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BIO-FABRICATION OF SILVER NANOPARTICLES USING LEAF EXTRACT OF *SAURURUS CHINENIS*

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Nanotechnology is gaining tremendous impetus in the present century due to its capability of modulating metals into their nanosize. Plant extracts are very cost effective and eco-friendly and thus can be an economic and efficient alternative for the large-scale synthesis of nanoparticles. The present study deals with the synthesis of silver nanoparticles using *Saururus chinensis* leaf extract. On challenging leaf broth of *Saururus chinensis* and aqueous AgNo3 (1mM) solution changed from yellowish to reddish brown, the final color appeared gradually with time. Formation of silver nanoparticles was confirmed by UV-visble spectroscopy, X-ray diffraction patterns, Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM) with Energy dispersive X-ray (EDX) patterns. The biomass of plants produces their nanomaterials by a process called biomineralisation. Biological methods are good competitors for the chemical procedures, which are environment friendly and convenient.

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1. Introduction

Nanotechnology is a new scientific field being developed since 1980s. Nano materials have a lot of different characters compared to the general materials with same components because of their small size effect, surface or interface effect, quanta tunnel effect, etc. With the development of new chemical or physical methods, the concern for environmental contaminations are also heightened as the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous byproducts. Thus, there is a need for 'green chemistry' that includes a clean, nontoxic and environment-friendly method of nanoparticle synthesis [1].

A number of approaches are available for the synthesis of silver nanoparticles for example, reduction in solutions [2], chemical and photochemical reactions in reverse micelles [3], thermal decomposition of silver compounds [4], radiation assisted [5], electrochemical [6], sonochemical [7], microwave assisted process [8] and recently via green chemistry route [9, 10, 11, 12].

The biosynthetic method employing plant extracts have drawn attention as a simple and viable alternative to chemical procedures and physical methods. Bioreduction of silver ions to yield metal nano particles using living plants, geranium leaf [13], Neem leaf [14]. Very recently, they have demonstrated synthesis of gold nanotriangles and silver NP using *Aloevera* plant extracts [15], *Emblica officinalis* (amla, Indian Gooseberry) [16]. . Nevertheless, the bioreduction of silver ions proceeded merely in the presence of ammonia. Most of the above research on the synthesis of silver or gold NP utilizing plant extracts employed broths resulting from boiling fresh plant leaves. The green synthesis of silver NP using *Capsicum annuum* L. extract has been reported [17]. According to previous reports, the polyol components and the water-soluble heterocyclic components are mainly responsible for the reduction of silver ions and the stabilization of the NP, respectively [18].

Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process [19, 20]. The most important application of silver and silver NPs is in medical industry such as topical ointments to prevent infection against burn and open wounds [21].

Saururus chinensis (Saururaceae) is a perennial herb distributed in China and southern Korea and has been used in folk medicine to treat edema, gonorrhea, and also as a uretic in Korean traditional medicine [22]. Previous chemical studies of the genus Saururus have shown the presence of more than 20 lignans [23], some of which exhibited neuroleptic [24], hepatoprotective [25], and antifeedant activities [26].

Here in, we report for the first time synthesis of silver NPs, reducing the silver ions presents in the solution of silver nitrate by the aqueous extract of *Saururus chinensis*.

2. Materials and methods

Plant material and preparation of extract and silver nanoparticles

Saururus chinensis leaves were collected from Gwangju oriental medical college, Gwangju, South Korea. The leaves were air dried for 10 days then were kept in the hot air oven at 600°c for 24- 48 hrs. The leaves were ground to a fine powder. 1 mM silver nitrate ($AgNo_3$ was purchased from Sigma-Aldrich Chemical Pvt. Ltd) was added to plant extract to make up a final solution 200 ml and centrifuged at 18,000 rpm for 25 min. The collected pellets were stored at -4°c. The supernatant was heated at 50°c to 95°c. A change in the color of solution was observed during the heating process.

UV-VIS spectra analysis

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-VIS spectrophotometer (Cary 4000 UV/ Vis spectrophotometer).

SEM (Scanning Electron Microscopic) and EDX (Energy dispersive X-ray spectrometer) analysis of silver nanoparticles

SEM analysis was done using Hitachi S-3500SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

EDX

Energy dispersive X-ray spectrometers take advantage of the photon nature of light.

In the X-ray range the energy of a single photon is just sufficient to produce a measurable voltage pulse X-ray, the output of an ultra low noise preamplifier connected to the low noise are a statistical measure of the corresponding quantum energy. By digitally recording and counting a great number of such pulses with in a so called Multi Channel Analyzer, a complete image of the X-ray spectrum is building up almost simultaneously. This digital quantum counting technique makes the energy dispersive spectrometry exceedingly reliable.

