Green synthesis and characterization of Silver nanoparticles (Ag NPs) using white water Lily flower extraction species *Nymphaea Alba* of Nymphaeaceae family

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**Abstract:** White Water lily flower, with the scientific name of Nymphaea Alba in the family of *Nymphaeaceae*, was collected from North of Iran (Sari, Farahabad) in spring, dried in shade and powdered. The powdered flower material was extracted in 70% (V/V) ethanol. Silver nanoparticles (Ag NPs) were synthesized by the reaction of Water lily flower extraction and silver nitrate solution. The progress of the reaction was monitored using UV-Vis spectra at 300-600 nm. The structure of nanoparticles identified through the FT-IR, TEM and XRD spectrum. UV-Vis spectrophotometric study showed the characteristic peak for Ag NPs at wavelength 420 nm ($\lambda_{max}$). The morphology of the samples was analyzed using TEM and the particles formed were characterized to be spherical. TEM micrographs revealed the formation of well-dispersed silver nanoparticles in the average size ranging from 5-30 nm.

**Keywords:** Green synthesis, Water lily, *Nymphaea Alba*, Extraction, Silver Nanoparticles.

**Introduction**

*Nymphaea Alba* belongs to Nymphaeaceae family to know as white Water lily or white Lotus is an aquatic flowering plant [1]. This plant grows in the wetland and slightly acidic Water. The roots and stems of Water lily are in the Water; the broad leaves are on the surface of Water toward the sun and flowers are in the air. Water lily was worshiped in ancient Egypt and Iran [2]. *Nymphaea Alba* contains some active chemical compounds like alkaloids, gallic acid, sterols, flavonoids, glycosides, tannins and polyphenolic compounds [3], because of them all parts of the plant have been the basis for medical treatments through much of human.

Nanoparticles can be synthesized environmentally using different methods [4]. The scientists are very interested in the green synthesis of metal nanoparticles using plants. The advantages of this method are to eliminate toxic chemical compounds, facile synthesis, decrease the cost of nanoparticles preparation, eco-friendly, simple work-up procedure [5-7]. Among the different metals, silver nanoparticles are very important because of their pharmaceutical properties [8-9]. Because of the above reasons, we have focused on the synthesis of silver nanoparticles using white Water lily flower extraction in good yield and under mild reaction conditions.

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Results and discussion

Optimization of different parameters

The effects of the reaction conditions such as the ratio of plant extract and silver nitrate, pH, reaction time and reaction temperature were studied to maximize the yield of Ag NPs. The resulting solutions of the reaction were monitored using a UV-Vis spectrophotometer. The influence of pH on the synthesis of silver nanoparticles was investigated to 3.0, 5.0, 7.0, 8.0, 9.0 and 11 (pH of Nymphaea Alba extract solution was about 3.0). The reaction completion time was studied by monitoring the absorption spectra of silver nanoparticles formed in the reaction media at different duration’s time (10 min to 48 h). Different concentrations of silver nitrate aqueous solution were investigated (0.5 mM, 1 mM, and 2 mM) in order to optimize the concentration of silver nitrate solution. Different ratios of plant extract and silver nitrate solution were investigated (1:4, 1:2, 1:1, 2:1 and 4:1) in order to find the maximum production of Ag NPs. The temperature of the reaction was set at 25°C, 45°C, 65°C and 85°C using the Water bath for optimization of the reaction temperature. The optimized condition of the reaction was obtained at room temperature in pH=9 after 24 h with the ratio 1:4 of plant extract and silver nitrate solution.

Transmission Electron Microscopy (TEM)

The morphology and particle size distribution of Ag NPs were studied using transmission electron microscopy (TEM). Figure 1 shows the TEM images of silver nanoparticles obtained by the reaction of Water lily flower extraction and silver nitrate solution. They confirmed that the small spherical shape particles with smooth surface dispersed very well and also are in nano range (10 to 30 nm).

X-Ray Diffraction (XRD)

X-ray diffraction (XRD) can be used to characterize the crystallization and average size of nanoparticles. XRD pattern of the plant derived Ag NPs shows four intense peaks in the whole spectrum of 2θ values ranging from 20° to 80°. Presence of four distinct high diffraction peaks at 2θ values of 38.07°, 44.25°, 64.39° and 77.29°, respectively, corresponding to the Bragg’s reflection planes (111), (200), (220), and (311) (JCPDS Number. 04-0783)[10, 11] confirmed that the silver nanoparticles had been formed using Nymphaea Alba extract (Figure 2). The other three diffraction peaks at 2θ values of 29.42°, 32.27°, and 44.26° (JCPDS File number 036-1474 and 04-0705) were also detected. The unassigned peaks could be due to some bioorganic chemical compounds crystals on the surface of the nanoparticle. The broad X-ray diffraction peaks
around their bases indicate that the silver particles are in nano sizes. With the XRD pattern, the average diameter which can be evaluated from Scherrer equation [12, 13] (\(D=K\lambda/\beta\cos\theta\), where \(K\) is constant, \(\lambda\) is X-ray wavelength and \(\beta\) is the peak width at half maximum) is obtained about 8 nm.

