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Synthesis and antibacterial activity of silver nanoparticles from the latex of *Calotropis gigantea*

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Abstract

Silver nanoparticles are being utilized in every phase of science along with engineering including medical fields and are still charming the scientists to explore new dimensions for their respective worth which is generally attributed to their corresponding small sizes. The growing demand in the chemical industries is due to the ample benefits of nanomaterials and preparation of nanomaterials include chemicals such as solvents, raw materials, reagents, and template materials. Such chemicals have engendered the noxious intermediates and products. To deminsh the undesirable products, the concept of green chemistry was brought in the chemical industries. The advantages of green synthesis of nanoparticles over the physical and chemical methods are Clean and eco-friendly approach, as toxic chemicals are not used; the active biological component itself act as reducing and capping agent, therefore reduction the overall cost of synthesis process; external experimental conditions like high energy and high pressure are not required, leads to energy saving process and can be used at large scale production of nanoparticles. The present study is to synthesis and to evaluate the antibacterial activity of silver nanoparticles from the latex of *Calotropis gigantea* . The results revealed that the silver nanoparticles synthesized from the latex of *Calotropis gigantea* showed the highest activity of against *E. coli* with 25 mm zone of inhibition. The diameters of the inhibition zones against *Klebsiella*, *B. cereus, S. aureus, and Streptococcus sp.*, were found to be 20 mm,15 mm,17 mm, and 15 mm respectively .

Introduction

Calotropis gigantea (crown flower) is a species of *Calotropis* native and it is a large shrub growing to 4 m (13 ft) tall. It has clusters of waxy flowers that are either white or lavender in colour. Each flower consists of five pointed petals and a small "crown" rising from the center which holds the stamens. The aestivation found in calotropis is valvate i.e. sepals or petals in a whorl just touch one another at the margin, without overlapping. The plant has oval, light fatty acids, and calcium oxalate, fatty acids, and calcium oxalate. *Calotropis* species is considered to possess anti-inflammatory components (David, 2010). Synthesis of silver nanoparticles is of much interest to the scientific community because of their wide range of applications. These silver nanoparticles are being successfully used in the cancer diagnosis and treatment as well (Popescu *et al*., 2010 and Baruwati *et al*., 2009]. The use of plants as the production assembly of silver nanoparticles has drawn attention, because of its rapid, eco-friendly, non-pathogenic, economical protocol and providing a single step technique for the biosynthetic processes. The reduction and stabilization of silver ions by combination of biomolecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpinoids and vitamins which are already established in the plant extracts having medicinal values and are environmental benign, yet chemically complex structures (Kulkarni and Muddapur, 2014). To collect and to prepare the plant extracts of *Calotropis gigantea* . The aim of the present study is to determine the antibacterial activity of silver nanoparticles synthesized using *C. gigantea* plant extracts against the selected pathogens.

2. Materials and Methods

2.1. Collection of *Calotropis gigantea*

The fresh , young disease free herbal plant *Calotropis gigantea* were collected from Palakkad. From fresh plant of *Calotropis gigantea* latex was collected.

2.2. Preparation of silver nitrate bulk

1mM Silver nitrate (A_gNO_3) solution was prepared using 0.01698 A_gNO_3 in 100 ml of distilled water.

2.3. Synthesis of silver nanoparticle using Milk

5 ml of raw milk from the plant was mixed with 100 ml of A_gNO_3 solution. Formation of silver nanoparticles were indicated by the brown- yellow colouration of the solution suggesting that aueous silver ions can be reduced by aqueous extract of plants part to generate extremely stable silver nano particles in water.

2.4. Characterization of silver nanoparticle

UV–Vis spectral analysis was done by using Shimadzu UV–visible spectrophotometer (UV-1800, Japan). UV–Visible absorption spectrophotometer with a resolution of 1 nm between 200 and 800 nm was used. One millilitre of the sample was pipetted into a test tube and subsequently analysed at room temperature. Dynamic light scattering (Spectroscatter 201) was used to determine the average size of synthesized silver nanoparticles. The suface groups of the nanoparticles were qualitatively confirmed by using Fourier transform infrared (FTIR) spectroscopy by a shimadzu FTIR spectrophotometer.

