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Green Synthesis and Characterization of Silver Nanoparticles using Ethanol Extract of *Myristica fragrans* (Nutmeg) and Its Biological Applications

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ABSTRACT

Silver nanoparticles have been synthesized from the ethanol extract of *Myristica fragrans* seed. The synthesized nanoparticles were characterized by UV-visible, FT-IR, XRD, SEM, TEM and AFM spectral studies. Formation of silver nanoparticles conform through colour change from pale yellow to brown when ethanol extract of *Myristica fragrans* was added dropwise to silver nitrate. The appearance of brown color in aqueous solution is due to excitation of surface plasmon vibrations of silver nanoparticles observed between 400 – 450 nm. Peak corresponding to 1741 cm^{-1} confirms the presence of aldehyde groups in the ethanol extract of *Myristica fragrans* which is responsible for the capping and stabilization of biosynthesised. AEM images of silver nanoparticles exhibit uniformly distributed, well defined regular nanostructures. Silver nanoparticles showed significant antibacterial action on both the gram classes of gram-negative *Escherichia coli* and gram-negative bacteria *Bacillus cereus.*

1. Introduction

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, radiation assisted, electrochemical, sonochemical, microwave assisted process and recently via green chemistry route [1-3]. The use of environmentally benign materials like plant leaf extract, bacteria, fungi and enzymes for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol.

Many research papers reported the synthesis of nanoparticles using various parts of plants extract such as leaves from *Helianthus annus*, *Oryza sativa* and *Zee mays* [4], seeds of Jujuba [5], *Jatropha curcas* [6], seed extract of *Myristica fragrans* (Nutmeg) [7], roots of *Trianthema decandra* [8], stems and roots of *Ocimum sanctum* [9] and Banana peel [10].

The present study is aimed to synthesis the silver nanoparticle using *Myristica fragrans* as biocatalyst with detailed spectral and morphological characterizations. Also, the antimicrobial activities of synthesized silver nanoparticles were investigated.

2. Experimental Methods

2.1 Materials

Freshnuts of *Myristica fragrans* was procured from a local supermarket. The silver nitrate was supplied by Sigma Aldrich Chemicals. All the chemicals and reagents were of analytical grade.

2.2 Preparation of Myristica fragrans Seed Extract

The *Myristica fragrans* seeds were collected from a local super market. The collected seeds were extensively washed with distilled water and air dried. Air dried seeds were transferred to round bottom flask with ethanol. The mixture was refluxed for 1-2 hours until the color of alcoholic solution changed from colorless to yellow. Then the mixture was cooled at room temperature and filtered using Whatman No.1 filter paper. The extract was stored in refrigerator for overnight.

2.3 Synthesis of Silver Nanoparticles

10 mL (0.1 mM) of silver nitrate was treated with 5 mL of *Myristica fragrans* alcoholic seed extract and stirred at 65 °C for 15 – 20 minutes. Color change from pale yellow to dark brown precipitate was observed. The brown precipitate was washed with ethanol several times. Then the precipitate was dried at 60 °C in vacuum oven and stored in a sealed bottle.

2.4 Characterization of Silver Nanoparticles

The synthesized silver nanoparticles were characterized using Shimadzu UV 2450 spectrophotometer. The XRD Spectrum of powdered sample of silver nanoparticles was obtained using a Philips P W 1710 diffractometer (λ= 0.15405 Å) with monochromatized CuKα radiation source. The diffractogram was then compared to diffraction data in well – known database to determine what species or molecules exist in a sample. Crystallite size was determined by using the diffraction peaks from the Debye Scherrer's equation [11], $d = K\lambda/\beta\cos\theta$, where K is the shape factor taken for a cubic system, λ is the X-ray wavelength, typically 1.54 Å, β is the full width at half maximum intensity (FWHM) in radians, and θ is angle of diffraction. The FTIR spectra of silver nanoparticles was recorded using Brucker model from 4000-400 cm^{-1} by mixing dried sample with KBr pellets. The surface morphology of silver nanoparticles was identified by scanning electron microscope. The surface morphology of silver nanoparticles was analyzed using Nanosurf easy scan 2 AFM in non-contact mode. Amplitude frequency was around 350 KHz, the mean length 120 nm and tip end diameter 10-15 nm.