A semiconductor material is used to detect the x-rays together with processing electronics to analyses the spectrum.

XRD analysis of silver nanoparticles

The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 5000 rpm for 20 min followed by redispersion of the pellet of silver nanoparticles into 10 ml of deionized water. After freeze drying of the purified silver particles, the structure and composition were analyzed by XRD **(**Rigaku RINT 2100 series**)**. The dried mixture of silver nanoparticles was collected for the determination of the formation of Ag nanoparticles by an X'Pert Pro x-ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation in a θ-2 θ configurations. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer formula.

$$D= 0.94\ \lambda / \beta \cos \theta \rightarrow (1)$$

where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle. To eliminate additional instrumental broadening the FWHM was corrected, using the FWHM from a large grained Si sample. β corrected = (FWHM2sample- FWHM2si)1/2 → (2)

This modified formula is valid only when the crystallite size is smaller than 100 nm [27]

FTIR analysis of silver nanoparticles

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 5000 rpm for 10 min and the resulting suspension was redispersed in 10 ml sterile distilled water. The centrifuging and redispersing process was repeated three times. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR.(Bruker model, TENSOR 37).

Particle size measurement

Particle sizing experiments were carried out by means of laser diffractometry, using Zetasizer nano series (Malvern). Measurements were taken in the range range between 0.1 and 10000 μm

3. Results and discussion

The synthesis of nanoparticles is in the lime light in modern nanotechnology. Bio synthesis of nanoparticles by plant extracts is currently under exploitation. The development of biologically inspired experimental process for the synthesis of nanoparticles is evolving into an important branch of nano technology. The present study deals with the synthesis of silver nanoparticles using leaf broth of *Saururus chinensis* (Saururaceae) (figure 1). Results noted at different timings immediately, and after 1 day.

Fig. 1. Photograph of Saururus chinensis leaf used in the synthesis.

Formation of the silver nanoparticles exhibit reddish brown in water [28]. Figure 2 shows the color changes before (a) and after(b) the process of reduction of Ag^+ to Ag nanoparticles. After 30 min of the conversion process silver nano particles in solution.

These colors arise due to excitation of surface Plasmon vibrations in the silver metal nano particles [29]

Fig a *Fig b*

Fig.2 a: Saururus chinensis L, b: Colour change of leaf extract containing Ag before and after synthesis of Ag nanoparticles.

UV-Visible spectral analysis of AgNPs

UV-VIS absorption spectrophotometer is used to investigate the LSPR phenomenon. Silver nanoparticles exhibits interesting optical properties directly related to Localized Surface Plasmon Resonance (LSPR) which is highly dependent on the morphology of the nanoparticles. Reduction of Ag+ ions during exposure to the extract of boiled *Saururus chinensis* leaves was easily followed by UV-spectroscopy. It was observed that silver nanoparticles exhibit reddish-brown color in water. The color appeared due to the excitation of the localized surface Plasmon vibrations of the metal nanoparticles. The evidence of LSPR was shown in Fig. 3. A smooth and narrow absorption bands were observed at 415 and 353nm which is a characteristic of mono dispersed silver nanoparticles.

