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Original Article

Eco-friendly synthesis and characterization of silver nanoparticles using Vitex leucoxylon linn leaf extract and their study of its antibacterial and anti-fungal activities

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ABSTRACT

Eco-friendly synthesis of stable Silver nanoparticles (AgNPs) was carried out using the aqueous extract of vitex leucoxylon leaves. The UV-Vis spectrum was recorded to monitor the formation of the nanoparticles, which exhibited a blue shifted absorption peak at 440 nm. FT-IR spectra were recorded for the Silver nanoparticles to identify the biomolecules involved in the synthesis process. The XRD analysis revealed well-defined peaks appearing at 2θ positions. From the full width at half maximum of XRD peaks the average size of the synthesized nanoparticles was 12 nm. Dispersity and morphology was characterized by Scanning Electron Microscope which showed the synthesized nanoparticles were Hexagen shape with the size range 10-20 nm. EDX analysis showed that the above route produced highly pure Silver nanoparticles. They were also evaluated for their antibacterial and anti-fungal activities. They do not contain any harmful chemicals.

Keywords: Silver nanoparicles, Vitex leucoxylon, FT-IR, XRD, SEM, Antimicrobial activity.

INTRODUCTION

Nowadays, the research on nanomaterials developed in all directions and influenced almost all disciplines of science and technology by unique properties that bulk materials never possess. Size, shape and crystalline nature of nanomaterials are the key parameters that control and determine the properties [1]. Among various nanomaterials studied yet, nanoparticles of noble metals like silver or gold have been found to possess attractive properties like optical [2], catalytic [3], anti-bacterial [4,5] and anti-viral [6] etc. Synthesis of these nanoparticles is the primary step for carrying out detail study of its various properties. Over the last decade, chemical [7, 8] and physical routes [9] had been used redundantly for nanoparticle synthesis.

The Silver nanoparticles have found to be used in many applications of different fields which also an effective antimicrobial agent [10] and have diverse in vitro and in vivo applications [11]. Although there are many routes available for the synthesis of Silver nanoparticles, such as thermal decomposition, electrochemical, microwave assisted process etc., Biological methods of nanoparticles synthesis using microorganisms, enzyme and plant extracts to offer numerous benefits of chemical and physical methods due to their cost effectiveness, environmental friendly nature, and can be easily scaled up for large scale synthesis. Here in, we report on the first time synthesis of Silver nanoparticles, reducing the Silver ions present in the solution of Silver nitrate by an aqueous plant leaf extract of Vitex leucoxylon. This work demonstrates that the reaction against Silver ions with Vitex Leucoxylon plant leaf extracts resulted in the extracellular of Silver nanoparticles at room temperature.

MATERIALS AND METHODS

To maintain a "green" synthesis of the nanoparticles, reaction medium chosen was distilled water. Leaf extracts were used as reducing agents. The reagent chosen for the synthesis was Silver nitrate (Himedia) which was analytical grade and does not involve any toxic hazard to the environment. The plant used was vitex leucoxylon (Nirnochi). The plant images were shown in Fig: 1.

Preparation Of plant leaf Extract: The Whole plant of Vitex Leucoxylon was collected from Puliarai, Tirunelveli District, Tamil Nadu and India. Taxonomic identification was made from Dr.

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V. Chelladurai, Research Officer-Botany (Scientist-C) Central Council for Research in Ayurveda & Siddha, Government of India (Rtd). The plant was dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. 5g of the collected plant powder was mixed with 100 ml of double distilled water and boiled to 60°-70° C for about 10-15 minutes. Then the extract was filtered using Whatmann No.1 filter paper to get a clear solution. The filtrate was stored at 4°C over night for further studies.

Fig: 1. Plant images of Vitex Leucoxylon

Synthesis of Silver Nanoparticles: 1mM aqueous solution of Silver nitrate ($AgNO_3$) was prepared and this solution was used for the synthesis of Silver nanoparticles. 5 ml of the leaf extract was added into 20 ml of aqueous solution of 1 mM Silver nitrate for the reduction of $Ag+$ ions. The beaker was wrapped with Silver foil and kept in the dark for 24 hours until the colour darkens the colour change indicates the formation of Silver nanoparticles. Then the solution was centrifuged at 5,000 rpm for 20 min [12], consequently dispersed in double distilled water to remove any heavy biological materials present in synthesized Silver nanoparticles [13].

