



Synthesis of silver nanoparticles using medicinal *Zizyphus xylopyrus* bark extract

Babu Sumi Maria · Aishwarya Devadiga ·
Vidya Shetty Kodialbail · M. B. Saidutta

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Abstract In the present paper, biosynthesis of silver nanoparticles using *Zizyphus xylopyrus* bark extract is reported. *Z. xylopyrus* bark extract is efficiently used for the biosynthesis of silver nanoparticles. UV–Visible spectroscopy showed surface plasmon resonance peaks in the range 413–420 nm confirming the formation of silver nanoparticles. Different factors affecting the synthesis of silver nanoparticles like methodology for the preparation of extract, concentration of silver nitrate solution used for biosynthesis and initial pH of the reaction mixture were studied. The extract prepared with 10 mM AgNO₃ solution by reflux extraction method at optimum initial pH of 11, resulted in higher conversion of silver ions to silver nanoparticles as compared with those prepared by open heating or ultrasonication. SEM analysis showed that the biosynthesized nanoparticles are spherical in nature and ranged from 60 to 70 nm in size. EDX suggested that the silver nanoparticles must be capped by the organic components present in the plant extract. This simple process for the biosynthesis of silver nanoparticles using aqueous extract of *Z. xylopyrus* is a green technology without the usage of hazardous and toxic solvents and chemicals and

hence is environment friendly. The process has several advantages with reference to cost, compatibility for its application in medical and drug delivery, as well as for large-scale commercial production.

Keywords Silver nanoparticles · Surface plasmon resonance · Biosynthesis · *Zizyphus xylopyrus* · pH conditions · Reflux extraction

Introduction

Nanoparticles are extremely small structures, the synthesis of which is much more complicated than that of their macroscopic counterparts. Research in the field of nanotechnology has gained significant momentum as the properties demonstrated by these structures at the nanoscale level are remarkable in terms of their applicability (Duncan 2011). Silver nanoparticles have been the prime focus of the nanoparticles' research industry due to their unique thermal (Moon et al. 2005), electrical (Chen et al. 2009) and optical (Kelly et al. 2003) properties and also because of the use of these structures in products that range from photovoltaics (Yoon et al. 2010) to biological and chemical sensors (McFarland and Van Duyn 2003). Also nanosilver has been used extensively as an anti-bacterial agent in the health industry (Jain and Pradeep 2005), food storage (Costa et al. 2011), textile coatings (Perelshtein et al. 2008) and also for a number of environmental applications (Li et al. 2008). Currently nanosilver is prepared by different methods including electrolysis, physical, chemical, and biological methods (Dubey et al. 2010; Usha Rani and Reddy 2011). Biological methods are the biomimetic methods which refer to the application of biological principles in material formation. Bioreduction of a precursor

B. Sumi Maria · A. Devadiga · V. Shetty Kodialbail (✉) ·
M. B. Saidutta

Department of Chemical Engineering, National Institute of
Technology Karnataka Surathkal, Srinivasnagar Post,
Mangalore 575025, Karnataka, India
e-mail: vidyaks68@yahoo.com; vidyaks95@nitk.ac.in

B. Sumi Maria
e-mail: sumimariyababu@gmail.com

A. Devadiga
e-mail: aishwarya.d.88@gmail.com

M. B. Saidutta
e-mail: mbs@nitk.ac.in

salt of silver by the bioactive compounds present in plants is one such biomimetic method in which, natural products are used as reducing agents (Bhainsa and D'Souza 2006). Biosynthesis of silver nanoparticles using plant extracts may be influenced directly or indirectly by phytochemicals in extracts such as phenols, flavonoids and antioxidants as well as the physicochemical factors governing the kinetics of the reaction. This route is preferably docile as it is ecofriendly, involves less energy intensive processes and is cost effective. Moreover, it is an efficient way of waste biomass utilization for the biosynthesis of silver nanoparticles.

