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Green synthesis of silver nanoparticles using the plant leaves of *Andrographis paniculata* (Burm. f.) Wall ex Nees and checked the efficacy of antimicrobial property, physical characterization, and cytotoxicity analysis

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Abstract

The green synthesis of silver nanoparticles (AgNPs) and the applications have used many fields as the AgNPs are used effectively in microorganisms. The purpose of this study is to synthesize and characterize silver nanoparticles used the plant leaves of *Andrographis paniculata* as well as to test their effectiveness in antimicrobial activity. In this study, at 0.1 mM concentration of silver nitrate ($AgNO_3$), stable AgNPs were synthesized and monitoring the colour change of the solution. The crystalline nature of these AgNPs was detected through an (XRD) pattern and confirmed the functional compunds as well the AgNPs were characterized through ZETA Potential to the study the morphology and size of the nanoparticles (NPs). And checked their effective of cytotoxicity test.

Key words : Silver nanoparticles (AgNPs), *Andrographis paniculata*, ZETA potential, XRD, FTIR, UV-Transmittance.

Nanotechnology is practical, and eco-friendly alternative method for creating nanoparticles has been introduced as the "green synthesis." In a standard green synthesis, biological substances (such plant extracts), microbes, or even eukaryotic cells operate as both a reducing agent and a stabilising agent, producing desired nanoparticles with predetermined properties. Silver nanoparticles are of tremendous interest because of their distinct and controlled properties. These include anti-bacterial, anti-fungal and antioxidant properties. Their mode of action against microbes as well as their impact on normal cells' toxicity have not been completely found.

In this study, investigated the viability of synthesising AgNPs utilising an aqueous extract from *Andrographis paniculata*. The

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herbal substance was extracted using a very gentle, solvent-free method at a low temperature. This method is biosafe extract functions both as a stabilising and reducing the nanoparticles. The antimicrobial characteristics and cytotoxicity of the synthesised nanoparticles were evaluated.

Plant review :

Andrographis paniculata (Burm f.) Wall ex Nees *:*

A vital medicinal plant belonging to the *Andrographis* genus is *Andrographis paniculata*. According to several reports, this plant's genus comprises a total of more than one species[2,6,9]. The exact number of species in the *Andrographis* genus has not yet been substantiated. In both the gametophytic and sporophyte counts, *Andrographis paniculata* chromosomes total 25 and 50.[8] The aerial parts of the plant are associated with protein, flavonoids, reducing sugar, antioxidants, and antidiabetics, and have been used traditionally in Asia for centuries to treat cancer, diabetes, blood pressure, ulcers, and other conditions.[7] This herbal remedy has the ability to inhibit microbial growth. The herbal plat is used in ayurveda medicine to create drugs and ointments. Herbs are used to get rid of toxins and to get rid of heat and fever in the body. The Ayurvedic medical system recommends using this herb for several illnesses, including dysmenorrhoea, leucorrhoea, pre- and post-natal care, severe illnesses including malaria, jaundice, and gonorrhoea, as well as general illnesses like wounds, cuts, boils, and skin conditions. This review focuses on the phytochemistry, therapeutic benefits, and impacts of the plant's many extracts and parts, including their anti-microbial, anti-infective, hepato-renal protecting, and liver enzyme properties.

Plant extract :

The plant leaves were manually separated and used by handpicking method then dried in a Sun shadow. This plant material was utilised in the extraction of a shaker apparatus after being dried into a good powder of the leaves (20 g). Starting with Aquas, the pure form of plant extract was carried out in a sequential solvent system. In a 24-hour period, 250 ml of Aquas solvents were used to extract the material.[12]

Green synthesis of AgNPs; by using A. paniculata extract :

In the characteristic synthesis of silver nanoparticles, 10 mL of leaf extract was treated with 140 mL of 1 mM silver nitrate solution and kept in room temperature. Afterward the synthesis of silver nanoparticles was initially notorious by brown colour formation and further monitored by measuring UV-vis spectra of the reaction mixture. To study the effect of parameters such as reaction time, silver nitrate concentration, pH, and temperature on the nanoparticles synthesis the reaction was carried out by the following experiments. Silver nitrate and leaf extract reaction mixture was kept at room temperature and formation of nanoparticles was recorded at different functional times. Influences of silver nitrate concentration (1 to 4 mM, pH: 5.5, temperature: 35°C), pH (3.7, 4.2, 4.8, 5.7, silver nitrate: 1 mM, temperature: 35°C), and temperature (20°C, 35°C, 45°C, silver nitrate: 1 mM, pH: 5.5.[13]

