www.amlett.com, www.vbripress.com/aml, DOI: 10.5185/amlett.2014.4563 Published online by the VBRI press in 2014

# Green syntheis of silver nanoparticles and its activity on SiHa cervical cancer cell line

**Anal K. Jha, K. Prasad***

*Aryabhatta Centre for Nanoscience and Nanotechnology, Aryabhatta Knowledge University, Patna 800001, India*

*Corresponding author. Tel: (+91) 9431690360; Fax: (+91) 6122351919; E-mail: k.prasad65@gmail.com

**Received: 13 April 2014, Revised: 12 July 2014 and Accepted: 16 July 2014**

## ABSTRACT

Biosynthesis and characterizations of nanoparticles has become an important branch of nanotechnology nowadays. In this paper, green synthesis of silver nanoparticles (AgNPs) using the alcoholic extract of *Argemone mexicana* Linn. as a reducing and stabilizing agent, has been discussed. This biosynthetic method is simple, cost-effective and reproducible. Formation of AgNPs was established by X-ray diffraction, transmission electron microscopy and UV-Visible spectroscopic techniques. Nanoparticles almost spherical in shape having a size of 2-6 nm are found. UV-visible study revealed the surface plasmon resonance at 414 nm. A possible involved mechanism for the biosynthesis of silver nanoparticles has also been proposed. Further, it was found that AgNPs sol when applied to the SiHa cancer cell line was found to inhibit the growth by 70-80%. It is cumulative effect of the unutilized plant extract and nanosilver. The work signifies the importance of medicinal plants in synthesis of nanomaterials as it bestows double benefit in terms of drug delivery as well as safety. It may open a fresh avenue in future cancer therapeutics. 

**Keywords:** Nano-silver; nanoparticle; nanobiotechnology; microstructure; cancer cells; green synthesis.

**Anal K. Jha**, M.Sc., M.Phil.(Cantab), Ph.D. is working as Assistant Professor in Aryabhatta Centre for Nanoscience and Nanotechnology, Aryabhatta Knowledge University, Patna, India He is on lien from T.M. Bhagalpur University, Bhagalpur. He has published more than 40 papers in reputed scientific journals and book chapters on Nanotechnology. His current research activities are concerned with the Natural Product Chemistry and biologically assisted synthesis of different nanomaterials for different biomedical applications. He is an active referee of various international journals. He has presented papers in many national/international conferences and delivered invited lectures.

**K. Prasad**, Ph.D., is Professor and Head at the Aryabhatta Centre for Nanoscience and Nanotechnology, Aryabhatta Knowledge University, Patna, India. He also served SLIET, Longowal, Punjab, T.M. Bhagalpur University, Bhagalpur and Central University of Jharkhand, Ranchi, India. His current research interests include synthesis and characterizations of eco-friendly ferroelectric/piezoelectric ceramics and ceramic-polymer composites and advanced nanomaterials for different industrial applications. He has authored/co-authored over 150 publications including a book, three book chapters and six XRD-reference data in ICDD, USA. He has presented papers at major national and international conferences and delivered invited lectures. Dr. Prasad has successfully guided six Ph.D. students. He is an active referee of various international journals and is a member of several professional bodies.

## Introduction

There is a prevailing belief in India that every plant in our ambience has a medicinal property and it is widely supported by many ancient Indian mythological literatures. Recent times have witnessed an up surge in plant or plant part (including fruits) mediated synthesis of variety of nanoparticles as it is a simple and economical procedure [**1–23**]. Plants are safe to handle and ensure a genuinely green option for synthesis of nanoparticles. They harbour many promising metabolites in order to circumvent the different environmental and pathogenic assaults which have been delved to bear medicinal properties. *Argemone mexicana* linn. known as mexican poppy, is a very common herbaceous weed in India. It belongs to the family *Papaveraceae* and bears medicinal properties. The plant is quite rich in isoquinoline alkaloids. The fresh latex contains protein dissolving constituents and is used externally to treat warts, tumours and cancer. Latex contains alkaloid berberine (0.74%), protopine (0.36%) and free amino acids. Sanguinarine is the toxic factor in seeds causing dropsy [**24-26**].

