International Journal of Current Engineering and Technology
ISSN 2277 - 4106

Available at http://inpressco.com/category/ijcet

Research Article

Sliver Nanoparticles Synthesis and Stabilization by different species of *Ocimum* and Characterization for its Antimicrobial Activity

T. Tejaswi[a*], K. Venakteswara Rao[a] Ch. Shilpa Chakara[a]

[a]Centre for Nano Science and Technology, Institute of Science and Technology, Jawaharlal Nehru Technological University Hyderabad

Accepted 5 May 2013, Available online 1June 2013, **Vol.3, No.2 (June 2013)**

Abstract

Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. Present work deals a cost effective and environment friendly technique for green synthesis of silver nanoparticles from Silver Nitrate solution by co-precipitation using the leaf extract of different species of Ocimum that acts as reducing as well as capping agent. The important ingredients responsible for the formation of silver nanoparticles present in the leaf extract are triterpenes, flavonoids and eugenol. UV–Vis absorption spectroscopy was used to monitor the formation of silver nanoparticles and Characterizations performed to study different properties of the obtained particles, crystalline size and shape analysis by X-ray diffraction, surface morphology by Scanning Electron Microscope, elemental analysis by Dispersive X-ray analysis and particle size by Dynamic Light Scattering. The Fourier Transform Infrared Spectroscopy was performed to identify types of chemical bonds, i.e. functional groups in a molecule. Antimicrobial activity of Silver Nanoparticles against Klebsiella and Staphylococcus has been tested by well diffusion technique.

Keywords: *silver nanoparticles, green synthesis, ocimum, antibacterial activity.*

1. Introduction

Nanotechnology encompasses biology, chemistry, material science, physics, and engineering working at the nano level along with creating materials on the atomic level and alterations at molecular level. Nanobiotechnology is the use of nanotechnology in life sciences, or the use of biological materials to manufacture technical nano systems. The silver particles that range from 1 to 100 nm are silver nanoparticles and are one of the most commonly utilized nanomaterials as they have unique optical, electrical, thermal and anti-microbial(E. Parameswari et al,2010) properties and are being incorporated into products that range from photovoltaic to biological and chemical sensors.

A number of approaches are available for the synthesis of silver nanoparticles for example, reduction in solutions, chemical and photochemical reactions in reverse micelles, thermal decomposition of silver compounds, microwave assisted process and recently via green chemistry route. The use of environmentally benign materials like plant leaf extract (V. Parashar et al,2009), bacteria (K. Govindraju et al,2009), fungi (E. David et al,2010) (N. Roy et al,2010) (N. Saifuddin et al,2009) and enzymes for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications (S. Shrivastavaet al,2007) (V.C. Verma et al,2010)(M. Rai et al,2009) as they do not use toxic chemicals for the synthesis protocol.

Ocimum (Tulsi) is considered to be a ubiquitous aromatic plant in india that plays a vital role in Indians everyday life and is said to be the queen of herbal plants. Nearly twenty four species of *Ocimum* is found in India with basil as common name. It is sweet, peppery and offers a slight anise-like aftertaste. It is traditionally thought to stimulate the appetite and ease stomach upset acts as strong antioxidant and antimicrobial activity. In classic Indian medicine, *Ocimum* has been used to treat everything from earaches and itching to malaria, arthritis and anorexia. Like tarragon, one of basil's major volatile oils is estragole. In addition to these uses researchers found Ocimum can be used to produce Silver Nanoparticles.

In present study different species of *Ocimum* namely *Ocimum basilicum, Ocimum sanctum, Ocimum gratissimum* (Fig1) are used to synthesize Silver Nanoparticles and the produced Nanoparticles are characterized by different techniques like UV-Visible Spectroscopy, X-Ray Diffraction, Particle Analyzer, Scanning Electron Microscope to find the morphology and size.

The importance of bactericidal nanomaterials is due to the increased resistant strains of bacteria against most

*Corresponding author: T. Tejaswi

potent antibiotics and has promoted research in the antibacterial properties of Silver Nanoparticles.

