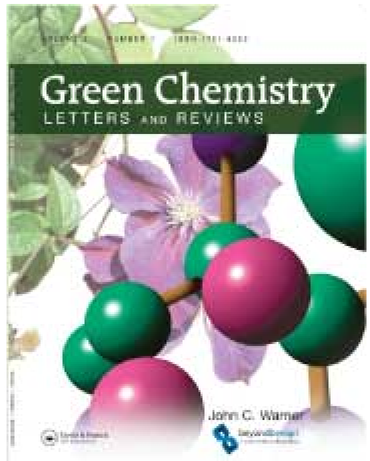

Synthesis and characterization of the photocatalytic and antibacterial properties of green silver nanoparticles

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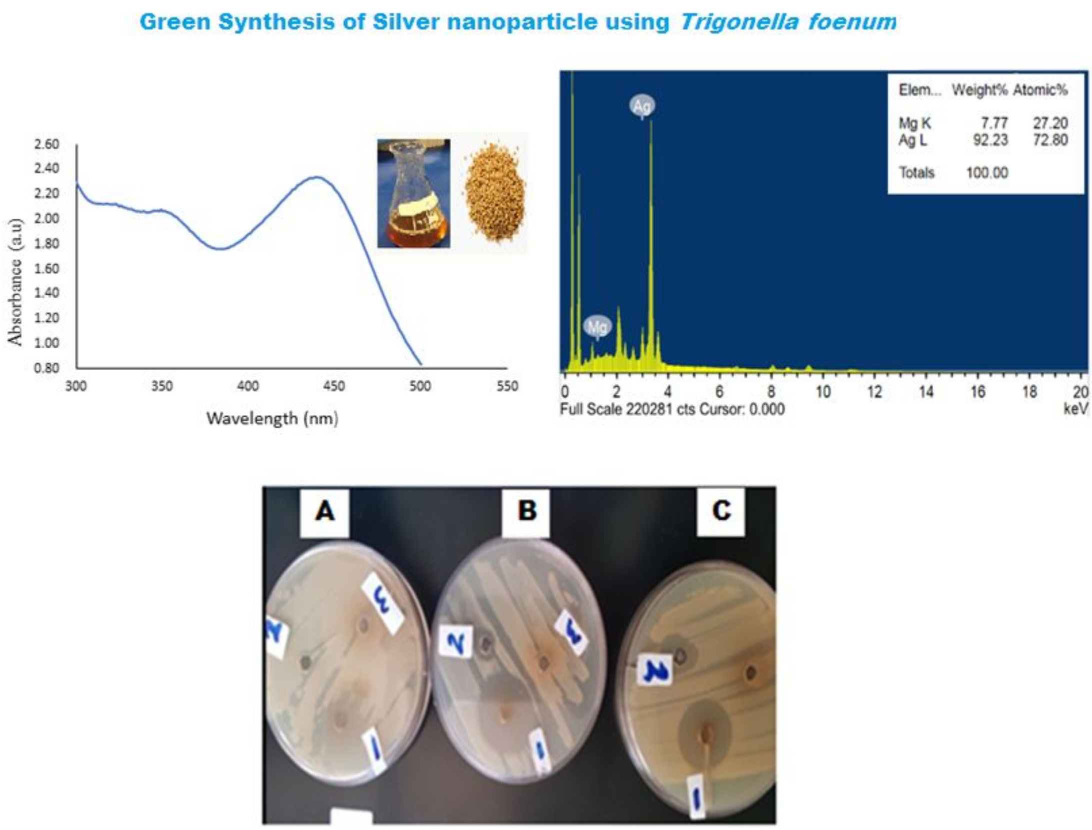

Green synthesis and characterization of photocatalytic and antibacterial properties of silver nanoparticles

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Abstract

The present study focused on exploiting of eco-friendly *Trigonella foenum* extract for the synthesis of silver nanoparticles with minimum scale size and ultra-stable features. The role of green synthesized nanoparticles was impact in improving and minimizing the environmental pollution by decreasing the use of hazardous chemicals and eliminating biological risks in biomedical applications. The as prepared silver nanoparticles were monitored by different spectroscopic and microscopic techniques. These include, UV-Vis spectrophotometry with maximum absorption peak at 443 nm, fourier transform infrared spectrophotometry and fluorescence analysis, transmission zetasizer, electron microscope and energy dispersive spectrometer. The morphology

of silver nanoparticles was predominantly spherical and aggregated into an irregular structure with an average diameter of 82.53 nm. Additionally, the prepared nanoparticles were employed for the screening of antibacterial activity and the photocatalytic degradation of hazardous *Rodamine B* dye.