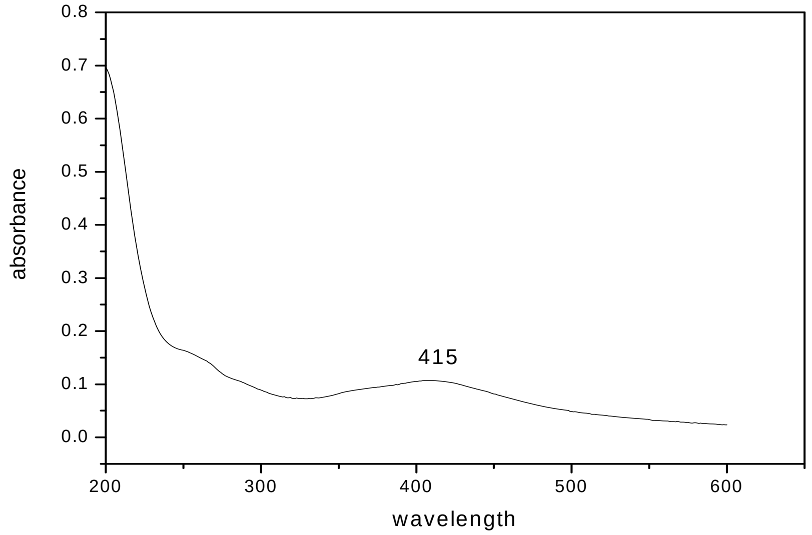


Fig.3a

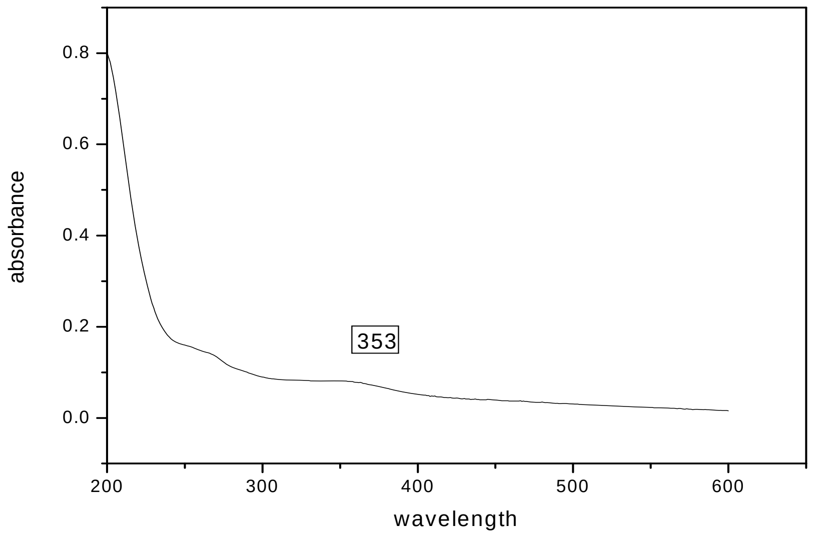


Fig. 3b

Fig. 3. UV–visible spectra of silver colloids:(a) immediately (b) after 1 day

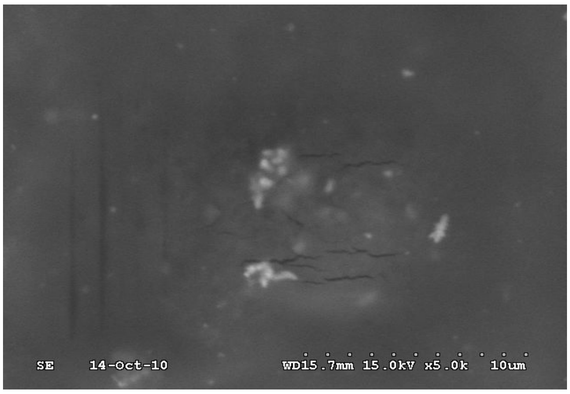


Fig.4a

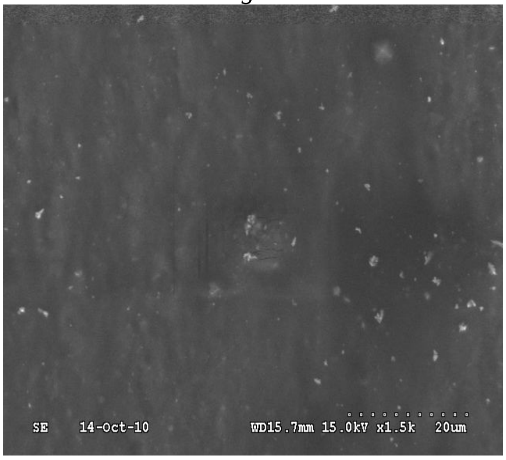


Fig. 4b

Fig. 4c

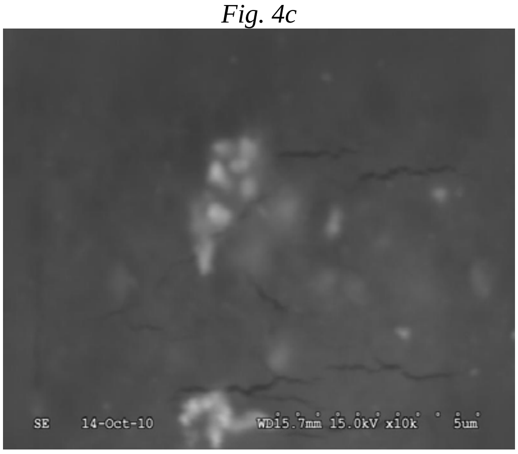


Fig.4d

Fig. 4: SEM images of Ag a, b, c and (b) under different magnification.