***Characterization of Silver nanoparticles*:** Synthesized Silver nanoparticles were observed by UV–Vis spectroscopy in 200 to 600 nm range. The absorption spectra of the synthesized nanoparticles were measured using UV-1800 SHIMADZU UV-spectrophotometer instrument. The possible photochemical involved in the synthesis and stabilization of nanoparticles was identified by performing FTIR analysis using SHIMADZU-8400S model instrument. Detailed analysis of the morphology, size and distribution of the nanoparticles was documented by Scanning Electron Microscopy using CARL ZEISS, EVO 18 model instrument. Crystalline size and structure of the silver nanoparticles were carried out by XRD using BRUKER, ECO D8 Advance model instrument. Elemental analysis of Silver was measured by EDX was carried out on BRUKER, X flash 6130 model instrument.

***Antimicrobial Studies*:** Synthesis of Silver nanoparticles using Vitex Leucoxylon leaf extract was tested for antimicrobial activities against different pathogenic microorganisms like Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Salmonella typhii. Mueller-Hinton agar (Himedia) was used for the bacterial sensitivity screening. The antibacterial screening of the nano extracts were evaluated by agar well diffusion method. In order to determine the antibacterial activity of the nanoparticle extracts of Vitex Leucoxylon at different concentration (5, 10 and 15 μl) by nutrient agar well diffusion method [16]. Using sterile micropipette 5, 10 and 15 μl of the sample of nanoparticles solution were loaded onto each of the wells at the centre in all the plates. In the middle a well-known antibacterial drug Ofloxin (1mg) was poured, and act as a positive control. The plates were incubated at 37^0C for 24 – 48 hours. After incubation different levels of zone of inhibition were measured as mm sensitivity [17].

Antifungal activity: The nano extracts of three different concentrations were screened for antifungal activity by agar well diffusion method [18] with sterile cork borer of size 6.0mm. The cultures of 48 hours old grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. An aliquot (0.02ml) of inoculum was introduced to molten PDA and poured into a petridish by pour plate technique. After solidification, the appropriate wells were made on agar plate by using cork borer. In agar well diffusion method 0.05ml of nano extracts of three different concentrations were introduced serially after successful completion of nano extract analysis. Incubation period of 24- 48 hours at 28^0C was maintained for observation of antifungal activity of nano extracts. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the plant extracts. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in triplicates.

RESULTS AND DISCUSSION

UV-VIS Spectra Analysis: UV–Visible spectroscopy is an important technique to determine the formation and stability of metal nanoparticles in aqueous solution. Surface plasmon resonance is a physical process that can occur when plane polarized light hits a metal film under total reflection conditions [14]. The colour change arises because of the excitation of surface plasmon vibrations in the Silver nanoparticles[15]. It shows a change of pale yellow to dark brown in colour. The dark brown colour of Silver colloid is accepted to be the surface Plasmon resonance arising when a group of free conduction electrons are induced by an interacting electromagnetic field [16]. The band appears at a range of 440 nm and the broadening of peak indicated that the particles are mono dispersed. The reduction of pure Silver ions into Silver nanoparticles was studied by the UV-Vis spectrum was shown in Fig.2.

FTIR Analysis: FTIR analysis was used for the characterization of synthesized Silver nanoparticles was shown in Fig.3. This Characterization was done to detect the functional organic molecules which cause the production and capping of biogenic Silver nanoparticles in the mixture. The FTIR spectrum shows a peak at 3282.62 cm^{-1} may be assigned to be O-H stretching of water, alcohol and phenols. The band at 1581.52 cm^{-1} corresponds to asymmetric stretching of nitro groups. The band at 1384.79 cm^{-1} shows to -N=O bend of nitro groups. From this study, it is clear that these functional groups are responsible for the bio reduction of Silver ions into Silver nanoparticles.

