Antioxidant, cytotoxic and catalytic degradation efficiency of controllable phyto-synthesised silver nanoparticles with high stability using Cordia myxa extract

Fayezeh Samari, Parinaz Parkharia, Ebrahim Eftekhar, Fatemeh Mohseni and Saeed Yousefinejad

ABSTRACT
This study aimed to report a one-pot, eco-friendly and room temperature procedure to prepare highly stable (at least 15 months) silver nanoparticles (AgNPs) using the aqueous leaf extract of Cordia myxa. The effects of different parameters, such as applied pH in the reaction mixture, amount of the leaf extract, silver ion concentrations, time of reaction and synthesis temperature, on the formation of NPs and their Surface Plasmon Resonance (SPR) spectra were studied. The characterisation of the prepared AgNPs was done using UV-Vis spectroscopy, Fourier Transform Infrared (FTIR), X-Ray Differentiation (XRD) and Transmission Electron Microscope (TEM) techniques. The shape of the synthesised AgNPs was spherical and it sized 3–10 nm with a face-centred cubic structure with SPR spectra at 410 nm. A possible reaction mechanism of AgNP formation by biomolecules of C. myxa was also introduced. The efficiency of the synthesised AgNPs as an excellent catalyst for the reduction of organic azo dyes using NaBH₄ was proved. Dose-dependent cytotoxic activity of the prepared AgNPs against SW480 and HCT116 human colon cancer cell lines was also shown using MTT assay. Furthermore, the Ferric-Reducing Antioxidant Power (FRAP) assay was utilised to confirm the antioxidant activity of NPs. Non-toxic reagents and low-cost synthesis were the main features that made these AgNPs more attractive for chemical/biomedical applications.

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1. Introduction

Nowadays, important applications of nanoscience in various chemical, industrial, medical and health/safety branches have made this field interesting and widespread [1–4]. On the other hand, noble metals Nanoparticles (NPs) have attractive physicochemical properties and, consequently, have found many applications in different fields such as medicine, chemistry, physics and biology [5, 6]. One of the attractive applications of noble metals NPs is in drug delivery and biosensing because of their unique physicochemical and optoelectronic properties. Amongst these NPs, silver nanoparticles (AgNPs) have attracted much interest because of their excellent biocompatibility, high electrical and thermal conductivity, optical properties, chemical stability, catalytic performance and electrochemical and antimicrobial activities [7–10]. On the other hand, increasing attention has been paid to the green synthesis of NPs over chemical and physical syntheses. These green approaches are very feasible, hygienic, economical, eco-friendly and harmless, include non-hazardous solvents, and can be done in safe reaction media [10–14]. The biosynthesis of NPs using fungi [13–16], bacteria [17, 18], algae [19] and plants [4, 20–25] is an important topic in nanotechnology. In this context, making use of plants has considerable benefits, such as their availability, low-cost, rapid single-step method, eco-friendliness, possibility in low pressure, not needing toxic compounds or high energy, and being safe for human therapeutic usages [10, 12, 26]. It has been approved that various water-soluble heterocyclic molecules and different polyol components are responsible for both protective and reductive roles.

*Cordia myxa* (C. myxa) belongs to *Boraginaceae* (Hound’s-Tongue) family and probably originates from an area stretching from the Middle East to tropical Africa [27]. The leaf extracts of *C. myxa* have shown significant anti-inflammatory, analgesic and antiarthritic properties. It has also been reported that *Cordia* extracts contain phenolics and flavonoids, such as robinin, rutin, datiscoside, hesperidin, aglycone, dihydrorobinetin, chlorogenic and caffeic acid [27–29]. Very recently, *C. myxa* leaf extract was utilised for the biosynthesis of AgNPs [30]. However, the effects of different experimental factors on the green synthesis of AgNPs have not been explored. Therefore, the present work aims at (i) biosynthesising AgNPs through application of the aqueous extract of dried *C. myxa* leaf for reduction of aqueous silver ions, (ii) proposing a possible reaction mechanism for AgNP preparation using phyto-molecules of *C. myxa*, (iii) investigating the impacts of
various empirical conditions such as pH, quantity of the leaf extract, concentration of the silver ion solution, time of mixing and temperature on the preparation of NPs, (iv) characterising the prepared AgNPs by UV-Vis spectrophotometry, Fourier Transform Infrared (FTIR) spectroscopy, X-Ray Diffraction (XRD) and Transmission Electron Microscope (TEM), (v) evaluating the antioxidant and cytotoxic activities of the green synthesised AgNPs towards biomedical applications and (vi) investigating the catalytic activity of the formed silver NPs on the degradation of Rhodamine B (RhB) and Methylene Blue (MB) in the presence of sodium borohydride (NaBH₄).

