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ORIGINAL ARTICLE

Low-cost and eco-friendly phyto-synthesis of Silver nanoparticles by using grapes fruit extract and study of antibacterial and catalytic effects

Saeed Farhadi[1]; Bahram Ajerloo[1]; Abdelnassar Mohammadi[2]*

[1]*Department of Chemistry, Lorestan University, Khoramabad, Iran*
[2]*Department of Biology, Lorestan University, Khoramabad, Iran*

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Abstract

In this research, silver nanoparticles (Ag NPs) were prepared by a low-cost, rapid, simple and ecofriendly approach using Grape fruit extract as a novel natural reducing and stabilizing agent. The product was characterized by UV-visible spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), field emission scanning electron microscopy (FESEM), energy-dispersive X-ray (EDX) spectroscopy and transmission electron microscopy (TEM). The reaction conditions including time, content of reducing agent and silver nitrate, temperature and pH were investigated. The optimum yield of Ag NPs was obtained when 10 mM of silver nitrate was reacted with 9 mL of Grape fruit extract at pH=9 and heated it to 55 °C within 25 minutes. The crystalline nature of Ag NPs was confirmed from XRD analysis. SEM and TEM images showed that the obtained Ag NPs were spherical in shape and their sizes were in the range of 25-85 nm. EDX analysis confirmed presence of the elemental silver. On the basis of FT-IR analysis, it can be stated that the hydroxyl, carbonyl and carboxyl functional groups present in bio-molecules of Grape fruit extract are responsible for the reduction of Ag^+ ions and stabilization of the obtained Ag NPs. The biosynthesized Ag NPs showed good antimicrobial activity against Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*) bacteria. In addition, the catalytic activity of the Ag NPs was studied for the reduction of nitro compounds by using $NaBH_4$.

Keywords: *Antibacterial activity; Catalyst; Grape fruit extract; Nitro reduction; Phyto-synthesis; Silver nanoparticles.*

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INTERODUCTION

In recent years, biosynthesis of silver nanoparticles (Ag NPs) has received considerable attention due to the growing need to develop clean and nontoxic chemicals, environmentally friendly solvents and renewable materials [1]. Although, the biosynthesis of Ag NPs has already been reported with micro-organisms such as bacteria [2-6], fungi [7–9], actinomycetes [10–12], yeast [13] and viruses [14-16], but it is more beneficial to use plant extracts than microorganisms, as they eliminate elaborate process for cell culture maintaining and also time consumption [17]. Moreover, plant-assisted synthesis has received considerable attention because of the high potential of plants in producing Ag NPs with different sizes and shapes, as well as the broad diversity of plant metabolites that may aid in the reduction. Because of the aforesaid reasons, the biosynthesis of Ag NPs has been reported by using a variety of plants such as *Azadirachta indica* [18], *Mimusops elengi* [19], *Macrotyloma uniflorum* [20], *marine macroalga Chaetomorpha linum* [21], *lemon* [22], *guava* [23], *Sorghum spp.* [24], *Justicia adhatoda* [25], *Allium cepa* [26], *Mentha piperita* [27], *Syzygium cumini* [28], *Anisochilus carnosus* [29], *Murraya keenigii* [30], *Olea europaea* [31], *Triticum durum* and *Aegilops tauschii* [32], *Svensonia hyderabadensis* [33], *Citrullus colocynthis* [34]. However, to enable the biosynthesis methods to compete with the chemical methods, there is still a need to achieve faster rates. The use of fruit extract of plants is

* Corresponding Author Email: *sfarhadi1348@yahoo.com*

an appropriate approach for this purpose. So far, the synthesis of Ag NPs using plant fruits such as *Terminalia chebula* [35], *Solanum trilobatum* [36], *Dillenia indica* [37], *Solanum lycopersicums* [38] *Averrhoa bilimbi* [39], *Crataegus douglasii* [40], and *Emblica Officinalis* [41] have been reported in the literatures. Grape fruit, one of the World's largest crops, has been part of human diet since ancient. It is estimated that the global Grape fruit production amounts to be over 60 million tons [42]. Among various countries, Iran is one of the largest producers of grape in the world. Then, the Grape fruit is considered to be an inexpensive and easily available important fruit in Iran. Grape fruit extracts exhibited strong antioxidant activity and prevented reactive oxygen species (ROS)-induced DNA damage [43, 44], cytotoxicity towards cultured human cancer cells [45] as well as inhibited tumor growth in animal models [46], cardiovascular disease [47], exhibit antimicrobial [48], antihypertensive [49], antiulcer activities [50], neurodegenerative disease aging [51,52]. In addition, previous investigations have demonstrated the presence of organic acids, polyphenols, flavonols, tannins, procyanidins, anthocyanins, lipids, enzymes, vitamins, carotenoids, terpenes, and reducing sugars in the grape fruits [53-55]. Due to the existence of these biomoleculs, Grape fruit extract is an ideal reducing and stabilizing agent for the biosynthesis of Ag NPs.

In this research, a simple and low-cost method for the synthesis of spherical Ag NPs by using Grape fruit extract is reported. The spherical Ag NPs were formed under mild conditions, without any additive protecting agents. The formation of Ag NPs was monitored by the UV-visible spectroscopy and the obtained product was analyzed by X-ray diffraction (XRD), energy-dispersive X-ray (EDX) spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and Fourier transform infrared (FT-IR) spectroscopy. Various important parameters (e.g. concentration of the reactants, reaction temperature, pH and time) were optimized that would increase the yield of Ag NPs. The antibacterial and catalytic activities of the biosynthesized Ag NPs were also investigated.

