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Research Article

BIOSYNTHESIS OF SILVER NANOPARTICLES USING SIMAROUBA GLAUCA SEED EXTRACT AND THEIR ANTI-MICROBIAL ACTIVITY

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ABSTRACT

In recent science Nanotechnology is a burning field for the researchers. Nanotechnology deals with the Nanoparticles having a size of 1-100 nm in one dimension used significantly concerning medical chemistry, atomic physics, and all other known fields. Nanoparticles are used immensely due to its small size, orientation, physical properties, which are reportedly shown to change the performance of any other material which is in contact with these tiny particles. These particles can be prepared easily by different chemical, physical, and biological approaches. But the biological approach is the most emerging approach of preparation, because, this method is easier than the other methods, ecofriendly and less time consuming. This study aims to investigate the green synthesis of silver nanoparticles (AgNPs) *Simarouba glauca,* and evaluation of their antimicrobial activities it is observed that seeds *Simarouba glauca* seeds extract can reduce silver ions into AgNPs. 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 seeds 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.

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 [1].

Silver nanoparticles are widely used for its unique properties in catalysis, chemical sensing, biosensing, photonics, electronics, and pharmaceuticals [2]. Silver nanoparticles have a great potential for use in biological including antimicrobial activity [3]. Antimicrobial capability of silver nanoparticles allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances, and medical devices. Silver is an effective antimicrobial agent which exhibits low toxicity. The most important application of silver and silver nanoparticles is in medical industry such as tropical ointments to prevent infection against burn and open wounds. Silver nanoparticles play a profound role in the field of biology and medicine due to their attractive physiochemical properties. Silver products have long been known to have strong inhibitory and bactericidal effects, as well as a broad spectrum of anti-microbial activities, which has been used for centuries to prevent and treat various diseases, most notably infections [4]. Silver nanoparticles are reported to possess antifungal, anti-inflammatory, anti-viral, anti-angiogenesis, and anti-platelet activity.

MATERIALS AND METHODS

Preparation of plant extract

The dried *Simarouba glauca* seeds 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 seed extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4°C for further use [10].

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Synthesis of silver nanoparticles using seed extract

For the silver nanoparticle synthesis, 5 ml of seeds extract was added to 45 ml of 1 mM aqueous $AgNO_3$ 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 control setup was also maintained without seed 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 [7].

Characterization of synthesized silver nanoparticles

UV-Visible analysis

The Seeds 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.

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).

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.

Determination of anti-microbial activity

Anti-biogram was done by disc diffusion method [5,6] using Silver nanoparticles seeds extracts. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. 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/fungi from a broth culture[5]. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis* specie of bacteria were spread on Nutrient agar plates for bacteria and *Candida albicans, Aspergillus niger* and *Aspergillus flavus was* spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl, 100 µl and 150 µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate [6].

RESULTS AND DISCUSSION

Synthesis of Silver Nanoparticles

The formation of Silver nanoparticles was initially confirmed visually, the change in color of the reaction mixture indicates formation of silver nanoparticles (Fig.1)

Fig 1 Synthesis of Silver nanoparticles colour observation

a) $AgNO_3$ (b) AgNPs

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

Characterization of synthesized silver nanoparticles

UV-Vis spectroscopy analysis

The nanoparticles were primarily characterized by UV-visible spectroscopy, which proved to be a very useful technique for the analysis of nanoparticles. Fig.2 shows the UV-visible spectra of reaction medium recorded as a function of reaction time using silver nitrate and seeds broth. It is observed that the maximum absorbance of silver nanoparticles occurs at 430 nm. Appearance of this peak, assigned to a surface Plasmon, is well-documented for various metal nanoparticles with size ranging from 2 nm to 100 nm.

