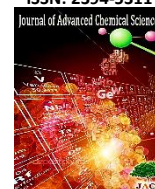




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## Green Synthesis of Silver Nanoparticles using the Leaf Extracts and Their Microbial Activity

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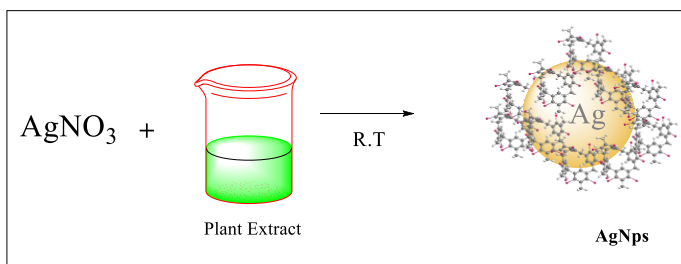
AgNps

Antimicrobial Studies

## ABSTRACT

Development of green nano technology is generating interest of researchers toward ecofriendly bio-synthesis of nano particles. In the present study, we report the bio synthesis of aqueous stable silver nanoparticles by using *argemone mexicana* (darudi) and *ocimum tenuiflorum* (tulsi) leaf extract as both reducing and capping agents. The plant extract capped silver nanoparticles (AgNps) were found to be highly stable over a long period of time. AgNps were characterized by UV/Vis-spectroscopy, particle size analyzer (PSA), transmission electron microscopy (TEM) and electron diffraction (EDX). Further, the AgNps showed high antimicrobial and bactericidal activity against bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* found highly strain.

## GRAPHICAL ABSTRACT



## 1. Introduction

Nanobiotechnology is one of the most promising areas in modern nanoscience and technology. This emerging area of research interlaces various disciplines of science such as physics, chemistry, biology and material science. Nanoparticles are usually  $\leq 100$  nm in each spatial dimension and are commonly synthesized using top-down and bottom-up strategies. In top-down approach, the bulk materials are gradually broken down to nanosized materials, whereas in bottom-up approach, atoms or molecules are assembled to molecular structures in nanometer range. Bottom-up approach is commonly used for chemical and biological synthesis of Nanoparticles [1]. The field of nanoscience has blossomed over the last twenty years and the need for nanotechnology will only increase as miniaturization becomes more important in areas such as computing, sensors, and biomedical applications [2]. Nanoscience has been established recently as a new interdisciplinary science. It can be defined as a whole knowledge on fundamental properties of nano-size objects [3]. Size and shape of nanoparticles provide an efficient control over many of the physical and chemical properties [4], and their potential application in optoelectronics [5], recording media [6], sensing devices [7], medicine [8] and catalysis hence, synthesis and characterization of nanoparticles is now a days an important area of research. Silver nanoparticles have unique electronic and optical properties because of this reason they have been used in a broad range of fields, including catalysis, biological labeling, photonics, and surface-enhanced raman scattering (SERS) [9].

Green chemistry is the utilization of a set of principles that will help reduce the use and generation of hazardous substances during the manufacture and application of chemical products. Green chemistry aims

to protect the environment not by cleaning up, but by inventing new chemical processes that do not pollute. It is a rapidly developing and an important area in the chemical sciences. Principles of green chemistry, developments in this field and some industrial applications are discussed [10, 11].

Biomolecules present in plant extracts can be used to reduce metal ions to nanoparticles in a single-step green synthesis process. This biogenic reduction of metal ion to base metal is quite rapid, readily conducted at room temperature and pressure, and easily scaled up. Synthesis mediated by plant extracts is environmentally benign. The reducing agents involved include the various water soluble plant metabolites (e.g. alkaloids, phenolic compounds, terpenoids) and co-enzymes. Silver (Ag) and gold (Au) nanoparticles have been the particular focus of plant-based syntheses. Extracts of a diverse range of plant species have been successfully used in making nanoparticles. Use of plant extracts in nanoparticle synthesis in producing nanoparticles using plant extracts, the extract is simply mixed with a solution of the metal salt at room temperature. The reaction is complete within minutes. Nanoparticles of silver, gold and many other metals have been produced this way [12].

During recent years' development and use of silver nanoparticles (AgNPs) has rapidly increased due to their unusual optical, chemical, electronic, photo electrochemical, catalytic, magnetic, antibacterial and biological labeling properties [13]. AgNPs have attracted intensive research interest because of their advantageous applications not only in biomedical [13] drug delivery [14], food industries [15], agriculture [16], textile industries [17], water treatment [18] as an antimicrobial and antifungal agent but also of their applications in catalysis, and in surface-enhanced Raman scattering [19].

