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Green Synthesis, Characterization and Antibacterial Activity of Silver Nanoparticles From Leaf Extract of Simarouba Glauca

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Abstract
Objective: This study aims to investigate the green synthesis of silver nanoparticles (AgNPs) Simarouba glauca, and evaluation of their antimicrobial activities it is observed that leaf Simarouba glauca extract can reduce silver ions into AgNPs.
Methods: The obtained particles were analyzed by UV-visible spectrophotometry, scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) spectroscopy, to understand the morphology of AgNPs. In addition, the antibacterial activity by Simarouba glauca leaf extract synthesized AgNPs was also investigated.
Results: The formation and stability of the reduced AgNPs in the colloidal solution were monitored by UV-Vis spectrophotometer analysis. SEM and FT-IR spectra of the leaf extract after the development of nanoparticles are determined to allow identification of possible functional groups responsible for the conversion of metal ions to metal nanoparticles.
Conclusion: Further, the AgNPs thus acquired showed highly antibacterial activity against Escherichia coli .
Keywords: Green synthesis, Nanoparticles, Simarouba glauca, Anti-bacterial activity.

I. INTRODUCTION

Nanotechnology is becoming a new area of increasing research and industrial interest since the 1980. Nanotechnology can be defined as the manipulation of atom by atom from the material world by the combination of engineering, chemical and biological approaches. In the past decade, considerable attention has been paid for the development of novel strategies for the synthesis of different kind of nano-objects. Most of the current strategies are usually working by the use of physical or chemical principles to develop a myriad of nano-objects with multiple applications. Main fields of nanotechnology applications range from catalysis, micro- and nano-electronics (semiconductors, single electrons transistors), non-linear optic devices, photo-electrochemistry to biomedicine, diagnostics, foods and environment, chemical analysis and others (Contescu and Putyera, 2009). Simarouba glauca is one of the important traditional medicinal plants due to the presence alkaloids, flavonoids, carbohydrates, glycosides phenolic compound, tannins, terpenoides, cardenolides, saponins, fixed oils which can usually account for their therapeutic action including anti-bacterial, anti-viral, anti-inflammatory, anti-protozoal and antitumor activities. But never synthesized and characterized silver nanoparticles by the extracts of Simarouba glauca.Hence, the present study was aimed to synthesize AgNPs rapidly using aqueous leaves extract of Simarouba glauca plant, to investigate the biomolecules responsible for the synthesis of AgNPs. The obtained nano particles were analyzed by UV-visible spectrophotometry, scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) spectroscopy, to understand the morphology of AgNPs. The physical, chemical and biological activity of the choosen compound is studied in detail experimentally for future pharmaceutical applications.

II. MATERIALS AND METHODS:

A. Preparation of plant Extract

The dried *Simarouba glauca* was pulverized well with mortar and pestle to make a powder. Five grams of powder sample was mixed into 100 ml of deionized water and the mixture was boiled for 10 min. After cooling the leaf extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4°C for further use.

B. Synthesis Of Silver Nanoparticles Using Plant Extract

For the silver nanoparticle synthesis, 5 ml of leaf extract was added to 45 ml of 1 mM $AgNO_3$ solution in a 250 ml Erlenmeyer flask. The flask was then incubated in the dark at 4 hrs (to minimize the photo activation of silver nitrate), at room temperature. A

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control setup was also maintained without plant extract. The silver nanoparticle solution thus obtained after five hours was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water for further use (Arunachalam*et al*., 2012)

C. Characterization of synthesized silver nanoparticles

1) *UV-Visible Analysis :* The extracts were examined under visible UV-Visible spectrum. The sample is dissolved in same solvent. The extracts were scanned in the wavelength ranging from 330-830 nm using Systronic Spectrophotometer. These solutions were scanned in turn at intervals of 50 nm and the characteristic peaks were detected. The peak value of the UV-Visible was recorded
2) *FT*-IR Spectroscopy: To determine Fourier transform infra-red (FT-IR) pattern of the sample *Simarouba glauca* filtrate containing the titanium nanoparticles was freeze-dried and the dried powder was diluted with potassium bromide in the ratio of 1:100 and recorded the spectrum in Perkin Elmer FT-IR Spectrum BX (Wellesley, MA, USA)
3) *SEM analysis of Silver Nanoparticle :* Scanning Electron Microscopic (SEM) analysis was done using JSM 6701F – 6701 machine (Japan). Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

