Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2017; 9(8): 610-616
doi: 10.25258/ijpcr.v9i08.9587
ISSN- 0975 1556

Research Article

Green Synthesis of Silver Nanoparticles from the Leaf Extract of *Volkameria inermis*

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Available Online:25th August, 2017

ABSTRACT

Nanoparticles are gaining interest in biomedical applications due to its importance such as anti-bacterial, anti-fungal and anti-cancer agents. Conventional methods for the synthesis of metal nanoparticles involves toxic reagents which produce harmful by-products and are hazardous to the environment. To overcome these limitations, green synthesis of nanoparticles was established. Eco-friendly methods using plant extracts are gaining popularity due to the abundance of raw materials and the production of non-toxic by-products threatening to the environment. Moreover, the nanoparticles synthesized from the plant extract are cost-effective. In addition, nanoparticles produced by green synthesis methods produces synergetic effect where both the nanoparticles as well as the natural bioactive constituents of the plant influence the biocidal properties. Different methods namely heating in water bath, microwave oven and exposure to bright sunlight were adopted for the synthesis of silver nanoparticles. Plant extract based synthesis of silver nanoparticles was eco-friendly and shows an alternative promise in bio-medical applications and it undertakes the negative effects of synthetic drugs.

Keywords: Nanoparticles; Green synthesis; Plant-extract; Synthetic drugs.

INTRODUCTION

Nanotechnology an emerging field of nanoscience deals with nano size particles having a size of 1-100nm. These nanomaterials are considered as "smart" materials used for constructing nanocarriers, which plays a vital role in drug delivery systems and possess high biocompatibility nature[1]. Nanoparticles have received special attention due to greater surface area to volume ratio and highly reactive than macromolecules[2]. Employing nanotechnology, green method for the synthesis of nanoparticles provides tremendous advantages as it is free of toxic chemicals and eco - friendly.

Among the various metal nanoparticles synthesized (such as silver, gold, iron, zinc and platinum), silver nanoparticles have gained more importance in the nanotechnology field. As, silver in the nano size is safe inorganic and non-toxic agents and encompasses a wide range of applications such as antibacterial and antifungal effects[3].

Silver nanoparticles synthesized from plant extract results that silver capped with the functional groups present in the active phytoconstituents of the plant extract, acts as antioxidant agents and enhance the biological activity like anticancer effect[4].

The plant selected for the synthesis of silver nanoparticles is *Volkameria inermis*. It is an evergreen sprawling shrub of 1-1.8m tall. It is widely distributed in the tropical regions of India, Nepal, Bangladesh, Sri Lanka, Southeast Asia and Mediterranean[5]. Its synonym is *Clerodendrum inerme* and it is commonly known as wild jasmine. Traditionally the roots and leaves of this plant were used as a febrifugal, uterine stimulant, a pest control agent and antiseptic, to arrest bleeding, treatment of asthma, hepatitis, ringworm and stomach pains[6].

MATERIALS AND METHODS

Collection of Plant Sample

Fresh leaves of *Volkameria inermis* (Plate 1) were collected in an area free of pesticides and other contaminants from Tamil Nadu Agricultural University (TNAU), Coimbatore district and authentication was done at Botanial Survey, Tamil Nadu Agriculture University (TNAU) Coimbatore, India

Plate 1*: Volkameria inermis.*

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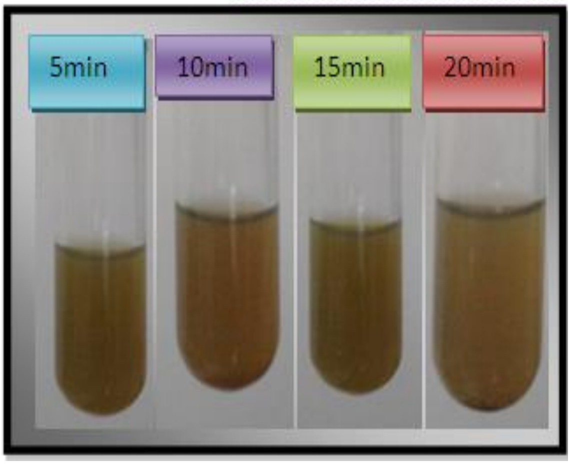

