Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [11] October 2023 :282-288

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Synthesis of Silver Nanoparticles from *Lumnitzera Racemosa* Leaf Aqueous Extract and Its Antioxidant Activity

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ABSTRACT

The utilization of plant-mediated biosynthesis for the production of nanoparticle is gaining significance due to its straightforward methodologies and utilization of non-toxic constituents. The utilization of Lumnitzera racemosa (LR) mangrove leaf extract as a bioreductor in the production of AgNPs was found to be efficient by modifying the concentration of silver nitrate and the volume of extract. The UV-Vis absorption spectra within the 439-453 nm range indicate the successful production of AgNPs in the colloid. The FT-IR absorption band analysis indicates that the biomolecules derived from L. racemosa (LR) responsible for the synthesis of silver nanoparticles consist of alcoholic compounds, alkyl halides, organic compounds, phenolic compounds, and aliphatic combinations. Based on the X-ray diffraction (XRD) pattern analysis, it can be inferred that the silver nanoparticles (AgNPs) were successfully synthesized and exhibited a face-centered cubic (FCC) crystal structure. The average size of these nanoparticles was determined to be approximately 30 nm. The transmission electron microscopy (TEM) analysis demonstrated that the synthesized silver nanoparticles (AgNPs) have a spherical morphology, with a size distribution ranging from 10 to 58 nm. When compared to pure plant extract, silver nanoparticles (AgNPs) exhibit a significantly elevated level of antioxidant activity.

Keywords: *Mangrove Silver Nanoparticles, Lumnitzera racemosa, Antioxidant*

Received 21.07.2023 Revised 20.08.2023 Accepted 26.10.2023

INTRODUCTION

Nanotechnology plays a crucial role in the manufacturing of nanoparticles (NP) that exhibit dimensions spanning from 1 to 100 nm. Nanoparticles have enhanced functionality due to their higher surface atom density compared to micro particles (1). These materials possess notable characteristics, including a substantial surface area, distinctive structural attributes, and an extended duration of viability. Nanotechnology facilitates the translation of advancements in nanoscience into the development of innovative materials and the acquisition of new functional capabilities. Currently, nanochemistry is experiencing rapid growth and is considered one of the most rapidly increasing domains within the field of nanoscience (2). Nanometer-sized metallic particles often display distinct and drastically modified physical, chemical, and biological properties in comparison to their macroscopic counterparts, mostly because to their high surface-to-volume ratio. Consequently, there has been a significant amount of research conducted on these nanoparticles in recent years (3). The development of ecologically sustainable bioprocesses for the synthesis of nanoparticles (NPs) is a very important field of study within material science and system engineering (4). Plant-mediated synthesis, commonly referred to as biosynthesis, has been recognized as a more efficient mode of synthesis in comparison to physical and chemical approaches. The key advantages of biogenesis in nanoparticle creation include the absence of dangerous substances, elevated temperatures, excessive energy consumption, and high pressure conditions (2). Numerous physical and chemical methodologies have been employed in the production of nanoparticles. The aforementioned procedures exhibit several limitations, including issues related to toxicity, high costs, and the utilization of hazardous chemicals as reducing agents (5). As stated in reference (6), biomolecules such as proteins, flavonoids, phenols, and saponins have the ability to convert ions into particles and encapsulate nanoparticles. There is a pressing requirement in the present time to advance a nanoparticle synthesis method that possesses characteristics of being non-toxic, cost-effective, and environmentally sustainable. Additionally, it is desirable for these nanoparticles (NPs) to exhibit significant antioxidant activity (7). The objective of this study is to explore the eco-friendly synthesis of multifunctional silver nanoparticles

