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Elaeagnus indica Mediated Green Synthesis of Silver Nanoparticles and its Potent Toxicity Against Human Pathogens

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Abstract: Present study, we utilized the bio–reductive potential of the Elaeagnus indica Servett for the synthesis of silver nanoparticles (AgNPs). The green synthesized AgNPs was achieved at 80°C and found to be highly stable in room temperature for a month. The AgNPs was found to be spherical in shape with an average size of ~30nm in diameter. The AgNPs were characterized using Ultraviolet–Visible (UV–Vis) absorption spectroscopy, Fourier Transform Infrared spectroscopy (FTIR), Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS). The anti-bacterial and anti-fungal activities of the synthesized AgNPs were tested against a few human pathogens. The results showed that the size and the shape can be easily controlled by employing the optimum concentrations ratio of AgNO3 solution and E. indica andalso varying temperature and incubation time. The synthesized AgNPs from E. indica shows wide range of toxic to human pathogens and can be used as a potent therapeutic agent.

Key words: Silver Nano Particles · Agnps · *Elaeagnus indica* · Human Pathogens

INTRODUCTION

Nanotechnology is the most promising field for generating new application in medicine [1]. The application of Silver Nanoparticles (AgNPs) is burgeoning day by day in all forms of human life. AgNPs applications have impressed by their diverse properties such catalysis, magnetic and optical polarizability [2], electrical conductivity [3] andsurface enhanced Raman scattering [4]. Also, it has some potential activities in medicine like biosensing [5], antimicrobial activity [6], biological labeling [7], detection of genetic disorders [8], drug delivery [9], gene therapy and DNA sequencing [10]. It is prepared by variety of chemical and physical methods such as chemical reduction, photochemical reduction, electrochemical reduction, heat evaporation, etc., Shankar *et al*. [11]. Most of these methods are very expensive and they also process by use of toxic, hazardous chemicals which are high risk for humans and not eco-friendly [12].

As an alternative to the chemical and physical methods, synthesis of nanoparticles by biological methods, using enzymes, microorganisms and plant or plant powder, have been suggested. Among these methods, the green route synthesis shows an advantageous over other biological methods by eliminating elaborate process of maintaining the microbial cultures. Jose-Yacaman and his co-workers first reported the formation of gold nanoparticles [13] and silver nanoparticles [14] by living plants. Already, AgNPs synthesized from leaf extracts of *Camellia sinensis* [15], *Pelargonium graveolens* [16], *Cymbopogon flexuosus* [17], *Cinnamommum camphora* [18], *Azadirachta Indica* [19], *Aloe vera* [20] etc., AgNPs are also very popular for their antimicrobial potential against several other bacteria [21] and fungus [22]. But so far there is no report on the synthesis of AgNPs from *Elaeagnus indica*.

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Elaeagnus indica Servett. (Elaeagnaceae) is a large, branched, usually scandent shrub, often running over trees. Its leaves are variable, broadly elliptiv or elliptic-lanceolate, obtuse or acuminate apex, upper surface pale green, clothed with small whitish seals, lower surface silvery white. Flowers usually many in cluster and its perianth covered with silvery scales. The fruits are nearly 2 cm long and edible. This is the new report about silver nanoparticles from *E. indica*. In this study, we first reported about green route synthesize of AgNPs from *E. indica* which characterized by FT-IR, TEM, UV-Vis spectroscopic study and their antimicrobial assessment was performed.

MATERIALS AND METHODS

Preparation of Plant Powder and Synthesis of Silver Nanoparticles: The fresh leaves of *E. indica* were collected from Kolli Hills, Tamil Nadu, India. The plant was identified and authenticated by Dr. S. Soosairaj, Assistant Professor, Department of Botany, St. Joseph's College, Trichy, India as *Elaeagnus indica* Servett. (Elaeagnaceae) with voucher specimen no: SJCBOT1286. The collected leaves were dried for 15 days and grinded into fine powder.

Synthesis of Silver Nanoparticles: With 10 mg of plant leaf powder, 3ml of 1mM $AgNO_3$ was added and mixed well. Then, the suspension was heated at 80°C until the color changes from yellowish to brown which indicates the formation of AgNPs. After cooling, the suspension was centrifuged at 3000rpm for 5 min and the colored supernatant was transferred and again centrifuged at 12000rpm for 10 min. The AgNPs pellet was re-suspended with sterile distilled water and used for further experiments.

