Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)
ISSN: 2349-2759
Available online at www.jipbs.com

Research article

Eco friendly green synthesized silver nanoparticle with *Ocimum basilicum leaves aqueous extract*

Deepa M K[1*], Suryaprakash T.N.K[2], Pawan Kumar[3]

[1]*Research Scholar, Department of Pharmacy and Medical Sciences, School of Pharmacy and Medical Sciences, Singhania University, Rajasthan, India.*
[2]*Department of Pharmaceutics, Al Shifa College of Pharmacy, Kizhattur, Poonthavanam Post, Perintalmanna, Kerala, India.*
[3]*Department of Life Sciences, Singhania University, Rajasthan, India.*

Key words: *Ocimum basilicum*, aqueous extract, Silver nanoparticles, TEM, XRD, Zeta-potential.

***Corresponding Author: Deepa M K,** Research Scholar, Department of Pharmacy and Medical Sciences School of Pharmacy and Medical Sciences, Singhania University, Rajasthan, India.

Abstract

Aqueous extract of *Ocimum basilicum* is used as reducing agent as well as capping agent for the environmentally friendly syntheses of silver nanoparticles. The nanoparticles were characterized by UV–Visible spectral analysis, Transmission electron microscopy (TEM), X –ray diffraction (XRD), zeta potential and FTIR analysis. Silver nanoparticle with in the size range 50-100 nm having symmetric SRD band centered on 436 nm are obtained for the colloid synthesized at room temperature. The crystallinity of the nanoparticles is confirmed from XRD pattern and which also showed a good result in zeta potential. From the FTIR study it is revealed that biomolecules present in extract are responsible for capping as well as stabilization of silver nanoparticle.

Introduction

Biosynthesis of metal nanoparticle using natural products is now an emerging area of nano science research [1]. There are several reports on use of medicinal plant extract for the synthesis of silver nanoparticles and also laboratory studies suggest that colloidal silver preparations possess antibacterial, antifungal, and anti-inflammatory properties, [2-5] although there is a lack of human research on the safety and effectiveness of oral colloidal silver preparations. In the present work, we chose leaf part of *Ocimum basilicum* plant for the green synthesis of silver nanoparticles.

Sweet Basil (*Ocimum basilicum*) is unusual among the many culinary herbs in the mint family Lamiaceae in that it is thought to have its origins in India. This plant has been cultivated in India and the Middle East since ancient. *Ocimum basilicum*, a well-known traditional medicinal plant on the Indian subcontinent, is incorporated in a number of herbal preparations for the treatment of various ailments. *Ocimum basilicum* extract, flavonoids, seed oil, phenolic compounds, root extract, leaf extract, aqueous extract, fixed oil, fresh leaf paste and leaves in the diet, have been studied and have shown promising results for bone marrow radioprotection chemoprotection, anti-ulcer activity, Hypoglycemic activity, and immunostimulant effects[7] .

In our study, we use of *Ocimum basilicum* for the Synthesis of Silver nanoparticles. From the study it revealed that chemical constituents such as proteins, carbohydrates, flavonoids and phenols might be contribute not only in capping but also play an important role in reducing the ions to the nanosize.

Experimental

Materials and Methods

Materials

All reagents used were of analytical grade. Deionized water was used throughout our study.

Plant Collection and Authentication

The leafy parts of the *Ocimum basilicum*is collected during March-April month and washed with water. Then 10 kg of the plant material was shade dried for 10 days until they were free from the moisture. The dried plant material was powdered using mechanical grinder to get uniform coarse particles. The powdered plant material was stored in polythene air tight containers at room temperature.

The leaves were collected from adjoining areas of Kalpathy, Palakkad district of Kerala, and authenticated by Dr. Udyan P.S., Assistant Professor, P.G. Department of Botany and Research Centre, Sree Krishna College, Ariyannur P.O., Guruvayur, Thrissur, Kerala, India. A Herberium specimen no. 115of the same was deposited in the P.G. Department of Botany and Research Centre, Sree Krishna College, Thrissur.

Methods

Phytochemical Screening

Phytochemical Screening of the aqueous extract of *Ocimum basilicum* was screened for presence of phytochemicals like

terpenoids, flavonoids, and alkaloids etc. using standard colour tests.

