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Green Synthesis of Silver Nanoparticles from leaf extract Jatropha gossypiifolia and to study its Antimicrobial Activities

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Abstract:

Biologically synthesized nanoparticles have been widely used in the field of medicine. The present study reports the green synthesis of silver nanoparticles using of Jatropha gossypiifolia leaves extract with silver nitrate solution as reducing and capping agent. The synthesized silver nanoparticles were analysed through UV-Visible Spectroscopy, X-ray diffraction, Fourier Transform Infrared Spectroscopy, Field Emission Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy. The synthesized Silver nanoparticles were spherical in shape with an average size of 50-90 nm. These silver nanoparticles were evaluated for antibacterial activity. The diameter of inhibition Zones around the disk two gram positive bacterial strains *Staphylococcus epidermidis* and *Bacillus subtilis*. One gram negative bacterial strains *Klebsiella pneumonia*. Antifungal activities showed good test organisms, from the result sample candida vulgaris was most effective and has the highest activity. It is concluded that the green synthesis of silver nanoparticles was very fast, easy, cost-effective, eco-friendly and without any side effect.

Keywords **-** Silver nanoparticles; jatropha Gossypiifolia; UV;XRD;FESEM;Antibacterial activity; Antifungal activity

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1. INTRODUCTION

Nanotechnology plays aprominent role in the recent day researches, as it involves the engineering and the manipulation of particles at the nano scale approximately ranging from 1-100 nm [1]. Nanotechnology is grasping importance in different fields like biomedical science, health care, environmental health, food and feed, drug and gene delivery, cosmetics, chemical industries, energy science,

electronics, mechanics, and space industries [2]. Moreover it also has been achieved for the treatments of cancer [3], diabetes [4], allergy [5], infection [6] and inflammation [7]. Green chemistry is a development, design and implementation of chemical products and processes to reduce the use and generation of substances that are hazardous to human health and environment [8]. In recent years the scientific community have been attracted by silver nanoparticles (AgNPs) in the field of nanotechnology due to their unique properties and biological applications. As compared to other physical and chemical methods, green synthesis of nanomaterials is recognized as a clean, nontoxic and environmental friendly method compared to other physical and chemical methods [9]. The benefits of green synthesized AgNPs in many biological applications such as antimicrobial [10] anticancer treatment [11] and in drug delivery [12].

Nanoparticles can be synthesized using several approaches such as chemical, physical, and biological approaches.The merit of chemicalmethodofsynthesisrequires short period of time for synthesis even for large quantity of nanoparticles, but this method uses capping agents for size stabilization are toxic and lead to non-eco-friendly by products. Due to the need for an environmental friendly and nontoxic synthetic protocols for nanoparticles synthesis, the biological approaches which are free from the use of toxic chemicals as by products grasp . Thus the demand for green nanotechnology are increasing [13]. In modern science, Nanotechnology is the prominent area of research. Nanoparticles exhibit totally new or improved properties based on specific characteristics such as size, distribution, and morphology [14]. The silver nanoparticles can be produced with the help of list of chemical and physical methods such as chemical reduction, electrochemical method and radiation methods [15-17], but the increasing need of eco-friendly and size control approach for synthesis of metal nanoparticles leads researchers to develop new approaches [18]. In biological activity like antibacterial activity, silver nanoparticles have an immense potential for use [19]. Silver nanoparticles which contains antimicrobial capabilities allowsthem to be employed suitably in enormous household products including home appliances, textiles, medical devices and food storage containers [20]. Silver exhibits less toxicity because it is naturally an effective antimicrobial agent [21]. In medical industry, one of the best application of silver and silver nanoparticles such as tropical ointments are used to prevent infection against open wounds and burns [22]. Moreover in the field of biology and medicine due to their attractive physiochemical properties of silver nanoparticles play a vital role [23]. Additionally silver nanoparticles are also

possessing activities such as antiviral, antifungal, anti-angiogenesis, anti-inflammatory and antiplatelet activities [24].

