WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 10, 1330-1340. Research Article ISSN 2277– 7105

BIOFABRICATION OF SILVER NANOPARTICLES USING AQUEOUS EXTRACT OF *OCIMUM SANCTUM* L.

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Article Received on
28 July 2015,

Revised on 18 Aug 2015,
Accepted on 08 Sep 2015

DOI: 10.20959/wjpr201510-4573

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ABSTRACT

Bio-Nanotechnology has gained an unavoidable attention because of the fiery properties of nano particles, this technology is emerging as a blazing area of present day research. Keeping in view the aforementioned facts in the present study phytofabrication has been done to synthesize silver nanoparticles by using the aqueous leaf extract of *Ocimum sanctum* L. (OLE) following an ecofriendly route via the Biological method. As this plant is having great medicinal potential, an attempt has been made to confirm whether the *Ocimum* extract & AgNPs aggregate can enhance the drug value of the plant. As a prerequisite for the above mentioned study biofabrication of Silver nanoparticles using natural reducing agents from (OLE) was done and were characterized by UV-Vis spectroscopy, (Scanning Electron Microscopy) SEM and Fourier Transform Infrared spectroscopy (FTIR).The synthesis of Ag NPs was confirmed by getting the SPR at~400nm by performing UV- VIS spectroscopy, size and shape of the synthesized Nanoparticles was determined by SEM, found to be spherical in shape with an average size of 250nm to 500nm. And the FTIR analysis revealed the presence of amines and phenolic compounds that act as a strong capping and stabilizing agent. This study offers a simple, cost effective, energy saving and green route of Ag NPs synthesis, used for effective drug preparation thereby serving the mankind and sounds to be a vital step in ebbing the pollution.

KEYWORDS: *Ocimum sanctum*, Biofabrication, Ag Nanoparticle, SEM, FTIR.

INTRODUCTION

Nature has bestowed man with innumerable resources that are uncountable. Among them medicinal plants are the most precious one. They have been a part and parcel of human society to combate diseases.[1] 'The royal herb'.[2] i.e. Tulsi derived from Sanskrit meaning "matchless one".[3] is known to have the most significant medicinal properties as it has been used in the treatment of various ailments since time immemorial. This is known as the Herb of heaven due to its curative capability and therapeutic potential. The whole plant has been reported to be used in medication of several disorders like juice of leaves are used as stimulant, demulcent and expectorant.[4] to cure upper respiratory tract infections, bronchitis.[5] a concoction of root is used as diaphoretic in malarial fevers.[6] seeds are mucilaginous prescribed for genito-urinary ailments.[7] and thus each and every part of it is equally important in treatment.

Another most excellent tool in the plethora of nature's treasure is 'Silver' which has been used for more than 150 years back.[8] as a biocidal agent. Its unbeatable versatile properties are well known in the present scenario as they have been found to be used since the dawn of civilization. Before the discovery of antibiotic during the First World War silver has been used as infection fighter/killer due to its antiseptic properties.[9]

Recently, nanotechnology has gained an unavoidable attention because of the fiery properties of nanoparticles (produced by atmospheric conversion in nature.[11] their high surface to volume ratio sharply distinguish them from their bulk counter parts. The fascinating and unique characteristic of these natures's essence elicits a novel concept of their combination. Exploiting these three gems in combination will leads to a more effective and efficient tool for mankind. Silver Nanoparticles have become the key to propel in every sector of society due to their innovative applications. They are not only confined to the medical sector involving drug delivery perhaps, proving as a pioneer of current consumer products ranging from the outer to inner objects of daily life by being used in cosmetics, clothing, shoes and detergents, in water purification system *i.e.* Aquapore .Being applied in a series of electronic gadets *i.e.* phones, laptops, toys etc.[10-12] Rapidly growing demand of nanoparticles to be consumed by smallest to largest section of society calls for its synthesis in bulk to meet the requirements. A number of techniques are available for their synthesis Viz. Physical (Physical vapor condensation (PVC).[13] Arc-discharge method.[14] Chemical (such as chemical reduction, electrochemical techniques, and photochemical reduction are most

