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BIOGENIC NANOSCALE SILVER PARTICLES: A STUDY ON THEIR ANTIMICROBIAL POTENTIAL

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ABSTRACT

A sustainable method for producing silver nanoparticles (AgNPs) using *Vitis vinifera* seed extract has been proposed. The formation of AgNPs was confirmed through spectroscopy, X-ray Diffraction (XRD), and electron microscopy. The UV-visible spectrophotometer revealed distinct absorbance peaks at 398.80 nm and 416.24 nm, signifying the presence of Surface Plasmon Resonance (SPR). XRD, quasi-elastic light scattering, and surface topography results exposed that the AgNPs were crystalline and had rod-like shapes. FTIR identified seed extract functional groups that reduced silver ions to AgNPs and acted as capping agents. The nanoparticles showed inhibition against gram-positive microorganisms like *Staphylococcus pneumonia* and *Mycobacterium tuberculosis*, surpassing their effectiveness against gram-negative microorganisms such as *Pseudomonas fluorescens*. This environmentally responsible approach not only offers a green synthesis method but also holds promise for combating specific pathogenic bacteria.

Keywords: *Vitis Vinifera*, Surface Plasmon Resonance, Green Synthesis, Antibacterial Properties, Sustainable Nanotechnology.

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INTRODUCTION

Nanotechnology, with its unique properties, has diverse applications from wastewater treatment to medicine[1, 2]. Nanoparticles, synthesized naturally or chemically, show promise in antibacterial research and have transformative effects in agriculture, improving crop productivity and soil health through precise nutrient delivery and pest control. In medicine, nanotechnology aids targeted drug delivery, enhances imaging, and allows rapid disease detection. Noble metal nanoparticles like silver and gold offer versatile potential in biomedicine, while plant-mediated synthesis provides a greener approach for biomedical nanoparticles.[34] Nanoparticles, with optical and magnetic properties, promise innovative solutions in medicine, while plant-mediated synthesis offers a safer and more cost-effective alternative for biomedical use. The documents from the literature reveal that numerous nanoparticles are synthesized via aerial plant parts, with *Ageratum conyzoides* and *Ageratum houstonianum*[5], *Ricinus communis var. carmencita* leaf extract[6,7], *Plumbago auriculata* leaf and calyx extracts[8], *Ziziphora tenuior* (Zt) extract[9], *Vitis vinifera*[10], beetroot[11], mangosteen[12], *Urtica dioica Linn* leaves.[13] Therapeutic potential in nanoparticle synthesis is attributed to plant secondary metabolites such as terpenoids, polyphenols, and flavonoids, offering precise control over size and properties. Silver nanoparticles (AgNPs) are notable for their antimicrobial properties and reactivity in medicine, biotechnology, and materials science through plant-mediated synthesis.[14,15] Silver nanoparticles, known for their antimicrobial properties, have shown promise in diverse biological activities, including anticancer, antifungal, antiviral, antioxidant, and antidiabetic actions.[16,17] At lower concentrations, AgNPs display stability and catalytic activity, aiding processes like pollutant degradation. Their inherent therapeutic potential, with potent antimicrobial properties, holds promise in combating infections.[18] They also exhibit biocompatibility, encouraging applications in drug delivery, wound healing, and antimicrobial treatments without harming healthy cells.[19] Grape-derived bioactive compounds have diverse benefits, from skincare to addressing broader health concerns and providing protection against cataracts, platelet aggregation, and certain cancers.[20] This underscores grapes' significance as a source of health-promoting compounds for preventive and therapeutic applications.[21]

This research aims to synthesize silver nanoparticles (AgNPs) using *Vitis vinifera*, commonly known as Concord grapes, its seed extract which contains natural agents for the process. Various analyses, like UV-visible, FTIR, quasi-elastic light scattering, x-ray diffractometry, and SEM, were performed to characterize the AgNPs. Additionally, the study assessed their antimicrobial properties against bacteria and fungi, shedding light on their potential as antimicrobial agents.