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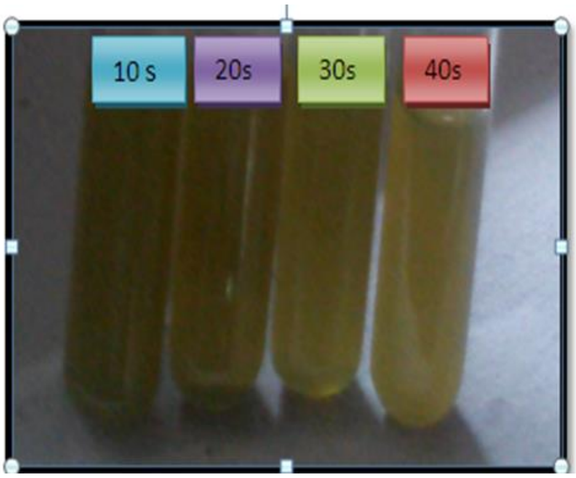

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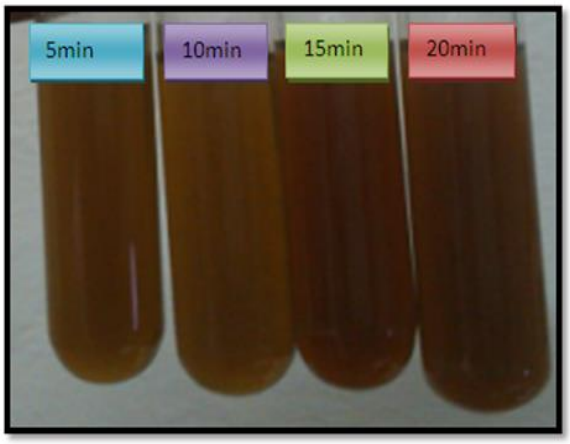

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Plate 2: Formation of silver nanoparticles from *Volkameria inermis* leaf extracta) Water bath heating at 60°C b) Microwave heating c) Sunlight Exposure method.

(BSI/SRC/5/23/2015/Tech/2082). The collected leaves were washed thoroughly in tap water, shade dried and powdered.

Preparation of Ethanolic Leaf Extract of Volkameria inermis

Table 1: Yield of silver nanoparticles from *Volkameria inermis* leaf extract.

S.No	Method	Duration of exposure	Yield (mg/ 100 ml)
1	Heating in Microwave	10 seconds	9
		20 seconds	13
		30 seconds	18
		40 seconds	20
2	Heating in water bath at 60°C	10 minutes	23
		20 minutes	27
		30 minutes	29
		40 minutes	30
3	Exposure to sunlight	10 minutes	35
		20 minutes	38
		30 minutes	42
		40 minutes	45

To 10g of powered leaf sample 100ml of ethanolic solvent was added (10g/100ml). Plugged with cotton wool and then kept on a rotary shaker at 190-220rpm for 24 hours. After 24 hours the extract was filtered and the filtrate was concentrated using flash evaporator and stored in air tight containers at 4°C and used for further experiments[7].

Synthesis of Silver Nanoparticles (AgNPs) from Ethanolic Leaf Extract of Volkameria inermis

Silver nanoparticles were prepared from ethanolic leaf extract of *Volkameria inermis*. To 10 ml of the ethanolic leaf extract 90 ml of 1mM silver nitrate solution was added[8]. The extent of nanoparticles synthesis was monitored by measuring the absorbance at 400-600nm.

Heating in Water Bath

The ethanolic extract of *Volkameria inermis* leaves in the presence of silver nitrate was heated for various durations (5, 10, 15 and 20 min) in a water bath at a temperature of 60 °C[9].

Heating by Microwave

The mixture of ethanolic extract of *Volkameria inermis* leaves with silver nitrate solution was heated in microwave for various durations namely 10, 20, 30 and 40 seconds[10].

Exposure to Bright Sun light

The ethanolic extract of *Volkameria inermis* leaves with silver nitrate solution was exposed to sunlight for various durations (5, 10, 15 and 20 min)[11].

Separation of Silver Nanoparticles

To separate the synthesized silver nanoparticles, samples were centrifuged at 13,000 rpm for 20 min under refrigeration and washed 3 times with deionized water. A dried powder of the silver nanoparticles was obtained by freeze drying.

Characterization of Silver Nanoparticles

UV- Visible Absorption Spectroscopy

The optical property of the AgNPs was analysed by UV – visible absorption spectroscopy (Shimadzu – BioSpec – nano, Japan). A volume of 100µl of synthesized AgNPs were diluted with 900 µl of distilled water and subjected to spectral analysis in the wavelength range from 220-800 nm.