EXPERIMENTAL

Materials and apparatus

Silver nitrate ($AgNO_3$), $NaBH_4$, 4-nitrophenol, 2-nitrophenol, 4-nitroanilin and 2-nitroanilin were obtained from Merck chemical company. All chemicals were of analytical grade and used as received without further purification. Double distilled de-ionized water was used for the experiments. All glass wares were properly washed with distilled water and dried in oven. The UV-vis spectra of samples were analyzed using a CARY 100 and double beam spectrophotometer operated at a resolution of 2 nm with quartz cells with path length of 1 cm in 200–800 nm range. The XRD pattern of the Ag NPs was obtained on an X-ray diffractometer (PANalytical/X'Pert Pro MPD) using Cu Kα (1.54059 Å) radiation. The particle size and shape were confirmed by using a scanning electron microscope (MIRA3 TESCAN) equipped with EDX attachment. Transmission electron microscopy (TEM) observations were conducted on a Philips CM120 microscope at the accelerating voltage of 80 kV. Infrared spectra were obtained by using a FT-IR 160 Schimadzu Fourier transform infrared spectrophotometer.

Preparation of Grape fruit extract

The fruit extract of Grape was used as a reducing and stabilizing agent for the synthesis of Ag NPs. The fresh Grape fruits were purchased from local supermarket in Iran and used for the synthesis of Ag NPs. They were washed repeatedly with distilled water to remove the dust and organic impurities. About 15 g of Grape fruits were crushed into fine pieces and taken into the 250 ml beaker containing 100 mL double distilled de-ionized water. The mixture was stirred for 30 min and filtered through Whatman No.1 filter paper twice. The obtained light yellow filtrate was stored in refrigerator at 4°C.

Phyto-synthesis of Ag nanoparticles

In a typical experiment, Ag NPs were prepared by using Grape fruit extract as follows: in a 50 mL round-bottom flask equipped with a magnet bar, 9 ml of aqueous solution of the extract was mixed with 25 ml of 10 mM aqueous silver nitrate solution. The mixture was then heated in an oil bath at 55 °C under constant stirring for an appropriate time (e.g. 25 min). The formation process and the optical properties of the Ag NPs were identified from both the color change and UV-Vis spectra of the solutions**.** In order to separate the Ag NPs product, the solution was centrifuged at 5500 rpm for 20 min. The supernatant was decanted and the precipitate was re-dispersed in double distilled water for another round of centrifugation.

The precipitate was then washed with deionized water for three times to remove any impurities. Finally, the washed precipitate was dried in an oven maintained at 60 °C for 2 h and ground into powder for characterization.

In addition, a series of experiments were conducted to investigate the effect of various parameters including reaction time, Ag^+ ion concentration, the amount of Grape fruit extract, pH and temperature on the reaction. The experimental procedures were similar to the above experiment. The reaction mixtures were monitored by a UV-vis spectrophotometer at different time intervals and the Ag NPs were characterized further. The effect of pH on the Ag NPs synthesis was determined by adjusting the pH of the reaction mixtures (10 mM silver nitrate, and 9 mL Grape extract) to 3, 5, 7, 9, 11 or 13 by using HCl (0.1 M) and NaOH (0.1 M) aqueous solutions. The effect of the silver salt was determined by varying the concentration of silver nitrate (0.1, 1, 10 and 100 mM). The Grape fruit extract content was varied to 1, 3, 5, 7, 9, 11 and 13 mL, while keeping the silver nitrate concentration at a level of 10 mM. To study the effect of temperature on Ag NPs synthesis, reaction mixtures containing 9 mL Grape extract, and 10 mM $AgNO_3$ at pH 9 were incubated at 25, 40, 55 or 70 °C.

Antibacterial tests

Antibacterial activity of the biosynthesized Ag NPs was evaluated against strains of Gram-positive bacteria: *Bacillus cereus* (PTCC 1015), *Staphylococcus aureus* (1431) and *Staphylococcus epidermidis* (PTCC 1114), and Gram–negative bacteria: *Escherichia coli* (PTCC 1330) and *Klebsiella pneumonia* (PTCC 1290) by modified Kirby-Bauer disk diffusion method. Bacteria were cultured for 18 h at 37 °C in Nutrient agar medium and then adjusted with sterile saline to a concentration of 2×10^6 cfu/mL. Bacterial suspension in Petri dishes (8 cm) containing sterile Mueller-Hinton agar were cultured using a sterile cotton swab. The compounds were dissolved in water and sterile paper discs of 6 mm thickness were saturated with 30 µl of Ag NPs and then placed onto agar plates which had previously been inoculated with the tested microorganisms. *Amikacin* (30 µg/disk) for Gram-negative and *penicillin* (10 µg/disk) for Gram-positive were used as controls. After incubation at 37 °C for 24 h, the diameter of inhibition zones were measured using a meter ruler, and the mean value for each organism was recorded and expressed in mm.

Catalytic tests

In a typical catalytic reaction, 3 mL of aqueous solution of 4-NP (0.1 mM) and 0.5 mL of aqueous $NaBH_4$ (15 mM) solution were mixed together in a test tube and then 1 mL of aqueous Ag suspensions (0.5 mg mL^{-1}) was added to the reaction mixture under magnetic stirring. Immediately after that, the solution was transferred to a standard quartz cell, and the UV–vis absorption spectra were recorded with a time interval of 2 min in a scanning range of 200-800 nm at ambient temperature. The reduction of 2-Nitrophenol, 2-Nitroaniline and 4-Nitroaniline was also investigated under the same conditions.