D. Determination Of Antibacterial Activity

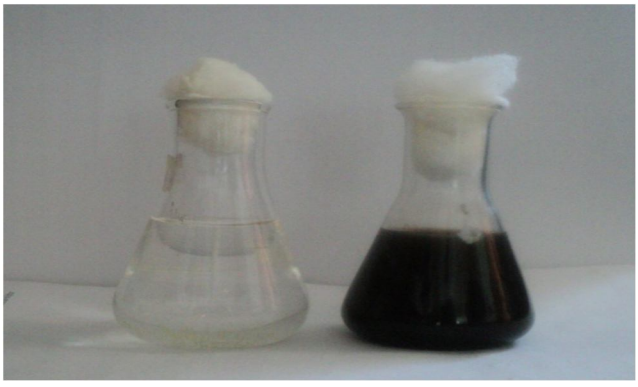
Antibacterial assay was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al*., 2007). Petri plates were prepared by pouring 30 ml of NA medium for bacteria the test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria from a broth culture. A sterile cotton swab is dipped into a standardized bacterial test suspension and used to evenly inoculate the entire surface of the Nutrient agar. Briefly, inoculums containing bacteria (Escherichia coli and Staphylococcus aureus) were spread on Nutrient agar plates. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the sample (30µl and 30µl for standard) was laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 h for the bacteria. Each sample was tested in triplicate. mercury lamp for 5 min.

III. RESULTS AND DISCUSSION

A. Synthesis of Silver Nanoparticles

Green synthesis of Silver nano particles through Simarouba glauca leaf extracts were carried out. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). During the visual observation, silver nitrate incubated with leaf extract showed a color change from yellow to brown within 4 hrs whereas no color change could be observed in silver nitrate without leaf extract (Fig.1). The appearance of brown color in leaf extract treated flask is clear indication for the formation of Ag nanoparticles. This color arises due to excitation of surface Plasmon vibrations in silver nanoparticles.

Fig 1: Synthesis of Silver nanoparticles colour observation



a) $AgNO_3$ (b) AgNPs

(a) Before adding the leaf extract and (b) After addition of leaf extract at 4 h.

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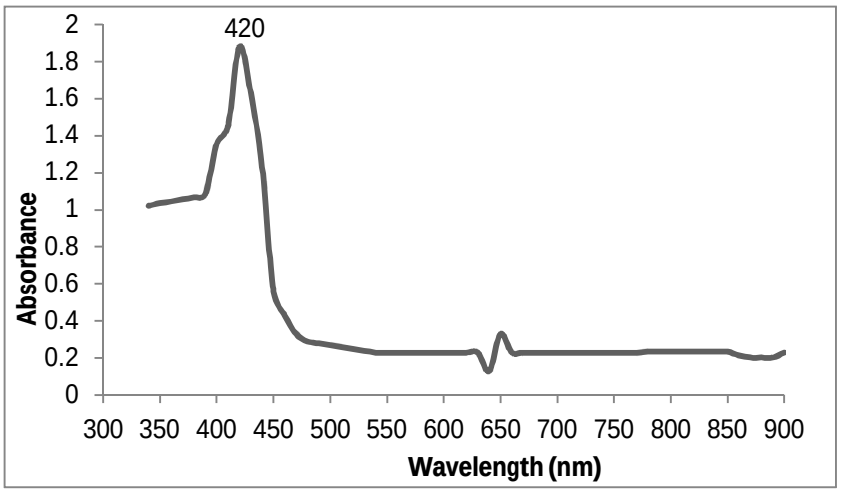
B. Characterization of synthesized silver nanoparticles

1) *UV-visible Spectrum Analysis:* Optical measurement is the prime technique for characterizing the biological synthesis of nanoparticles. (Figure 2) displays the UV-visible spectra of the samples periodically withdrawn and exposed to UV-visible spectroscopy during the experiment. A broad peak located at 420 nm was observed after 5 min of exposure in sunlight. The height of the peak, i.e. the absorbance, increased with time till the end of the experiment. However, a gradual shift in the peak from 420 to 400 nm characteristic of surface plasmon resonance of AgNPs formation is observed that remained constant till the end of the experiment.