Klebsiella is a genus of non-motile, Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule. This is a frequent human pathogen which can lead to a wide range of disease states, notably pneumonia, urinary tract infections, septicemia, and soft tissue infections. *Klebsiella* species are ubiquitous in nature

Figure 1: *Ocimum basilicum*, *Ocimum gratissimum* and *Ocimum sanctum*

Staphylococcus is a genus of Gram-positive bacteria. Under the microscope, they appear round (cocci), and form in grape-like clusters. *Staphylococcus* can cause a wide variety of diseases in humans and other animals through either toxin production or penetration. Staphylococcal toxins are a common cause of food poisoning, as they can be produced by bacteria growing in improperly-stored food items. The most common sialadenitis is caused by staphylococci, as bacterial infections.

2. Experimentation

Plant Extract Preparation

Fresh *Ocimum sanctum* leaf was washed several times with de-ionized water. 10g of the leaf was finely cut and were crushed and stirred with 100 ml de-ionized water at 300K for 1min and filtered by Whatman No.1 filter paper to get the extract. In the same way the leaf extracts of *Ocimum gratissimum and Ocimum basilicum* are synthesized. The filtrates are used as reducing agent and stabilizer.

Synthesis of Silver Nanoparticles

Appropriate amount of *Ocimum sanctum* leaf extract was taken in a burette. Appropriate amount of Silver nitrate solution was taken in a beaker. Beaker was placed on a magnetic stirrer. Titration was carried out by drop wise addition of leaf extract to $AgNO_3$ solution for reduction into Ag^+ ions. The mixture is thoroughly mixed for about 15 minutes on magnetic stirrer. As the titration started immediately color changes were observed from watery to yellowish brown indicating the formation of silver nanoparticles. Similarly using *Ocimum gratissimum and Ocimum basilicum* leaf extract, Silver Nanoparticles (AgNPs) were synthesized.

The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 4000 rpm for 30 mins. The supernatant was transferred to a clean dry beaker for further settlement of particles and repeated centrifugation was carried to purify AgNPs. The sample so obtained was dried in an incubator. The particles obtained were used for further characterizations. Thus the silver nanoparticles are synthesized in a simple and green approach when compared with other wet chemical hazardous methods(P.K. Sahoo et al,2009).

3. Characterization

UV-Visible absorption spectra to monitor the formation of silver nanoparticles were recorded using a double-beam UV-Vis spectrophotometer UV-2450 (Shimadzu) with the wavelength ranging from 200-1100nm. X-ray diffraction (XRD) scans were performed with Bruker D8 advanced X-ray diffractometer using CuKα radiation (λ=1.5418˚A). Dynamic Light Scattering (Model no: HORIBA Nano particle analyzer SZ-100) was used to analyse particles size distributions. The surface morphology of the obtained silver nanoparticles was examined by using Hitachi VP-SEM S-3400N machine. Dispersive X-ray analysis (EDX) was performed to obtain the elemental analysis of the obtained sample. The Fourier Transform Infrared Spectroscopy (FTIR) was performed to identify types of chemical bonds, i.e. functional groups in a molecule

(Model no: Bruker FT-IR spectrometer) over the wave number range of 4000-500 cm^{-1}.

4. Well diffusion method

The sterilized nutrient agar was poured (20ml/plate) onto the Petri plates & left for a while till the agar gets solidified. 2 pathogenic strains *Klebsiella* and *Staphylococcus* were taken .Fresh overnight cultures of inoculum (100 µl) of each culture were spread on to Nutrient agar plates surface evenly in 4 different petri plates respectively.

The wells were casted by porer. The samples (containing 50mg/litre silver nanoparticles) along with standard antibiotic were loaded with equal volume on the plates. Control plate contained distilled water; antibiotic.The square plates were incubated at room temperature for 19-24 hrs. The activity was clearly visible from19-24 hrs on the plates. The zone of inhibition was measured & the sample of the silver nanoparticles showing maximum antimicrobial activity was noted.

5. Results and discussion

UV-Visible Spectroscopy

The reduction of pure Ag^{+} ions was monitored by measuring the UV-Vis spectrum of the reaction medium by diluting a small aliquot of the sample in distilled water. From UV results obtained, it is evident that the silver nanoparticles were formed & this was confirmed by the surface Plasmon resonance exhibited & the UV range obtained lies in the range of 367.2-444[11,12]. For *Ocimum sanctum*, Silver nano particles exhibited maximum absorption peak at 329 nm, In the case of *Ocimum gratissimum*, the absorption band of silver nano particles shifted to 333.2nm. For *Ocimum basilicum*, Silver nano particles exhibited absorption peak at 372.8 nm. The flat curves of the graph represent the polydispersed particles in the solution. (Fig2)

XRD Analysis

The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherer formula.

