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GREEN SYNTHESIS OF SILVER NANOCCLUSERS VIA *MELIA AZEDARACH* PLANT AND THEIR POTENTIAL TOWARDS CATALYTIC REDUCTION OF 4-NITROPHENOL

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ABSTRACT. Biomolecules present in the plant extracts have potential to reduce metal ions to nanoclusters by a single-step green synthesis approach. In the current study, we have synthesized the silver nanoclusters (AgNCs) from a medicinal plant, *Melia azedarach* and studied their catalytic activity toward the reduction of 4-nitrophenol to 4-aminophenol and organic dyes. Moreover, the phytochemical analysis of the plant extract was carried out in order to determine the bioactive compounds present in it. Metallic nature of the synthesized AgNCs was verified by X-ray diffraction study, while their morphology and size of was confirmed by transmission electron microscopy and Zetasizer, respectively. The study revealed that they were 56±2 nm in size and formed clusters. Fourier transformed infrared spectroscopy gives information about the different functional groups present in synthesized these NCs. Furthermore, the important catalytic applications, such as catalytic reduction of 4-nitrophenol in the presence of mild reducing agent NaBH₄ and the catalytic degradation of organic dyes was monitored by FTIR. Therefore, these results indicate that the obtained nanomaterials have important applications in industrial areas.

KEY WORDS: Green synthesis, Silver nanoclusters, Catalytic reduction, Characterization

INTRODUCTION

Nano-biotechnology is comparatively new emerging field of biotechnology working simultaneously on the synthesis, manipulation and applications of nano-sized particles [1]. The aspect that makes nanosciences different from science of macroscopic object is the nano-sized range of the particles. The microscopic objects ranging from 1 μm to 100 nm, while nano sized particles are below this range, i.e. from 1 nm to 100 nm [2]. There are two ways to synthesize nanoparticles of the above mentioned range. The first one is the, top-down approach leads to breakdown of larger macroscopic molecules into nano-sized particles, while the second one is the bottom-up approach that leads toward the gathering of microscopic molecules into range of nano-sized particles [3].

The most essential of these is to synthesis nanoparticles in way utilizing less toxic chemicals, low reaction rate, and ecofriendly environment, controlled size and morphology of nanoparticles. Use of costly physical and chemical methods lead toward synthesis of nano sized particles may result in usage of toxic chemicals, solvents, and reducing agents to stop agglomeration of synthesized particles sometimes causing environmental hazards as well. All of these limitations can be overcome by synthesizing nanoparticles via biological methods. These biological methods include synthesis by microorganisms as well as by plants extract [1, 4]. The use of plant extract is favorable in a sense that it is easy to use, safe handling [5], easily available and contains variety of molecules that are bioactive, and also important for reduction of metallic salt into metallic nanoparticles. Metallic nanoparticles synthesized by plant extract

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showed their potential toward catalysis, pharmacological, therapeutic, anti-cancer, anti-diabetic and many other applications. From almost all of the alkali and transition metals, silver is believed to be less toxic for both humans and animals [6]. Potential of size dependent silver Nano sized particles toward catalytic reduction of nitro compounds into amino compounds is gaining importance day by day [7]. Researchers worked on the synthesis of silver nano particles by using plant extract of *Gliricidia sepium* [6], *Cinnamomum camphora* [8-9], *Magnolia kobus* and *Diopyros kaki* leaf [10], *Aloe vera* [11], *Acalypha indica* leaf [12], neem [13] and *Geranium* leaf [14]. The home grown medications involve unmistakable position ideal from the crude time frame to display day. *Melia azedarach* is indigenous plant of Pakistan used for several therapies like for cough, skin diseases, and kidney diseases from decades. Leaf extract of *M. azedarach* represent a broad spectrum of antimicrobial activities [15]. The phytochemistry of *Melia azedarach* showed that main constituents are triterpenes and limonoids ample in oil. The extract of *Melia azedarach* has about 20 chemical constituents; which have 2 or 3 main compounds, and others are also useful [16]. It was reported that the alkaloids, terpenes, tannins, saponins, and various phenolic compounds were present in *M. azedarach*. Tannins acted as a reducing agent for reducing Ag^{+1} into Ag^0 [17-19]. Bio-reduction of silver nitrate by plant extract resulted into nucleation of metallic particles, bio-reductant and other phytochemical's behaving as a stabilizing and capping agents step forwarding toward accumulation of these materials in order to synthesize stabilized and capped silver nano sized particles [20]. It was reported that the nano sized particles of silver has shown antifungal activities [21], antimalarial activities [22], antioxidant properties [23], antiviral [24] and antibacterial activities [25].

