

Role of *Ribes khorassanicum* in the biosynthesis of AgNPs and their antibacterial properties

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Abstract: Silver nanoparticles (AgNPs) have been biosynthesised through the extracts of *Ribes khorassanicum* fruits, which served as the reducing agents and capping agents. Biosynthesised AgNPs have been found to be ultraviolet–visible (UV–vis) absorption spectra since they have displayed one surface plasmon resonance peak at 438 nm, attesting the formation of spherical NPs. These particles have been characterised by UV–vis, field-emission scanning electron microscopy, energy-dispersive X-ray spectroscopy, X-ray diffraction, Fourier transform infrared spectroscopy, and transmission electron microscopy analysis. The formation of AgNPs at 1.0 mM concentration of AgNO₃ has resulted in NPs that contained mean diameters in a range of 20–40 nm. The green-synthesised AgNPs have demonstrated high antibacterial effect against pathogenic bacteria (i.e. *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*). Biosynthesising metal NPs through plant extracts can serve as the facile and eco-friendly alternative for chemical and/or physical methods that are utilised for large-scale nanometal fabrication in various medical and industrial applications.

1 Introduction

Silver nanoparticles (AgNPs) synthesis is an expanding research area due to their unique chemical, physical, and biological properties [1–4] that can lead to a wide range of applications in spectroscopy, sensors, electronics, catalysis, and pharmaceutical sciences [5–8]. Although several approaches (physical- and chemical-based methods) have been reported in order to prepare AgNPs [9–12], yet the biosynthesis of NPs can eliminate the requirement of using harmful chemicals while reducing the need for downstream procedures, and as a result make the biosynthesis protocol further low cost, fast, and less energy consuming [13–15]. For this purpose, utilising plant extracts are potentially advantageous over other biosynthesis methods, mainly microorganisms, due to the ease in improving and the lack of requiring an elaborate process of maintaining cell cultures [16].

Several plant extracts such as palm kernel [17], *Prosopis fraxa* [18], *Malva sylvestris* [19], *Coffea arabica* [20], *Moringa oleifera* [21], *Descurainia sophia* [22], *Clerodendrum phlomidis* [23], *Justicia adhatoda* [24], *Panax ginseng* [25], *Rheum turkestanicum* [26], *Diospyros paniculata* [27], and other particular species have been used for the synthesis of AgNPs. Within this context, the present work describes the biosynthesis of AgNPs through employing the aqueous fruit extract of *Ribes khorassanicum* (*R. khorassanicum*) for the first time. *R. khorassanicum* is a unique medicinal plant with a large number of natural antioxidants, mainly anthocyanins – a group of natural compounds and secondary metabolites belonging to the flavonoid family – as suitable reducing agents in the reduction process of Ag⁺ to AgNPs [28]. Herein, AgNPs are easy to produce along with short preparation

time and in moderate temperature. Different characterisation instruments including ultraviolet–visible (UV–vis) spectrophotometry, transmission electron microscopy (TEM), field-emission scanning electron microscopy (FESEM), energy-dispersive X-ray spectroscopy (EDX), and Fourier transform infrared spectroscopy (FT-IR) were performed to analyse the structure and formation of AgNPs. We have also examined the antibacterial effects of biosynthesised AgNPs by following disc diffusion Mueller–Hinton agar method on Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The difference in antibacterial effects between Gram-positive and Gram-negative bacteria was attributed to the structure of their perspective cell walls [29]. For example, the cell wall of Gram-negative bacteria is included of a thin peptidoglycan layer and a lipopolysaccharide layer [30]. In the end, observations have strongly recommended the use of ‘green’ AgNPs to prevent bacterial biofilms in medical applications.

2 Materials and methods

2.1 Materials

Fresh *R. khorassanicum* fruits were collected from an eastern–southern area in Iran, and Ag nitrate (AgNO₃, pre-analysis) was procured from Carlo Erba (France). The *R. khorassanicum* fruits were cleansed and dried for 3 days at room temperature. The laboratory experiments were applied by double distilled water (ddw).