$D = 0.94 \lambda / \beta \cos \theta$

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.

The XRD pattern showed four intense peaks in the whole spectrum of 2θ value ranging from 35 to 80 and revealed that the sample contains Face centered cubic lattice structures of silver nanoparticles and the average crystalline size range from 21nm to 27nm. (Fig3)(K. Mallikarjuna et al,2009)

Dynamic Light Scaterring

Silver nanoparticles synthesized using different leaf extracts were characterized by DLS for particle size distribution analysis. DLS was performed for samples synthesized by various leaf extracts i.e *Ocimum sanctum, Ocimum gratissimum, Ocimum basilicum.* From the DLS results (Fig4) it can be concluded that particle size is nearly large (42.8nm) in *Ocimum sanctum*. This may be because the particles are not protected from agglomeration. Whereas *Ocimum gratissimum, Ocimum basilicum* synthesized particles has the average particle size smaller around 35.9, 34.8, respectively. But however, it is observed that the synthesized silver nanoparticles are maximum distributed within the range of 30-50 nm notably & also shows polydispersity. The dispersity of the silver nanoparticles was given for the first time.

SEM with EDAX

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine and the image showing the high density silver nanoparticles synthesized by the leaf extract further confirmed the development of silver nanostructures. The nature of silver nanoparticles was found to be amorphous. The SEM images also showed the formation of different structures of silver nanoparticles like porous surfaced with spherical and rod shaped nanoparticles along with rocky surfaced spherical nanoparticles and flake surfaced spherical and whiskers in *Ocimum sanctum, Ocimum gratissimum and Ocimum basilicum* synthesised silver nanoparticles respectivley. (Fig5a, 5b)

Energy Dispersive X-ray analysis (EDX) is available along with SEM. The peaks obtained from EDX gives the elemental composition of the sample and found to have silver. The EDX analysis shows that the samples of AgNPs produced by *Ocimu sanctum, Ocimum gratissimum, Ocimum basilicum* are 93.96%, 93.14% and 98.4% respectively of silver element in its composition i.e., The energy-dispersive spectroscopy (EDS) of the nanoparticles dispersion confirmed the presence of elemental silver with traces of Oxygen as impurities. These compounds are assumed to be present as components due to oxidation of metal nanoparticles. Among all the samples of *Ocimum basilicum* is found to be pure with less impurities. In the present synthesis EDAX shows high percentage of Silver indicating the purity of synthesized sample.

FTIR Analysis

Fourier-transform infrared spectroscopy was used for the analysis of the reduced silver. The spectrum was taken in the mid-IR region of 400–4000 cm-1 with 16 scan speed. The spectrum was recorded using attenuated total reflectance (ATR) technique. The samples were mixed with pure KBr crystals in the ratio of 1:100 and the pellets were fixed in the sample holder for the analysis.

The FTIR spectrum of silver nanoparticles is shown in Fig. 6. The bands obtained for different AgNPs produced from different species of *Ocimum* are almost same with

little variations at absorbed wavelengths and percentage transmittance. The band near 3400 cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols. The peak around 1635 cm^{-1} corresponds to N-H bend primary amines. The peak around 1384 cm^{-1} corresponds to C-N stretching of aromatic amine group.

Antibacterial Activity

The antibacterial activity of the sample was identified by the formation of Zone of Inhibition. Zone of Inhibition is the area on an agar plate where growth of a control organism is prevented by an antibiotic usually placed on the agar surface. If the test organism is susceptable to the antibiotic, it will not grow where the antibioitic is present. The size of the zone of inhibition is a measure of the compound's effectiveness, the larger the clear area around the antibiotic, the more effective the compound.

The activity of the sample was observed by the formation of Zone of inhibition after 24hours (Fig.7) The Zone of Inhibitions of different bacteria is given in the figure. The control plates show the growth of bacteria in the absence of antibacterial agents. The clear zone surrounding the sample in the remaining plates shows the activity of the sample. The zone surrounding the sample is clear that shows complete zone of inhibition. The space surrounding the complete zone of inhibition is partial zone of inhibition where the activity decreases than complete zone of inhibition. Comparing the two, inhibitory activity of silver nanoparticles on both the organisms is almost same.