Biosynthesis of silver nanoparticles has been carried out successfully using the extracts of plants such as, *Lippia citriodora* (Cruz et al. 2010), *Mollugo nudicaulis* (Anarkali et al. 2010), *Prunus armeniaca* (Dauthal and Mukhopadhyay 2013), *Azadirachta indica* (Renugadevi and Venus 2012), *Desmodium gangeticum* (L.) DC. (Thirunavoukkarasu et al. 2013) *Citrus sinensis* (Kaviya et al. 2011), *Mangifera indica* (Philip 2011), *Alternanthera dentata* (Kumar et al. 2012) *Murrayya koenigii* (Philip et al. 2011), *Magnolia kobus* (Kim et al. 2014), *Malva parviflora* (Mervat et al. 2012) and *Ocimum Tenuiflorum* (Patil et al. 2012a). *Zizyphus xylopyrus* (Family: Rhamnaceae) is found throughout northwestern India, Pakistan and China, a large, straggling shrub or a small tree, armed with spines. The major chemical compositions of *Z. xylopyrus* are Quercetin, Kempferol-4'-methylether and Kempferol, Cyclo peptide alkaloids Amphibine-H and Nummularine-K (Vimal et al. 2009). The root bark of this plant is reported to have an anti-convulsant and anti-inflammatory activity (Kumar et al. 2011). It also possess anxiolytic, anticancer, antifungal, antibacterial, antiulcer, anti-inflammatory, antispastic, and wound healing properties (Saima et al. 2013). As it is rich in flavonoids, it can serve as an ideal candidate for reduction of silver salt to nanosilver.

The current paper presents the biosynthesis of silver nanoparticles by bioreduction of the precursor salt using extracts of the stem bark of *Z. xylopyrus*. The conditions under which the biosynthesis occurs have also been studied. To the best of our knowledge, this is the first study that uses *Z. xylopyrus* bark extract for the synthesis of silver nanoparticles.

Materials

Silver nitrate was purchased from Merck Specialities Private Ltd., India and used as received. *Z. xylopyrus* stem barks were purchased from a local market in Surathkal, Karnataka, India.

Methods

Preparation of plant extract

Zizyphus xylopyrus bark was thoroughly washed with tap water followed by a series of rinses with distilled water to remove any impurities and dried in shade for a week to completely remove the moisture. The bark was cut into small pieces, powdered in a motor operated domestic mixer and stored in airtight dark bottles for further use. For the preparation of extract three different methods of (1) open heating, (2) reflux extraction and (3) ultrasonication extraction were used. (a) For extraction by open heating, 5 g of the stored powder was mixed with 100 ml of distilled water and heated until boiling and then boiled for a duration of 5 min. (b) For reflux extraction, 5 g of the plant bark powder was mixed with 100 ml of distilled water and extraction with water was carried out at a temperature of 100 °C under reflux condition for a period of 1 h. (c) For extraction by ultrasonication, the slurry of 5 g of the plant bark powder mixed with 100 ml of distilled water was subjected to sonication for a time period of 10 min under conditions of 90 % amplitude and at the rate of 20 pulses per second using Vibra Cell (Sonics, USA) probe sonicator. The bark decoction hence obtained after the extraction was allowed to cool till 28 ± 2 °C filtered using Whatman No. 1 filter paper and stored at 4 °C for future use.

Preparation of silver nitrate solution

Different concentrations of silver nitrate solutions (1–100 mM) were prepared and stored in amber-colored bottles.

Biosynthesis of silver nanoparticles

For biosynthesis of silver nanoparticles, 1 ml of the prepared extract was added to 4 ml of 1 mM aqueous silver nitrate solution. The reaction was carried at 28 ± 2 °C for a period of 24 h. The visual colour change in the reaction mixture from light yellow to dark brown was observed at regular intervals with reference to control. The formation of silver nanoparticles was confirmed by spectrophotometric determination.

UV–Visible spectrophotometric studies on biosynthesized silver

The biosynthesis of silver nanoparticles was confirmed by carrying out spectral studies on the reaction mixture after 24 h of reaction. Spectrum studies were carried out in the range of 350–700 nm at a resolution of 1 nm using dual

wavelength UV–Visible spectrophotometer (Labomed, USA).