Transmission Electron Microscope (TEM) with Energy Dispersive X-ray Spectroscopy (EDX)

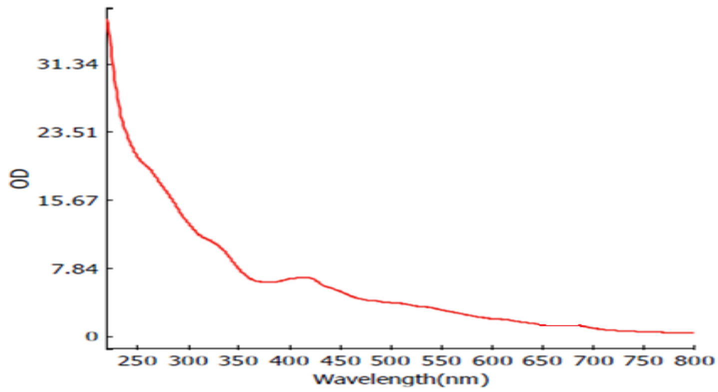


Figure 1: Absorption Spectrum of Silver nanoparticles.

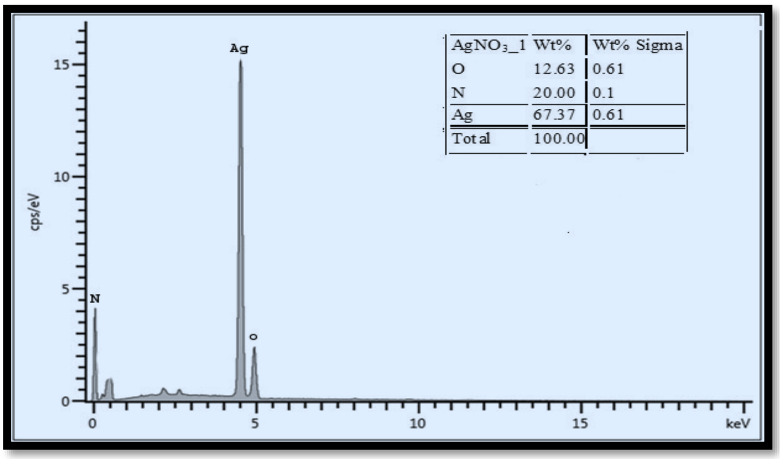


Figure 2: EDX composition of AgNPs, i)AgNPs.

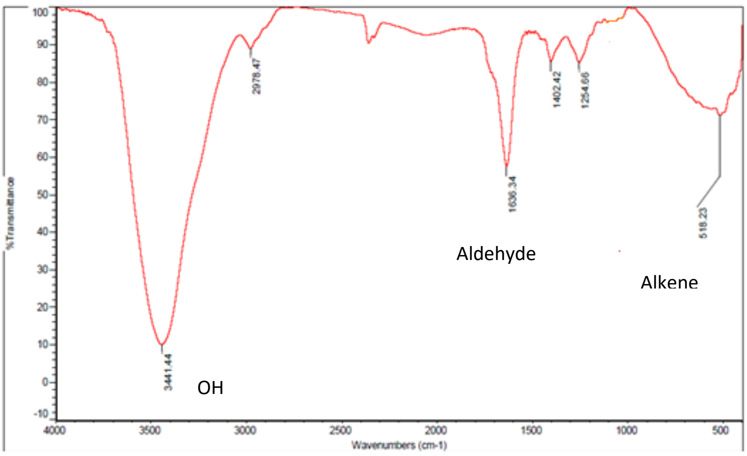


Figure 3: FTIR spectrum of *Volkameria inermis* leaf extract.

The morphology and size of the silver nanoparticles were examined by Hitachi 7000H, Tokyo, Japan Transmisssion Electron Microscopy operated at an accelerating voltage of 120 KV. The colloidal dispersion of the samples were prepared and placed on a carbon coated copper grid and evaporated under vacuum conditions. The TEM images were obtained and the energy dispersive X-ray analysis (TECNAI F30, Genesis Rev.3.0 software) was performed to determine the composition of the synthesized AgNPs.

Fourier – Transform Infrared (FTIR) Analysis

The FTIR spectrum was used to identify the functional groups in the plant extract responsible for the reduction of silver ions for the synthesis of silver nanoparticles. The FTIR spectrum was recorded for the plant extract, silver nanoparticles (AgNPs) using FTIR spectroscopy (Sigma, infinity).

