NON-TOXIC SYNTHESIS OF SILVER (AG) NANOPARTICLES FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT

Silver nanoparticles were synthesized by an ecofriendly method utilizing *Nicotiana rustica* leaves extract as a reducing agent. The UV-VIS, EDX, SEM and XRD techniques were used to study the composition, morphology, and crystalline nature of the nanoparticles. The synthesized silver nanoparticles were tested for their antibacterial activity against two gram positive and two gram-negative bacteria. The nanoparticles show good activity against all examined bacterial species however their activity is low than levofloxacin used as standard drug.

Keywords: **antibacterial, silver, ecofriendly, Tobacco, Nicotine**

1. INTRODUCTION

Green synthesis has got the attention of many researchers due the simplicity, ecofriendly nature, and low cost [1-4]. A number of metal and metal oxide nanoparticles were synthesis previously using plant leaves extract for the reduction of precursor salts [5-7]. In green method enzymes, microorganism, and plants leaves, roots and fruits extract were used as reducing agents and better alternate chemical and physical methods [8-17]. Beside other methods, silver nanoparticles were synthesized by consuming plants leaves, roots and fruits extract and were utilized for various catalytic and antimicrobial applications [18-20].

Silver nanoparticles have many applications as textile, fabrics, plastics, catalytic and antimicrobial agent [21-24]. However, it is known for their inhibitory activity against microbes since for long time and shows good activity against multi-drug resistant human pathogen. Silver

nanoparticles is very potent antimicrobial agent with very low toxicity especially in treatment of burn wounds [25]. Due broad spectrum of antimicrobial action and low toxicity, it is used for many therapeutic applications.

The present work was designed to synthesized silver nanoparticles using *Nicotiana Rustica* leaves extract as a reducing agent. EDX, SEM and XRD techniques were used to study the composition, purity, morphology, and crystalline nature of silver nanoparticles. The synthesized silver nanoparticles were screened against two gram positive and gram-negative bacterial species.

2. EXPERIMENTAL SETUP

Analytical grade silver nitrate (AgNO3) were purchased from Sigma Aldrich and used without further purification. The fresh leaves of *Nicotiana Rustica* (Tobacco) were collected from tobacco fields in Baffa distract Mansehra and were identified by department of Botany, Hazara University, Mansehra, Pakistan. All the solutions and extract were prepared in distilled water.

2.1 PREPARATION OF NICOTIANA RUSTICA LEAVES EXTRACT

The Nicotiana Rustica leaves extract were prepared by a common method previously reported by [26]. The collected plant leaves were washed with distal water twice and then cut down into small piece. For typical preparation of extract, 10 grams of cut leaves were added into 250ml distilled water and boiled for 30minutes. The extract was then faltered and centrifuged at 4000rmp for 15 minutes to remove uncoordinated biomass. The upper layer of extract was collected and store at 4˚C for further use.

2.2 SYNTHESIS OF SILVER NANOPARTICLES

3 and 5mM Stock solutions of silver nitrate was prepared by dissolving 0.128g and 0.213g in 250ml of distal water. 50ml of 3mM solution of silver nitrate solution was with 5ml of plant leaves extract and vigorously stirred at 50˚C. The reaction mixture was stirred till the appearance of dark brown color and centrifuged at 4500rmp for 30 minutes to remove uncoordinated biomaterial. The centrifuged materials were edged for overnight followed by washing with distilled water and ethanol. The silver nanoparticles were dried at 120˚C and store in polyethylene bottle for further use. The same process was repeated for 5mM solution for the synthesis of silver nanoparticles.

2.3 CHARACTERIZATION

The composition and purity of silver nanoparticles was studied through energy dispersive X-ray Model INCA 200 (UK). The morphological studied was conducted through scanning electron microscopy model 5910 (JEOL Japan) while X-ray diffraction model Panalytical X-Pert Pro was utilized to study the XRD pattern of silver nanoparticles.

2.4 ANTIBACTERIAL ASSAY

The synthesized silver nanoparticles were conducted *against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* using agar well diffusion method. Agar media was used for the growth of bacterial species while a stable suspension of silver nanoparticles was prepared in distilled water by adding few drops of nitric acid. A sterile borer was used to bore wells in agar media and bacterial culture were spread over that media. The wells were equipped with 90µl of silver nanoparticles suspension as well as levofloxacin was used as standard drug and incubated at 37˚C. The inhibition zone was measure in millimeter after 24h is the activity of silver nanoparticles against these bacterial species.

3. RESULTS AND DISCUSSION

The confirmation of silver nanoparticles was monitored by measuring the UV–Vis spectra of the reaction salt solution and plant extract (aqueous silver nitrate solution with leave extract) as mentioned in the graph below.