Recently reported green synthesis of silver nanoparticles with different extracts such as tea, *Berberis vulgaris* leaf, *Fritillaria* flower, *Rosa* and formation of silver NPs with different shapes. AgNPs have good application for nanomedicine, and mediated biogenic cytotoxicity evaluation, antibacterial activity, their morphological, biological photocatalytic [27-33].

The recent study on the green synthesis of AgNCs by *Melia azedarach* was compared with reported work of *Raman Sukirtha* [34] and applications of synthesized silver particles as a reducing agent was also studied [35]. With all of these facts, here we did the green synthesis of silver nano sized particles by *Melia azedarach* leaf broth, and their characterizations would be analysed by FTIR Spectrophotometer, XRD, TEM, Zeta-Sizer and their catalytic applications.

EXPERIMENTAL

Preparation of Melia azedarach leaf extract

Fresh leaves of *Melia azedarach* were collected from the premises of University of Wah, Pakistan and washed with distilled water several times. The dried leaves were crushed and ground into fine powdered form. Fine powdered leaves in 100 mL distilled water were allowed to stir at room temperature.

Phytochemical analysis of Melia azedarach

Active bio-components of *Melia azedarach* was confirmed by conventional methods used for confirmation of tannins, terpenoids, glycosides, and alkaloids. Bromine water test, sodium nitrite test and ferric chloride test was performed for tannins. For glycosides and alkaloids, Keller Kiliani's test Wagner's test were performed respectively in different organic solvents like dimethyl chloride (DMC), ethanol, methanol, ethyl acetate and n-butanol.

Green synthesis of silver nano-clusters

Analytical grade silver nitrate salt bought from DAEJUNG which was used as a precursor for silver metal. 1 g silver nitrate was put into the leave extract mixture and allowed to stir at 280

rpm for one and a half hour until all of the bio-reductants starts reduction of silver ion. After that it was allowed to stand for almost 3 days in darkness. After 72 hours the sample was took out and filtered. Yellow color of filtrate obtained the evidence for reduction of silver metal into silver nano form. Yellow liquid mixture was placed in oven at 95 °C for 60 min to dry. When it dried, the jelly like thick paste was then calcined in furnace for 3 hours and 30 min at 550 °C. The obtained product was grinded then stored in a sample bottle for further characterization and applications.

RESULTS AND DISCUSSION

Phytochemical screening of Melia azedarach leaf extract

The phytochemical screening reveals that tannins are present in leaf extract of plant (Table 1), which are responsible for the reduction of silver ion into silver particles [36].

Table 1. Phytochemical analysis of *Melia azedarach*.

S. No.	Chemical components	Methanol extract	Ethanol extract	n-Butanol extract	Ethyl actate extract	DMC extract
1	Alkanoids	-	-	+++	++	+++
2	Phenol	+++	+++	+++	+	++
3	Glycosides	-	-	-	-	-
4	Flavonoids	++	+++	++	-	-
5	Tannins	+++	+++	+++	-	-

Confirmation of green synthesized silver nanoclusters

Melia azedarach is rich with tannic acid in its leaf extract. Synthesized silver nanoparticles were first confirmed by the physical induction of yellow color (Figure 1). The biosynthesis of silver particles were initiated by hydrolyses of tannic acid into gallic acid and glucose under slight acidic or basic condition. Both tannic acid and gallic acid were hydrolysable phenolic acids present in the leaves of plant. Gallic acid could act as a reducing agent in order to reduce silver ions into silver particles. The suggested result for this study was strictly in accordance with Raman Sukirtha and Sivaraman *et al.* [17, 35].