EXPERIMENTAL

Materials and Methods

The study employed thorough cleaning and drying of glassware and utilized deionized water for solution preparation. High-quality silver nitrate from Sigma Aldrich was used for nanoparticle synthesis. UV-Vis on a Shimadzu UV-1700, FT-IR with a Perkin Elmer Nicolet 510P, SEM on a JEOL IT100LA, and dynamic light scattering on a Malvern Zetasizer NanoZS. Powder X-ray diffraction measurements utilized a Rigaku MiniFlex 600c. The study sourced *Vitis vinifera* seeds from Thiruvanaikovil, Tiruchirappalli, Tamilnadu, India, ensuring consistent starting material.

Vitis Vinifera Seed Extract

The *Vitis vinifera* seeds were collected and thoroughly washed with distilled water to eliminate any impurities. Then it was dried for a week and powdered into granules. About 10 grams of the powdered seed was taken in a 500 ml beaker containing 200 ml of de-ionized water. The mixture was boiled with stirring for 2 hours, cooled, then filtered, centrifuged, and stored.

Silver Nanoparticle Fabrication with *Vitis vinifera* Seed Extract

The procedure was initiated by introducing *Vitis vinifera* seed extract into a burette, followed by a controlled, dropwise addition to a 0.05N silver nitrate solution prepared using deionized water. Over the course of an hour, the mixture underwent agitation facilitated by a magnetic stirrer. The incorporation of NaOH played an additional role in stabilizing the synthesized silver nanoparticles, and managing electrostatic repulsion between them. This, in turn, prevented agglomeration and maintained nanoparticle dispersion within the solution. The change in color to a deep brown specified the silver nanoparticle formation (Fig.-1). The colloidal solution was centrifuged at 3,000 rpm for 10 minutes and then dispersed in deionized water. The varied phytochemicals in the seed extract facilitated the conversion of silver ions (Ag^{+}) into silver metal (Ag^{0}) nanoparticles. Remarkably, this method adhered to a hazard-free, fresh, harmless, and naturally sustainable approach.

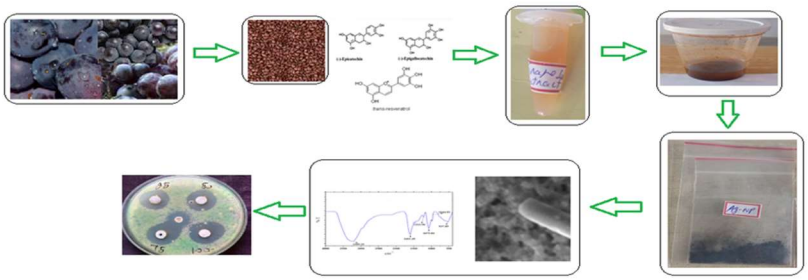

Fig.-1: Scheme for Production of Silver Nanoparticles from *Vitis vinifera* Seed Extract

Analysis of an Aqueous *Vitis vinifera* Extract by GCMS

The principal phytochemical constituents identified through mass spectrometry encompass a range of compounds. The chemical components include Dasycarpidan-1-methanol acetate (ester), Pseudosolasodinediacetate, 5a-Pregn-16-en-20-one, 3a, 12a-dihydroxy-diacetate, Cyclohexanecarboxamide, Oxacycloheptadec-8-en-2-one, 6-Methyl-2, (4-bromophenyl)-7-phenylmethylindolizine, and Triamcinolone acetonide.[22] Furthermore, in the context of black grape seeds, exposure led to the presence of various compounds including flavonol glycosides, resveratrol, and anthocyanidins[23,24] This indicates the diverse range of compounds that can be found in black grape seeds, contributing to their potential health benefits and applications. Various phenolic compounds like ferulic

acid, fertaric acid, catechin, epicatechin, etc., reported in the seed are recognized for their antioxidant and health-promoting properties, and their presence in grape seed extract underscores its potential as a valuable source of bioactive compounds[25] shown in Fig.-2.

Fig.-2: Structure of Few Chemical Components in Grape Seed Extract[25]

RESULTS AND DISCUSSION

UV–Vis Spectroscopy

The UV peaks at 398.80 nm and 416.24 nm are characteristic of the synthesized AgNPs, and the values "0.344 AU" and "0.360 AU" represent the absorbance intensity of the peaks, which can be related to the concentration or size distribution of the nanoparticles in the sample (Fig.-3 and 4).