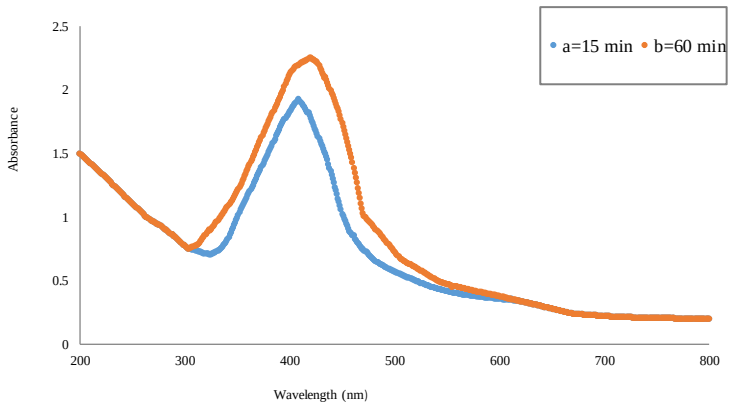

Fig. 1. UV-VIS Spectroscopy of Ag nanoparticles

The EDX analysis of synthesized silver nanoparticles were performed in order to study its composition and purity. The EDX spectra given in figures 2 and 3 shows that the maximum emission took place at 3 KeV, which confirmed the presence of silver nanoparticles in samples. Beside silver nanoparticles, EDX spectra possess some low intensity peaks for Aluminum, Carbon, Nitrogen, Oxygen, and Phosphorus.

The SEM analysis of silver nanoparticles were perfumed to study the detailed morphology are given as insets in figure 2 and 3 shows that particles are highly agglomerated however some particles with distinct boundaries are also seen.

The XRD pattern of silver nanoparticles was performed to calculate crystallite size and geometry. The Figure 4 are the diffractogram for silver nanoparticles, shows the diffraction peaks in 2θ range from 10˚ to 70˚. The diffractogram shows two sets of diffraction peaks for silver nanoparticles synthesized by using 3 and 5mM solution. The diffraction peaks with hkl planes are positioned at 2θ values 26.32(111), 27.96(400), 29.38(110), 32.31(420), 35.03(040), 37.98(240), 39.00(202), 41.47(311), 42.53(102), 46.17(620), 47.81(212), 48.28(211), 54.87(222) and 57.68(800).

3.1 ANTIBACTERIAL ACTIVITY

The antibacterial activity of silver nanoparticles was carried out against the bacterial species mention in experimental section using agar well diffusion method and levofloxacin was used as standard drug shown in figure 5. The synthesized Silver nanoparticles are active against both gram positive and gram-negative bacterial species while both the silver nanoparticles (3 and 5mM) have nearly same activity against same bacterial species.

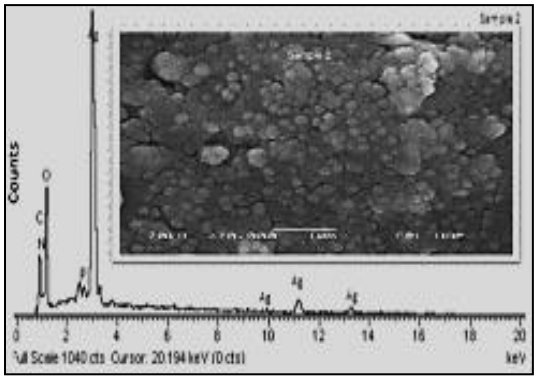

Fig. 2. EDX spectra and SEM micrograph in inset of AgNPs (3mM)

The chemistry of green process is not clear, but the synthesis of silver nanoparticles was confirmed various characterization techniques. The EDX study of silver nanoparticles shows weak peaks for C, N, O and P, other Ag are due the use of plants extracts as a reducing agent while oxygen through atmosphere as the reactions were carried out in open air. However, % weight of these elements is very less and considered insignificant as compared to silver as shown in table 1. The silver nanoparticles are unevenly distributed with high degree of agglomeration while the particles exhibit irregular size and shape and the grain seen in SEM micrographs are ranging from 85 to 97nm.

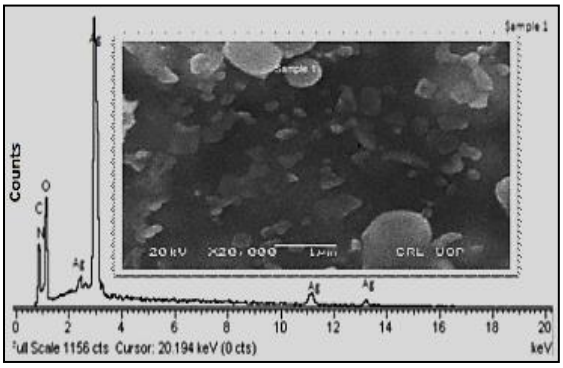

Fig. 3. EDX spectra and SEM micrograph in inset of AgNPs (5mM)

