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SYNTHESIS OF SILVER NANOPARTICLES USING *PELTOPHORUM PTEROCARPUM* FLOWER EXTRACT AND EVALUATION OF THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

The current study manifested that the aqueous flower extract of *Peltophorum pterocarpum* contain bioactive compounds, is found to be responsible for bioreduction during the synthesis of silver nanoparticles The synthesis of silver nanoparticles occurred under the exposure of the flower extract to 1mM silver nitrate aqueous solution .During this process the complete reduction was observed nearly 48 hours at room temperature. During the incubation period the color change was observed .Synthesized silver nanoparticles were characterized by UV-Visible spectroscopy, FTIR were carried out to assess the formation of silver nanoparticles. The formation of Ag-NPs was confirmed by TEM, XPS and XRD studies. The synthesized Ag-NPs flower extract was carried out against the fish pathogenic microorganism by agar- well diffusion method. It exhibited good antibacterial activity. The effect of antibacterial activity depends upon the concentration of Ag-NPs.

Keywords: Aqueous Flower extract, UV-Vis spectroscopy, FTIR, TEM, XPS, XRD and antibacterial activity.

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INTRODUCTION

Peltophorum pterocarpum belongs to family Fabaceae native to tropical south eastern Asia. It is popularly ornamental tree grown around the world including India. It is a deciduous tree growing too tall. The flowers are yellow, 2.5-4cm, and produced in large compound racemes up to 2cm long. Different parts are used to treat many diseases like Stomatitis, Insomnia, Skin troubles, Constipation and ringworm; especially the flower extract is known to be a good sleep inducer and used in insomnia treatment [1-3]. Flowers are used as an astringent to cure or relieve intestinal disorders after pain at child birth, sprains, bruises and swelling or as a lotion of eyes troubles, muscular pain and sores. The bioactive compounds obtained from medicinal plants have been used to treat various ailments caused by microorganism [4-8].the bioactive compounds act as self defense against pests and pathogens [9]. Nano particles are mostly prepared from Nobel metals such as Gold, Silver, Platinum and Lead. Among the nobel, Silver (Ag) is the metal of choice in the field of biological systems, living organisms and medicin[10].Here the advantage of using flowers for the synthesis of silver nano scaffold is that, they are easily available and safe. A number of plants are being currently investigated for their major role in the synthesis of nanoparticle[11]. Since nanoparticles exhibit completely new properties based on their specific characteristics such as size, distribution and morphology. The size dependent use of silver nano particles as carrier molecules in applications, such as drug delivery, diagnostics, nano biosensors, etc. are increasing with the advancement in technology [12].The chemical and physical procedures, numerous organism and plants have also been found to synthesize nanoparticles endogenously and exogenously [13]. The biosynthesis of silver nanoparticles using *Lantana camera* fruit extract and *Datura metel* flower extract has been studied[14]. The microbial enzymes and Phytochemical with antioxidant/ reducing properties are usually responsible for the reduction of plant compounds in to their respective nanoparticles. However the green synthesis approaches of producing Ag-NPs are an alternative source of conventional method and posses' excellent antimicrobial activity[15].

MATERIALS AND METHODS

Collection of Plant material and synthesis of silver nanopparticles

Peltophorum pterocarpum flowers were collected from VELS University campus, Pallavaram, Chennai. The taxonomic identification and voucher specimen was numbered (PARC|2015|3033) by Prof.P.Jayaraman, Ph.D. Institute of Herbal Botany, Plant Anatomy Research centre, Tambaram, Chennai. Flowers were collected freshly and washed with running tap water and finally with sterile distilled water. 20 g flowers were immersed with 150 ml of distilled water and boiled (~ 80 °C) for about 30 minutes and then kept at room temperature. The filtrate was done by using Whatman grade I filter paper and stored at 4 °C until further use. In a typical experiment, 90 ml of 1 mM aqueous AgNO3 was added to 10mL of aqueous flower extract and kept it for sun light for 20 minutes. A colour change was obtained from yellow to reddish brown and it confirmed the reduction of Ag+ ions in the aqueous medium Solution [18,19]. The pH of the working solution was adjusted to 7. The AgNPs were separated by centrifugation at 10,000rpm for 30 minutes at cooling temperature and washed twice with distilled water to remove the impurities [10]. Finally the AgNPs were filtered and concentrated. The Ag NPs were stored at 4 °C before being used for further analysis.