Synthesis of Silver Nanoparticles

Ocimum basilicum leaves are washed several times with de-ionized water. The leaves of the plants were subjected to size reduction to get a coarse powder by passing through sieve number 40 to get uniform size. These coarse powders were subjected to standardization with different parameters as per standard procedures. 100 g of powder was infused and boiled with 1 liter of de-ionized water at 60^{o}C for 5 min, followed by filtration (Whatman grade 1) and evaporation of the solvent under water batch at 60^{0}C until getting a semisolid material. The resultant green color residue (crude extract) was stored in a refrigerator at 6^{o}C for further studies. Plant extract was added to Silver nitrate solution (10^{-3} M) to make up to a final volume of 100ml and it was centrifuged at 18000 rpm for 25 min. Collected pellets were stored at -40^{o}C. The supernatant was heated at 100^{0}C.A change in the color of solution was observed during heating process.

Characterization of silver nanoparticles

UV-VIS Spectral Analysis

The reduction in Ag+ ions was monitored by UV-VIS spectra of the silver nanoparticle solution after diluting a small aliquot of the sample into de-ionized water and the UV-VIS spectra were recorded by Shimadzu UV-1800 Spectrophotometer from 200-800 nm.

Particle size distribution

The average particle size and polydispersity index (PDI) of the nanoparticles synthesized with the extract is determined by using Zeta-sizer DST ver.5.03(Malvern instrument).the samples of nanoparticle dispersions were diluted to four times their volume with 0.05M NaCl. The particle size and PDI are represented by the average diameter of Gaussian distribution function in the logarithmic axis mode.

Zeta potential

Zeta potential of nanoparticles from both the species was determined by the electrophoretic mobility applying the Helmholfz-Smoluchowsky equation for the measurement of Zeta potential using Zetasizer DST ver.5.03 (Malvern instrument) was used. Samples were adjusted to a conductivity of 80µs/cm with a solution of 0.05 M NaCl at the field strength of 20 V/cm.

Particle shape

TEM technique was employed to visualize the size and shape of Ag nanoparticles. HR-TEM measurements were made using a 200 kV Ultra High Resolution Transmission Electron Microscope (Joel/JEM 2100). TEM grids were prepared by placing a drop of particle solution on a carbon-coated copper grid and drying under a lamp.

XRD Analysis

XRD analysis of the prepared sample of Ag nanoparticles was done using a Bruker AXS D8 Advance diffractometer, using Cu-Kα X-rays of wavelength (λ)=1.54056 Å as source and operated at a voltage of 40 kV and a current of 35 mA. The sample was scanned in the 2θ range from 10° to 80° with a step size 0.02° and step time 32.8 s. XRD patterns were analyzed to determine peak intensity, position and width. Full width at half-maximum (FWHM) data was used with the scherrer's formula determine to mean particle size. Scherrer's equation is given by

$D=0.9\lambda/\beta\cos\theta$ Where d is the mean diameter of the nanoparticles, λ is the wavelength of X-ray radiation source, β is the angular FWHM of the XRD peak at the diffraction angle θ.

Results and Discussion

Preliminary phytochemical analysis

The preliminary phytochemical analysis of leaf extracts Ocimum *basilicum*. Revealed the presence of amino acids, flavonoids, sterols, terpenoids, proteins, and phenolic compounds, whereas saponin and tanins were not detected in aqueous extract of *Ocimum basilicum*.

FTIR analysis

Fourier transform infrared (FTIR) technique was used to study the presence of phytochemical constituents. FTIR spectrum of Silver nanoparticle was recorded using FTIR spectrometer Thermo Nicolet; Avatar 370.The instrument is equipped with a deuteratedtriglycinesulphate (DTGS) detector. The Alpha-P ATR accessory is equipped with a single-reflection diamond ATR hemisphere and a spring-loaded mechanical press for compacting solid samples at the ATR waveguide surface with uniform and reproducible pressure. Data were recorded in the MIR spectral range from 4000–375 cm^{-1} at a spectral resolution of 2 cm^{-1}. 200 scans were averaged for background and sample spectra, respectively.

In our study also we use of *Ocimum basilicum* for the Synthesis of Silver nanoparticles. The plant extract may act as reducing and capping agents in silver nanoparticles synthesis.

UV-VIS Spectral analysis

The synthesized nanoparticles were primarily characterized by UV Visible Spectroscopy, which proves to be a very useful technique for the analysis of nanoparticles. When extract is added to silver nitrate solution, a colour change is observed from golden yellow colour to red. This is due to the excitation of the surface plasma vibrations, which indicates the formation of the Silver nanoparticles [8]. UV-Visible Spectrograph of Silver nanoparticles has been recorded as a function of time intervals such as 10 min, 20 min up to 70 min by using quartz cuvette with deionized

water as the reference. A stable UV spectrum absorption is recorded at 436 nm with in 30min (Figure 1).

