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Green Synthesis of Silver Nanoparticles Using Leaf Extract of *Canavalia rosea* and Its Antibacterial Properties

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Abstract: Nanobiotechnology is the most active area of research in modern material science. Silver nanoparticles have a great potential for use in biological including antimicrobial activity. The purpose of the study is to synthesize and characterize the plant mediated silver nanoparticles from Beach bean (*Canavalia rosea*) an herbaceous vine that trails along beaches and coastal areas, which is useful in fundamental research and also has significant implications in various practical applications like catalysis, drug delivery systems, and antimicrobial agents. Biosynthesis, characterization and antibacterial potential of the CrSNPs was done by following standard methods. The studies have the potential to significantly impact the development of novel antimicrobial agents, contributing to the ongoing efforts in combatting antimicrobial resistance and improving public health.

Keywords: Antibacterial, *Canavalia rosea*, Silver Nanoparticles

INTRODUCTION

Nanoparticles serve as fundamental building blocks of nanodevices which are used in various practical applications.[1] Nanotechnology is a science that deals with manipulation of materials within size range of 0.1–100 nm. Nanomaterials are known to possess unique properties such as electrical conductance, chemical reactivity, magnetism, optical effects and physical strength, which differentiate them from bulk materials. The unique properties possessed by nanomaterials are as a result of their small size.[2] Production of nanoparticles can be achieved through physical, chemical and biological methods. Of these three methods, chemical method is the most common method which involves the use of toxic chemicals in the production of nanoparticles. Some of the physical and chemical methods include reduction, arc discharge, electrochemical and reduction processes. The desire of nanotechnology in the production of eco-friendly nanoparticles which is different from the previous ones has given rise to the biosynthesis of nanoparticles.[3] The wide use of synthesized nanoparticles in human activities create the need for a method which will not pose threat to the environment and humans as a result of their exposure to the nanoparticles, hence the biological method.[4-5]

Canavalia rosea is a leguminous, sand dune plant, commonly known as “beach bean or jack bean” in India. The plant shows the pantropical distribution, in America, Asia, Antilles, Africa, and Oceania. It has bioactive compounds, namely tannins, phlorotannins, saponins, flavonoids, alkaloids, cardiac glycosides and phenolics. Most of its sections including roots, stem, leaves, pods, seeds and flowers are utilized as medicine against flu, viruses, bacteria and fungi. The plant is traditionally explored for several ailments, rheumatism, tuberculosis, leprosy, haemorrhage, and also exhibits anti-microbial, anti-inflammatory, anti-cancer, anti-plasmodial, activities.[6] Silver nanoparticles are reported to possess antifungal, anti-inflammatory, antiviral, antiangiogenesis, and antiplatelet activity.[7] The study of characterization and antimicrobial properties of silver nanoparticles is a multidisciplinary field that involves materials science, nanotechnology, chemistry, microbiology, and biomedical research.

MATERIALS AND METHODS

Collection Plant Material and bacterial strains: The leaf sample of *Canavalia rosea* was collected from the seashore of Batapady Beach, Someshwara village, Ullala, Mangalore. The test pathogens *Escherichia coli* ATCC 11775 and *Lactobacillus acidophilus* ATCC 700396 were obtained from the Microbiology Lab, Microbiology Programme, Department of Biosciences.

Fig 1: *Canavalia rosea*

Preparation of Plant Materials: Fresh leaves of *Canavalia rosea* plants which is free from diseases were collected from Someshwar beach and then washed thoroughly 2-3 times with tap water and once with sterile water. The sample was sun dried until it becomes powdery. 20 grams of fresh leaves was finely chopped and added to 100 mL of distilled water and stirred at 60 °C for 1 hr. After boiling, the mixture was cooled and filtered with Whatman paper number 1. Filtrate was collected. This filtrate extract is used for phytochemical screening of *C. rosea* leaf. Phytochemicals are chemical compounds that take place naturally in plants.

Biosynthesis of Silver Nanoparticles: 0.1 M of aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 5 mL of leaf extract of *Canavalia rosea* was added to 45 mL of 0.1 M $AgNO_3$ solution for bio reduction process at room temperature.