Fig 2: UV–visible spectroscopy analysis of Silver nanoparticles



2) *Fourier Transform Infrared (FT-IR) Spectroscopic Analysis:* FT-IR measurements were carried out in order to identify the presence of various functional groups in biomolecules responsible for the bioreduction of Ag^{+} and capping/stabilization of silver nanoparticles. The observed intense bands were compared with standard values to identify the functional groups. FT-IR spectrum shows absorption bands at 3905, 3466, 3434, 2832, 2368, 2088, 1363, 1016, 773 and 670 cm^{-1}indicating the presence of capping agent with the nanoparticles (Figure 3).

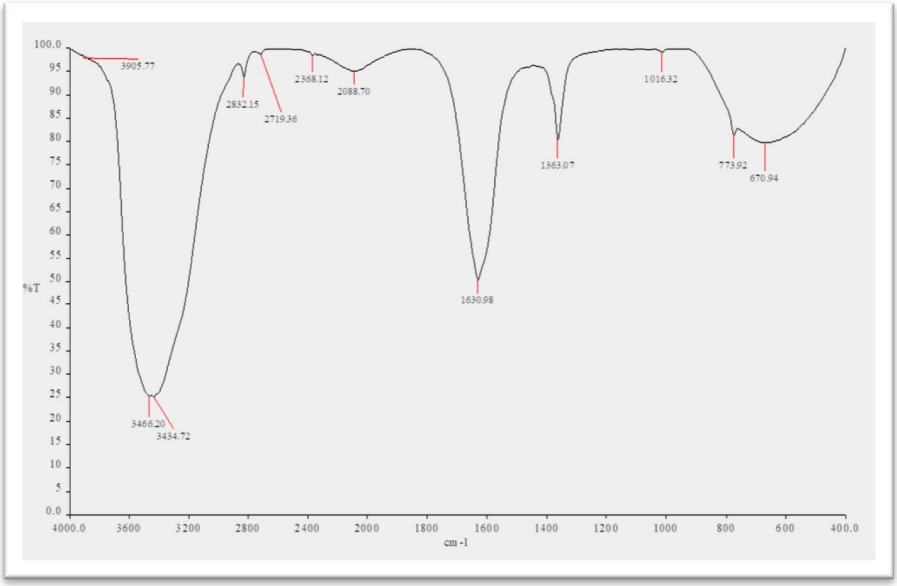


Fig 3: FT-IR Spectroscopic analysis of silver nanoparticle

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3) *Sem Analysis:* SEM analysis was carried out to understand the topology and the size of the Ag-NPs, which showed the synthesis of higher density polydispersed Ag-NPs of various sizes. The SEM image showing the high density silver nanoparticles synthesized by the leaf extract further confirmed the development of silver nanostructures. Most of the nanoparticles aggregated and only a few of them were scattered, as observed under SEM. The SEM analysis showed the particle size between 08-32nm as well the spherical structure of the nanoparticles (Figure 4).