(AgNPs) using Lumnitzera racemosa leaf extract as a novel bioreductant. The aim is to evaluate the possible applications of these nanoparticles in the field of nanomedicine. The extracts derived from mangrove plant leaves serve as capping and reducing agents, thereby inducing the formation of crystals and subsequently affecting the characteristics of silver nanoparticles. The present study suggests utilizing a sustainable source of mangrove plants derived from natural resources as a means to incorporate an antioxidant agent in conjunction with AgNPs. This study aims to investigate the potential of secondary metabolic components found in mangrove extract, specifically flavonoids, to serve as both reducing agents for Ag+ ions, converting them to Ag0, and as capping agents to stabilize the resulting Ag0 nanoparticles. The goal of this study is to investigate the potential of mangrove *Lumnitzera racemosa* leaf extract to synthesize and characterize silver nanoparticles, including particle size, crystalline structure, and sustainability. In this study, variables such as the percentage of the preparation and the amount of extraction were altered. This study is a pioneering investigation that only employs *L.racemosa* aqueous leaf extracts as a reducing agent for the synthesis of silver nanoparticles.

MATERIAL AND METHODS

PLANT MATERIAL COLLECTION

Mangrove leaves of L. racemosa (LR) were obtained from a forest near Pichavaram, Tamil Nadu, India. First, the leaves were thoroughly cleaned with tap water to remove any soil particles that could affect the final result. After 15 days of shade drying, the leaves were ground into powder. The leaf specimen was identified at Annamalai University's Marine Science Department mangrove herbarium lab in Tamil Nadu, India.

PREPARATION OF AQUEOUS EXTRACT FROM MANGROVE AND BIOSYNTHESIS OF SILVER NANOPARTICLES

A mixture of 10 grams of finely crushed, dried L. racemosa powder and 100 ml of double-distilled water was boiled at 65°C for 1 hour. The mixture was then filtered using Whatman paper No. 1. This sample was stored at 4°C in an airtight container for future use. L .racemosa leaf extract was used to produce AgNP nanoparticles using approved procedures. The experiment varied silver nitrate concentration by 1, 5, and 10 mM. The three AgNO3 concentrations were used to identify the metal concentration that maximized productivity while decreasing AgNP size. The relationship between silver nanoparticle stability and response time was also examined. Silver nitrate solution was mixed with different amounts of mangrove leaf extracts. This mixture is allowed to approach equilibrium at room temperature for up to 3 months, during which time a UV-Vis spectrophotometer measures its absorbance.

PHYTOCHEMICAL SYSTEMS AND THEIR ACTIVITY

Following the reference (8), L. racemosa leaf extract alkaloid, flavonoid, phenolic, saponin triterpene, and tannin components were qualitatively evaluated. The qualitative results of this study are tabulated and indicate a favorable (+) or negative (-) outcome.

SILVER NANOPARTICLE CHARACTERIZATION

A reaction mixture of 20 ml of a 1 mM silver nitrate (AgNO3) solution and 2 ml of a leaf aqueous extract was used to create silver nanoparticles (AgNPs). The reaction mixture's absorbance spectra were scanned frequently using a UV-Vis spectrophotometer (Hitachi-U-2001) from 300 to 800 nm to monitor AgNPs production. The AgNPs solution was applied to a glass substrate utilizing the XPERT-PRO D-8 apparatus at 30 kv, 40 mA, and Cu kα radiation. At a 2θ angle, X-ray diffraction (XRD) observations were taken. X-ray diffraction (XRD) is an effective analytical method for identifying phases in crystalline materials and measuring unit cell size. The crystallite size was calculated using Scherrer's formula: $D = K\lambda/\beta\cos\theta$. K is the shape factor, or Scherrer's constant, in this equation. Where λ is the X-ray wavelength, β is the Full Width Half Maximum, and θ is the angle of diffraction. For FT-IR measurement, nanoparticles (NPs) were dispersed in deionized water and centrifuged at 10,000 rpm for 15 minutes. To remove biological impurities, the leftover material was suspended in sterile de-ionized water again. The pure residue was dried in a 70 °C oven for a long time. After drying, nanoparticles were mixed with KBr powder to make pellets. The samples' transmittance was measured using a Perkin-Elmer spectrum instrument with a 4 cm−1 resolution, operating in transmission mode (4000–400 cm−1). The surface properties of AgNPs were examined using a BRUKER-INDIA FESEM. A small amount of sample was put onto the copper grid using aluminum foil to make a thin layer. The reduced Ag+ ions were dried on a copper grid using aluminum foil. The material was then examined using FESEM with an EDX attachment.