Characterization of synthesized Silver nanoparticles :

Synthesis of silver nanoparticles was primarily characterized by ZETA potential. which are sensitive to concentration, size, shape, and agglomeration state. The surface plasmon resonance of the electrons on the nanoparticle surface is responsible for the exceptional of a distinctive peak at a particular wavelength of light.[2]

Particle size and charge analysis :

Hydrodynamic diameter (2062.099781 nm) and Polydispersity and surface (ZETA potentials) analysed by the ZETA Sizer (Litesizer 500) The particle size analysis was performed at Side Scatter angle and medium Viscosity 0.890 mPa.s and Count rate 417kcps at 25°C.[10]

UV–vis spectroscopy :

UV-Vis spectroscopy is the simple, effective, and primary characterization technique used to determine the stability & optical properties. the synthesis reaction conditions such as time, temperature, and pH. The free-electron oscillates and produces charges over the surface of nanoparticles under electromagnetic radiations as a result of the SPR effect. The process of AgNPs synthesis is the coloured reaction and shows strong and sharp absorption bands under the visible region in the range of 300–800 nm. *Andrographis paniculata* extract loaded green synthesized nanoparticles has shown spectrum curve in UV-Vis analysis showed absorbance spectra at -- nm, -- nm, and -- nm for A0, A1, A2 respectively. Similarly, the change in the colour of the reaction and reduced silver ions can and has been measured using UV-Vis spectroscopy in many studies.[15]

XRD – analysis :

XRD is an analytical technique broadly used to observe the structure of crystalline metallic nanoparticles by penetration of X-rays deeply into the material. The resulting diffraction pattern confirms the formation of nanoparticles with crystalline structure.[4]

Fourier transform infrared (FTIR) spectroscopy:

Investigation of Ag Nanoparticles associated molecular that the FTIR measurement were done. The Ag Nanoparticles solution of 100ml was centrifuged at 8000rpm, for 40mins. The pellets were washed 5 times with 10ml of distilled water. The dried powder samples and ground with de-ions water pellets and analysed on FTIR-4700 type A model in the diffuse reflectance mode operating a resolution $4cm^{-1}$. In order to verify the possible effect of the AgNPs on the surface modification of the synthesized nanoparticles using in the range of (655.79 to 3306.36). [11,14]

Cytotoxicity evaluation of AgNPs :

To determine the cytotoxic and antidiabetic effects of AgNPs, cell viability study was conducted using the conventional MTT reduction assay. 24–26 Briefly, Mouse insulinoma MIN6 cells were density of 2 × 103 cells/well in 96-well plates. In the presence of 100 µL cell culture medium in DMEM media supplemented with 10% FBS, penicillin

(100 units/ml) and streptomycin (100µg/ml). Cells were incubated for 24 hours in an incubator at 37°C. After 24 hours of seeding, the medium inside the wells was replaced with fresh medium along with concentrations of the AgNPs (0.45 mg/mL) and incubated for 4 hours at 37°C. To detect cell viability, the old medium was replaced with 100 µL of fresh medium, then 10 µL of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well, and the plates were incubated further for another 4 hours. The MTT solution was then discarded, and 100 µL of MTT Assay was added to each well followed by incubation for 40 minutes in dark. The solution was then pipetted and its absorbance was recorded at 570 nm using a microplate reader (Synergy H1MFG, Biotek, USA).[5]

Antimicrobial activity :

The antibacterial activity of the green synthesis of silver nanoparticles (AgNPs) were evaluated. The test solution of AgNPs were using in different concentrations 50µl, 100µl, 250µl, 500µl was evaluated by MHA-agar well diffusion method. The Sterile plates were allowed to solidify for 15 minutes and wells of 6 mm were punctured using a well borer. inoculums suspension of *Bacillus* sp., *Staphylococcus aureus, Klebsiella* sp., *Escherichia coli, Streptococcus* sp., *Pseudomonas aeruginosa* were swabbed uniformly over the surface of the agar. Based on the listed concentration the samples were loaded into the well and the plates were kept for incubation at 37ºC for 24 hours. The antibacterial activity was evaluated in terms of zone of inhibition, measured and recorded in millimetres. After 24hrs of incubation, Zones were measured and susceptibility was expressed as sensitive when the diameter is ≥ 17.5; Moderately sensitive (12.5 – 17.4 mm); resistant ≤ 12.4 mm. [3,4.6,9]
(Figs. 3-10).