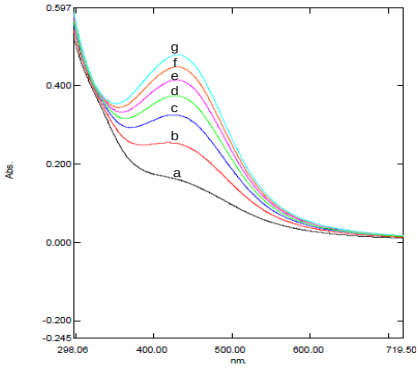

Figure 1. UV-visible spectra of Silver nanoparticles of *Ocimum basilicum* at different time (a-10min, b-20min, c-30min, d-40min, e-50min, f-60min and g-70min).

XRD analysis

The crystalline nature of silver nanoparticles with *Ocimum basilicum* was confirmed by the X-ray diffraction (XRD) analysis. Five diffraction peaks observed at 28.025^0,37.825^0, 40.340^0, 49.915^0 and 66.144 and 73.5345 in the 2θ range 20^o- 80^0 can be described to the (111), (200), (220), (311) and (222) reflection planes of a face- centered cubic (fcc) structure of Ag phases(JCPDC, file no:04-0783).Similar results were reported by Daizy Philip *et.al* [8]. XRD pattern of dried powder of gold nanoparticles was shown in the Figure.2 In addition, residual peaks were also observed at 28.239, 46.214, 66.229 and 73.534. These peaks are due to the constituents present in *Ocimum basilicum* extract. Generally, the broadening of peaks in the XRD patterns of solids is attributed to particle size effect. The broader peaks represent small size particles and that reflect the effects of experimental condition on the nucleation and crystal growth. The average crystalline size was calculated using Debye scherrer equation with the width of the (111) peak was found to be 31.8 nm [10].

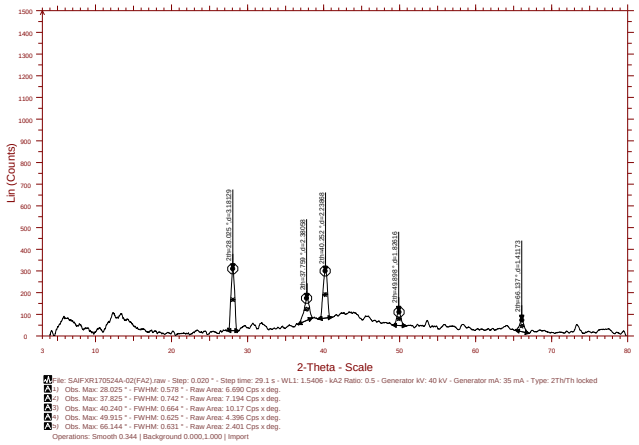

Figure 2. XRD pattern of synthesized silver nanoparticles of *Ocimum basilicum.*

FTIR analysis

FTIR measurements were carried out to study the possible chemical constituents contribute for the capping and efficient stabilization of silver nanoparticle synthesized by the *Ocimum basilicum* extract. FTIR spectra of the *Ocimum basilicum* extracts and biosynthesis silver nanopartcles synthesized using *Ocimum basilicum* extract with 10^{-3}M Ag NO_3 solution are given in Figure 3 and Figure 4 respectively.

According to Figure 4 strong peak at 3392 cm^{-1} clearly indicate the –N-H-stretches, whereas which was not found in Figure 3 due to the absence of Ag NO_3 solution. The band at 2933 cm^{-1} for carboxylic acid O-H stretching and the band at 1589 cm^{-1} in silver nanoparticles may attribute to –c=c- stretching mode which is already reported [12]. FTIR analysis corresponding to vibrational bands such as –C=C(ring),-C-O,-C-O-C are derived from water soluble compounds such as flavonoids and phenol present in *Ocimum basilicum* leaves. Hence those biomolecules might be responsible for capping and efficient stabilization.