Collection of Culture

Fish pathogenic organism such as *Aeromonas hydrophila, Pseudomonas aeruginosa, Edwardsiella tarda and Vibrio cholerae* are used in this experiment.These organism were isolated from the infected fish. The isolates were identified .The sub cultured was maintained in nutrient agar slant in the lab.

Agar-well diffusion method

The Muller-Hinton agar plates were prepared and 100 µl of overnight pure cultures were plated using sterile L-rod. The synthesized AgNPs and the crude plant extract in different concentration (75 µl and 100µl) were loaded onto each well and placed in each culture plate. Tetracycline used as a control. All the plates were incubated at 37°C for 24 h and the zone of inhibition was observed.

Characterization of silver nanopparticles

UV-Visible Spectroscopy analysis

UV-Vis spectral analysis was performed on a DU 800 Spectrophotometer. It is a foremost technique to preview the morphology and stability of nanoparticles. Synthesis of silver nanopparticles by reducing the silver ions solutions with *Peltophorum pterocarpum* flowers extract may be easily absorbed by UV-visible spectroscopy by using small aliquot of the sample diluted with distilled water. The absorption spectra of flower extract concentration were measured using 400-800nm at a resolution of 1nm.

FTIR

FTIR spectra were obtained using a Perkin–Elmer Spectrum 100 spectrophotometer, operated at the resolution of 4 cm-1. The sample was drop cased on a silicon wafer and the material was analyzed and the spectra were recorded in diffuse reflectance mode.

TEM

The size and morphology of NPs were examined using performed using a JEOL 1010 TEM instrument operated at an accelerating voltage of 100 kV. The samples were prepared by placing a drop of AgNPs on a carbon coated copper grid.

XRD

XRD measurement was carried out on a Bruker AXS X-ray diffraction system operating at a voltage of 40 kV and current of 40 mA with Cu Kα radiation(λ = 1.54060 Å). The crystallite size of the silver nanopparticles was calculated using line broadening information and Scherrer's formula[17].

$D = 0.9\lambda/\beta\cos\theta$.

XPS

XPS measurements were obtained with a Thermo K-Alpha XPS instrument at a pressure better than than 1 x 10–9 Torr (1 Torr = 1.333 × 102 Pa). The core level binding energies (BE) were aligned with adventitious carbon (C 1s) binding energy of 285 eV.

The general scan and C 1s, N 1s, O1s, and Ag3dcore level spectra for the samples were recorded.

RESULTS

Figure 1-show the color change in the reaction mixture. (*i e*. Silver solution + flower extract) from yellow to brown due to reduction of silver ion, which indicates the formation of silver nanoparticles. It was recorded through the visual observations.

Figure 1
Peltophorum pterocarpum flowers extract (A), Aqueous solution of 1 mM AgNO3 with Peltophorum pterocarpum flowers extract (B) .

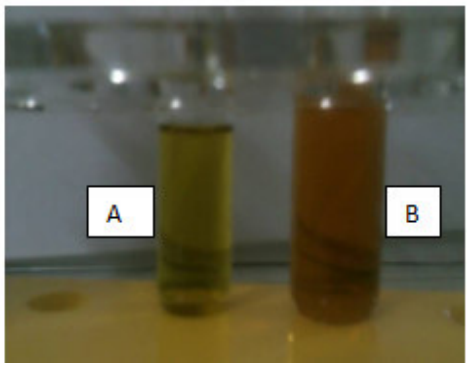


Figure: 2- Shows the bio reduction of Ag^{+} in aqueous solution was monitored by UV-vis spectroscopy. The absorbance spectra of synthesised nanoparticles were detected at various absorbances 400nm to 800nm.The synthesised silver nanoparticles from the aqueous solution found at the absorption peak around 550nm that indicates the particles are completely dispersed in the aqueous solution.