Keywords: *Rhodamine B*; *Trigonella foenum*; Green synthesis; Catalysis; Antibacterial activity

1. Introduction

Nanoscience and nanotechnology are the manipulation and the study of extremely small scale matter and can be employed in various applications across other science fields, such as physics, biology, chemical engineering and material science (*1*). Nanoparticles (NPs) are almost the final results of physicochemical and biological processes (*2*). The unique features of metal NPs such as, large surface energy, high surface area and quantum confinement, etc. might open expanding areas for researchers to exploit their potential applicability in various fields such as analytical chemistry (*3*), electronics (*4*), fuel cell (*5*) and biomedical applications (*6*). Thus, it is promising to use green and environmentally friendly methods for synthesis of NPs. Due to the presence of various natural chemical compounds such as polyphenols, flavonoids, sterols, triterpenes, and sugars in the plants, the literature survey revealed several green methods based on the employment of plant extracts (*7, 8*), or naturally capping materials (*9*) for the synthesis of metal NPs such as gold and silver NPs. The synthesized silver NPs (AgNPs) exhibited effective inhibitory activity against waterborne pathogens and microbes (*10*). Beside the valuable properties of AgNPs in various science fields such as optical, electronic, chemical, photoelectrochemical, catalytic, magnetic, they have significant antimicrobial activity and can be applied to the biomedical applications such as blood collection vessels, and wound bandages

(*11*). On the other hand, due to their cytotoxicity features, AgNPs have been reported as nontoxic agents for animal cells, but highly toxic for bacterial cells such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Therefore, they can be act as effective bactericidal agents (*12-13*).

Organic dyes are considered as the major source of pollutants widely used in many fields such as in industry of plastic, textile and medicine. The scientific researchers started to concern with the hazardous influence of organic dyes as a major environmental pollution problem (*14*). These industries required large quantities of dyes that cause toxicity. Therefore, the necessary of new techniques to reduce their accumulation and sever harmful effect is the main attention now.

Nowadays, the use of nanocparticles of nobel metals in photocatalytic degradation provides many advantages over the conventional methods. Many researchers started to study how to improve the photocatalytic degradation using nanometals such as silver and gold (*15, 16*). The physical and chemical features of nanoparticles including their reduced size, large surface area and high reactivity, play an important role in the degradation of dyes (*17*). Therefore, the removal of non-biodegradable dyes from the environment is considered as a crucial ecological problem (*18*). Although, there are numerous available techniques to remove these dyes; recently, metal NPs have been used to face this problem.

The present study aimed to suggest a new green synthesized AgNPs using *Trigonella foenum* extract to degrade and illuminate the dye *Rhodamine B* (RB). Furthermore, the antibacterial activity of the green synthesized AgNPs was screened against different strains of bacteria.

2. Materials and methods

2.1. Green synthesis of AgNPs

Trigonella foenum seeds were purchased from a local market in Riyadh City, Saudi Arabia. The seeds were initially washed in distilled water and dried on paper towels. Approximatelly, 10 g sample was grinded to powder and boiled in 100 mL distilled water for 30 min. The extract was filtered using a Whatman filter paper No.1 and the filtrate was kept at 40ºC for further usage. Pure analytical grade of 2.0 mmol L^{-1} silver nitrate (Techno Pharmchem, Delhi, India) was dissolved in 50 mL distilled water under vigorous stirring at 100 ºC for 10 min. Then, approximately, 10 mL of *Trigonella* extract was added to the silver nitrate solution. The color of the colloidal solution was changed within ~30 min, which indicated the reduction of Ag ions into Ag metal and confirmed the formation of green AgNPs. The green AgNPs solution was centrifuged at 9000 rpm for 15 min and AgNPs pellets were collected, lyophilized and recovered in powdered using a Heto-Holten A/S, DK-3450 freeze dryer (Allerd, Denmark).