This study deals with the synthesis of silver nanoparticles using the medicinal plant called Jatropha gossypiifolia. Jatropha gossypiifolia commonly known as bellyache bush, black physicnut or cotton leaf physicnut. This plant is a species of flowering plant and belongs to spurge family, Euphorbiaceae. Jatropha gossypiifolia grows around the height of 2.2to 4 m (8.2 to 13.1 ft) high. When the plant is young, three lobed leaves are purple and sticky and become bright green with age. The flowers are small red in colour with yellow centres appear in clusters and are followed by cherry-sized seed pods that are poisonous.

In the present study, we have investigated the synthesis of silver nanoparticles and characterized them using UV-visible spectroscopy, Fourier Transform Infrared spectroscopy, X-ray Diffraction, Field Emission Scanning Electron Microscopy and Energy Dispersive Spectroscopy and Dynamic Light Scattering. Likewise, against Staphylococcus epidermidis, Bacillus subtilis and Klebsiella pneumonia, the antibacterial activity of synthesized Silver nanoparticles was assessed.Antifungal activities showed candida vulgaris and candida albicans.

2. MATERIALS AND METHODS

2.1. Collection of the plant materials

The fresh leaves of Jatropha gossypiifolia Figure. 1 were collected from Trichy, India. The leaves were rinsed with water thrice continued by deionized water to get rid of the fine dust particles and then plant leaves were dried in shadow area for a week to thoroughly eliminate the moisture.

Figure.1 Jatropha gossypiifolia

2.2. Preparation of leaves extract

To make fine powder, the dried leaves were pulverized well with a domestic grinder. The powder sample of 10 grams was blended into 100 ml of deionized water and the mixture was boiled on a heating mantle at 60 °C for 15 minutes. The leaves extract was cooled and then filtered with Whattman No.1 paper.

2.3. Determination and synthesis of Silver Nanoparticles

To perform the synthesis of silver nanoparticles, the 10 ml of the aqueous extract of Jatropha gossypiifilia leaf extract was added

into Erlenmeyer flask. Housing 90 ml of 1mM silver nitrate. The complete reaction process was carried out in a dark room condition. The colour of the solution turns into a brown after the time period of one hour as illustrated in the Figure.2. The colour change of the reaction solution was observed for the characterization of silver nanoparticles. After the completion of synthesis and reaction, the resultant solution was centrifuged at 10,000 rpm for 15 minutes. The transparent solution was discarded and the pellets of silver nanoparticles were collected and the final solution was dried.

Figure.2 The Solution change after the addition of Silver nanoparticles

3. CHARACTERIZATION OF SILVER NANOPARTICLES

UsingUV-VISIBLE SPECTROPHOTOMETER LAMBDA 35 PERKIN ELMER spectrophotometer instrument, the formation of silver nanoparticles was confirmed by UV-Visible Spectroscopy.The biomolecules present in the leaf extract was determined by carrying out FTIR analysis for the reduction of Ag ions having spectral range around400-4000 Cm^{-1}. To form a pellet, the sample was centrifuged at 10,000 rpm for 15 minutes. Then the sample was dried using hot air oven and ground with the help of Kbr. The pellet was analysed by FTIR SPECTRUM 1000 PERKIN ELMER SPECTROMETER instrument. The X-ray Diffraction analysis using an XRDX "X" PERT PRO Diffractrometer instrument was used to determine the crystalline structure of the silver nanoparticles. FESEM images were recorded by using FEI QUANTA-250 FEG instrument. For qualitative elemental analysis, an energy dispersive spectroscopy BRUKER analysis was performed on the prepared sample. Nanoplus has been utilized to calculate the average particle size of the synthesized silver nanoparticles.

3.1 Screening of Antibacterial Activities:

3.1.1 Antibacterial activity of silver nanoparticles (disc diffusion method)

Antibacterial activity of Jatropha gossypiifoliasilver nanoparticles was determined using the disc diffusion method. The petridishes (diameter 60 mm) was prepared with Muller Hinton Agar and inoculated with test organisms. Sterile disc of six millimeter width were impregnated with 10 µl of various samples respectively. Prepared

discs were placed onto the top layer of the agar plates and left for 30 minute at room temperature for compound diffusion. Positive control was prepared using the 10 µl of Amoxicillin as standard antibiotic disc. The dishes were incubated for 24 h at 37ºC and the zone of inhibition was recorded in millimeters and the experiment was repeated twice.