Characterization of AgNPs: The UV–VIS absorbance spectra of the synthesized AgNPs was recorded in UV-VIS spectrophotometer (Shimadzu UV 2450), 200-900 nm wavelength, operated at a resolution of 1nm resolution. The size distribution was determined using Dynamic light scattering system (Nano-ZS90, Malvern). The hydrodynamic size distribution was evaluated several times. The size and shape homogeneity of the synthesized AgNPs was analyzed in High resolution Transmission electron microscopy (HRTEM) using a Philips CM-10 TEM. Fourier transform infrared (FTIR) spectroscopy measurements were done over the range of 400 – 4000 cm^{-1} under transmittance mode using Spectrum-RXI (Perkin Elmer).

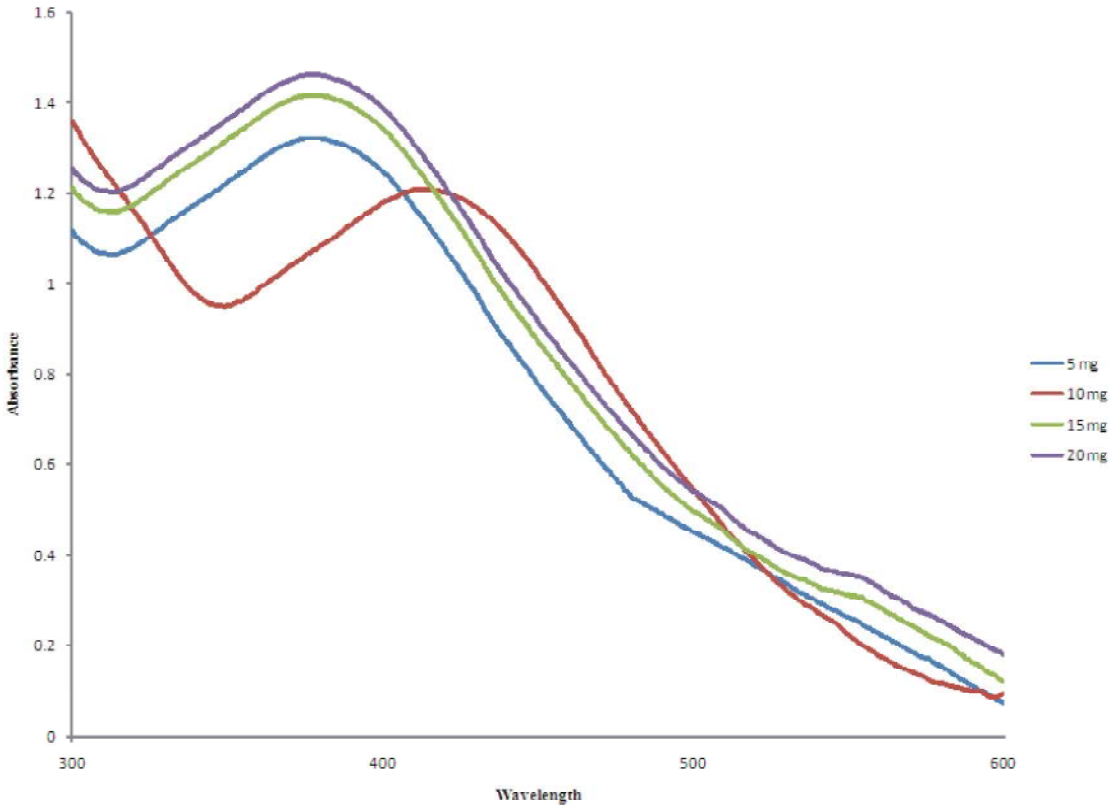
Antimicrobial Activity: The human pathogenic strains were obtained from the DST-FIST, GOI sponsored Culture Collection Centre Department of Botany, National College, Tiruchirappalli. Culture strains were maintained in sterile conditions and sub-cultured at regular intervals.

Antibacterial Activity: The antibacterial activities of AgNPs were carried out by disc diffusion method [23]. Nutrient agar medium plates were prepared, sterilized and solidified. Then, bacterial cultures were swabbed on these plates. The sterile discs were dipped in AgNPs solution and placed in the nutrient agar plate and kept for incubation at 32°C± 1°C for 24 hours. Zones of inhibition for AgNPs, silver nitrate and Streptomycin (10 mcg) were measured.

Antifungal Activity: The antifungal activities of AgNPs were done by agar incorporation method [24]. The fungal strains were individually inoculated on Sabouraud Dextrose Agar (SDA) medium and Czapek Dox Agar Medium in Petri dish in triplicate. The plates were inoculated at 28 ± 1°C for five days to attain good growth in control. The fungal colony growth in AgNPs, Silver Nitrate andwith Bavistin was compared.

