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Biosynthesis of silver nanoparticles using murraya koenigii (curry leaf): An investigation on the effect of broth concentration in reduction mechanism and particle size

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ABSTRACT

Biological synthesis of silver nanoparticles using *Murraya koenigii* leaf extract was investigated and the effect of broth concentration in reduction mechanism and particle size is reported. The rapid reduction of silver (Ag^+) ions was monitored using UV-visible spectrophotometry and showed formation of silver nanoparticles within 15 minutes. Transmission electron microscopy (TEM) and atomic force microscopy (AFM) analysis showed that the synthesized silver nanoparticle are varied from 10-25 nm and have the spherical shape. Further the XRD analysis confirms the nanocrystalline phase of silver with FCC crystal structure. From this study, it was found that the increasing broth concentration increases the rate of reduction and decreases the particle size. Copyright © 2011 VBRI press.

Keywords: Silver nanoparticles; biosynthesis; *murraya koenigii*; leaf extract.

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Introduction

Silver nanoparticles (AgNPs) have become the focus of much research interest due to their wide variety of applications [**1**]. Their ability to alter the physical, optical and the electronic properties of compounds [**2**] have found applications in various fields including electronic devices [**3**], chemical/biological sensing [**4**], and surface enhanced

Raman spectroscopy [**5**]. In contrast, a promising usage of AgNPs as antimicrobial agent is well known and has already found applications in antimicrobial paint coatings [**6**], textiles, water treatment, medical devices [**7**], and HIV prevention as well as treatment [**8**]. Traditional chemical methods of synthesizing silver nanoparticles include the use of ethylene glycol [**9, 10**], pyridine [**11**], and sodium borohydride [**12**]. The chemicals used in these methodologies can be toxic and highly reactive posing a risk to the environment and humans, or the procedures are too expensive to be feasible at an industrial scale. Therefore there has been a search for inexpensive, reliable, safe, and "green" approach to the synthesis of stable metal nanoparticles with controlled size and shape. As the result, some novel methods have recently developed using (i) biologically derived reducing agents such as chitosan [**13**], glucose [**14**] and polysaccharides [**15**], (ii) microbes such as bacteria and fungus [**16, 18**], and (iii) a variety of plant (seed, leaf as well as tuber) extracts [**19-23**] for the synthesis of metal nanoparticles. Among them, plant leaf extract mediated biological process has been widely investigated due to their inexpensive and simple protocol.

We have recently reported the successful synthesis of silver nanoparticles and their impregnate in carbon nanotubes using soy leaf extract [**24, 25**]. We have also explored various plant leaf extracts for the bioreduction of metal ions including silver, gold and palladium. A promising result was obtained for the *Murraya koenigii* (curry leaf, depicted in **Fig. 1a**) extract that actively reduces the silver ions in to silver nanoparticles. Recently, Philip et. al also reported the rapid synthesis of silver and gold nanoparticles using Murraya koenigii leaf extract [**26**]. Curry leaf is well known as a spice but has also been used in traditional medicine as a treatment for a variety of ailments [**27**]. Curry leaf has recently been found to be a potent antioxidant due to high concentrations of carbazoles [**27-30**], a water soluble heterocyclic compound which has been reported by Rai et al. [**31**] may be responsible for the reduction and stabilization of metal ions. Detailed investigation is necessary in order to extend the reduction mechanism of *Murraya koenigii* leaf extract for further applications. Hence, the present manuscript deals with the investigation on the effect of *Murraya koenigii* leaf broth concentration in reduction mechanism of silver ions in to silver nanoparticles and their size.

Experimental

Broth extraction

The curry leaf extract was prepared with 10g of fresh curry leaves, obtained from East Indian Food & Spices in Guelph, ON, which were thoroughly rinsed with deionized water and cut into small pieces. The chopped leaves were boiled in 75mL of deionized water for 3 minutes. The leaf broth was then cooled and filtered yielding 50mL of broth. The colour and translucency of the broth can be seen in **Fig. 1b** and it was stored in a refrigerator.