3.2 Screening of Antifungal Activities:

Culture Media

The media used for antifungal test was Sabouraud's dextrose agar/broth of Hi media Pvt. Bombay, India.

Inoculum

The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 h and the suspensions were checked to provide approximately 10^5 CFU/ml.

Fungal strains used

The clinical fungal test organisms used for study are *Candida albicans* (*MTCC*-3498) and *Candida vulgaris* (MTCC 227),were procured from National Chemical Laboratory (NCL), Pune, Maharashtra, India.

Determination of antifungal activity

Antifungal activity of sample was determined using the disc diffusion method. The petridishes (diameter 60 mm) was prepared with Sabouraud's dextrose agar (SDA) and inoculated with test organisms. Sterile disc of six millimeter width were impregnated with 10 µl of various samples. Prepared discs were placed onto the top layer of the agar plates and left for 30 minute at room temperature for compound diffusion. Positive control was prepared using the 10 µl of Fluconazole as standard antibiotic disc. The dishes were incubated for 24 h at 37ºC and the zone of inhibition was recorded in millimeters.

4. RESULTS AND DISCUSSIONS

4.1 UV-Visible Spectroscopy analysis

UV-Visible spectroscopy analysis has confirmed the reduction of silver ions to silver nanoparticles as shown in Figure.3. It was a common knowledge that the Silver nanoparticles shows dark brown colour and this colour comes as a result of the surface plasmon excitations in the metal nanoparticles. When the extract of plant was included into the $AgNO_3$ solution, the brown colour solution was received. In another 1 hour of time, the colour changes to dark brown from brown. At the range of 200-500 nm, the formation of absorption spectra of silver nanoparticles takes place in the reaction mixture was achieved by the UV-Visible analysis. The formation of silver nanoparticles in the silver nitrate reaction medium was confirmed UV-Visible absorption Spectroscopy by creating peaks at 364, 369 and 378 nm. During the experiment the absorbance band of AgNPs was 369 nm whereas the sharp absorbance with highest peak at 378 nm [25].

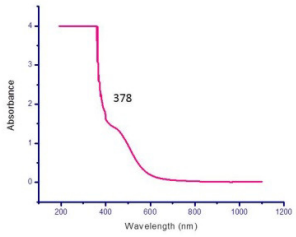

Figure.3 UV-Visible Spectroscopy analysis

4.2 Fourier Transform Infrared Spectroscopy

The biosynthesized silver nanoparticles using Jatropha gossypiifolia has FTIR spectrum. The absorption peaks at 3423 cm^{-1}, 2924 cm^{-1}, 2854 cm^{-1}, 2073 cm^{-1}, 1626 cm^{-1}, 1384 cm^{-1}, 1072 cm^{-1}, 1041 cm^{-1}, 668 cm^{-1}, 604 cm^{-1} was illustrated in Figure.4. The presenceof N-H stretching vibration indicating the presence of amine was revealed at the peak 3423 cm^{-1}, whereas 2924 cm^{-1}reveals the presence of C-H stretching vibration indicating the presence of alkane. The peak 2073 cm^{-1} reveals the presence of N=C=S stretching vibration indicating the presence of isothiocyanate. The peaks 1626 $cm^{-1}$and 1384cm^{-1} has revealed the presence of C=C stretching vibration indicating the presence of alkane and presence of C=H bending vibration indicating the presence of aldehyde respectively. At 1072cm^{-1} the presence of S=O stretching vibration indicating the presence of sulfoxide was confirmed. The presence of C-O-CO stretching vibrations indicating the presence of C=C bending vibration indicating the presence of alkane was revealed at1041cm^{-1}. Finally the peak 604 cm^{-1} reveals the presence of C=Br stretching vibration indicating the presence of halo compound. From the above results we came to know that the nano capping of the Jatropha gossypiifolia leaf extract is responsible for the reduction and subsequent stabilization of the AgNPs [26].