RESULTS AND DISCUSSION

Characterization of Silver Nanoparticles: The green synthesized AgNPs is obtained due to reduction of the $AgNO_3$ with the exposure of the plant leaf powder. The rate of synthesis can be easily observed by the change in color from pale yellowish to brown. AgNPs synthesize with the 1mM solution of silver nitrate and different concentrations of powder of *E. indica* are shown in Fig. 1a. It is observed that the synthesized AgNPs exhibit yellowish to brown color in aqueous solution due to excitation of surface plasmon vibration in the silver particles [11]. Fig. 1a. showed the UV-Vis spectra recorded from the reaction medium as a function of reaction using *E. Indica* leaf broth together with spectra of pure AgNPs. It is observed surface plasmon resonance peak at 412nm with 10 mg concentration of plant powder which confirms the synthesis of AgNPs and with 5 mg concentration of plant powder is insufficient for nanoparticles synthesis. The optimum level of green synthesis of AgNPs is observed with 10 mg of plant powder. Based on the UV-Vis spectra the sharpness of the absorption peak is dependent on the concentration of leaf powder, which gets further sharpened at still higher concentrations. Dubey (2010) correlated increased leaf powder concentration with decreased size of nanoparticles.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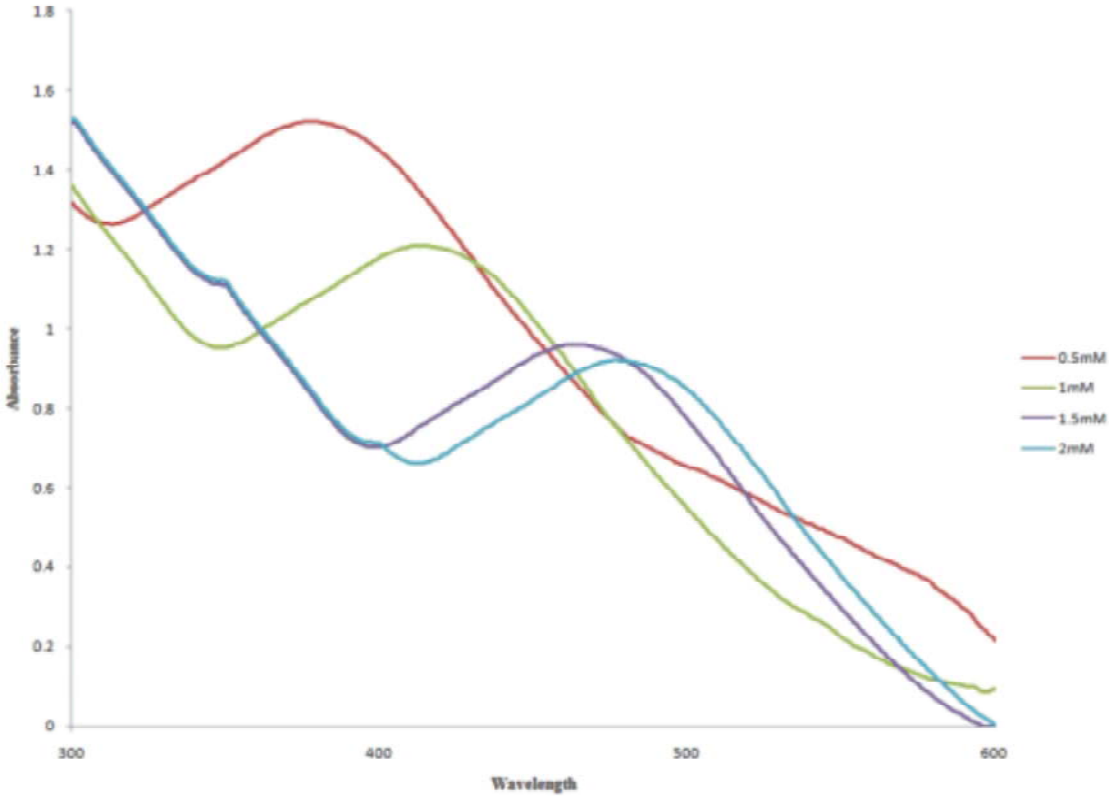
(a)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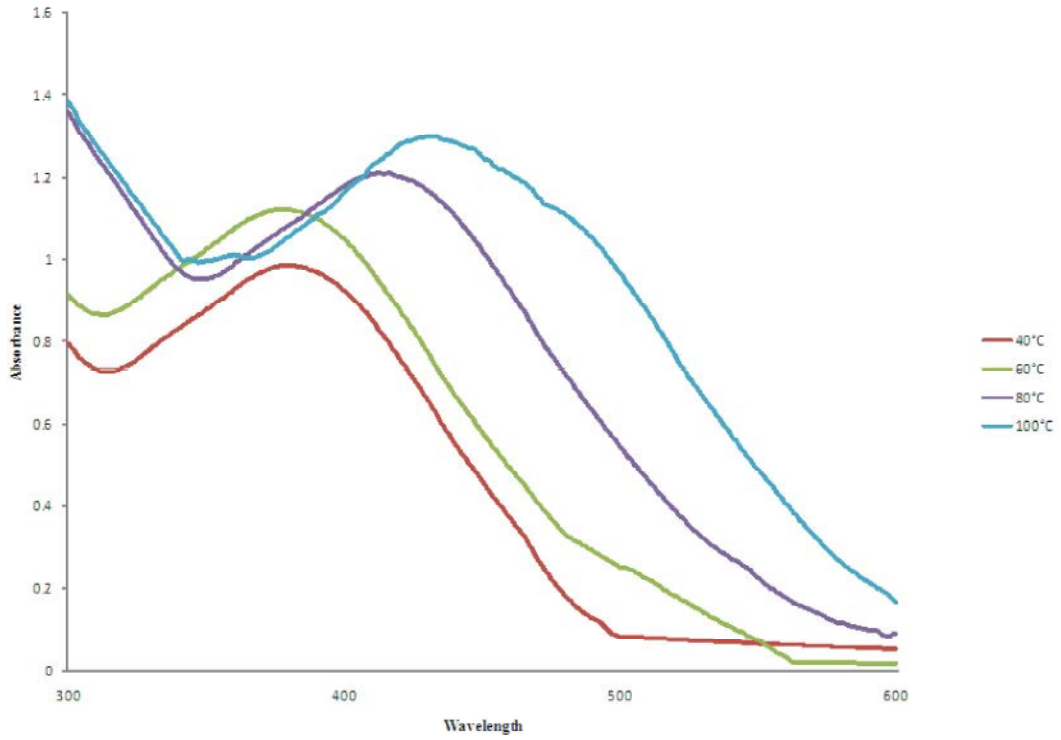