Synthesis of silver nanoparticles

Silver nitrate ($AgNO_3$) was obtained from Sigma *Aldrich* and used as received without further purification. 5mL curry leaf broth was added to 100mL 10^{-3} M silver nitrate and allowed to react at ambient conditions. The observed colour change of reaction mixture from transparent yellow to dark brown indicates the formation of silver nanoparticles. Further the reduction of the Ag^+ ions was monitored over time by UV-visible spectral analysis. The suspension of silver nanoparticles was allowed to settle and the excess liquid was removed. The particles were then rinsed to remove any organic residue and re-suspended in 95% ethanol (Fisher scientific) for further characterization.

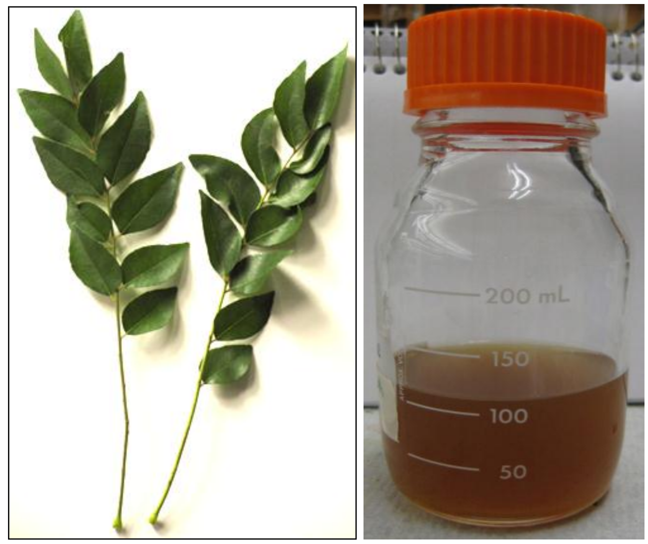

Fig. 1. Photograph of Murraya koenigii leaf bunch (curry leaf) and the extracted broth.

Effect of leaf broth concentration on nanoparticle synthesis

To investigate the effect of leaf broth concentration for the synthesis of silver nanoparticles, the reaction mixtures with the ratios of 1:25, 1:50, 1:100, 1:200 leaf broth to 10^{-3} M silver nitrate were prepared. The reduction mechanism of silver ions in to nanoparticles was investigated through the UV-visible spectrophotometer analysis.

Characterization

The periodic scans of the optical absorbance between 200 and 800nm with a UV-visible spectrophotometer (Varian Cary 300 Bio) at a resolution of 1nm were performed to investigate the reduction rate of silver ions by curry leaf extract. The reaction mixture was diluted 20 times and used for UV-Visible analysis. Transmission electron microscopy (TEM) analysis of the synthesized silver nanoparticles was performed on a LEO model 912AB instrument at an accelerating voltage of 100kV. A drop of the nanoparticle suspension was placed on carbon coated copper grids and allowing the solvent to evaporate prior to analysis. In order to perform AFM analysis, the particles were deposited on a silicon slide and the solvent evaporated. The sample was analyzed using a Veeco diCaliber set to tapping mode with a Veeco TESP silicon tip. The average particle size of synthesized silver nanoparticles was identified using Zeta plus particle analyzer, from Brookhaven Instruments Corporation (BIC). The crystalline structure of the synthesized silver nanoparticles were investigated through powder x-ray diffraction using a Rigaku Multiflex X-ray powder diffractometer using Cu K_α radiation operating between 10º and 80º at the scanning rate of 2º per minute. The silver nanoparticles were distributed over a glass slide and the solvent was evaporated to form the thin film of silver nanoparticles for XRD analysis. The crystalline size was calculated using line broadening profile and *Scherrer's* formula.