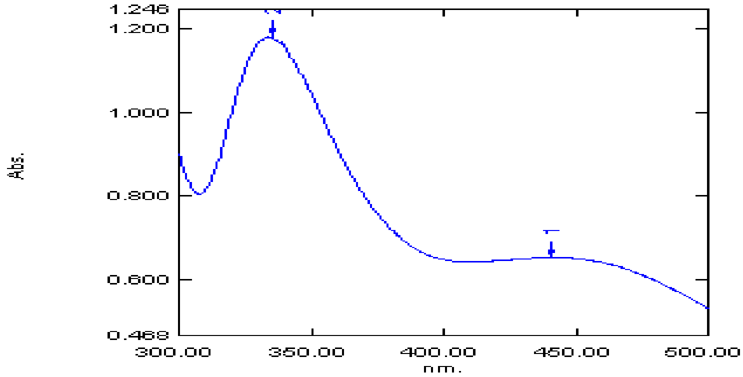

Fig.2. UV-Vis spectrum of synthesized Silver nanoparticles

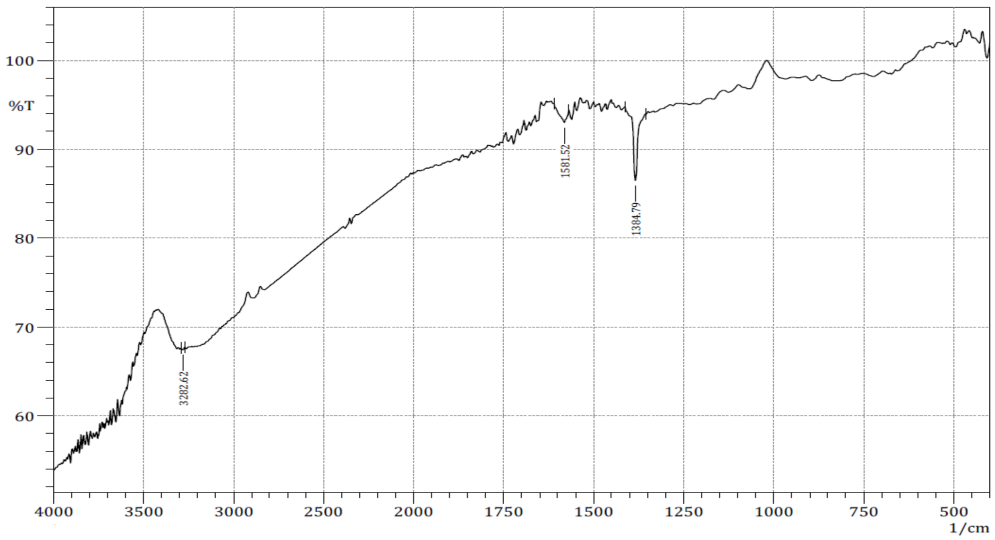

Fig.3. FTIR spectrum of Silver nanoparticles using Vitex Leucoxylon leaf extract

XRD analysis: The XRD spectrum analysis indicated different diffraction peaks at 32.74°, 38.61°, 44.64°, 46.71º, 58.00º, 64.89º, 77.85º which was shown in Fig.4. All diffraction peaks correspond to the characteristics of cubic face centred Silver nanoparticles. These diffraction lines are obtained at 2θ angle, it have been indexed as (111), (122), (200), (231), (103), (220), (311). The Debye-Scherrer equation is used to determine the average grain particle size of the synthesized Silver nanoparticles. D = K λ / β cosθ Where, D is the crystalline size of nanoparticles, λ is the wavelength of the X-ray source (1.54 nm) used in XRD, β is the full width at half maximum of the diffraction peak, (FWHM) K is the Scherrer constant with a value of 0.9 and θ is the Bragg angle. According to Debye Scherrer equation the average particle size was found to be 12 nm [19].

Scanning Electron Microscope (SEM): SEM image shows the size and shape of the biosynthesized Silver nanoparticles using Vitex leucoxylon leaf extract. Size of the nanoparticles was observed at different magnifications. Hexagen shape of nanoparticles was noted with the size range from 10-20 nm. In this SEM image, some of the nanoparticles show large size due to the aggregation of small size of nanoparticles. Poly dispersed nanoparticles were observed in SEM image. The surfaces of aggregated nanoparticles were shown in fig.5. Aggregation of nanoparticles took place due to the in sufficiency of capping agent in the leaf extract to the synthesis of nanoparticles.