The treatment of cancer is quite an agony for the patients as the treatment causes many side effects which sometimes lead to death. Nanomedicine has revolutionized the procedure and has to scale greater heights in days to come. Employing plants with reported medicinal and/or anticancer properties is quite advantageous in the sense that in one hand they negotiate the nano synthetic cues while the

 

unused metabolites further enhance its therapeutic efficacy. Taking this novel idea in mind; in the present investigation, we have reported synthesis of silver nanoparticles (abbreviated hereafter as AgNPs) applying bionanotechnological procedure by utilizing a common medicinally promising weed, *A. mexicana* and its application on cervical cancer cell line SiHa with an approach to suggest a herb based nano-fortified cure for cancer having no and/or least side effects. The procedure is highly reproducible, economical and safe for future scaling up and may open a new chapter in cancer treatment in future.

## Materials and methods

*Synthesis of silver nanoparticles*

Prior to commencing the experiment, *A. maxicana* plant was collected from a clean area and was taxonomically authenticated. Leaves were then collected and prickles were removed with the help of a sterilized scissor and rinsed by deionized water. These leaves were thoroughly washed, dried and cut into fine pieces. Thereafter, leaf extract was prepared by taking 25 gm of leaf and 100 mL of 50% ethanol in a 250 mL Erlenmeyer flask and this mixture was boiled for 15 min before decanting till the colour changes from clear transparent to light straw indicating extraction. The leaf mass was pressed by wrapping in serene cloth and 50 mL extract was collected under laminar flow. It was doubled in volume by adding 50% ethanol and was treated as source extract.

For reduction of silver ions, 5 mL of *A. maxicana* leaf extract was added drop-wise into 25 mL of 0.025 molar aqueous solution of $AgNO_3$ (E. Merck, Mumbai, India, purity: 99.95%) with constant stirring at 50–60ºC on steam bath for 20 minutes. As soon as, *A. maxicana* extract was mixed in aqueous solution of silver ion, it starts to change color from deep straw to yellowish brown and finally black due to excitation of surface plasmon resonance which indicated the formation of silver nanoparticles. Once the synthesis is complete, the nanoparticles settle down leaving faint straw supernatant at the top (inset Fig. 4). The synthesized nanoparticles by *A. maxicana* leaf extract were centrifuged at 5500 rpm for 15 min and subsequently re-dispersed in de-ionized water to get rid of any uncoordinated biological molecules.

*Characterizations*

The X-ray diffraction (XRD) pattern of AgNPs was recorded to check the formation of single-phase nature with XPERT-PRO, PW3050/60X-ray diffractometer at room temperature using $CuK_\alpha$ radiation ($\lambda$ = 1.5406Å) over a range of Bragg angles 35° to 85°. The $2\theta$ *vs*. intensity data obtained from this experiment were plotted with the WinPLOTR program and the angular positions of the peaks were obtained with the same program. The dimension of the unit cell, *hkl* values and space group was ascertained using a program TREOR in the Full Prof 2000 software and then refinement was carried out through the profile matching routine of FullProf. The Bragg peaks were modeled with pseudo-Voigt function and the background was estimated by linear interpolation between selected background points. The average crystallite size ($D$) and the lattice strain of AgNPs were estimated by analyzing the broadening of XRD peaks, using Williamson-Hall approach: $B\cos\theta = (K\lambda/D) + 2(\Delta\xi/\xi)\sin\theta$, where $B$ is diffraction peak width at half intensity (FWHM) and $\Delta\xi/\xi$ is the lattice strain and $K$ is the Scherrer constant (0.89). The term $K\lambda/D$ represents the Scherrer particle size distribution. The transmission electron microscope (TEM) image of AgNPs was obtained using a Bruker high resolution transmission electron microscope operated at an accelerated voltage of 200 keV. The absorption spectrum was obtained by a computer interfaced UV-visible spectrophotometer (Hitachi U-2800, Japan).

*Cell lines and culture conditions for the study anticancer property*

Human cervical cancer cell line (SiHa) was obtained from National Centre for Cell Sciences (NCCS) Pune, India. The cell line was grown as monolayer culture in Dulbecco's modified Eagle's medium containing 10% foetal bovine serum and antibiotics (100 units/ml penicillin and 100 mg L-1 streptomycin) in a humidified atmosphere of 5% $CO_2$ at 37°C in T-75 flasks and was sub cultured twice a week.