ANTIOXIDANT PROPERTIES

The antioxidant activity of the sample was assessed by employing 1,1-diphenyl-2-picrylhydrazyl hydrate (DPPH, Merck). The DPPH free radical scavenging activity of silver nanoparticles was assessed using the methodology (9) with minor modifications. In summary, 3.8 mL of DPPH solution at 30 g/mL was combined

with 0.2 mL of silver nanoparticles at 30-70 g/mL. The mixture was incubated in darkness for 30 minutes. Sample absorbance was measured at 517 nm using a Spectrophotometer UV-Vis. DPPH in methanol without sample was used as a control. Ascorbic acid was the experiment's reference standard. As a control, leaf extract antioxidant activity was measured. The measurements were duplicated. The formula's free radical scavenging was measured to determine antioxidant activity. The lowest antioxidant needed to scavenge 50% of DPPH free radicals was estimated as the IC50 value.

REUSULTS

PHYTOCHEMICAL ANALYSIS OF EXTRACTS OF L. racemosa LEAF

The L. racemosa (LR) leaf extract was easily extracted using double-distilled water, and the presence of several bioactive components tested positive. The presence of flavonoid, polyphenol, steroid, triterpenoid in the leaf extract is suggested by phytochemical analysis. These secondary metabolite groups should decrease Ag+ to Ag0. (Table 1)

BIOSYNTHESIS OF AgNPS

A thorough examination was carried out to evaluate the extracellular production of silver nanoparticles (AgNPs), with particular emphasis on the mangrove leaf extract. The aim of this research was to examine the interaction between mangrove leaf extract, at different concentrations of 1%, 2%, and 3% v/v, and a solution of silver nitrate with concentrations of 1 mM, 5 mM, and 10 mM. The experimental procedure involved the utilization of a synthesis solution with a total volume of 50 ml. The specimens were designated as AgNPs-A-1-1, AgNPs-A-5-1, and AgNPs-A-10-1, representing silver nanoparticles with precursor concentrations of 1 mM, 5 mM, and 10 mM, correspondingly. Furthermore, the concentrations of the extracts employed in this study were standardized at 1% for all samples, with the exception of certain samples which were subjected to concentrations of 2% and 3%. After the addition of the leaf extract and AgNO3, a discernible alteration in color was seen. The color underwent a transformation from its initial state to a subdued yellow and deep brown shade. The observed change in hue signifies a gradual decline in the AgNO3 concentration as a result of the leaf extract's presence. The change in hue that is observed can be related to the occurrence of surface Plasmon Resonance Excitation (SPR) in silver nanoparticles (AgNPs) (10).

UV-VIS SPECTROPHOTOMETER ANALYSIS SPECTRUM

A high-concentration leaf extract (3%) produced a more pronounced absorbance peak, probably due to more biomolecules participating in the reduction activity. The 3% leaf extract concentration enhanced aggregation after one month. This is supported by the SPR peak's redshift and greater particle size. The concentration of silver nanoparticles and the extract used to make them—AgNPs-A-1-1, A-5-2, and A-10-1—are critical for optimal results. Nanoparticles generated at precursor concentrations of 1, 5, and 10 mM have maximum absorption peaks at 440, 448, and 452 nm. The observed spectra shifted towards redshift or blueshift due to particle size, shape, aggregation state, and dielectric medium (11). The lifetime of silver nanoparticle colloids was tested for three months. The stability of nanoparticles is higher at 1 and 5 mM than 10 mM. This is because concentration directly affects agglomeration, reducing nanoparticle absorption. UV-visible spectroscopy is advised for preliminary assessment of nanoparticle synthesis with a spherical morphology. Transmission electron microscopy (TEM) examination will explain the topic.

