

Rajeev Kumar*, Priyanka Sharma, Aditi Bamal, Sushma Negi and Savita Chaudhary

A safe, efficient and environment friendly biosynthesis of silver nanoparticles using *Leucaena leucocephala* seed extract and its antioxidant, antimicrobial, antifungal activities and potential in sensing

DOI 10.1515/gps-2016-0146

Received August 23, 2016; accepted October 26, 2016; previously published online January 18, 2017

Abstract: One step green synthesis of silver nanoparticles (AgNPs) from silver nitrate (AgNO_3) using *Leucaena leucocephala* seeds extract as the reducing agent at room temperature was performed. The bioreduced NPs were characterized by UV-vis spectroscopy, dynamic light scattering (DLS), transmission electron microscopy (TEM), scanning electron microscopy (SEM) coupled with energy dispersive spectrophotometry, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and thermogravimetric analysis (TGA) techniques. Qualitative information of major components in the seed extract was obtained through its phytochemical screening. The phytochemical data of *L. leucocephala* revealed the presence of terpenes, flavonoids, coumarins and sterols. The reaction was optimized for AgNO_3 , extract concentration and time duration for the reaction. The obtained NPs showed a characteristic UV peak of AgNPs at 420 nm. TEM and SEM images showed the spherical shaped NPs over which the extract coating was very prominent. The binding of *L. leucocephala* seeds extract onto NPs was tested using FTIR and TGA. The antifungal activity of the as-synthesized NPs against two fungal species, namely *Phlebiopsis gigantea* and *Echinodontium taxodii*, was studied. The antimicrobial effect of the as-synthesized NPs was ascertained against *Escherichia coli* and *Staphylococcus aureus*. The antioxidant potential of the AgNPs was tested with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging.

Also, the sensitivity of the NPs towards Fe^{3+} ions was tested in aqueous media.

Keywords: bioreduction; DPPH; *Escherichia coli*; *Echinodontium taxodii*; *Leucaena leucocephala*; *Phlebiopsis gigantea*; sensing; silver nanoparticles; *Staphylococcus aureus*.

1 Introduction

Green or sustainable chemistry is an area of chemistry that focuses on the design of products and processes that use and generate minimum hazardous substances [1–14]. One approach of green chemistry is to synthesize the products with very little or no chemicals. The substitute to chemicals could be some natural or waste products such as rice husk [15] for energy production, refuse derived fuel [16] as an additive to coal to reduce coal consumption, fermentation to produce secondary metabolites such as ethanol [17], antibodies [18], antibiotics [19] etc. and plant parts as reagents [20]. All of these methods have their own significance according to their functional areas. The extracts of plant parts as reducing agents have gained much attention in recent years due to their easy and more availability, simple extraction process, multiplicity, and variability [21]. Multiplicity and variability are related as there are a number of plant species, with each one having many plant parts from which the extract can be made. Also, there is no need of maintenance and no risk of biohazard, as in the case of microbes based synthesis [22, 23].

Green synthesis of nanoparticles (NPs) covers a large area in the literature in today's research [24–31]. The chemical moieties present in the extract act as reducing agents that reduce the metals to metallic NPs. Much work has been done in this direction by many scientists. Saravanakumar et al. [32] synthesized silver NPs using *Cassia tora* leaf extract, Rajan et al. [33] synthesized AgNPs using *Areca catechu* nut, and ethanolic petal extract of *Rosa indica* also acts as a reducing agent for AgNPs as reported

*Corresponding author: Rajeev Kumar, Department of Environment Studies, Panjab University, Chandigarh-160014, India, e-mail: rajeev@pu.ac.in

Priyanka Sharma, Aditi Bamal and Sushma Negi: Department of Environment Studies, Panjab University, Chandigarh-160014, India
Savita Chaudhary: Department of Chemistry and Centre of Advanced Studies in Chemistry, Panjab University, Chandigarh-160014, India

by Ramar et al. [34]. Nasrollahzadeh and Sajadi [35] used *Euphorbia heteradena* Jaub root extract to synthesize titanium oxide NPs and Venkateswarlu et al. [36] used *Vitis vinifera* stem extract to synthesize Fe_3O_4 -Ag core-shell recyclable NPs. AgNPs have promising applications in the field of nanotechnology due to their excellent electrical conductivity [37], chemical stability [38], catalytic [39], antioxidant [40] and antibacterial [41] properties.

Leucaena leucocephala (family: Fabaceae) is a small, fast-growing mimosoid tree. *L. leucocephala* is widely used for a variety of purposes, such as firewood, fiber, and livestock fodder [42]. *L. leucocephala* secretes phytotoxic allelochemicals, such as mimosine and phenolic compounds like p-hydroxycinnamic acid, protocatechuic acid, and gallic acid [43]. The plant also shows bioherbicidal activity towards many terrestrial plants [44] and aquatic weed water hyacinth [45]. Various medicinal properties of *L. leucocephala* include cure of stomach disorders, cancer chemoprevention, antiproliferative properties and in contraception and abortion [46]. Various parts of leucaena genus have been reported to contain epicatechin-3-O-gallate, quercetin-3-O-arabinofuranoside, quercetin-3-orhamnoside, apigenin, hydrocyanic acid, leucaenine, tannic acid and galactomannan mucilage in endosperm of the seeds [47]. Also, the plant parts contain fatty alcohol, fatty acids, terpenes, coumarins, flavonoids, sterols and other volatile oil compounds [48].

In the present work, we demonstrated biosynthesis of AgNPs by reduction of aqueous silver nitrate (AgNO_3) using aqueous *L. leucocephala* seed extract. According to our knowledge, this kind of work has been not done using this plant part and it is novel in this category. The method provides a new, cost-effective, non-toxic, and green reducing agent for the synthesis of AgNPs. Also, the antioxidant, antimicrobial and antifungal properties of as-synthesized AgNPs were examined. The as-synthesized NPs were also tested for their sensing behavior towards different metal ions.

2 Materials and methods

2.1 Materials

All the reagents used for the experiment were of analytical grade and used without any further purification. Silver nitrate (AgNO_3) was purchased from Qualigens, Mumbai, India. Malt extract agar (MEA) and Muller Hinton agar were purchased from Sigma-Aldrich, Germany. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) was provided by Sisco Research Laboratories, Maharashtra, India. Absolute ethanol was provided by Changshu Yangyuan Chemicals, China. Reagents used for phytochemical screening viz. Molisch's reagent, concentrated sulphuric acid, ferric chloride, sodium hydroxide, concentrated sulphuric acid,

chloroform, ammonia solution, Ninhydrin, glacial acetic acid and acetone were purchased from Central Drug House Pvt. Ltd., New Delhi, India. The fungal cultures (*Phlebiopsis gigantea* and *Echinodontium taxodii*) were collected from detritus wood from Kinnaur Himachal Pradesh, India. *Escherichia coli* and *Staphylococcus aureus* strains were collected from Microbiology Department Panjab University, Chandigarh. All the reactions were carried out in deionized water.