2.2. Characterization of AgNPs

The synthesized AgNPs were characterized using a Shimadzu UV 2450 UV-Vis Spectroscope (Shimadzu Corporation, Kyoto, Japan) over 300 –550 nm. The fluorescence and optical properties were characterized by a fluorescence spectrofluorophotometer (XRF, RF-5301(PC) S Shimadzu Corporation). Fourier Transform Infrared Spectroscopy (FTIR) for the prepared NPs was performed using Perkin Elmer FT-IR spectrophotometer (PerkinElmer Ltd., Yokohama, Japan). The size of the synthesized green AgNPs was analyzed using a dynamic light scattering (DLS) technique through a zetasizer (Nano series, HT Laser, ZEN3600 Malvern Instruments, Malvern, UK). The particle size, shape and morphology of the green synthesized AgNPs were investigated using TEM (JEM-1011, JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 100 kV. Energy-dispersive spectrometer (EDS) analysis was carried using a JEOL LTD JSM-7610F scanning electron microscope to confirm the presence of silver in the particles as well as to detect other elementary components of the particles.

2.3. Photocatalytic activity

In order to evaluate the photocatalytic activity of the green synthesized AgNPs, RB dye was depredated under light irradiation. A sample of ~ 5 mg of AgNPs was mixed with 20 mL of 10 mgL^{-1} RB dye solution using a magnetic stirrer in the dark to maintain the equilibrium constant. After, exposing the solution to a UV lamp with constant stirring, the supernatant solution was measured using UV-Vis spectrophotometer.

Optical absorption spectra were determined for different durations of light exposure. A Perkin Elmer (Waltham, MA, USA) UV-Vis spectrophotometer was used to monitor the rate of degradation by recording the reduction in absorption intensity of dye at the maximum wavelength (λ_{max} = 552 nm). The degradation efficiency (DE) was calculated by the following equation:

$$DE\% = \frac{A_0 - A}{A_0} \times 100$$

Where, A_0 is the initial absorption intensity at λ_{max}= 553 nm and A is the absorption intensity after photocatalytic degradation.

2.4. Assessment of antibacterial activity

Antibacterial activity of synthesized green AgNPs was performed by using the agar well diffusion method (*19, 20*). Approximately 20 mL of sterile nutrient agar (Scharlau Microbiology, Barcelona, Spain) was poured into the sterile petri dishes. Triplicate plates were swabbed with overnight cultures of human pathogens *E. coli*

ATCC 25922 S. aureus ATCC 25923*, and Bacillus cereus* ATCC 11778 (clinical isolates obtained from King Khalid University Hospital Riyadh, Saudi Arabia). The solid medium was gently punched with a sterile cork borer to make a well (3 wells/plate). Finally, the aqueous *Trigonella fornum* AgNPs (5, 15 μgmL^{-1}) were added to each well and incubated for 24 h at 37°C. After complete incubation, the diameter of the zone of inhibition was measured in cm.

3. Results and Discussion

3.1. UV-Vis spectral analysis of green synthesized AgNPs

UV-visible spectroscopy is a widely applied technique used to characterize and determine the formation of nanoparticles and their optical properties. Due to the influence of NPs spectrum by size, shape, interparticle interaction, free electron density, it is considered as efficient tool for monitoring the electron transfer and aggregation of NPs (*21*). The as prepared AgNPs using *Trigonella foenum* seeds extract as reducing agent (*22*) was characterized using UV-Vis spectrophotometry. It was observed that the initial pale yellow color was changed to dark brown due to the excitation of the surface plasmon resonance (SPR) vibrations of the noble metal NPs, indicating the reduction of Ag^{+} in silver nitrate to the noble AgNPs. Figure 1 showed the UV-Vis spectrum of AgNPs, with an absorption/extinction peak band at 443 nm. The variation of intensity and bandwidth was resulted from the variation in size and size distribution of the NPs. Furthermore, it was been found that there is an increase in the integrated peak area of the band, which indicated a decrease in interparticle spacing, due to the particle aggregation (*23-25*).

