

Green synthesis of silver nanoparticles using *Cassine glauca* bark extract and its antibacterial activity

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Abstract

Nanotechnology is a field that is burgeoning day by day making an impact in all spheres of human life. Eco friendly methods of green mediated synthesis of nanoparticles are the present research in the limb of nanotechnology. The present work leads to the synthesis of nanoparticles from 1mM AgNO₃ solution through aqueous bark extract of *Cassine glauca* as reducing as well as capping agent. Synthesised nanoparticles are characterized by UV-Visible spectroscopy, Scanning electron microscopy, Energy dispersive X-ray analysis, Fourier transform infrared spectroscopy and X-ray diffraction. Green synthesized silver nano particle and aqueous extract of plant showed zone of inhibition against *Escherichia coli*, *Staphylococcus aureus* species of bacteria and *Candida albicans* fungi specie.

Keywords: Biosynthesis, Silver nanoparticles, *Cassine glauca* bark extract, Characterization, Antibacterial activity.

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INTRODUCTION

In recent years, nanoparticle biosynthesis is the active area of research. The most effectively studied nanoparticles in the recent past are those made from the noble metals such as silver^{1,2}. The most important and distinct property of nanoparticles is that it exhibit larger surface area to volume ratio³. Physical and chemical methods are more popular for nanoparticle synthesis but the use of toxic compounds limit their application⁴. An array of physical, chemical and biological methods have been used for synthesis of noble metal nanoparticles of particular shape and size for various applications, but they remain expensive and involve the use of hazardous chemicals⁵. The biosynthesis method employing plant extracts have drawn attention as a simple and viable alternative to chemical procedures and physical methods.

Bio reduction of silver ions to yield metal nanoparticles using *Pinuseldarica baric*, *Thespesiapopulnea* stem bark, *Zeylanicum* bark extract.⁶⁻⁸ Silver nanoparticles are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organism at low concentrations and without any side effects.⁹ The most important application of silver and silver nanoparticles is as tropical ointments to prevent infection against burns and open wounds¹⁰. *Cassine glauca* commonly known as Karuvali belongs to the family Celastraceae. Roots and leaves of *Cassine glauca* are used in treating cough with phylegm, swellings¹¹ and menstrual disorders¹². The plant paste is used on skin wounds and cuts¹³ and bark powder is used in treating rheumatism¹⁴. Hence, the aim of present study is to synthesize silver nanoparticles by using aqueous *Cassine glauca* extract, characterization of these silver nanoparticles and the antimicrobial activity.

MATERIALS AND METHODS

Collection of plant material

Cassine glauca (Family: Celastraceae) was collected freshly on the road sides in July, from Papanasam, Tamilnadu, India. The plant was identified and authenticated by personnel of Rapinet Herbarium, St. Joseph's college, Thiruchirappalli, Tamilnadu, India. The bark of the plants were collected and shade dried.



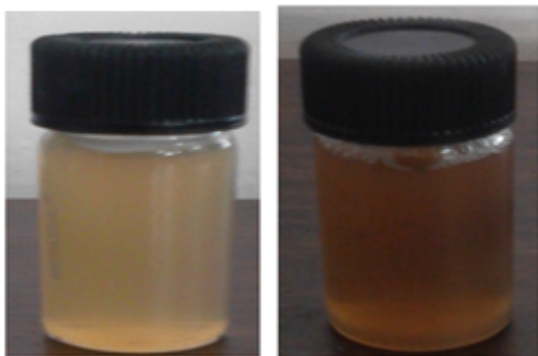
Figure 1: Voucher specimen photo copy of *Cassine glauca*

Preparation of bark extract

The plant bark was rinsed with water thrice to remove the fine dust materials. The plant bark was air dried for 20 days and then they were dried in air hot oven at 60⁰ for 36 hours. The plant bark was ground to a fine powder. 25g of bark powder was added in 250ml deionized water in 500ml Erlenmeyer flask and it was boiled for approximately 15 minutes and were filtered. The filtrate was used as plant extract.