*Cytotoxicity assay*

Cytotoxicity of AgNPs was evaluated by the assessment of its in-vitro impact on the human cervical cancer cells. Cell counts of $1\times10^4$cells/well were seeded in plate containing 96 wells (50µl/well). After 24 hrs cells were treated with different concentrations of AgNPs (0-100 µg/ml) for 72 hrs. After 72 hrs medium was removed and cells were incubated with 20µl of MTT (5mg/ml in PBS) in fresh medium for 4 hrs at 37°C. Farmazon crystals, formed by mitochondrial reduction of MTT, were stabilized in DMSO (150µl/well) and absorbance was read at 570 nm after 10 min incubation on the *i*Mark Microplate Reader (BioRad, USA). Percentage of cytotoxicity was calculated as a fraction of control. $1\times10^6$ SiHa cells were plated in 30 mm culture plate. When the cells reached approximately 70% confluency, cells were treated with AgNPs and incubated for 48 hrs. After treatment, cells morphology was analyzed under the Motic inverted Microscope.

## Results and discussion

The crystalline nature of AgNPs was confirmed from XRD analysis. Rietveld refinements of the XRD data of AgNPs were carried out. The observed, calculated and difference XRD profiles of *A. maxicana* leaf extract negotiated AgNPs is presented in **Fig. 1**. It can be seen that the profiles for observed and calculated one are perfectly matching ($\chi^2$ = 0.0532). The profile fitting procedure adopted was minimizing the $\chi^2$ function. Five peaks of XRD data were assigned to the diffraction planes: {111}, {200}, {220}, {311} and {222} of face-centered cubic (*fcc*) silver. The unit cell edge was estimated to be 4.0783 Å with the space group of *Pm*3*m* (221) in Hermann-Mauguin symbol, which is in excellent agreement with the literature report (PCPDF No. #89-3722). A little difference of 0.0067 Å between the cell parameter of bulk and nanoparticles has been observed. Consequently, a lowering

in unit cell volumes from 68.19 $Å^3$ (for bulk Ag) to 67.8321$Å^3$ (for AgNPs) has been noticed which could be due to the nanosizing effect. Inset of **Fig. 1** illustrates the Williamson-Hall plot for AgNPs. A linear least square fitting to $B\cos\theta$–$\sin\theta$ data yielded the values of average crystallite size to be 14 nm.

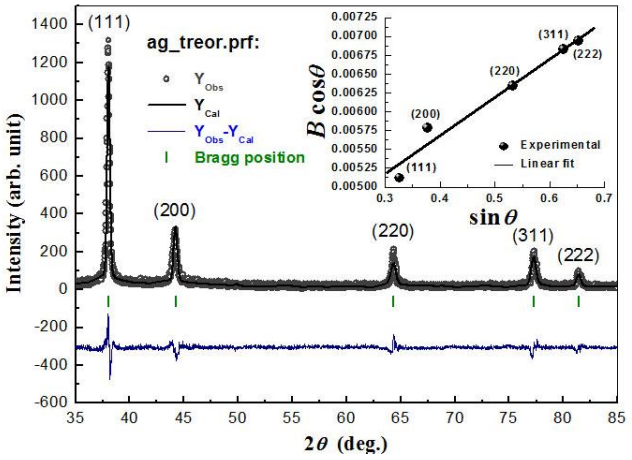


**Fig. 1**. Indexed Rietveld refined XRD pattern of silver nanoparticles using *Argemon maxicana* leaf extract in the space group *Pm*3*m*(221). Inset: Williamson-Hall plot.

**Fig. 2** illustrates typical TEM image of AgNPs synthesized after reduction of $AgNO_3$ with *A. maxicana* leaf extract. The micrographs clearly show individual nanoparticles which are almost spherical in shape. The sizes of particles are found to be in the range of 2 to 6 nm.