3.2. Fluorescence studies of green synthesized AgNPs

The fluorescence properties of green synthesized AgNPs were investigated at λ_{ex} 450 nm and λ_{em} 662 nm. To the best of our knowledge, the surface activity of metal NPs tends to react with hydroxide molecules could be enhanced by the large surface area per unit mass of metal NPs. Therefore, the hydroxyl groups of the extract exhibited a strong affinity towards the electrons. This phenomenon can be confirmed by the SPR of the AgNPs. It was observed that the SPR signal and the fluorescence were enhanced after the adsorption process (Figure 2). Furthermore, electromagnetic excitation induces electrons at the higher occupied energy level (HOEL) undergo electronic transitions to the lowest unoccupied energy level (LUEL) or excited states, the observed fluorescence was affected by the interaction between the missive centers and the interface environment. This can be explained that partial oxidation of metal NPs causes the formations of metal oxide clusters on the metal NP surface, which photoactivated by light excitations (*26*).

3.3. TEM and particle size distribution measurement

The green prepared AgNPs using *Trigonella foenum* seed extract was investigated microscopically using TEM. The morphology and size details of the formed AgNPs were provided and the TEM image depicted in Figure 3A, showed the appearance of spherical shaped AgNPs in individual form as well as aggregated particles.

Additionally, DLS is the most facile technique for evaluating the diffusion coefficient of particles in liquids. The average size distribution and hydrodynamic diameters of the nanoparticles can be calculated (*27*). Figure 3B, showed monodispersed AgNPs with an average diameter ~ 82 nm, with polydispersity index (PDI) of 0.134 revealing good colloidal dispersion and long term stability. The aggregation of AgNPs also estimated using PDI, values ranged from 0-1. Generally, values of ≤ 0.3 are considered to be acceptable and detect a homogenous distribution, and values > 0.7 indicate a heterogeneous dispersion of the samples and are usually not suitable to be measured by DLS (*28*).

3.4. EDS analysis of green synthesized AgNPs

Due to surface plasmon resonance, the metallic silver nanocrystals showed a typical optical absorption peak at the ~ 3 KeV (*29, 30*). Silver (92.23%) was the most common constituent element compared to magnesium (7.77%) as demonstrated in Figure 4. The EDS profile displayed a sharp signal for silver particles, which may be attributed to the biomolecules of *Trifonella foenum* extract bound to the surface of AgNPs, revealing their reduction of elemental silver.

3.5. FT-IR analysis of green AgNPs

The presence of biomolecules in the in the dispersed AgNPs was estimated using FT-IR. A comparative study between the FT-IR spectra of AgNPs (A) and *Trigonella foenum* seeds extract (B) was carried out and it was observed that synthesized AgNPs exhibited significant absorption bands at 3264.32, (2166.98 and 2144.50), 1636.98 and 1961.83 cm^{-1} were corresponding to -OH group, -C ≡ C-, N-H, and =C-H, respectively (Figures 5A and 5B). On the other hand, the previous FT-IR comparative spectra investigation showed a significant peak position shift, which may be attributed to the presence of the residual *Trigonella foenum* extract as reducing and stabilizing or capping agents for AgNPs *(31)*. Furthermore, the presence of alkyne (-C ≡ C-) and amine (−NH) groups in *Trigonella foenum* extract were mainly involved in the reduction process of silver ions to AgNPs, which also confirmed that the presence of protein in *Trigonella foenum* extract could be acted as a stabilizing and reducing agents for AgNPs and preventing their agglomeration.