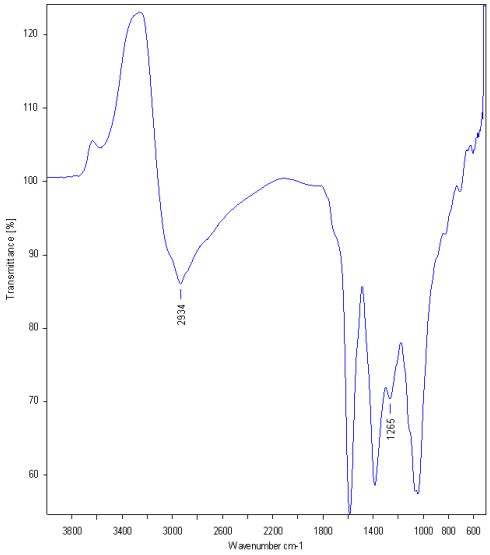

Figure 3. FTIR Spectrum of aqueous extract of *Ocimum basilicum.*

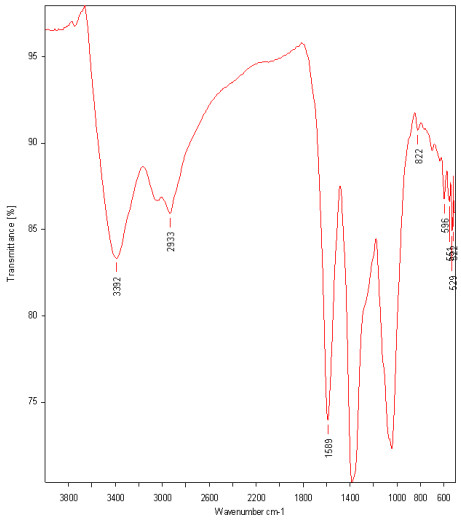

Figure 4. FTIR Spectrum of Silver nanoparticle synthesized *Ocimum basilicum* extract with 10^{-3}M Ag NO_3 solution.

Transmission electron microscopy (TEM)

In continuation, we characterized the morphology of AgNP by TEM analysis. The obtained micrograph of AgNp is shown in Figure 5. It is found to be that AgNP were successfully prepared in spherical shape. The particle size distribution was found to be with and it also matches with data obtained from XDR pattern Transmission electron microscopy (TEM) has provided further insight into the morphology and size details of the silver nanoparticles. A representative TEM image recorded from the silver nanoparticles solution is shown in Figure 5.

The Figure 5 shows individual silver particles as well as a number of aggregates. . In this micrograph, the major nanoparticles were spherical in shape and they were distributed in the range of 10-100nm diameter.

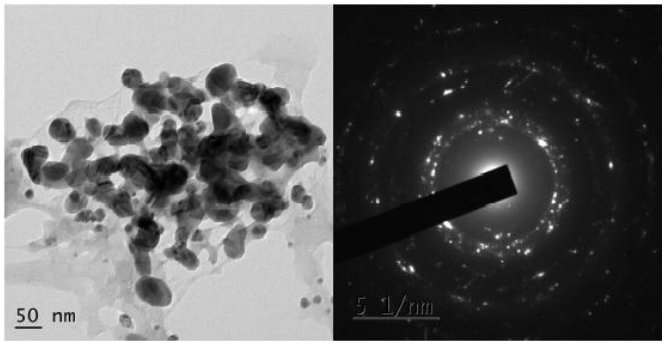

Figure 5. TEM Image of Silver nanoparticle with *Ocimum basilicum.*

Zeta potential

The zeta potential of silver nanopaticles dispersed in 0.0005M phosphate buffer of pH 6.8 was determined by a zeta meter as shown in Figure 6. The directional movement of AgNPs from the formulation was observed and averaged from 3 determinations was calculated as -17.4±9.11 mV.

The hydrodynamic diameter of the aqueous solution of AgNP as shown in Figure 7 with a mean diameter of 95.47nm. However, the size of silver nanoparticles obtained by zeta sizer is greater than that obtained through transmission electron microscopy (TEM). This might be due to the fact that the particle size in dynamic light scattering is augmented substantially by the hydrated capping agents (probably protein) or from solvation effects. In such cases the hydrodynamic diameter could be as high as 3 times the original diameter of the capped particles [10]. Similar results were also reported [11].

The result obtained for zeta potential and poly-dispersed index (PDI) are -17±9.11 mv and 0.256 respectively, those indicate that colloidal dispersion of silver nanoparticle are not aggraded much and also confirmed the stabilization of nanoparticles by capping agent.