Studies on the factors affecting the biosynthesis of silver nanoparticles

Effect of concentration of silver nitrate

To study the effect of concentration of silver nitrate solution on biosynthesis of Ag nanoparticles, during the synthesis, 4 ml of different concentrations of silver nitrate (1, 5, 10, 20, 50, 70 and 100 mM) was taken in different test tubes. 1 ml of extract was added to each of the test tubes and the reaction was allowed to occur at 28 ± 2 °C over duration of 24 h. Formation of silver nanoparticles was confirmed through observation of surface plasmon resonance (SPR) by UV–Visible spectrophotometric studies.

Effect of pH

In order to study the effect of pH, biosynthesis of nanoparticles was carried out at three different initial pH's of 3, 7 and 11. The biosynthesis was carried out according to the procedure explained in “Biosynthesis of silver nanoparticles” but with silver nitrate at optimum concentration obtained using the results of experiments performed as per “Effect of concentration of silver nitrate” section. The pH was adjusted by using 0.1 N HNO₃ and 0.1 N NaOH.

Recovery of silver nanoparticles

The colloidal reaction mixtures containing the nanoparticles were centrifuged at 15,000 rpm for 15 min. The pellets thus obtained after centrifugation were washed with 70 % ethanol and dried in hot air oven at a temperature of 120 °C for 24 h. The dried powder was separated carefully from centrifuge tubes and stored in sample vials for further analysis. The supernatant after centrifugation was analyzed for residual silver content using atomic absorption spectroscopy (AAS) for determination of conversion of silver ions to silver nanoparticles.

Conversion of silver ions to silver nanoparticles

After recovery of silver nanoparticles, concentration of residual silver ions in the supernatant was analyzed using atomic AAS (GBC 932 plus) and there by conversion of silver nitrate to silver was calculated. AAS was operated with fuel flow rate of 2 (l/min) and air flow rate of 10 (l/min) running at 338.3 nm. The conversion of silver ions to silver was calculated using Eq. (1)

$$X = \frac{C_0 - C_f}{C_0} \times 100 \quad (1)$$

where X denotes the conversion of silver ions to silver nanoparticles; C₀ is the initial concentration of silver ions in the solution; and C_f is the final concentration of silver ions in the solution (Jiale et al. 2011).

Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analysis of the biosynthesized silver nanoparticles

Silver nanoparticles biosynthesized under optimum conditions (concentration of 10 mM silver nitrate and at alkaline initial pH) using *Z. xylopyrus* bark extract, were recovered after centrifugation at 15,000 rpm for 15 min and the solid portion after removal of supernatant, was washed with 70 % ethanol and then dried in hot air oven at a temperature of 120 °C for 24 h. The silver nanoparticles hence obtained, were drop coated onto carbon tapes and gold sputtering (JFC-1600 Auto fine coater, JEOL, Japan) was performed before carrying out the SEM analysis. EDX analysis was performed to confirm the elemental composition of the nanoparticles.

Results and discussions

The efficacy of the extract of *Z. xylopyrus* to reduce silver nitrate to form silver nanoparticles was tested by using the extract prepared by open heating method. UV–Vis absorption spectroscopy is one of the most widely used simple and sensitive techniques for the observation of metal nanoparticle synthesis. Synthesis of silver nanoparticles was carried out by the methodology described in “Biosynthesis of silver nanoparticles”, using the extract prepared by open heating method and aliquots were taken from the reaction mixture and analyzed for surface plasmon peak by spectrophotometric analysis. In order to monitor the formation of silver nanoparticles, the absorption spectra of the biosynthesized silver nanoparticles were recorded against distilled water as the blank. UV absorption spectra of biosynthesized silver nanoparticles demonstrated SPR peaks characteristic of silver nanoparticles. The SPR spectra of biosynthesized silver nanoparticles obtained from the reaction medium containing *Z. xylopyrus* extract prepared with open heating and silver nitrate solution of 1 mM concentration is shown in Fig. 1, which has its λ_{max} at 414 nm and broadening of the peak indicates that the nanoparticles are polydispersed. Shifting of peak from the standard 420 nm corresponding to silver nanoparticles may be attributed to the presence of bioactive components as capping agents on the surface of