Synthesis of silver nanoparticles

10 ml of plant extract was added to the aqueous solution of 1 m M silver nitrate. The appearance of reddish brown colour after 3h indicates the formation of silver nanoparticles. The completion of the reaction was monitored by UV-Visible spectroscopy. The synthesized silver nanoparticles were separated by centrifuging at 13,000 rpm for 15 mins and filtered. The filtrate was stored at 4⁰C for further use.



Legend

Figure 2: The conversion of silver nitrate to nano silver by *Cassine glauca* (2.A) At starting stage, (2.B). after three hours

Figure 3: UV-Vis spectra of silver nanoparticles

The SEM image (Figure-4) showed relatively spherical shape silver nanoparticles formed with diameter ranging from 11.4 nm to 40.7 nm, different sizes of nanoparticles

Characterization of silver nanoparticles

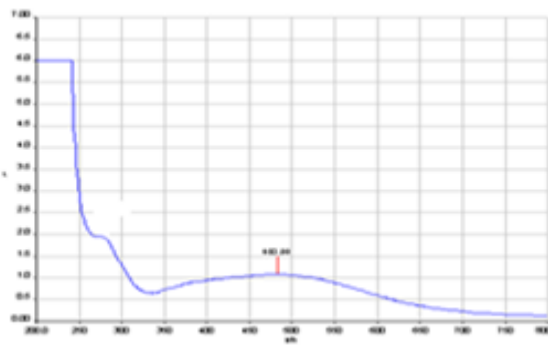
UV-Vis absorption spectra was measured using a Lambda 25 spectrophotometer. Scanning electron microscopy (SEM) analysis of synthesized silver nanoparticles was done using a Hitach S-4500 SEM machine. Crystalline metallic silver nanoparticles were examined using an X-ray diffractometer (Shimadzu, XRD-6000) equipped with Cu α radiation source using Ni as filter at a setting of 30 KV/30 mA. FTIR spectra of the sample was analyzed using a perkin-Elmer spectrum2 instrument by KBr pellet method.

Antimicrobial activity

Antimicrobial activity of aqueous extract was determined by disc diffusion method[15] for bacterial species like *Staphylococcus aureus*, *Escherichia coli* and fungal specie like *candida albicans*. The culture was inoculated by spread plate method. Chloromphenical and Fluconazole were used as Standard Solutions. The Plates were then incubated for 24 hours at 37⁰ C.

RESULTS AND DISCUSSION

There was a visible colour change seen after the addition of plant extract to the silver nitrate solution. Initially the plant extract was yellow. On addition of silver salt, it turned brown. The presence of nanoparticles was confirmed by obtaining a spectrum in visible range of 200nm to 600nm. A typical peak at 483nm (Figure.3) was obtained due to the surface Plasmon resonance of silver nanoparticles.¹⁶



present in *Cassine glauca*. The average size of nanoparticles was 22.8 nm in basic extract of *Cassine glauca*.