Silver nanoparticle synthesis (Green method)

Figure 1: Silver Nitrate ($AgNO_3$)

Figure 2: Herbal Extract + Silver nitrate

Antimicrobial Activity :

Figure 3

Figure 4

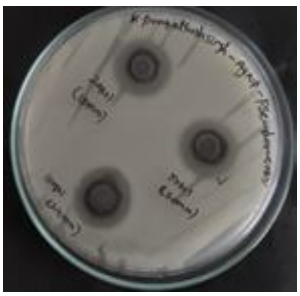

Figure 5

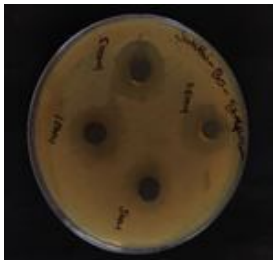

Figure 6

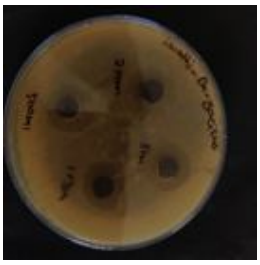

Figure 7

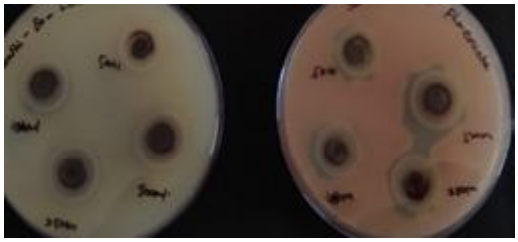

Figure 8 & 9

Antibacterial activity results

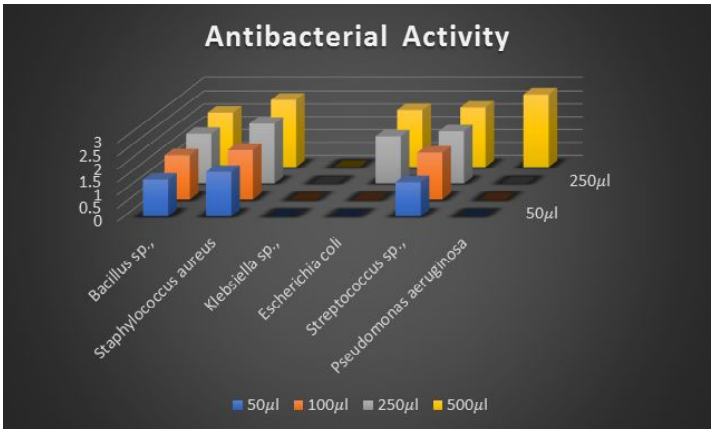

Figure: 10 Antibacterial Activity

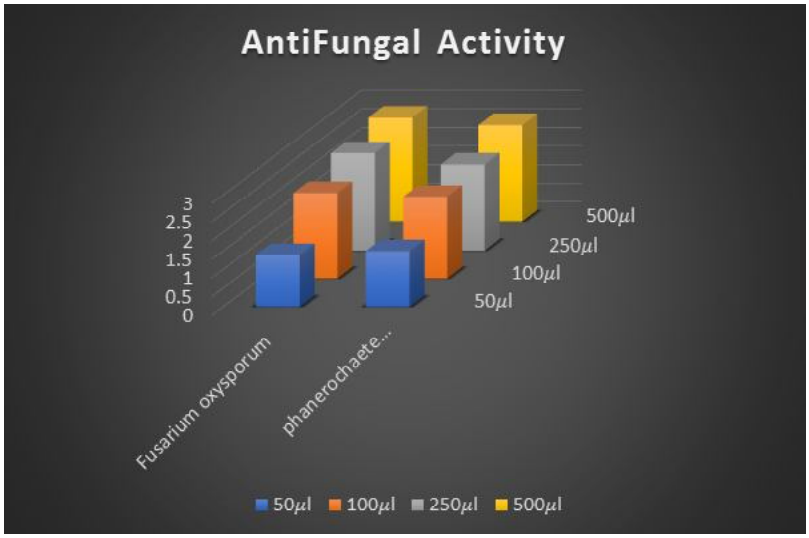

Figure 11. Antifungal Activity

Table - 1. Zone of incubation

Test organisms	50µl	100µl	250µl	500µl
Bacillus sp.,	1.4	1.7	1.9	2.1
Staphylococcus aureus	1.7	1.9	2.3	2.6
Klebsiella sp.,	0	0	0	0
Escherichia coli	0	0	1.8	2.2
Streptococcus sp.,	1.3	1.8	2	2.3
Pseudomonas aeruginosa	0	0	1.8	0

Test Fungai	50µl	100µl	250µl	500µl
Fusarium oxysporum	1.4	2.3	2.6	2.8
phanerochaete chrysosporium	1.5	2.2	2.3	2.6

Particle size analysis of silver nanoparticle:

The results show the particle size distribution of silver nanoparticles synthesized the analysis was performed only on Litesizer 500. The mean particle size of silver nanoparticles synthesized was 200 nm with 90% of nanoparticles having a diameter less than 282nm. Supernatant showed a mean particle size of 282 nm with 90% of nanoparticle with diameter under 2107 nm. It indicates that the centrifugation process, even with a high rotation (15,000 rpm), is not capable of separating nanoparticles with a size less than 10 nm, segregating only the bigger nanoparticles (≥ 50 nm). The size and homogeneity of nanoparticles interferes directly in the quality of the formed conductive layers. The smaller

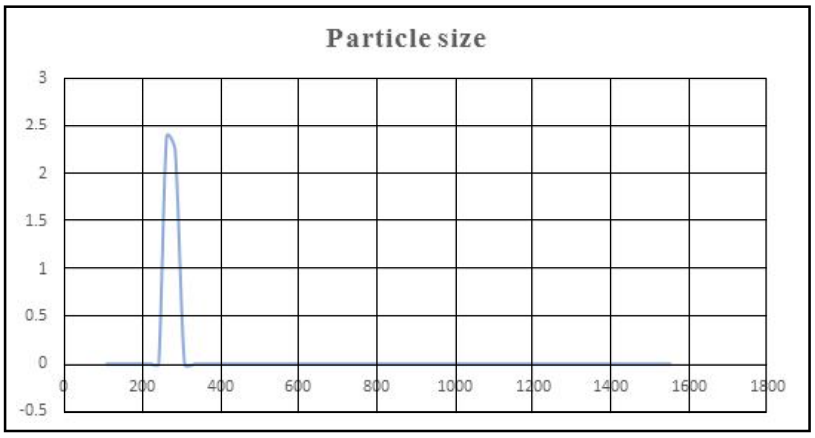

Figure 12. ZETA – Potential Particle size