Results and discussion

Fig. 2 shows the photographs of the reaction mixture of curry leaf broth and silver nitrate solution as a function of time. A visible colour change from transparent to yellow within 15 min indicates the formation of silver nanoparticles which was confirmed by UV-visible, XRD analysis. Further the colour change to dark orange-brown is due to increased concentration as well as growth of silver nanoparticles. After 90 minutes there was no significant colour change, which is evidence for the completion of reduction reaction. **Fig. 3** shows the results of the UV-visible spectrophotometer scan at various time intervals. The peak occurs at 435nm (λ_{max}) which corresponds to the absorbance of silver nanoparticles [**25**]. The intensity of the peak at 435nm was increased with time until the reduction completes. **Fig. 3** inset shows the absorbance at λ_{max} as a function of time. From the inset, the formation of silver nanoparticles slows after 120 min.

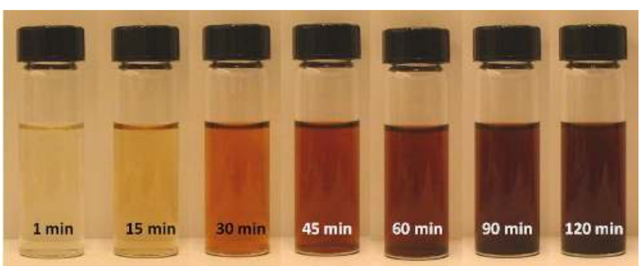

Fig. 2. Photographs of the mixture of curry leaf broth and silver nitrate solution over a two hours time period.

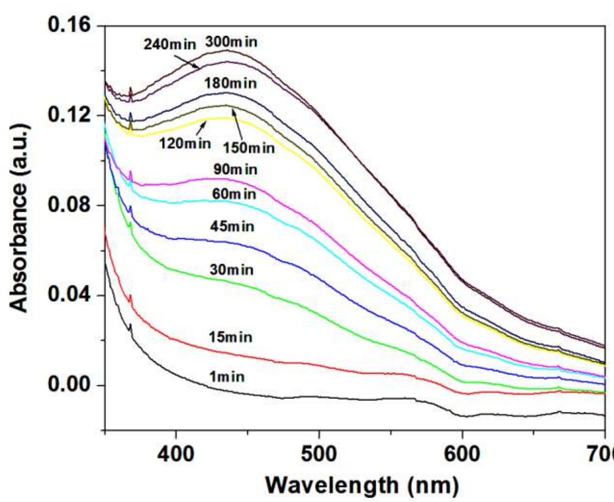

Fig. 3. UV-visible spectra of silver nitrate and curry leaf broth mixture over a 5 hours period. Inset: the absorbance of the sample at λmax, 435nm, which is associated with silver nanoparticles.

Fig. 4. Photographs of the varying concentrations (A-1:25, B-1:50, C-1:100 and D-1:200) of leaf broth to silver nitrate.

Fig. 4 shows photographs of varying concentrations of leaf broth in silver nitrate solution. The observed darker colour, which can be attributed to silver nanoparticle formation, in the 1:25 ratio sample at all time points indicates an increased rate of silver nanoparticle formation and a higher final concentration of nanoparticles achieved after 4 hours. **Fig. 5** shows the change in absorbance at the wavelength associated with silver nanoparticles λ_{max}, as a function of time for the varying concentrations. From **fig. 5** there is a reduction in the rate of formation after 120min in the 1:20 sample which suggests that all the Ag^+ ions are completely reacting to form silver nanoparticles. The complete reaction together with the rapid rate of reduction shows that the reaction is optimized at the 1:20 leaf broth to silver nitrate solution ratio.

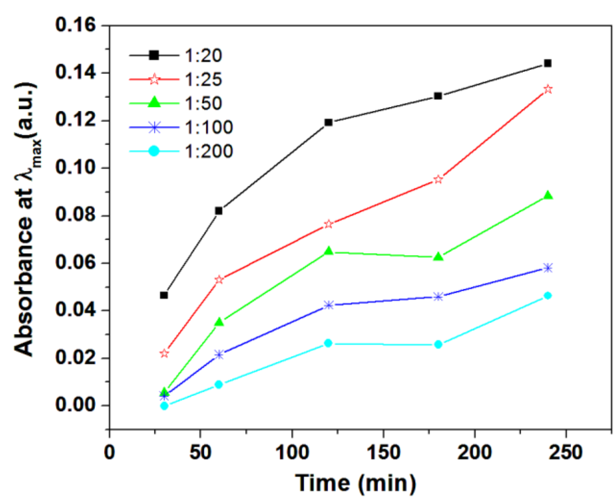

Fig. 5. A graph of the absorbance at 435nm of samples of varying concentrations of leaf broth.