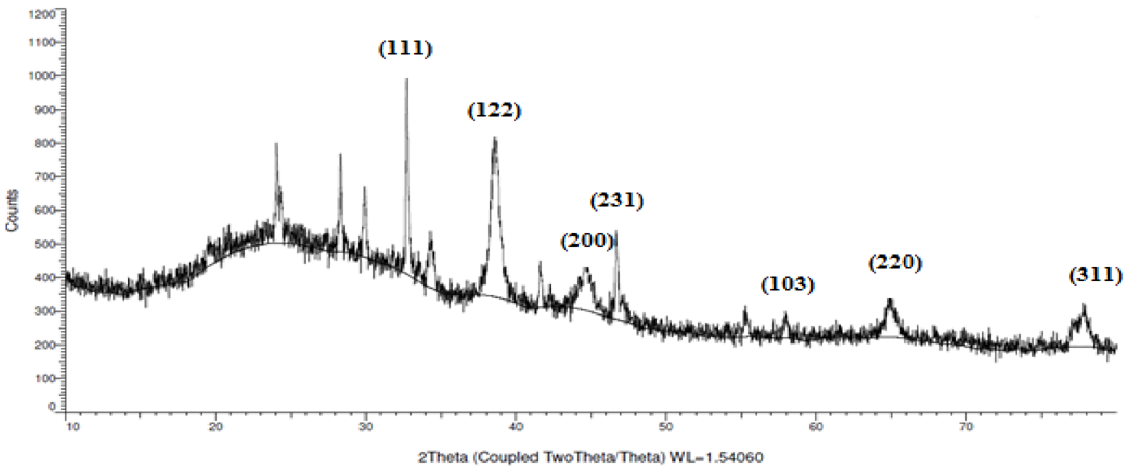

Fig.4. XRD Graph of Silver nanoparticles using Vitex Leucoxylon leaf extract

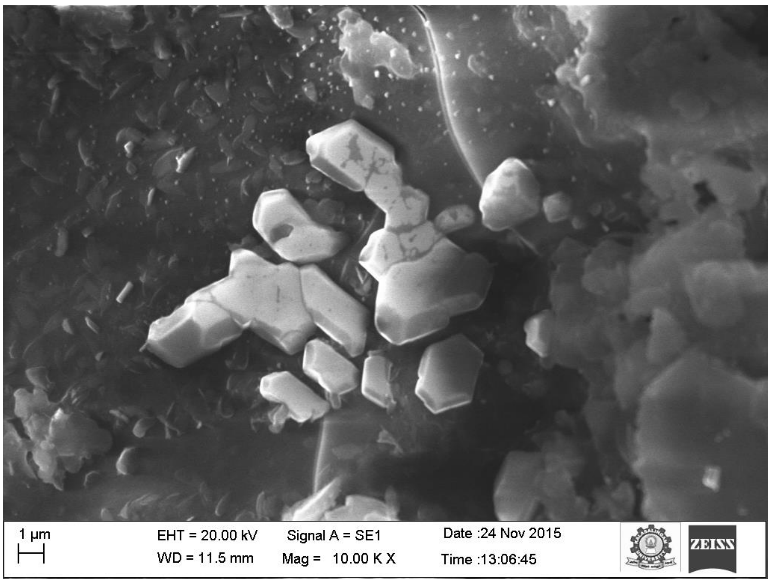

Fig.5. SEM image of AgNPs using Vitex Leucoxylon leaf extract

Energy Dispersive X-ray Spectroscopy (EDX) analysis: Elemental analysis of Silver was measured by EDX spectrum was shown in Fig.6. EDX spectra revealed that strong signals in the Silver region at 3 keV and confirm the formation of nano Silver and its elemental nature. This signal was formed due to the excitation of surface plasmon resonance of Silver nanoparticles. Some of the weak signals were also observed. These signals were found due to the presence of impurity from the biological molecules of leaf extract.