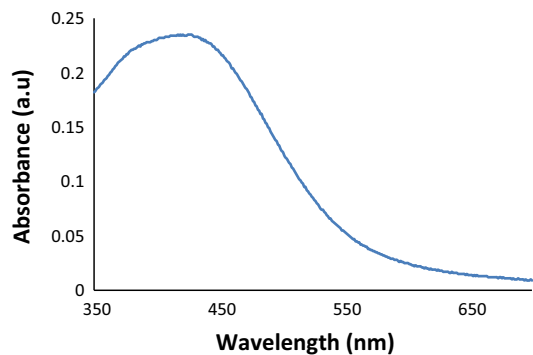


Fig. 1 Surface plasmon resonance peak for biosynthesized silver nanoparticles

nanoparticles. The surface plasmon peak confirms the formation of silver nanoparticles and hence the bark extract of *Z. xylopyrus* is shown to have the ability to reduce silver ion to nano Ag in zerovalent state. This simple process for the biosynthesis of silver nanoparticles using aqueous extract of *Z. xylopyrus* is a green technology with no use of hazardous and toxic solvents and chemicals and hence is environment friendly and economical.

Factors affecting the biosynthesis of silver nanoparticles

Different factors affecting the conversion of silver ions to silver nanoparticles by biosynthesis using the extracts prepared by each of the different extraction methods (open heating, reflux extraction and ultrasonication extraction) were studied. The effect of parameters such as precursor salt concentration and initial pH upon the biosynthesis was also studied.

Effect of silver nitrate concentration

Synthesis of silver nanoparticles was carried out as described in “Biosynthesis of silver nanoparticles” with different concentrations of AgNO_3 solution added to the extract. Visual confirmation of the synthesis of silver nanoparticles was obtained by the development of the characteristic brown colour of the reaction mixtures in each case. The extracts obtained by different methods were added to increasing concentration of AgNO_3 solution from 1 to 100 mM and the nanoparticles were synthesized. The synthesized nanoparticles demonstrated SPR peaks characteristic of silver nanoparticles as shown in the Fig. 2a–c. Figure 2a and b show the UV–Vis spectra of nanoparticles synthesized (Sastry et al. 1998) with extracts prepared by open heating and ultrasonication. In both the cases, the synthesis mixture prepared with 10 mM silver nitrate solution showed SPR peak at 418 nm and the peak