The field of nanotechnology is the most active area of research in modern materials science. Though there are many chemical as well as physical methods, green synthesis of nanomaterials are the most emerging method of synthesis. We report the synthesis of antibacterial silver

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nanoparticles (AgNPs) using leaf broth of medicinal herb, *argemone mexicana* (darudi) and *ocimum tenuiflorum* (tulsi) (Fig. 1) [20].

*Ocimum tenuiflorum* (tulsi) is a medicinal herb abundantly found and cultured in India, Malaysia, Australia, West Africa, and some of the Arab countries. Tulsi leaves have been traditionally used for treatment of many infections. The antibacterial activity has been reported to be the upshot of essential oil components, mostly eugenols found in it. The present study aims at the synthesis of silver nanoparticles from the aqueous extract of tulsi leaves. We also attempt to combine the inherent antimicrobial activities of silver metal and tulsi extract for enhanced antimicrobial activity [21].



Fig. 1 *Ocimum tenuiflorum* (tulsi) and *argemone mexicana* (darudi) plant

## 2. Experimental Methods

### 2.1 Material and Physical Methods

All the reagents and metal salts of AR grade were purchased from Sigma-Aldrich and used without further purification. Solvents used for spectroscopic studies were purified and dried by standard procedures before use. All aqueous solutions were prepared from quartz distilled deionized water which was further purified by a Millipore Milli-Q water purification system (Millipack 20, Pack name: Simpak 1, Synergy). Absorption spectra were studied on a Jasco V-570 UV-Vis recording spectrophotometer. pH of the solutions was measured using pH analyzer LI 614- Elico. The particle size and zeta potential were determined by using the Malvern Zetasizer (Model; ZEN3600) as such without dilution. TEM images were recorded in MACK/model JEOL, JEM 2100 at an accelerated voltage of 200 kV. A drop of dilute solution of a sample in water on carbon coated copper grids was dried in vacuum and directly observed in the TEM. The antimicrobial susceptibility of nanoparticles was evaluated using the disc diffusion or Kirby-Bauer method and zones of inhibition were measured after 24 hours of incubation at 35 °C. The bacterial interaction with the AgNPs has been proposed as shown in Fig. 2.

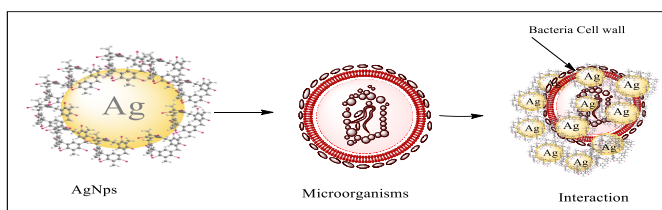


Fig. 2 proposed bacterial interaction with silver Nanoparticles (AgNPs)

### 2.2 Preparation and Characterization of Silver Nanoparticles

In a typical experiment of synthesis of silver nanoparticles, 5 mL of plant extract was rapidly added to the 25 mL of 1 mM boiling solution  $\text{AgNO}_3$  and the heating was continued for 1 hour. The colour of the solution changed to yellow. The particle size, morphology and composition of the nanoparticles were performed by means of transmission electron microscopy (TEM), energy dispersive X-ray Analysis (EDX). Samples for TEM studies were prepared by placing drops of the silver nanoparticles solutions on carbon-coated TEM grids using by just dropping very small amount of the sample on the grid and allowed to dry for analysis [22, 23].

### 2.3 Time, pH and Temperature Dependent Stability Study of AgNPs by UV/Visible Spectroscopy Measurements

It is generally believed that pH and temperature greatly affects the stability of nanoparticles. The time, pH, and temperature dependent stability of AgNPs were studied by monitoring UV/Vis-spectra upto four months, at different pH and different temperature (0-100 °C). The pH of

silver nanoparticles dispersion was adjusted using 0.1 N hydrochloric acid and 0.1 M sodium hydroxide solution (pH 4, 5, 6, 7, 8, 9 and 10) using calibrated pH meter. Change in surface plasmon resonance (SPR) of nanoparticles dispersion was recorded using UV/Visible spectrophotometer. It is generally believed that the reaction temperature will have a great effect on the rate and shape of particle formation. Therefore, we try to fabricate CPH-stabilized silver colloids at different temperatures. The reaction temperature increasing in the range 0–80 °C, the maximum absorption wavelengths of silver colloids take on a red shift (from 406 nm to 450 nm). When the reaction system reaches low temperature (0°C) and high temperature (80°C) the maximum absorption wavelength (408 nm) appears a little red shift and the stability of as-prepared Silver colloid decreases a little, which predicts that some other particles with larger sizes and different morphologies. The results showed that silver nanoparticles were stable at room temperature.