the nanoparticle formed, the more complex the separation process through centrifugation without irreversible agglomeration (Fig. 12).

UV-Transmittance :

The developed Lambert-Beer law model's estimation of the transmission of nanoparticle suspension was one of its other functions. A domain with a side length of 1 cm was constructed, and the incident light's intensity was 1 MW/m2 at a wavelength of 600 nm. the volume fraction of 0.1 to 10 minutes for 600 nm AgNPs nanoparticle suspensions with the incoming light intensity. It was bare that the transmission light's intensity reduced as the medium's thickness increased throughout the course of 0.1min to 30mins. in line with the increased to decreased scattering and absorption of AgNPs nanoparticles. The transmission of the dispersion of AgNPs nanoparticles significantly decreased with increasing particle size. It's interesting to note that other nanoparticle suspensions did not exhibit the particle size dependence during transmission. The simulation model created in this section can generally be used to investigate the optical transmission properties of suspensions of nanoparticles. A thorough theoretical approach to studying the optical properties of nanoparticles and their suspensions was made possible by the entire integration of models on UV-Vis spectra, scattering, absorption, and transmission (Fig. 13).

Fourier transform infrared (FTIR) spectroscopy :

The interaction between different functional groups and charges in chemical composition of the nature undergone during AgNPs Production was investigated by FTIR Spectroscopy. The AgNPs Showed strong absorption peaks of 3306.36cm^{-1} which refers to O-H stretching (Phenols/hydroxyl group) C=O Groups at 1770.33CM^{-1} (Carboxylic acid groups) 1644.98 CM^{-1} corresponded to the C=C functional groups 1055.84 cm^{-1} Correspond (C-O) functional group (Fig. 14).