Fig. 6a-e shows the TEM images of silver nanoparticles synthesized with different concentration of curry leaf extract for 2h. TEM images shows that the synthesized silver nanoparticles are polydisperse and ranges approximately from 10-25nm. The shape of the nanoparticles are spherical with few exceptional as ellipsoidal. From **Fig. 6** it is found that decreasing leaf extract concentration in reaction mixture reduces the particle size and also their agglomeration tendency. Also, from **Fig. 6** it can be seen that the particles cluster together without much physical contact. This confirms the possibility of secondary material that bind the silver nanoparticles in clusters,which was further confirmed by XRD analysis. AFM analysis of the synthesized silver nanoparticles was made and typical AFM image is shown in **Fig. 7**, which confirms the spherical shape of the particles and again the tendency for particles to aggregate.

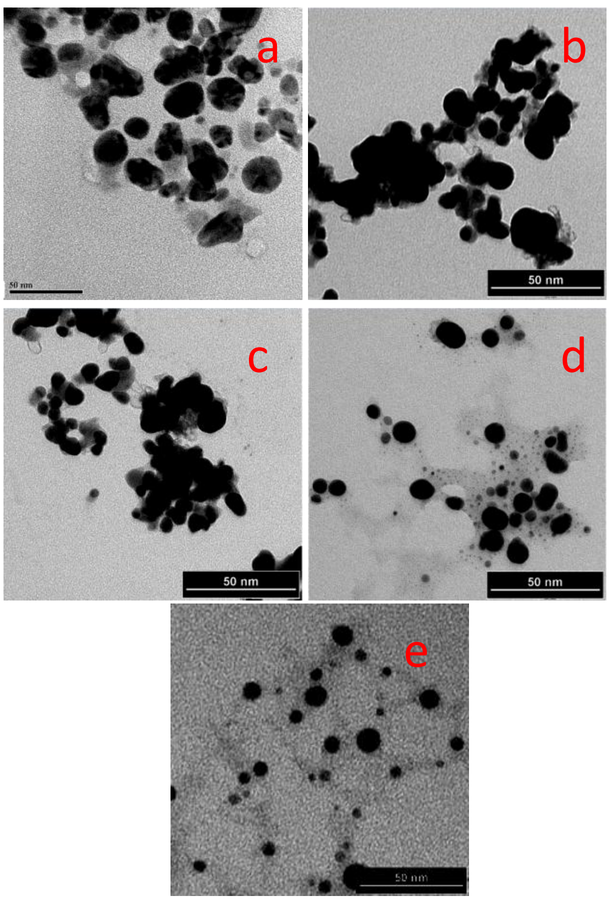

Fig. 6. TEM images of the biosynthesized silver nanoparticles using concentrations of leaf broth (A-1:20, B-1:25, C-1:50, D-1:100 and E-1:200).

Particle size analysis was performed for the silver nanoparticles synthesized using different concentration of curry leaf extract and the obtained results are shown in **Fig. 8.** The result indicates that the average particle size of the synthesized silver nanoparticles is highly influenced by the concentration of leaf broth. Increasing leaf extract concentration in the reaction mixture decreases the particle size. The similar trend was observed in TEM analysis as shown in **Fig. 6**. The smallest particle size was found to be 65nm, which was obtained using the leaf broth to silver nitrate solution ratio of 1: 200.