2. Materials and methods

2.1. Chemicals

Silver nitrate, RhB, MB and NaBH₄ were procured from Merck Company, Germany. All other utilised compounds were at the reagent grade and were used with no further purification. All glassware was washed with aqua regia, rinsed several times using freshwater and re-washed with Deionised (DI) water. DI water from Millipore was used for reagent preparation. In order to adjust the pH, diluted nitric acid (HNO₃) and sodium hydroxide (NaOH) solutions were used.

2.2. Preparation of C. myxa leaf extract

Cordia myxa L. (Boraginales order, Boraginaceae family) leaves were collected from Rooydar, Hormozgan province, Iran (57°6’E, 60°3’N). The taxonomy of the plant was confirmed and authenticated by Dr. Mansoore Shamili (https://orcid.org/0000-0001-7967-438X. . . . . . . . ), Faculty of Agriculture and Natural Science, University of Hormozgan, Iran, assigning voucher specimen number 2356. The collected leaves of C. myxa were thoroughly cleaned in tap water for several times in order to remove the dust particles followed by washing with distilled water and drying with air for 4–6 days. The dried leaves were pulverised into a fine powder using an electric grinder. The C. myxa leaf solution was made by putting 10 g of dried powder in a 250 mL Erlenmeyer flask along with 150 mL of DI water. Then, the mixture was heated at 80 °C for 30 min. After cooling, the mixture was first filtered through normal filter paper to remove the leafy particles. Then, it was filtered by Whatman No. 1 paper, and the filtrate was collected and kept in the refrigerator and utilised within a week.

2.3. Synthesis of AgNPs using C. myxa leaf extract

In order to synthesise AgNPs, 2.0 mL of C. myxa leaf extract was added to 25 mL of 1.0 mM aqueous solution of silver nitrate in a 100 mL Erlenmeyer flask with continuous stirring. The synthesis of AgNPs was clarified by the alternation in solution colour from watery yellow to dark-brown.

2.4. Optimisation studies for the biosynthesis of AgNPs

The effects of different experimental parameters were studied during the preparation of AgNPs, which have been explained as follows:
2.4.1. pH

To study the effect of pH on the synthesis of NPs, *C. myxa* leaf aqueous extract (2 mL) interacted with the AgNO₃ solution (25 mL, 7.0 mM) at room temperature. Then, the reaction pH was adjusted to 7.0, 9.0 and 11.0, and its effect on the Surface Plasmon Resonance (SPR) peaks was measured after 3 h at each pH level. A pH meter/potentiometer (Metrohm, model 654) with a combined glass electrode was used for the measurement of pH.

2.4.2. Concentration of silver nitrate solution

Herein, 25 mL of different concentrations of AgNO₃ (0.5, 1.0, 5.0, 7.0 and 10.0 mM) were used to determine the optimum concentration for the reaction at room temperature and pH = 11.0. After 3 h, absorption spectra of the AgNPs suspension were obtained using a UV-Vis spectrophotometer.