2.2 Plant material

Leucaena leucocephala seeds were collected from Botanical garden, Panjab University, Chandigarh, India. The plant was identified and confirmed as *L. leucocephala*. The collected seeds were thoroughly washed in running tap water to completely remove foreign matter. Then, the seeds were finely crushed to powder in mortar. Some 5 g of this powder was added to 100 ml of deionized water and heated to 250°C for 5 min to make aqueous extract. The mixture was stirred overnight to extract out maximum components from the seeds. Then, the extract was filtered using Whatman filter paper followed by centrifugation in order to obtain a transparent solution.

2.3 Synthesis of AgNPs

The AgNPs from *L. leucocephala* seeds extract was prepared by dissolving 5 mm AgNO_3 solution in deionized water followed by the addition of seed extract in a 2:3 ratio. Constant stirring of 300 rpm was provided throughout the mixing process. After 1 h of stirring, the solution was kept in the dark for 8 h. The obtained dark colored solution was separated using a centrifuge at 7500 rpm and washed several times with deionized water followed by ethanol to remove unreacted impurities. Then, it was dried in an oven at 50°C to obtain dark AgNPs that were further subjected to characterizations.

2.4 Characterizations

The dried AgNPs were dispersed in deionized water and subjected to UV-vis spectroscopy and dynamic light scattering (DLS) to validate their formation. The UV vis spectroscopy was done using a LABINDIA spectrophotometer and DLS studies were carried out using a Malvern zetasizer. The size and morphology of the NPs was studied using Hitachi H-7500 transmission electron microscopy (TEM) and Hitachi FE-scanning electron microscopy (SEM) SU8010, along with energy dispersive spectrophotometry to test the elemental composition. Fourier transform infrared spectroscopy (FTIR) spectra were recorded in Perkin-Elmer (RX1) in the range 4000–400 cm^{-1} . The crystalline size and phase of the NPs was studied with Panalytical D/Max-2500 X-ray diffraction (XRD). The synchronized thermal degradation of the NPs was studied with the help of thermogravimetric analysis (TGA)–DTG instrument model SDTQ-600 from TA Instruments. For the centrifugation purposes, a REMI R-24 centrifuge was used.

2.5 Phytochemical screening

Qualitative phytochemical screening of the plant extract was performed in order to identify the major biochemical components of

the extract. Tests for carbohydrates, tannins, saponins, flavonoids, quinones, glycosides, cardiac glycosides, terpenoids, phenols, coumarins, proteins, phytosteroids, anthraquinone, oils and fats and resins were performed. Methods of identification are listed in Supplemental Table 1.

2.6 Antibacterial activity

The antibacterial potential of biofunctionalized AgNPs was tested against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria by an antimicrobial susceptibility test. Approximately, 25 ml of autoclaved and cooled Muller Hinton agar media was poured into radiation sterilized disposable Petri plates. The Muller Hinton agar was allowed to cool down and left overnight for any contamination to appear. The inoculums of bacteria were spread over the agar surface, and wells of 7 mm diameter were punctured. AgNPs of different concentration were poured in to these well and the plates were incubated at 37°C for 24 h. After the incubation period, the zone of inhibition (in mm) was noted and the effective zone of inhibition was calculated by subtracting the well diameter from each reading.

2.7 Antifungal activity

Antifungal activity was evaluated by growing *P. gigantea* and *E. taxodii* on media (MEA) containing different concentrations of AgNPs. Different concentrations of AgNPs (10 ppm, 25 ppm, 50 ppm and 100 ppm) were mixed in 25 ml of 5 g MEA. The solutions were autoclaved to maintain sterilized conditions. After cooling the solution down to room temperature, the liquid medium was transferred to Petri plates and allowed to solidify inside laminar air flow. In every Petri plate, 2–3 discs of pure fungus culture were inoculated and kept in a BOD incubator at 28°C. The radial growth of the fungal mycelium was recorded at regular intervals of time. A negative control was set to calculate radial growth inhibition. Mathematically it is calculated as:

$$\text{Inhibition rate (\%)} = \frac{R - r}{R} \quad (1)$$

where R is the radial growth of fungal mycelia on the control plate and r is the radial growth of fungal mycelia on the plate treated with AgNPs.

2.8 DPPH free radical scavenging assay

The antioxidant activity (AA) of the AgNPs was evaluated using a DPPH assay [49]. Some 2 ml of various AgNP concentrations was mixed with 3.5 ml of freshly prepared 6×10^{-5} M DPPH free radical in ethanol. Deionized water (without AgNPs) was used as a control. The reaction mixtures were kept in incubation at 50°C in the dark for 30 min. Afterwards the samples were subjected to UV-vis spectroscopy for the quantitative estimation of DPPH ($\lambda = 520$ nm) using ethanol as blank. AA was determined using the following equation [49]:

$$\text{AA \%} = \left(\frac{\text{Ab. of control} - \text{Ab. of sample}}{\text{Ab. of control}} \right) \times 100 \quad (2)$$

3 Results and discussion

3.1 UV-vis spectroscopy

AgNPs display characteristic absorption or surface plasmon resonance (SPR) (Figure 1), which is the result of coherent oscillation of conduction electrons induced by the interacting electromagnetic field [50]. To find out the optimum concentration of the reactants, various trial batches were carried out. Different concentrations of AgNO₃ were taken to ascertain the optimum concentration of the starting salt with 3 ml of prepared extract (Figure 1A). The results show that the intensity of the SPR increased as the concentration of starting salt was increased up to 5 mM, but beyond this concentration, the SPR intensity decreased due to lesser availability of reducing extract

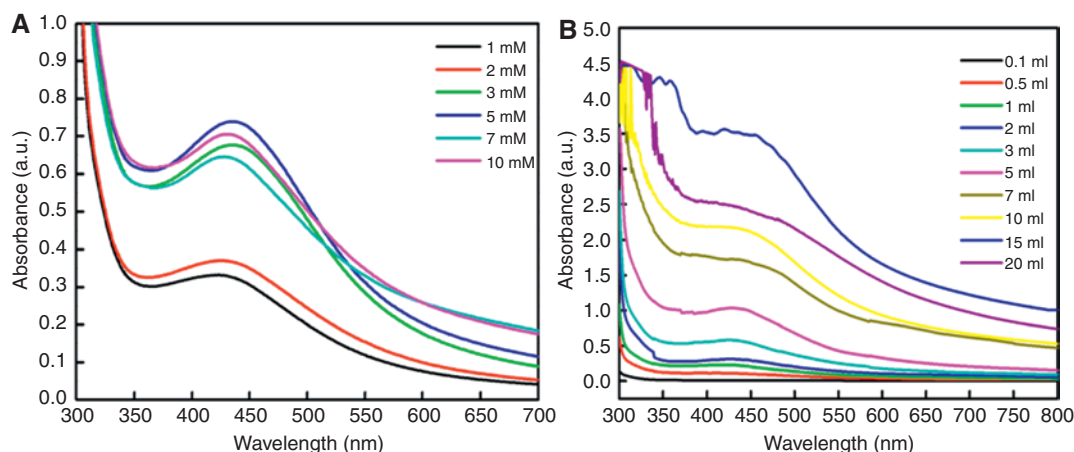


Figure 1: UV-vis spectra of as-synthesized silver nanoparticles (AgNPs) as a function of (A) varying AgNO₃ concentration and (B) plant extract concentration in 25 ml total solution.

and high unreacted AgNO_3 gradient. Therefore, 5 mm concentration of AgNO_3 was optimized for further studies.