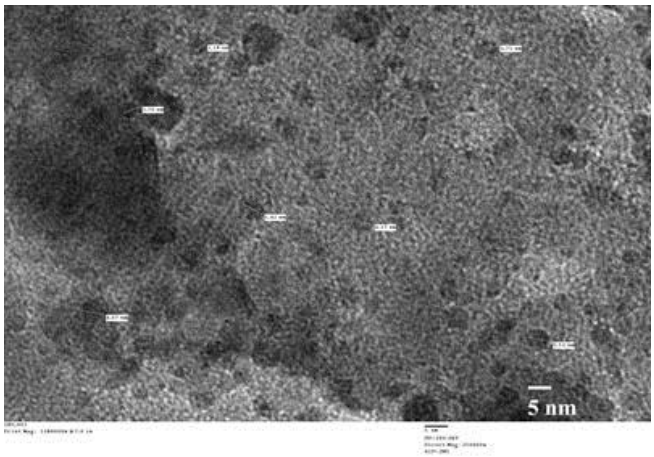


**Fig. 2.** TEM micrograph of silver nanoparticles synthesized using *Argemon maxicana* leaf extract.

UV-vis spectroscopy is an important technique to ascertain the formation and stability of nanoparticles in aqueous solution. **Fig. 3** shows the UV-vis spectrum recorded for AgNPs. It is well known that colloid of AgNPs exhibits lovely straw-yellow color which arises due to excitation of surface plasmon vibrations. The surface plasmon resonance was observed in the range of visible region at 414 nm. Also, the plasmon bands are broadened with an absorption tail in the longer wavelengths, which could be due to the size distribution of the particles. This observation supports the distribution of particle sizes observed in TEM image (**Fig. 2**). Besides, the biosynthesized AgNPs was laid aside at room temperature in the laboratory and inspected after three months. There was obviously no observed aggregation in the solution, and its absorption value showed almost the same. This simply suggested that the AgNPs synthesized by *A. maxicana* bears very good stability which might be due to encapsulation by the different proteins present in the leaf parenchyma.

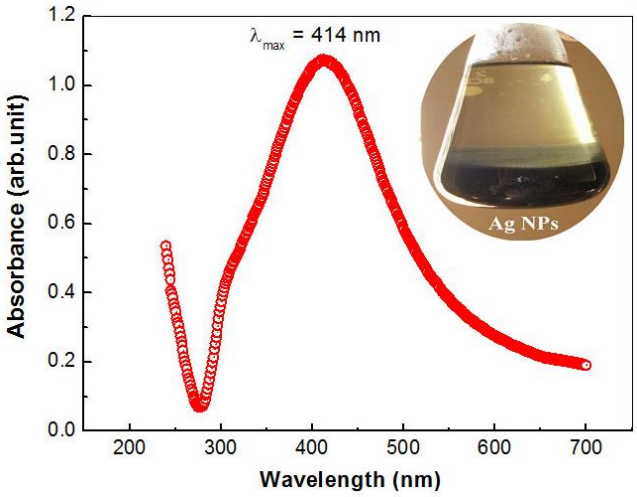


**Fig. 3.** UV-vis spectrum of Ag-NPs synthesized using *Argemon maxicana* leaf extract. The inset shows AgNPs as deposit and above which is colloids of AgNPs in *Argemon maxicana* leaf extract medium.

Plants, whether wild or cultivated have their own weaponry (Primary or Secondary metabolites) against environmental stresses. Phytochelatins (PCs) are naturally occurring peptides found in plants and fungi. PCs have a general structure of (g-Glu-Cys-)n-Gly (n¼1–11) and have been shown to chelate heavy metals with higher affinity and binding capacity than Metallothioneins (MTs). PCs are synthesized by phytochelatin synthase (PCS) via the transfer of g-Glu-Cys from glutathione (GSH) to another GSH or other PCs and can be easily fine-tuned for high-level production [**27-29**]. MTs are the other low molecular weight proteins which bind heavy metals and are found throughout the animal and plant kingdoms. These proteins also play an important role in detoxification by sequestering metals in plant cells. Plants have been found to contain a number of genes encoding MT-like proteins having sequence similarity to animal MT proteins. Treatment of plants with Cu was found to induce MT mRNAs, notably in tissues where the normal level of expression is low [**30,31**]. These are triggered against any stress as primary response from plants.

*A. maxicana* is quite rich in many secondary metabolites ranging from alkaloids, amino acids, phenolics to fatty acids. Several isoquinoline alkaloids *viz.* cheilanthifoline, berberine, coptisine, cryptopine, uramine, protopine, scoulerine, sanguinarine, stylopine and thalifoline have been reported from the plant. The synthesis of AgNPs might have resulted due to other primary metabolites (like organic acids, fatty acids and quinones in this case) or metabolic fluxes and other oxido-reductively labile metabolites like ascorbates or catechol/ protocatacheuic acid *etc.* [**32, 33**]. Quick transformation

  

and appreciable reduction of particle size might have taken place as a cumulative effect of all the above mentioned factors. The probable biosynthetic mechanism is detailed in **Fig. 4**.