2.4.3. Quantity of *C. myxa* leaf extract

To examine the effect of the *C. myxa* extract quantity on the synthesis of NPs, different volumes of *C. myxa* leaf extract were added to 25 mL silver nitrate (7.0 mM) at pH = 11.0 and room temperature. After 3 h, the SPR peaks were measured using a UV-Vis spectrophotometer.

2.4.4. Temperature

The effect of different temperatures (room temperature, 40, 60 and 80 °C) on the synthesis of NPs was investigated. The quantity of *C. myxa* leaf extract (2.0 mL), AgNO₃ solution concentration (25 mL of 7.0 mM), and incubation time (3 h) were kept constant at pH = 11.0, and the suspension was analysed using a UV-Vis spectrophotometer.

2.4.5. Time

The time of the reaction process was optimised with the incubation of the reaction solution at specific time intervals (from 10 to 260 min). The resulting AgNPs were analysed using a UV-Vis spectrophotometer.

The synthesised mixture at the optimum condition was subjected to centrifugation at 10,000 rpm for 30 min following which the pellet was re-dispersed in DI water for the elimination of uncoordinated biological species. Re-dispersion and centrifugation of NPs in DI water were repeated three times to separate free objects from the metal NPs completely. Afterwards, the purified pellets were dried and an aliquot dried powder of AgNPs was utilised for XRD and FTIR analyses.

2.5. Characterisation of silver nanoparticles

2.5.1. Absorbance spectroscopy

Preliminary characterisation of the as-synthesised AgNPs was done by a Scinco UV-Vis spectrophotometer (S-3100, Korea) equipped with a 1.0 cm quartz cuvette at room temperature. For all UV-Vis spectrophotometry analyses, the synthesised AgNPs solution was diluted by DI water at 1:35 ratio.
2.5.2. XRD analysis

XRD pattern of AgNPs was obtained using a powder X-ray diffractometer (Bruker D8 Advance powder diffractometer) with Cu-Kα radiations ($\lambda = 1.5406 \text{ nm}$) in 2θ range from 20° to 80°.

2.5.3. FTIR spectroscopy

FTIR analysis of the dried powder of the leaf extract and synthesised AgNPs was carried out on a Bruker alpha FTIR spectrometer (Bruker, Germany) using a diamond Attenuated Total Reflection (ATR) accessory by scanning in the range of 4000–600 cm$^{-1}$ at a resolution of 4 cm$^{-1}$ to reveal the distribution of functional groups related to the phyto-compounds on the surface of the NPs.

2.5.4. TEM

The fine configuration of the constructed AgNPs was analysed using TEM. For this purpose, the sonication of colloidal AgNPs was done first and then, a thin film of AgNPs was made on the carbon-coated grid Cu Mesh 300. All TEM images were collected at an accelerating voltage of 80 kV.

2.5.5. Dynamic light scattering (DLS)

The particle size distribution of the synthesised AgNPs was determined by DLS particle size analyser (Horiba SZ-100) at 25°C. The intensity of scattering was measured at a 173° angle in kilo-counts per second.

2.6. Homogenous catalytic potential of AgNPs

The efficacy of the phyto-synthesised AgNPs for degradation of pernicious azo dyes, MB and RhB was evaluated. In a typical reduction protocol, 10 mL of 4.0 × 10$^{-5}$ M MB aqueous solution was mixed with the freshly made NaBH$_4$ aqueous solution (0.05 M, 0.5 mL) and the reaction mixture was stirred at room temperature for 2 min. Then, 100 μL of AgNPs colloid was added and stirring was continued for one more minute. Scans were started 1 min after the addition of the NPs, and the solution was left untouched until completion. The reduction reactions were monitored using a UV-Vis spectrophotometer at regular time intervals (1 min). The same procedure was repeated with 10 mL of 2.3 × 10$^{-5}$ M RhB, 0.5 mL of NaBH$_4$ (0.05 M) and 0.5 mL of the synthesised AgNPs.

Absorbance was monitored at the wavelength of maximum absorption of the respective dye (554 nm for RhB and 664 nm for MB). Similar experiments were also done in the absence of a nanocatalyst as the reference (control) runs.

