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MICROWAVE ASSISTED SYNTHESIS OF SILVER NANOPARTICLE BY USING AQUEOUS ROOT EXTRACT OF *OCIMUM SANCTUM* AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Development of green nanotechnology is creating interest of researcher towards synthesis of nanoparticles. In the present study, microwave assisted synthesis of stable silver nanoparticles by using aqueous root extract of *Ocimum sanctum*. The biosynthesized nanoparticles were characterized by using UV–Vis, Scanning electron microscopy (SEM), X-ray diffraction (XRD) and FTIR analysis. The observed peaks in XRD pattern corresponding to (111), (200) and (220) planes. The size of the synthesized silver nanoparticles in the range from 36-45 nm. Further Crystallinity of nanoparticle was confirmed by XRD pattern. The FTIR measurement was carried out to identify the possible bio molecules responsible for efficient stabilization of silver nanoparticles. The surface morphology reveals that silver nanoparticles have spherical shape. The antimicrobial activity of silver nanoparticle was screened against gram positive and gram negative microorganisms

KEYWORDS: Silver nanoparticle, ocimum sanctum, antibacterial activity.

1. INTRODUCTION

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level. The biosynthesis of nanoparticles has been proposed as a cost-effective and environmentally friendly alternative to chemical and physical methods. Plant-mediated synthesis of nanoparticles is a green chemistry approach that connects nanotechnology with plants. Novel methods of ideally synthesizing NPs are thus thought that are formed at ambient temperatures, neutral pH, low costs. Keeping these goals in view nanomaterials have been synthesized using various routes. Among the biological alternatives, plants and plant extracts seem to be the best option. Plants are nature's "chemical factories". They are cost efficient and require low maintenance. The advantages and disadvantages of nanotechnology can be easily enumerated. This study attempts to review the diversity of the field, starting with the history of nanotechnology, the properties of the nanoparticles, various strategies of synthesis, the many advantages and disadvantages of different methods and its application.

Currently, there is a need to develop environmental suitable metal nanoparticle synthesis processes that do not use toxic chemicals in the synthesis protocols to avoid adverse effects in medical applications. Silver nanoparticles (Ag NPs) have received considerable attention owing to their diverse application like catalysis, magnetic, optical polarizibility[1] electrical conductivity and antimicrobial activity.[2] Physical and chemical methods of nanoparticle synthesis[3-9] are not environmentally friendly. Nature is a rich source of crude untreated extracts of plants and microorganisms. Therefore it is crucial to investigate their various properties. In the present study, to develop a simple, rapid and green approach of synthesizing AgNPs using aqueous root extract of *Ocimum sanctum*. The synthesis was carried out in aqueous medium by microwave heating. The advantage of microwave heating over conventional heating is rapid and uniform internal heating to the solution.

2. MATERIALS AND METHODS

Silver nitrate ($AgNO_3$) was procured from high media chemicals, Mumbai. 15g of dry powder of *Ocimum Sanctum* roots (Tulsi). The solutions were prepared in de ionized water. All the glass wares were cleaned thoroughly with water and dried in a micro oven.

2.1. Preparation of root extracts: The fresh roots of medicinal plants, *Ocimum sanctum* were thoroughly cleaned with tap water followed by distilled water to remove dust particles and were further dried. About 25 g of roots were grinded into fine powder and transferred into two beakers, each containing 100 ml distilled water. The broth was boiled for about 20 min. The extract was filtered through whatman filter paper No. 1 to remove particulate matter. The filtrate was cooled and stored in refrigerator.

2.2. Green synthesis of silver nanoparticles (AgNPs)

In typical preparation, aqueous solution (0.01M) of silver nitrate ($AgNO_3$) was prepared in 250 ml Erlenmeyer flasks. Certain amount of Silver nitrate was added drop wise to root extract of *Ocimum sanctum*. Then the reaction mixture was placed in micro oven (RAGA'S Microwave system, 700 W) for a period of 10 min at 60^0C for complete reduction of silver ions. During the heating process, the colour of reaction mixture changed slowly from yellow to dark brown due to reduction of Ag^+ to Ag^0. Filter and dry it. The dry powder (AgNPs) was calcinated for 300^0 C for 3 hrs in Muffle furnace.