![XRD pattern](image)

**Figure 2:** XRD of Ag NPs synthesized with Water lily flower extraction.

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FT-IR spectroscopic studies

The possible interaction between silver nitrate and *Nymphaea Alba* extraction was investigated using FT-IR spectroscopy, which leads to the reduction of silver ions and stabilization of silver nanoparticles. FT-IR spectra of silver nanoparticles (Figure 3) exhibits absorption peaks at wavelengths 767, 823, 1036, 1207, 1351, 1449, 1613, 1762, 2400, 2926 and 3312 cm\(^{-1}\). The broad line at 3312 cm\(^{-1}\) is characteristic of the hydroxyl functional group in alcohols, phenols, or the strong interaction of Water with the surface of Silver. The bands at 2926 cm\(^{-1}\) could be due to alkane C-H stretch, which is associated with lipid molecules in the flower. The bands at 2400 cm\(^{-1}\) can be assigned to C=N. The carboxyl, carbonyl and NO\(_2\) groups commonly exhibit vibrations at 1207 to 1762 cm\(^{-1}\) region.

![Figure 3: FT-IR spectrum of silver nanoparticles synthesized with Waterlily flower](image)

The bands at around 1351 and 1449 cm\(^{-1}\) indicate C-N, amino or aminomethyl groups stretching vibrations respectively. The protein and some chemical compounds from plant extraction might play an important role in the stabilization of silver nanoparticles by binding or encapsulate them. This action forms a layer around nanoparticles and protects them from agglomeration [14, 15].

UV-Vis Studies

Completion of the reaction between Water lily flower extraction and AgNO\(_3\) was monitored and optimized by taking absorption spectrum in UV-Vis spectrophotometer at different reaction condition. As the Water lily flower extraction was added to aqueous silver nitrate solution, the color of the solution changed from light to yellow and finally to brown with silver colloids formation. Similar changes in color have also been observed in previous studies for other plants [16, 17]. The UV-Vis spectra recorded after 10 min, 20 min, 60 min, 16 h and 24 h at different temperatures (25°C, 45°C, 60°C, and 90°C) from the initiation of reaction at the wavelength of 300-600 nm. Absorption UV-Vis spectra of biosynthesized Ag NPs showed \(\lambda_{\text{max}}\) at 420 nm. The UV-Vis spectra showed that Ag NPs were obtained rapidly within the first 10 min only and the Ag NPs in solution remained stable even after 24 h of completion of reaction at room temperature.

![Figure 4: UV-Vis spectrum of biosynthesized silver nanoparticles.](image)

Conclusions

The reaction was carried out in Water: ethanol, 70:30 as a green solvent. Biosynthesized Ag NPs have been appropriately characterized using UV-Vis spectroscopy, FT-IR, XRD, and TEM analysis. FT-IR analysis revealed the efficient capping and stabilization properties of Ag NPs. Based on TEM and XRD analysis, the silver nanoparticles were synthesized with an average size of 5-30 nm, with spherical structures. The results showed Water lily flower extract to be an excellent agent for reducing of Ag\(^+\) in comparison to some other plants extract. The advantages of this reaction are low cost, facile, simple and eco-friendly biological method for synthesis of silver nanoparticles using *Nymphaea Alba*. This plant may have the potential for production of different nanometals under mild conditions.
Experimental

Chemicals

The chemical materials and solvents were purchased from Merck and Aldrich Chemical Company.

Collection and preparation of white Water lily flower extracts

White Water lily flower, with the scientific name of Nymphaea Alba, was collected from the wetland in the north of Iran (Mazandaran, Sari, Farahabad, Kharra village) in spring 2016 (Figure 1). The flower was washed, dried at room temperature for one week and then ground in a blender before extraction.

Figure 5: Water lily flower was picked up from wetland in Iran (Mazandaran, Sari, Kharra village)

The powdered Water lily flower (50 g) was weight in a beaker and percolated with 70% (V/V) ethanol (150 ml). This beaker was properly covered with aluminum foil and left for 72 hours. The solution was then filtered using a funnel filled in a filter paper and the extract obtained. It was concentrated using rotary evaporator and stored in the refrigerator at 4°C prior to use.

Synthesis of silver nanoparticles (Ag NPs)

Water lily flower extract (1 g) solved in Water: ethanol, 70: 30 (100 ml), the pH was changed from 3 to 9 using NaOH (1M). Flower extract solution (5.0 ml) was added to aqueous precursor AgNO₃ solution (20 ml, 0.01 M). The reaction was carried out in darkness (to avoid photoactivation of AgNO₃) at room temperatures. The colloidal solution was kept aside at room temperature for 24 h to complete the bio-reduction. Completion of the reaction was confirmed by the change in color from yellow to colloidal brown and monitored by UV-Vis spectra at 300-600 nm (λmax=420 nm). The Ag NPs synthesized by Nymphaea Alba at different conditions were purified by repeated centrifugation at 8000 rpm for 20 min, followed by washing in deionized Water.

Characterization of Ag NPs:

The reduction of pure Ag⁺ ions was monitored by measuring the UV-Vis spectrophotometer (JENWAY 650), having a resolution of 1 nm in the wavelength range of A300 to A600 nm. Functional characterization of the Ag NPs was carried out using Fourier transform infrared (FT-IR) spectroscopy. The FT-IR spectra were recorded on a Bruker IFS-88 instrument (the samples as KBr disks for the range 400–4000 cm⁻¹). The morphology of Ag NPs was carried out using an energy filtering transmission electron microscope (TEM, CM120, Philips). Nanoparticles size was determined by powder X-ray diffraction (XRD) PW 3040/60 X’Pert PRO diffractometer system, using Cu Ka radiation with (λ = 1.5418 Å) in the range of 2θ = 20–80° at room temperature.

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References