Characterization of the biosynthesized CrSNPs (*Canavalia rosea* Silver Nanoparticles): Visual detection of the greenly synthesized CrSNPs was done by observing the mixture for a change in colour in comparison to the control samples. UV-Visible spectrophotometric analysis of the CrSNPs solutions were determined at room temperature using UV–Visible spectrophotometer (a Lambda 25-Perkin Elmer, Waltham, MA, USA) with a resolution of 0.5 nm. The absorbance of the sample was read at the wavelengths of 200–800 nm.[8] The chemical structure of the CrSNPs samples was analyzed using FTIR spectroscopy (Shimadzu). Two milligram of the dried samples was taken ground with KBr salt at 25 °C and pressed into a mold to form pellet. The spectra were recorded at a wave range of 500–4000 cm^{-1} and at resolution of 4 cm^{-1}.[9]

Antibacterial potential of the CrSNPs: The antibacterial potential of the CrSNPs was evaluated using the Agar Well Diffusion method.[10] The antibacterial assays were done against nosocomial pathogens such as *Lactobacillus acidophilus* and *Escherichia coli*. Luria Bertani (LB) broth/agar medium was used to cultivate bacteria. Fresh overnight culture of inoculum (100 μL) of each culture was spread on to Mueller Hinton Agar (MHA) plates. Silver nanoparticles along with aqueous solution taken as control in each plate. The plates were incubated at 37 °C overnight. Next day the inhibition zones around the well were measured. Uniform wells were cut on the dried agar plate using a sterile cork-borer of diameter 7 mm. Each well was filled with 25, 50, 75, 100 µl of the biosynthesized CrSNPs. $AgNO_3$ solution (1 mM) and the aqueous and ethanol extract of *C. rosea* were put into respective wells as negative controls. The inoculated plates were incubated at 37 °C for 24 hrs. After incubation the plates were observed for zones of inhibition (ZOI) around the wells. ZOI diameters (mm) greater than 1 mm were considered positive.[11]

RESULTS AND DISCUSSION

Qualitative phytochemical screening

The qualitative phytochemical screening analysis of *C. rosea* leaf extract showed the presence of tannin, xanthoproteins, quinones, coumarins, phenolic compounds, flavonoids, saponins and alkaloids contents. These compounds have potentially significant application against human pathogens, including those that cause enteric infections.[12]

Fig 2: Visual Observation of SNPs formation: (a) Canavalia rosea extract (b) Silver nitrate solution and (c) SNPs.

Visual Observation of the nanoparticle's biosynthesis

Formation of silver nanoparticles through reduction of silver nitrate into silver ion by the reducing agent is known to be associated with colour change (Fig 2). The reaction mixture of $AgNO_3$ and the aqueous and ethanol extract of *C. rosea* leaf powder turned brown indicating formation of silver nanoparticles. The intensity of the colour increases after 24 hrs of incubation. Silver nanoparticles formation was identified visually by colour change. The immediate colour changes as observed in formation of silver nanoparticles biosynthesized using *C. rosea* extract was due to the excitation of Surface Plasmon Resonance (SPR) of nanoparticles

in the reaction mixture. This was also reported by Rajeshkumar [13] who observed colour change in the reaction mixture during silver nanoparticles formation biosynthesized from *Padina tetrastromatica*.

UV-vis spectrophotometry analysis

The biosynthesized CrSNPs were characterized at different incubation hours using UV-Visible Spectrophotometer. At different hours of incubation Surface Plasma Resonance (SPR) peak was observed at 500 nm and broad-spectrum range was at 400–600 nm. The results obtained from the UV-Visible spectra suggested the formation of silver nanoparticles.

Fig 3: UV-Visible spectra of silver nanoparticle biosynthesized at different hours of incubation.