RESULTS AND DISCUSSION

Phytoreduction of silver ions

In this work, phytosynthesis of the Ag NPs by using the aqueous extract of Grape fruit was investigated. During the visual observation, silver nitrate treated with Grape fruit extract showed a color change from yellow to brown within 25 min whereas no color change could be observed in silver nitrate solution without Grape extract. As can be seen in Fig. 1, the appearance of brown color is a clear indication for the formation of Ag NPs. This color arises due to excitation of surface plasmon resonance (SPR) band in Ag NPs.

UV–visible absorption studies

Initially, the reaction mixtures containing silver nitrate and Grape fruit extract were characterized by UV–Visible spectroscopy. Based on UV–vis spectroscopy, various parameters (concentration of the fruit extract and silver salt, pH, temperature and reaction time) were optimized for the reduction Ag^+ ions to Ag NPs using Grape fruit extract.

To optimize the reaction time, a time variation study was carried out using 10 mM $AgNO_3$ and 9 mL of aqueous Grape extract. Fig. 2 shows the UV–vis absorption spectra of Ag NPs synthesized at different times. It is observed that the intensity of SPR bands increases as the reaction time progresses and within 25 min a considerable intensity of the SPR bands is achieved. However, the intensity of SPR band was hardly changed after 25 min. The optimal time for the reduction Ag^+ ions to Ag NPs is 25 min. As shown in the inset of Fig. 2,

after the reaction between Ag^+ and Grape extract, the color was changed from yellow to dark brown which shows the formation of Ag NPs.

Fig. 3 shows the UV–Vis absorption spectra of Ag NPs obtained at different concentrations of $AgNO_3$ (0.1, 1, 10 and 100 mM). At 0.1 mM $AgNO_3$ concentration, an observable SPR band was not appeared, indicating very low yield of Ag NPs formed (Fig. 3, curve i). With increasing $AgNO_3$ concentration to 1 mM, the SPR of Ag NPs appears at 411 nm and its intensity remarkably increases with the increase of $AgNO_3$ concentration to 10 mM with increasing in the peak wavelength to 415 nm (Fig. 3, curves i and ii, respectively). High intensity of the 410 nm SPR band indicates increasing concentration of nanoparticle. However, further increasing the concentration of $AgNO_3$ from 10 to 100 mM increase the intensity of SPR band slightly and give a broad SPR band shifted to longer wavelength (~425 nm). This phenomenon may be due to the fast growth of the particles at high concentration.

In order to get control growth and smaller particle size, we have used 10 mM $AgNO_3$ for the further study and hereafter the reactions were carried out under the above mentioned condition.

The effect of the Grape extract amount on

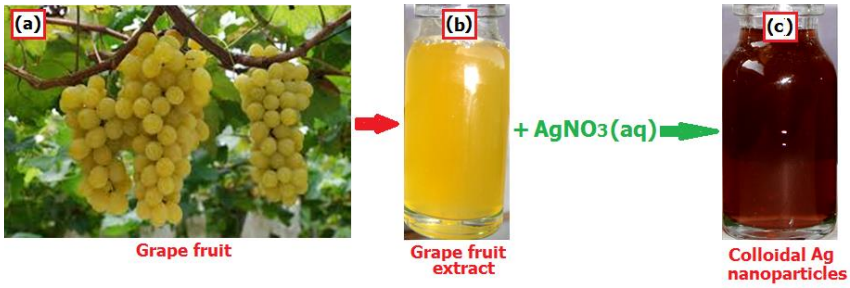

Fig. 1: Photographs of: (a) Grape fruits, (b) aqueous extract of Grape fruits and (c) Colloidal aqueous Ag NPs solution formed by reduction of $AgNO_3$ with Grape fruit extract.

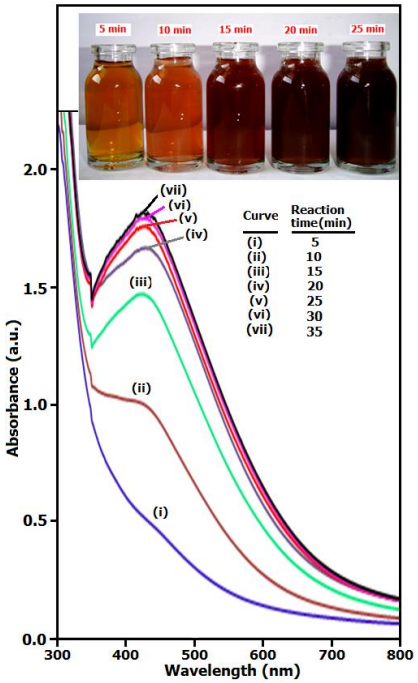

Fig. 2: UV–vis absorption spectra of Ag NPs synthesized by treating 10 mM aqueous $AgNO_3$ solution with 9 mL Grape fruit extract at various times. The inset photo shows the color change of solution with time of reaction.