## 3. Results and Discussion

### 3.1 Characterization of Silver Nanoparticles by Spectroscopy and TEM-EDX

It shows the change in colour of the reaction medium as an effect of presence of any type of reducing substance. As per this qualitative assessment of reducing potential of tulsi extract, presence of significant amount of reducing entities was attested therein. In case of bacteria and fungi mediated synthesis of AgNPs, reduction of silver nitrate to elemental silver has been attributed to the presence of reductive enzymes. But there is controversy regarding the plant extract components involved in reduction of silver nitrate to elemental silver.

In another recent study, it has been suggested that different compounds such as caffeine and theophylline bring out the reduction processes and thus silver nanoparticles synthesis. As Tulsi possess a potent antioxidant activity, we attribute the reduction process to their presence of high quantity of antioxidants in the leaves extract.

The reduction of silver nitrate in the presence of plant extract *argemone mexicana* (darudi) and *ocimum tenuiflorum* (tulsi) results in the formation of plant extract capped silver nanoparticles (AgNPs). The synthesised AgNPs were confirmed by their colour change to yellow. The colour of synthesised silver nanoparticles was due to excitation of surface plasmon vibrations in the metal nanoparticles. It was observed that the nanoparticles were found to be stable for more than one months, with no noticeable variation in SPR band indicating that the particles are dispersed in the aqueous solution, with no evidence for aggregation [23].

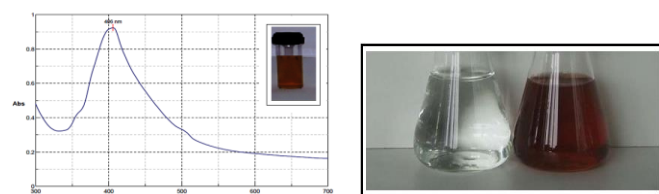


Fig. 3 SPR band of silver nanoparticles and picture of flask containing silver nitrate (1mM) before (left) and after (right) reduce by plant extract (AgNPs)

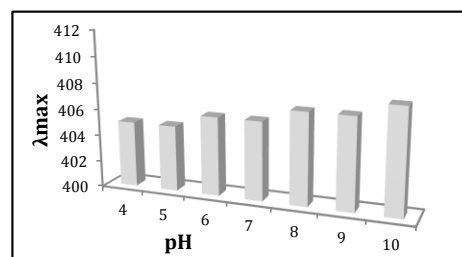


Fig. 4 Effect of pH treatment on nanoparticles

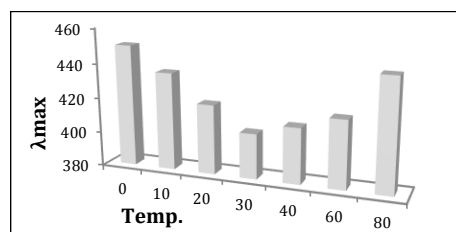


Fig. 5 Effect of temperature on nanoparticles

Also the synthesised nanoparticles did not showed any considerable change in their SPR band at different pH (4-10) (Fig. 4). But, when temperature was increased or decreased, their SPR band changed due to increase in the heat movement of molecules with the reaction temperature, while the bonding degree of CPH molecules to Ag ions decreased with the increasing temperature (Fig. 5).

A representative TEM showed that the sample was composed of a large quantity of silver nanoparticles (Fig. 6). The particle size histogram of silver nanoparticle (Fig. 6) showed that particles ranged in size from 2 to 5 nm and possessed an average size of 5 nm. The EDAX (energy dispersive analysis of X-rays) spectrum recorded in the spot-profile mode from one of the densely populated silver nanoparticle regions on the surface of film, strong signals from silver atoms in the nanoparticles were observed, while weaker signals from C, O, Si, Cu and Ca atoms were also recorded (Fig. 7).

