes respectively of silver crystal. All the diffraction peaks can be indexed to the planes of face centred cubic structure of metallic silver ions respectively revealing that the synthesized silver nanowires are composed of pure crystalline silver. No impurities were detected from this pattern within the resolution limit of XRD. The crystallite size was found to be 38 nm and it was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Debye Scherrer formula.

$$D = 0.94 \lambda / \beta \cos \theta$$

Where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the Full Width at Half Maximum (FWHM) and θ is the diffraction angle.

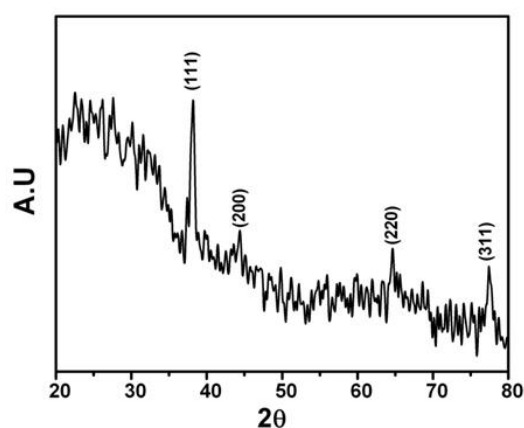


Figure 2: XRD pattern of the synthesized silver nanowire
SEM analysis of synthesized silver nanowires

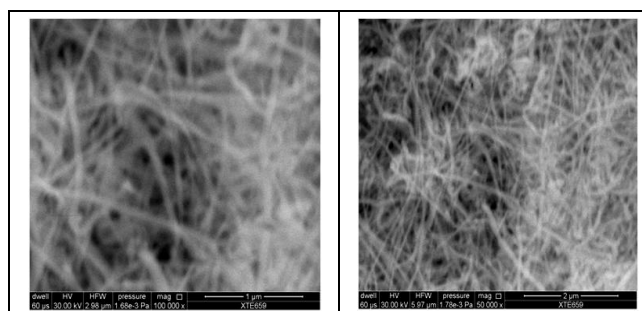


Figure 3: SEM image of synthesized silver nanowires

A typical SEM image of the synthesized silver nanowires is presented in Fig. 3. It was clearly seen that the prepared samples consisted of an abundance of nanowires. The average diameter of the nanowires is about 40-60 nm on average but the length of the nanowires cannot be measured due to the interlacing of their ends.

FTIR spectrum analysis of synthesized silver nanowires

For FTIR spectrum analysis, the silver nanowires synthesized using the *piper betle* leaves extract were centrifuged at 10000 rpm for 20 min to remove free proteins or other compounds present in the solution if any. The centrifuged and vacuum dried particles were

made in a KBr pellet and the spectrum was recorded. FTIR measurements were carried out to identify the possible biomolecules responsible for the capping leading to the efficient stabilization of the silver nanowires. Fig. 4 shows the FTIR spectrum of the silver nanowires synthesized using the leaf broth of *piper betle* leaves. The medium intense band at 1069.33 cm^{-1} is assigned to the C-N stretching mode of amine group. The sharp band at 1631.35 cm^{-1}

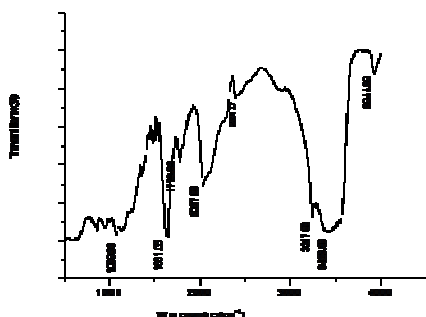


Figure 4: FTIR spectrum of silver nanowires synthesized using *piper betle* leaf extract

arises from C=O (amide I band). The absorption bands located at 3217.62 cm^{-1} and 3423.56 cm^{-1} may be attributed to O-H stretching mode of alcohols and phenols. The presence of these active functional groups in leaf extract results in the swift reduction of silver ions to silver nanowires.