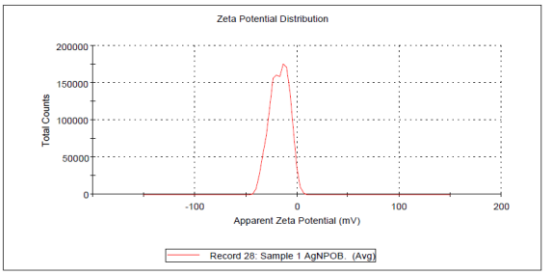

Figure 6. Zeta potential of silver nanoparticle synthesized with *Ocimum basilicum*

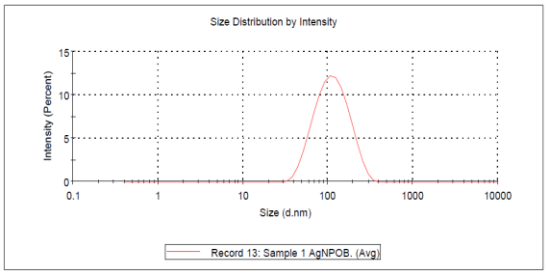

Figure 7. Particle size distribution of silver nanoparticle synthesized with *Ocimum basilicum*

Conclusion

An Eco friendly green synthesized silver nanoparticles with aqueous leaf extract of *Ocimum basilicum*. The synthesized Silver nanoparticle was characterized by UV visible spcrophotometry, TEM, XRD, Zeta potential, particle size distribution and FTIR. The characterization parameters reveal that the synthesized silver nanoparticles are in range of 10-100nm and are stable. From TEM analysis the bright spots in the SAED pattern shows the crystalline nature of the nanoparticles and also confirmed by the peaks in the XRD pattern. From the FTIR study it was found that biomolecules from the plant extract are responsible for capping and stabilization of green synthesized silver nanoparticle. From this study it can be conclude that the synthesized nanoparticles are in the Nano range and are stable, crystalline nature. These properties enhance the penetration in to the cells. It can be used for further *in vitro* and *in vivo* pharmacological studies.

Acknowledgements

The authors thank the SAIF, Cusat, Cochin, Kerala for SEM, XRD and FTIR measurement. Also thanks to Dr. Udyan P.S., Professor, Sree Krishna College, Guruvayur, Thrissur, Kerala, India for identification and authentication of the plant.

Conflict of Interest

All the authors declare that there is no conflict of interest regarding the publication of this article.

References

1. Kalaiarasi R, Jayalakshmi N and Venkatachalam P: Phytosynthesis of Nanoparticles and Its Applications - A Review. Plant Cell Biotechnology and Molecular Biology 2010; 11:1–16.
2. Prabakaran M, Subha K, Thennarasu V, Merinal S: Biosynthesis of silver nanoparticles using Sphaerulina albispiculata and evaluation of antibacterial activity. European Journal of Experimental Biology 2012; 2 (1):297-303.
3. Jain D, Daima HK, Kuchhwaha S, Kothari SL: Plant mediated silver Nanoparticles using papaya fruit extract and evalution of their antimicrobial activates. Digest Journal of Nanomaterials and Biostructures 2009; 4: 557-563.
4. Virender K S, Ria AY, Yekaterina L: Silver nanoparticles: Green synthesis and their antimicrobial activities. Advances in Colloids and Interface Science 2009; 145: 83-96.
5. Dasgupta T, Rao A R and Yada P K: Chemomodulatory efficacy of Basil leaf (Ocimumbasilicum)on drug metabolizing and antioxidant enzymes, and on carcinogen-induced skin and fore stomach papillomagenesis. Phytomedicine2004; 11:139–151.
6. Anitha P, Sakthivel P: Microwave Assisted Synthesis and Characterization of Silver Nanoparticles Using Ocimum basilicum and its Anti-Inflammatory Activity against Human Blood Cells. International Journal of Science and Research 2016; 5(1):1422-1428.
7. Surender Singh: Evaluation of gastric anti-ulcer activity of fixed oil of Ocimumbasilicum Linn. and its possible mechanism of action. Indian Journal of Experimental Biology 1999;36:253–257.
8. Daizy Philip, Unni C: Extracellular biosynthesis of gold and silver nanoparticles using Krishna tulsi (Ocimum sanctum) leaf. Physica E: Low-dimensional Systems and Nanostructures 2011;43 (7):1318-1322.
9. Leff DV, Brandt L and Heath J R: Synthesis and characterization of hydrophobic, organically soluble gold nanocrystals functionalized with primary amines. Langmuir 1996;12(20):4723-4730.
10. Mukherjee P, Roy M, Mandal BP, Dey GK, Mukherjee PK, Ghatak J, Tyagi AK, Kale SP: Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus T. asperellum. Nanotechnology 2008; 20; 19(7): 075103.doi: 10.1088/0957-4484/19/7/075103.
11. Maliszewska K, Szewczyk K and Waszak J: Biological synthesis of silver nanoparticles. Journal of Physics Conference Series 2009; 146:120-126.