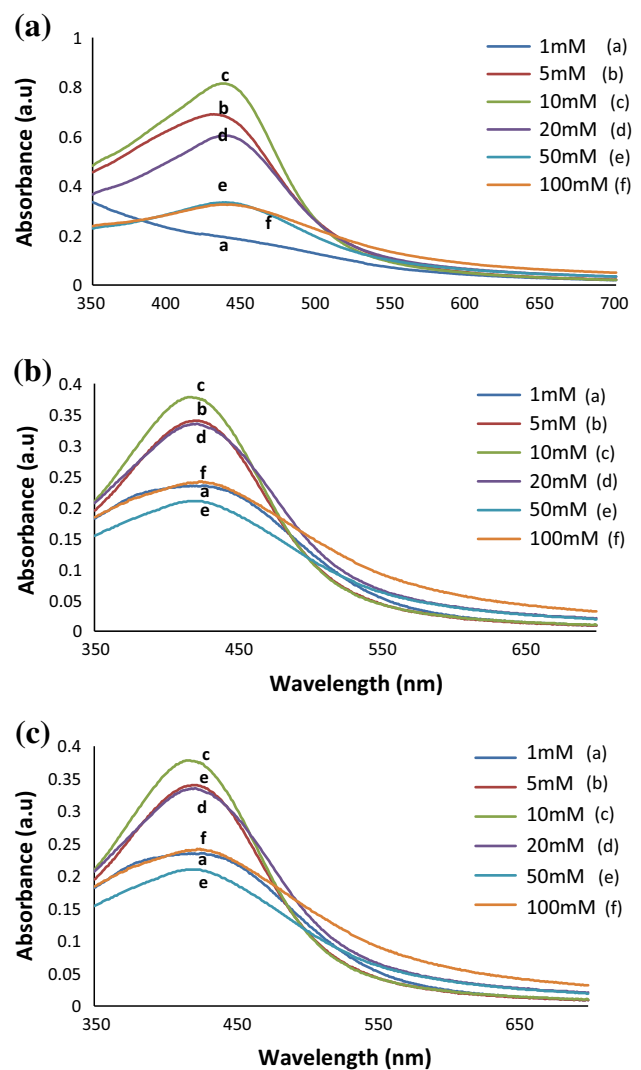


Fig. 2 Effect of silver nitrate concentration on synthesis of silver nanoparticles **a** using extract prepared by open heating, **b** using extract prepared by ultrasonication and **c** using extract prepared by reflux method

intensity increased with the increase in silver nitrate concentration from 1 to 10 mM indicating faster rate of bioreduction with increased concentration of precursor salt (Zaki et al. 2011). The rise in peak intensities with increasing salt concentration was also observed by Dubey et al. (2010). The rise in SPR peak intensities is due to the longitudinal vibrations (Prathna et al. 2011). But further increase in silver nitrate concentration to 20 mM and above lead to peaks with lower intensity which can be accounted by the formation of agglomerated nanoparticles and their settling. The settling of particles at higher concentrations may be due to the presence of large number of silver particles in small volume of solution which creates higher coalescence between silver nanoparticles and hence results in agglomeration and formation of particles with

large size at micro scale. The presence of very large particles was the reason of faster settling of particles (Safekordi et al. 2011). Reaction mixtures prepared with 50 and 100 mM silver nitrate solutions demonstrated broadened peaks with less intensity because of the settling of particles.

In case of extract prepared by reflux method, synthesis increased with the increase in concentration of silver nitrate from 5 to 10 mM, indicated by the rise in SPR peak intensity as shown in Fig. 2c. Lower precursor salt concentration of 1 mM, did not display an SPR peak; this may be due to inadequate number of silver ions for bio-reduction in the sample. From Fig. 2a–c it can be deduced that owing to highest intensity obtained with the synthesis mixture prepared with 10 mM of silver nitrate solution, the optimum concentration of the salt required for formation of stable colloidal nanoparticles is 10 mM for biosynthesis using extracts prepared by all the three different methods.

Effect of pH

Effect of initial pH on the biosynthesis of nanoparticles with the extracts of *Z. xylopyrus* prepared by three different methods of open heating, ultrasonication and reflux heating were studied as per the methodology described in “Effect of pH”. SPR spectra of the synthesized nanoparticles using the extracts prepared by three different methods and at different pH conditions are presented in Fig. 3a–c. At pH 3, a flat absorption spectrum was observed. No SPR peak was formed under acidic conditions hence terming it as unsuitable for promoting biosynthesis of silver nanoparticles while neutral and alkaline conditions demonstrated SPR peaks indicating that these conditions of pH are suitable for biosynthesis of Ag nanoparticles. Higher SPR peak intensities at alkaline condition, indicates increased number of smaller silver nanoparticles. The peak signifies uniformly shaped nanoparticles. A possible explanation for alkaline condition to be favorable for this would be that due to alkalinity, hydroxides get