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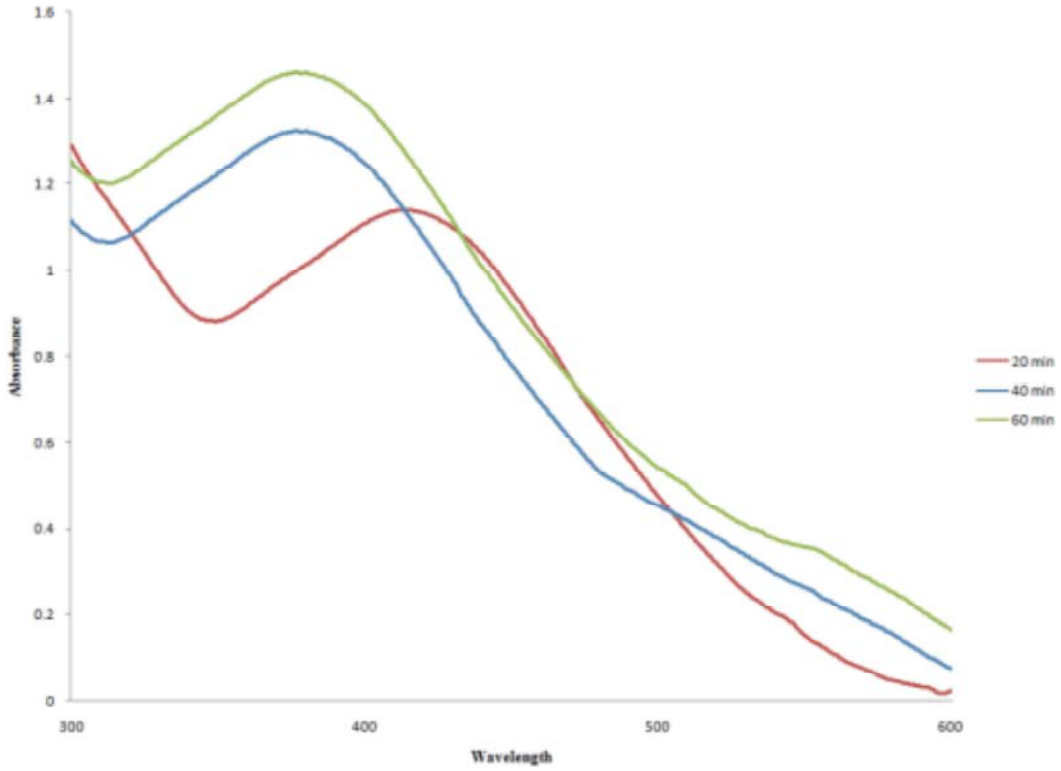

(d)

Fig. 1: UV-Vis absorption spectra of a) 1mM $AgNO_3$ with different plant powder concentration b) 10mg Plant powder concentration with different $AgNO_3$ concentrations c) 1mM $AgNO_3$ and 10mg Plant powder concentration with various temperatures d) 1mM $AgNO_3$ and 10mg Plant powder concentration at 80°C in different time intervals

The amount of leaf powder used for the synthesis of AgNPs was also found less among many reported research [25].