The appropriate ratio of AgNO_3 to extract was found by varying the extract concentration in 25 ml of reaction solution (Figure 1B). With the increase in quantity of extract, the absorbance was observed to increase up to 15 ml and afterwards it started decreasing. The possible reason for this could be that beyond the limit of 15 ml of seed extract, the growth of NPs is favored over the nucleation and the agglomerated large particles showed reduced absorbance. Therefore, we may interpret that optimum dose of starting salt as well as seed extract play a vital role in concentration and size of as-synthesized AgNPs (Supplemental Figures 1 and 2).

3.2 Size and morphological studies

TEM images of plant reduced AgNPs gave a clear idea about the shape and morphology of the as-synthesized AgNPs (Figure 2A). The images validate the presence of spherical polydispersed AgNPs in the range of 6–25 nm that are

effectively covered and stabilized by the phytochemicals present in the *L. leucocephala* seed extract. Detailed morphological analysis attempted using the TEM images is in good agreement with the SEM (explained later). SEM image of the AgNPs in Figure 2B shows spherical bead like NPs, which are coated by plant extract giving it a rough texture. The large agglomerated NPs are the result of a solvent drying process during sample preparation. This further imparts changes in variation in particle sizes. The elemental composition of the sample is shown in Figure 3A and reveals a strong signal in the silver region and confirmed the formation of AgNPs. The peaks shown by C and O are the result of the coating of the plant extract adsorbed over the NPs surface. DLS spectra have provided the hydrodynamic diameter of the NPs and size distribution in Figure 3B. As expected, the hydrodynamic diameter is much larger than the primary particle size, due to the presence of surface layer of the stabilizing agent and the hydration sphere. The z-average of the NPs was found to be 212.6 nm with less polydispersity index. The DLS spectra showed two peaks of size <100 nm and >500 nm. The sharpness in intensity of the first peak indicates the presence of

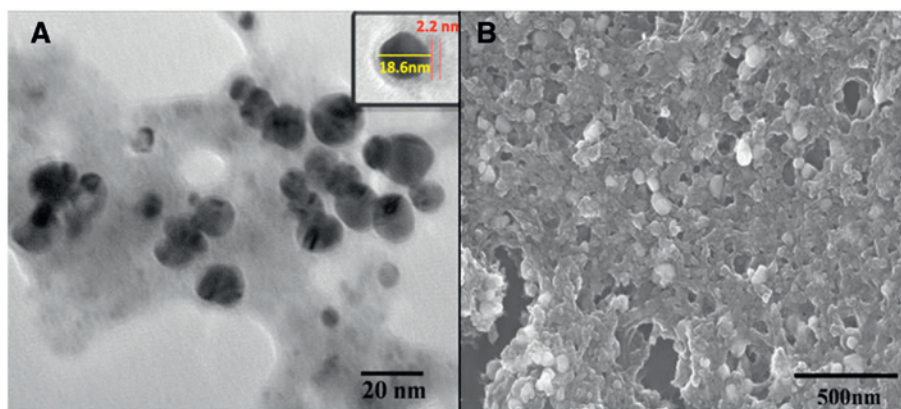


Figure 2: (A) transmission electron microscopy (TEM) and (B) scanning electron microscopy (SEM) images of silver nanoparticles (AgNPs) clearly demonstrating the nanoscale size and plant extract coating over NPs.

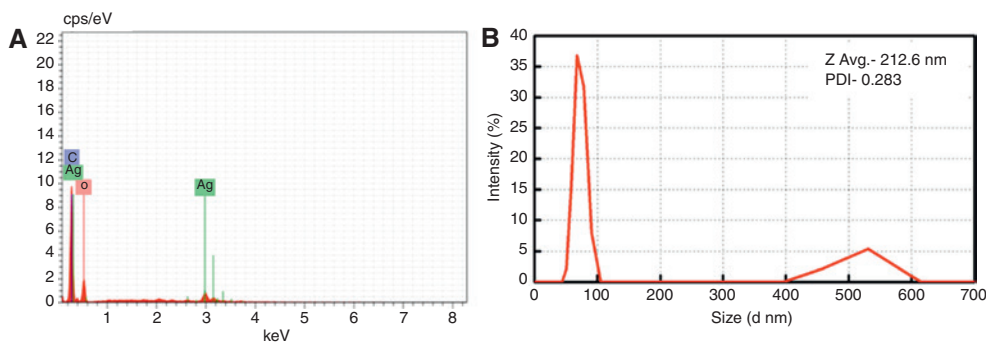


Figure 3: Representative (A) energy dispersive X-ray spectroscopy (EDX) and (B) dynamic light scattering (DLS) intensity versus size spectra of silver nanoparticles (AgNPs).

more small sized NPs as compared to larger agglomerates. The reason for less agglomeration and high dispersion is because of photochemical layering over the NP surface.

3.3 Phytochemical screening

Phytochemical screening for *L. leucocephala* seed extracts was performed and showed positive results for many biochemical compounds listed in Table 1. The qualitative phytochemical screening data of the seed extract when correlated with FTIR spectra could result in better understanding of its interaction with NP surface.

3.4 Surface functionalization and crystalline phase

The plant extract not only reduces the metal into metallic NPs, but also act as a stabilizing agent, because it covers the surface of NPs. The extract may bind to the AgNP surface by many attractive forces such as chemisorption, electrostatic attraction and/or hydrophobic interaction and gives rise to an NP-extract corona that is stable in itself and avoids aggregation. FTIR analysis offers an insight into the interactions among NPs surface and the plant extract. The plant extract is a mixture of many organic components having different functional groups.