3.6. Photocatalytic degradation

The photocatalysis of the green synthesized AgNPs to induce RB degradation was studied. It was observed that the photodegradation of RB dye was increased by passing time and the complete photodegradation nearly (93%) with decolorization was achieved after 216 h. Regarding to Beer's-Lambert law, a linear relationship was obtained between the concentrations of RB vs. the intensity of the absorption peak at 443 nm. This can be attributed to the stable number of the photons available for photodegradation. The layer of reducing agent on the surface of AgNPs may also promote the effective adsorption between AgNPs and RB molecules. As a consequence, the oxidation-reduction between the active RB and reducing agent can occur more easily, effectively, and rapidly for particles of a smaller size (*32*). The results indicated that the high reactivity of large surface area of AgNPs acted as an efficient photocatalysis for dye degradation under UV light and confirmed the high efficiency of AgNPs to minimize and eliminate the dye pollution. Owing to the reaction mechanism is not fully known; oxidation-reduction reaction can be occurred involving intermediates such as hydroxyl radicals (*33*).

3.7. Antibacterial activity of green AgNPs

The antibacterial activity of AgNPs synthesized by *Trigonella foenum* extract was investigated using two concentrations 5 μgmL^{-1} (Ag 2) and 15 μgmL^{-1} (Ag 1) against pathogenic organisms *S. aureus*, *B. cereus,* and *E. coli*. The well diffusion method was applied and the diameters of the zone of inhibition /cm using AgNPs were shown in Figure 7. The AgNPs synthesized by *Trigonella foenum* extract were found to have higher antibacterial activity at both high and low concentrations against *E. coli* (1.4, 0.2 cm) and *B. cereus* (1, 0.09 cm), respectively, while less antibacterial activity for the green AgNPs was found against *S. aureus* (0.5, 0.2 cm). Additionally, there was no inhibition zone observed in the *Trigonella foenum* extract (Figure 7).

Several studies have proposed the mechanisms underlying the bactericidal action of AgNPs, one of them suggested that, due to their high surface area, the AgNPs showed efficient antibacterial activity compared to the extract. This high surface area provides better contact with microorganisms. The NPs is attached to the cell membrane and penetrate the bacterial cell, through the interaction of AgNPs with sulfur-containing proteins contained in the bacterial membrane, as they do with phosphorus-containing compounds such as DNA. Another study suggested that when AgNPs penetrate the bacterial cell, they form a low molecular weight region in the center of the bacteria that conglomerates, protecting DNA from the silver ions. A third study suggested that, the NPs prevent cell division, which ultimately leading to cell death, by preferentially attack the respiratory electron transport chain. The NPs enhance their bactericidal activity, by release silver ions in the bacterial cells. Also, the action of AgNPs on bacteria may also occur via interaction with thiol group-containing respiratory enzymes of bacterial cells that inhibits the respiration process. In addition to that, it has been shown that AgNPs can enter into bacterial cells and hence condense DNA, thus preventing DNA from replicating and cells from reproducing (*34-38*).

Conclusion

The present study described a simple, rapid, and efficient method for the green synthesis of AgNPs using *Trigonella foenum* seed extract as a reducing agent. The optical properties, structure, morphology, photocatalytic activity and antibacterial activities of these AgNPs were examined. The optical analysis resulted in the appearance of a significant peak at 443 nm which confirmed the formation of AgNPs in the reaction mixture. FTIR spectrum identified the potential biomolecules in the *Trigonella foenum* extract which enhanced the reduced of Ag^+ ions to Ag^0. Spherical-shaped AgNPs with an average diameter of 82 nm were observed using

TEM, a zetasizer, and metal Ag signatures were confirmed by EDS. The antibacterial activity of green synthesis AgNPs was exhibited to be effective against both Gram-negative and Gram-positive strains. Finally, the obtained results revealed the suitability of AgNPs as active material in the photocatalytic degradation of RB dye.