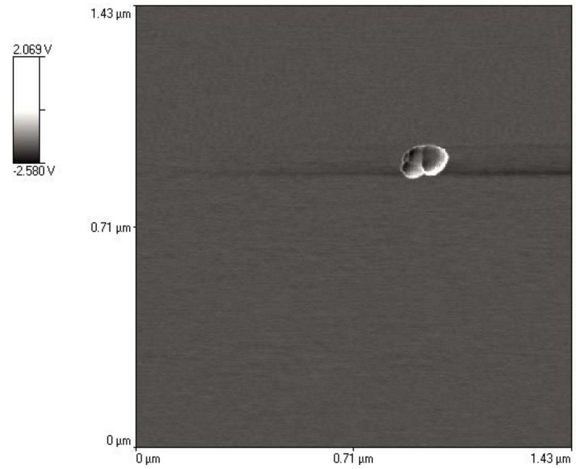

Fig. 7. Typical AFM image of the biosynthesized silver nanoparticles.

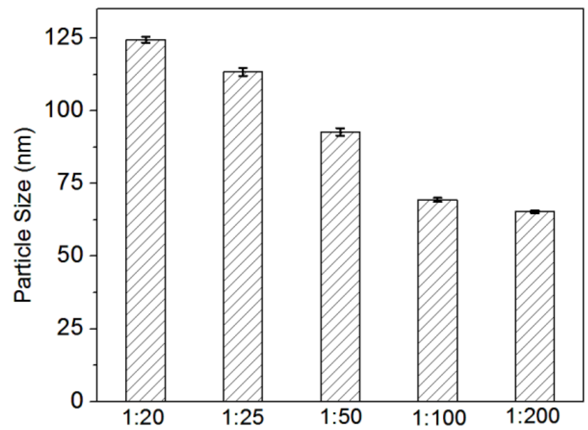

Fig. 8. Particle size of silver nanoparticles synthesized using different curry leaf extract (1:20, 1:25, 1:50, 1:100 and 1:200).

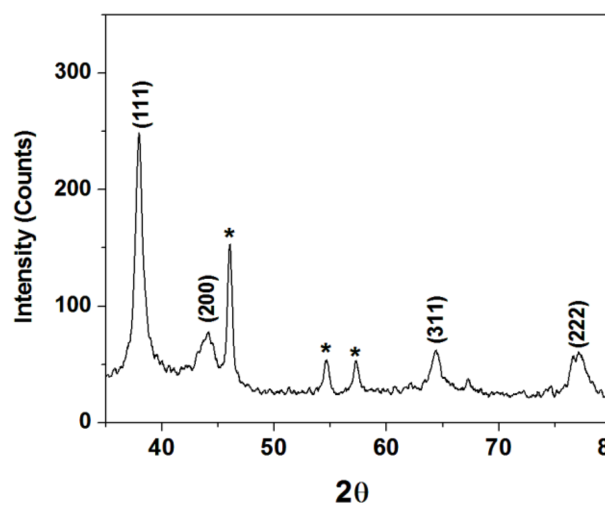

Fig. 9. Typical XRD pattern of silver nanoparticles synthesized using curry leaf extract.

The XRD pattern of synthesised silver nanoparticles using curry leaf extract were recorded and typical XRD pattern is shown in **Fig. 8**. The peaks are indexed as (111), (200), (220), (311) and (222) plans of FCC silver by comparing with JCPDS data. Apart from theese peaks responsible for silver nanoparticles, the recorded XRD pattern shows additional unassigned peaks,noted with stars. This may be due to the formation of the crystalline bio-organic compounds/metalloproteins that are present in the curry leaf broth. Similar observations were reported by S. Shiv Shankar et al. [**19**] for the silver nanoparticles synthesized using *P. graveolens* leaf broth. The deatiled investigation on this crystalline phase existing with the silver nanocrystals are in progress.