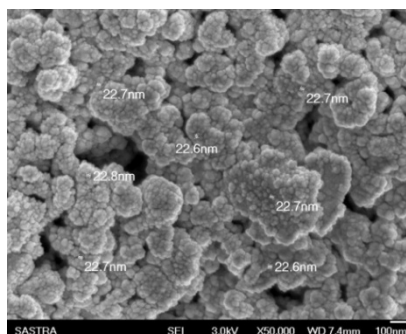


Figure 4: SEM image of silver Nanoparticles

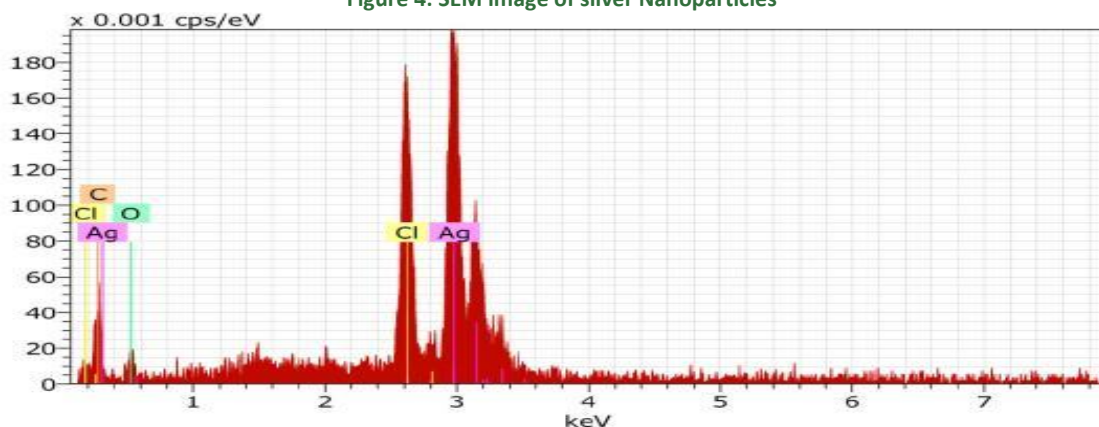


Figure 5: EDX spectra of silver Nanoparticles formed in *cassineglauca* bark extract

FTIR Analysis

Results of FTIR study of bio synthesized silver nanoparticles showed sharp absorption peaks 3425,2947,2592,2359,2075,1652,1599,1458,1421,1338,1119,1234,1022,794,650 cm^{-1} . Three absorption peaks located around 650,794 and 1022 cm^{-1} can be assigned as the absorption peaks of -C-N stretching vibrations of the amine, -C-O-C OR -C-O groups respectively¹⁷. The bonds or functional groups such as -C-O-C or -C-O- and -C=C derived from heterocyclic compounds are presents in stem bark extract are the capping ligands of the nanoparticles¹⁸. The broad and strong bonds at 3425 to 2947 cm^{-1} were due to bonded hydroxyl (-OH) or amine groups (-NH) and aliphatic C-H of the *cassineglauca* bark extracts respectively. The peak at 1652 cm^{-1} is attributed to the carboxyl group (-C=O) stretching vibration. These

results imply that the carboxyl (-C=O), hydroxyl (-OH) and amine (-NH) groups of *cassineglauca* bark extracts are mainly involved in fabrication of silver nanoparticles.

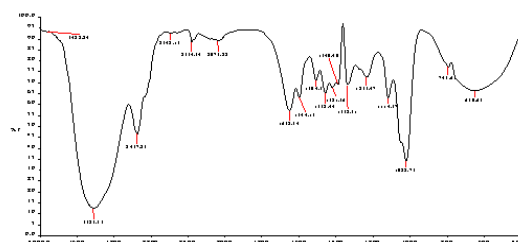


Figure 7: FTIR spectrum of silver nanoparticles formed by *cassineglauca* bark extract

XRD STUDIES

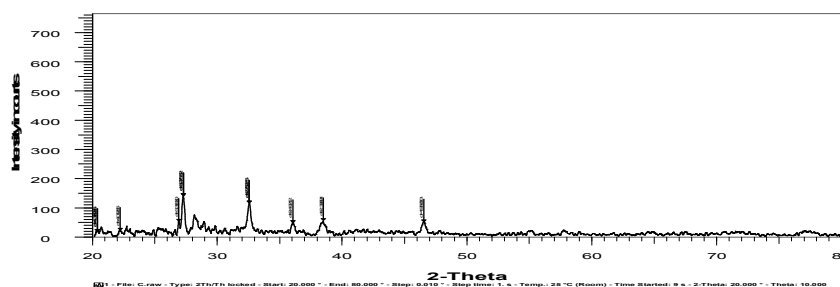


Figure 8: XRD spectrum of silver nanoparticles formed by *cassineglauca* bark extract

The XRD patterns confirm that the synthesized silver nanoparticles from *Cassineglauca* bark extract have cubic structure according to JCPDS data. The peaks are observed at $2\theta \approx 26.84, 32.50, 36.00, 46.51$ corresponding to the plane of 110, 111, 200, 211 show the presence of cubic phases in the final product. Apart from these peaks responsible for silver nanoparticles, the recorded XRD pattern shows additional unassigned peaks also noted. This may be due to the formation of the crystalline bio organic compounds/ metalloproteins that are present in the *Cassineglauca* bark extract. Similar observations are reported^{19,20}