Figure 1. Color intensity of synthesized (AgNCs).

The tannic acid has two other entities present in their structure, i.e. gallic acid and glucose which can be hydrolysable phenolic acids.

X-Ray diffraction analysis

The XRD analysis showed four distinct peaks for 2θ at 37.9°, 44.2°, 64.3°, and 77.3° indices at 111, 200, 220 and 311 hkl, respectively. The crystalline nature of synthesized silver

nanoparticles were confirmed by XRD results. Sharp peaks showed that plant have some stabilizing agents that capped the synthesized AgNCs [35, 36].

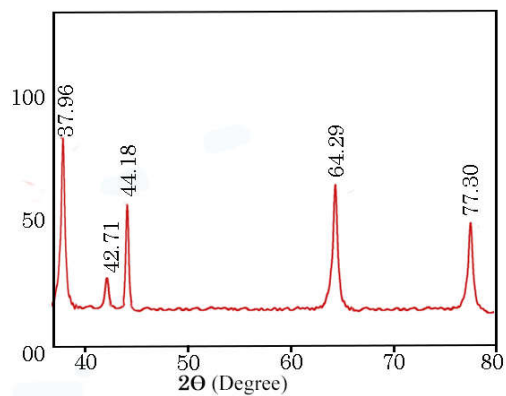


Figure 1. XRD of AgNPs by *Melia azedarach* plant.

Table 2. XRD data for AgNPs.

Sr #	Peak (2θ)	Θ	Sin ² θ	Indices (h,k,l)	S	a ²	a (Å)
1	37.9°	18.95	0.10	1, 1, 1	3	17.77	4.21
2	44.2°	22.09	0.14	2, 0, 0	4	16.90	4.11
3	64.3°	32.14	0.28	2, 2, 0	8	16.74	4.09
4	77.3°	38.65	0.39	3, 1, 1	11	16.71	4.08

By using Scherrer equation, the estimated size of NCs was determined, i.e.

$$D = K\lambda/\beta\cos\theta \quad (1)$$

$$D = 0.9 \times 180^\circ / \beta\cos\theta\pi$$

The average size of synthesized silver nanoparticles was 35 nm.

FTIR spectra analysis

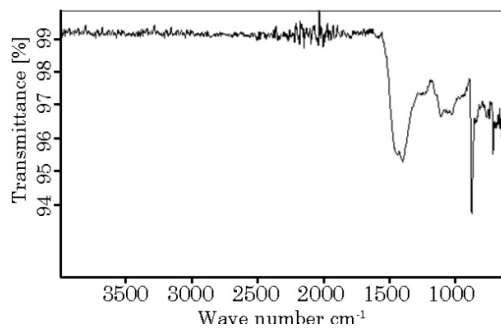


Figure 2. FTIR Spectra of silver nanoparticles.

The FTIR spectroscopy was taken to determine the functional group of active bio molecules of *Melia azedarach* taking part to reduce silver ions into silver particles. FTIR spectra analysis data was shown in Table 3. The spectra showed that the finger print region has sharp peaks but remaining peaks were not sharp as shown in Figure 2.

Table 3. FTIR data of synthesized silver nanoparticles.

Sr. No.	Peak cm^{-1}	Functional group
1	3523	Phenolic/alcoholic –OH stretch
2	3405	Amine N-H stretch
3	1632	N-H bend
4	2874	Aldehyde C-H stretch
5	1522, 1396	Aromatic C=C stretch
6	1107	C-O-C stretch
7	1046	Germinal CH_3 bend
8	657	Primary amine

Transmission electron microscopy (TEM)

Surface morphology [37] was studied via transmission electron microscopy (TEM). The TEM images reveal that the surface of AgNCs (Figure 3) is rough and irregular. The average size of the Ag-NCs are almost 56 nm as given in right side of Figure 3. The size of the AgNCs distributed in a wide range, i.e. 40–80 nm which clearly explains the nucleation, maturation and aggregation of the Ag nanoparticles. The high catalytic activity of the Ag-NCs is also explained on the basis of difference in the surface area of the NCs.