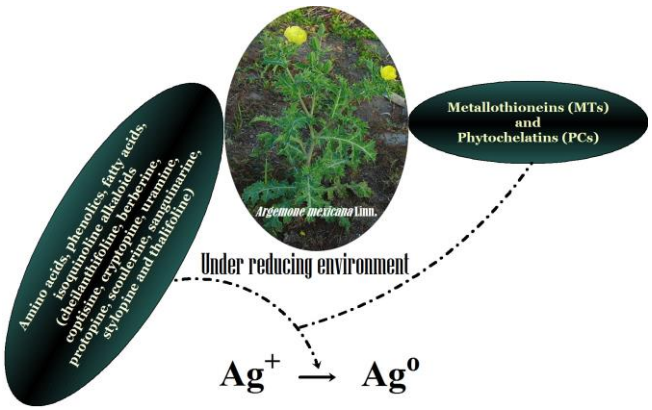


**Fig. 4**. Schematics for the biosynthesis of AgNPs using *Argemon maxicana* leaf extract.

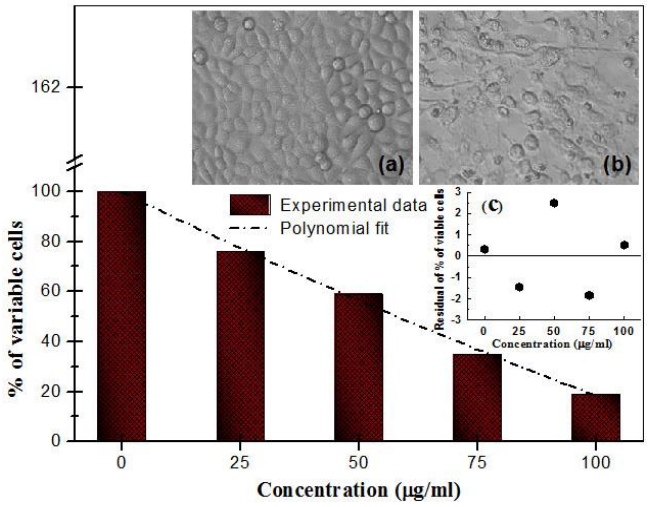


**Fig. 5**. Dose-dependent effect of *Argemon maxicana* leaf extract mediated silver nanoparticles on SiHa cervical cancer cell viability (%). Inset: **(a)** Monolayer culture of SiHa cervical cancer cells (Control), **(b)** with 100 µg/ml AgNPs and **(c)** Residue plot.

**Fig. 5** illustrates the MTT assay of AgNPs on SiHa cervical cancer cell line. It is observed that with an increase in the dose of *A. maxicana* mediated AgNPs the cell viability (%) decreases appreciably. A second order polynomial fitting to concentration ($x$) dependent SiHa cell viability data yielded a relation: $y = 98.68571 - 0.98486x + 0.00103x^2$ with $r^2 = 0.99415$. **Fig. 5c** (inset) shows the residue plot. The (untreated) control of SiHa cells (**Fig. 5a**) appeared to be mostly elliptical, closely aligned or associated with each other and growing well. Further, the SiHa cells treated with AgNPs have altered morphology these cells got constricted, fragmented, and round-shaped, detached from the base and floated in their tissue culture media (**Fig. 5b**). Morphological changes caused by the AgNPs treatment were quite significant. As observed in the present preliminary investigation the degree of inhibition of SiHa cells is quite encouraging and is up to 70-80% for the application of 100 µg/ml. The ethanol extract of *A. mexicana* was reported to exhibit inhibitory activity against different human cancer cell lines such as Hela-B75 (48%), HL-60 (20.15%) and PN-15 (58.11%) [**34**], while in another work *in vitro* cytotoxicity of *A. mexicana* against different human cancer cell lines had been studied [**35**]. Compared to earlier studies involving this plant, the present investigation is quite significant and this has motivated us to take this work further for lower mammalian trials and our effort in that direction is on. Reduction in number of cancer cells is quite encouraging and may be considered a significant step towards cancer chemotherapy rather it has potential to change its face from painful chemotherapy to a highly amenable natural nanotherapy. If applied in future, it can ensure a drastic reduction in the number of patient mortality caused by conventional procedures. Additionally, we are taking use of a common roadside weed to synthesize the nanomaterial for medical applications this may further add to the importance of the plant and might lead to its effective utilization as well as subsequent future conservation.