SEM

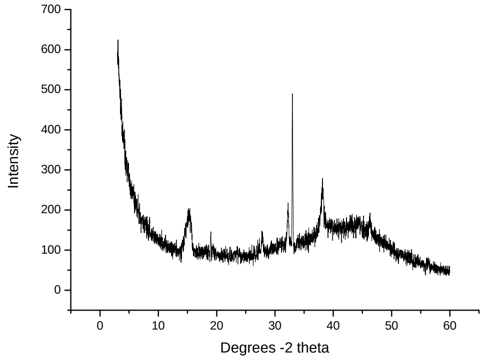
The SEM micrographs showing (figure 4) polydispersed silver nanoparticles with varied sizes. After 1 day of reaction of plant extract with the silver ions, the reduction and stabilization process was completed and clear mono dispersed and comparatively smaller particles are observed in the SEM micrograph and this result is in good agreement with the particle grain size 38 and 54 nm (Table 1).

Table 1 Grain size estimated by Scherrers's formula

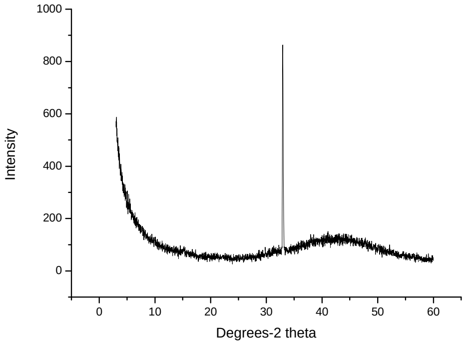
Sample name	Grain size (nm)
Ag immediately)	38
Ag (after 1 day)	54

XRD analysis of silver nanoparticles

X-ray diffraction (XRD) confirmed the existence of silver colloids in the sample. The Braggs reflections observed in the XRD pattern at 2θ = 15.3, 32.22, 32.96, and 38.08 (immediately) and 32.94 (after 1 day). A strong diffraction peak located at 38.2 was ascribed to the (111) facets of silver respectively (Figure 5a). XRD pattern thus clearly illustrate that the silver nanoparticles formed in this present synthesis are crystalline in nature. There are no peaks observed for the impurities in the XRD pattern is a sign of the purity of the silver nano particles (Figure 5b)



a immediately



b after 1 day

Fig. 5: XRD pattern of silver nanoparticles.

FTIR analysis of silver nanoparticles

The FTIR spectrum was taken at two times ie., immediately after formation of silver nanoparticles and after 1 day of formation. It is interesting to note that comparatively some of the peaks were absent in the FTIR spectrum taken after 1 day of formation of silver nanoparticles. The amide I band is primarily of carbonyl (C=O) stretching of the peptide backbone. The energy of this vibration is sensitive to the secondary and tertiary structure of protein. The observed band at 1637 cm^{-1} with the solution with Ag (after 1 day) probably arises from carbonyl groups in α-helices that are present in the plant extract whereas the peak observed at 1645cm^{-1} likely to results from carbonyl groups in the α-helices that are less exposed to the solution. The representative spectra of nanoparticles obtained (Figure 6b) manifests absorption peaks located at about 1638, 2200 and 3460 $^{-1}$. The band observed at 2200 cm^{-1} with the solution Ag (immediately) attributes to the side chain vibrations consisting of C-H stretching of aliphatic group and interestingly found missing with the solution Ag (after 1 day) (Figure 6c) It may be due to the proteins participation in reduction and stabilization of silver nanoparticles. 3460^{-1} is characteristic of –NH stretching of amide (II) band. The weaker band at 1638^{-1} corresponds to amide I, arisen due to carbonyl stretch in proteins.

Table 2a. Particle size analyzed by volume (%)

Ag-0 hours	Diam (nm)	Volume (%)	Width (nm)
Peak 1	248.8	86.4	137.1
Peak 2	4904	13.6	894.9