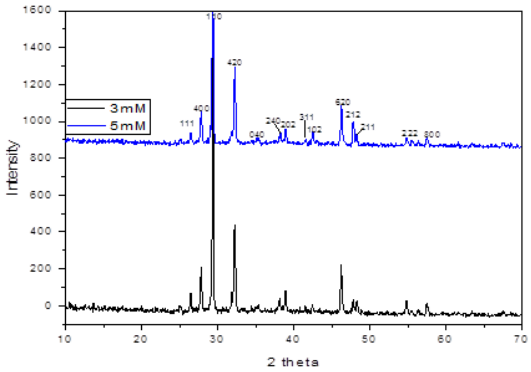

Fig 4. XRD spectra of AgNPs (3 and 5mM)

Table. 1. The elements detected in EXD analysis of silver nanoparticles

Element	Weight%	Atomic%
C K	10.45	30.08
N K	5.24	2.11

O K	18.78	43.16
P K	0.54	0.65
Ag L	70.22	24.00
Totals	100.00	

The diffraction peaks at 2θ values with hkl planes are 26.32(111), 27.96(400), 32.31(420), 35.03(040), 37.98(240), 41.47(311), 46.17(620), 54.87(222) and 57.68(800) corresponding to reference card 01-077-1289 with orthorhombic geometry. Other peaks at 2θ values with corresponding hkl planes are positioned at 29.38(110), 39.00(202), 42.53(102), 47.81(212) and 48.28(211) matched with JCPDS file no 00-043-1038 with monoclinic geometry. The peaks for silver nanoparticles with 3mM solution are sharp, intense and a bit board confirm highly crystalline nature with small crystallite size [27]. The Dedye-Scherrer equation was used to calculate crystallite size for both detected geometries. The crystallite size for orthorhombic geometry is 44nm while those for monoclinic is 52nm are two time smaller than the grain size calculated from SEM micrographs.

The silver nanoparticles show good activity against gram positive bacteria as compared to gram negative are may be due the difference in outer membrane of bacterial species. As it is investigated previously silver nanoparticles can have disturbed growth bacterial cells by interacting with surface of bacterial species. The surface of silver nanoparticle release Ag^{+} ions which interact with negatively charge Teichoic acid and phospholipids of the outer membrane of gram positive and gram-negative bacteria respectively and inhibit cellular functions [28]. However, the activity of silver nanoparticles is very less than the standard drug while solvent have no activity. In table 2, (B.S) means B. Subtilis, (S.A) means S. Aureus, (E.C) means E. Coli and (P.A) means P. Aeruginosa.

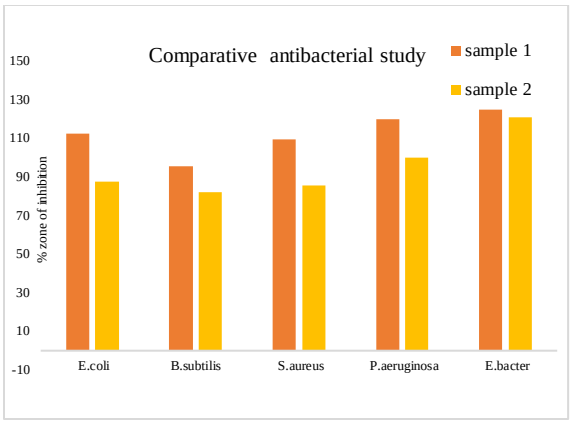

Fig. 5. The anti-bacterial activity of the samples 1 & 2 marked by different colors.

Table. 2. *Antibacterial activity of silver nanoparticles synthesized via Nicotiana Rustica leaves extract*

Samples	**Gram positive bacteria**		**Gram negative bacteria**	
	B.S	S.A	E.C	P.A
Solvent	0.00	0.00	0.00	0.00
Ag NPs (3mM)	8.03	7.37	6.47	5.74
Ag NPs (5mM)	6.61	5.96	4.99	4.03
STD drug	18.31	17.85	19.45	16.64

4. CONCLUSION

The silver nanoparticles are successfully synthesized by using *Nicotiana Rustica* leaves extract and was confirmed by EDX analysis. Other peaks in EDX spectrum are due to the use of plant leaves extract as a reducing agent. The SEM micrographs revealed that the particles are agglomerated with irregular size and shape however few identical particles are also seen. The X-ray diffractogram of both samples shows similar pattern though peaks for silver nanoparticles with 3mM solution are sharp and intense. Two geometries orthorhombic and monoclinic are detected while the crystallite size calculated from XRD data are smaller than the grain size seen in SEM

micrographs. Both the samples are active against gram positive and gram negative bacteria however its activity against gram positive bacteria is slightly higher than gram negative bacteria.

Acknowledgement. It was great pleasure to work under the supervisor of Dr. Kishwar Sultana (Assistant Professor, and Dr. Muhammad Tauseef Qureshi (Assistant Professor, the author also like to thank another contributor of this research paper, Dr. Nighat Sultana (Assistant Professor, Department of Bio-Chemistry Hazara University Mansehra).

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