Figure2
2-UV-spectra of synthesized silver nanoparticles from Peltophorum pterocarpum flowers extract

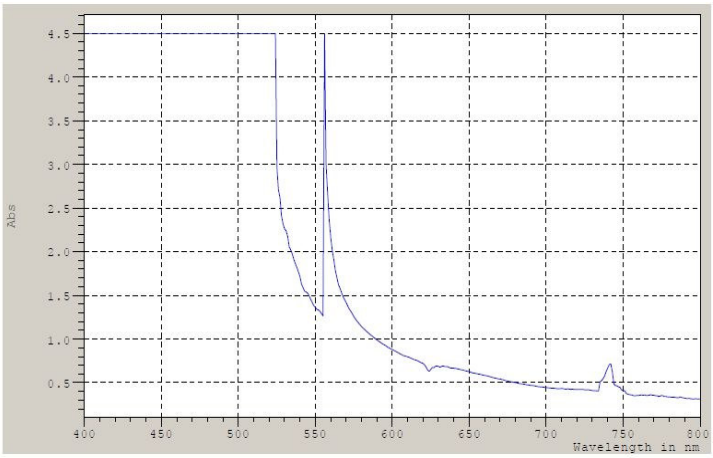


Figure 3- show the strong IR bands were observed at 3397.5 cm^{-1} , 2917.4 cm^{-1}, 2849.6 cm^{-1}, 1666.7 cm^{-1}, 1451.4 cm^{-1}, 1217.4 cm^{-1}, 1108.0 cm^{-1} and 873.5-610.5 cm^{-1}. The bands appeared which appeared at 3397.5 cm^{-1}, 2917.4 cm^{-1}, 2849.6 cm^{-1} corresponds to O-H and aliphatic –C-H stretching respectively C=O group, the peak at 1666.7 cm $^{-1}$could be assigned to the vibrations due to amide group. The absorption peak at 1666.7 cm $^{-1}$ is close to the native proteins which suggest that protein are intercalating with biosynthesized nanoparticles. The IR bands shows 1451.4cm^{-1} and 1217.4cm^{-1} may be related to –C-O and –C-O-C stretching respectively. The Minor bands 1108.0cm^{-1} corresponds to C-N stretching vibration of amine. The peak at 873.5 to 610.5cm^{-1} region for C-H out of plane bends which are characteristics of aromatic phenols.

Figure 3
FTIR spectra for Silver nanoparticles synthesized from Peltophorum pterocarpum flower extract

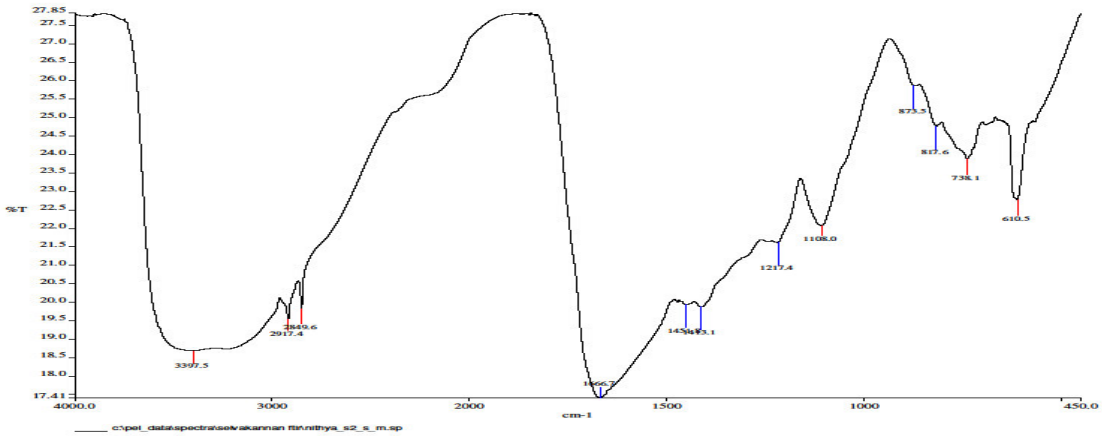
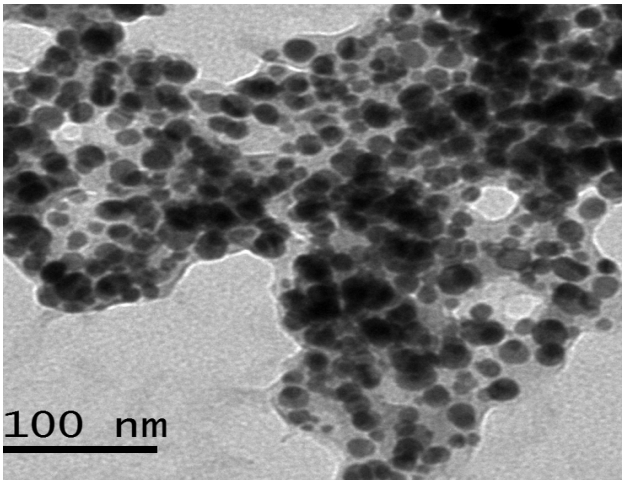