The FTIR spectrum of seed extract and AgNPs (Figure 4A) showed a shift in the peaks of 2968 cm^{-1} to 2899 cm^{-1} , 1650 cm^{-1} to 1607 cm^{-1} and 1362 cm^{-1} to 1336 cm^{-1} . The absorption peak at 2968 cm^{-1} observed

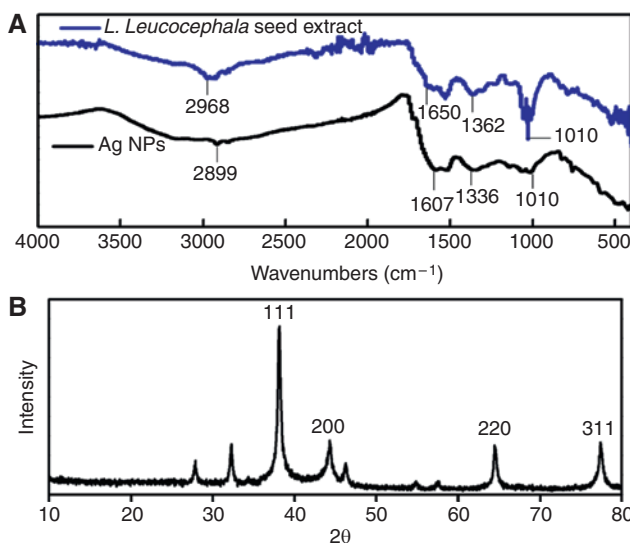


Figure 4: (A) Fourier transform infrared spectroscopy (FTIR) spectra of *Leucaena leucocephala* seed extract and silver nanoparticles (AgNPs), (B) X-ray diffraction (XRD) spectra of bioreduced AgNPs.

in seed extract is due to OH stretching vibration which is shifted to lower frequency regions (2899 cm^{-1}) in AgNPs. This peak widens up to a band ending around 1800 cm^{-1} and covers C-H stretching. The peak at 1650 cm^{-1} in seed extract is due to the presence of amide I vibrations which shifted to 1607 cm^{-1} in AgNPs. These shifts in peak indicate the interaction of different functional groups of seed extract with AgNPs surface, i.e. the shifting in 2968 cm^{-1} indicates the interaction through the phenolic group, and shifting of 1650 cm^{-1} peak can be correlated with binding of extract proteins through the amine group. The shifting of the 1362 cm^{-1} band in plant extract to 1336 cm^{-1} in AgNPs indicates band shift at the binding of the C=O functional group with AgNPs. There was no significant difference seen in the peak at 1010 cm^{-1} denoting C-N and C-O stretching, except for the reduction in its intensity indicating the removal of uncombined excessive phytochemicals during washings of the NPs. XRD analysis was carried out to study the crystal structure of the biosynthesized AgNPs (Figure 4B). The NPs showed the Bragg's reflection peaks at positions 38.07° , 44.31° , 66.25° , and 77.35° indexed to be $\langle 111 \rangle$, $\langle 200 \rangle$, $\langle 220 \rangle$, and $\langle 311 \rangle$ planes of fcc structure. The d-spacing of the corresponding planes were found to be 2.33 \AA , 2.03 \AA , 1.41 \AA , and 1.24 \AA , respectively (JCPDS card No: 04- 0783).

The unassigned peaks are thought to be due to the presence of phytochemicals adsorbed over the surface. The peak ratio of the $\langle 111 \rangle$ plane is highest among other planes indicating the dominance of NPs growth in this plane. Also, the broadened peaks show the nanometric

Table 1: Qualitative response of various components in *Leucaena leucocephala* seed extracts.

| Sr. no. | Extract component | Response |
|---------|--------------------|----------|
| 1. | Carbohydrates | + |
| 2. | Tannins | + |
| 3. | Saponins | + |
| 4. | Flavonoids | + |
| 5. | Quinones | + |
| 6. | Glycosides | + |
| 7. | Cardiac glycosides | – |
| 8. | Terpenoids | + |
| 9. | Phenols | + |
| 10. | Coumarins | + |
| 11. | Proteins | + |
| 12. | Phytosteroids | + |
| 13. | Anthraquinone | – |
| 14. | Oils and fats | + |
| 15. | Resins | + |

+, present; –, absent.

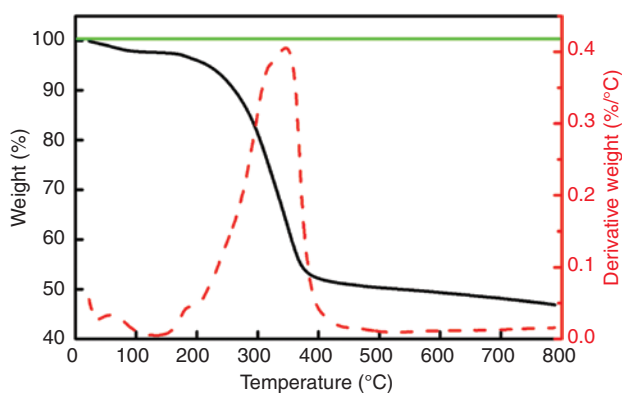


Figure 5: Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) graph of bioreduced silver nanoparticles (AgNPs).

size of obtained particles. The mean crystallite size was calculated using the Debye-Scherrer's equation:

$$D = \frac{K\lambda}{\beta \cos \theta} \quad (3)$$

where D is the average particle size, K is the shape-dependent Scherrer's constant, λ is the X-ray wavelength, and β is full width at half maxima of the diffraction peak of the planes. The mean size of AgNPs with all planes was found to be 13.84 nm, which is in close agreement with the size calculated from SEM and TEM analysis. However, the

size calculation using Debye-Scherrer's equation provides the average size of the polydispersed NPs. The TGA plot of the bioreduced AgNPs (Figure 5) showed a steady weight loss in the temperature range of 200–400°C. The slight weight loss up to 200°C was due to moisture loss by the sample. The major weight loss (48.96%) was due to desorption and evaporation of organic phytochemical compounds adsorbed over AgNPs. Afterwards, a small weight loss continued until 800°C (4.16%) due to heat resistive phytochemicals such as oils.

3.5 Antimicrobial, antifungal and AA of AgNPs

The as-formed AgNPs exhibited good antimicrobial activity against *E. coli* and *S. aureus* showing the potential of these NPs in disinfection and in solving multi-drug resist problems, as the microbes were not found to become resistant to the NPs (Figure 6). The extent of their activity against both Gram-positive and Gram-negative bacteria also strengthens the broad range of application. Supplemental Table 2 shows the effective zone of inhibition (zone of inhibition of the treatment-zone of inhibition of the control) by AgNPs.