Ag-24 hours

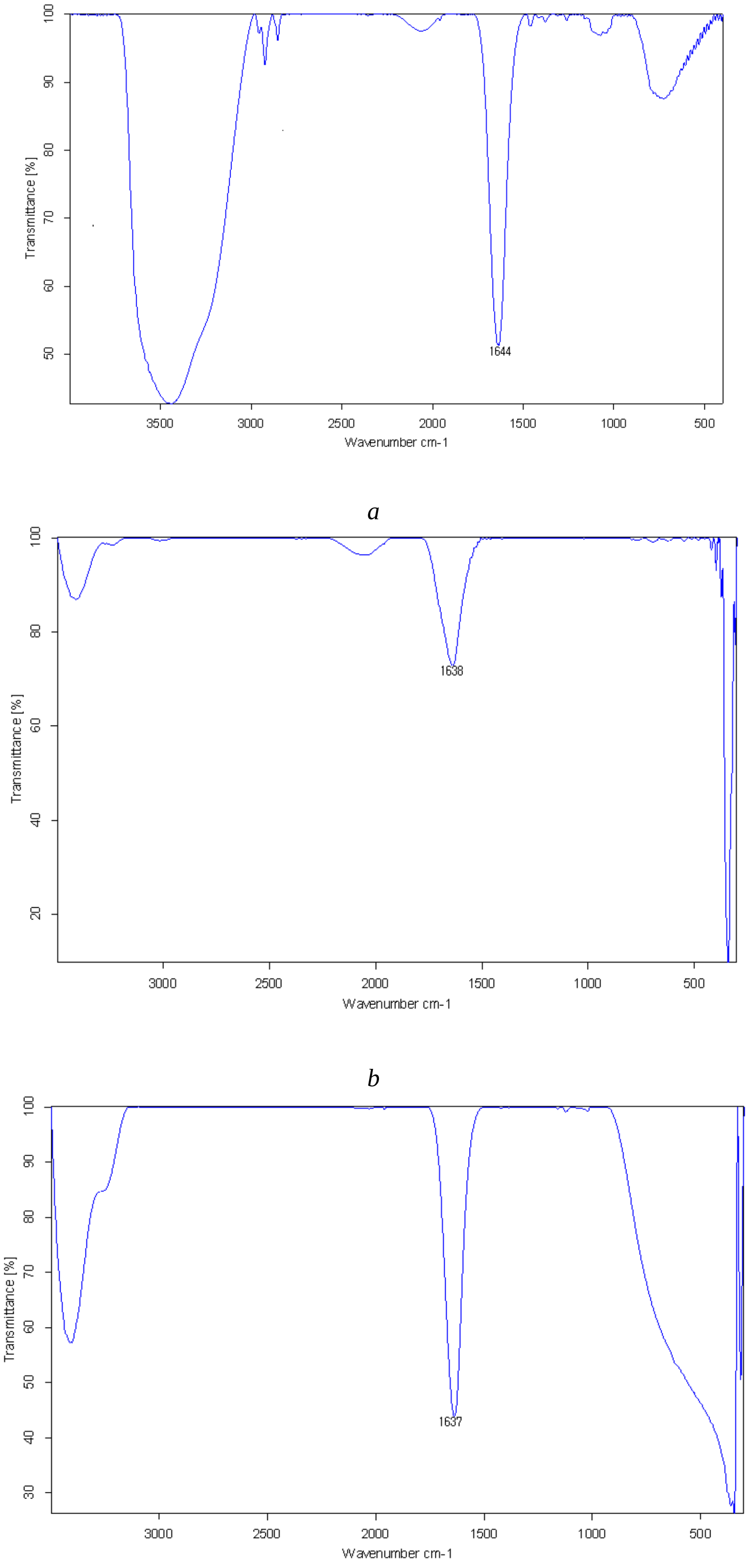
	Diam (nm)	Volume (%)	Width (nm)
Peak 1	109.9	12.5	82.13
Peak 2	1445	87.5	5.885

Table 2b. Particle size analyzed by intensity (%)

Ag-0 hours	Diam (nm)	Intensity (%)	Width (nm)
Peak 1	226.6	98.9	105.6
Peak 2	4647	1.1	807.9

Ag-24 hours

	Diam (nm)	Intensity (%)	Width (nm)
Peak 1	148.7	95.2	82.56
Peak 2	18.03	4.8	5.605



a

b

c

Fig. 6 FTIR spectra of (a).Saururus chinesis leaf extract (b) immediately and (c). after 1 day

Particle size determination

Particle size determination of the formulated nanoparticles was shown under different categories like size distribution by volume, by intensity(Figure 7,8). First and second peaks, the average diameter of the particles were found to be 226.6 and 4667 (Table 2a); 148.7 and 18.03 nm (Fig 7b), and the peaks intensity were found 98.9 and1.1% ; 95.2 and 4.8 respectively. The size distribution by volume gives a bell shaped pattern which indicates the wide range size distribution of nanoparticles in the sample formulation (Figure 8a, b). The volume% of the sample was found to be in the range of 10-10000d.nm (Table 2b). The formed silver nanoparticles are well distributed with respect to volume and intensity is an indication of the formation of well built silver nanoparticles and their monodispersity.