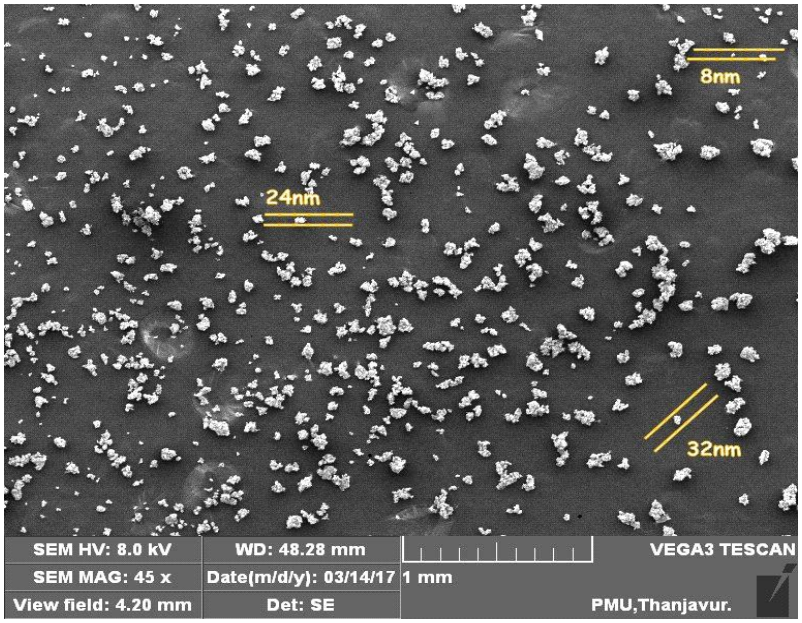

Fig 4: SEM Analysis of Silver nanoparticles

C. Antibacterial activity

The synthesized AgNPs showed antibacterial activity against the tested microorganisms which was confirmed by the formation of inhibition zones of various diameters as shown in Figure 6. The results of these bacterial bioassays were given in (Table 3)**.** This antibacterial assay revealed that the ethanol leaf extract of simarouba glauca posses highest antibacterial activity against Escherichia coli, even though the significant antibacterial activity was observed the other bacteria such as Staphylococcus aureus. The MIC value of the active extract against the strains showing results of antibacterial potential of the simarouba glauca. The results of these bacterial bioassays were given in (Table 3)**.** This antibacterial assay revealed that the ethanol leaf extract of simarouba glauca posses highest antibacterial activity against Escherichia coli, even though the significant antibacterial activity was observed the other bacteria such as Staphylococcus aureus. The MIC value of the active extract against the strains showing results of antibacterial potential of the simarouba glauca.

Table- 1: Antimicrobial activity of Silver nanoparticles

Microorganisms	Leaf extract (30µl)	$AgNO_3$ (30µl)	Silver nanoparticles (30µl)	Standard (30µl)	Control (Distilled water - 30µl)
Escherichia coli (mm)	5.20±0.34	2.14±0.147	8.20±0.56	12.13±0.56	0
Staphylococcus aureus (mm)	2.21±0.14	1.10±0.07	8.60±0.36	11.42±0.70	0

Values were expressed as Mean ± SD

Bacterial standard : Chloromphenicol

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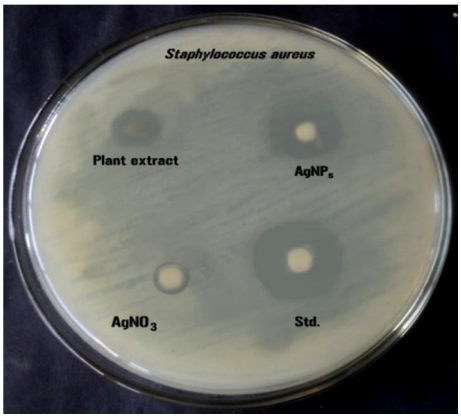
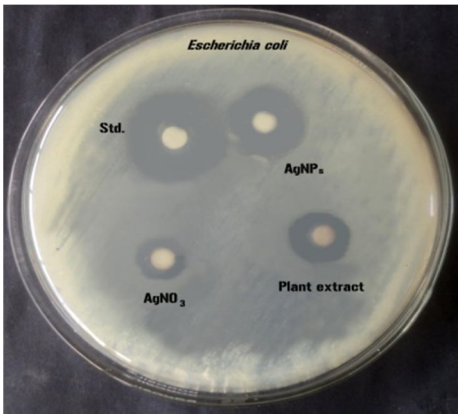
Escherichia coli



Staphylococcus aureus



IV. CONCLUSION

Plants are the important source for the development of new chemotherapeutic agents. Now a days the interest in study of natural products is growing rapidly especially, as a part of drug discovery programs. Silver nanoparticles were synthesized and its antimicrobial assays shows that the leaves of Simarouba glauca have significant medicinal properties. So in this present study it is found that leaves extract of Simarouba glauca express good biological capacity which indicates that the substance with powerful biological effect exists in the extract and must be isolated and purified to confirm its pharmacological and medicinal use. Further studies are necessary for the isolation of the compound from leaves extract to detect its medicinal potentials.