2.5. Antibacterial activity of *Calotropis gigantea n*

The milk extracts were screened against pathogens such as *E.coli* , *Staphylococcus aureus, Proteus vulgaricus, Bacillus cereus* , *Salmonella typhi, Streptococcus sp., Klebsiella pneumonia* .

2.6. Agar well diffusion method

Agar well diffusion method (Perez *et al* ., 1990) was performed to detect the antimicrobial effect off plant extracts . Muller Hinton Agar plates were swabbed with pathogenic bacteria using sterile cotton swab. Using Cork borer three wells were made on agar plates. 100µl of plant and milk extracts were added to each well and all the plates were kept undisturbed for 1 hour for proper diffusion. Finally, all the plates were incubated at 37° C for 12 hours. After incubation, antibacterial activity was observed.

3. Results and Discussion

3.1 UV-Vis spectrum of silver nanoparticle synthesized from the latex of *Calotropis gigantea*

Reduction of silver ions in the aqueous solution of silver during the reaction with the ingredients present in the latex was observed by the UV-vis spectroscopy. The change in colour was noted by visual observation in the *Calotropis gigantea* latex when it was incubated with A_gNO_3 did not show any change incolour. The colour of the extract changed to light brown within an hour and then later changed to dark brown during the 2 hours incubation period. There were no significant change occurred 2 hours. The brown colour could be due to the excitation of surface plasmon vibrations, typical of the silver nanoparticles (Ahmad *et al*., 2003 and Krishnaraj *et al*., 2010). The figure shows the UV-vis spectra of latex solution as a function of reaction time. The strong resonance centered at 410 – 445 nm was clearly observed and increased in intensity with tim. It might arise from the excitation of longitudinal plasmon vibrations in silver NPs in the solution. Reduction of silver ions present in the aqueous solution of silver complex during the reaction with the ingredients present in the leaves of *C. gigantea* latex observed by the UV-vis spectra.These changes were attributed to the excitation of surface plasmon bands are broadened (SPR) in the metal nanoparticles (Natarajan *et al*., 2010). Silver nanoparticle was observed to be stable in solution and show very little aggregation. Besides , the plasmon bands are broadened with an absorption tail in the longer wave lengths which may be due to the size distribution of the particles (Ahmad *et al*., 2003).

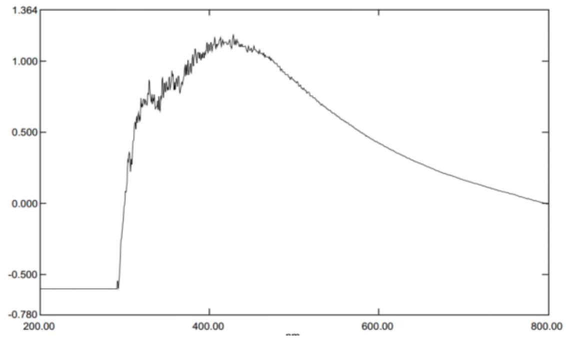

Fig 1. UV-Vis spectrum of silver nanoparticle synthesized from the latex of *Calotropis gigantea*

3.2. FTIR spectral analysis for the silver nanoparticle synthesized from latex of *Calotropis gigantea*

FTIR spectroscopy analyses were carried out to identify the biomolecules responsible for capping of the bioreduced AgNPs synthesized using latex. The FTIR spectra of aqueous silver nanoparticles are prepared from the *Calotropis gigantea* latex shows transmittance peaks at 732.95 , 785.74 and 866.04 (C-Cl stretch alkyl halides, C-H “oop” Aromatics and N-H wag primary and secondary amines); 1010.70 , 1124.50 and 1246.02 (C-O stretch Alcohols ,carboxylic acid, esters, and ether; C-N stretch Aliphatic amines); 1483.97 (C-C stretch aromatics in ring; N-O asymmetric stretch nitro compounds); 1641.42 (N-H bend primary amines; -C=C-stretch alkenes); 2627.05 (O-H stretch carboxylic acid); 2852.82 (C-H stretch alkanes); 3267.41 (O-H stretch carboxylic acid;