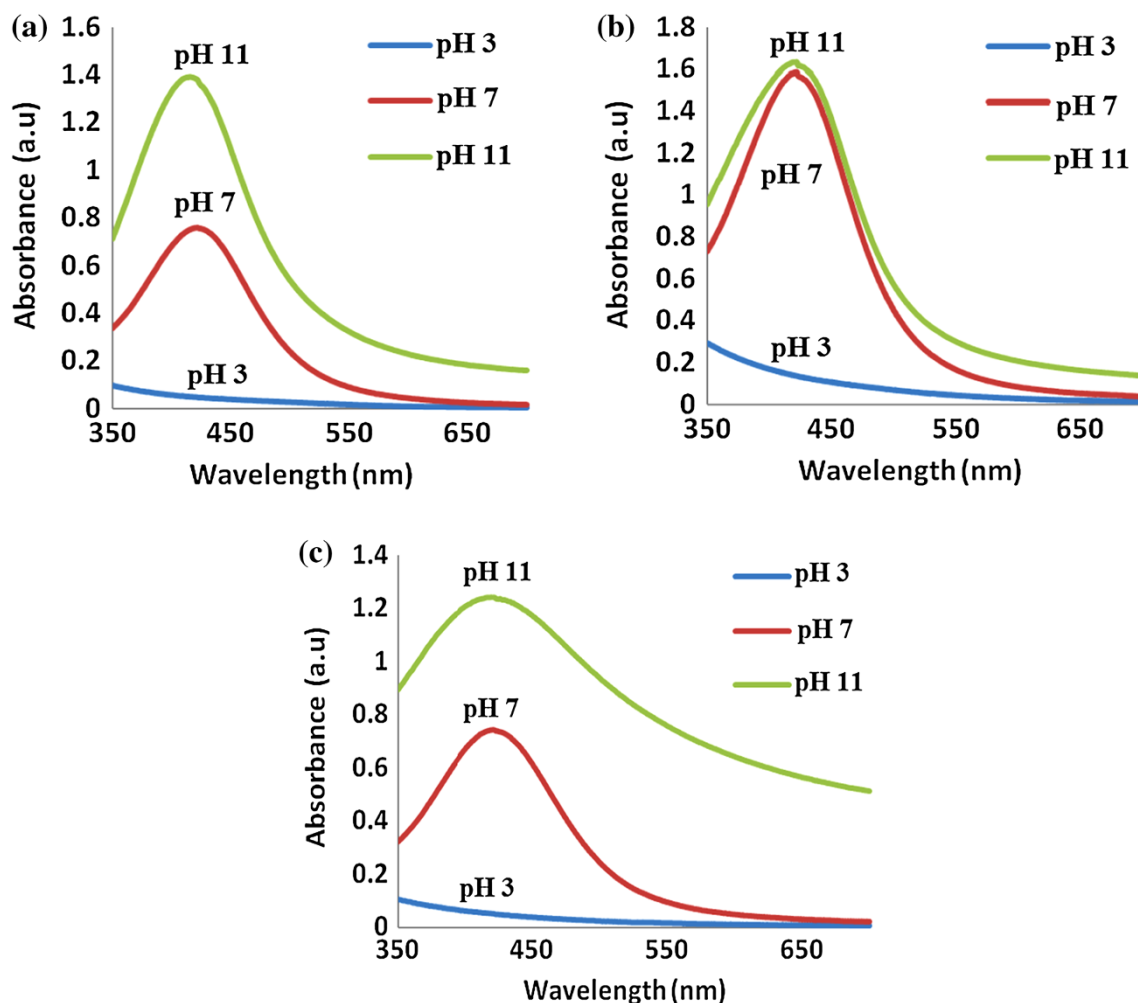


Fig. 3 Effect of pH on synthesis of silver nanoparticles **a** using extract prepared by open heating, **b** using extract prepared by reflux method and **c** using extract prepared by ultrasonication

deposited on the silver nanoparticle. It may be hypothesized in the similar lines as that by Oza et al. (2012) in their explanation on biosynthesis of gold nanoparticles, that at alkaline pH both reducing as well as capping agents are efficiently reducing the particles and further encapping them at specific facets. This allows growth of spherical nanoparticles due to vulnerable deposition of silver atoms on all the facets forming thermodynamically favorable spherical nanoparticles. Due to very high proton concentration at acidic pH, all the functional groups responsible for biosynthesis of nanoparticles possess positive charge. Thus even if the nanoparticles are formed, they are not stable enough to prevent agglomeration. The reducing power of these functional groups at lower pH is less, but as the pH increases to alkalinity range, the reduction potentials of all these functional groups are enhanced, thus allowing the formation of thermodynamically favorable structures. Hence alkaline condition is favorable for the biosynthesis. Sathishkumar et al. (2009) in their studies on effect of pH on nanoparticle synthesis have also observed small and stable nanoparticles being formed at alkaline pH. According to them, Lower pH values promote the nucleation of the nanoparticles while higher pH caused electrostatic repulsion among them thereby leading to formation of smaller nanoparticles. In the present study, in all the three cases of utilizing the extracts prepared by different methods, it is observed that acidic condition suppressed the formation of silver nanoparticles and the basic condition enhanced the formation of silver nanoparticles.