widely used.[15]& Dispersion of preformed polymers.[16] Polymerization of monomers.[17] Ionic gelation or coacervation of hydrophilic polymers.[18] and Biological (employing both biological microscopic creatures such as bacteria.[19] and fungus.[20] or plants extract.[21-23] Among them Nano- Biotechnology is emerging as a blazing area of present day research. Phytofabrication of NPs is the burning and most efficient way. As this implies simple, easy, time saving, eco-friendly and quick route of synthesis. The green factories are available in abundance and easy to harvest too. The chances of contamination and other strict laboratory conditions like culture preservation, culture maintainance and transfer on particular time period (as in case of microbial synthesis method) saves the time, labour and cost. Additionally the bioactive compounds present in the plants offer a much easier and safer path for the nanoparticle to get synthesized in comparatively short span of time.

Keeping in view the aforementioned facts the present fraction of work is focused on the synthesis of AgNPs by employing Biological method which involves biosynthesis of silver nanoparticles using the leaf extract of *Ocimum sanctum* L. and will indirectly leads to a forward step in ebbing the environmental pollution.

MATERIALS AND METHODS

Silver nitrate ($AgNO_3$) AR grade was purchased from Thermo fischer scientific in the present study. Distilled water and well sterilized glass wares were used.

Selection and Collection of Plant (*Ocimum sanctum* L.)

10 gms of freshly plucked leaves were cleaned with the help of fine edge brush to remove the adhered dust/dirt present if any. Also, intrusive particles were removed by introducing the leaves to gentle flow of running tap water thrice followed by distilled, double distilled(dd/w) water and sterile dd/w twice and singly respectively then washed leaves were cut into fine pieces.

Preparation of *Ocimum* leaf extract (OLE)

In a 250ml Erlenmeyer flask; 100ml of distilled water was heated till it reaches 50-60^{0}C and then finely chopped leaves were added to the flask. The mixture was stirred for 15 min by maintaining the temperature, allowed to cool and upon cooling filtered using Whatman filter paper No. 1. Filtrate was used and the remaining was stored at 4^{0}C for further reference.

Preparation of 1mM $AgNO_3$ Solution

Stock solution of 1 M concentration of $AgNO_3$ was prepared by dissolving 1.69 gms of $AgNO_3$ salt in 10 ml of distilled water, followed by constant stirring and kept in dark to prevent the photo activation phenomenon. 0.1 ml of this stock solution was added in a 250 ml Erlenmeyer flask (covered with foil) containing 100 ml of distilled water followed by constant stirring to facilitate a uniform distribution of the solute particles in the medium.

Synthesis of Silver Nanoparticle from (OLE)

5 ml of filtrate was added to 45ml of 1mM $AgNO_3$ solution and was kept in sunlight for 30 sec. to enhance the bioreduction phenomenon and then left undisturbed for 30 minutes. Afterwards, the solution was subjected to different analysis.

Characterization

Visual Observation

After addition of the leaf extract colour change was detected by visually observing the mixture solution (OLE and $AgNO_3$ solution).

UV-VIS Spectra

Preliminary analysis of AgNPs analysis was performed by using double beam UV-Vis Spectrophotometer model no. Systronics-2203. λ- Max and was carried out at a resolution of 1 nm from 200 to 800 nm range wavelength.

Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier transform infrared spectroscopic (FTIR) measurement was performed by using Shimadzu IR Affinity. Test solution containing Ag NPs was dried in fine powder for the analysis. Sample was prepared by mixing the dried powder of Ag NPs synthesized by OLE with KBr.

Scanning Electron Microscopy (SEM) Analysis

Synthesized NPs were characterized on morphological grounds by Scanning Electron Microscopy (SEM). Sample was dried following the precipitation techniques.

RESULTS AND DISCUSSION

Visual Inference

Phytofabricated metal nanoparticle was firstly analyzed by Visual Inference. Fig .1 clearly indicates the change of colour from light yellow to reddish brown within 15 minutes which confers the bioreduction of Ag ions by the bioactive compounds present in the (OLE).

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Fig.1: Indicates the Bioreduction phenomenon.

UV – Vis spectroscopy

It is quiet useful technique in characterizing the metal nano particles. A strong surface Plasmon peak is seen commonly while observing metal nanoparticles in the range from 2 – 100 nm size. Figure. 2 shows a strong surface Plasmon resonance band observed at ~ 400nm which confirms the formation of Ag nano-particles due to the reduction carried out by the reaction.