3. RESULTS AND DISCUSSION

3.1. X Ray Diffraction

XRD analysis was performed to confirm the crystal phase of the synthesized AgNPs. Fig 3.1 shows typical XRD pattern of the dried nanoparticle obtained from silver colloid. The five diffraction peaks at 38.5°, 44.2^0, 64.3^0 and 77.4^0 are assigned to reflection from (111) (200) (220) (311) planes of silver crystal respectively. (JCPDS, file no.04-0783). The (2 0 0), (2 2 0), (3 1 1) Bragg reflections are weak and broadened relative to the intense (111) reflection. Which confirms the existence of silver and further on this basis they can be indexed as face centered crystal(FCC) structure of silver. The (111) peaks shows variation in the intensity at 38.5° Bragg's angle. The grain size of AgNPs was determined using the Debye Scherrer equation**.**

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

Where K is constant equal to 0.94, D and λ are the particle size in manometer and wavelength of the radiation (1.54056 Å for Cu Kα radiation) respectively. β and θ are the peak width at half-maximum Intensity (FWHM) and peak position. The average crystallite size of AgNPs was found to be 44-50 nm. Similar results were reported in silver nanoparticles synthesized using geranium leaf extract[10] and mushroom extract.[11]

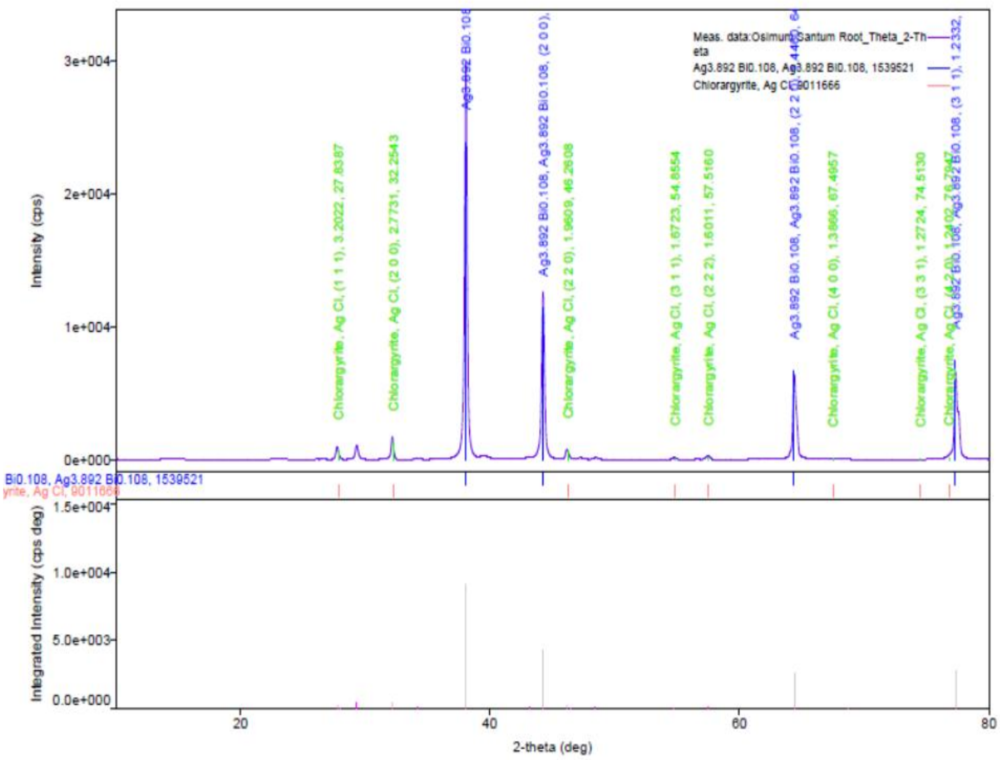

Fig. 4.1: XRD pattern of dry powder Ag NPs.