O-H stretch H bonded Alcohols and phenols); 3361.93 (N-H stretch primary and secondary amines and amides; O-H stretch H – bonded Alcohols and phenols). These peaks indicate that the carboxyl group formed aminoacid residues and that these residues "capped '' the silver nanoparticle to prevent agglomeration, thereby stabilizing the medium (Sathyavathi *et al.*, 2010) . When the metal nanoparticle forms in solution, they must be stabilized against the van der waalsforces of attraction which may otherwise cause coagulation. Physisorbedsurfactant and polymers may cause steric or electrostatic barriers or purely electrostatic barriers around the particle surface and may thereby provide stabilization (Mulvaney, 1996). FTIR peaks that were corresponding to aromatics rings, ether linkages and O-H stretch indicates the presence of flavonoids, terpanoids and phenols responsible for the stabilization of the AgNPs synthesized by *Calotropis gigantea* latex extract.

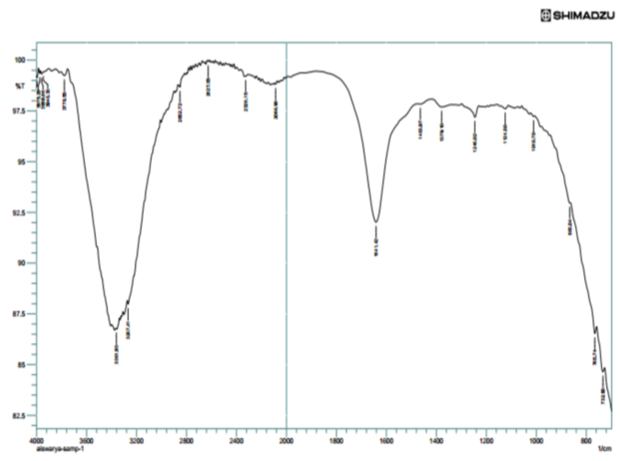

Fig 2. FTIR spectral analysis for the silver nanoparticle synthesized from latex

3.3. Antibacterial activity of silver nanoparticles from *Calotropis gigantea* latex

The antibacterial activity of silver nanoparticles produced from *Calotropis gigantea latex* was studied against both gram positive and gram negative bacteria. The average antibacterial activity of silver nanoparticles against selective bacterial strains ranged from 15 to 25 mm (the zone of inhibition. The maximum activity of silver nanoparticles was found against *E. coli* with 25 mm in diameter. The diameters of the inhibition zones against *Klebsiella*, *B. cereus, S. aureus , and Streptococcus sp.,* were found to be 20 mm,15 mm,17 mm, and 15 mm respectively . The results were tabulated in the Table -1. *Salmonella* and *Proteus* didn't show any inhibition zone, this indicates that there was no activity of *Calotropis gigantea* latex against these selective pathogens.

Gaurav *et al* (2010) studied the aqueous extract of *C. gigantea* leaves exhibited the antibacterial activity against six clinical isolates of bacteria. Extracts showed maximum antibacterial activity against *E. coli* (16±0.15 mm) and followed by *K. pneumoniae* (16±0.52 mm). *Staphylococcus aureus* showed an antibacterial activity of 13±0.15 mm.

4.Conclusion

In this study, we demonstrated the synthesis and antibacterial activity of silver nanoparticles from the latex of *Calotropis gigantea agianst* selective pathogen. We found that this particular nanoparticle may used as a potent antibacterial agent. Further studies can be done in future to identify the active compound from the latex of *Calotropis gigantea.*

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