At different concentrations of $AgNO_3$ (0.5 to 2 mM) with 10 mg of plant powder was carried out to optimize the $AgNO_3$ concentrations. Fig. 1b. showed that the Surface Plasmon Resonance (SPR) band increased progressively with increased concentration of $AgNO_3$ and 458 and 473nm are appeared for 1.5 and 2 mM of $AgNO_3$ respectively. The peak with 1mM of $AgNO_3$ exhibited suitable range of peak at 412nm. Increasing intensity of SPR band indicates increasing concentration of particle and the appearance of red shifted band at 458 and 473 nm at higher concentration of $AgNO_3$ indicated the formation of larger particles.

To optimize the temperature, a temperature variation study was carried out using the optimized concentration of $AgNO_3$ (1mM) with aqueous plant powder (10 mg). Fig. 1c. showed the SPR band width of AgNPs synthesized at different temperatures (40, 60, 80 and 100°C). It is observed that both synthesis rate and final conversion to AgNPs increase with increased temperatures. We observed that at 40, 60 and 100°C are not optimum to rate the synthesis of AgNPs and 80°C was produced AgNPs at same wavelength 412nm with optimum concentrations of $AgNO_3$ and plant powder. Rai *et al*. [26] also observed the reduction rate increase with increase in the reaction temperature for the gold nanotriangles using lemongrass extract. As the reaction temperature increases, the reaction rate increases and thus most silver ions are consumed in the formation of nuclei, stopping the secondary reduction process on the surface of the performed nuclei.

To find an optimum time for AgNPs synthesis from *E. indica*, the optimum $AgNO_3$ (1mM), plant powder (10 mg) and temperature (80°C) was used with different times (20, 40 and 60 min). Fig. 1d. showed the UV-Vis absorption spectra of AgNPs synthesized at different time durations. It is observed the intensity of SPR bands increases as the reaction time progresses and after 60 min of rate reaction was declined. From the results, it is observed that 20min was optimum time to synthesize controlled AgNPs from *E. indica*. Throughout this study we have used 10 mg plant powder, 1mM of $AgNO_3$ maintained at 80°C in 20min to control the growth and rate of reaction to produce the small nanoparticles.

FTIR Spectroscopy: TIR analysis confirmed that the green synthesis of AgNPs is due to the reducing by capping material of plant powder. The FTIR spectra of untreated and treated leaf powder samples containing AgNPs is depicted in Fig. 2. The band intensities in different regions of spectrum for the control and test sample were analyzed. There was a shift in the following peaks: 2140-2150, 1646-1648, 1048-1052, 741-755 cm^{-1}. The peak located at around 2140cm^{-1}, was attributed to the weak terminal alkynes stretching. The peak shift from 2140–2150 cm^{-1} implicated that these groups may be involved in the process of nanoparticle synthesis. The peak located at 1646 cm^{-1} could be assigned to the medium stretching in alkenes or medium bending in the primary amine group. A shift in this peak (from 1646-1648 cm^{-1}) indicated the possible involvement of carboxyl or amino groups of the plant powder in nanoparticle synthesis. The vibration shift around 1048-1052 cm^{-1} was suggestive of the involvement of the medium stretch of C-N and aliphatic amine groups in the reductive process. The leaf powder of *E. indica* are mainly composed secondary metabolites and the functional groups associated with these polymers as well as proteinaceous matter may be involved in reducing the silver salt to Ag^0. Comparison between spectra of untreated sample to the treated samples AgNPs reveal only less alterations in the positions as well as on the magnitude of the absorption bands; wave number varying typically about 1-10 cm^{-1}.