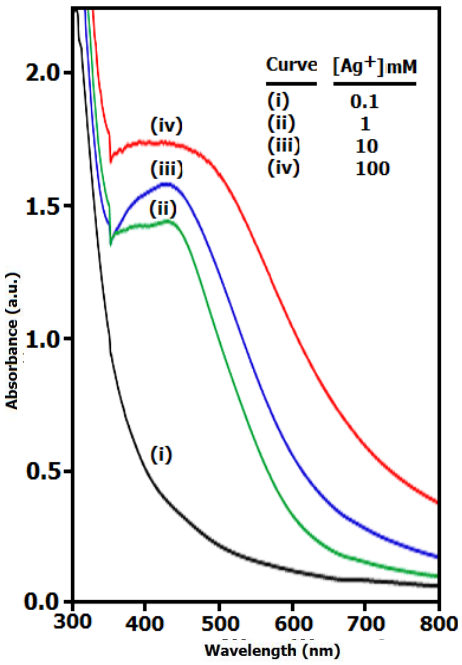

Fig. 3: UV–vis absorption spectra of Ag NPs synthesized at 55 ◦C from 9 mL Grape fruit extract for 25 min with different concentrations of aqueous $AgNO_3$ solutions.

the synthesis of Ag NPs was investigated under the provided reaction conditions, and the results are shown in Fig. 4. As observed with increasing the Grape extract quantity from 1 to 9 mL in 25 mL of 10 mM $AgNO_3$ solution, the intensity of characteristic SPR absorption bands of Ag NPs increases (Fig. 4, curves i-v); and then keep constant when the Grape extract increases further (Fig. 4, curves vi and vii). The maximum absorption was found at an amount of 9 mL fruit extract. From the UV-vis absorption spectra in Fig. 4, a slight redshift in wavelength from 400 to 412 nm is observed. Accordingly, it can be concluded that with the increase in Grape extract amount, the size of Ag nanoparticles increases.

The effect of reaction temperature was also evaluated with varying temperature form 25 to 75 ℃. As shown in Fig. 5 (curves i and ii), the reaction mixtures incubated at room temperature (25 ℃) and 40 ℃ showed less pronounced SPR peaks during a long time of 60 min while by heating the reaction mixtures at 55 and 70 ℃, the intense peaks were developed within a short time of 25 min (Fig. 5, curves iii and iv). This indicates that higher temperature facilitates the formation of Ag NPs due to the increase in the reaction rate [56]. However, a slight increase in SPR peak intensity occurs at 70 °C. Then, the lower temperature of 55 °C is preferred for further study.

Among the various parameters, the initial pH of solution plays a significant role in the synthesis of Ag NPs [35, 57]. Thus, the effect of pH on the synthesis of Ag NPs was studied under acidic, natural and basic conditions using 10 mM $AgNO_3$ and 9 ml Grape fruit extract. As can be seen in Fig. 6 (curves i and ii), no characteristic Ag NPs peak was observed at acidic pH of 3. A weak SPR peak was appeared at pH = 5. Under the acidic conditions, biomolecules are likely to be inactivated [58].

This suggests that acidic pHs are not favorable for the Ag NPs synthesis. At pHs of 5.65 and 7, the Ag NPs formation was observed at relatively high concentration, as confirmed by the appearance of absorbance bands at about 420 nm (Fig. 6, curves iii and iv). However, Ag NPs were readily obtained at pH higher than 7, as evidenced through progressive evolution of the characteristic SPR band in the 400-450 nm spectral region. As can be seen in Fig. 6 (curves v), the SPR band intensity increased significantly upon increasing the pH to 9, indicating that correspondingly higher yields of Ag NPs were obtained, probably due to the presence of a considerable number of reactive ionized functional groups to bind with silver ions. However, the SPR band intensity decreased with further increasing pH to 11 and 13 (Fig. 6, curves vi and vii). The optimal pH for Ag NPs synthesis was chosen to be pH 9, which is in good agreement

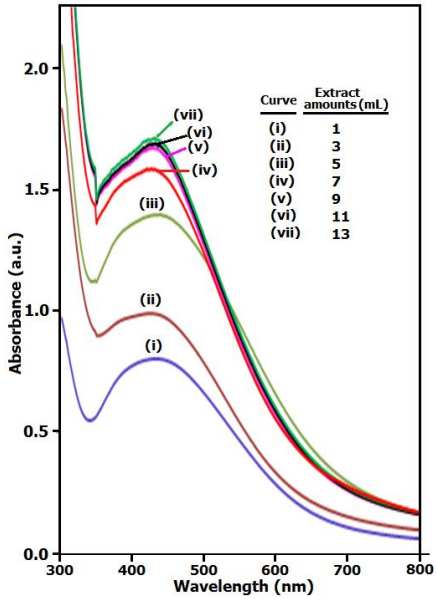

Fig. 4: UV-vis absorption spectra of Ag NPs synthesized at 55 ◦C by treating 10 mM aqueous $AgNO_3$ solution for 10 min with different amounts of Grape fruit extract.

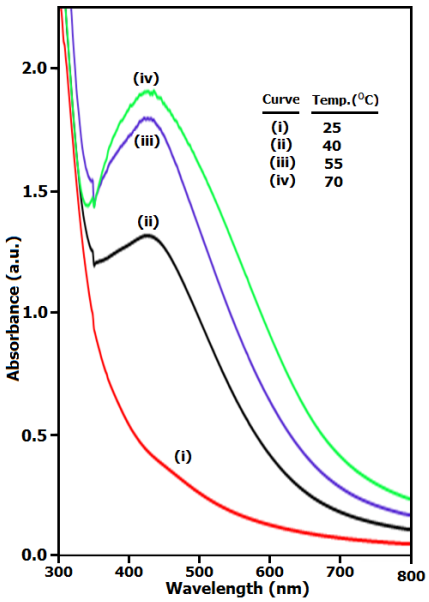

Fig. 5: UV–vis absorption spectra of Ag NPs synthesized by treating 10 mM aqueous $AgNO_3$ solution with 9 mL Grape fruit extract at different temperatures.