Zeta potential

The stability of AgNPs were studied using zeta potential. The Zeta potential measurement was done using a Zetasizer Nano ZS (Malvern instruments) in a disposable

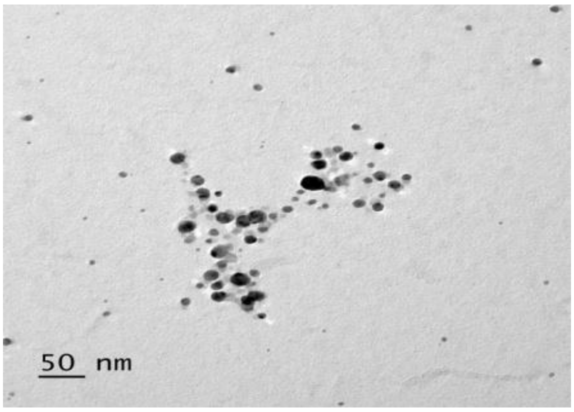


Plate 3: TEM of AgNPs, i) AgNPs.

cell at 25°C, and the results were analyzed using Zetasizer 7.01 software.

X-RAY Diffraction Analysis (XRD)

XRD is an analytical technique used to determine phase crystallinity of the material. The XRD pattern was obtained by placing the prepared samples on a glass slide and dried under hot air oven at 50°C. The samples were dried and analyzed under the XRD instrument (PAN analytical, XPERT- PRO diffractometer) with a Cu source at 1.5406 A^o wavelength as X - ray source in thin film mode.

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles

Different methods namely heating in microwave for 10, 20, 30 and 40 seconds, heating in water bath for 5, 10, 15 and 20 minutes at 60°C and exposure to sunlight for 5, 10, 15 and 20 minutes were carried out.

Formation of silver nanoparticles

Rapid synthesis of silver nanoparticles occurred in all the three different methods namely heating in water bath, heating in microwave and exposure to sunlight. The efficiency of *Volkameria inermis* leaf to synthesize silver nanoparticles was confirmed by the change in colour from yellow to intense brown, which was recorded by visual observation.

The results arrived shows that all the three different methods were efficient in the synthesis of silver nanoparticles from the leaf extract of *Volkameria imermis* (Plate 2). Of all the three different methods a notable increase in the intensity of colour was observed in exposure to sunlight for 5 to 10 minutes. These observations confirmed that sunlight exposure for 20 minutes was considered as the best method for the synthesis of silver nanoparticles from the leaf extract of *Volkameria imermis*.

Synthesis of silver nanoparticles from natural sources has gained importance in recent days. The synthesis exhibited a notable change in colour from yellow to brown and the intensity of brown colour is directly proportional to the increase in incubation period and temperature, which indicated the reduction of silver nitrate by the extract [12].

Yield of silver nanoparticles

Followed by the intensity of colour change, the yield in the silver nanoparticles synthesized from the leaf of *Volkameria inermis* was calculated. Table 1 lists the yield of silver nanoparticles synthesized by various methods from *Volkameria inermis* leaf extract. The synthesis was found to be increased in all the methods with the increase in the duration of exposure. Conversely, there was a tremendous increase in the nanoparticle synthesis pattern from *Volkameria inermis* leaf under sunlight exposure, which showed the utmost yield at 20 minutes. Based on the above results, the synthesis of silver nanoparticles from *Volkameria inermis* leaf extract using the three different methods, an intense colour change and increase in yield was more pronounced in sunlight exposed samples than the other methods, retelling that this was the best method among the methods tested.

The synthesis of nanoparticles using light was found to be highly advantageous than chemical synthesis method[13]. Thus, our results showed that exposure to sunlight generates rapid and high yielding silver nanoparticles than the other methods, which is an inexpensive , potential and the best method for the synthesis of silver nanoparticles.

Characterization of AgNPs

Among the different methods used for the synthesis of silver nanoparticles, the maximum intensity of colour observation and yield was observed in twenty minutes exposure to bright sunlight. Hence, this method was used for the synthesis of silver nanoparticles for further study and characterization.

UV- Visible Absorption Spectroscopy

The optical properties of the synthesized silver nanoparticles were analyzed by this technique. The silver nanoparticles synthesized from *Volkameria inermis* leaf extract showed a distinct peak at 430 nm, which are characteristic for AgNPs.

Similar characteristic peak at 430nm has been reported using *Lactobaciluus acidophilus* 01 strain[14]. Silver nanoparticles synthesized from *Andrographis paniculata* showed an absorption band at 410 nm.