2.5 Antibacterial Activity

Antibacterial activity of silver nanoparticle against gram negative *Escherichia coli* and gram-positive bacteria *Bacillus cereus* were observed using an agar well diffusion and disk diffusion method respectively. Mueller Hinston agar was prepared, sterilized and kept ready in molten condition. Various fractions of silver nanoparticles loaded discs were dispersed on the solidified Mueller Hinston agar with test organisms and standard organism. The plates were incubated at 37 °C for 24 hours. MIC was recorded based on the growth of the organism.

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3. Results and Discussion

3.1 Visual Confirmation

Color change from pale yellow to brown when ethanol extract of *Myristica fragrans* was added dropwise to silver nitrate formed the primary evidence for the formation of silver nanoparticles (Fig. 1). The formation of silver nanoparticles is due to the reduction of Ag^{+} into Ag^{0} by the polyphenols present in the *Myristica fragrans* seed [12]. The appearance of brown color was noticed for 10% ethanol extract of *Myristica fragrans*. The surface plasmon vibrations of silver nanoparticles was observed between 400 – 450 nm [13]. Fig. 2 illustrates a wide absorption peak. No surface plasmon vibrations were seen with varying the concentration of the ethanol extract of *Myristica fragrans* and silver nitrate. This is in good agreement with the report of green synthesis of silver nanoparticles by *Aegle marmelos* leaf extract [14].

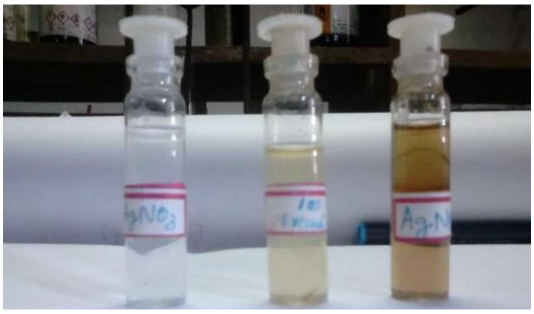

Fig. 1 Color change of leaf extract from yellow (without silver nitrate) to brown (with silver nitrate)

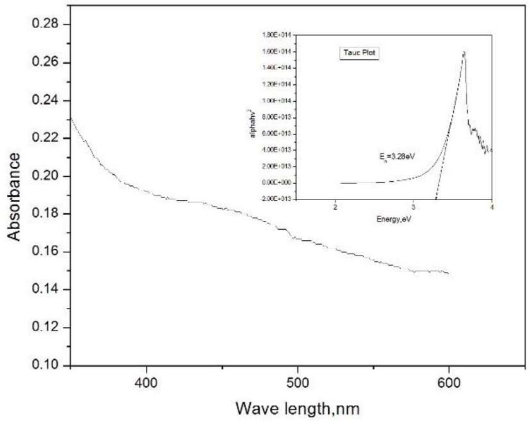

Fig. 2 UV-visible spectrum of AgNPs

The band gap of semiconductor materials increases with the decrease in particle size, which leads to the shift of the absorption edge towards high energy, this is the so-called quantum size effect. The band gap Eg of the sample was determined from the Tauc plot, $(\alpha h\nu)^2 = k(h\nu - Eg)$, where α is the absorption coefficient, hν is the incident energy, k is the energy independence constant and Eg is the band gap energy. Inset image of Fig. 2 shows the plot of $(\alpha h\nu)^2$ vs. hν. The band gap energy was calculated to be 3.28 eV.