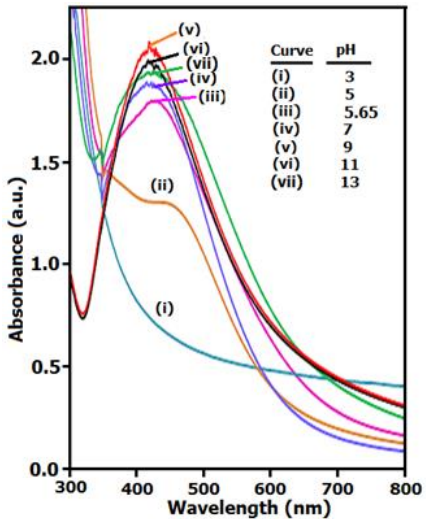

Fig. 6: UV-vis absorption spectra of Ag NPs synthesized by treating 10 mM aqueous $AgNO_3$ solution with 9 mL Grape fruit extract at different pHs.

with the reported literature [41]. The differences in the amount of Ag NPs obtained over the range of pH could be ascribed to a variation in the ionization of functional groups (OH and COOH) on the biomolecules that are involved [59].

XRD analysis

Fig. 7 shows the XRD pattern of the biosynthesized Ag NPs after the complete reduction of Ag^+ to Ag under the optimized conditions (10 mM $AgNO_3$, 9 mL Grape extract, pH 9 at 55 °C for 25 min). In the XRD pattern, the four characteristic diffraction peaks at 2θ values of 38.12°, 44.20°, 64.68°, and 77.55° can be indexed to the (111), (200), (220), and (311) reflection planes of faced center cubic (fcc) structure of silver (JCPDS card no 04.0784). The considerable broadening of the diffraction peaks demonstrates the nanometer nature of the Ag particles. The average crystallite size of the Ag product is approximately 62.5 nm as estimated by the Debye–Scherrer equation [60]: $D_{XRD} = 0.9\lambda/(\beta\cos\theta)$, where D_{XRD} is the average crystallite size, λ is the wavelength of Cu Kα radiation, β is the corrected full-width at half-maximum of the main diffraction peak of (111), and θ is the Bragg angle.

SEM, TEM and EDX analysis

The morphology and size of the Ag NPs were determined via SEM and TEM images. Fig. 8 shows the SEM images of the as-prepared Ag NPs. From the SEM images at different magnifications (Fig. 8(a)-(c)), it is clearly evident that the product consists of extremely fine particles with sphere-like morphologies that appreciably aggregated as clusters due to the extremely small dimensions and high surface energy of the obtained nanoparticles. We also can find from the images that the morphology of the particles is almost homogeneous. The resulting images show the presence of large number of spherical nanoparticles. The EDX was used to further characterize the composition of the product. Fig. 8 (d) shows the EDX spectrum of the obtained Ag NPs. The peaks around 3.40 keV and 3.45 keV are correspond to the binding energies of Ag $K_{L\alpha}$ and Ag $K_{L\beta}$, respectively.

The TEM images and size distribution of the Ag NPs are shown in Fig. 9. The TEM sample was

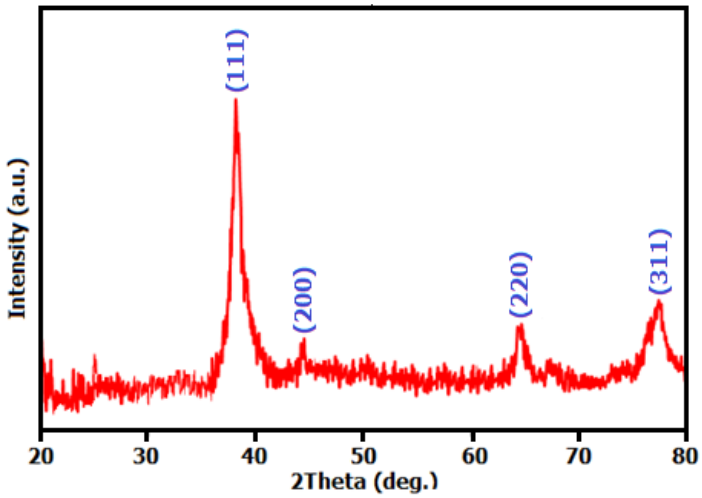

Fig. 7: XRD pattern of Ag NPs synthesized by Grape fruit extract.

prepared by dispersing the powder in ethanol by ultrasonic vibration. It can be seen from Fig. 9(a) and (b) that the Ag particles show approximately sphere-like morphologies with a uniform size. To investigate the size distribution of the Ag NPs nanoparticles, the particle size histogram was also determined from the TEM images. The inset of Fig. 9(b) shows the size distribution of the Ag NPs. It is clear that the sizes of the Ag NPs are in the range of 25 to 85 nm with a narrow size distribution. The average particle size was estimated to be approximately 58 nm, which is in agreement with the result calculated for the half-width of diffraction peaks using the Scherrer's formula.