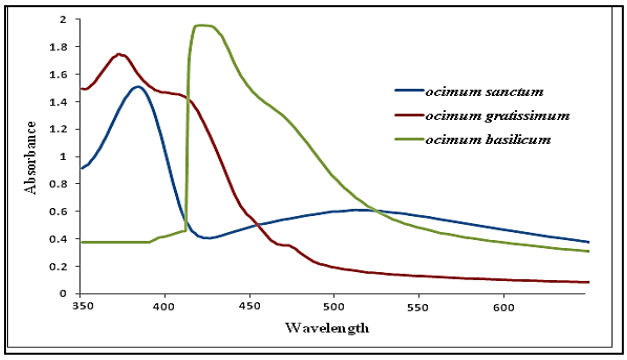


Figure2: UV-Visible analysis

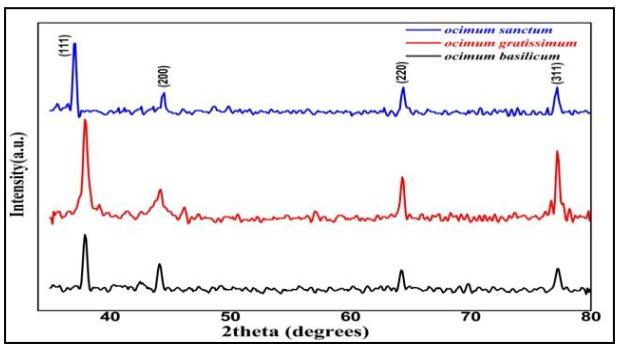


Figure3: XRD results

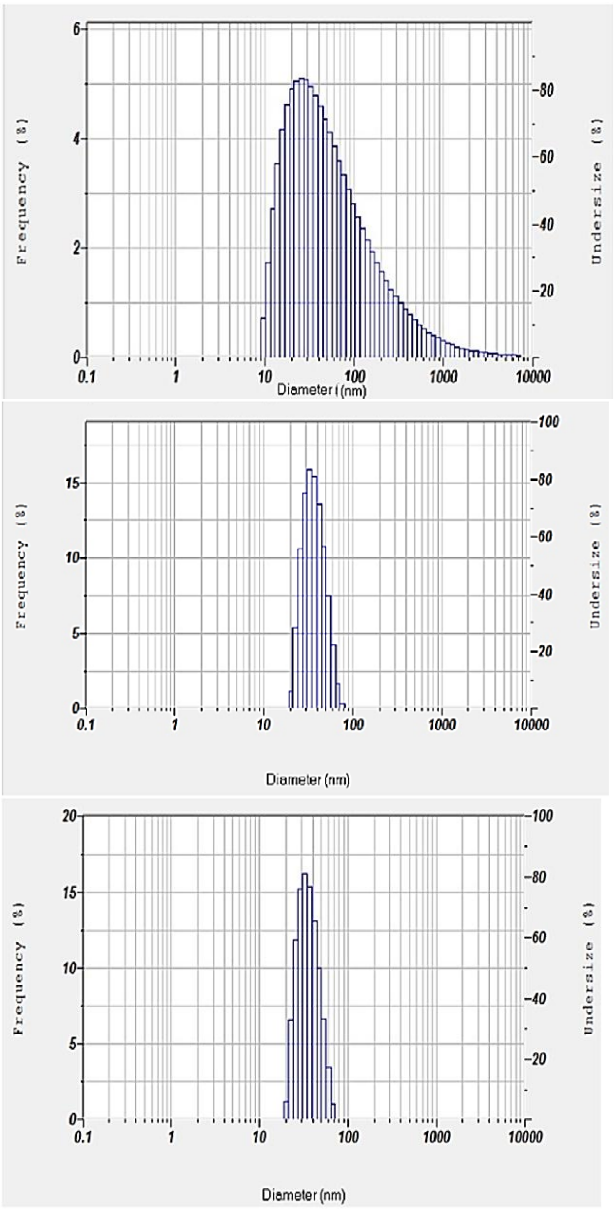
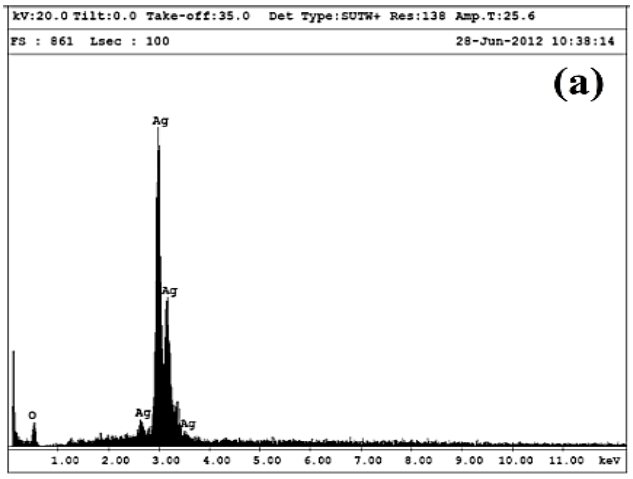