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Conflict of interest

The authors declare that this work not associated with any conflict of interest

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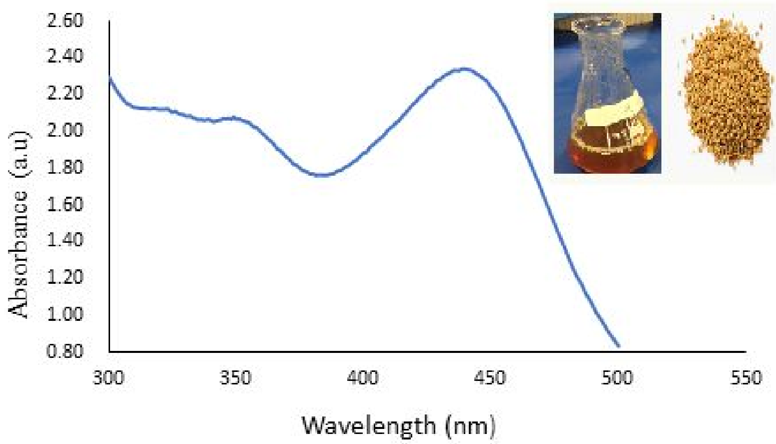

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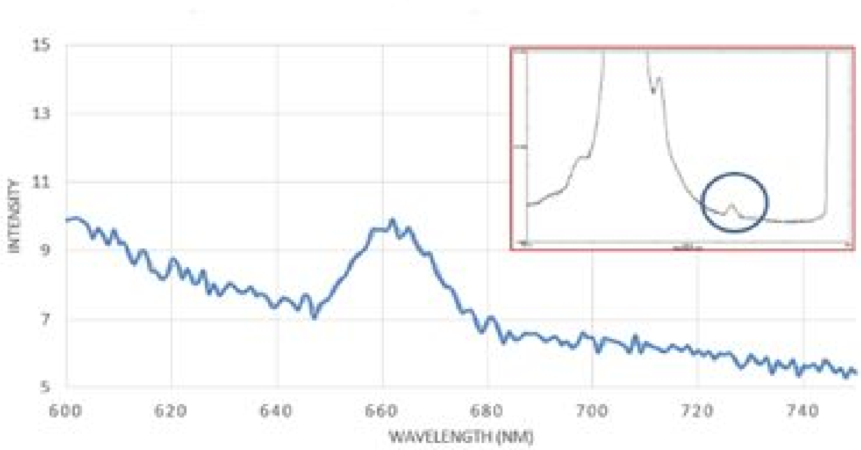
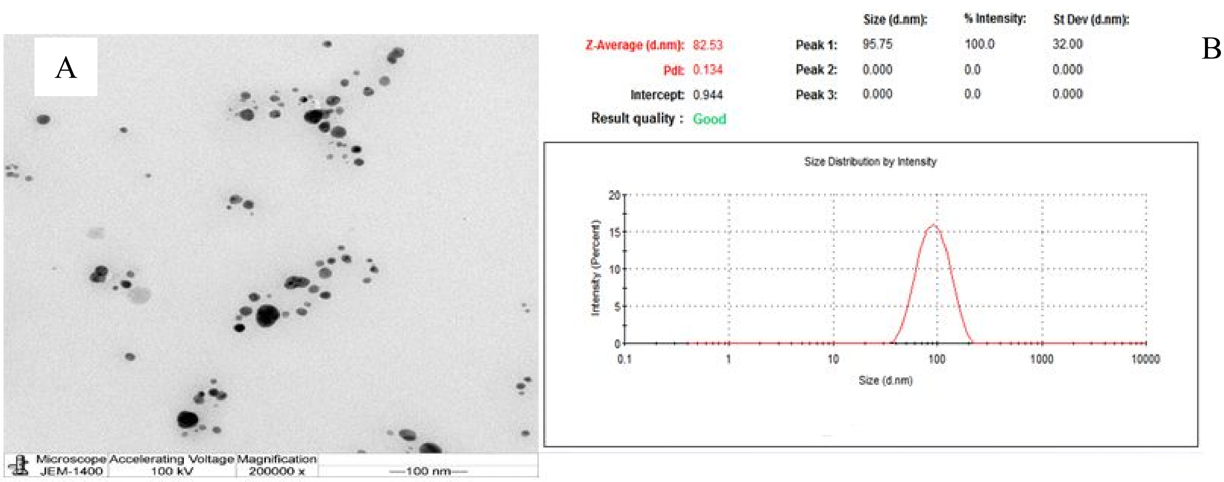