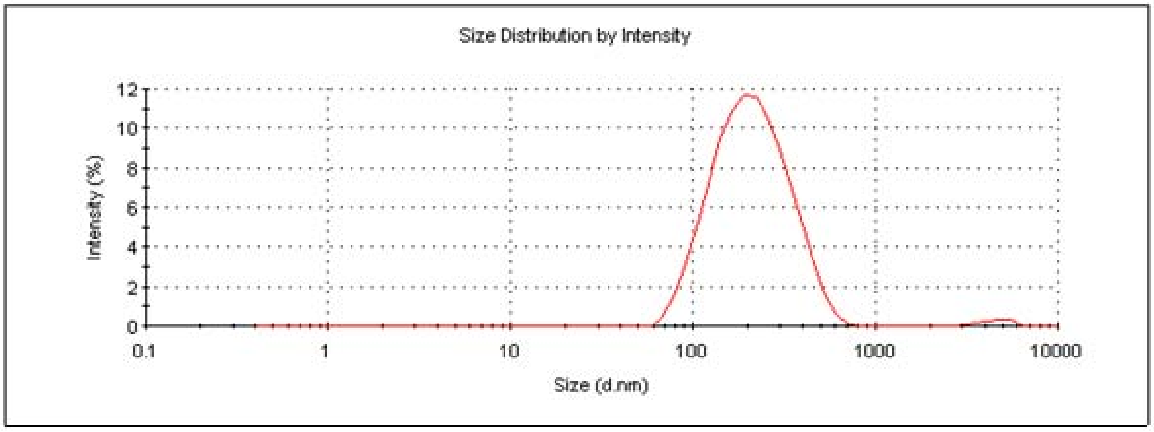


Fig.7a

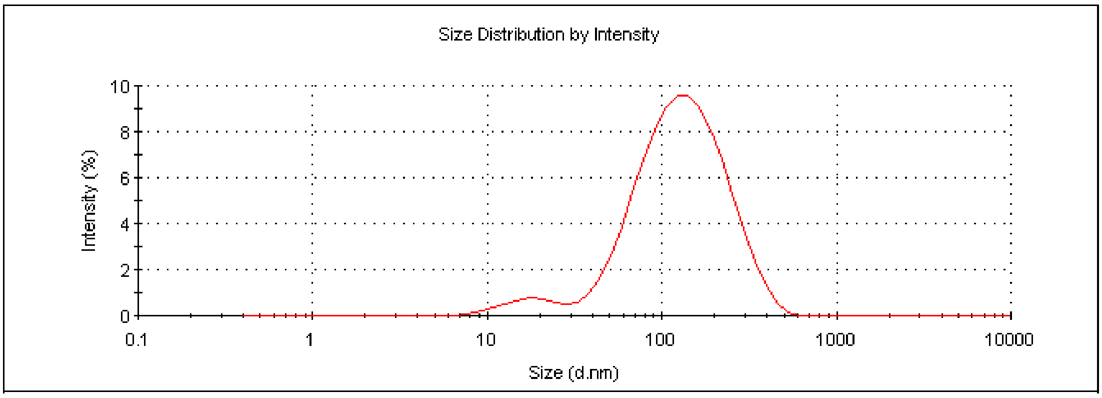


Fig.7b

Fig. 7. Particle size distribution of Ag-NPs.by volume (a) immediately (b) after 1 day