According to Mie's theory (1908), only a single SPR band expected in the absorption spectra of spherical nanoparticles, whereas anisotropic particles could give rise to two or more SPR bands depending on the shape of the particles. The spherical nanoparticles, disks and triangular nanoparticles of silver show one, two and more peaks, respectively (Mukherjee et al. 2012). The single SPR band noticed in the UV–Visible spectroscopic studies in the current study also substantiates the spherical nature of silver nanoparticles.

Comparison of extraction methodology in terms of conversion of silver ions to silver nanoparticles

Conversion of silver nitrate to silver nanoparticles during biosynthesis with reaction mixture at optimum pH of 11 and at natural pH of the extract (without any pH adjustment), with all the three types of extracts was calculated. In these studies, silver nanoparticles were biosynthesized using 10 mM AgNO_3 solution. From Fig. 4, it can be seen that with the extract prepared by reflux method higher conversion of silver ions to nanoparticles was achieved, followed by that with ultrasonication method and open heating. In reflux heating method, solvent loss by evaporation is minimal and the desired temperature is maintained throughout the reflux

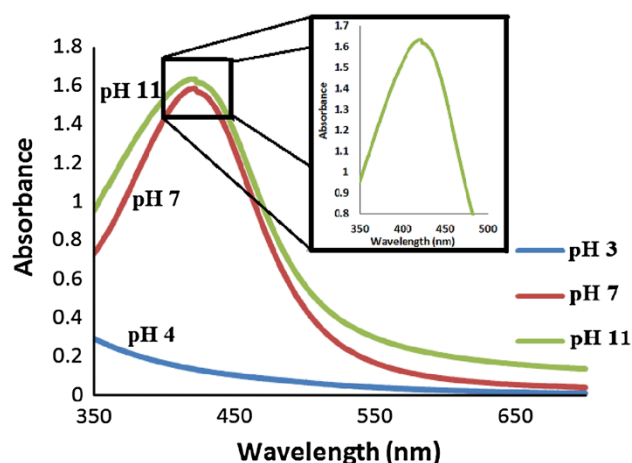


Fig. 4 SPR peak obtained under the optimized conditions (*inset*) at 10 mM precursor salt concentration, at pH 11 by reflux extraction method

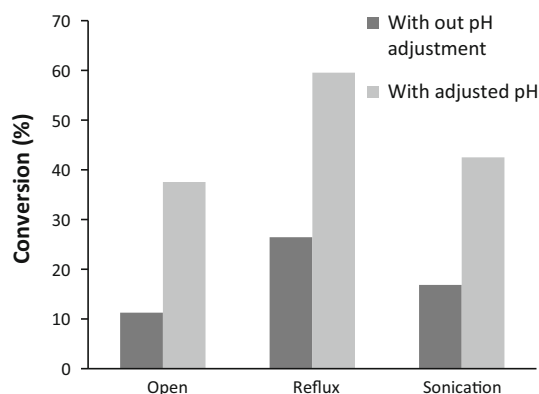


Fig. 5 Studies on yield of silver nanoparticles

operation. This leads to effective extraction of different bioactive components of the bark like antioxidants, phenolics, etc., by the solvent. But in open heating extraction method, solvent loss may occur by evaporation and temperature is lower than the boiling temperature of the solvent throughout the extraction process, which leads to less efficient extraction of bioactive components. Ultrasonication method seems to be more efficient than open heating, as it accelerates the release of bioactive components into the solvent. Solvent extraction with reflux (Fig. 5) is the best method among the others studied. Biosynthesis at optimum pH condition of 11 has been proven to be favorable as compared to the natural pH conditions, as indicated by higher conversion to silver nanoparticles at pH of 11.