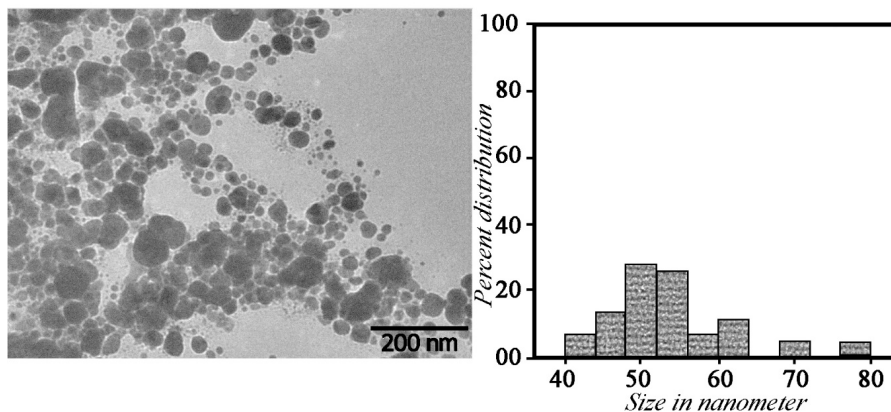


Figure 3. Transmission electron microscopy of silver nanoparticles.

Zetasizer analysis

Size distribution of synthesized silver particles was carried out by Nano-Zetasizer from National Institute of Biotechnology and Genetic Engineering, Faisalabad, Pakistan. The results uncovered the size of particles.

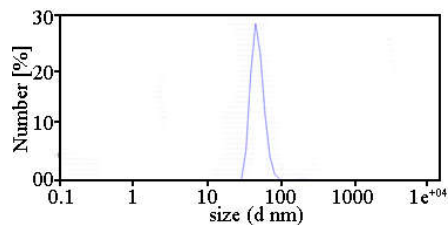
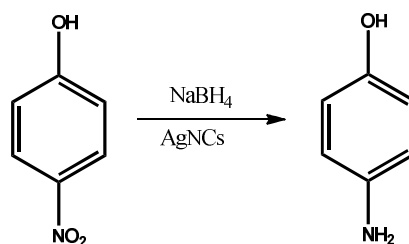


Figure 4. Nano-ZS of AgNCs.

It is shown in Figure 3 that the synthesized particles average size of 56.2 nm and are poly-dispersed in nature.

Catalytic reduction of 4-nitrophenol into 4-aminophenol

AgNCs synthesized by *Melia azedarach* leaf broth showed their potential catalytic reduction of 4-nitrophenol into 4-aminophenol followed by using mild reducing agent NaBH_4 as a source of hydrogen and AgNCs as a catalyst but in absence of AgNCs the reaction was not occurred. The Scheme 1 showed the complete reaction for catalytic reduction of 4-nitrophenol into 4-aminophenol.



Scheme 1. Catalytic reduction of 4-nitrophenol into 4-aminophenol.

FTIR analysis spectra of catalytic reduction of 4-nitrophenol into 4-aminophenol showed in Figure 5 express that there was broad peak at 3323 cm^{-1} due to $-\text{OH}/-\text{NH}$ stretching vibrations and 1635 cm^{-1} N-H bending and aromatic $\text{C}=\text{C}$ stretching. It uncovered the result that nitro group was being reduced into amino functional group.

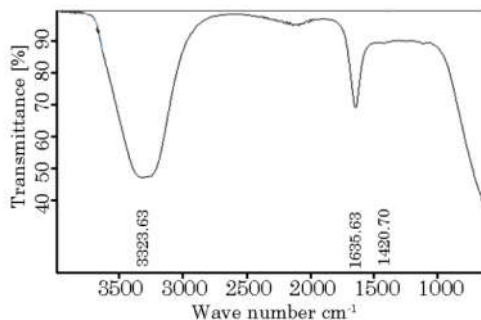


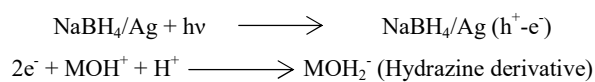
Figure 5. FTIR spectra of 4-aminophenol.