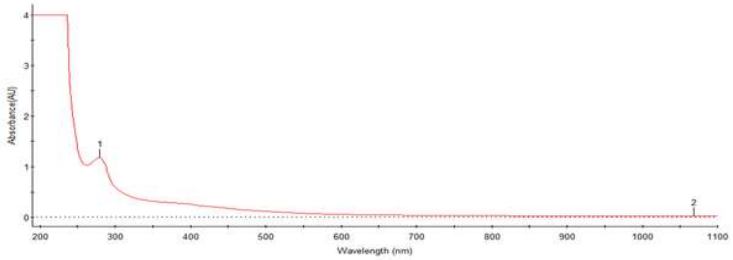

Fig.-3: UV absorbance spectrum for Grape Seed Extract

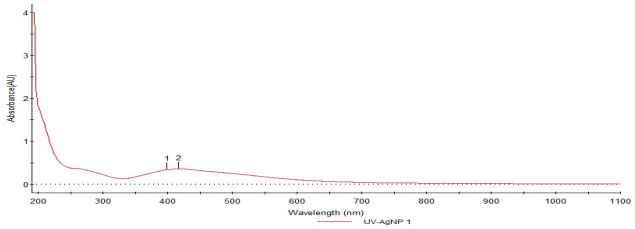

Fig.-4: UV Absorbance Spectrum for Silver Nanoparticles from Grape Seed Extract

FTIR Assay

The FTIR spectrum of AgNPs synthesized using grape seed extracts exhibits several distinctive peaks that shed light on their composition and characteristics (Fig.-5). The peak at 3424.76 cm^{-1} signifies the hydroxyl groups (-OH), potentially originating from the grape seed extract, which often contains phenolic compounds. Peaks at 2924.65 cm^{-1} and 2855.23 cm^{-1} are indicative of aliphatic C-H stretching vibrations, suggesting the involvement of organic molecules from the grape seed extract. The existence of a peak at 1638.74 cm^{-1} is a strong indicator of carbonyl groups (C=O), likely arising from ketones or aldehydes, highlighting the role of these functional groups in the stabilization of the AgNPs. The peak at 1399.60 cm^{-1} shows C-H bending vibrations, while 1121.33 cm^{-1} suggests the presence of alcoholic and ether C-O stretching vibrations. Additionally, the peaks at 1015.87 cm^{-1}, 838.99 cm^{-1}, and 689.98 cm^{-1} are indicative of aromatic C-H bending vibrations, pointing to the presence of aromatic compounds in the synthesized AgNPs.

XRD Assay

The *Vitis vinifera* seed extract was subjected to XRD and the structure pattern is shown (Fig.-6). The planes (111), (200), (220), (311), (222), and (400) to the diffraction peaks at 2Ɵ of 35.6126, 38.3150, 39.3117,

40.3030, 43.7116 and 44.3581, designated the face-centered cubic (fcc) structure as reported in the reference (JCPDS: file No. 96-901-1608).

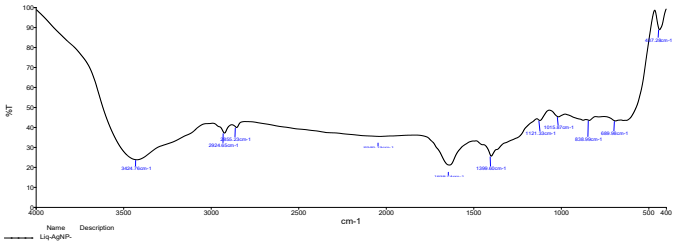

Fig.-5: Fourier Transform Infra-Red Spectrum of AgNPs

Estimation of the size of crystalline domains was calculated by Scherrer's Equation:

$$D = K\lambda/\beta \cos \Theta$$

Where D is the crystallite size, k is the shape factor, which is assumed to be 0.89 for silver, X is the X-ray wavelength (1.5406 A), is the half-height width of the XRD peak, and Θ is the diffraction angle.