Fourier transform infrared (FTIR)

The FTIR spectra of the biosynthesized CrSNPs showed 10 major bands each of which suggested the presence of different functional groups of compounds present (Fig 4). The UV-vis spectroscopy is a technique used to confirm the formation and stability of nanoparticles based on the optical properties of the nanoparticles and it also serves as an indirect method used to determine the reduction of silver nitrate to silver nanoparticles in the aqueous solution. The optical property of silver nanoparticles is dependent on size and shape.

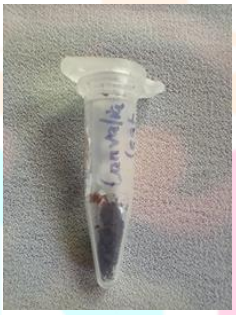
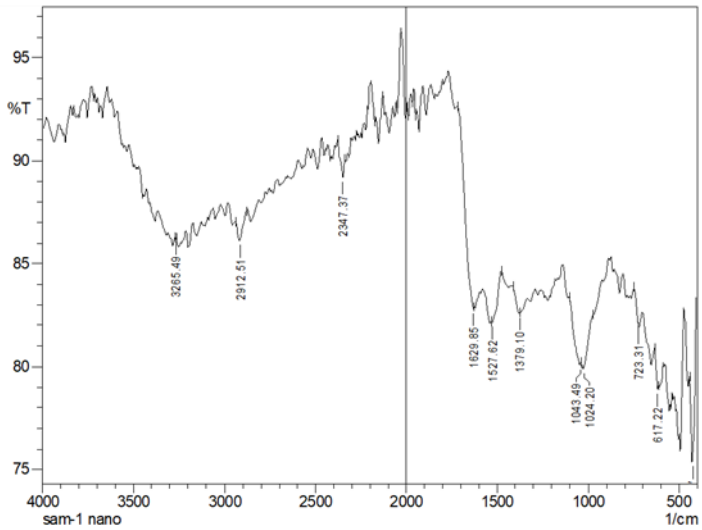


Fig 4: Crystals of Siver Nanoparticles extracted from aqueous extract of *C. rosea*

The result obtained as shown by the UV-vis spectra of the biosynthesized silver nanoparticles was formed after 24 hrs of incubation, a drop in the surface Plasmon peak was observed at 46 hrs and maximum increase in the resonance was observed after 67 hrs of incubation. This shows that complete nanoparticles formation occurred after 67 hrs of incubation and it implies that nanoparticles formation is associated with incubation time. The FTIR spectrum of the nanoparticles is shown in (Graph 1). The results of FTIR analysis of this study show different stretches of bonds shown at different peaks. The peak at 3265.49 cm^{-1} was more prominent than other peaks and it showed the presence of alcohol and phenols of -OH stretching. The peak 2912.51 cm^{-1} suggested the presence of alkanes C-H stretching. The peaks 2347.37 cm^{-1} suggest the presence of O=C=O stretching of carbon dioxide and 1629. 85 cm^{-1} indicate the presence of -C=C- stretching of alkenes, α, β-unsaturated ketone compounds in which 1527.62 cm^{-1} is an alkanes and alkyl groups with N-O stretching nitro compound. While 1379.62 cm-1 CH_3CH bending of alkanes. However, the peak 1043.49 cm^{-1} is CO-O-CO stretching with anhydride group. Peak 1024.20 cm-1 is an ether =C-O-C symmetric stretching and peak 723.31 cm-1 and 617.22 indicates the presence of an alkyl halides compound with C–Cl and C-Br stretching respectively.[14]



Graph 1: FTIR spectrum of the biosynthesized silver nanoparticles.

Antibacterial potential of the CrSNPs

The antibacterial activity of the biosynthesized CrSNPs was tested against two clinical pathogenic bacteria shown in table 1 and 2. All the test pathogens were susceptible to the nanoparticle as shown Fig 5 and 6. After 24 hrs, *Escherichia coli* and *Lactobacillus acidophilus* had the highest susceptibility with zone of inhibition 17 mm (75µl) and 12 mm (100µl) respectively.