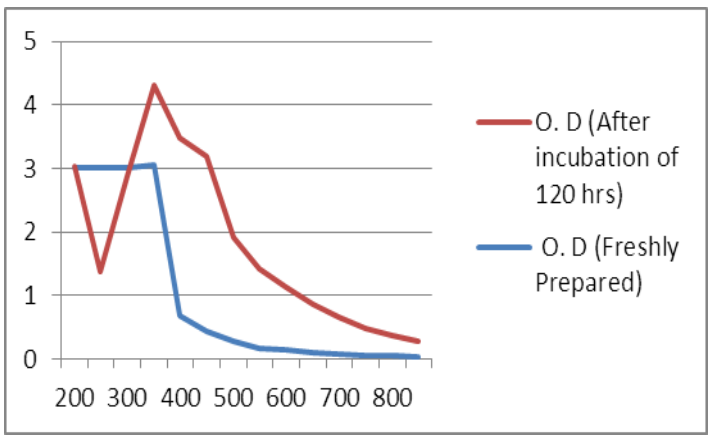

Fig.2: Showing the UV–Vis absorption spectra of extract (blue) and synthesized AgNPs (red).

Fourier Transform Infrared Spectroscopy (FTIR)

Plants have been considered as a huge reservoir of a variety of primary and secondary metabolites. The active principle found in them are supposed to be responsible in capping phenomenon that occurs during the nano particle synthesis. Single spectra of *Ocimum* leaf extract treated Ag NPs is stated in (Fig.3) that clearly reflects the contribution of phytochemicals in their Capping and stabilization. Spectra confers the chemical composition of the surface of AgNPs. The absorbance peak at 1024 cm^{-1} might be associated with =C-H (out of the plane) or C-O stretch. 1384 cm^{-1} might be associated with –C-N, stretching vibrations of aromatic amine.[24] This peak reflects the presence of protein components in the reaction mixture that act as a ligand and are responsible for the stability of Ag NPs.[25] Peak at 1639 cm^{-1} might be correspond to C=O aromatic carbonyl stretch.[26] 2914 cm^{-1} and 2777 cm^{-1} expected to be of C-H- alkyl groups (side chain hydrocarbons). 3421 cm^{-1} corroborates the probable existence of –OH groups that may be attributed to the presence of alcohols and phenols. In the cocktail of bioactive compounds it has been reported that the terpenoids and flavonoids plays very active role in the phenomenon of bioreduction as suggested by.[27]

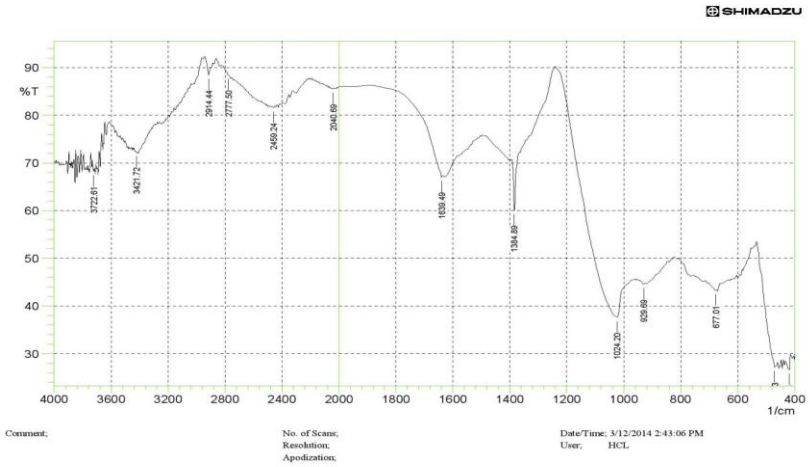

Fig. 3: FTIR Spectrum of Ag NPs synthesized from (OLE).

Scanning Electron Microscopy

This technique is used to get the pictorial information of prepared nano particles. (Fig.4) depicts the probable size (diameter) of the silver nanoparticles was found in between the range of 250 nm to 500nm. Silver nanoparticles of spherical and cubic shaped were found.