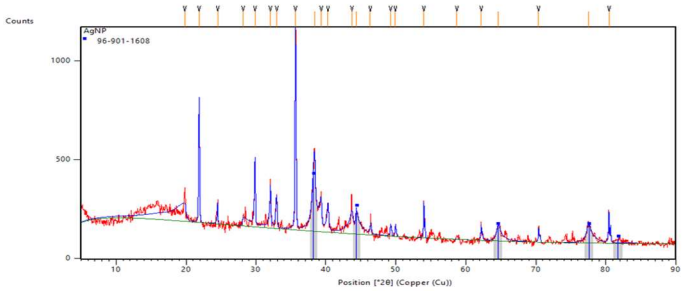

Fig.-6: X-Ray Diffraction Spectrum of Powder Silver NP

The AgNPs have an estimated average size of 35.99 nm. The size, crystalline nature, shape, and stable properties of the AgNPs are enhanced due to the biomolecules. These biomolecules, both large and small, convert silver ions (Ag^+) to neutral silver atoms (Ag^0), forming nanosilver particles. This approach is environmentally friendly and holds promise for creating AgNP colloidal dispersions.[26]

Structural Characteristics of the Silver Nanoparticles (AgNPs)

Figures-7 and 8 present SEM images, capturing surface features and revealing the magnitude of silver NPs produced with the specific concentration of grape seed extract. Figure-8 shows rod-like shapes, confirming successful AgNP synthesis. This results from crystal growth mechanisms, anisotropic growth, capping agents, reaction conditions, kinetics, thermodynamics, and potential bio-templating. These factors guide preferential growth along crystallographic directions, yielding elongated structures.[27]

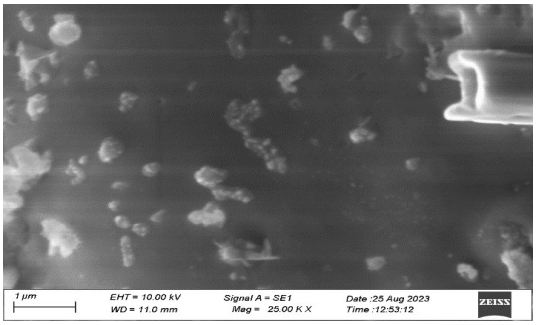

Fig.-7: Image of the AgNP at the Scale 1 Micrometer

Size Distribution Profile of Silver NPs

Quasi-electric scattering analysis was used to regulate the hydrodynamic size and aggregation state of the produced AgNPs. The average hydrodynamic size of the AgNPs was measured to be 195.4 nm (Fig.-9).

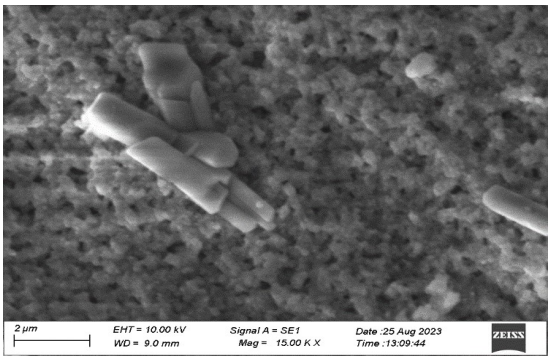

Fig.-8: Image of the AgNP at the Scale 2 Micrometer

The Polydispersity Index is 0.346, indicating a moderately varied size distribution with some AgNPs being smaller or larger than the average size. The hydrodynamic size obtained through DLS is influenced by the Brownian motion of the AgNPs in the solution. The motion of the nanoparticles, along with the motion of solvent molecules forming a hydrodynamic sphere around the particles, contributes to the overall observed fluctuations in scattered light intensity. The incorporation of the hydrodynamic sphere in the measurement allows for the consideration of the solvent layer's influence on the particle's movement. This method provides valuable insights into the behavior of nanoparticles in solution, taking into account their hydrodynamic properties.[28]