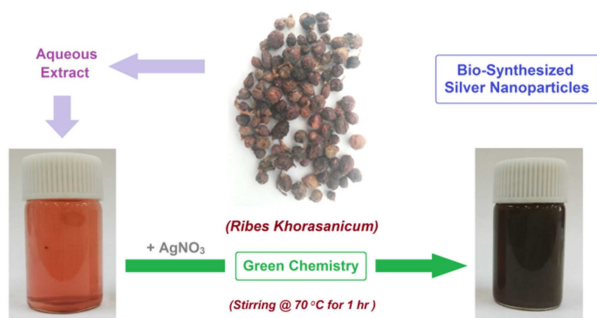


Fig. 1 Schematic plan for biosynthesis of AgNPs using *R. khorasanicum* seeds extracts

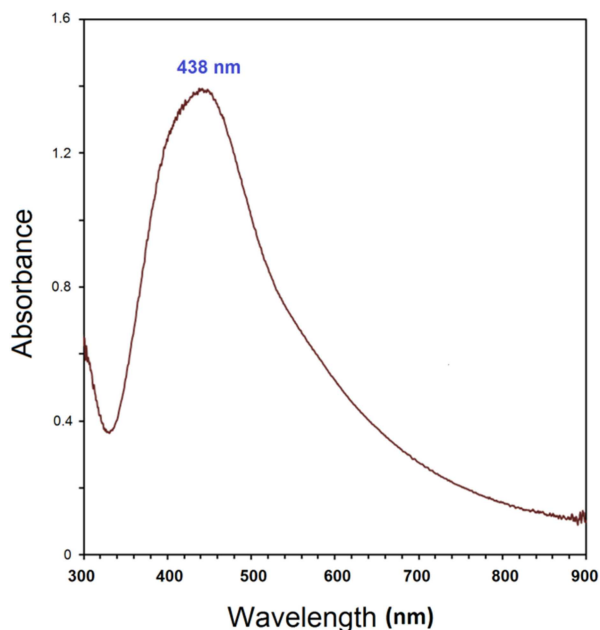


Fig. 2 UV-vis spectrum of biosynthesized AgNPs using *R. khorasanicum* seeds extracts

2.2 Preparation of *R. khorasanicum* fruits extracts

R. khorasanicum fruits extract (using 3.0 g of dried shoots) was prepared in 250 ml of ddw. Then, it was placed in a shaker for 24 h and was sieved using a Whatman #1 filter. The obtained extract (red colour) was reserved in a refrigerator (4°C).

2.3 Biosynthesis of AgNPs

The fruit extract (10.0 ml) was added to 90 ml of AgNO₃ (1.0 mM) and was vigorously stirred at 70°C for 1 h. The obtained AgNPs solution (dark brown colour) was lyophilised for 24 h and it was scrapped out for forthcoming studies.

2.4 Characterisation of AgNPs

The bio-prepared AgNPs was analysed using many tools, e.g. UV-vis (double beam spectrophotometer, CECIL, CE 9500, UK), FESEM + EDX (TESCAN, MIRA 3), TEM (Leo 912 AB, 120 kV, Zeiss, Germany), X-ray diffraction (XRD) (D8, ADVANCE-BRUKER, Germany), and FT-IR (Shimadzu 8400, Japan).

2.5 Antibacterial activity

Method of disc diffusion was applied to the antibacterial evaluation of AgNPs. The bacterial culture was modified to the 1.5×10^8 colony (equivalent to 0.5 McFarland) for an overnight. Then, discs that were soaked by AgNPs (15 µl) were placed on the immunised agars [31]. Besides the discs soaked by extract (0.1% v/v), the rest was labelled as the control (-). All of the plates were incubated

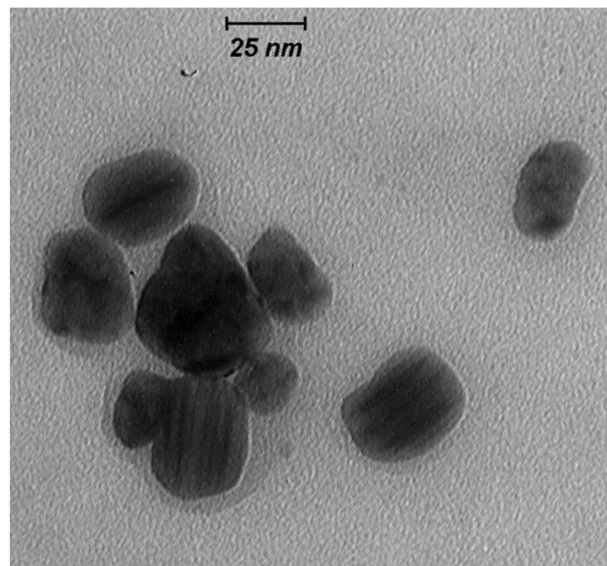


Fig. 3 High-magnification ($\times 80,000$) TEM image of biosynthesized AgNPs using *R. khorasanicum* seeds extracts

(37°C) for 24 h. Gentamicin (GM) and streptomycin were used as standards in this work.

3 Results and discussion

The schematic plan of AgNPs biosynthesis is shown in Fig. 1. The fruit extract of *R. khorasanicum* was used as a natural reducing and capping agent. As shown in Fig. 1, mixing the extract with Ag⁺ solution has resulted in the change of solution colour from light red to dark brown due to the bio-preparation of AgNPs [32].