The antimicrobial activity of AgNPs could be related to many factors; one such factor is the electrostatic

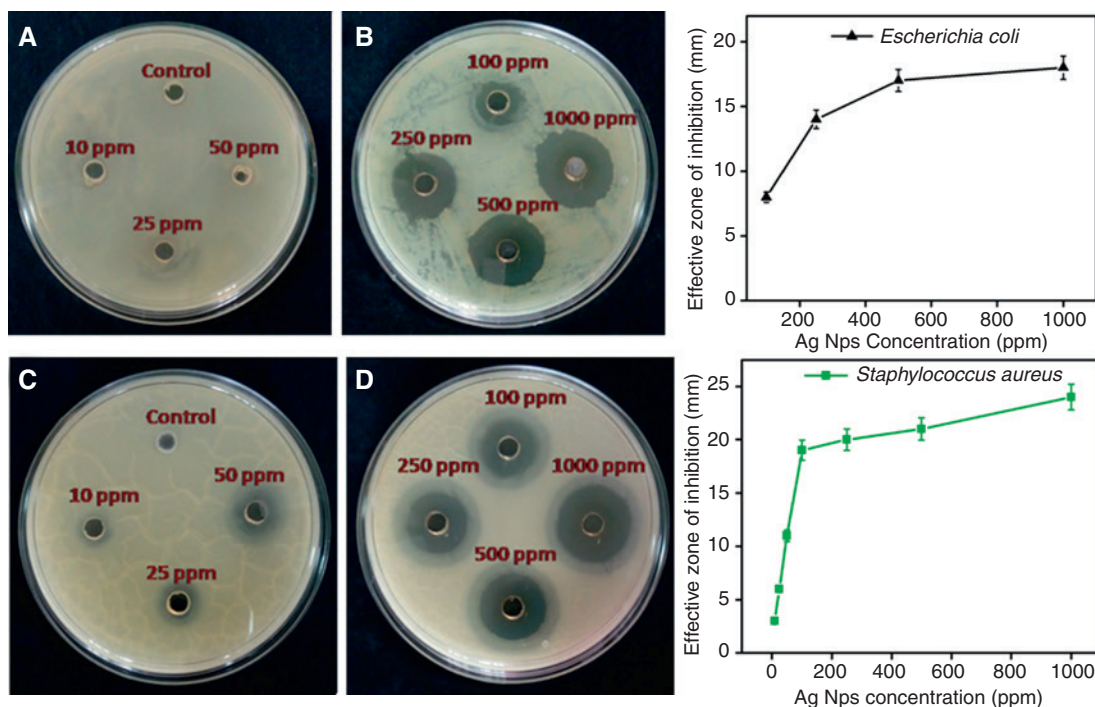


Figure 6: Antimicrobial activity of *Escherichia coli* (A), (B) and *Staphylococcus aureus* (C) and (D) as a function of different concentrations.

interaction between the negatively charged NPs surface and positively charged proteinaceous components of the integral membrane of bacteria [51]. Another reason could be substitution of vital biomolecules by AgNPs which could lead to physicochemical changes in the bacteria. Thirdly, the AgNPs may block the cellular channels and disturb the bacterial osmoregulation, leading to its death. Inside the bacteria, AgNPs may inactivate or disturb the operon system of its genome. Also, the AgNPs have the potential of reactive oxygen species (ROS) generation, which further adds to antimicrobial activity. It has been also reported by some researchers that NPs below 10 nm may release Ag^+ ions, which is also lethal to the microorganisms [52]. To summarize the antimicrobial mechanism, we hypothesized that initial AgNPs-bacterial membrane interact and cause damage to it, followed by their entry into the bacteria where damage is caused to organelles and genome, possibly due to ROS generation and release of Ag^+ ions (Scheme 1). Further studies are needed to explore the above possibilities.

It is seen from the results that the AgNPs synthesized by our method show better antimicrobial activity when compared with AgNPs reported in the literature at the same concentrations. The enhanced antimicrobial activity could be related to the medicinal and antiseptic properties of the *L. leucocephala* plant. The seed is thought to

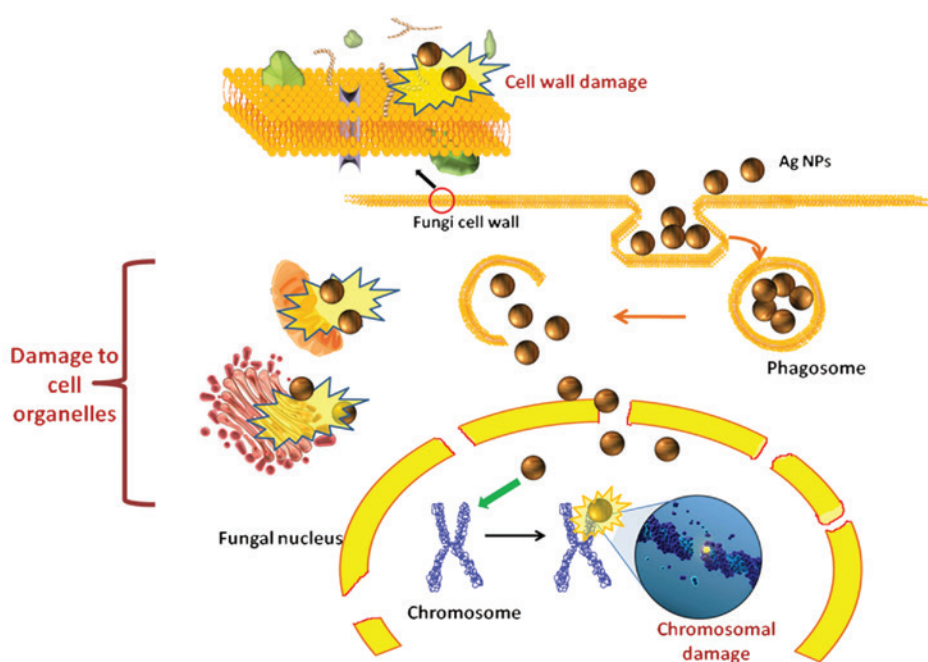
contain many phytochemicals that alone are very effective antimicrobial agents [53, 54].

The AgNPs were tested as antifungal agents against two fungal species – *Phlebiopsis gigantea* and *Echinodontium taxodii*. The pictorial results are shown in Figure 7. The results tabulated in Supplemental Tables 3 and 4 indicate that the AgNPs inhibit the growth of fungal mycelium even at a very low concentration of 10 ppm and 100% inhibition was observed at 100 ppm concentration.

DPPH is a stable radical that combines with other compounds or radicals and starts decolorizing. The results in Figure 8 show the effect of different concentrations of AgNPs on DPPH radical scavenging activity. DPPH accepts hydrogen or electrons from AgNPs and changes into a colorless stable compound. The DPPH radical scavenging activity or AA% is shown in Table 2. It may be seen from the data that AA% of AgNPs tends to increase with increasing the concentration of AgNPs. The maximum AA% observed in AgNPs is 68.91%.