TEM and DLS Analysis: HR-TEM analysis reveals the synthesized AgNPs was found to be mono-dispersive in nature with the shape homogeneity. The average size distribution was found to be 30 nm in diameter. HR-TEM images recorded from drop coated films of the silver nanoparticle synthesized by treating 1mM silver nitrate solution with 10 mg plant powder at 80°C in 20 min are shown in Fig. 3a. Fig. 3b shows the size distribution of DLS analysis and was found to be 30nm in diameter which is in agreement with the TEM results. Table 1 shows also the hydrodynamic diameter of the synthesized AgNPs was found to be 30nm in diameter with the poly dispersive index of ~0.432 (PDI<1) resembles the actual size of particles in TEM measurements. The zeta potential of the synthesized AgNPs shows -27mV suggests that the particles are caped with negative charge plant biomolecules like amino acids, flavanoids, etc.

Antimicrobial Activity: Biological synthesis of metal nanoparticles is a traditional method and the use of plant powder has a new awareness for the control of disease, besides being safe and no phyto-toxic effects [14].

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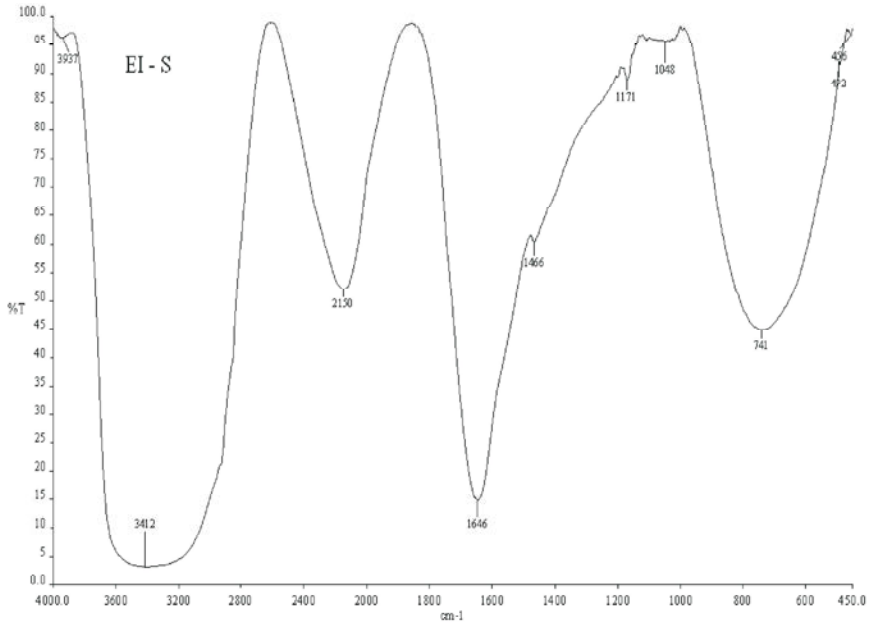

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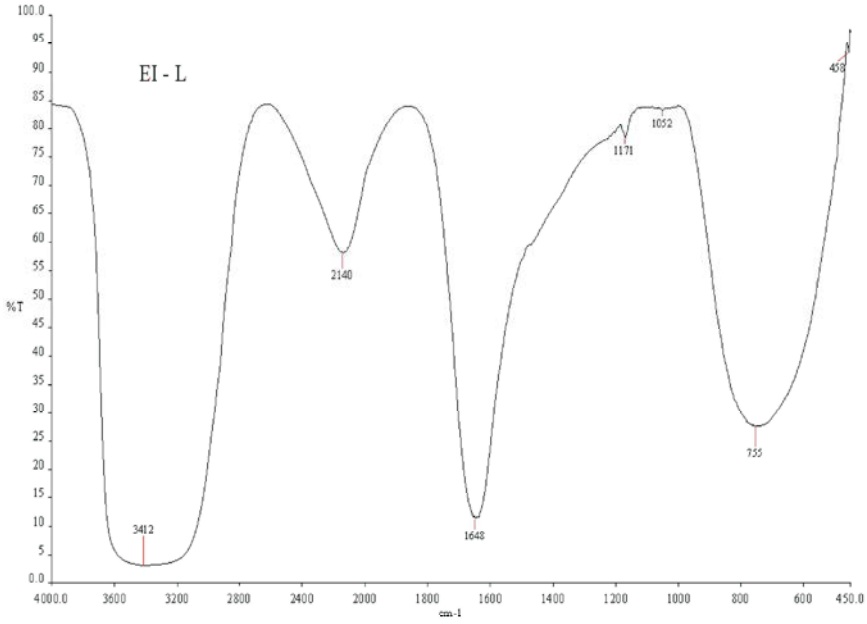
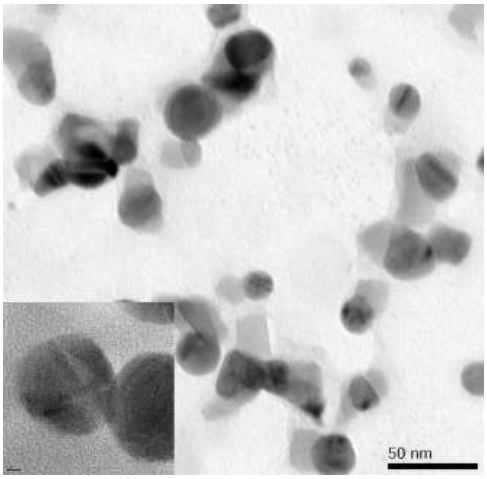