FOURIER TRANSFORMS INFRARED SPECTROSCOPY (FTIR)

The observed absorption spectrum of the RS dry powder extract exhibited a characteristic absorption peak at 3372.87 cm-1, indicating the involvement of hydroxyl (OH-) groups. This peak is attributed to the presence of alcoholic, phenolic, and flavonoid groups, which serve as capping agents on the surface of the nanoparticles. The Fourier Transform Infrared (FTIR) spectra of the LR dry powder exhibited a characteristic peak corresponding to the hydroxyl (OH-) functional group, which was observed as a broad and intense band at a wavenumber of 2929.83 cm-1. The presence of absorption bands at 2364.91 cm-1 and 1637.00 cm-1 in the leaf extract indicated the presence of different phytochemicals, which served as capping agents. The observed peak at 1379.91 cm-1, which corresponds to the stretching vibration of the alcoholic group, exhibited a downward shift to a lower wavenumber of 1041.01 cm-1. This shift coincides with the stretching band associated with the aromatic amine group's CN stretching vibration. The potential cause of the phenomenon might be attributed to the reduction of silver ions into silver nanoparticles, facilitated by the presence of carboxyl groups and amine groups acting as stabilizers on the nanoparticle surface (12). The leaf extract and AgNPs' wavenumber change suggests that the biomolecules' functional groups touch the silver cation. During silver nanoparticle production, oxidation and reduction processes cause this interaction. The evidence suggests that biological molecules can create and stabilize colloidal silver nanoparticles in aqueous settings, controlling their size and preventing agglomeration (12).

XRD (X-RAY DIFFRACTION)

X-ray diffraction (XRD) analysis was employed to characterize the structural determination of RS-AgNPs. The X-ray diffraction pattern has distinct peaks at 2θ values of 38.21◦, 44.65◦, 64.65◦, and 77.52◦, indicating the presence of a face-centered cubic (FCC) structure in the silver sample (ICSD N0 4068). Furthermore, the experimental results indicate the presence of a peak at around 44.65 degrees in the 2θ angle, which can be attributed to the LR extract. This finding supports the previous study (13) that suggests the existence of a stabilizing agent in the AgNPs sample. Additionally, previous research conducted by (14) has yielded comparable results, suggesting that the extract plays a role in diminishing, limiting, and maintaining the size of particles. The size of the silver nanoparticles that were generated during the reaction was determined to be within the range of 25-32 nm, as calculated using the Scherrer equation.

TRANSMISSION ELECTRON MICROSCOPY (TEM)

The transmission electron microscopy (TEM) images provide evidence that the produced silver nanoparticles (AgNPs) exhibit a spherical morphology, with a size distribution ranging from 9 to 57 nm. The average size of the nanoparticles was determined to be 30 ± 2 nm using the "Image J" software. The periphery of the nanoparticle exhibits a higher level of brightness compared to its central region. The silver nanoparticles exhibit a surrounding thin layer, indicating their encapsulation by biomolecules, including proteins and other secondary metabolites present in the extract.