The amount of dye residual was determined by following each dye absorbance ($A$) at 554 nm for RhB and at 664 nm for MB. The degradation efficiency ($R$) of each investigated dye by AgNPs was measured using Eq. (1) [31, 32]:

$$R = \left( \frac{A_0 - A_t}{A_0} \right) \times 100$$

(1)

where $A_0$ and $A_t$ represented absorbance values at the 0th and tth minutes at 554 nm for RhB and 664 nm for MB.
2.7. Cytotoxicity against human Colon cancer cell lines

The human colon cancer cell lines SW480 and HCT116 used for this part were prepared from the National Cell Bank (NCBI, Pasteur Institute, Tehran, Iran). The utilised cells were cultured in RPMI-1640 (SW480) or DMEM (HCT116) supported with 10% Fetal Bovine Serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin at 37°C in 5% CO₂ atmosphere with 95% humidity.

To investigate the cytotoxic effect of the green synthesised AgNPs, Multi-Target Tracking (MTT) assay was performed against SW480 and HCT116 cell lines. In doing so, 8 × 10³ colon cancer cells were seeded in each well of a 96-well plate and were then incubated at 37°C with 5% CO₂ in 100 μl of the culture medium for 24 h. After the growth of the cells, they were treated with various concentrations of the prepared AgNPs (0, 12.5, 25 and 50 μg/mL), which were freshly synthesised and incubated for 24 h. For the control runs, the media of the cells were replenished without synthesised AgNPs. After that, 20 μl of the MTT solution (5.0 mg/mL) was added to each well and was then incubated for four hours. Afterwards, the media were aspirated and DMSO (150 μl/well) was applied to dissolve the crystals. The absorbance value of the crystals solution was recorded at 570 nm by a microplate reader (Biotech, USA). The percentage of cell viability was reported as the mean of three-independent experiments. The cell viability was calculated based on Eq. (2) and the Optical Density (OD) of the control and main samples at 570 nm [33]:

\[
\text{Viability (\%)} = \frac{OD_{570(\text{sample})}}{OD_{570(\text{control})}} \times 100
\]

2.8. Antioxidant potential of the synthesised AgNPs

The antioxidant potential of C. myxa extract and phyto-synthesised AgNPs was checked using Ferric-Reducing Antioxidant Power (FRAP) [34], which is based on the reduction of ferric tripyridyltriazine complex (Fe(III)-TPTZ) to ferrous tripyridyltriazine (Fe(II)-TPTZ) using a reductant (here, AgNPs) at a low operating pH accompanied with monitoring the change in its absorbance at 593 nm. The reagent of the FRAP assay was prepared daily by mixing 300 mM acetate buffer (pH 3.6) with 10 mM TPTZ solution in 40 mM HCl and 20 mM FeCl₃ · 6H₂O at a 10:1:1 ratio and at a temperature equal to 37°C. Fifty microlitres of the tested samples (AgNPs or C. myxa extract dissolved in DI water) at various concentrations (125, 250, 500, 750 and 1000 μg/mL) were properly mixed with 1.5 mL of the FRAP reagent followed by incubation in the dark at 37°C for 10 min. To identify the FRAP value for each sample, the initial absorbance of the blank sample (only FRAP reagent) was subtracted from the final absorbance of the main sample (FRAP reagent added to AgNPs and C. myxa extract). The standard curve was constructed using a set of known concentrations of ferrous sulphate (FeSO₄) (0.125–2 mM) and the obtained values were expressed as Fe(II) ion equivalent (mM).