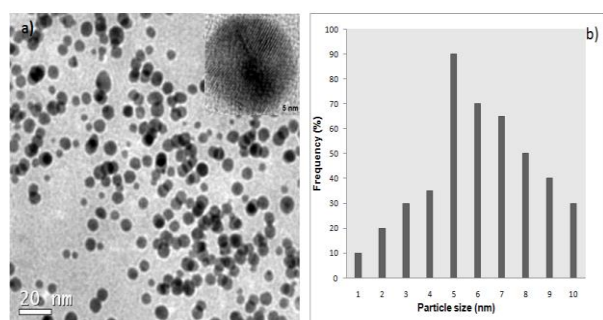


Fig. 6 (a) TEM image of AgNPs and b) particle size distribution graph

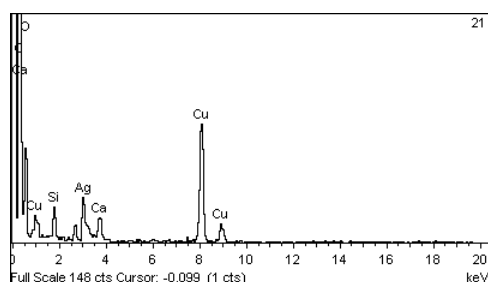


Fig. 7 EDAX spectrum recorded from drop-coated films of silver nanoparticles. Different X-ray emission peaks are labelled.

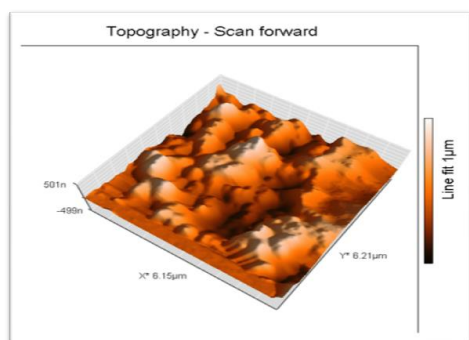


Fig. 8 Characterization of AgNp by atomic force microscopy (AFM)

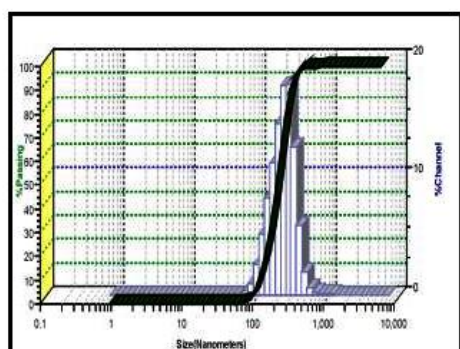


Fig. 9 Characterization of AgNp by dynamic light scattering (DLS)

### 3.2 Characterization of Silver Nanoparticles by Atomic Force Microscopy (AFM)

The particle dimensions were calculated by AFM measurements, in which a drop of the particle solution was spread onto a freshly peeled mica surface and dried under a gentle nitrogen stream. The AFM micrographs exhibit very visible and well-dispersed nanosized particles. Because of tip convolution, particle core diameter was estimated by the heights in AFM measurements. The AFM result for AgNp shows the average particles size around 185 nm and roughness is around 905 pm (Fig. 8).

### 3.3 Characterization of Silver Nanoparticles by Dynamic Light Scattering (DLS)

Dynamic light-scattering (DLS) profiles show that the average diameters of silver nanoparticles around 70 nm whereas the average diameter of (AgNps) *Ocimum tenuiflorum* (tulsi) and *argemone mexicana* (darudi) plant capped silver nanoparticles was found around 195 nm (Fig. 9).

### 3.4 Anti-bacterial Assay Study of Silver Nanoparticles

The antibacterial activity of silver nanoparticles was evaluated using the disc diffusion and Kirby-Bauer method. The antimicrobial application of the AgNPs obtained by *argemone mexicana* leaf and *ocimum tenuiflorum* was carried out using both gram positive *Staphylococcus aureus* and gram negative *Escherichia coli* and *Pseudomonas aeruginosa*. The well diffusion method was used to study the antibacterial activity of the synthesized silver nanoparticles. All the glassware, media and reagents used were sterilized in an autoclave at 100 °C for 20 min. Bacterial suspension was prepared by growing a single colony overnight in nutrient broth and by adjusting the turbidity to 0.5 McFarland standards. Mueller Hinton agar (MHA) plates were inoculated with this bacterial suspension. Sterile paper disc of 10 mm diameter containing silver nanoparticles and standard antibiotic chloramphenicol (100 μg/mL) containing discs were placed in each plate as control. The plates were incubated at 30 ± 4 °C overnight and the inhibition zones around the discs were measured and shown in Table 1.