3.6 Sensing of metal ions

The as-synthesized AgNPs were tested for their ability as analytical sensors. Some 3 ml aqueous solution of NPs (25 ppm) was mixed with 0.2 ml of different salts (3 mM) without adjusting the pH and showed visual and



Scheme 1: Diagrammatical representation of expected damage caused to fungi and bacteria by silver nanoparticles (AgNPs) (keeping the fungi cell as reference).

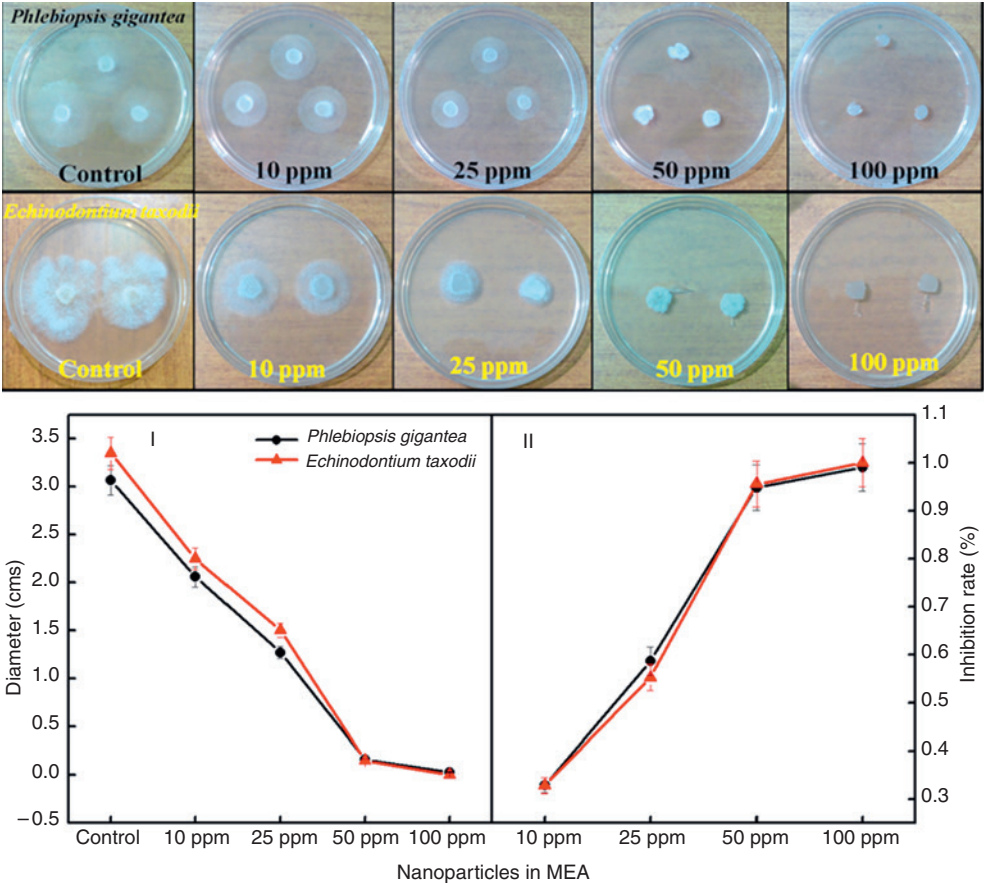


Figure 7: Fungal growth response of *Phlebiopsis gigantea* (black labelled) and *Echinodontium taxodii* (yellow labelled) inoculated on media containing different concentrations of silver nanoparticles (AgNPs) and (I) fungal growth diameter and (II) percentage inhibition of fungal growth (right) with increasing AgNPs concentration.

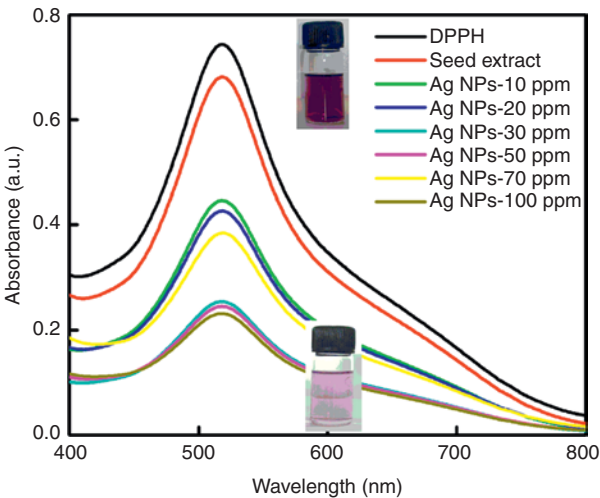


Figure 8: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) extinction responses of plant extract and silver nanoparticles (AgNPs) at different concentrations.

Table 2: Percentage antioxidant activity (AA%) of plant extract and silver nanoparticles (AgNPs).

| Sample | AA% |
|---|-------|
| <i>Leucaena leucocephala</i> seed extract | 8.42 |
| AgNPs (10 ppm) | 39.81 |
| AgNPs (20 ppm) | 42.49 |
| AgNPs (30 ppm) | 48.23 |
| AgNPs (50 ppm) | 65.46 |
| AgNPs (70 ppm) | 66.99 |
| AgNPs (100 ppm) | 68.91 |

AgNPs, silver nanoparticles.

spectroscopic sensitivity towards iron (Fe^{3+}) (Figure 9). A solution of AgNPs after mixing with ferric chloride showed an intense red color, while the color remained unchanged with other salts. The UV-vis spectra of these samples were recorded and all showed a reduction in characteristic