3. Results and discussion

3.1. The ability of C. myxa extract in the phyto-synthesis of silver nanoparticles

In this study, the reducing and stabilising agent for the phyto-synthesis of AgNPs was the leaf extract of C. myxa without the need for any other chemical or external reducing
agents. The applied approach was totally non-hazardous, non-toxic, clean and environmentally friendly. It has been reported that the leaf extracts of certain species of *Cordia* contain flavonoids and phenolic derivatives, such as robinin, rutin, datiscoside, hesperidin, dihydrorobinetin, chlorogenic and caffeic acid [27–29], which could probably aid the phyto-synthesis of AgNPs. Phenolic compounds via ketonic and hydroxyl groups are capable to bind to metals and demonstrate chelate effects [35]. A possible mechanism for the synthesis of AgNPs during the reduction of Ag$^+$ by the leaf extract of *C. myxa* has been illustrated in Scheme 1. In order to monitor the functional groups existed in the *C. myxa* leaf extract, FTIR and UV-Vis spectroscopy were carried out and the recorded spectra have been depicted in Figure 1(a,b). The absorption spectra of the *C. myxa* leaf extract (Figure 1(a)) demonstrated specific signals of phenolic compounds, which could be found inside the plants; the bands at $\lambda_{\text{max}}$ 322 nm (band I) originated from the transition within the cinnamoyl system ring, whereas the other absorbance band around 282 nm (band II) was due to the absorbance of the $\pi \rightarrow \pi^*$ transitions in the benzoyl ring system. It should be noted that these two absorbance bands illustrated the presence of polyphenolics in the utilised plant extract [36, 37]. Therefore, these absorbance bands along with previous reports about the constituents of the extract [28, 29] revealed the capability of the extract for application in the biosynthesis of AgNPs. Furthermore, FTIR analysis was performed to confirm the presence of polyphenolics in the *C. myxa* leaf extract. A number of bands that are indicators of the complex feature of the applied leaf extract have been shown in Figure 1(b). A band at 3232 cm$^{-1}$ showed the free OH in the molecule and OH group of hydrogen bonds. Besides, the small sharp band presented at 2929 cm$^{-1}$ might indicate the C–H stretching of a methylene group. The absorption band at 1573 cm$^{-1}$ corresponded to the stretching band of C=O in amides. In addition, the IR band appearing at 1382 cm$^{-1}$ could be attributed to the stretching C=C aromatic ring. Finally, the band at 1062 cm$^{-1}$ corresponding to C–N stretching indicated the presence of an amine group [38–40]. Because of the presence of these functional groups, the spectrum could show the presence of phenolic compounds in the *C. myxa* extract. These results confirmed that the bioactive compounds present in the *C. myxa* aqueous leaf extract had the upper hand in the synthesis of AgNPs.

**Scheme 1.** Mechanism of synthesised AgNPs using the aqueous leaf extract of *C. myxa.*
3.2. Biosynthesis of silver nanoparticles

In this research, the *C. myxa* leaf extract was applied for reduction of $\text{Ag}^{+}$ to $\text{Ag}^{0}$ and formation of AgNPs. Adding the leaf extract of *C. myxa* to an AgNO$_3$ solution within 10 min caused the colour of the mixture to change from watery yellow to brown, showing the synthesis of AgNPs due to its SPR \[9\]. The reaction was further allowed to progress for 180 min to achieve complete reduction.

3.2.1. The effect of pH

UV-Vis spectra of the phyto-synthesised AgNPs at different initial pH values (7.0, 9.0 and 11.0) have been depicted in Figure 2(a), which demonstrated the strong dependence of the construction of AgNPs on the pH of the reaction medium. By increasing the pH of the mixture from 7.0 to 11.0, the absorbance spectra increased, as well. This might be related to the higher formation rate of AgNPs in alkaline pH than in neutral pH because of the ionisation of the phenolic functional group present in the leaf extract \[41, 42\]. Additionally, by increasing the pH value, the $\lambda_{\text{max}}$ of the synthesised AgNPs shifted to a shorter wavelength from 420 to 410 nm and became sharper, indicating the formation of small sized, highly dispersed NPs. No agglomeration was observed at pH $= 11.0$ within 24 h. Therefore, pH $= 11.0$ was favourable and was chosen for NP formation.
3.2.2. The effect of AgNO₃ concentration