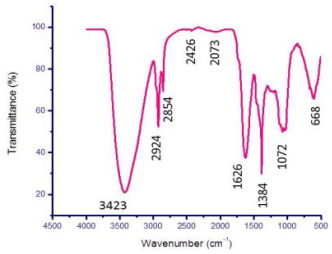

Figure.4 Fourier Transform Infrared Spectroscopy

4.3 X-ray Diffraction

In Figure.5 The powder Diffraction (XRD) patterns of the $AgNO_3$ were shown. The diffraction peaks of $AgNO_3$ which indicated characteristic of metallic face centred cubic structure at 38.1, 44.2, 64.5, 77.4, 81.5. It can be observed that the potential Silver nitrate corresponded diffraction peaks. The results correspond to the (111) and (311)

Bragg's reflection respectively. In addition, the formation of dark brown colour has confirmed the biosynthesis of silver nanoparticles and it happened as a result of excitation of the surface plasmon vibration of the synthesized silver nanoparticles [27]. Furthermore, the synthesis of silver nanoparticles with sharp bands of Bragg peaks was substantiated by the results of XRD patterns. This might be due to the stabilization of the synthesized nanoparticles by the Jatropha gossypiifolia leaves extract which holds various reducing agents and thus conceded the crystallization nature of the Silver nature of the silver nanoparticles [28].

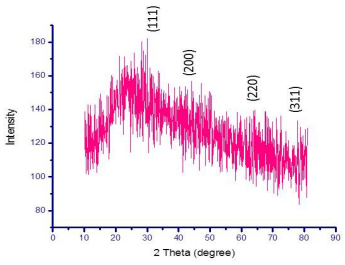

Figure.5 X-ray Diffraction

4.4 Field Emission Scanning Electron Microscopy

FESEM showed the morphology of green synthesized AgNPs. The figure.6(a). represented FESEM image which showed that the formed AgNPs formed were well dispersed having spherical in shape and particle sizes ranging from 50 to 90 nm. It is came to know that the shape of nanoparticles strongly affects the optical and electronic properties of metal nanoparticles [29].

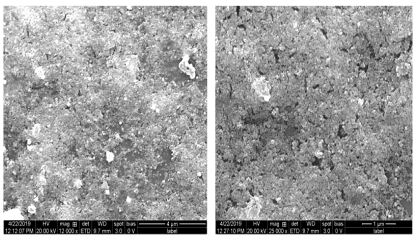

Figure.6(a) Field Emission Scanning Electron Microscopy

Energy Dispersive X-ray Spectroscopy

The result of EDAX clearly exposed the elements present in the biosynthesized nanoparticles. The crystalline property was indicated by EDAX profile of phyto-capped SNPs showing strong signal of the Ag atoms as shown in Figure.6(b). At 3KeV it achieves the optical absorption peak, which is typical for the absorption of metallic silver nanocrystallites [30].

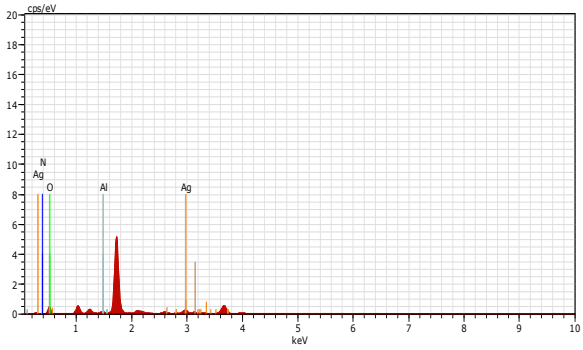

Figure 6(b) Energy Dispersive X-ray Spectroscopy

4.5 Dynamic Light Scattering

Dynamic Light Scattering measurements were performed to persuade the size of the formed silver nanoparticles [31]. The particle size distribution curve of the synthesized AgNPs. In the same figure the particles ranging from 77.5 nm had an average particle size of 163.9 nm as shown in Figure 7.