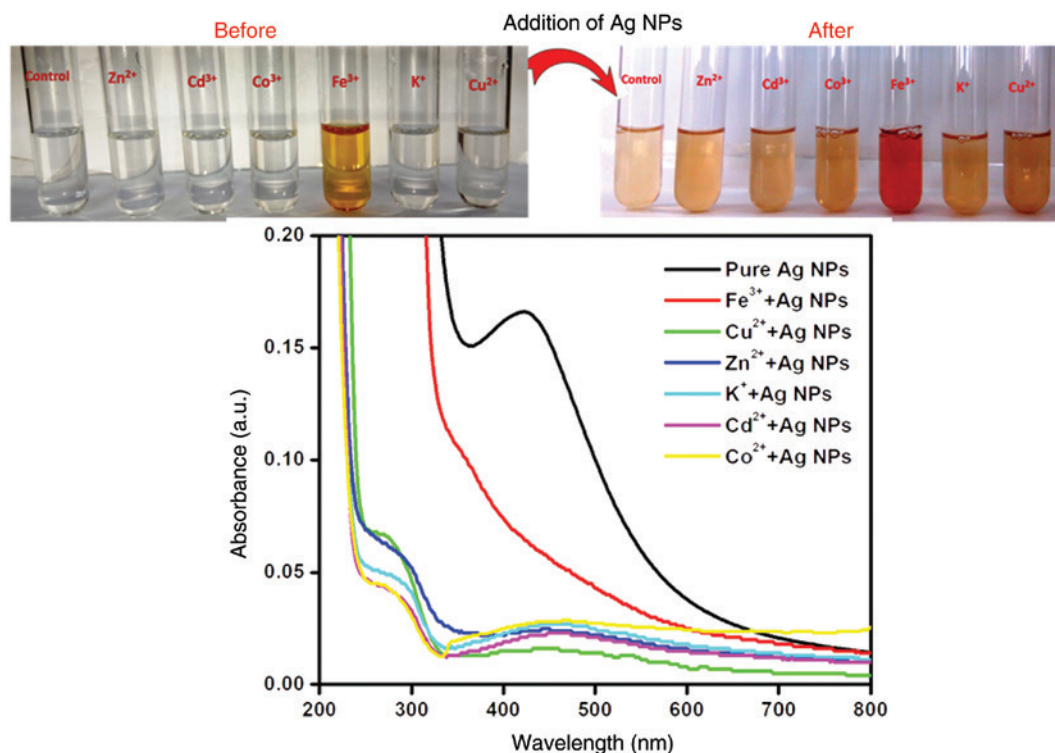


Figure 9: Visual and spectroscopic sensing of different metal ions by silver nanoparticles (AgNPs).

peak of AgNPs. The exception was seen in the case of Fe^{3+} where the Ag peak merged and became a broad shoulder peak. This trial shows the potential of AgNPs in the field of visual and quick sensing. However, further studies need to be considered for quantitative analysis of the sensed metal ion and its selective sensing.

4 Conclusion

In the present study, AgNPs were synthesized using *L. leucocephala* seed extract in deionized water. The method was green, chemical free, easy and cheap. The as-synthesized AgNPs were spherical in shape, well dispersed and stable as confirmed using TEM, SEM, DLS, XRD and UV-vis spectroscopy. The plant extract itself acted as a stabilizing agent, hence no external chemicals were required for capping the NPs. The mode of interaction of the plant extract with the AgNPs surface was studied using FTIR and TGA. The phytochemical screening of the plant extract confirmed the presence of organic natural reducing agents in seed extract. The synthesized NPs exhibited significant antimicrobial and antifungal activity. They also showed excellent AA of 68.91% at as low as 100 ppm concentration. A trial for sensing property

of AgNPs towards common ions showed the extraordinary ability of AgNPs towards Fe^{3+} . This piece of work shows the effectiveness of *L. leucocephala* seed extract as a reducing agent for the synthesis of AgNPs. The seed extract not only stabilized the NPs, but also enhanced its antimicrobial, antifungal and antioxidant properties when compared to previous studies in the literature. It has also been found that the extract did not overshadow the properties of core AgNPs, as it shows selective optical sensing towards Fe^{3+} ions in aqueous solution.

Acknowledgments: Rajeev Kumar is thankful to DST, SERB/F/8171/2015-16 as well as UGC (F. No. 194-2/2016 IC) for providing financial support. Savita Chaudhary is also thankful to DST Inspire Faculty award (IFACH-17) and DST Purse grant II for financial assistance, and Priyanka Sharma is thankful to DST INSPIRE for SRF (IF 140267).

References

- [1] Anastas PT, Kirchhoff MM. *Acc. Chem. Res.* 2002, 35, 686–694.
- [2] Momeni SS, Nasrollahzadeh M, Rustaiyan A. *J. Colloid Interface Sci.* 2016, 472, 173–179.
- [3] Nasrollahzadeh M, Sajadi SM, Hatamifard A. *Appl. Catal. B* 2016, 191, 209–227.