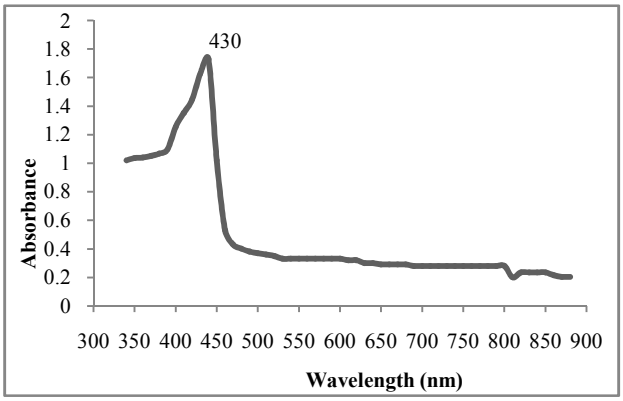


Fig 2 UV–visible spectroscopy analysis of Silver nanoparticles

Fourier Transform Infrared (FT-IR) Spectroscopic Analysis

The FT-IR Characterization is used to find the molecules and their functional group present in the synthesized Silver nanoparticles. Figure 3 represent the FT-IR spectra which shows peaks at 3464, 2767, 1313, 1407, 2767, 1645, 1531 and 668 cm^{-1}. The FT-IR spectra revealed the presence of different functional groups like Alcohol (OH stretch H-bonded, free), Alkane (C-H stretch, -C-H bending) Alkene (=C-H bending, C=C stretch) Amine(C-N, stretch) Nitro compounds (N-O stretch) Acid (OH, stretch) Ester(C-O,

stretch). These functional groups play a very important role in these Silver nanoparticles synthesis[9].

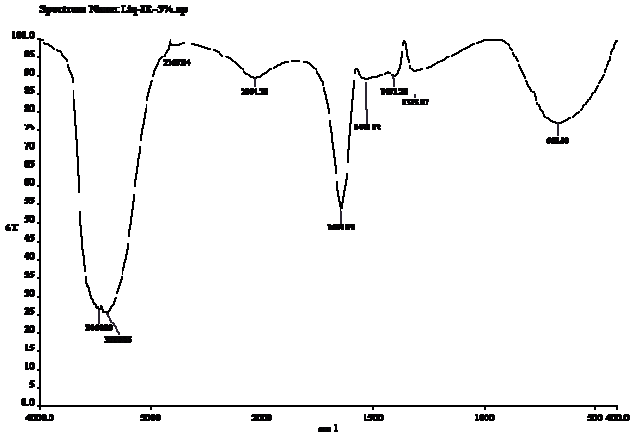


Fig 3 FT-IR Spectroscopic analysis of silver nanoparticles

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 poly dispersed Ag-NPs of various sizes. The SEM image showing the high density silver nanoparticles synthesized by the seeds 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 20-63nm as well the spherical structure of the nanoparticles (Figure 4).

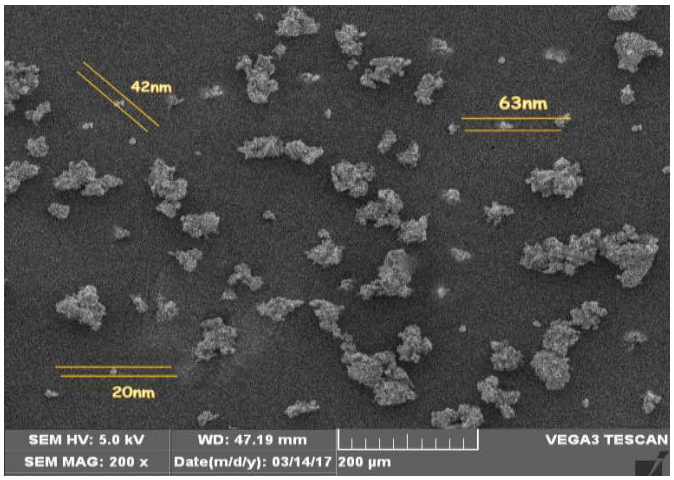


Fig 4 SEM Analysis of Silver nanoparticles

Antimicrobial 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[8]. The results of these bacterial bioassays were given in (Table 1). This antibacterial assay revealed that the silver nanoparticles seed 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*.