FT-IR analysis

The identification of the possible biomolecules responsible for the reduction and stabilization of Ag NPs can be achieved by the FTIR studies. It has been reported that the Grape fruit is rich in phytochemicals like organic acids, polyphenols, flavonols, tannins, procyanidins, anthocyanins, lipids, enzymes, vitamins, carotenoids, terpenes, and reducing sugars (mainly glucose, sucrose and fructose) [53-55]. These components are containing carboxyl (-COOH), phenolic –OH, carbonyl (C=O) and amino (NH_2) functional groups. Fig. 10 shows FT-IR spectra recorded for the Grape fruit extract and the Ag NPs synthesized with the Grape extract before and after washing. The FT-IR spectrum of Grape fruit extract in Fig. 10(a) shows phenolic/acidic/ O-H, C=O/NH_2, and C-OH stretching bands, corresponding to a number of bands at 3400, 1637, and 1062 cm^{-1}, respectively. The absorption bands at 2935, 1423, and 1338 cm^{-1} are related to the C-H stretching bands in Grape

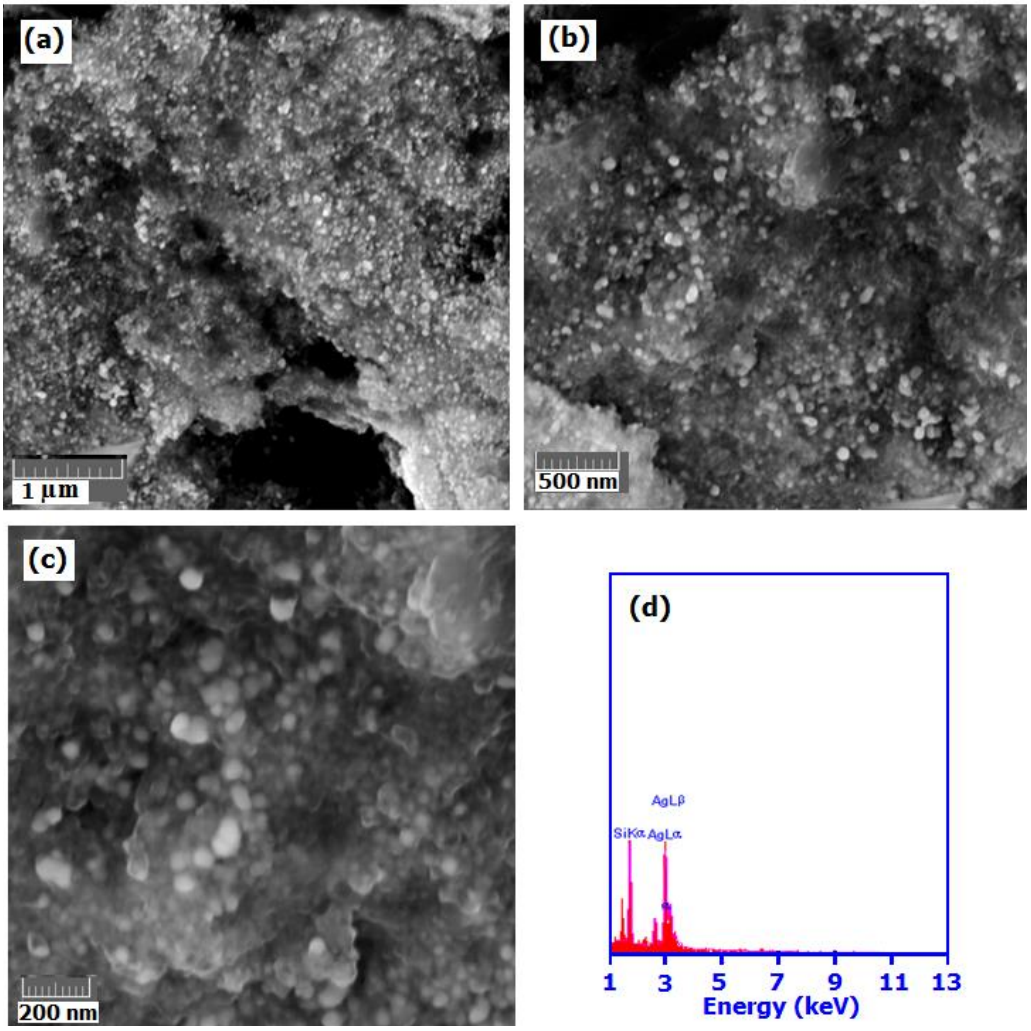

Fig. 8: (a – c) SEM images of the as-prepared Ag NPs, and (d) EDX elemental spectrum of the Ag NPs.

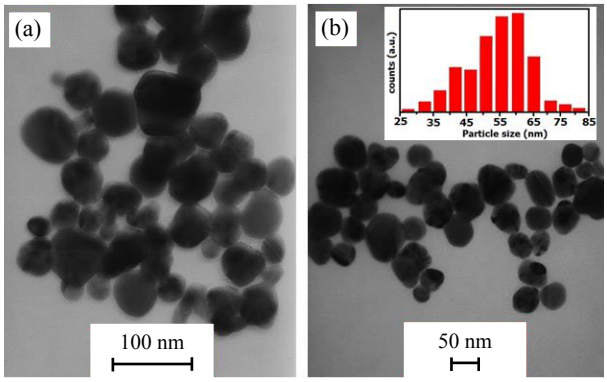

Fig. 9: a) and b) TEM images of the Ag NPs. The inset in Figure 9(b) shows the size distribution of the Ag NPs.