Figure 4: Particle size distribution of different Silver Nanoparticles produced by *Ocimum sanctum*(a), *ocimum gratissimum*(b), *ocimum basilicum*(c)



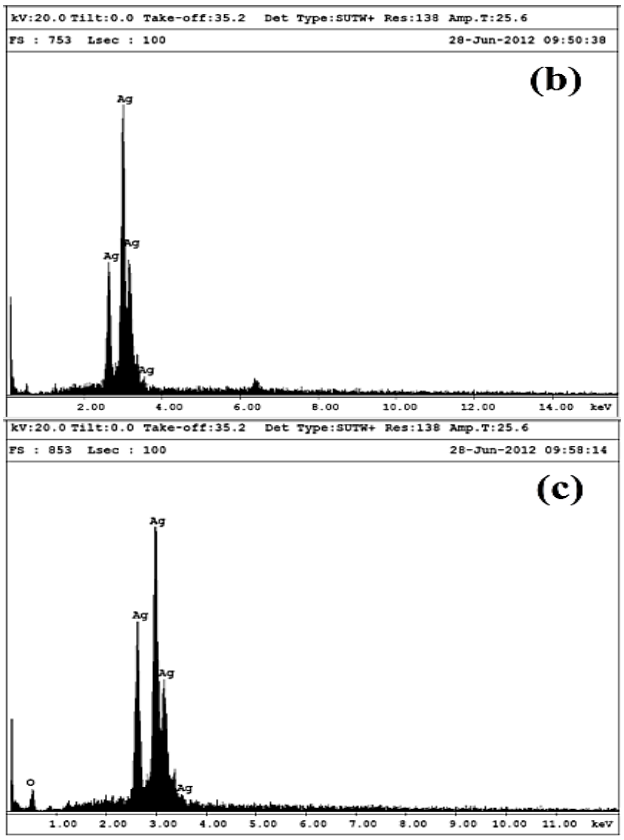
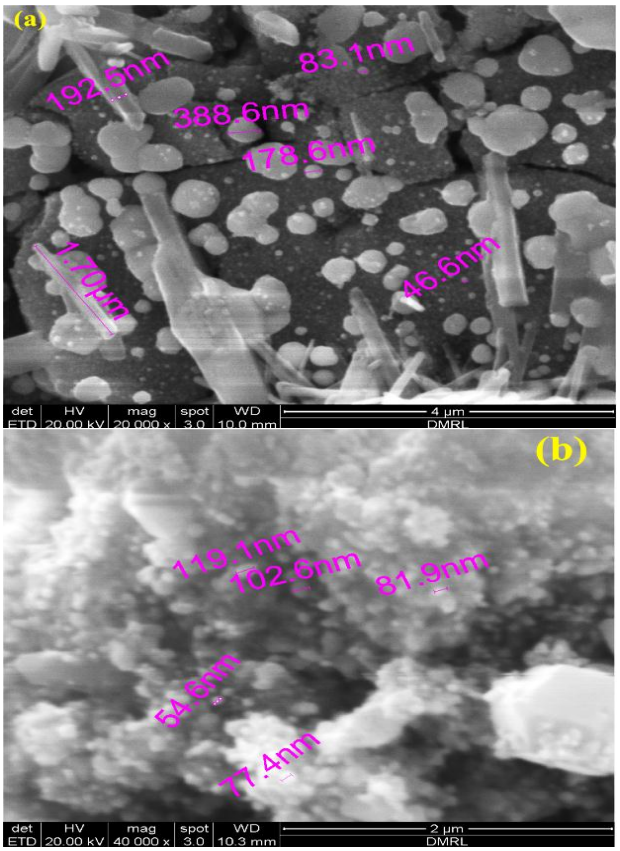