- [4] Nasrollahzadeh M, Atarod M, Jaleh B, Gandomi M. *Ceram. Int.* 2016, 42, 8587–8596.
- [5] Nasrollahzadeh M, Sajadi SM. *J. Colloid Interface Sci.* 2016, 469, 191–195.
- [6] Nasrollahzadeh M, Sajadi SM, Maham M. *J. Colloid Interface Sci.* 2016, 469, 93–98.
- [7] Nasrollahzadeh M, Atarod M, Sajadi SM. *Appl. Surf. Sci.* 2016, 364, 636–644.
- [8] Nasrollahzadeh M. *Tetrahedron Lett.* 2016, 57, 337–339.
- [9] Nasrollahzadeh M, Sajadi SM, Rostami-Vartooni A, Alizadeh M, Bagherzadeh M. *J. Colloid Interface Sci.* 2016, 466, 360–368.
- [10] Atarod M, Nasrollahzadeh M, Sajadi SM. *J. Colloid Interface Sci.* 2016, 465, 249–258.
- [11] Nasrollahzadeh M, Sajadi SM. *J. Colloid Interface Sci.* 2016, 462, 243–251.
- [12] Nasrollahzadeh M, Sajadi SM. *J. Colloid Interface Sci.* 2016, 465, 121–127.
- [13] Nasrollahzadeh M, Sajadi SM. *J. Colloid Interface Sci.* 2016, 464, 147–152.
- [14] Nasrollahzadeh M, Sajadi SM, Rostami-Vartooni A, Hussin SM. *J. Colloid Interface Sci.* 2016, 466, 113–119.
- [15] Sankar S, Sharma SK, Kaur N, Lee B, Kim DY, Lee S, Jung H. *Ceram. Int.* 2016, 42, 4875–4885.
- [16] Rigamonti L, Grosso M, Biganzoli L. *J. Ind. Ecol.* 2012, 16, 748–760.
- [17] Abbas A, Ansumali S. *Bioenergy Res.* 2010, 3, 328–334.
- [18] Adivitiya A, Dagar VK, Devi N, Khasa YP. *Int. J. Biol. Macromol.* 2016, 83, 50–60.
- [19] Zhu X, Yang S, Wang L, Liu Y, Qian F, Yao W, Zhang S, Chen J. *Environ. Pollut.* 2016, 211, 20–27.
- [20] Bar H, Bhui DK, Sahoo GP, Sarkar P, De SP, Misra A. *Colloids Surf. A* 2009, 339, 134–139.
- [21] Ahmed S, Ahmad M, Swami BL, Ikram S. *J. Adv. Res.* 2016, 7, 17–28.
- [22] Vizuete KS, Kumar B, Vaca AV, Debut A, Cumbal L. *J. Photochem. Photobiol. A* 2016, 329, 273–279.
- [23] Pugazhendhi S, Kirubha E, Palanisamy PK, Gopalakrishnan R. *Appl. Surf. Sci.* 2015, 357, 1801–1808.
- [24] Suresh D, Shobharani RM, Nethravathi PC, Pavan Kumar MA, Nagabhushana H, Sharma SC. *Spectrochim. Acta, Part A* 2015, 141, 128–134.
- [25] Huang L, Weng X, Chen Z, Megharaj M, Naidu R. *Spectrochim. Acta, Part A* 2014, 130, 295–301.
- [26] Alvarez RAB, Cortez-Valadez M, Bueno LON, Britto Hurtado R, Rocha-Rocha O, Delgado-Beleño Y, Martinez-Núñez CE, Serrano-Corrales LI, Arizpe-Chávez H, Flores-Acosta M. *Physica E (Amsterdam, Neth.)* 2016, 84, 191–195.
- [27] Tajbakhsh M, Alinezhad H, Nasrollahzadeh M, Kamali TA. *J. Alloys Compd.* 2016, 685, 258–265.
- [28] Rostami-Vartooni A, Nasrollahzadeh M, Alizadeh M. *J. Alloys Compd.* 2016, 680, 309–314.
- [29] Rostami-Vartooni A, Nasrollahzadeh M, Alizadeh M. *J. Colloid Interface Sci.* 2016, 470, 268–275.
- [30] Hatamifard A, Nasrollahzadeh M, Sajadi SM. *New J. Chem.* 2016, 40, 2501–2513.
- [31] Atarod M, Nasrollahzadeh M, Sajadi SM. *J. Colloid Interface Sci.* 2016, 462, 272–279.
- [32] Saravanakumar A, Ganesh M, Jayaprakash J, Jang HT. *J. Ind. Eng. Chem.* 2015, 28, 277–281.
- [33] Rajan A, Vilas V, Philip D. *J. Mol. Liq.* 2015, 207, 231–236.
- [34] Ramar M, Manikandan B, Raman T, Arunagirinathan K, Prabhu NM, Basu MJ, Perumal M, Palanisamy S, Munusamy A. *Spectrochim. Acta A* 2015, 138, 120–129.
- [35] Nasrollahzadeh M, Sajadi SM. *Ceram. Int.* 2015, 41, 14435–14439.
- [36] Venkateswarlu S, Natesh Kumar B, Prathima B, Anitha K, Jyothi NVV. *Physica B.* 2015, 457, 30–35.
- [37] Alshehri H, Jakubowska M, Młodziński A, Horaczek M, Rudka D, Free C, Carey JD. *ACS Appl. Mater. Interfaces* 2012, 4, 7007–7010.
- [38] Heinonen S, Huttunen-Saarivirta E, Nikkanen JP, Raulio M, Priha O, Laakso J, Storgards E, Levanen E. *Colloids Surf. A* 2014, 453, 149–161.
- [39] Jiang ZJ, Liu CY, Sun LW. *J. Phys. Chem. B* 2005, 109, 1730–1735.
- [40] Abdel-Aziz MS, Shaheen MS, El-Nekeety AA, Abdel-Wahhab MA. *J. Saudi Chem. Soc.* 2014, 18, 356–363.
- [41] Smekalova M, Aragon V, Panacek A, Pucek R, Zboril R, Kvitek L. *Vet. J.* 2016, 209, 174–179.
- [42] Jones RJ. *World Anim. Rev.* 1979, 31, 13–23.
- [43] Hassan RA, Tawfik WA, Setta LMA. *Afr. J. Tradit. Complementary Altern. Med.* 2014, 11, 67–72.
- [44] Chou H, Kuo YL. *J. Chem. Ecol.* 1986, 12, 1431–1448.
- [45] Chai TT, Ooh KF, Ooi PW, Chue PS, Wong FC. *Bot. Stud.* 2013, 54, 8–13.
- [46] Sastry MS, Singh R. *Indian J. Anim. Sci.* 2008, 78, 251–253.
- [47] Afza N, Kalhor MA, Khan RA, Anwar MA. *Pak. J. Pharmacol.* 2007, 24, 13–16.
- [48] Nehdi A, Sbihi H, Tan CP, Al-Resayes SI. *Ind. Crops Prod.* 2014, 52, 582–587.
- [49] Subbaiya R, Lavanya RS, Selvapriya K, Selvam MM. *Int. J. Curr. Microbiol. App. Sci.* 2014, 3, 600–606.
- [50] Ankamwar B, Kamble V, Sur UK, Santra C. *Appl. Surf. Sci.* 2016, 366, 275–283.
- [51] Agnihotri S, Mukherji S, Mukherji S. *RSC Adv.* 2014, 4, 3974–3983.
- [52] Boopathi S, Gopinath S, Boopathi T, Balamurugan V, Rajeshkumar R, Sundararaman M. *Ind. Eng. Chem. Res.* 2012, 51, 5976–5985.
- [53] Mohammed R, Souda S, Taie H, Moharam ME, Shaker K. *J. Appl. Pharm. Sci.* 2015, 5, 138–147.
- [54] Aderibigbe SA, Adetunji OA, Odeniyi MA. *Afr. J. Biomed. Res.* 2011, 14, 63–68.

Supplemental Material: The online version of this article offers supplementary material (DOI 10.1515/gps-2016-0146).

Bionotes



Rajeev Kumar

Rajeev Kumar obtained his PhD degree from the Department of Chemistry, Panjab University, Chandigarh in 2010 and since then

has worked as an assistant professor in the Department of Environment Studies, Chandigarh. His main research areas include organo-metallic and nano chemistry. He also focuses on bioremediation of the environment with the help of fungi. He has over 15 publications and more than 150 citations; his h index=6 and his i10 index=4.



Priyanka Sharma

Priyanka Sharma works at the Department of Environment Studies, Panjab University, Chandigarh as a senior research fellow. Priyanka obtained her Master's in Environment Science from Panjab University. Her primary research areas include nanotechnology in relation to the environmental remediation. In particular, she is focused on the synthesis, characterization and environmental applications of metallic nanoparticles (NPs).



Aditi Bamal

Aditi Bamal graduated from Delhi University, India and went on to the Department of Environment Studies, Panjab University, Chandigarh. During her Master's, she started her research work with Dr. Rajeev Kumar. Aditi Bamal's interests include sustainable and green chemistry based on chemical free synthesis of NPs and their role in environmental remediation.



Sushma Negi

Sushma Negi is a Junior Research Fellow in the Department of Environment Studies, Panjab University. She completed her Master's in Environment Science. Her interest areas are isolation and identification of indigenous fungi and their physiological studies. She also works in the bioremediation of various industrial pollutants using indigenous fungi.



Savita Chaudhary

Savita Chaudhary is currently working as an assistant professor in the Department of Chemistry, Panjab University, Chandigarh. She received her Hons. School BSc and MSc degrees in Chemistry from Panjab University, Chandigarh and obtained her PhD degree in 2010 with specialization in the metallic and semiconducting NPs synthesis and characterization. She has published more than 50 publications in peer reviewed journals and reviewed for many international SCI journals. She has 427 citations to her credit, an h index value of 11 and an i10 index of 11.