Fig. 2: FTIR spectra of *E. indica* before (a) and after (b) reaction with 1mM $AgNO_3$ at 20°C in 20 min

Antibacterial Activity: The AgNPs from medicinal plants were highly toxic against different pathogenic bacteria. Here, the AgNPs of *E. indica* showed highest antibacterial activity against all tested human pathogens such as *E. coli* (Fig. 4a), *Pseudomonas putida* (Fig. 4b), *Bacillus subtilis* (Fig. 4c) and *Staphylococcus aureus* (Fig. 4d). Similar observation was found in *Allium cepa* [27], *Argimone Mexicana* [28] *Artocarpus heterophyllus* [29].

The exact role of mechanism and mode of action of AgNPs against bacteria are remains unknown. Recent reports stated that the silver ions treated bacteria controlled by stopping of its DNA replication process. Hence, the toxicity effect of AgNPs to the human pathogens can be attributed to their stability in the medium as a colloid, which changes the phosphor-tyrosine of the pathogen proteins and arrests its growth. The removal of free silver ions, due to the teichoic acid or

(a)



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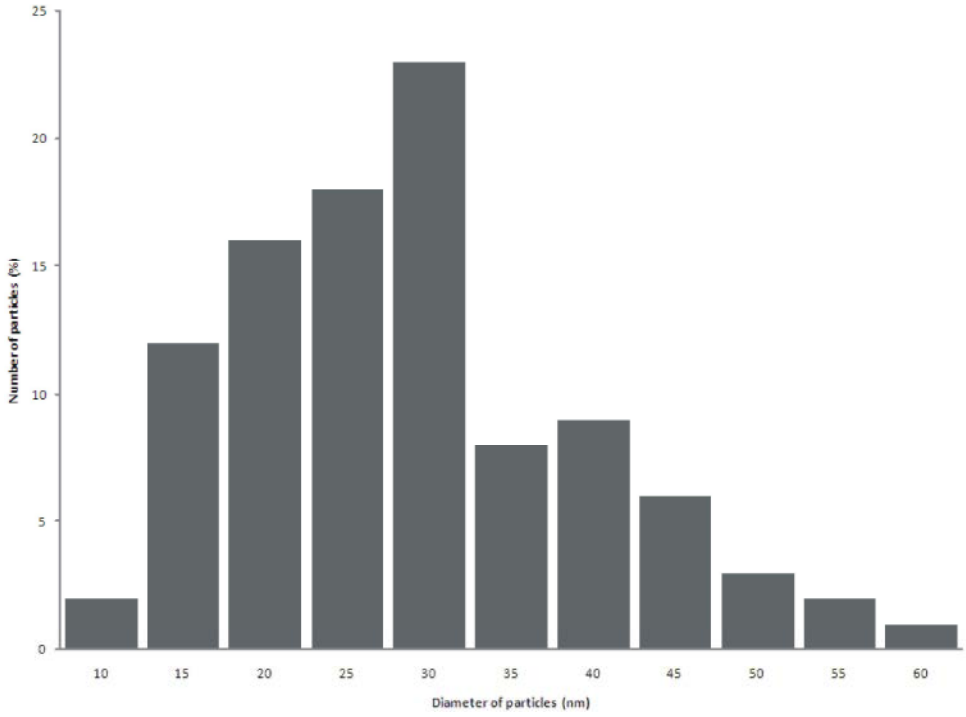

Fig. 3: (a) HRTEM micrograph of AgNPS synthesized from 1mM $AgNO_3$ solution, 10mg of *E. indica* powder at 20°C in 20 min. (b) Particle size distribution histogram of AgNPS determined using Image J

liptoeichoic acid present in peptidoglycan, may be control the growth of pathogen. Hence, gram negative bacteria allow more silver to penetrate the cytoplasmic membrane than the gram positive bacteria [12]. Pal *et al*. found that AgNPs have the potential to interfere the metabolic pathways [30]. A recent study stated that the AgNPs also control the bacterial growth by inhibiting the oxidation based biological process by entering of metallic nano sized particles through the microsomal membrane.