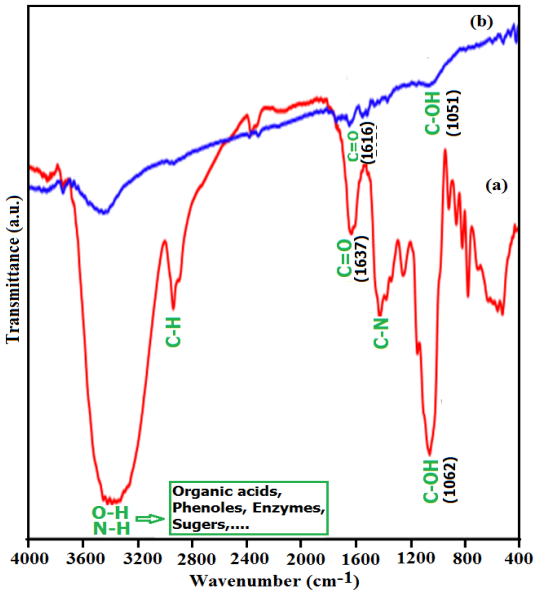

Fig. 10: FT-IR spectra of: (a) Grape fruit extract, and (b) Ag NPs capped with Grape fruit extract solution.

fruit. As shown in Fig. 10 (b), after the reduction of $AgNO_3$, the C-OH band shows a red-shift from 1062 to 1051 cm^{-1}, signify the involvement of OH groups in the stabilization process of Ag NPs.

On the other hand, the shift of a band from 1637 to 1616 cm^{-1} is attributed to the binding of C=O/NH groups with Ag NPs. On the basis of FT-IR analysis, it can be stated that the hydroxyl, carboxyl and carbonyl functional groups present in carbohydrates, flavonoids, tanins and phenolic acids of Grape fruit extract play a crucial role for the reduction of Ag+ to Ag as well as stabilizing the obtained Ag NPs.

Antibacterial activity of Ag nanoparticles

The antibacterial activity of Ag NPs was analyzed against five bacteria: *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, and *Escherichia coli* by disk diffusion method. The results of the antibacterial activity of silver nanoparticles were showed in Fig. 11. The results show that Ag NPs have good antibacterial activity; bacteria cells have been killed at the concentration of 30 µg/ml. Table 1 represents the inhibition zone of these bacteria. Highest activity of Ag NPs was obtained against *Staphylococcus epidermidis*, while lowest activity was observed against *Bacillus cereus* and *Escherichia coli*. Biosynthesized Ag NPs exhibit more antimicrobial activity on gram-positive microorganism than gram-negative. The potential antimicrobial activities showed by Ag NPs have made them encouraging candidates as novel generation antimicrobials.

The antimicrobial effects of Ag have been known since ancient times [61]. The clear mechanism of Ag NPs interaction with bacteria is not well known. However, several main mechanisms underlie the biocidal properties of Ag NPs against microorganisms. First, Ag NPs attach to the negatively charged cell surface, alter the physical and chemical properties of the cell membranes and the cell wall and disturb important functions such as permeability, osmoregulation, electron transport and respiration [62,63]. Second, Ag NPs can cause further damage to bacterial cells by permeating the cell, where they interact with DNA, proteins and other phosphorus- and sulfur-containing cell constituents [64]. Third, Ag NPs release silver ions, generating an amplified biocidal effect, which is size- and dose-dependent [65].

Catalytic activity of Ag nanoparticles

To investigate the catalytic performance of the biosynthesized Ag NPs, the reduction of some aromatic nitro compounds by $NaBH_4$ in aqueous solutions was used as the model systems. The catalytic process was monitored by UV–vis spectroscopy as shown in Fig. 12. Fig. 12(a) shows the reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP). 4-NP shows an absorption peak at about 320 with a shoulder at about 400 nm. It was seen that this peak undergoes a red shift from 320 to 400 nm immediately upon the addition of aqueous solution of $NaBH_4$, corresponding to a significant change in solution color from light yellow to yellow-green due to formation of 4-nitrophenolate ion. In the absence of Ag NPs catalyst, the absorption peak at 400 nm remained unaltered for a long duration, indicating that the $NaBH_4$ itself cannot reduce 4-nitrophenolateion without a catalyst. In the presence of Ag NPs catalyst and $NaBH_4$ the 4-NP was reduced, and the intensity of the absorption peak at 400 nm decreased gradually with time and after about 20 min it fully disappeared.

At the same time, a new absorption peak appeared at about 297 nm and increased progressively in intensity. This new peak is attributed to the typical absorption of 4-AP. This result suggests that the catalytic reduction of 4-NP exclusively yielded 4-AP, without any other side products. Furthermore, it was found that the present catalyst could also catalyze the reduction of some other aromatic nitro compounds including 2-nitrophenol (2-NP) and 4-nitroaniline (4-NA) and 2- nitroaniline to corresponding amines. Fig. 12 (b)-(d) shows the concentration changes of the three aromatic nitro-compounds with the time in the presence of Ag NPs. The reductive reactions were finished within 24 min (for 2-NP), 20 min (for 4-NA) and 44 min (for 2-NA), respectively. Since the reductive reactions were carried out under the same experimental conditions, the difference rates can be related to the structures of organic compounds.