The effect of silver nitrate concentration was investigated by changing its concentration from 2.0 to 10.0 mM at room temperature. The influence of silver nitrate concentration on the preparation of AgNPs has been shown in Figure 2(b). Accordingly, the absorbance intensity enhanced drastically by increasing the concentration of AgNO₃, because of the bioavailability of functional groups for reducing silver ions to AgNPs and stabilising the constructed NPs. The most stable AgNPs formation was found at the 7.0 mM concentration of AgNO₃ solution, which showed no agglomeration for a long time. It is noteworthy that agglomeration is enhanced with an increase in the concentration of metal ions [41]. Hence, 7.0 mM was selected as the optimum concentration.

3.2.3. The effect of the quantity of C. myxa leaf extract

The influence of the volume of the utilised extract on the formation of AgNPs was studied by exposing 1.0–5.0 mL of the C. myxa leaf extract to 25 mL of 7.0 mM AgNO₃ for 2 h (Figure 2(c)). As the volume of the leaf extract increased from 1.0 to 2.0 mL, the absorption intensity of AgNPs increased drastically due to the formation of more AgNPs. However, the absorption intensity decreased at 3.0 and 5.0 mL of the leaf extract. The regular decrease in SPR band intensity at 3.0 and 5.0 mL confirmed the formation of large-sized AgNPs. Similar results were obtained by Kokila et al. in the synthesis of AgNPs using Carica Papaya peel extract [43]. It appears from the above discussion that
the optimised volume of the leaf extract for the preparation of AgNPs was 2.0 mL for 25 mL of AgNO₃ (7.0 mM).

### 3.2.4. The effect of the reaction temperature

The effect of temperature on the reduction process was investigated by carrying out the reaction at different temperatures (room temperature, 40, 60 and 80 °C). The results have been presented in Figure 2(d). The absorption spectra showed a gradual enhancement in the absorption intensity as the temperature increased, without any shift in the peak position and peak sharpness (the SPR bands centred at 410 nm). Despite a gradual increase in the intensity of absorption with increasing the reaction temperature from room temperature to 80 °C, these changes were not significant. Therefore, for energy saving, the room temperature was chosen as the optimum temperature.

### 3.2.5. The effect of the reaction time

The progress of the reaction was followed by considering colour changes as well as following the UV-Vis spectrum (Figure 3(a,b)). According to Figure 3(a), by increasing the incubation time, the intensity of absorption peaks was enhanced and SPR got shaper and shifted to a shorter wavelength, indicating smaller sized and monodispersed AgNPs. After 170 min of incubation, no change was observed in absorbance, which confirmed the complete reduction of silver ions to AgNPs (Figure 3(b)).

### 3.2.6. Stability investigation

The stability of the prepared NPs was evaluated by exposing them to the optimum condition for several months. The results were very interesting and showed that the NPs dispersion was very stable. In fact, no obvious changes were detected in the shape, symmetry and position of the absorption peaks even after 15 months. In addition, no signs of aggregation were observed at the end of this period, which demonstrated that the synthesised NPs were highly stable (Figure 3(c)).

### 3.3. Characterisation of the biosynthesised AgNPs

#### 3.3.1. TEM analysis

TEM images were applied to represent the surface morphology and size distribution of the as-prepared AgNPs using C. myxa extract (Figure 4). TEM micrographs of the AgNPs confirmed that the particles were spherical in shape, were well-distributed in the solution without any aggregation, and sized 3 – 10 nm with the average size of 5.8 nm. The TEM images also indicated that the synthesised AgNPs were much smaller than the synthesised AgNPs with C. myxa leaf extract in a previously reported work [30].