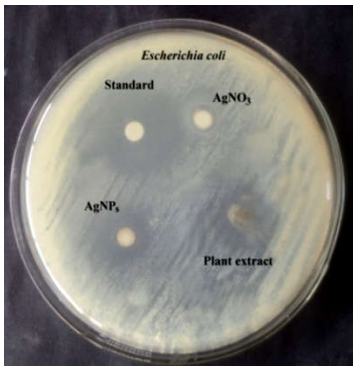
Table 1 Anti-bacterial activity of Silver nanoparticles

Microorganisms	Seeds extract (30µl)	$AgNO_3$ (30µl)	Silver nanoparticles (30µl)	Standard (30µl)
Escherichia coli (mm)	4.32±0.28	1.23±0.14	7.63±0.50	13.12±0.85
Staphylococcus aureus (mm)	2.80±0.16	0.98±0.06	6.94±0.43	7.21±0.50

Values were expressed as Mean ± SD

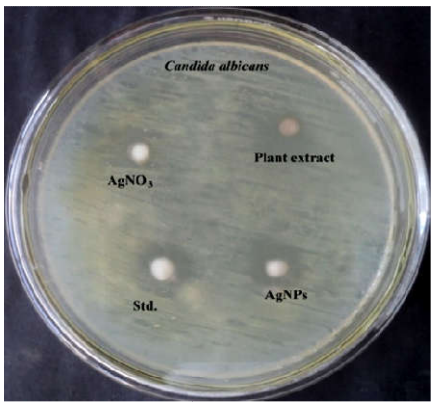
Bacterial standard: Chloromphenicol

Escherichia coli

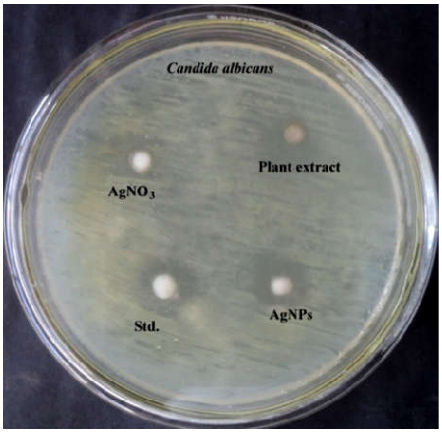


Staphylococcus aureus

The results of these fungal bioassays were given in (Table- 2)**.** This anti-fungal assay revealed that the silver nanoparticles seeds extract of *simarouba glauca* posses highest anti-fungal activity against *Candida albicans,* even though the significant antibacterial activity was observed the other bacteria such as *Aspergillus flavus.* The MIC value of the active extract against the strains showing results of anti-fungal potential of the *simarouba glauca*.



Candida albicans



Aspergillus flavus

Table 2 Anti-bacterial activity of Silver nanoparticles

Microorganisms	Seeds extract (30µl)	$AgNO_3$ (30µl)	Silver nanoparticles (30µl)	Standard (30µl)
Candida albicans(mm)	1.97±0.11	2.02±0.15	7.72±0.58	7.86±0.56
Aspergillus flavus(mm)	1.46±0..013	1.88±0.14	9.32±0.65	8.42±0.58

Values were expressed as Mean ± SD

Fungal standard : Fluconazole

CONCLUSION

In the present study, a simple and economic approach has been attempted to obtain a green eco-friendly synthesis of silver nanoparticles which was obtained from bio-reduction of simarouba glauca seed extracts with $AgNO_3$ solution. Silver nanoparticles synthesized by the green chemistry approach reported in the present study may have potent applications in functional textiles. Synthesized AgNPs from the plant extracts are characterized specifically using UV-Visible spectroscopy, FT-IR and SEM, whereas protocol to produce uniform sized nanoparticles has to be standardized for specific applications.

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