Transmission Electron Microscope (TEM)

The morphology and size of the synthesized silver nanoparticles (AgNPs) was analyzed by this technique. The AgNPs synthesized from *Volkameria inermis* extract showed spherical shape (Plate 3). The size of the AgNPs was 50 nm. The nanoparticles were monodispersed without aggregation.

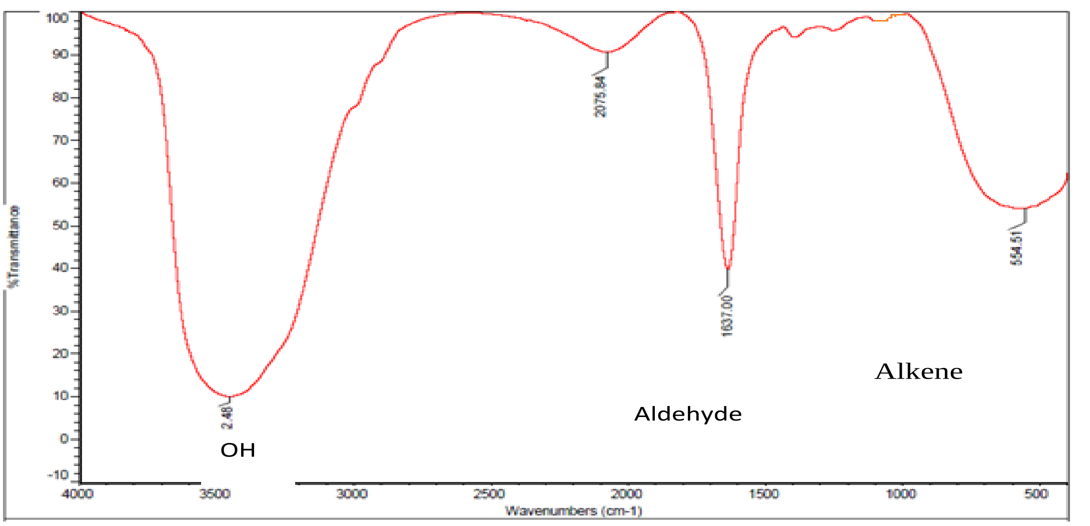
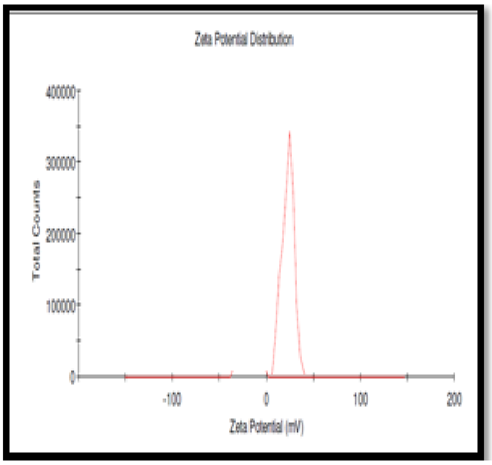


Figure 4: FTIR spectrum of AgNPs.



	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -18.5	Peak 1: 18.5	100.0	6.58
Zeta Deviation (mV): 6.58	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 1.17	Peak 3: 0.00	0.0	0.00
Result quality : Good			

Figure 5: i) Zeta potential analysis of AgNPs.

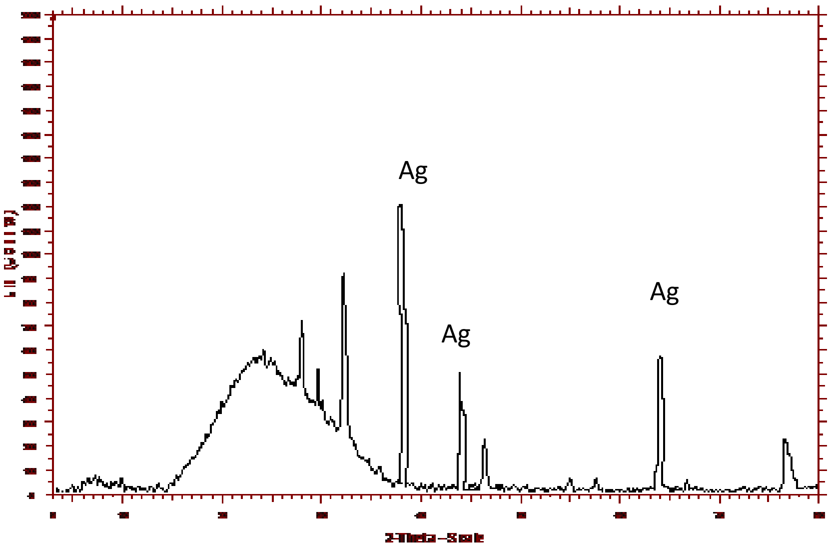


Figure 6: XRD pattern of AgNPs.