3.1 UV-vis spectral analysis

UV-vis spectrophotometric analysis has provided sufficient evidence for NPs preparations. AgNPs that have been biosynthesized by *R. khorasanicum* fruits extract have illustrated a sharp characteristic surface plasmon resonance (SPR) peak at 438 nm, which confirms their biosynthesis (Fig. 2). Previous works have reported that AgNPs give an absorption peak between 410 and 450 nm due to the SPR phenomenon [33–37].

3.2 TEM micrograph analysis

The morphology and size of biosynthesized AgNPs were observed through a high-magnification TEM image (Fig. 3). AgNPs seemed to be mainly spherical and nano-sized particles with diameters in the range of 20–40 nm. It is noted from this image that the NPs are very fine, while covered by little organic material as residual biomolecules of the fruit extracts.

3.3 FESEM and EDX analyses

The FESEM image of AgNPs demonstrated in Fig. 4 displays the spherical AgNPs with a mean diameter in a range of 15–45 nm, which stands in agreement with the particle size illustrated in the TEM image. As represented in Fig. 4 (inset), EDX result reveals a sharp signal in the Ag region and confirms the formation of AgNPs. Owing to SPR, AgNPs generally show a characteristic absorption peak at ~ 3 keV [38]. EDX spectrum has displayed a strong absorption peak for Ag along with a weak oxygen peak that may have originated from the extract (biomolecules) bound to the surface of AgNPs, indicating on the reduction of Ag⁺ to elemental Ag. The other peak corresponding to gold (Au) in the EDX would be an artefact of Au coating on the sample. There were no extra peaks observed for Ag compounds due to the complete reduction of Ag ions and/or compounds to AgNPs, as shown in the spectrum.

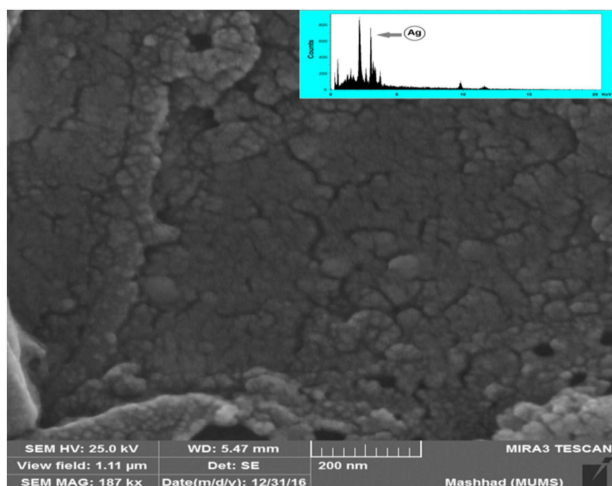


Fig. 4 FESEM and EDX spectrum of biosynthesised AgNPs

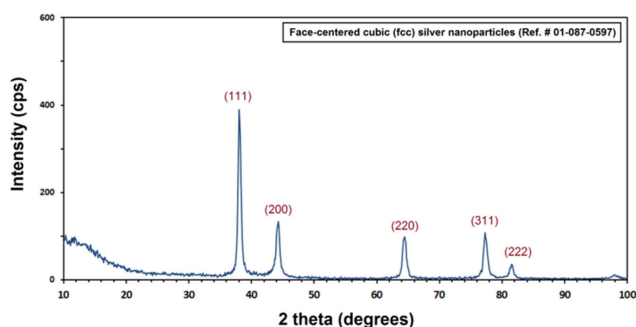


Fig. 5 XRD pattern of biosynthesised AgNPs

3.4 XRD pattern

Fig. 5 illustrated the XRD result of the biosynthesised AgNPs. The characteristic Miller indices at 38.1° , 44.2° , 64.2° , 77.4° , and 81.8° are corresponding to the (111), (200), (220), (311), and (222), respectively. Consistently, it approves that the obtained AgNPs have face-centred cubic structure (JCPDS # 01-087-0597).

3.5 FT-IR spectral analysis

The spectral analysis of FT-IR result was employed in recognising the existence of various organic functional groups due to their reducing, capping, and stabilising functions. FT-IR spectrum (Fig. 6b) has demonstrated bands at 3417 , 2918 , 1719 , 1607 , 1384 , and 1072 cm^{-1} , representing the presence of biomolecules along with AgNPs. The strong band at 3417 cm^{-1} corresponds to O–H (ν_{st}) due to existing phenolic compounds in ddw [39]. The characteristic bands in 2918 cm^{-1} are accredited to the C–H (ν_{st}) of aromatic compounds [40]. The band in 1719 cm^{-1} seem to be assigned for C–C (ν_{st} ; non-conjugated), while a band in 1607 cm^{-1} correlates to C=O (ν_{st} ; amide) [41]. The peak of C=C group (at 1398 cm^{-1}) absent for AgNPs is possible, due to the reduction of Ag^+ to Ag^0 [42]. The peak at 1070 cm^{-1} , which is absent for the AgNPs FT-IR spectrum, is the bending vibration of the C–O stretch and could be due to the stabilisation of AgNPs through this group [43]. Similarly, the same phytochemical compounds and biomolecules that are present in plant extracts have been reported to be capable of acting as the important roles of reducing and capping agents in the synthesis of AgNPs [37].