BACTERIA		**Inhibition zone at different concentrations (in µl)**				
		Distilled water	25	50	75	100
Escherichia coli (mm)	1	-	8	9	17	13
	2	-	5	7	8	11
	3	-	9	10	11	12

Table 1: The antibacterial activity in *C. rosea* extract against *E.coli*

BACTERIA		**Inhibition zone at different concentrations (in µl)**				
		Distilled water	25	50	75	100
Lactobacillus acidophilus (mm)	1	-	-	-	-	-
	2	-	7	9	9	12
	3	-	4	7	11	12

Table 2: The antibacterial activity in *C. rosea* extract against *L. acidophilus*

Fig 5: Antibacterial activity in *C. rosea* extract against *E. coli*

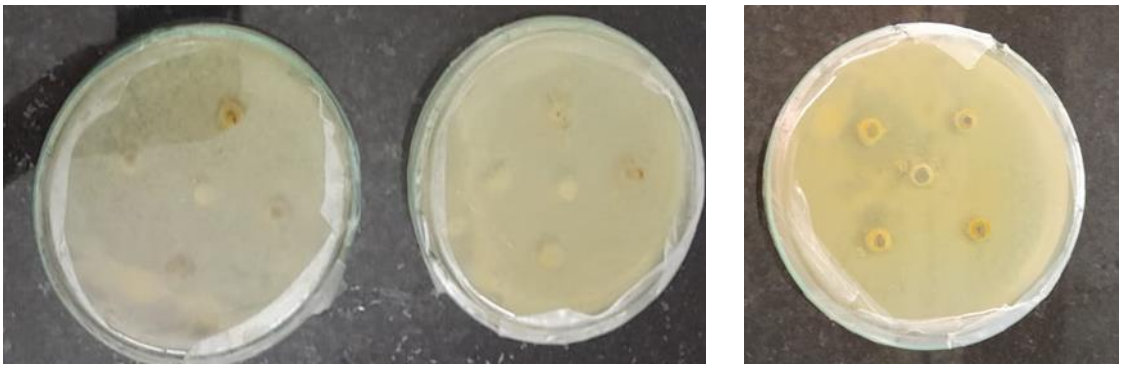


Fig 6: Antibacterial activity in *C. rosea* extract against *L. acidophilus*

The antimicrobial activity of the sample is determined by measuring the zone of inhibition of bacteria at different concentration. As a result, maximum zone of inhibition is observed in the culture where *E. coli* is used against the extract of *C. rosea* at 75µl (17 mm). In the culture containing *L. acidophilus* against extracts of *C. rosea*, the maximum zone of inhibition is at 100 µl (12 mm). The antimicrobial effect of silver nanoparticles is attributed to their small size and large surface area, which enable efficient interactions with microorganisms. They can disrupt microbial cell membranes, generate reactive oxygen species (ROS), and interfere with essential enzymes, ultimately leading to the death of bacteria, viruses, and fungi. Additionally, the combination of silver nanoparticles with the inherent antimicrobial compounds present in leaf extracts, such as phenols, flavonoids, terpenoids, and alkaloids, can create a synergistic effect, enhancing their overall antimicrobial potency. This promising antimicrobial activity opens up potential applications in medical, food preservation, and environmental remediation fields.

CONCULSION

Canavalia rosea, commonly known as Baybean or Beach Bean, is a fascinating flowering plant belonging to the Fabaceae family, native to tropical regions of the Americas. It displays a remarkable appearance, with herbaceous vines that trail or climb and form thick mats on sandy beaches and dunes. The synthesis of silver nanoparticles using leaf extracts of *C. rosea* offers a green and sustainable approach to nanoparticle production. The bioactive compounds present in the leaf extract act as effective reducing and stabilizing agents, facilitating the conversion of silver ions into silver nanoparticles. This eco-friendly method is cost-effective, simple, and has a reduced environmental impact compared to conventional chemical synthesis approaches. The characterization of the synthesized silver nanoparticles using various analytical techniques provides valuable insights into their properties. UV-Visible spectroscopy confirms the formation of silver nanoparticles. Fourier Transform Infrared Spectroscopy (FTIR) helps identify the functional groups present in the leaf extract, responsible for the reduction and stabilization of silver nanoparticles. The synthesized silver nanoparticles exhibit promising antimicrobial activity against various bacterial strains, owing to the inherent properties of silver nanoparticles to disrupt bacterial cell membranes and interfere with cellular processes. The combined findings of the phytochemical analysis, nanoparticle characterization, and antimicrobial activity evaluation highlight the potential of *Canavalia rosea* leaf extract as an eco-friendly and potent source for green synthesis of silver nanoparticles with potential antimicrobial applications.