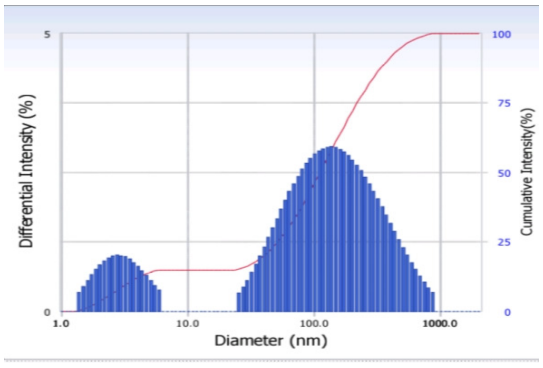

Figure. 7 Dynamic Light Scattering

4.6 Antibacterial activity

To examine the antibacterial activity of silver nanoparticles, two gram positive bacterial strains *Staphylococcus epidermidis* (MTCC 737) and *Bacillus subtilis* (MTCC 2451). Two gram negative bacterial strains *Klebsiella pneumonia* (MTCC 3384) were prepared as test organisms Figure.8 . All the strains were procured from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India. Bacterial strains were cultivated at 37ºC and maintained on nutrient agar (Difco, USA) slant at for 4ºC.

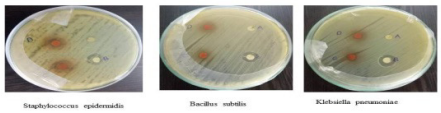

Figure.8 Antibacterial activity

Samples	Concentrations (µl/ml)	Organisms/ Zone of Inhibition (mm) *Staphylo Bacillus Escherihiacoli Coccus subtilis aureus*		
A(Silver Nitrate)	10 µl	0	0	0
B (Amoxicillin)	10 µl	9	9	9
C(Plant Extract)	10 µl	8	5	3
D (Nanoparticles)	10 µl	7	6	5

4.7 Antifungal activity

The experiment represents the outcomes of the antifungal susceptibility test of the various samples and against the test organisms. As shown in the Figure.9, the experiment outcome demonstrates that the sample D was the most effective and has the highest activity against Candida vulgaris (4 mm zone of inhibition) as shown in table.2.

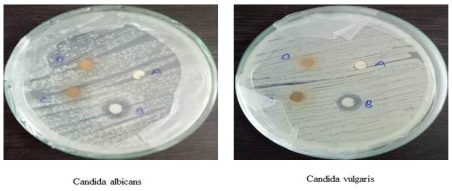

Figure.9 Antifungal activity

Table 2: Antifungal activity

Samples	Concentrations (µl/ml)	Organisms/ Zone of Inhibition (mm) Candida Vulgaris	Candida Albicans
A(Silver Nitrate)	10 µl	0	0
B (Amoxicillin)	10 µl	8	8
C (Plant Extract)	10 µl	3	1
D (Nanoparticles)	10 µl	2	0

Conclusion

Jatropha gossypiifolia leaves extract was found suitable for the green synthesis of silver nanoparticles. The reduction of silver nanoparticles by the leaves extract resulted in the formation of stable nanoparticles with spherical morphologies. The concentration of leaves extract and metal ions play an important role in the green synthesis of AgNPs. The spectroscopic characterization using UV-Vis, XRD, FESEM, EDAX and particles size analyzier were useful in proving the formation of nanoparticles and also in confirming their size and shape. FTIR evidenced the formation and stability of the biosynthesized AgNPs which can be studied further to understand the chemical and molecular interaction which could be responsible for the nanoparticle synthesis. The antibacterial activity of silver nanoparticles, two gram positive bacterial strains *Staphylococcus epidermidis* (MTCC 737) and *Bacillus subtilis* (MTCC 2451). Two gram negative bacterial strains *Klebsiella pneumonia* (MTCC 3384). Antifungal activities showed good test organisms, from the result sample candida vulgaris was most effective and the highest activity.

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