Similar studies were reported by[15] the synthesis of silver and gold nanoparticles from the extract of Erigeron annus (L.) pers flower extract showed spherical shape with size ranging from 15 to 60 nm and 20 to 100 nm. In another study the average diameter of the silver nanoparticle synthesized from *Cynodon dactylon* under sunlight exposure was found to be 8-10 nm[16].

Energy Dispersive X- ray Spectroscopy (EDX)

The EDX is a reliable tool to determine the elemental composition of the synthesized AgNPs from *Volkameria inermis* extract. The EDX profile of the AgNPs from *Volkameria inermis* extract showed the presence of typical peaks for silver (Figure 2). Additional peaks also observed which indicated the presence of nitrogen and oxygen, representing the existence of other elemental compounds in the AgNPs.

EDX technique is used to determine the elemental analysis and quantitative determination of available minerals in the nanoparticles[17]. Geetha *et al*., 2012[18] reported that silver nanoparticles formed from the leaf extract of *Chromolaena odorata* showed the presence of silver signals on EDX analysis.

FTIR analysis

The FTIR spectrum was documented for the biosynthesized silver nanoparticles (AgNPs) and the non – nano source (leaf extract of *Volkameria inermis*). The FTIR spectra (figure 4, 5) of *Volkameria inermis* leaf extract showed the characteristic peaks of OH, alkene, aldehyde groups which may be involved in the reduction and stabilization of silver nanoparticles. The presence of OH, aldehyde and alkene in the extract acted as the capping and stabilizing agent for the synthesis of silver nanoparticles. The silver nanoparticles synthesized from *Annona muricata* leaf extract showed the presence of phytoconstituents namely amides,alkenes, aliphatic amines, alkanes and alkyls, responsible for the reduction of silver nitrate[19]. The FTIR spectrum of silver nanoparticles synthesized from the fruit extract of European black elderberry (*Sambucus nigra*) revealed the presence of quinoidal, a polyphenol (OH)[20].

Our results are in accord with the above studies showing the presence of several functional groups in *Volkameria inermis* leaf extract which were expressed in the synthesized silver nanoparticles possibly rendering capping and stabilization to the particles.

Zeta potential analysis

The surface charge of the synthesized silver nanoparticles was determined by zeta potential measurements. The zeta potential values of the biosynthesized silver nanoparticles was 18.5. These values were found to be within the normal range (-30 to + 30).

The zeta potential of silver nanoparticles synthesized by green method was equal to 54.5 ± 7.8 mV which strongly indicates the stability of silver nanoparticles due to electrostatic repulsion[21].

X- Ray diffraction

The phase purity and the crystallinity of the biosynthesized AgNPs from the *Volkameria inermis* leaf extract was examined by XRD technique. The XRD profiles of the synthesized AgNPs are depicted in figure 7. The diffraction peaks at 38.12°, 44.313°, 64.464° and 77.424° can be index to (111), (002), (022) and (113) planes of face centered cubic structure of AgNPs. This confirmed the presence of silver and the highly crystalline nature of the particles. The pattern of AgNPs synthesized from *Volkameria inermis* extract showed some additional peaks, which might be due to the presence of organic molecules in the etract. Thus, the results of X-ray diffraction pattern corroborated with our EDX profile results, which validated the presence of organic molecules that facilitate the synthesis of nanoparticles.

The XRD technique is used to identify the crystalline nature by comparing the obtained pattern with the reference library and assigning its nanocrystalline nature[22]. In the present research work, the XRD patterns of the silver reaffirmed the presence of silver. It also proved the crystalline nature of the material.

CONCLUSION

The study concluded that, all the different techniques used to characterize AgNPs showed the successful capping of phytoorganic components on silver, to form well – dispersed, spherical nanoparticles. The functional groups present on their surface confirm the bioactive potential. The AgNPs showed a good stability profile. The ideally suited structures for AgNPs was successfully characterized.

ACKNOWLEDGEMENT

The authors are grateful to the Supervisor Dr. Mrs. Annapurani Professor and Head, Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for providing the necessary support and guidance.

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