Figure 2. Fluorescence spectra of *Trigonella foenum* AgNPs



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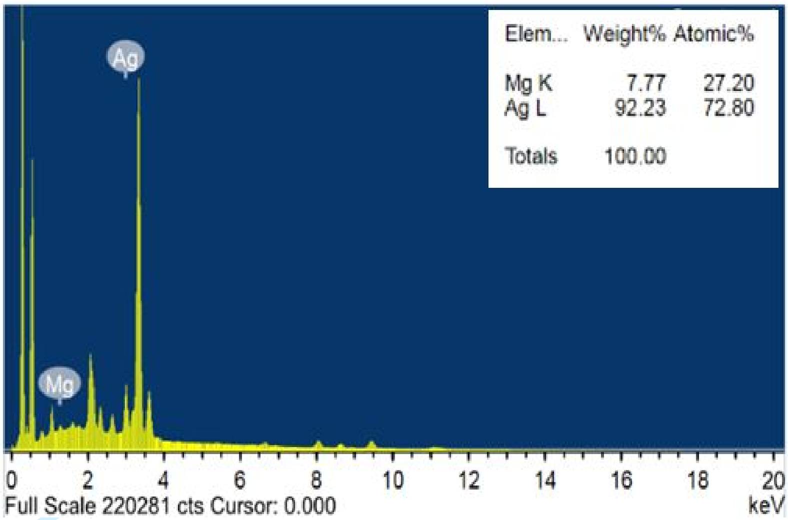

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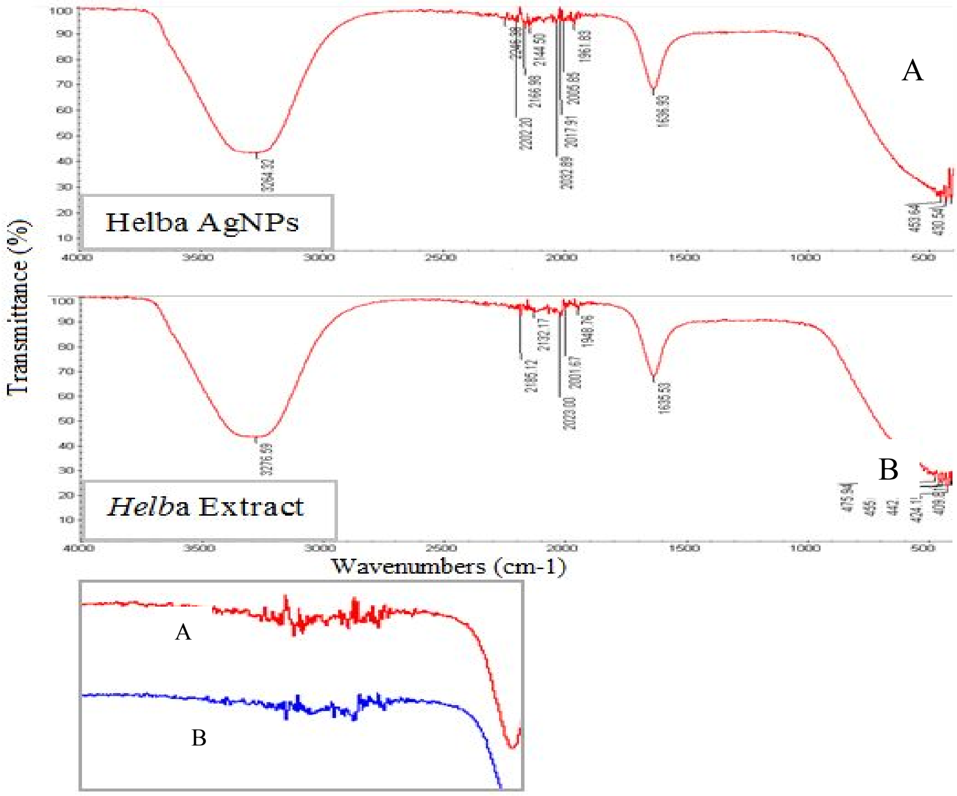