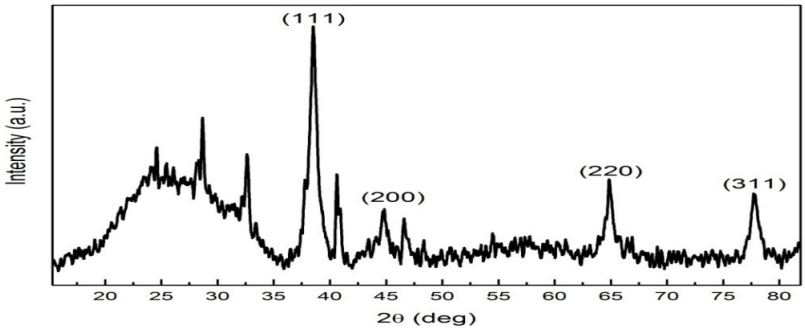
Figure: 4- shows the TEM image and it is evident that the morphology of AgNPs are nearly spherical and some non-spherical in nature having size 100 nm. It is known that spherical as well as non-spherical nanoparticles exhibits better physical properties if they are produced small in size, as the antibacterial properties of silver nanoparticles are size dependent.

Figure4
TEM image of silver nanoparticles formed by Peltophorum pterocarpum flower extract



X-ray diffraction pattern (XRD) was recorded for the synthesized Ag NPs (Figure 5). It shows four distinct diffraction peaks in the whole spectrum of 2θ values ranging from at 38.04°, 44.23°, 64.37° and 77.34° were indexed with the planes (111), (200), (220) and (311) for the face-centered cubic silver confirming crystalline nature. .The well resolved and intense XRD pattern clearly showed that the Ag NPs formed by the reduction of Ag+ ions using *Peltophorum pterocarpum* flowers extract are crystalline in nature. Similar results were reported for Ag NPs in the literature[14]. The low intense peak at 77.34° belongs to (311) plane. The presence of minor peaks suggests that the prepared silver nanoparticles are biphasic in nature.

Figure 5
XRD pattern of synthesized silver nanoparticles



The X-ray photoelectron spectroscopy (XPS) analysis confirmed the formation of metallic silver and elucidates the surface state composition of AgNPs. The general scan spectrum shows the presence of strong Cls, Ols, Nls, and Ag3d core levels (shown in Figure 6). The Ag3d core level spectrum (shown in Figure 6-a) is resolved into two spin-orbit components, which occurred at binding energy of (Ag3d3 scanA) 368.0 and (Ag3d5 scanA) 375.0ev respectively.