Antimicrobial activity evaluation of synthesized silver nanowires

The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. Sterile 6mm diameter cork borers were pierced in the agar at equidistant spots. $20\mu\text{l}$ of the diluted solution ($16\mu\text{g/ml}$) was deposited on the inoculated well and left for 10 min at room temperature for the compound diffusion. Amphotericin-B (Hi-Media) for fungi and Ciprofloxacin (Hi-Media) for bacteria were used as control. The plates inoculated with bacteria were incubated at 37°C for 24 hr and for fungal cultures at 30°C for 24-48 hr. The experiment was repeated thrice and the average results were recorded. The antimicrobial activity was determined by measuring the diameter of the inhibition zone (mm) around the well (Table 1 and Table 2).

Table 1: Antifungal activity (zone of inhibition)

	Zone of inhibitions (mm)									
	<i>Aspergillus flavus</i>		<i>Aspergillus fumigates</i>		<i>Aspergillus niger</i>		<i>Candida albicans</i>		<i>Candida krusei</i>	
	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5
Silver nanowire	-	-	-	-	-	-	-	-	-	-
Amphotericin B ($16\mu\text{g/ml}$)	32		34		38		38		40	

Table 2: Antibacterial activity (zone of inhibition)

	Zone of inhibitions (mm)									
	<i>Proteus mirabilis</i>		<i>Klebsiella pneumonia</i>		<i>Escherichia coli</i>		<i>Salmonella paratyphi</i>		<i>Pseudomonas aeruginosa</i>	
	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5
Silver nanowire	14	11	12	10	12	11	12	10	10	-
Ciprofloxacin ($16\mu\text{g/ml}$)	32		34		36		36		34	

The susceptibility of microbial was determined by minimum inhibitory concentration determination method.¹² The minimum inhibitory concentrations (MICs) of the prepared silver nanowires were determined by serial dilution against the micro-organisms. The minimum concentrations at which no visible growth was observed were defined as the MICs, which were expressed in mg/ml (Table 3 and Table 4).

DISCUSSION

The formation of silver nanoparticles was primarily confirmed by the colour changes from pale yellow to dark brown due to the Surface Plasmon Resonance (SPR) property of silver nanoparticles. SPR in nanometer-sized structures is called Localized Surface Plasmon Resonance

(LSPR). LSPRs are the collective electron charge oscillations in metallic nanoparticles that are excited by incident light. For nanoparticles, LSPRs can give rise to intense colours of suspensions or sols containing the nanoparticles. Nanoparticles or nanowires of noble metals exhibit strong absorption bands in the ultraviolet-visible light regime that are not present in the bulk metal. In the present study a single but strong surface plasmon resonance band absorption peak was observed at 420 nm which indicates the formation of silver nanoparticles. In the beginning of the biosynthesis, silver ions were reduced to metallic silver by certain components existing in the broth of fresh piper betle leaf extract solution. Silver nuclei were formed in the initial stage. By gathering the surrounding silver atoms, the nuclei gradually grew

into relatively small particles which may act as the seeds for the growth of larger nanoparticles. The surface energy of larger particles is lower than that of the smaller ones, so these small nanoparticles were apt to dissolve into the solution and grow into larger ones via an Oswald ripening process. Afterwards the adjacent large nanoparticles grew and joined together because of their Brownian motion in the solution, forming wire like structures. By prolonging reaction time, the newly formed silver atoms deposit onto the concave regions of the connected nanoparticle through capillary phenomenon, leading to the formation of long nanowires. Furthermore, biomass proteins perform multiple tasks as a reducing agent of silver ions and capping agent for nanoparticles. Hydroxide ions also played a key role in the production of nanowires. In spite that the capping agent was available, the competition between the biomolecules of the biomass and hydroxide ions for silver ions favored the aggregation of nanoparticles due to the lower ability of biomolecules to stabilize the nuclei formed. In turn, the nanoparticles aggregation led to the formation of nanowires. Phenolics possess hydroxyl and carboxyl groups, able to bind to heavy metals. They may inactivate metal ions by chelating. Antioxidant action of phenolic compounds is due to their high tendency to chelate metals. Antioxidant action of phenolic compounds is expressed not only through its scavenging reactions but also due to the formation of H⁺ radicals which in turn reduces the size of silver particles to nanosize. Hydroxychavicol is a major phenolic compound present in the aqueous extract of piper betle leaf. The compound is better known for its antioxidant and anticancer properties. The potent antioxidant capacity exhibited by the *piper betle* leaf extract may be due to the phenolic compounds in this extract such as chevicol, chevibetol, chevibetol acetate and eugenol. Lastly, toxicity studies on pathogens open a door for nanotechnology applications in medicine.