Figure 5b: EDX images of different Silver Nanoparticles produced by *Ocimum sanctum*(a), *ocimum gratissimum*(b), *ocimum basilicum*(c)



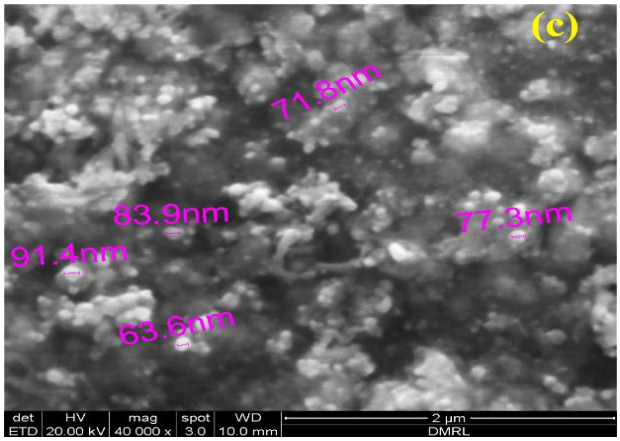


Figure 5a: SEM images of different Silver Nanoparticles produced by *Ocimum sanctum*(a), *ocimum gratissimum*(b), *ocimum basilicum*(c)

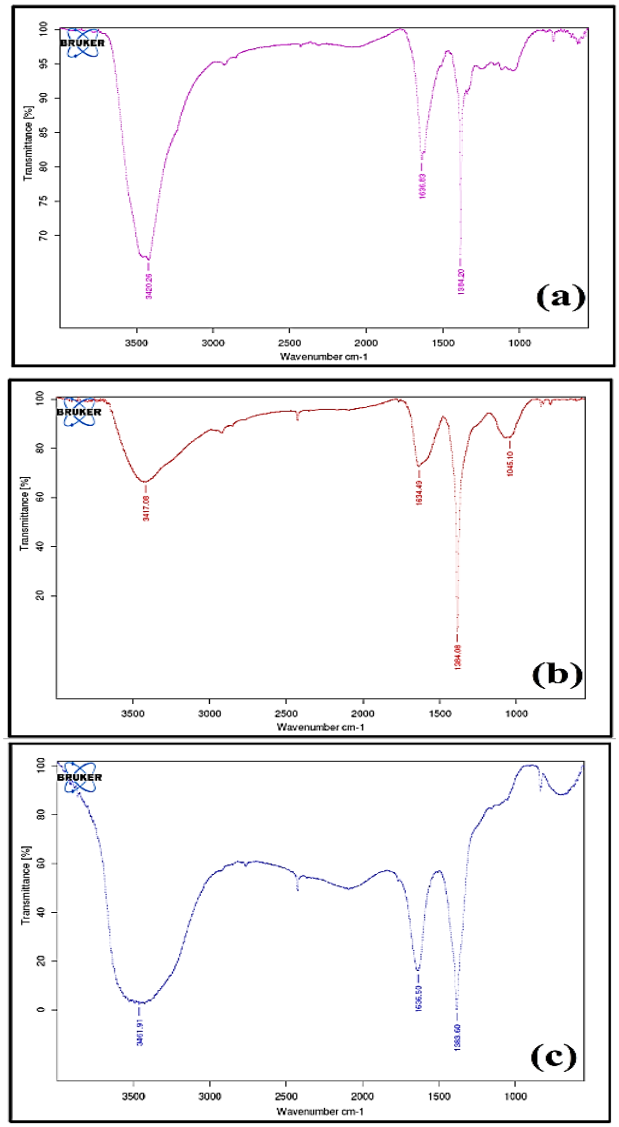


Figure 6: FTIR spectra of different Silver Nanoparticles produced by *Ocimum sanctum*(a), *ocimum gratissimum*(b), *ocimum basilicum*(c)