## Conclusion

The present biosynthesis method is a green low cost approach, capable of producing silver-nanoparticles in laboratory ambience. The synthesis of AgNPs might have resulted due to different metabolites (like organic acids, fatty acids and quinones) or metabolic fluxes and other oxido-reductively labile metabolites like ascorbates or catechol/protocatacheuic acid along with different alkaloids. Present investigation has an additional advantage in the sense that it suggests an effective utilization of a commonly ignored medicinally promising weed, *A. maxicana* in synthesizing nanoparticles like silver, consequently addressing the environmental issues. Further, an encouraging response in terms of SiHa cervical cancer cell line inhibition might have occurred due to AgNPs and other metabolites present in the plant. Inhibition of cancer cells is quite encouraging and may be considered a significant step towards cancer chemotherapy. If applied suitably and methodically in future, it can ensure a drastic reduction in the number of patient mortality caused by conventional procedures.

### Reference


1. Mie, R.; Samsudin, M.W.; Din, L.B.; Ahmad, A.; Ibrahim, N.; Adnan, S.N.A.; *International Journal of Nanomedicine*, **2014**, *9*, 121. **DOI:** 10.2147/IJN.S52306
2. Rai, V.; Yadav, A.; *IET Nanobiotechnology*, **2013**, *7*, 117. **DOI:** 10.1049/iet-nbt.2012.0031
3. Jha, A.K.; Prasad, K.; *International Journal of Green Nanotechnology: Physics and Chemistry*, **2012,** *4*, 219. **DOI:** 10.1080/19430892.2012.706070
4. Umashankari, J.; Inbakandan, D.; Ajithkumar, T.T.; Balasubramanian, T.; *Saline Systems*, **2012**, *8*, 11. **DOI:** 10.1186/2046-9063-**8**-**11**
5. Aromal, S.A.; Babu, K.V.D.; Philip, D.; *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **2012**, *96*, 1025. **DOI:** 10.1016/j.saa.2012.08.010
6. Jha, A.K.; Prasad, K.; *International Journal of Green Nanotechnology: Physics and Chemistry*, **2011,** *3*, 92. **DOI:** 10.1080/19430892.2011.574560
7. Jha, A.K.; Prasad, K.; *Digest Journal of Nanomaterials and Biostructure*, **2011**, *6*, 1717. **ISSN:** 1842-3582
8. Jha, A.K.; Kumar, V.; Prasad, K.; *Journal of Bionanoscience*, **2011**, *5*, 162.