3.6 Assessment of antibacterial effects

The antibacterial effects of biosynthesised AgNPs have been assessed by disc diffusion technique [44]. As represented in Fig. 7, indicating the high antibacterial properties of these biosynthesised particles. AgNPs have demonstrated high antibacterial effects in opposition to Gram (+), i.e. *S. aureus* and Gram (–), i.e. *E. coli*

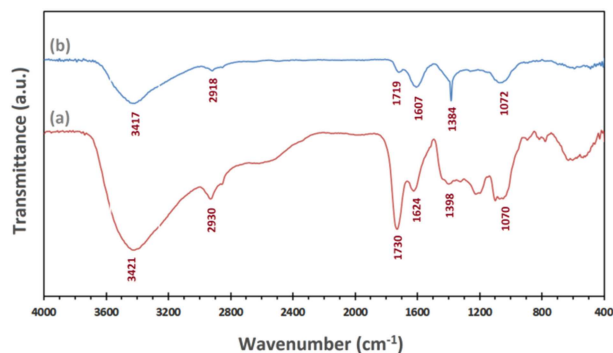


Fig. 6 FT-IR spectra of (a) Biosynthesised AgNPs, (b) *R. khorasanicum* fruits extracts

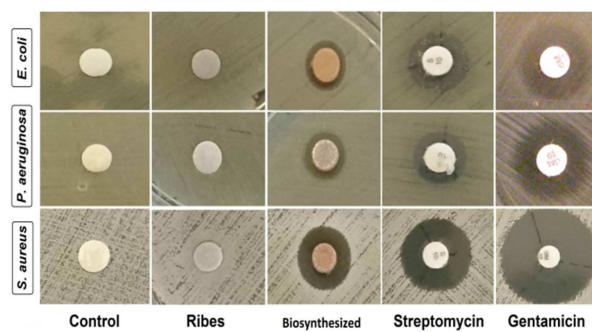


Fig. 7 Antibacterial activity of biosynthesised AgNPs against human pathogenic bacteria

Table 1 Diameter of IZs by biosynthesised AgNPs

Bacteria	IZ, mm			
	Test AgNPs	Control (–) Water	Control (+) S GM	
<i>E. coli</i>	8.9	NA	11.3	15.0
<i>P. aeruginosa</i>	9.1	NA	9.8	15.1
<i>S. aureus</i>	12.6	NA	16.3	26.0

NA = not appearing, GM = gentamicin, and S = streptomycin.

and *P. aeruginosa* bacteria, which has been reported previously as well [45, 46]. The inhibition zones (IZs) have been recognised in Table 1, while each experiment has been performed three times. The precise mechanism of antibacterial effects in AgNPs is not clearly known and yet remains as a debated topic. However, there are many theories on this matter such as the ability of AgNPs to get attached to the bacterial cell wall, and finally penetrate (through changing the structure of cell membrane) it, leading to the cells annihilation [47]. In this manner, the formation of free radicals by AgNPs and releasing Ag^+ from the surface of AgNPs could be considered as another procedure that destroys the cells [48, 49]. In addition, AgNPs are talented to inhibit bacterial growth due to their small mean diameter (large surface area) that provides adequate contact to bacteria [50].

4 Conclusion

We have proposed a novel approach in this paper regarding the biosynthesis of AgNPs from fruits extract of *R. khorasanicum*. The biosynthesised particles contained a spherical shape and the size that ranged around 20–40 nm as observed in TEM/FESEM and UV–vis/EDX analyses. These particular NPs have shown antibacterial activity against Gram-negative and Gram-positive human pathogenic bacteria. Thus, it is concluded that the biosynthesis of AgNPs in plant extracts and through the use of *R. khorasanicum* fruits can stand as a ‘green’, cost-effective, facile, and eco-friendly method that excludes the hazards that may arise out of utilising harmful reducing and/or capping agents. Furthermore, this process has the potential of scaling up for the

industrial applications to increase the yield of NPs significantly, which doubtlessly would begin its commercial viability in medicine.

5 Acknowledgment

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