Figure 7: Inhibitory Zones of Silver Nanoparticles produced by different species of *Ocimum sanctum*(1), *ocimum gratissimum*(2), *ocimum basilicum*(3) aainst *klebsiella* and *staphylococcus*

Conclusion

The bio-reduction of aqueous Ag^{+} ions by the leaf extract of different species of *Ocimum* plants has been demonstrated. The reduction of the metal ions through leaf extracts leading to the formation of silver nanoparticles of fairly well-defined dimensions. This green chemistry approach towards the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, etc. XRD, UV-Visible spectroscopy, results states that the size of particles differs as the species of leaf extract changes. According to SEM images *Ocimum santum* produced a mixture of rods and spherical nanoparticles and according to EDX, *Ocimum basilicum* sample is found to be the purest containing highest % of silver with very less impurities and the other species with less amount impurities. From the analysis of FTIR peaks we can conclude that the AgNPs were surrounded by terpenoids, flavanoids and eugenol having functional groups of alcohols, phenols, amines, carboxylic acids, ethers and esters. The antibacterial activity of silver nanoparticles was confirmed by Zone of inhibition. As the diameter of the zone of inhibition is high, we can conclude that silver is a very effective antibacterial agent. The activity of silver is effective against both the bacteria which gives a conclusion that it is effective against gram +ve and gram –ve bacteria. The further activity of silver on other micro organisms like fungi, virus, etc should be studied. Basing on these results silver can be used to cure the diseases caused by bacteria especially. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications makes this method potentially exciting for the large-scale synthesis of other nano materials. Toxicity studies of silver nanoparticles on human pathogen opens a door for a new range of antibacterial agents.

References

E. Parameswari (Oct. 2010), The bactericidal potential of silver nanoparticles. International Research Journal of Biotechnology, vol. 3, pp. 044-049,.

V. Parashar (Mar. 2009), Parthenium leaf extract mediated synthesis of silver nanoparticles: a novel approach towards weed utilization. Digest Journal of Nanomaterials and Biostructures, vol. 4(1), pp. 45 -50

K. Govindraju (Sep. 2009), Extracellular synthesis of silver nanoparticles by a marine alga, Sargassum wightii Grevilli and their antibacterial effects. Journal of Nanoscience and Nanotechnology, vol. 9(9), pp. 5497 – 5501

E. David (2010), Green synthesis of silver nanoparticle using Euphorbia hirta L and their antifungal activities. Archives of Applied Science Research, vol. 2(6), pp. 76-8.

N. Roy. and A. Barik (2010) Green synthesis of silver nanoparticles from the unexploited weed resources. International Journal of Nanotechnology and Applications, vol. 4(2), pp. 95-101

N. Saifuddin(2009),Rapid Biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. The Electronic Journal of Chemistry, vol. 6(1), pp. 61-70

S. Shrivastava (April. 2007),Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnology, vol. 18(22), pp. 9

V.C. Verma (Jan. 2010), Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus Aspergillus clavatus. Journal of nanomedicine, vol. 5(1), pp. 33-40

M. Rai.(Feb. 2009), Silver nanoparticles as a new generation of antimicrobials. Biotechnology advances, vol. 27(1), pp. 76-83

P.K. Sahoo(July. 2009), Synthesis of Silver Nanoparticles using Facile Wet Chemical Route. Defence Science Journal, Vol. 59(4), pp. 447-455

Qingzhi. Wu. (2008),Biomolecule-Assisted Synthesis of Water-Soluble Silver Nanoparticles and Their Biomedical Applications Inorganic Chemistry, Vol. 47(13), pp. 5882-58888

M. Mano priya, B. Karunai selvi, J.A. John paul (June 2011), Green synthesis of silver nanoparticles from the leaf extracts of euphorbia hirta and nerium indicum. Digest Journal of Nanomaterials and Biostructures, Vol. 6(2), pp. 869 – 877

K. Mallikarjuna. G. Narasimha. G. R. Dillip. B. Praveen. et.al.(March 2011), Green synthesis of silver nanoparticles using *ocimum* leaf extract and their characterization. Digest journal of nanomaterials and biostructures, vol. 6(1), pp. 181 – 186.