Conclusion

The biological synthesis of silver nanoparticles using *Murraya koenigii* extract was shown to be rapid and produce particles of fairly uniform size and shape. Following the addition of curry leaf broth to the silver nitrate solution, silver nanoparticles began to form within 15 minutes and the reaction neared completion at 2 h, as shown by the UV-Vis spectrophotometry. It was found that the increasing broth concentration increases the rate of reduction and reduces the particle size as well as their agglomeration. The reduction of silver ions to silver nanoparticles was found to be optimized at a ratio of 1:20 of leaf broth to 10^{-3} M silver nitrate solution. The synthesized particles ranged in size from 10-25 nm and were spherical in shape, as shown by the TEM and AFM analysis. The particles also tended to aggregate which suggests they may be useful in applications requiring the coating of materials.

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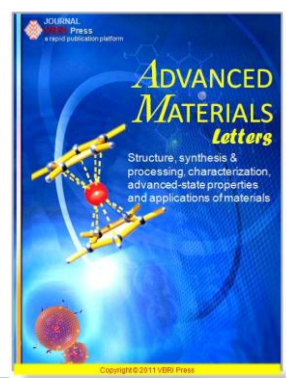
Reference

1. Cobley, C. M.; Skrabalak, S. E.; Campbell, D. J.; Xia Y. *Plasmonics* **2009**, *4*, 171. **DOI:** 10.1007/s11468-009-9088-0
2. Karimzadeh, R.; Mansour, N. *Opt. Laser Technol.* **2010**, *42*, 783. **DOI:** 10.1016/j.optlastec.2009.12.003
3. Wang, T.; Kaempgen, M.; Nopphawan, P.; Wee, G.; Mhaisalkar, S.; Srinivasan, M. *J. Power Sources* **2010**, *195*, 4350. **DOI:** 10.1016/j.jpowsour.2009.12.137
4. Sun, R. W. Y.; Chen, R.; Chung, N P. Y.; Ho, C. M.; Lin, C. L. S.; Che, C.M.; *Chem. Commun.* **2005**, 5059. **DOI:** 10.1039/B510984A
5. Dick, L. A.; McFarland, A. D.; Haynes, C. L.; Van Duyne, R. P. *J. Phys. Chem. B* **2002**, *106*, 853. **DOI:** 10.1021/jp013638l
6. Kumar, A.; Vemula, P. K.; Ajayan, P.M.; John G., *Nature* **2008**, *7*, 236. **DOI:** 10.1038/nmat2099
7. Khaydarov, R. A.; Khaydarov, R. R.; Gapurova, O.; Estrin, Y.; Scheper, T. *J. Nanopart. Res.* **2008**, *11*, 1193. **DOI:** 10.1007/s11051-008-9513-x
8. Murphy, C. J.; Gole, A. M.; Hunyadi, S. E.; Stone, J. W.; Sisco, P. N.; Alkilany, A.; Kinard, B. E.; Hankins, P. *Chem. Commun.* **2008**, 544. **DOI:** 10.1039/B711069C
9. Sun, Y.; Gates, B.; Mayers, B.; Xia, Y., *Nano Lett.* **2002**, *2*, 165. **DOI:** 10.1021/nl010093y
10. Wiley, B.; Herricks, T.; Sun, Y.; Xia, Y. *Nano Lett.* **2004**, *4*, 1733. **DOI:** 10.1021/nl048912c
11. Creighton, J. A.; Blatchford, C. G.; Albrecht, M. G., *J. Chem. Sot. Faraday II* **1979**, *75*, 790. **DOI:** 10.1039/F29797500790
12. Kim, J. S., Kuk, E.; Yu, K. N.; Kim, J. H.; Park, S. J.; Lee, H. J.; Kim, S. H.; Park, Y. K.; Park, Y. H.; Hwang, C. Y.; Kim, Y. K.; Lee, Y. S.; Jeong, D. H.; Cho, M. H. *Nanomed- Nanotechnol.* **2007**, *3*, 95. **DOI:** 10.1016/j.nano.2006.12.001