Figure 6

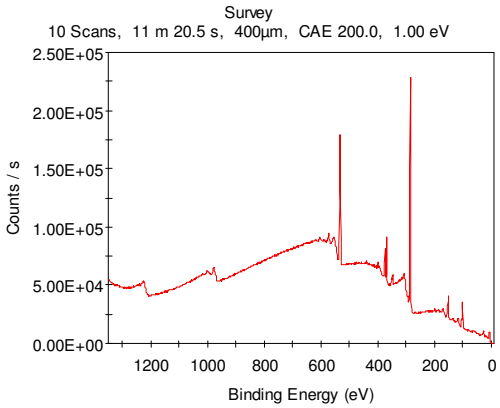
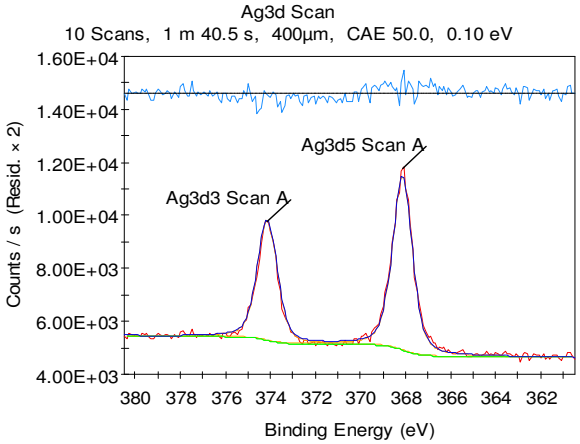


Figure 6a

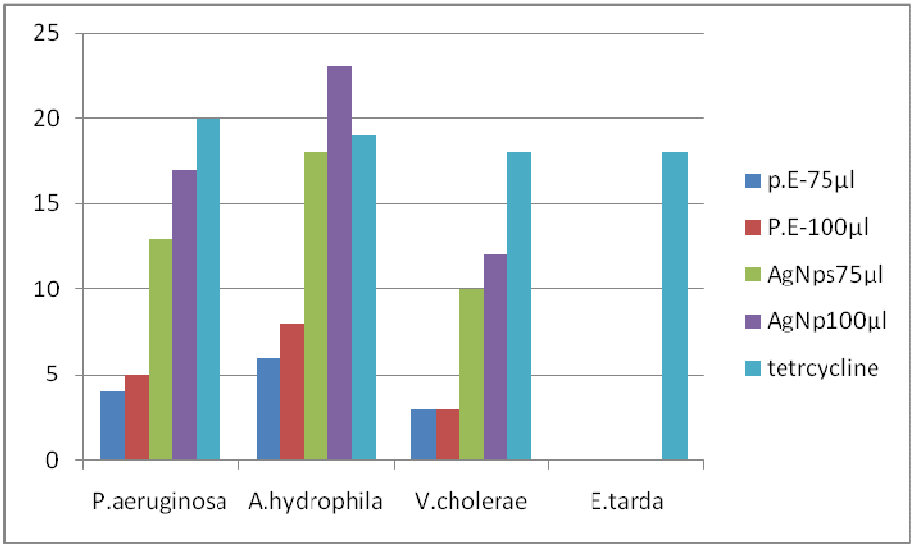


Antibacterial test

The antibacterial activity of silver nanoparticles and plant extracts was tested against fish pathogenic organism using the well diffusion method. Different concentrations of flower extract and synthesized silver nanoparticles were used for the study of antibacterial activity reported in graph-1. 75µl concentration showed less inhibition zone when compared to 100µl flower extract showing the most antibacterial activity against *Aeromonas hydrophila, Pseudomonas aeruginosa* but not showed to the other two species**.** In 75µl and 100µl of synthesized nanoparticles showed maximum inhibition zone against *Aeromonas hydrophila*(18mm and 23mm)*, Pseudomonas aeruginosa*(16mm and 20 mm) and *Vibrio cholerae* (10mm and 12mm) but not showed to *Edwardsiella tarda* in both concentration. Tetracycline drug showed high inhibition zone against bacterial species because it is a standard antibiotics. The AgNPs synthesized from plant species are toxic to multidrug resistant microorganisms. From this study it showed that they have great potential in biomedical applications.

Graph1

Plant extract and synthesis of AgNps plant extract using different concentration



CONCLUSION

In this study we have proved the *Peltophorum pterocarpum* flowers extract is suitable for the synthesis of silver nanoparticles. UV–Vis spectroscopic, FTIR,TEM XPS and XRD techniques confirmed the formation of silver nanoparticles by flower extract. The biosynthesized silver nanoparticles using flower extract showed the excellent antibacterial activity against fish pathogenic microorganism.

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