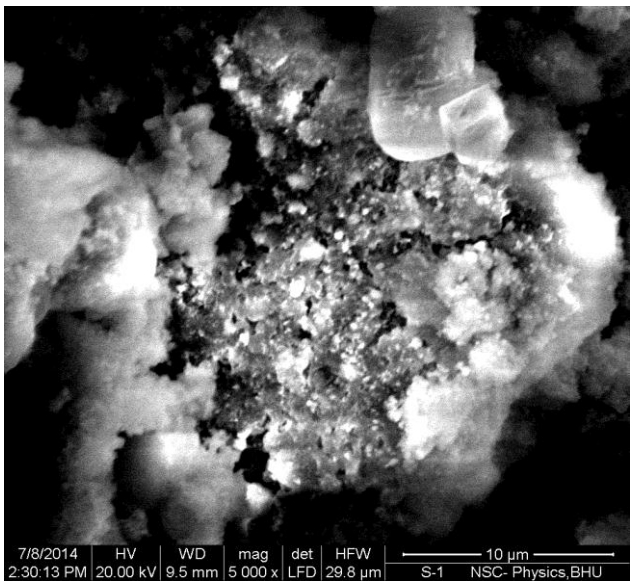

Fig.4: Showing SEM micrographs of Ag NPs synthesized by using *Ocimum sanctum* leaf broth.

Silver Nanoparticles are in great demand because they are used in almost every sector of society. In agricultural practices for root elongation in plants, as strong antimicrobial agent, used in batteries of cell, various electronic gadets, for keeping the fabric dust and microbe free, in water purification unit etc. Due to its versatile nature it has been produced by so many researchers following the green route by using the plant extracts.[28-30] Green factories offers a green route justifying the slogan of Go Green. phytonanofabrication is performed by a variety of methods i.e. microwave assisted synthesis.[31] biological reduction method.[32] Phenolic and flavanoid compounds play an important role in the formation of nanosilver.[33]

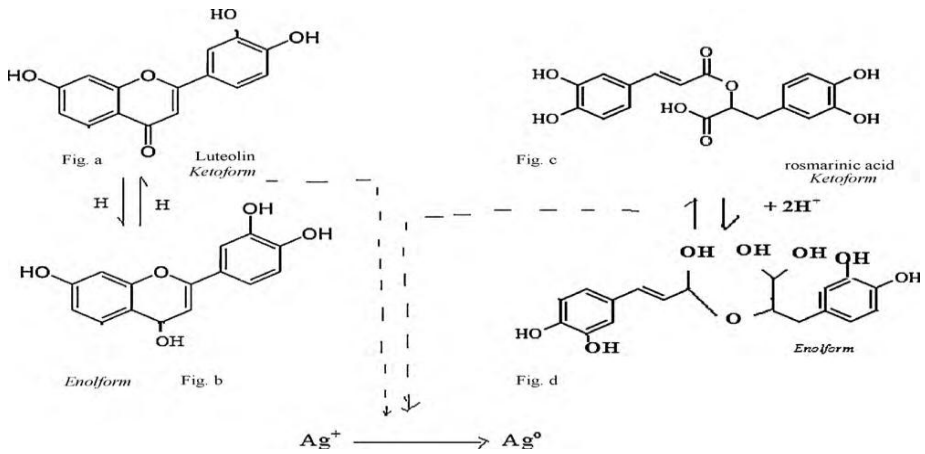

Fig.5: Mechanism of biosynthesis of Ag nanoparticles followed by[33]

CONCLUSION

Phytonanofactories has been a promising alternative in nanoparticle synthesis. The method employed in the present fraction of work is feasible and cheap as compared to other used methods on the basis of limited resources requirement at normal laboratory condition. No need of sophisticated instrument of high price value is required. Also, its rapid, cheaper,less time consuming and less labour intensive, efficient and above all a green approach of nanoparticle synthesis spreading the message of preserving environment for nature and mankind.

ACKNOWLEDGEMENT

The authors wish to thank MANF- a grant by UGC New Delhi, India for the financial assistance, Dept. of Physics, BHU Varanasi for getting SEM analysis, Dept. of Pharmaceutical Sciences, GGV, Bilaspur for getting FTIR.

CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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