3.2. FTIR Spectroscopy

The FTIR studies have been carried out to identify the presence of possible vibrational modes of different molecules responsible for the reduction and capping of the Ag NPs. Fig.3.2 shows FTIR spectra of the AgNPs have been recorded in 400 - 4000 cm^{-1}. (Shimadzu IR Affinity -1) The spectra contain various vibrational modes centered at 428.22, 470.65, 509.23, 535.52 cm^{-1} due to the presence of Ag-O bond. The band at 1041 cm^{-1} can be assigned as absorption peaks of -C-O-C-. The vibrational band corresponding to the bond such as C=O (ring), C-O, C-O-C, C=C (2202.0 cm^{-1}) are derived from soluble compound such as terpenoids and flavonoid present in tulsi plant. The peaks centered around 1539.6 cm^{-1}, 1689.2 cm^{-1} can be attributed to asymmetric stretching vibration of N-O compounds.[12-14] Therefore it was assumed that these biomolecules are work as capping agent and efficient stabilization.

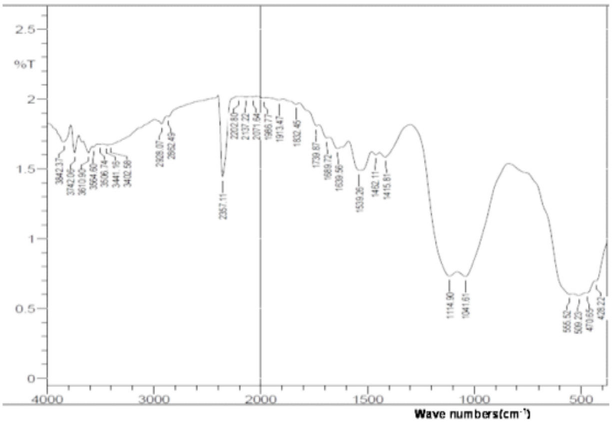

Fig. 3.2. FTIR spectra of AgNPs.

3.3. UV- Visible Absorbance studies

UV-vis spectroscopy is a simple and most sensitive technique for the characterization of AgNPs. To observe the optical property of biosynthesized silver nanoparticle, sample was periodically analyzed with UV-Vis spectroscopic studies (Shimadzu UV-1800) at room temperature operated at a resolution of 1 nm between 200 and 600 nm ranges. Cuvette of path length 10 mm was used. Fig 3.3 shows UV-Vis absorption spectrum of silver nanoparticle. It is observed that the silver SPR occurs at 463.0 nm. There is no change in peak position, suggesting that nucleation of silver nanoparticle starts with initiation of reaction time only, and the size remains unchanged throughout the course of reaction.[15] Broadening of the absorption peak at 463.0 nm with increase in time indicated the polydispersity of the nanoparticles.

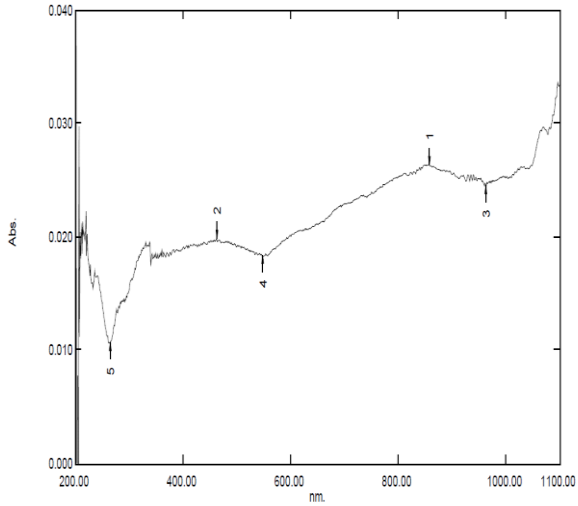

Fig. 3.3: UV spectra of dry powder AgNPs.

3.4. Scanning Electron Microscopy (SEM)

SEM studies were carried out to visualize the size and morphology of the AgNPs. Fig.3.4 shows typical bright sea fossils type SEM micrograph of the synthesized AgNPs. It was observed that AgNPs were spherical in shape with maximum particles in size range within 44-50 nm. Agglomerated silver nanoparticles were observed in many places, thereby indicating possible Sedimentation at a later time.