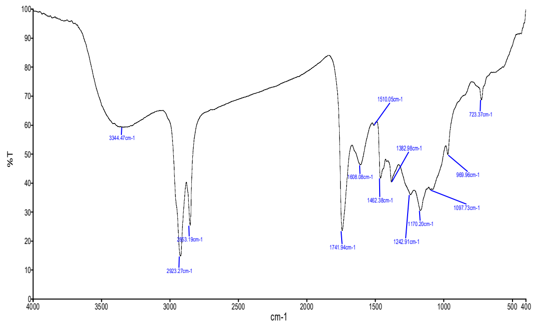

Fig. 3 FT-IR spectrum of AgNPs

3.2 FTIR Spectral Study

The FTIR spectra images of silver nanoparticle using ethanol extract of *Myristica fragrans* were recorded and shown in Fig. 3. The peaks at 3344 and 2923 cm^{-1} is due to the –OH stretching vibrations of phenols. Peaks corresponding to the wave numbers 2853, 1741 and 1382 cm^{-1} shows the presence of C-H stretching. Wave numbers corresponding to 1242, 1097, 987 and 723 cm^{-1} confirms the presence of aromatic hydrocarbons. In addition to that, peak corresponding to 1741 cm^{-1} confirms the presence of aldehyde groups in the ethanol extract of *Myristica fragrans* which is responsible for the capping and stabilization of biosynthesized silver nanoparticles.

3.3 XRD Characterization

Fig. 4 shows the XRD pattern of the synthesized silver nanoparticles using CuKα radiation source. (λ=0.15405 Å). All the different peaks can be well indexed to the face centered cubic structure of silver nanoparticles with lattice constant of a = 4.689 Å. Three intense peaks at 2θ = 38.94°, 44.98°and 64.17° corresponding to (111), (200) and (220) planes respectively confirmed the cubic face–centered structure (JCPDS-84-0713) of silver nanoparticles. The average size of Silver Nanoparticles was calculated using Debye-Scherrer's equation, $D = 0.94\lambda/\beta\cos\theta$, where D is the average crystallite domain 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 determined the full width at half maximum and found to be approximately ~11 nm (Table 1).

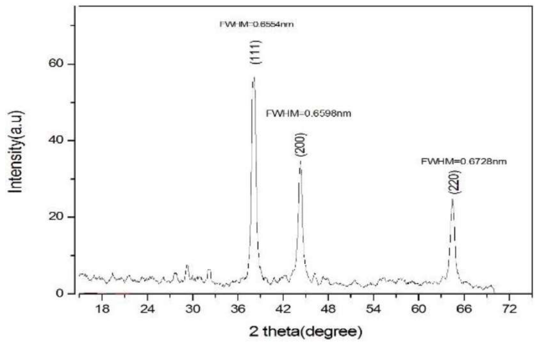

Fig. 4 XRD pattern of AgNPs

Table 1 Summary of XRD characterization

2θ (degree)	hkl	FWHM (degree)	Crystallite Size (nm)
38.10	111	0.65544	11.87
44.29	200	0.65985	11.90
64.62	220	0.67285	11.14

3.4 SEM and TEM Study

SEM image of the silver nanoparticles were recorded and are shown in Fig. 5. SEM micrograph showed that the silver nanoparticles formed were uniform and crystalline. This result is in good agreement with the crystallite size calculated using the XRD data. Fig. 6 also reveals no characteristic peaks of impurities or other precursor compounds are observed. TEM image of the silver nanoparticles were recorded and are shown in Fig. 6. TEM micrograph has been used to identify the size, shape and morphology of nanoparticles. TEM showed the silver nanoparticles formed are ~10 nm.