ANTIOXIDANT ACTIVITY

The antioxidant activity test was conducted on L. racemosa (LR) AgNPs that were generated using precursor concentrations of 1, 5, and 10 mM, and a 2% extract. The results were evaluated based on IC50 values. The determination of the inhibition percentage involved the comparison of the absorbance values at a wavelength of 517 nm between pure DPPH and the tested AgNPs (Table 2). The relationship between the DPPH free radical scavenging activity of silver nanoparticles facilitated by marine plants and the hydroxyl group concentration is postulated. According to the findings presented by (15), compounds exhibiting hydroxyl groups in close proximity on the B-ring demonstrate enhanced activity. Based on the aforementioned facts, it can be inferred that the primary constituent accountable for the antioxidant efficacy of the L. racemosa (LR) extract is a derivative of flavanol, as shown by the presence of phenolics, flavonoids, and polysaccharides. The Biological processes, such as green nanoparticle production, are preferred due to society's goal for environmental sustainability. Naturally existing antecedents like plants, fungus, bacteria, and others have advantages for the production of nanoparticles. Antibacterial silver nanoparticles (AgNPs) are used in food preservation, textile coatings, healthcare, and environmental applications. Silver's toxicity remains unproven after decades of use. Silver nanoparticle (AgNP) products have been approved by the US FDA, US EPA, SIAA in Japan, the Testing and Research Institute for Chemical Industry in Korea, and FITI (16-20). Silver nanoparticles (AgNPs) are antioxidants and antibacterial agents used to clean medical devices, home appliances, and water (21-25). This process has also accelerated the incorporation of silver nanoparticles (AgNPs) into textile fabrics. The present study created silver nanocomposite fibers with silver nanoparticles inserted in the fabric matrix. This Silver nanoparticles accelerate the bleaching of organic pigments with potassium peroxodisulphate in an aqueous solution at room temperature. The effects of nanoparticle size, shape, metallic species, and medium on plasmon resonant peaks and line widths are well known. Nanoclusters of 2–8 silver atoms could provide the basis for a new optical data storing method. Additionally, the clusters' fluorescent emissions could be used in biological labeling and electroluminescent displays (26-30).

CONCLUSION

The current study synthesized AgNPs using L. racemosa as stabilizing and capping agents. The green AgNPs were characterized by XRD, FESEM, FT-IR, and EDX. The structural morphology, functional groups, and elemental composition of AgNPs were examined using these methods. AgNPs averaged 20 nm and were face-centered cubic. In vitro antioxidant and antibacterial tests investigated silver nanoparticles (AgNPs) biological potential. A bio-friendly production of silver nanoparticles (AgNPs) was achieved using L. racemosa aqueous extract. This synthesis used the extract as a fast, reliable, and non-toxic stabilizer, reducing agent, and capping agent. At 1mM and 5mM concentrations, AgNPs are stable for three months. Biomedical uses are possible with nanoparticles.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF LEAF EXTRACT

SECONDARY METABOLITES	DISTILLED WATER
Alkaloid	-
Flavonoid	+
Polyphenol/ tannin	+
Saponin	-

Steroid	+
Triterpenoid	+

TABLE 2: IC_{50} VALUES OF AGNPS SYNTHESIZED

SAMPLES	IC_{50} DPPH (µg/ml)
AgNPs-A-1-2	49.52 ± 0.42
AgNPs-A-5-2	47.84 ± 0.20
AgNPs-A-10-2	44.13 ± 0.37
Leaf Extract	52.66 ± 0.60
Ascorbic Acid	24.58 ± 0.38