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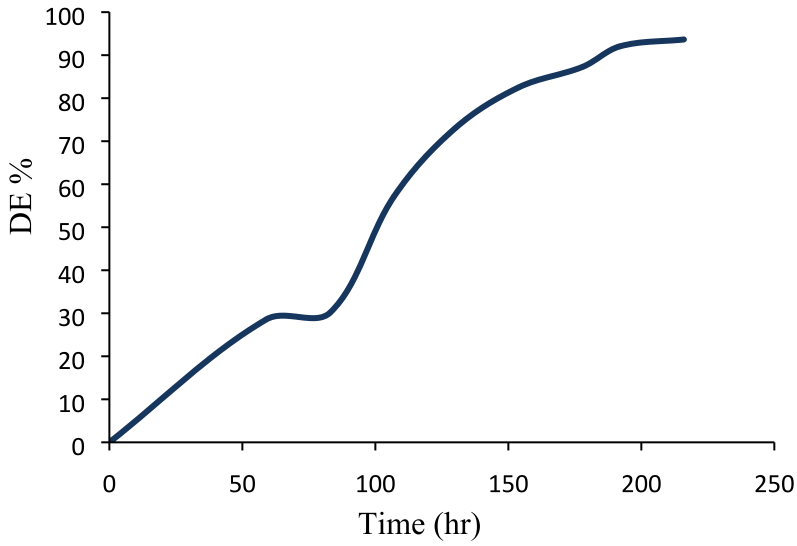
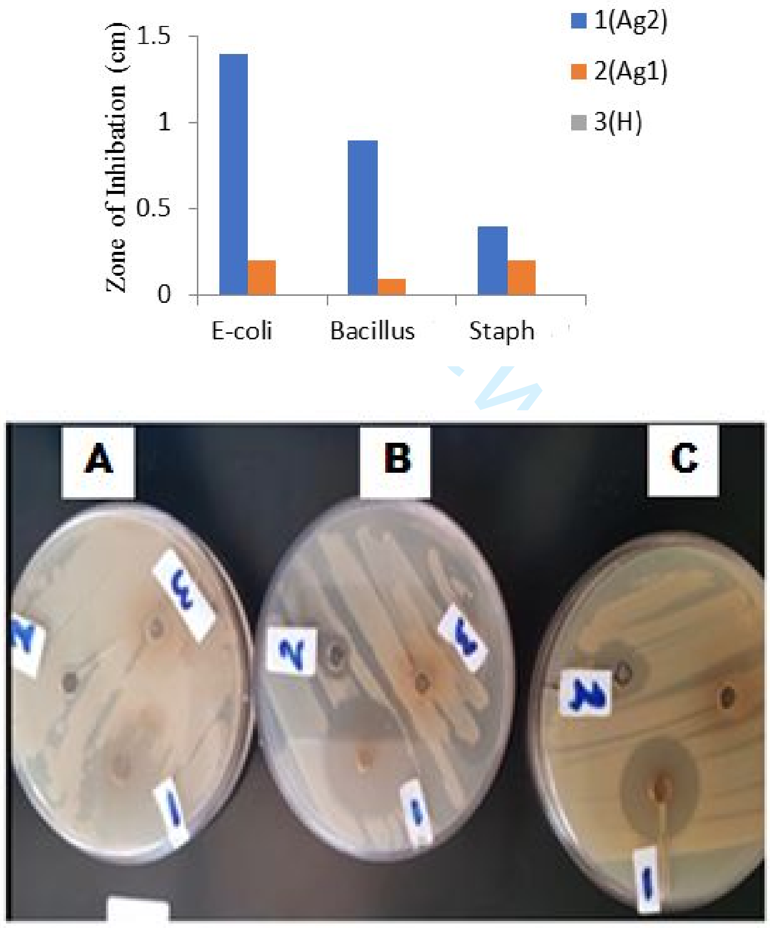


Figure 6. Percentage of dye degradation at time of exposure



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