Table 3: MIC for antifungal activity

	Fungal	MIC (mg/ml)
Silver nanowire	<i>Aspergillus flavus</i>	>1
	<i>Aspergillus fumigates</i>	>1
	<i>Aspergillus niger</i>	>1
	<i>Candida albicans</i>	>1
	<i>Candida krusei</i>	>1

Table 4: MIC for Antibacterial activity

	Bacteria	MIC (mg/ml)
Silver nanowire	<i>Proteus mirabilis</i>	<0.0625
	<i>Klebsiella pneumonia</i>	>0.0625
	<i>Escherichia coli</i>	<0.0625
	<i>Salmonella paratyphi</i>	=0.0625
	<i>Pseudomonas aeruginosa</i>	>0.125

Biosynthesis of metal nanowires is a traditional method and opened a new awareness for the control of disease. In the present investigation, the biologically synthesized silver nanowires are found to be moderate toxic against the tested bacterial pathogens but not in fungal pathogens. The best inhibitory activity was recorded against *Proteus mirabilis* and *Escherichia coli* among the tested bacterial pathogens.

CONCLUSION

The present study confirmed a simple, efficient biological method at room temperature for the synthesis of silver networked nanowires with diameters in the range of 40-60 nm using *piper betle* leaf extract and testing of their antimicrobial activities. Biomolecules with carbonyl, hydroxyl and amine functional groups have the potential for metal ion reduction and for capping the newly formed nanowires. Antimicrobial activities were assayed and exhibited a moderate antibacterial activity against the tested bacterial pathogens but not in fungal pathogens. The best inhibitory activity was observed against *Proteus mirabilis* and *Escherichia coli* among the tested bacterial pathogens. The flexibility of silver nanowires that could be tuned could find applications in microelectronics, optoelectronics, nanoscale electronic devices and other fields.

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REFERENCES

- Jin R, Cao Y, Milkin CA, Kelly KC, Scharz GC, Zheng JG, Photo induced conversion of silver nanospheres to nanoprisms, *Science*, 2001, 294, 1901-1903.
- Aizpurua J, Hanarp P, Sutherland DS, Kall M, Bryant GW, Abajo FJG de, Optical properties of gold nanorings, *Phy Rev Lett*, 2003, 90, 057401-0574014.
- Sun Y, Xia Y, Shaped controlled synthesis of gold and silver nanoparticles, *Science*, 2002, 298, 2176-2179.
- Murphy CJ, Gole AM, Hunyadi SE, Orendorff CJ, One dimensional colloidal gold and silver nanostructures, *Inorg Chem*, 2006, 45, 7544-7554.
- Wang MCP, Gates BD, Direct assembly of nanowires, *Mater today*, 2009, 12, 34-43.
- Laura Castro, M Luisa Blazquez, Jesus A Munoz, Felisa Gonzalez, Camino Garcia- Balboa, Antonio Ballester, Biosynthesis of gold nanowires using sugar beet pulp, *Process Biochemistry*, 2001, 46, 1076-1082.
- He S, Zhang Y, Guo Z, Gu N, Biological synthesis of gold nanowires using extract of *Rhodospseudomonas capsulate*, *Biotechnol Prog*, 2008, 2, 476-480.
- Lin L, Wang W, Jiale Huang, Li Q, Sun D, Xin Yang, et al., Nature factory of silver nanowire: plant mediated synthesis using broth of *Cassia fistura* leaf, *Chem Eng J*, 2010, 162, 852-858.



9. Reeves DS, Phillips I, Williams JD, Laboratory Methods in Antimicrobial Chemotherapy, Longman Group Ltd Edinburgh, 1979, 20.
10. Prashant Singh, R Balaji Raja, Biological synthesis and characterization of silver nanoparticles using the fungus *Trichoderma Hargianum*, Asian J Exp Sci, 2011, 2(4), 600-605.
11. D Ashok kumar, Rapid and green synthesis of silver nanoparticles using the leaf extracts of *Parthenium hysterophorus*: A novel biological approach, International Research Journal of Pharmacy, 2012, 3(2), 169-173.
12. Cheruiyot KR, Olila D, Kateregga D, In-vitro antibacterial activity of selected medicinal plants from Longisa region of Bomet district, Kenya, Afri Health Sci, 2009, 9(S1), S42 – S46.

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