**DOI:** 10.1166/jbns.2011.1053
9. Zhan, G.; Huang, J.; Lin, L.; Lin, W.; Emmanuel, K.; Li, Q.; *Journal of Nanoparticle Research*, 2011, *13*, 4957. **DOI:** 10.1007/s11051-011-0476-y
10. Kumar, V.G.; Gokavarapu, S.D.; Rajeswari, A.; Dhas, T.S.; Karthick, V.; Kapadia, Z.; Shrestha, T.; Barathy, I.A.; Roy, A.; Sinha, S.; *Colloids and Surfaces B: Biointerfaces*, **2011,** *87*, 159. **DOI:** 10.1016/j.colsurfb.2011.05.016
11. Sangeetha, G.; Rajeshwari, S.; Venckatesh, R.; *Materials Research Bulletin*, **2011,** *46*, 2560. **DOI:** 10.1016/j.materresbull.2011.07.046
12. Prathna, T.C.; Chandrasekaran, N.; Raichur, A.M.; Mukherjee, A.; *Colloids and Surfaces B: Biointerfaces*, **2011**, *82*, 152. **DOI:** 10.1016/j.colsurfb.2010.08.036
13. Philip, D.; Unni, C.; Aromal, S.A.; Vidhu, V.K.; *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **2011**, *78*, 899. **DOI:** 10.1016/j.saa.2010.12.060
14. Santhoshkumar, T.; Rahuman, A.A.; Rajakumar, G.; Marimuthu, S.; Bagavan, A.; Jayaseelan, C.; Zahir, A.A.; Elango, G.; Kamaraj, C.; *Parasitology Research*, **2011**, *108*, 693. **DOI:** 10.1007/s00436-010-2115-4
15. Singhal, G.; Bhavesh, R.; Kasariya, K.; Sharma, A.R.; Singh, R.P.; *Journal of Nanoparticle Research*, *13*, 2981. **DOI:** 10.1007/s11051-010-0193-y
16. Jha, A.K.; Prasad, K.; *International Journal of Green Nanotechnology: Physics and Chemistry*, **2010**, *1*, 110. **DOI:** 10.1080/19430871003684572
17. Tripathy, A.; Raichur, A.M.; Chandrasekaran, N.; Prathna, T.C.; Mukherjee, A.; *Journal of Nanoparticle Research*, **2010**, *12*, 237. **DOI:** 10.1007/s11051-009-9602-5
18. Jha, A.K.; Prasad, K.; Kumar, V.; Prasad, K.; *Biotechnology Progress*, **2009**, *25*, 1476. **DOI:** 10.1002/btpr.233
19. Jha, A.K.; Prasad, K.; Prasad, K.; Kulkarni, A.R.; *Colloids and Surfaces* B: *Biointerfaces*, **2009**, *73*, 219. **DOI:** 10.1016/j.colsurfb.2009.05.018
20. Song, J.Y.; Jang, H.-K.; Kim, B.S.; *Process Biochemistry*, **2009,** *44*, 1133. **DOI:** 10.1016/j.procbio.2009.06.005
21. Haverkamp, R.G.; Marshall, A.T.; *Journal of Nanoparticle Research*, **2009,** *11*, 1453. **DOI:** 10.1007/s11051-008-9533-6
22. Mohanpuria, P.; Rana, N.K.; Yadav, S.K.; *Journal of Nanoparticle Research*, **2008,** *10*, 507. **DOI:** 10.1007/s11051-007-9275-x
23. Jha, A.K.; Prasad, K.; *Journal of Bionanoscience*, **2013**, *7*, 245. **DOI:** 10.1166/jbns.2013.1139
24. Khare, C.P. Indian Medicinal Plants an illustrate Dictionary. Springer, **2007**, 60. **ISBN:** 978-0-387-70637-5
25. Priya, C.L.; Rao, K.V.B.; *International Journal of Pharmaceutical Sciences and Research*, **2012**, *3*, 2143. **ISSN:** 0975-8232
26. Rajvaidhya, S.; Nagori, B.P.; Singh, G.K.; Dubey, B.K.; Desai, P.; Jain, S.; *International Journal of Pharmaceutical Sciences and Research*, **2012**, *3*, 2494. **ISSN:** 0975-8232
27. Mehra, R.K.; Winge, D.R.; *Journal of Cellular Biochemistry*, **1991**, *45*, 30. **DOI:** 10.1002/jcb.240450109
28. Vatamaniuk, O.K.; Mari, S.; Lu, Y.P.; Rea, P.A.; *Proceedings of National Academy of Sciences USA*, **1999**, *96*, 7110. **DOI:** 10.1073/pnas.96.12.7110
29. Kang, S.H.; Singh, S.; Kim, J.Y.; Lee, W.; Mulchandani, A.; Chen, W.; *Applied and Environmental Microbiology*, **2007**, *73*, 6317. **DOI:** 10.1128/AEM.01237-07
30. Zenk, M.H.; *Gene*, **1996**, *179*, 21. **DOI:** 10.1016/S0378-1119(96)00422-2
31. Memon, A.R.; Aktoprakligül, D.; Zdemür, A.; Vertii, A.; *Turkish Jurnal of Botany*, **2001**, *25*, 111.
32. Bhattacharjee, I.; Chattarjee, S.K.; Chattarjee K.; Chandra, G.; *Memórias do Instituto Oswaldo Cruz*, **2006**, *101*, 645.
33. Brahmachari, G.; Gorai, D.; Roy, R.; *Revista Brasileira de Farmacognosia - Brazilian Journal of Pharmacognosy*, **2013**, *23*, 559. **DOI:** 10.1590/S0102-695X2013005000021
34. Gacche, R.N.; Shaikh, R.U.; Pund, M.M.; *Asian Journal of Traditional Medicines*, **2011**, *6*, 127.
35. Verma, S.K.; Singh, S.K.; Singh, S.; Mathur, A.; *International Journal of Chemical, Environmental and Pharmaceutical Research*, **2010**, *1*, 37.



  