13. Huang, N. M.; Radiman, S.; Lim, H. N.; Khiew, P. S.; Chiu, W. S.; Lee, K. H.; Syahida, A.; Hashim, R.; Chia, C. H. *Chem. Eng. J.* **2009**, *155*, 499. **DOI:** 10.1016/j.cej.2009.07.040
14. Le, A. T.; Huy, P. T.; Tam, P. D.; Huy, T. Q.; Cam, P. D.; Kudrinskiy, A. A.; Krutyakov, Y. A. *Curr. Appl. Phys.* **2010**, *10*, 910. **DOI:** 10.1016/j.cap.2009.10.021
15. Leung, T. C. Y.; Wong, C. K.; Xie, Y. *Mater. Chem. Phys.* **2010**, *121*, 402. **DOI:** 10.1016/j.matchemphys.2010.02.026
16. Saravanan, M.; Nanda, A. *Colloid. Surface. B* **2010**, *77*, 214. **DOI:** 10.1016/j.colsurfb.2010.01.026
17. Sintubin, L.; Windt, W. D.; Dick, J.; Mast, J.; Van der Ha, D.; Verstraete, W.; Boon, N. *Appl. Microbiol. Biot.* **2009**, *84*, 741. **DOI:** 10.1007/s00253-009-2032-6
18. Sanghi, R.; Preeti, V.; *Adv. Mat. Lett.* **2010**, *1(3)*, 193. **DOI:** 10.5185/amlett.2010.5124
19. Shahverdi, A. R.; Minaeian, S.; Shahverdi, H. R.; Jamalifar, H.; Nohi, A. A.; *Pro. Biochem.* **2007**, *42*, 919. **DOI:** 10.1016/j.procbio.2007.02.005
20. Shiv Shankar, S.; Ahmad, A.; Sastry, M.; *Biotechnol. Prog.* **2003**, *19*, 1627. **DOI:** 10.1021/ bp0501423
21. Shiv Shankar, S.; Rai, A.; Ahmad, A.; Sastry, M. *J. Colloid Interf. Sci.* **2004**, *275*, 496. **DOI:** 10.1016/ j.jcis.2004.03.003
22. Ankamwar, B.; Chaudhary, M.; Sastry, M.; *Synth. React. Inorg. Met.-Org. Chem.* **2005**, *35*, 19. **DOI:** 10.1081/SIM-200047527
23. Nadagouda, M. N.; Varma, R. S. *Green Chem.* 2008, 10, 859. **DOI:** 10.1039/b804703k
24. Vivekanandhan, S.; Misra, M.; Mohanty, A. K. *J. Nanosci. Nanotechnol.* **2009**, *9*, 6828. **DOI:** 10.1166/jnn.2009.2201
25. Vivekanandhan, S.; Misra, M.; Mohanty, A. K. *Nanosci. Nanotechnol.* Let. **2010**, *2 (3)*, 240. **DOI:** 10.1166/nnl.2010.1087
26. Philip, D; Unni, C; Aromal, S. A.; Vidhu, V. K.; *Spectrochimi. Acta A.* **2011**, *78*, 899. **DOI:** 10.1016/j.saa.2010.12.060
27. Kumar, V. ; Yadav, S. K. *J. Chem. Technol. Biot.* **2009**, *84*, 151. **DOI:** 10.1002/jctb.2023
28. Adebajo, A. C.; Olayiwola, G.; Verspohl, J. E.; Iwalewa, E. O.; Omisore, N. O. A.; Bergenthal, D.; Kumar, V.; Adesina, S. K. *Pharm. Biol.* **2004**, *42(8)*, 610. **DOI:** 10.1080/13880200490902518
29. Tachibana, Y.; Kikuzaki, H.; Lajis, N. H.; Nakatani, N. *J. Agr. Food Chem.* **2001**, *49*, 5589. **DOI:** 10.1021/jf010621r
30. Tachibana, Y.; Kakusaki, H.; Lajis, N. H.; Nakatani, N. *J. Agr. Food Chem.* 2003, 51, 6461. **DOI:** 10.1021/jf034700+
31. Rai, M.; Yadav, A.; Cade, A. *Crit. Rev. Biotechnol.* 2008, 28, 277. **DOI:** 10.1080/07388550802368903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