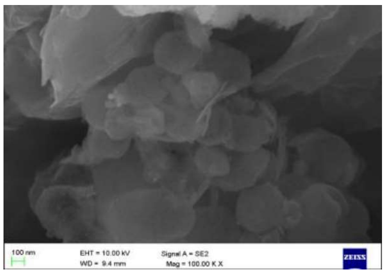

Fig. 5 SEM image of AgNPs

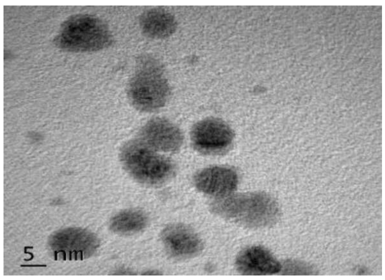

Fig. 6 TEM image of AgNPs

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3.5 AFM Study

Fig. 7 shows the surface topography of silver nanoparticles synthesised using ethanol extract of *Myristica fragrans* seeds. AFM images of silver nanoparticles exhibit uniformly distributed, well defined regular nanostructures.

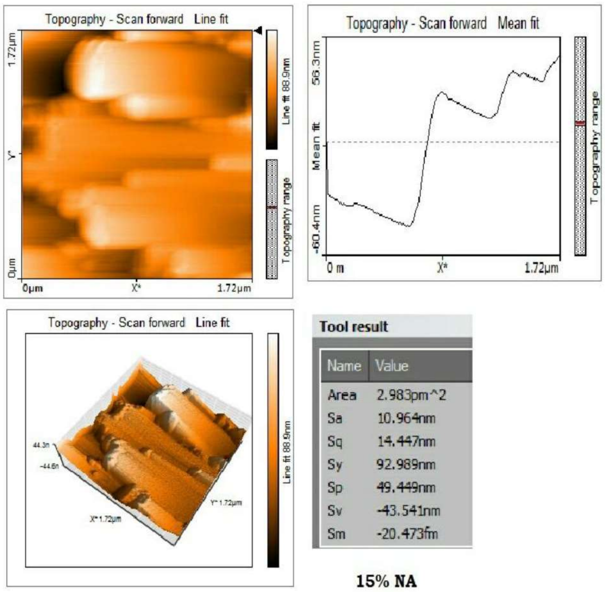

Fig. 7 AFM image of AgNPs

Table 2 Zone of Inhibition observed for *Escherichia coli* and *Bacillus cereus*

AgNPs (mg)	Gram Negative Bacteria *Escherichia coli* (mm)	Gram Positive Bacteria *Bacillus cereus* (mm)
50	22	20
100	25	25
150	28	30
200	29	32
250	30	30
300	30	27
350	30	34

Fig. 8 Zone of Inhibition of AgNPs for gram negative bacteria *Escherichia coli* and gram positive bacteria *Bacillus cereus*

3.6 Antibacterial Activity

Nanoparticle synthesis by green route are found to be highly effective against multi-drug resistant human pathogenic bacteria. Antibacterial activity of silver nanoparticles against *Escherichia coli* and *Bacillus cereus* were observed and compared. As the concentration of silver nanoparticles increases the zone of inhibition also increases (Fig. 8) [15, 16]. The maximum activity was found to be 35 mm and 30 mm for 400 mg gram of both negative and 350 mg of gram positive respectively. The antibacterial activity has been calculated by measuring the zone of inhibition and listed in Table 2.

4. Conclusion

AgNPs were prepared by the ethanol extract of *Myristica fragrans* seeds. The synthesized AgNPs were characterized with UV-visible spectroscopy, FT-IR, XRD, SEM and AFM studies. UV-Vis spectrum exhibits excitation of surface plasmon vibrations of silver nanoparticles observed between 400 – 450 nm. Three intense peaks at 2θ = 37.94°, 45.98°and 64.17° corresponding to (123), (312) and (404) planes respectively confirmed orthorhombic structure of silver nanoparticles. Morphological behaviour of SEM, TEM and AFM confirm the size and shape of silver nanoparticles.

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About the Conference…

"International Conference on Advanced Materials and Their Applications" has been convened by Dr. C. Vedhi, Assistant Professor, PG and Research Department of Chemistry, V.O. Chidambaram College, Thoothukudi at his designated venue on 27th December 2018.

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