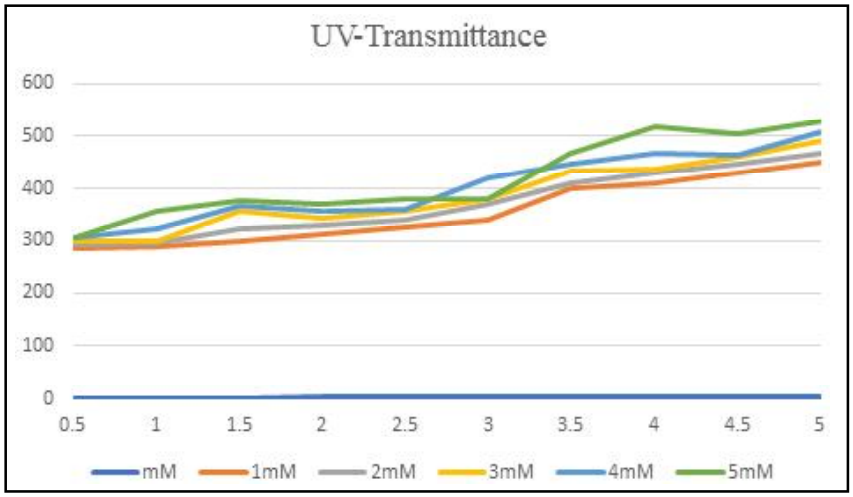

Figure 13. Zeta Potential – UV Transmittance

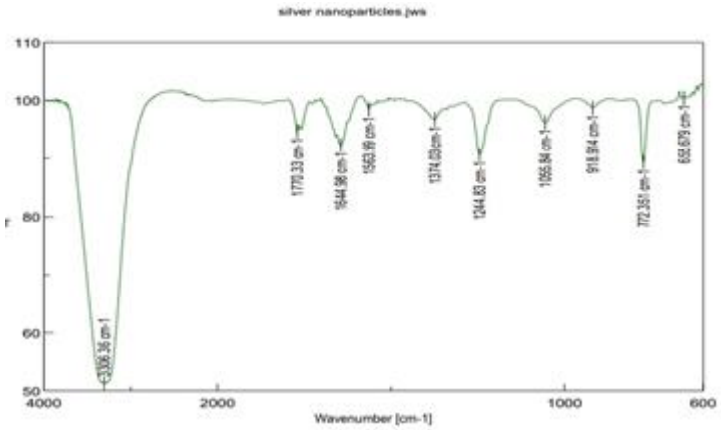

Figure: 14 FTIR Analysis – *Andrographis paniculata*

XRD – analysis :

XRD is a powerful characterization technique for both qualitative and quantitative analyses of nanoparticles. XRD analyses are used to confirm the formation of nanoparticles and determine their crystal structure. In addition, this technique has been used to calculate the crystalline nanoparticle size and measure the degree of crystallinity. The analysis of materials using this technique depends on the diffraction patterns because each material has a unique diffraction beam. Thus, a material can be defined and identified by comparing the diffraction beams to the reference database of the Joint Committee on Powder Diffraction Standards - (JCPDS). This technique is also useful for determining purity as it can easily indicate whether the material is pure or contains impurities. The working principle of XRD is Bragg's law, which helps determine the Bragg reflection of AgNPs. XRD patterns