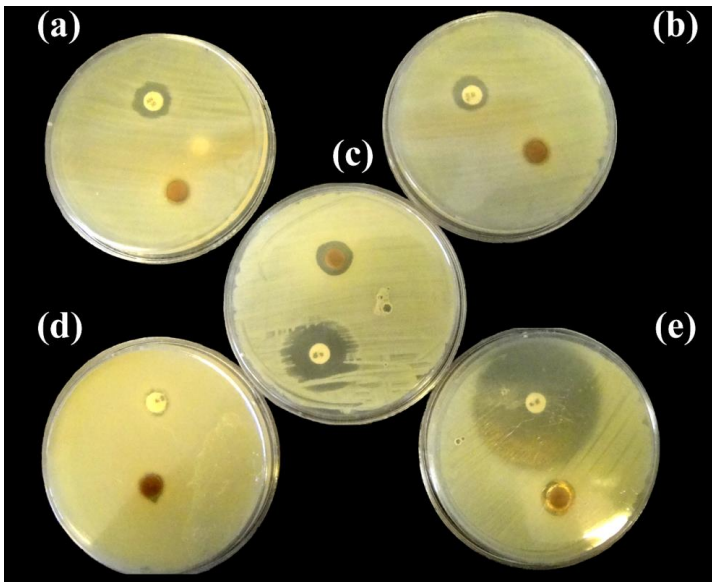

Fig. 11: Images of antibacterial activities of discs 30 μg/mL Ag NPs on (a) E. Coli, (b) Klebsiella Pneumonia, (c) S. Epidermidis, (d) Bacillus Cereus, and (e) S. Aureus.

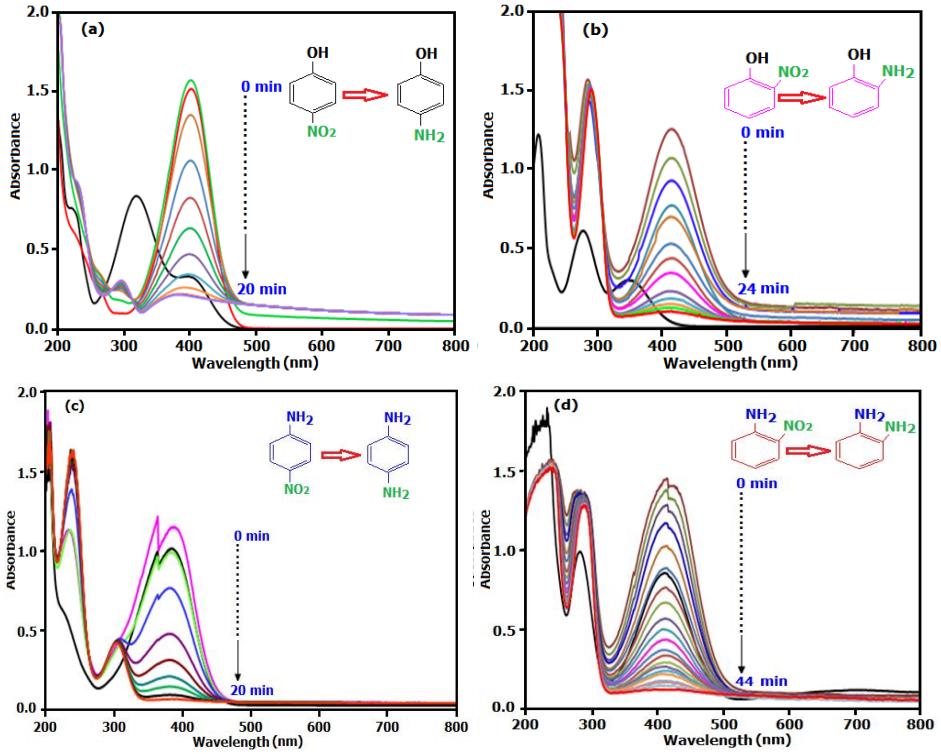

Fig. 12: UV–vis spectra of the reduction of nitro compounds with NaBH4 in the presence of Ag NPs as a catalyst: (a) 4-Nitrophenol, (b) 2-Nitrophenol, (c) 4-Nitroaniline, and (d) 2-Nitroaniline

Table 1: Average of inhibition zone of the Ag NPs synthesized using grape fruit extract against different bacterial species.

Entry	Bacteria species	Type	Inhibition zone diameter (mm)	
			Ag NPs	Disc standard[a]
1	*E. Coli*	Gram-negative	10	13
2	*K. Pneumonia*	Gram-negative	10	12
3	*S. Epidermidis*	Gram-positive	13	21
4	*B. Cereus*	Gram-positive	12	9
5	*S. Aureus*	Gram-positive	13	40

[a] Amikacin (30 µg/disk) and penicillin (10 µg/disk) were used for Gram-negative and Gram-positive bacteria, respectively.

CONCLUSIONS

In the present work, Grape fruit extract was used as an effective reducing as well as capping agent for the biosynthesis of Ag NPs in aqueous solution. The synthesis of Ag NPs was affected by the variation in reaction conditions such as time, temperature, concentration of Grape fruit extract and silver solutions and pH. The synthesized Ag NPs were spherical, 25-85 nm in size, crystal in nature and showed absorption spectrum at ~400-420 nm. The formed Ag NPs were quite stable, showed good antimicrobial activity and were utilized as a catalyst for rapid reduction of several aromatic nitro-compounds into their corresponding amine derivatives. Further experiments for the synthesis of other metal nanoparticles such as Au, Pd, and Cu using Grape fruit extract are in progress in our laboratory. Synthesis of metallic nanoparticles using green resources like Grape fruit extract is a challenging alternative to chemical synthesis, since this novel green synthesis is cost effective, pollutant free and eco-friendly synthetic route.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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