Table 1 Antimicrobial activity (Zone of inhibition in mm) of Compounds 1-2

| Name of compound | Zone of inhibition (mm) |         |                 |         |               |         |
|------------------|-------------------------|---------|-----------------|---------|---------------|---------|
|                  | <i>Pseudomonas</i>      |         | <i>S.aureus</i> |         | <i>E.coli</i> |         |
|                  | 50 ppm                  | 100 ppm | 50 ppm          | 100 ppm | 50 ppm        | 100 ppm |
| Control          | 0                       | 1       | 0               | 1       | 0             | 1       |
| A*               | 12                      | 16      | 15              | 18      | 10            | 12      |
| 1                | 8                       | 12      | 7               | 11      | 3             | 7       |
| 2                | 10                      | 13      | 6               | 13      | 5             | 9       |

A\* - Chloramphenicol; 1 - AgNp (Tulsi); 2 - AgNp (darudi)

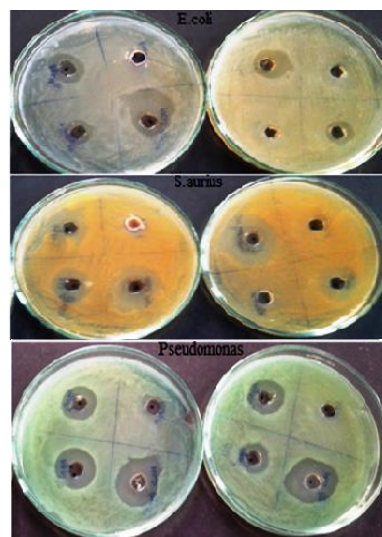


Fig. 10 optical image of anti microbial activity of antibiotics and compound against various microorganisms.

The silver nanoparticles show efficient antimicrobial property (Fig. 10) compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria. The bacterial membrane contains sulfur-containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. When silver nanoparticles

enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions [24, 25]. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity. All of the compounds have significant and potent antibacterial activity against bacteria. While silver nanoparticles is the most effective and inhibited bacterial growth more than other compound. When the obtained results are compared with standard antibiotics (chloramphenicol), silver nanoparticles have higher antimicrobial activity compared to other but slight less than standards [19,20,23,26].

#### 4. Conclusion

Synthesis of silver nanoparticles from the leaf extract of *argemone mexicana* (darudi) and *ocimum tenuiflorum* (tulsi) was confirmed by the colour changes from colourless to yellow colour, which indicated the formation of silver nanoparticles. Therefore, the growing need of developing an ecofriendly nanoparticles synthesis is possible and it can be used for various biomedical application to avoid the adverse effect chemically synthesized nanoparticle in the field of medical applications. Rapid and green synthetic methods using extracts of plant of (tulsi) and (darudi) have shown a great potential in AgNP synthesis and their antimicrobial activity also. Silver nanoparticles exhibited antibacterial activity against the common pathogens. The results of the antibacterial activity study clearly demonstrated that the colloidal silver nanoparticles inhibited the growth and multiplication of the tested bacteria, including highly multiresistant bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Such high antibacterial activity was observed at very low total concentrations of silver nanoparticles [8].