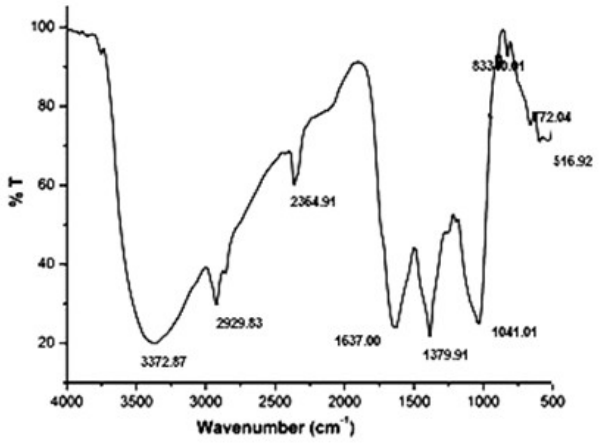

FIGURE 1: FTIR SPECTRUM OF GREEN SYNTHESIZED AGNPS

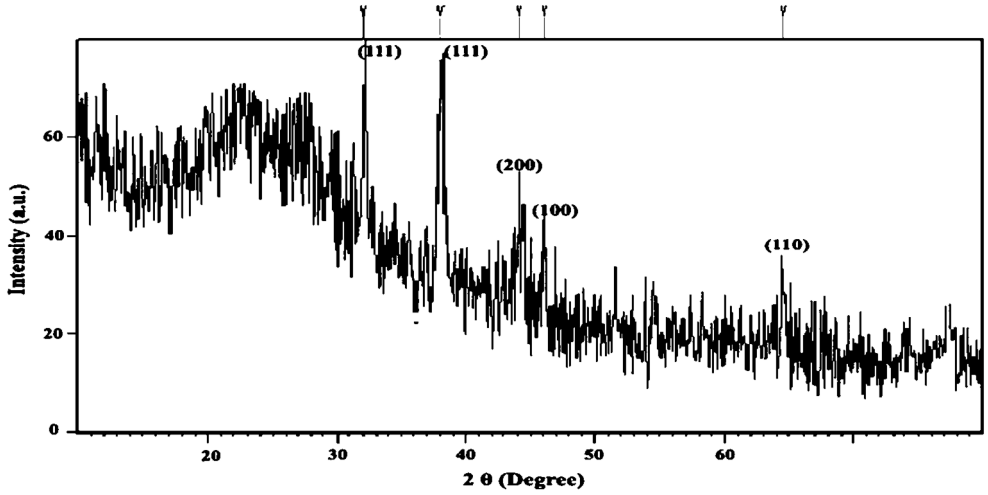

FIGURE 2: XRD PATTERN OF SYNTHESIZED AgNPS

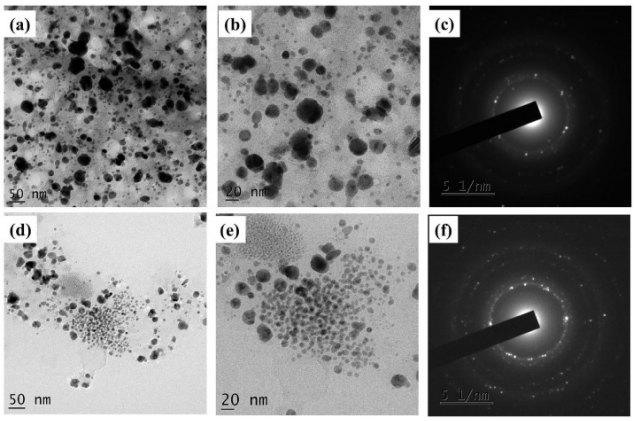

FIGURE 3: TEM FOR SYNTHESIZED AgNPS
(a, b, c –Bio-synthesised nanoparticles; d,e,f - Leaf extract)

ACKNOWLEDGMENTS
The authors are thankful to the College Principal and Head Department of Zoology, Thanthai Periyar government arts and Science College, for providing all the necessary facilities related to the present work and Vice Chancellor and Research director, Bharathidasan University, Trichirappalli for providing the official support for the research work.

CONFLICT OF INTEREST
There is no conflict of interest between authors regarding academic, commercial, financial, personal and professionally relevant to the work.

AUTHOR'S CONTRIBUTION
Synthesis, preliminary characterization and antitumor studies were performed by the Samivel Celliah and Vasugi Swamivel while the review and interpretations were performed by Krishnamoorthy Palaniyandi.

FUNDING
No Funding from any Govt. and Non Govt. Organizations.

ETHICS STATEMENT
Not Applicable as the study performed in-vitro and in-silico.

INFORMED CONSENT
Not Applicable

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CITATION OF THIS ARTICLE

Samivel Celliah, Vasugi Swamivel Rathinavelu and Krishnamoorthy Palaniyandi. Synthesis of Silver Nanoparticles From *Lumnitzera Racemosa* Leaf Aqueous Extract And Its Antioxidant Activity. Bull. Env.Pharmacol. Life Sci., Vol 12 [11] October 2023: 282-288