#### 3.3.2. DLS analysis

In order to distinguish the particle size distribution of AgNPs in the solution, DLS measurements were performed. As Figure 4(b) depicts, the mean size of the phyto-synthesised AgNPs was 15.3 nm with a Polydispersity Index (PDI) of 0.946. The mean particle size was higher compared to the average particle size of the TEM analysis, which might be due to the fact that DLS measurements were based on the hydrodynamic radius of the particles [43].
Figure 3. (a) Time-dependent UV-Vis absorption spectra of the formation of AgNPs. (b) Optimisation of the reaction time for the synthesis of AgNPs. (c) Spectral changes of the synthesised AgNPs after more than 10 months.
3.3.3. XRD analysis

The XRD patterns of the synthesised AgNPs have been presented in Figure 5. Notable Bragg reflections were observed at 2θ values of 38.10, 44.25, 64.55 and 77.20, indicating the (111), (200), (220) and (311) of the Face-Centered Cubic (FCC) AgNPs, respectively [44, 45]. These results obviously showed that the AgNPs were composed of highly crystalline nanostructures. The mean crystalline size of the AgNPs was estimated via the Debye-Scherrer equation using the Full Width at Half Maximum (FWHM) of the most intense reflection peak at 2θ value of 38.10 related to (111) from the XRD data [46]:

$$d = \frac{K \times \lambda \times 180^\circ}{\beta \pi \cos \theta}$$

where $K$ was the shape factor (0.9), $\lambda$ showed the used wavelength of X-ray Cu-Kα radiation (1.5406 Å), $\theta$ indicated the Bragg angle and $\beta$ represented the FWHM. The average particle size was found to be 8.1 nm, which was in line with the observed TEM image.

Figure 4. TEM image of the biosynthesised AgNPs.

Figure 5. XRD patterns of AgNPs synthesised by C. myxa leaf extract.
3.3.4. FTIR analysis

FTIR measurements of the biosynthesised AgNPs were carried out to identify the possible interaction between the phyto-compounds of the aqueous leaf extract of *C. myxa* and NPs (for reduction and stabilisation) [47, 48]. The FTIR spectra of the synthesised AgNPs have been shown in Figure 1(b). The FTIR band at 3157 cm$^{-1}$ indicated OH stretch.
3.4. The catalytic activity of the biosynthesised AgNPs

The catalytic potential of the synthesised AgNPs was tested for the reduction reactions of MB and RhB in the presence of NaBH₄. The catalytic activity of the biosynthesised AgNPs to reduce MB and RhB was monitored by spectrophotometry and the results have been presented in Figure 6(a,b), respectively. Pure MB and RhB had λ_max values of 664 nm and 554 nm, respectively. By adding AgNPs to the mixture containing dye (MB or RhB) and NaBH₄ and increasing the time, an intensive decrease was observed in the absorption peaks of the dyes. After a few minutes, the absorbance reached zero and the solution became colourless. Blank runs were also performed (without catalysts), which revealed no significant degradation. This indicated that no degradation could occur in the absence of the catalyst (data not shown). The degradation efficiency (R) of AgNPs as a function of time has been depicted in Figure 6(c,d). Accordingly, the complete reduction of MB and RhB in the presence of the silver nanocatalyst was accomplished in less than 5 and 8 min, respectively. The catalytic reduction of dyes by this catalyst was done faster with a smaller amount of the catalyst in comparison to many other works that synthesised AgNPs using other plant extracts [49, 50]. This might be due to the smaller size and higher surface to volume ratio of the AgNPs. To confirm the catalytic activity of the synthesised AgNPs, the process was monitored in a reaction of mixture without silver nanocatalysts and only in the presence of NaBH₄ as a reference (control solution). In the absence of NPs, the control experiments showed no significant changes, suggesting no self-degradation of these tested organic dyes under equal conditions (data shown in Figure 6(e) for MB and Figure 6(f) for RhB). Compared to the results presented in Figure 6(a–d), it can be concluded that the green synthesised AgNPs could be effective in degradation of noxious MB and RhB dyes in environmental remediation applications.