Catalytic degradation of organic dyes

Synthesized AgCPs also showed their potential catalytic activity of degradation of organic dyes. Methyl orange, methyl red, bromocresol green and eosin Y were degraded using NaBH₄ as a reducing agent and AgCPs as a catalyst.

Reduction adopted the absorption phenomena firstly reduced the dyes into nontoxic compounds. It is somehow believed that the NaBH₄ put its layer upon silver nanoclusters so that the absorption takes place more effectively. In this way oxidation reduction reaction and absorption takes place in short time.

Above phenomena followed electron transfer mechanism in which electron transfer from reducing agent to organic dye intermediate specie as shown is Scheme 2.



Scheme 2. Electron transfer mechanism.

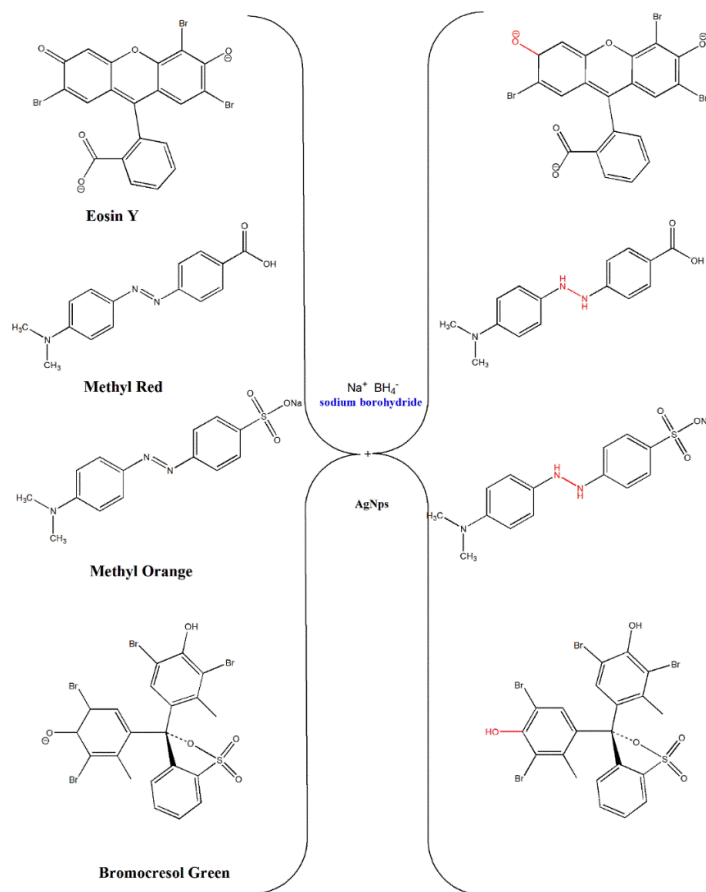


Figure 6. Catalytic degradation of organic dyes.

CONCLUSION

The *Melia azedarach* is rich with bio-reductants important for the reduction of metal ions, such as silver ions. The mechanism for synthesis of silver nanocluster using the extracts with an average size of 30 to 80 nm has been given. TEM showed that the synthesized clusters were poly-disperse in nature and are spherical in shape. Furthermore, the characterization confirmed the bio-reduction of silver ion into silver nanoparticles. Moreover, the obtained NCs showed their potential towards catalytic reduction of 4-nitrophenol into 4-aminophenol, which is toxic air pollutant released from industrial waste. Comparative study showed using mild reducing agent to reduce 4-nitrophenol into 4-aminophenol which are very slow process, while using silver nanoparticles as a catalyst the reaction was completed in 15 min. Similarly, another effective application of AgNCs was the catalytic degradation of organic dyes into nontoxic derivatives. Hence, it could be envisaged that the green synthesized NPs could be an effective tool for future industrial catalytic applications.

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