SEM and EDX analysis of the biosynthesized silver nanoparticle

Biosynthesized nanoparticles (under optimum conditions using 10 mM silver nitrate solution and alkaline initial pH)

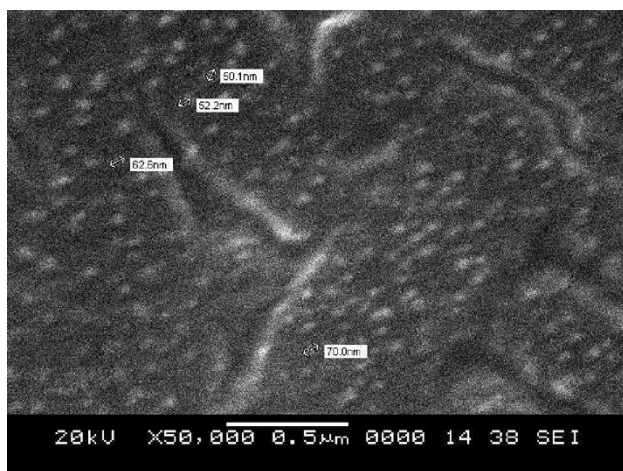


Fig. 6 Scanning electron microscopy image indicating the size of the silver nanoparticle biosynthesized

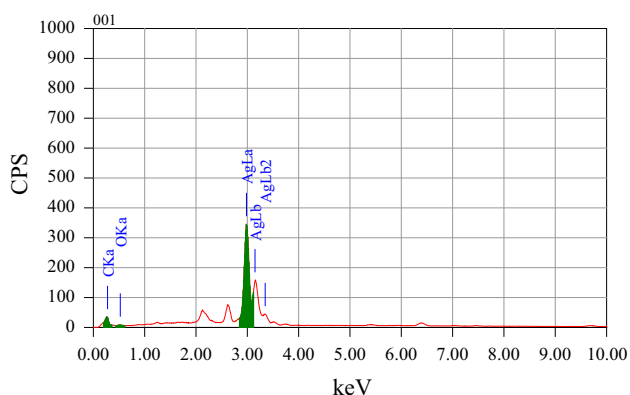


Fig. 7 EDX analysis revealing the presence of silver nanoparticles

were found to be spherical in nature as shown in the SEM image (Fig. 6) and ranged from 60 to 70 nm in size. The EDX spectrum shown in Fig. 7 displays the presence of silver in the sample, further confirming the presence of silver nanoparticles and also the presence of carbon and oxygen suggesting that the silver nanoparticles must be capped by the organic components present in the plant extract. AgNPs are established as antimicrobial agents, while the presence of plant bioorganic capping material upon the AgNPs enable them to exhibit enhanced antibacterial activity (MubarakAli et al. 2011; Prabhu and Poulouse 2012) and can be used as antioxidant agents (Niraimathi et al. 2013).

Conclusions

Zizyphus xylopyrus bark extracts can be efficiently used for the biosynthesis of silver nanoparticles. UV–Visible spectroscopy showed peaks in the range 413–420 nm confirming the formation of silver nanoparticles. Different

factors affecting the synthesis of silver nanoparticles like methodology for the preparation of extract, concentration of silver nitrate solution used for biosynthesis and initial pH of the reaction mixture were studied. Silver nitrate solution of 10 mM concentration and alkaline initial pH of 11, which resulted in SPR peak of high intensity indicating enhanced synthesis of silver nanoparticles can be considered the optimum conditions for the biosynthesis. The extract prepared by reflux extraction method at optimum initial pH resulted in higher conversion of silver ions to silver nanoparticles as compared with those prepared by other extraction methods. SEM analysis showed that the biosynthesized nanoparticles are spherical in nature and ranged from 60 to 70 nm in size. EDX showed the presence of carbon and oxygen with Ag, suggesting that the silver nanoparticles must be capped by the organic components present in the plant extract for elucidating the bioconversion of the precursor salt into AgNPs (Patil et al. 2012b). This simple process for the biosynthesis of silver nanoparticles using aqueous extract of *Z. xylopyrus*, is a green technology with no use of hazardous and toxic solvents and chemicals and hence is environment friendly. The process has several advantages with reference to cost and compatibility for the bioorganic compound capped AgNPs to be used as antimicrobial agents, in water purification systems, for its application in medical and drug delivery, as well as for large scale commercial production.

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