ANTIBACTERIAL ANALYSIS

The Silver nano particles synthesized by *Cassineglauca* bark extract showed growth inhibitory effects against *Escherichia coli*, *staphylococcus aureus* species of bacteria and *candida albicans* fungi specie. Silver ions and silver salts are used as antimicrobial agents²¹. However, the high concentrations of silver salts restrict the use of them in present day medicine. Use of metal nanoparticles decreases the concentration of silver and other metal salts. The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio which allows them to interact closely with microbial membranes and is not merely due to release of metal ions in solution or in

culture plates²². The mode of action of both silver nanoparticles and silver ions were reported to be similar, although the nanoparticles were reported to be effective at significantly lower concentration than that of the ions²². According to Lok²³, the attachment of both silver ions and nanoparticles to the cell membrane caused accilimization of envelope protein precursors causing dissipation of the protein motive force. SNPs also exhibited destabilization of the outer membrane and rupture of the plasma membrane thereby causing depletion of intracellular ATP. According to Lin *et al*²⁴, rupture of bacterial cell membrane may lead to cell death. Siva Kumar *et al*²⁵ proposed that oxygen associated with silver reacts with the sulphhydryl (-S-H) groups on the cell membrane to form R-S-S-R bonds causing inhibition of respiration resulting in cell death. The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the extracts were measured using a millimeter scale. The diameter sizes in mm of the zone of inhibition are shown in the table 1 and Fig. 9. From the results, silver nano particles exhibited significant antibacterial activity. Both silver nano particles and aqueous plant extract show antibacterial and antifungal potential. Among these both have better result in antibacterial than antifungal. Silver nano particles gave better than plant aqueous extract.

Table 1: Antimicrobial activity of Silver nitrate nano particles, AgNO₃ and Plant extract

Sample	Concentrations	<i>Escherchia Coli</i> (mm)	<i>Staphylococcus aureus</i> (mm)	<i>Candida albicans</i> (mm)
AgNO ₃	30µl	2 ± 0.14	2 ± 0.14	2 ± 0.14
Plant extract	30µl	4 ± 0.28	3 ± 0.21	2 ± 0.19
Silver nano particles	30µl	6±0.42	6 ±0.42	5 ± 0.35
Standard (Chloromphenical)	30µl	10± 0.70	11± 0.77	---
Standard (Flucanazole)	30µl	---	---	9 ± 0.63

Values are expressed as Mean ± SD for triplicates

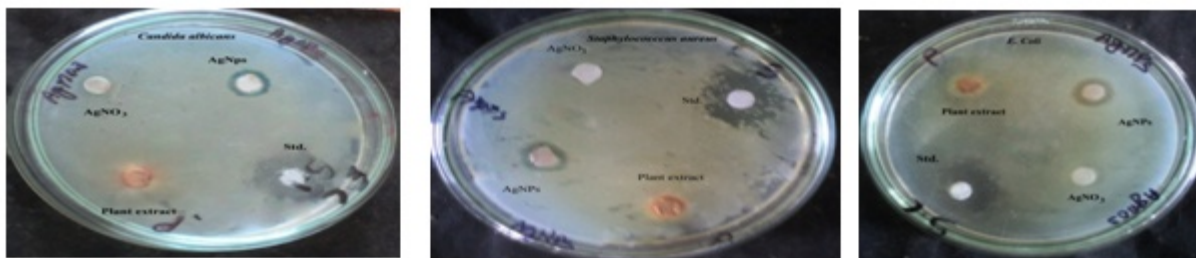


Figure 9: Antimicrobial activity of silver nano particles, AgNO₃ and Plant extract

CONCLUSIONS

The silver nano particles have been produced by *Cassineglauca* bark extract, which is an economical, efficient and eco-friendly process. UV-vis spectrophotometer, XRD, SEM, EDX and FTIR

techniques have confirmed the reduction of silver nitrate to silver nanoparticles. The results indicated that silver nanoparticles have good antibacterial activity against different microorganisms and hence has a great potential in the preparation of drugs used against fungal diseases.

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