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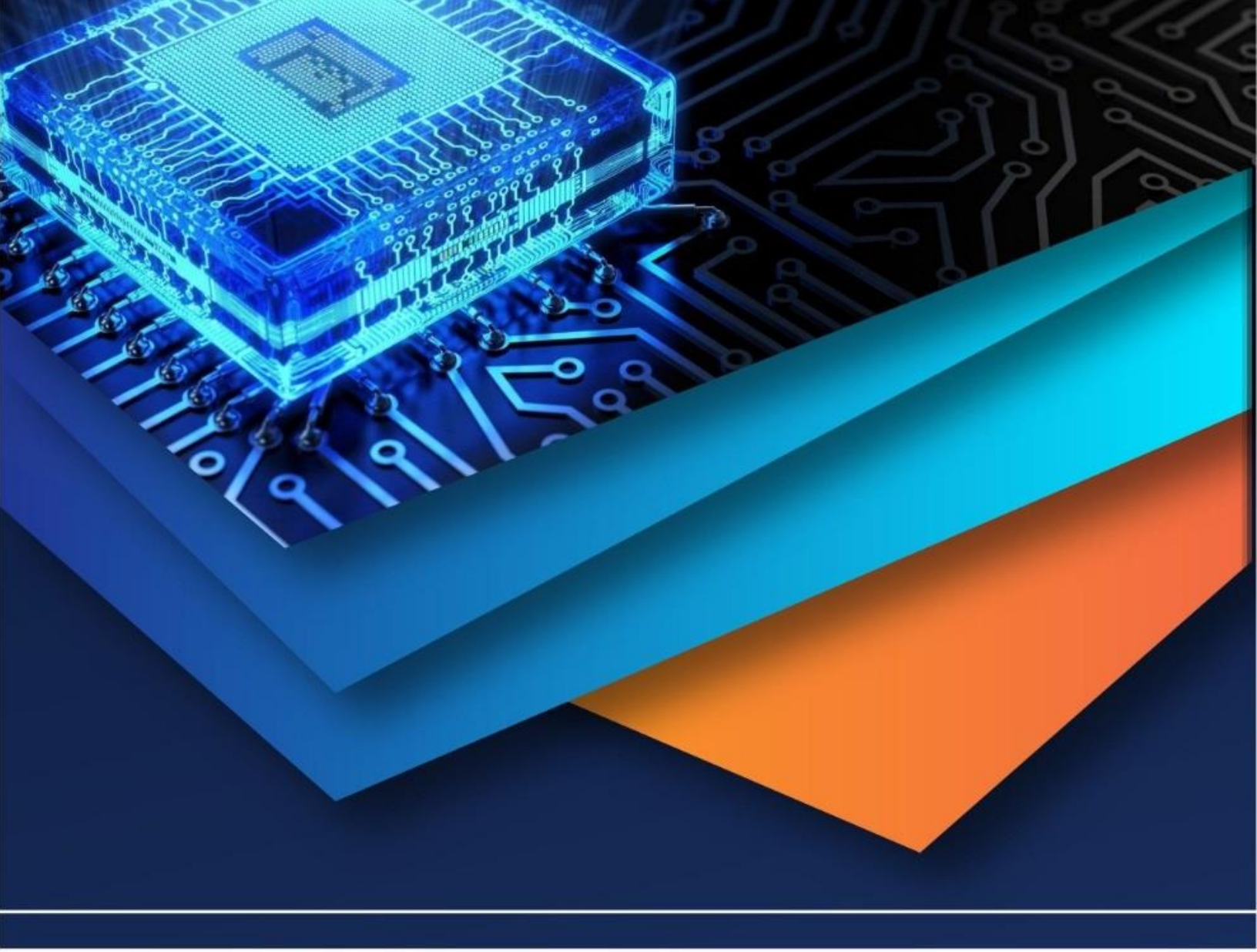

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