3.5. Cytotoxicity analysis of the green synthesised AgNPs against SW480 and HCT116 cell lines

The anticancer potential of the green phyto-synthesised AgNPs with C. myxa leaf extract against two human colon cancer cell lines (SW480 and HCT116) was investigated by MTT assay. The cell viability was assessed following the treatment of cells with several concentrations of AgNPs (12.5, 25.0 and 50.0 μg/mL). The results of the MTT assay revealed that the phyto-synthesised AgNPs inhibited the growth of two cell lines (HCT116 and SW480) in a dose-dependent passion as depicted in Figure 7. The cell viability decreased with increase in the concentration of the synthesised AgNPs. This might be attributed to two effects: (1) intracellular Reactive Oxygen Species (ROS) generation by AgNPs and (2) AgNPs activity on the key cellular components, leading to cell death [51, 52]. The cytotoxic effect of the synthesised AgNPs was more pronounced on the SW480 cancer cell line, especially at lower concentrations. The HCT116 and SW480 cells treated by AgNPs, respectively, showed 29.3% and 28.3% viability at the highest concentration depicting the presence of phenolics. Similarly, bands procured at 2921 cm⁻¹ denoted C–H stretching peak of a methylene group. The bands at 1556 cm⁻¹ and 1350 cm⁻¹ corresponded to C=O stretching and C=C stretching of an aromatic ring, respectively. The band at 957 cm⁻¹ corresponded to C–N stretching, which indicated the presence of an amine group. From the obtained spectra, it is obviously apparent that polyphenolic compounds, such as flavonoids and phenolic acids, are responsible for producing and capping AgNPs.
On the basis of the results, the synthesised AgNPs with C. myxa leaf extract showed a clear cytotoxic effect on human colon cancer HCT116 and SW480 cell lines. Thus, the synthesised AgNPs might be an effective candidate for treating cancerous cells.

3.6. Reducing the potential of the biosynthesised AgNPs using C. myxa

The antioxidant abilities of the C. myxa extract and phyto-synthesised AgNPs were determined using FRAP assay [53]. The results demonstrated that the reducing potential steadily increased in direct proportion to an increasing amount of the C. myxa extract or AgNPs; means-tested samples showed a dose-dependent antioxidant activity (Figure 8). Thus, the results suggested that the synthesised AgNPs using C. myxa leaf extract possessed an efficient antioxidant potential due to encapsulation of phytochemical compounds on the surface of the synthesised AgNPs [54] and plain leaf extract of C. myxa.
However, the phyto-synthesised AgNPs had a lower reducing ability compared to the crude leaf extract due to the higher concentration of phenolics in the leaf extract.

4. Conclusion

The current work presented an environmentally friendly approach without the application of harsh, expensive and toxic chemicals to the synthesis of AgNPs by C. myxa leaf extract at room temperature. The flavonoids and phenolics of the C. myxa leaf extract could act as a reducing and stabilising agent in the preparation of NPs. This study also assessed the impact of different empirical parameters, such as the pH of the reaction mixture, concentrations of the silver precursor, quantity of C. myxa leaf extract, time of mixing and temperature, on the construction of AgNPs. The prepared AgNPs at the optimum condition were characterised by various analytical techniques, including UV-Vis, TEM, XRD, FTIR and DLS. The crystalline size of the phyto-synthesised AgNPs was 3–10 nm. Indeed, it was spherical in shape, had FCC structure, and its SPR band was centred at 410 nm. DLS showed that the hydrodynamic radius of the particles was ~15.3 nm. The prepared AgNPs represented high catalytic activity during the reduction of two well-known azo dyes (RhB and MB) in the presence of NaBH4 in water at room temperature. The comparison of the catalytic effect in the presence and absence of AgNPs confirmed the effectiveness of AgNPs. Antioxidant and cytotoxicity activities of AgNPs were assessed, as well. The results of MTT assay proved the substantial cytotoxic activity of AgNPs against SW480 and HCT116 human colon cancer cell lines. Hence, the phyto-synthesised AgNPs could act as an effective catalytic agent and might have biomedical applications.

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Disclosure statement

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