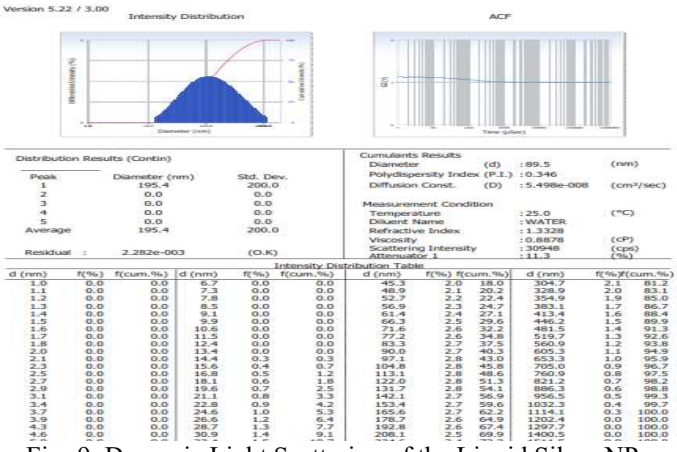

Fig.-9: Dynamic Light Scattering of the Liquid Silver NPs

Antioxidants Evaluation

Reactive oxygen species (ROS), unstable oxygen-containing molecules, readily interact with cellular components, resulting in cell death and contributing to oxidative stress. This stress is linked to disorders such as inflammation, cancer, aging, and neurodegenerative conditions. The DPPH free radical scavenging experiment was used in this investigation to assess the Vv-Ag_2ONPs' in vitro antioxidant properties (Fig.-10). Vv-SE and Vv-Ag_2ONPs demonstrated notable inhibition, reaching 67.47% and 82.63% against DPPH radicals at 200 mg/ml. Vv-Ag_2ONPs exhibited higher scavenging activity than the standard ascorbic acid, highlighting their potent antioxidant properties.[29, 30]

Antibacterial Activity

The agar well diffusion method was employed to assess the antimicrobial nature of AgNPs. Different microorganisms were evenly swabbed onto agar plates, and wells with a 6 mm diameter were generated. Twenty microliters of silver nanoparticle solutions at varying concentrations (0.25, 0.50, 1.0, and 2.0 mM) were introduced into the respective wells. To assess the combined impact of silver nanoparticles and Gentamicin, a mixture containing 15 ml of 0.5 mM Gentamicin and 15 ml of 0.5 mM silver nanoparticles solution was placed in the designated well on agar plates inoculated with the target microorganisms. After a day of incubation at 37°C, the inhibition diameter size was determined and related to the effects of Gentamicin and silver nanoparticles individually.

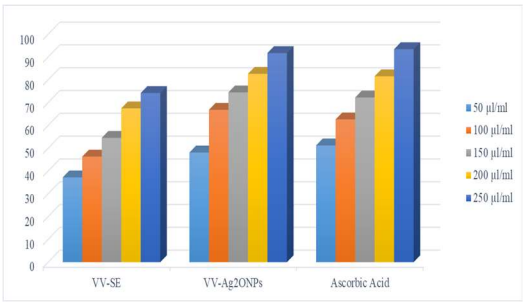

Fig.-10: Antioxidant Activity of the Grape Seed Extract, Silver Nps, and Ascorbic Acid

AgNPs exhibit a strong attraction to bacterial cell surfaces, particularly Gram-negative types. AgNPs are highly attracted to bacterial cell surfaces, particularly Gram-negative ones, and can penetrate these cells more effectively due to their thinner cell walls and electrostatic interactions. AgNPs interact with cell membranes, altering composition and permeability, disrupting proton motive force, and causing degradation. Studies using plant extracts confirm AgNPs' physical interaction with bacterial surfaces, impacting functions like adhesion, penetration, organelle disruption, and oxidative stress.[31,32]