Antifungal Activity: With the fungal treatment, we observed the highest activity against *Aspergillus flavus* (Fig. 5a) and *Fusarium oxysporum* (Fig. 5b). The AgNPs via green route are highly toxic towards fungal species when compared to bacterial species. The antifungal effects and the mode of action of AgNPs against fungi have also still unknown. Since it is observed the treated fungal cells with AgNPs expressed significant damage like pit in their cell walls and pores in their plasma membrane

Fig. 4: Antibacterial activity of AgNPs from *E. indica* - (a) *E. coli*, (b) *Pseudomonas putida* (c) *Bacillus subtilis* (d) *Staphylococcus aureus*

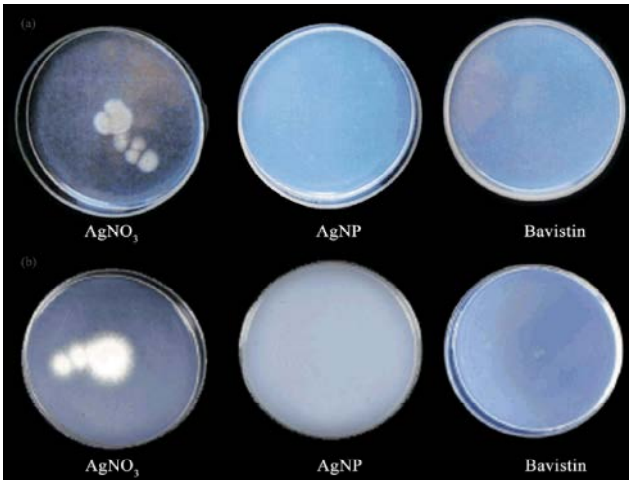

Fig. 5: Antifungal activity of AgNPs from *E. indica* - (a) *Aspergillus flavus* (b) *Fusarium oxysporum*

Table 1: Dynamic light scattering analysis of Synthesised silver nanoparticles using

S.No	Hydrodynamic Size (d/nm)	Zeta potential (mV)	Poly dispersive index (PDI)
1.	30±3.2	-27.6±0.8	0.432±0.26

and also noted that in the presence of AgNPs, 15% cells increased in G2/M phase while 20% of cells decreased in the G1 phase [31].

The green syntheses of nanoparticles open a new possibility of conveniently synthesizing pure metallic or bimetallic nanoparticles using natural products. However, the mechanism of green route to synthesis of nanoparticles is not completely understood. Biological molecules are interact with metal salts through these functional groups and mediate their reduction to nanoparticles [32]. A recent research stated the additional valuable note that Caffeine and theophylline catalyzes synthesis of fewer nanoparticles [33]. With Neem leaf broth, it was reported that terpenoids are believed to be the surface-active molecules stabilizing the nanoparticles andreaction of the metal ions is possibly facilitated by reducing sugars and/or terpenoids present in the Neem leaf broth [11]. Recent results with *Capsicum annuum* L. powder indicated that the proteins which have amino groups played a reducing and controlling role during the formation of AgNPs in the solution andthe secondary structure of the proteins changed after reaction with silver ions [20]. The result from a study alleged that the enzyme reductase together with electron shuttling compound and other peptides/proteins may be responsible for the reduction of Ag^+ ions and the continues formation of silver nanoparticles [34]. In *B. licheniformis*, the nitrate reductase enzyme is found at the membrane that may result in the formation of silver nanoparticles over the invaginated cell membrane. In all the organisms, nitrate reductase that synthesizes silver nanoparticles is found to be an integral part of it. Extracellular synthesis of nanoparticles could be highly advantageous from the point of view synthesis in large quantities and easy downstream processing [35]. More elaborate studies are required to elucidate the exact mechanism of biological nanoparticles synthesis.

CONCLUSION

At first time, we reported that *E. indica* leaf powder is found to be suitable for the synthesis AgNPs with 20 min and found that the optimum quantity of leaf powder, silver nitrate concentration, temperature and time for the rapid and controlled formation of AgNPs. The spectroscopic characterization from UV-Vis, FT-IR, TEM and DLS support the stability of green synthesized AgNPs. Very importantly, the antimicrobial study also discover the toxic nature of AgNPs against human pathogens such as *E. coli, Pseudomonas putida, Bacillus subtilis* and *Staphylococcus aureus, Aspergillus flavus* and *Fusarium oxysporum*. This study supports the simple, rapid and economical green route synthesis of AgNPs with eco-friendly manner and their capability of rendering the antimicrobial efficacy. Moreover the synthesized AgNPs enhance the therapeutic efficacy and strengthen the medicinal values of these plants.

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