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#### References

- [1] H. Brune, H. Ernst, A. Grunwald, W. Grünwald, H. Hofmann, H. Krug, P. Janich, M. Mayor, W. Rathgeber, G. Schmid, Nanotechnology: assessment and perspectives, Springer Science & Business Media, 2006.
- [2] D.D. Evanoff, G. Chumanov, Synthesis and optical properties of silver nanoparticles and arrays, Chem Phys Chem, 6 (2005) 1221-1231.
- [3] K.M.A. El-Nour, A.A. Eftaiha, A. Al-Warthan, R.A. Ammar, Synthesis and applications of silver nanoparticles, Arabian J. Chem. 3 (2010) 135-140.
- [4] R. Jin, Y. Cao, C.A. Mirkin, K. Kelly, G.C. Schatz, J. Zheng, Photoinduced conversion of silver nanospheres to nanoprisms, Science 294 (2001) 1901-1903.
- [5] Y. Sun, Y. Xia, Shape-controlled synthesis of gold and silver nanoparticles, Science 298 (2002) 2176-2179.
- [6] R. Sathyavathi, M.B. Krishna, S.V. Rao, R. Saritha, D.N. Rao, Biosynthesis of silver nanoparticles using Coriandrum sativum leaf extract and their application in nonlinear optics, Adv. Sci. Lett. 3 (2010) 138-143.
- [7] A.J. Haes, R.P. Van Duyne, A nanoscale optical biosensor: sensitivity and selectivity of an approach based on the localized surface plasmon resonance spectroscopy of triangular silver nanoparticles, J. Am. Chem. Soc. 124 (2002) 10596-10604.
- [8] J.S. Kim, E. Kuk, K.N. Yu, J.H. Kim, S.J. Park, H.J. Lee, S.H. Kim, Y.K. Park, Y.H. Park, C.Y. Hwang, Antimicrobial effects of silver nanoparticles, Nanomed. 3 (2007) 95-101.
- [9] B.A. Makwana, D.J. Vyas, K.D. Bhatt, V.K. Jain, Y.K. Agrawal, Highly stable antibacterial silver nanoparticles as selective fluorescent sensor for Fe<sup>3+</sup> ions, Spectrochim. Acta Part A 134 (2015) 73-80.
- [10] A. Shukla, B.A. Makwana, Facile Synthesis of Silver Nanoparticle and Their Potential Application, Am. J. Nanosci. Nanotech. 2 (2014) 84-92.
- [11] Z. Wang, Q. Zhang, D. Kuehner, A. Ivaska, L. Niu, Green synthesis of 1-2 nm gold nanoparticles stabilized by amine-terminated ionic liquid and their electrocatalytic activity in oxygen reduction, Green Chem. 10 (2008) 907-909.
- [12] C. Panneerselvam, S. Ponarulselvam, K. Murugan, Potential anti-plasmodial activity of synthesized silver nanoparticle using Andrographis paniculata Nees (Acanthaceae), Arch Appl Sci Res. 3 (2011) 208-217.
- [13] U.B. Jagtap, V.A. Bapat, Green synthesis of silver nanoparticles using Artocarpus heterophyllus Lam. seed extract and its antibacterial activity, Ind. Crops Prod. 46 (2013) 132-137.
- [14] W. Siangproh, W. Dungchai, P. Rattanarat, O. Chailapakul, Nanoparticle-based electrochemical detection in conventional and miniaturized systems and their bioanalytical applications: a review, Anal. Chim. Acta 690 (2011) 10-25.
- [15] X. Li, H. Xu, Z.S. Chen, G. Chen, Biosynthesis of nanoparticles by microorganisms and their applications, J. of Nanomater. 2011 (2011) 26 pages. DOI: 10.1155/2011/270974
- [16] R. Nair, S.H. Varghese, B.G. Nair, T. Maekawa, Y. Yoshida, D.S. Kumar, Nanoparticulate material delivery to plants, Plant Sci. 179 (2010) 154-163.
- [17] F.M. Kelly, J.H. Johnston, Colored and functional silver nanoparticle- wool fiber composites, ACS Appl. Mater. Interfaces 3 (2011) 1083-1092.
- [18] M. Sweet, I. Singleton, Silver nanoparticles: a microbial perspective, Advances in applied microbiology, 77 (2011) 116-127.
- [19] V.K. Sharma, R.A. Yngard, Y. Lin, Silver nanoparticles: green synthesis and their antimicrobial activities, Adv. Colloid Interface Sci. 145 (2009) 83-96.
- [20] G. Singhal, R. Bhavesh, K. Kasariya, A.R. Sharma, R.P. Singh, Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity, J. of Nano. Res. 13 (2011) 2981-2988.
- [21] K. Mallikarjuna, G. Narasimha, G. Dillip, B. Praveen, B. Shreedhar, C.S. Lakshmi, B. Reddy, B.D.P. Raju, Green synthesis of silver nanoparticles using Ocimum leaf extract and their characterization, Dig J Nanomater Bios. 6 (2011) 181-186.
- [22] A.K. Mittal, Y. Chisti, U.C. Banerjee, Synthesis of metallic nanoparticles using plant extracts, Biotechnol. Adv. 31 (2013) 346-356.
- [23] A. Singh, D. Jain, M. Upadhyay, N. Khandelwal, H. Verma, Green synthesis of silver nanoparticles using Argemone mexicana leaf extract and evaluation of their antimicrobial activities, Dig J Nanomater Bios. 5 (2010) 483-489.
- [24] M. Rai, A. Yadav, A. Gade, Silver nanoparticles as a new generation of antimicrobials, Biotechnol. Adv. 27 (2009) 76-83.
- [25] H. Song, K. Ko, I. Oh, B. Lee, Fabrication of silver nanoparticles and their antimicrobial mechanisms, Eur Cells Mater. 11 (2006) 58.
- [26] S. Pal, Y.K. Tak, J.M. Song, Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli, Appl. Environ. Microbiol. 73 (2007) 1712-1720.