Antibacterial Activity: Pathogenic bacteria isolated from the clinical specimens were used in this study. The antibacterial activities of synthesized Silver nanoparticles against the Gram negative pathogens like Esherichia Coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhii and gram positive bacteria Staphylococcus aureus were shown in Fig.7. The zone of inhibition values obtained from the assay was presented in table.1

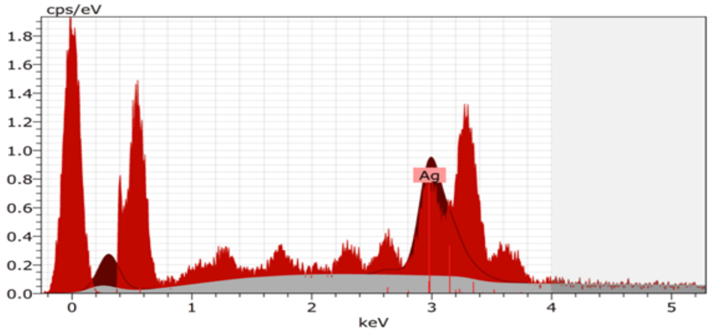

Fig.6. EDX spectrum of Synthesized Silver nanoparticles

Fig.7.Antibacterial activities of synthesized Silver nanoparticles against different pathogens.

Table 1: Antibacterial activity of biosynthesized Silver nanoparticles at different concentrations against pathogenic species

Bacterial Strains	Standard Ofloxin (1mg)	Zone of inhibition in diameter(mm)		
		S_2		
		5µL	10µL	15µL
Staphylococcus aureus	12	6	8	10
Escherichia coli	16	10	14	20
Pseudomonas aeruginosa	14	8	10	16
Klebsiella pneumoniae	12	5	8	12
Salmonella typhi	16	10	16	20

Antifungal Activities: Regarding the antifungal activity of all four fungal strains used in this study are found to be sensitive to the green synthesized Silver nanoparticles as well as to the commercially available antifungal drug ketoconozole. The antifungal activities of Silver nanoparticles was shown in Fig. 8. The zone of inhibition values were presented in Table 2. The fungal species candida albicans and Aspergillua niger had shown high sensitivity to synthesized Silver nanoparticles with a concentration of 15 µl, whereas candida tropicalis had medium sensitivity to synthesized Silver nanoparticles. The remaining Aspergillus fumigates showed resistance to the synthesized Silver nanoparticles with a concentration of 5µl, and 10µl.

CONCLUSION

We established an eco-friendly, rapid biological approach for the biosynthesis of Silver nanoparticles by using Vitex Leucoxylon plant leaves, which provides easy, cost effective, proficient way for the synthesis of Silver nanoparticles. The biological synthesis involves the utilization of plant leaves. The biosynthesized Silver nanoparticles can be used in various biological applications for human benefit. Further, the above Silver nanoparticles revealed to possess an effective antibacterial and antifungal activity. In this study, the antibacterial assays revealed that the Gram-negative pathogens were sensitive to the synthesized Silver nanoparticles. Similarly the effective inhibitions of the fungi by the AgNPs were comparable to that of positive control.

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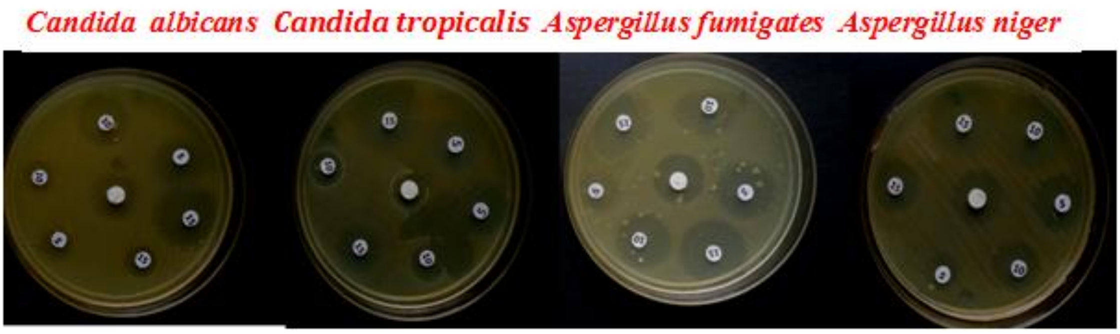

Fig.8. Antifungal activities of synthesized silver nanoparticles against different pathogens

Table 2: Antifungal activity of biosynthesized Silver nanoparticles at different concentrations against pathogenic species

Fungal strains	Standard Fungicide	Zone of inhibition in diameter(mm) Concentration of AgNPs		
	Ketoconazole(10µg/disc)	S_2		
		5µL	10µL	15µL
Candida albicans	16	8	12	16
Candida tropicalis	14	6	8	10
Aspergillus fumigatus	12	R	R	4
Aspergillus niger	14	6	10	14

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