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REFERENCES

1. Takeshima, T., Tada, Y., Sakaguchi, N., Watari, F., & Fugetsu, B. (2015). DNA/Ag nanoparticles as antibacterial agents against gram-negative bacteria. *Nanomaterials*, 5(1), 284–297.
2. Nikalje, A.P. (2015). Nanotechnology and its application in medicine. *Medicinal Chemistry*, 5(2), 81–89.
3. Massironi, A., Morelli, A., Grassi, L., Puppi, D., Braccini, S., Maisetta, G., Esin, S., Batoni, G., Pina, C.D., & Chiellini, F. (2019). Ulvan as novel reducing and stabilizing agent from renewable algal biomass: application to green synthesis of silver nanoparticles. *Carbohydrates Polymers*, 203, 310–321.
4. Prasad, T.N., & Elumalai, E.K. (2011). Bio fabrication of Ag nanoparticles using Moringa oleifera leaf extract and their antimicrobial activity. *Asian Pacific Journal of Tropical Biomedical*, 1(6), 439–442.
5. Nair, B., & Pradeep, T. (2002). Coalescence of nanoclusters and formation of submicron crystallites assisted by Lactobacillus strains. *Crystal Growth and Design*, 2(4), 293–298.
6. Vasanthi, R. & Balamurugan, V. (2022). A review on pharmacological aspects of Canavalia rosea. *Science Progress and Research*, 2(2), 567- 579.
7. Wiley, B. J., Im, S. H., Li, Z. Y., McLellan, J., Siekkinen A and Xia, Y. (2006). Maneuvering the surface plasmon resonance of silver nanostructures through shape-controlled synthesis. *Journal of Physical Chemistry*, 10(32), 15666–15675.
8. Ashok kumar, T., & Kuppusamy, V. (2016). Brown seaweed-mediated biosynthesis of gold nanoparticles. *Journal of Environmental Biotechnology Research*, 2(1), 45–50.
9. Bhat, R., Deshpande, R., & Ganachari, S.V. (2011). Photo-irradiated bio-synthesis of silver nanoparticles using edible mushroom *Pleurotus florida* and their antibacterial activity studies. *Bioinorganic Chemistry and Application*, 36, 168–171.
10. Shivashankar, M., Premkumari, B., & Chandan, N., (2013). Biosynthesis, partial characterization and antimicrobial activities of silver nanoparticles from Pleurotus species. International Journal of Integrative Sciences, *Innovation and Technology*, 2 (3), 13–23.

11. Prabhu, S.S., Mohan, R, K., & Sanhita, P. (2014). Production of bacteriocin and biosynthesis of silver nanoparticles by lactic acid bacteria isolated from yoghurt and its antibacterial activity. *Scrutiny International Research Journal of Microbiology and Biotechniques*, 1(3), 7–14.

12. El-Mahmood Muhammad Abubakar. (2009). Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections. *Journal of Medicinal Plants Research*, 3(7), 498-505.

13. Rajeshkumar, S., Malarkodi, C., & Venkat Kumar, C. (2017). Synthesis and characterization of silver nanoparticles from marine Brown seaweed and its antifungal efficiency against clinical fungal pathogens. *Asian Journal of Pharmaceutical Clinic Research*, 10 (2), 190–193.

14. Marimuthu, S., Rahuman, A.A., Rajkumar, G., Santhoshkumar, T., Vishnu, K.A., Jayasheelan, C., Bagavan, A., Zahir, A.A., Elano, G., & Kamaraj, C. (2011). Evaluation of green synthesized silver nanoparticles against parasites. *Parasitology Research*, 108(6), 1541–1549.