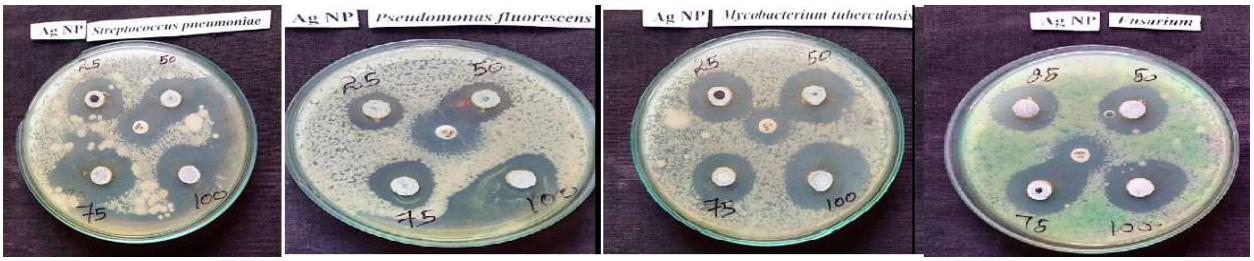

Fig.-11: Microbial Inhibition Zones of the Green Synthesised AgNPs and Gentamicin

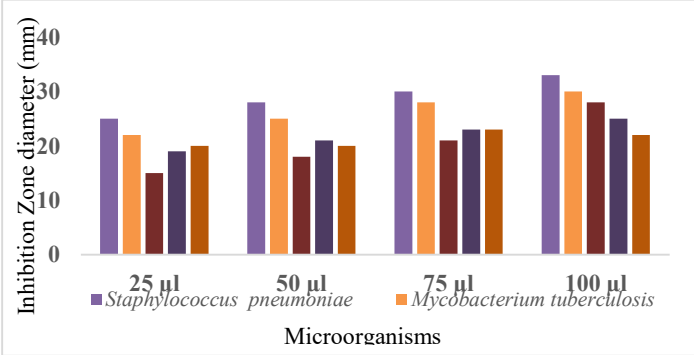

Fig.-12: Concentration-Dependent Changes in Inhibition Zones

When tested against the bacterium *Staphylococcus pneumoniae*, AgNPs demonstrated a substantial zone of inhibition measuring 33 mm/ml surpassing the control, which was treated with Gentamicin and had a zone of 20 mm/ml, underscoring their robust antimicrobial activity (Fig.-11 and 12). This potency can be attributed to the nanoscale dimensions of AgNPs, which provide an expanded surface area for interactions with bacterial cells, as well as the release of silver ions (Ag^{+}) that can disrupt bacterial cell membranes.[33] Similarly, in the context of *Mycobacterium tuberculosis*, AgNPs displayed effective antimicrobial activity, resulting in a 30 mm/ml zone of inhibition outperforming the control Gentamicin treatment, which had a zone of 20 mm/ml. The nanoscale size of AgNPs, along with the release of silver ions, contributes to their ability to hinder the growth of this pathogenic bacterium. Furthermore, against the bacterium *Pseudomonas fluorescens*, AgNPs exhibited strong antimicrobial activity, evident from the 28 mm/ml zone of inhibition. The nanoscale size of AgNPs and the release of silver ions were pivotal in their effectiveness against this bacterium. Lastly, in the case of the fungus *Fusarium*, AgNPs demonstrated antimicrobial activity, generating a zone of inhibition measuring 25 mm/ml slightly higher than the control's 22 mm/ml treated with Gentamicin. The nanoscale size and the release of silver ions were key factors in their capacity to

inhibit the growth of this fungus. The nanoscale size, coupled with the release of silver ions, contributes to their ability to disrupt the growth and functionality of these microorganisms, offering promising applications in antimicrobial strategies.

CONCLUSION

The exploration of synthesized nanoparticles for antibacterial applications has showcased their potential as effective agents against bacterial infections. Their ability to breach bacterial membranes with minimal adverse effects holds promise for revolutionizing infection control strategies. The surge in nanomaterial applications across diverse industries underscores the transformative nature of nanotechnology, driven by the unique properties that emerge at the nanoscale. The adoption of green chemical technologies signifies a collective shift towards sustainable practices. By prioritizing renewable resources, efficient processes, and waste reduction, these approaches align with the urgent need for environmentally conscious solutions. Using *Vitis vinifera* seed extract in silver nanoparticle synthesis reflects sustainable practices. Characterization techniques like UV-Vis spectroscopy, FTIR, XRD, and DLS provide essential insights for tailoring nanoparticles to specific applications.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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