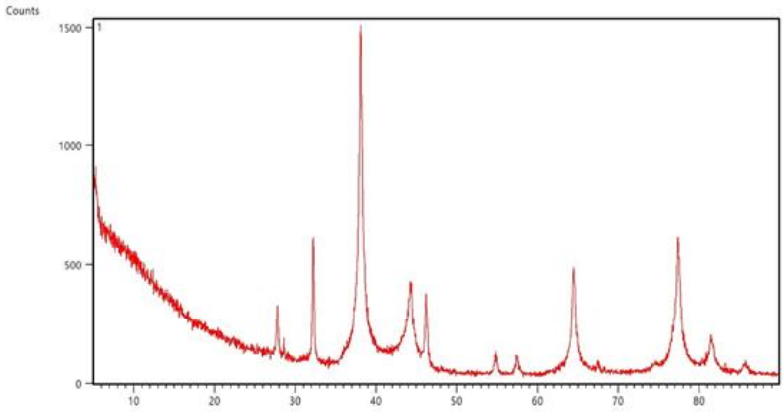

Figure 15. XRD Analysis: Silver nanoparticles

reveal remarkable peaks such as the (110), (215), (323), (550), (1500) crystallographic peeks that are specific for AgNPs. The diffraction peaks (111), (215), and (1500) represent the cubic silver, while the sharpness of these peaks indicates the development of nano-sized particles (Fig. 15).

Cytotoxicity analysis :

To evaluate the dose dependent effect of silver NPs on Mouse insulinoma MIN6 cells viability. In this study, silver NPs induced significant ($P<0.05$) level of cytotoxicity from 5 and 40 µg/ml dosage range. Whereas dosage range of 10-20 µg/ml does not cause any significant ($P<0.05$) cytotoxic effects. Hence, based on the current study in Mouse insulinoma MIN6 cells, the silver NPs are considered safe up to the 10-20 µg/ml dosage range in cells (Fig. 16).

Effect of NPs on cytotoxicity :

Results of MTT assay in Mouse insulinoma MIN6 cells are represented as bar diagram. Data represented are mean ± SD of three identical experiments. Values labelled with distinct letter indicate statistically significant difference with each other ($P < 0.05$).

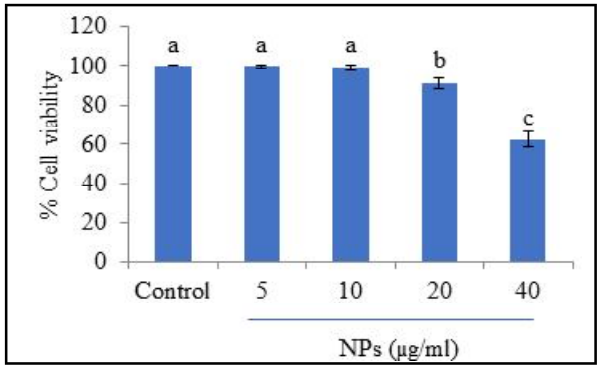

Figure: 16 Cytotoxicity analysis – *Andrographis paniculata*

The silver nanoparticles are synthesized by numerous methods and fuctional materials on reduced scale. Environmental and medical friendliness to protection of human health, are the reasons researches are considering the green manufacturing of AgNPs, the area of nanotechnology is used in medicinal plants to sysnthesize AgNPs, which can stimulate the development of medicinal area. The optimization conditions, mechanisms, characterization for AgNPs, and used particularly medicinal plants extracts, in consort with the effect of different parameters of AgNPs. AgNPs were prepared by reduction method and the nanoparticles analysis was done by qualitative analysis, *i.e*., AgNPs appeared as a reddish-green color and characterization of NPs was shown by particle size analyzer. ZETA potential analysis showed that the nanoparticles size was found the range of 178 nm and 282.99 nm for AgNPs. The FTIR analysis was confirmed the functional groups, were the XRD confirms the peaks with purity of sample and range. The cyctoxicity showed the good results in $AgNP_S$ against the Mouse insulinoma MIN6 cells. The antibacterial and antifungal tests showed, $AgNP_S$ have the capacity to control the mictrobial growth and determined the nanoparticles can be used as a therapeutic drug for various diseases such as antibacterial, antifungal treatment. Smaller sizes of nanoparticles have great capacity to eradicate the DFU disease due to the high surface area. The uses of AgNPs in various fields and areas is expected to develop in the future. In addition, *in vivo* testing of AgNPs should be performed to determine their cytotoxicity. which can assist in the development of novel and potentially beneficial antimicrobial agents.

The research has carried from Hindustan College of Arts & Science. Coimbatore. The Anti-bacterial and Anti-fungal activity test done by Department of Microbiology, Hindustan College of Arts & Science. Coimbatore. FTIR and Zeta potential, UV-Visible and Cytotoxicity activity test were done by Department of Nanotechnology, Department of Botany and Department of Environmental Sciences, Bharathiar university, Coimbatore. & The plant has authenticated by TNAU, Coimbatore.

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