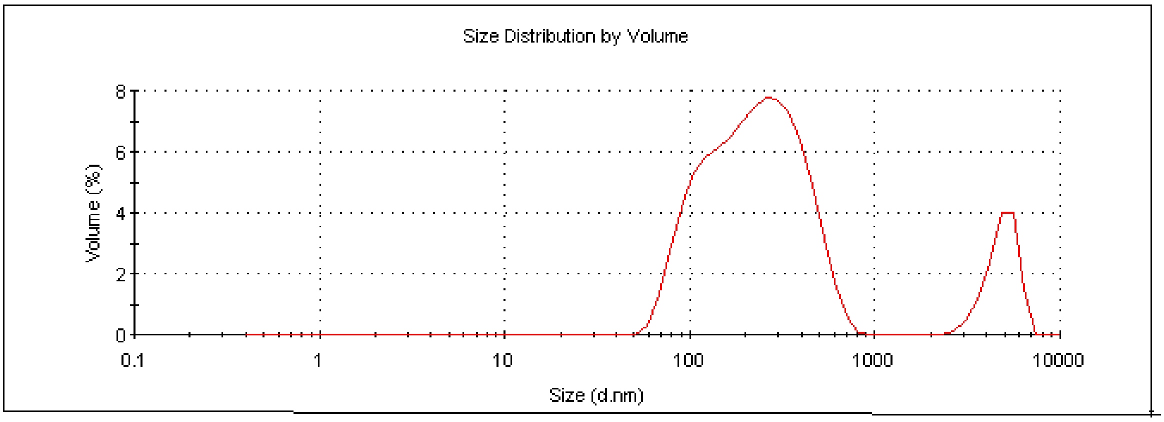


Fig. 8a

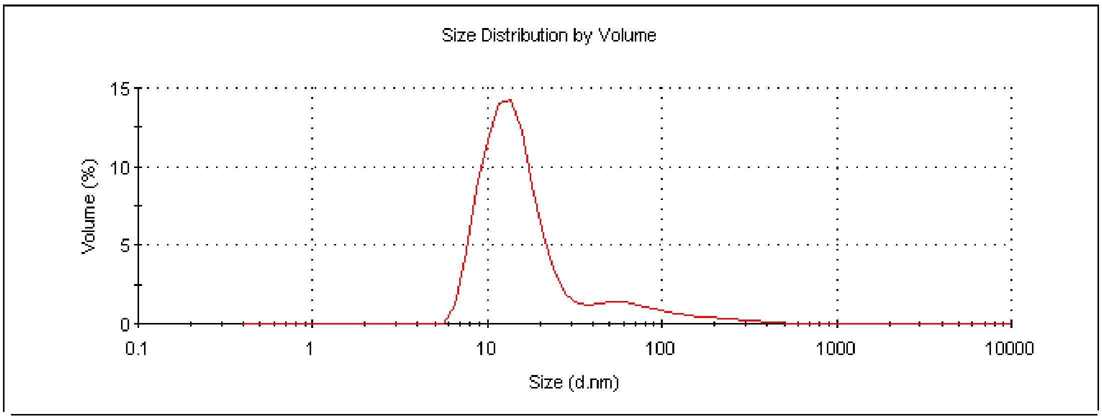


Fig. 8b

Fig.8: Particle size distribution of Ag-NPs.by intensity (a) immediately (b) after 1 day

EDAX analysis of silver nanoparticles

The energy dispersive X-ray analysis (EDX) reveals strong signal in the silver region and confirms the formation of silver nanoparticles (Figure 9). Metallic silver nanocrystals generally show typical optical absorption peak approximately at 3 keV due to surface plasmon resonance [30]..There were other EDAX peaks for Pt, Cl, K and Al suggesting that they are mixed precipitates present in the plant extract. This analysis revealed that the nano-structures were formed solely of silver (Table 3).

Table 3. EDX elemental micro-analysis of the silver nanoparticles

Element	Weigh (%)	Atomic (%)
O	10.40	38.91
Al	8.02	17.79
Cl	0.35	0.59
K	2.36	3.61
Ag	59.99	33.30
Pt	18.89	5.80

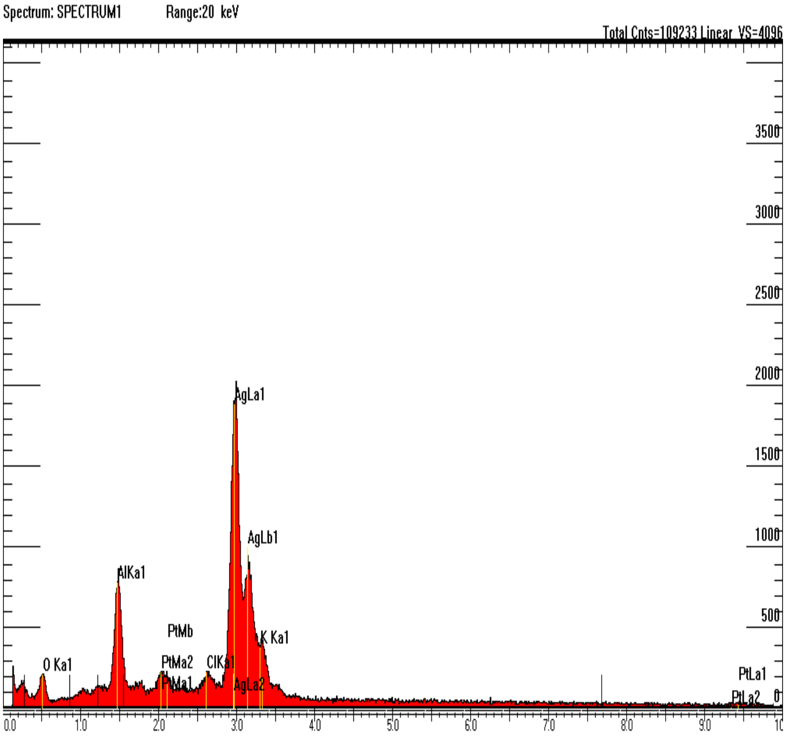


Fig. 9. EDX spectrum recorded from a film, after formation of silver nano particles.

4. Conclusions

The present study demonstrated the extracellular biosynthesis of anisotropic silver nanoparticles using the leaf extract *Saururus chinensis.* The formation of AgNPs at immediately and after 1 day of reaction time was well studied. UV-Vis spectroscopy recorded LSPR of the AgNPs. The AgNPs characterization and morphology was studied with XRD, SEM and particle size analyzer techniques. The FTIR examination of the samples confirms the involvement of Proteins and amino groups in the reduction and stabilization of the AgNPs. This procedure is easy, cost-effective, energy saving and environment friendly.