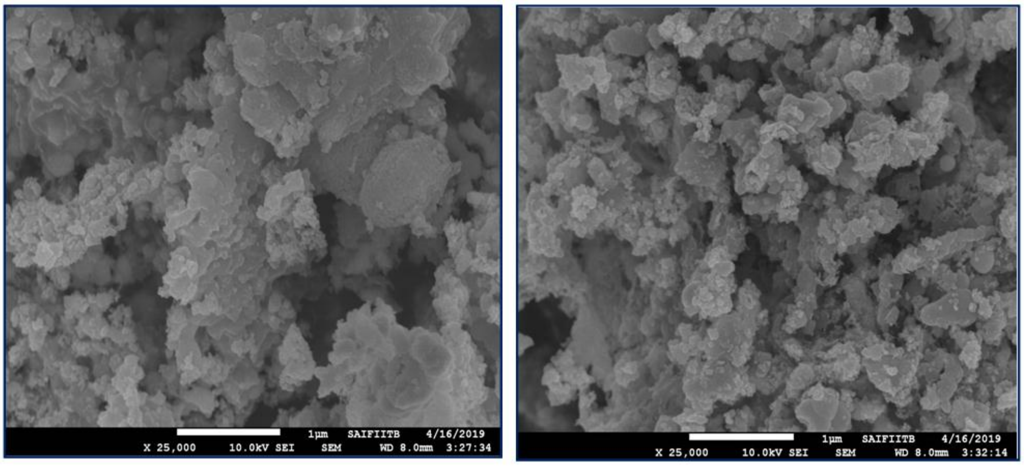

Fig. 3.4: SEM images of silver nanoparticle.

3.5 Antibacterial activity

The antimicrobial activity of silver nanoparticle synthesized by root extracts of tulsi plant was investigated against the various bacterial organisms included both gram positive and gram negative stains like *E. coli* (MTCC 118), *K. pneumoniae* (MTCC 109), *S. aureus* (MTCC 1430), *E. faecalis* (MTCC 2729). Silver nanoparticle shows remarkable antibacterial activity, due major changes in the membrane structure of bacteria as a result of the interaction with silver cations lead to the increased membrane permeability of the bacteria. Another reason for enhanced activity of AgNPs, is due to decrease in size, nanoparticles are able to adhere on cell wall of organism, thus causing its destruction and killing.

For experimental antibacterial susceptibility testing of silver nanoparticle, in the disc diffusion test, sterile Whatman filter paper (No. 1) disc were impregnated with 20µl of different samples, (1000 mg/ml) in DMSO. The disc was placed at the centre Mueller Hinton Agar seeded plates (M173) procured from Himedia Pvt. Ltd.

Mumbai, with bacterial inoculums nearly 106 CFU/ml, incubated at 37°C for 24 hrs. Then the growth free "zone of Inhibition" of respective disc was measured. The assay was performed in triplicate and mean value was considered as inhibition zone.

In this study, the synthesized AgNPs exhibit good antibacterial activity against both Gram-negative and Gram-positive bacteria (Table 1), but it shows higher antibacterial activity against E. coli (Gram-negative) then S. aureus (Gram-positive)

Table 1: Zone of inhibition (nm) of silver nanoparticle against bacterial pathogens.

Test organism	Silver nanoparticle in 20µl
E. coli	9
K. pneumoniae	4.5
S. aureus	5.8
E. faecalis	5.1

4.0 CONCLUSIONS

In this study, a facile and efficient method for the green synthesis of spherical AgNPs was developed. The synthesis was carried out by using root extracts of (*ocimum sanctum*) tulsi plant and aqueous solution of silver nitrate by microwave irradiation. As synthesized silver nanoparticle has FCC crystal and size about 45 nm. Further the nanoparticle was characterized by UV-vis, XRD, SEM and FTIR measurement has confirmed the reduction of silver nitrate to silver nanoparticle. The zones of inhibition were formed in the antimicrobial screening test indicated, that the Ag NPs synthesized in this process has the efficient antimicrobial activity against ***E. coli***.

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