References

[1] P.Mukherjee, A. Ahmad, D.Mandal, S. Senapati, S.R. Sainkar, M.I. Khan, R. Parishcha, P.V. Ajaykumar, M.Alam, R.Kumar, M.sastry, Nano Lett.**1**, 515(2001).
[2] D.V. Goia, E. Matijevic N. J. Chem. **22,** 1203 (1998)
[3] C. Taleb, M. Petit, P. Pileni Chem. Mater. **9,** 950 (1997)
[4] K. Esumi, T. Tano, K. Torigoe, K. Meguro Chem. Mater. **2,** 564 (1990)
[5] A. Henglein Langmuir **17,** 2329 (2001)
[6] L. Rodriguez-Sanchez, M. C. Blanco, M. A. Lopez-Quintela J. Phys. Chem. B **104,** 9683 (2000)
[7] J. J. Zhu, S. W. Liu, O. Palchik, Y. Koltypin, A. Gedanken Langmuir. **16,** 6396 (2000)
[8] Pastoriza-Santos, L. M. Liz-Marzan Langmuir **18,** 2888 (2002)
[9] N. A. Begum, S. Mondal, S. Basu, R. A. Laskar, D. Mandal Colloids and Surfaces B: Biointerfaces **71,** 113-118 (2009)
[10] H. Bar, D. K. Bhui, G. P. Sahoo, P. Sarkar, S. P. De, A. Misra. Colloids and Surfaces A: Physicochem. Eng. Asp. **339,** 134 (2009)
[11] J. Y. Song, B. S. Kim. Bioprocess Biosyst. Eng. **32,** 79–84 (2009).

[12] A. Singh, D. Jain, M. K. Upadya, N. Khandelwal, H. N. Verma. Digest. J. Nanomat. Biostru. **5**, 483 (2010)
[13] S. S. Shankar, A. Ahmad, R. Pasricha, M. Sastry. J. Mater. Chem. **13**, 1822 (2003).
[14] S.S. Shankar, A. Rai, A. Ahmad, M. Sastry, J. Colloid. Interface. Sci. **275**, 496 (2004).
[15] S.P. Chandran, M. Chaudhary, R. Pasricha, A. Ahmad & M. Sastry, Biotechnol. Prog. **22,** 577 (2006).
[16] B.Amkamwar, C. Damle, A. Ahmad, M. Sastry. J. Nanosci. Nanotechnol. **5**,1665 (2005).
[17] S. Li, Y. Shen, A. Xie, X. Yu, L. Qiu, L. Zhang, O. Zhang. Green. Chem. **9,** 852 (2007).
[18] L. Arangasamy, V. Munusamy, Afr. J. Biotech. **7,** 3162 (2008).
[19] R. M. Jose, L. E. Jose, C. Alejandra. Nanotechnology **16**, 2346–2353. (2005)
[20] C. Lok, C. Ho, R. Chen, Q. He, W. Yu, H. Sun, P. K. Tam, J. Chiu, C. Che J. Biol. Inorg. Chem. **12**, 527 (2007)
[21] M. Ip, S. L. Lui, V. K. M. Poon, I. Lung, A. Burd J. Medical Microbiol. **55**, 59–63 (2006)
[22]J.G. Kim. Illustrated Natural Drugs Encyclopedia. Nam san dang, Seoul, 1989.
[23] K.V. Rao, N.S.P.J. Rao. Nat. Prod. **53**, 212 (1990).
[24] K.V. Rao, V.N. Puri, P.K. Diwan, F.M. Alvarez. Pharmacol.. Res. Commun. **19**, 629. (1987)
[25]S.H. Sung, Y.C. Kim. J. Nat. Prod. **63**, 1019 (2000).
[26] J. Kubanek, W. Fenical, M.E. Hay, P.J. Brown, N. Lindquist. Phytochem. **54**, 281 (2000).
[27] F. Boulch, M. C. Schouler, P. Donnadieu, J. M. Chaix, E. Djurado ,Image Anal Stereol. **20**, 157 (2001).
[28] M.Sastry, V.Patil, S.R.Sankar, J.Phy. Chem B. **10**2, 1404 (1998)
[29] P.Mulvaney, R. Langmuir. **12**,788 (1996)
[30] P. Magudapatty, P. Gangopadhyayrans, B.K. Panigrahi, K.G.M. Nair, S. Dhara. physica B .**299**, 142 (2001).