GREEN SYNTHESIS, CHARACTERIZATION, ANTIBACTERIAL, ANTIFUNGAL AND ANTICANCER ACTIVITY OF SILVER NANOPARTICLES FROM NIGELLA SATIVA SEED EXTRACT.

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Abstract

Nanotechnology has rapidly advanced in the 21st century, creating nanomaterials, especially silver nanoparticles (AgNPs), attracting a lot of interest because of their remarkable qualities and broad applications. This study investigates the use of black seed (Nigella sativa) extract in the synthesis of (AgNPs) through natural and heat-assisted methods. The antimicrobial efficacy of the synthesized AgNPs was tested against pathogens such as Escherichia coli, Streptococcus spp., Aspergillus n., and Candida auris, showing potent inhibitory effects with varying susceptibility. Characterization of the nanoparticles through UV spectrophotometry and HR- FESEM revealed their size, shape, and distribution, with heat-assisted synthesis producing nanoparticles with enhanced antimicrobial properties. The research suggests that black seed- synthesized AgNPs could be a sustainable, natural antimicrobial agent, particularly for combating drug-resistant pathogens. Additionally, an anti-cancer

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study using the MTT assay demonstrated a dose-dependent cytotoxic effect on the HepG2 cell line, highlighting potential biomedical applications. This study merges traditional knowledge with modern nanotechnology, opening the door for the development of eco-friendly antimicrobial drugs. Future studies should focus on optimizing synthesis methods and exploring broader microbial targets, along with investigating the mechanisms behind the nanoparticles' activity.

1 Introduction

Open nanotechnology refers to the design, synthesis, characterization, and use of materials and devices where at least one dimension is on the nanometre scale (one billionth of a meter). At these scales, understanding the behaviour of individual molecules is essential. By controlling molecular structure, the chemical and physical properties of materials or devices can be precisely enabled, facilitating advancements across various fields, including clinical applications.[1–3]

Nanomaterial's (NMs) are synthesized using two main approaches: top-down and bottom-up. In the top-down approach, larger structures are reduced into Nano scale forms, while the bottom-up approach builds nanostructures atom-by-atom or molecule-by-molecule. Nanomaterial's consist of three layers: the core, which is chemically distinct; the shell; and the surface layer, typically made of polymers or small molecules for stability. These structural layers allow for control over the size and functionality of the nanomaterial, supporting a wide range of applications. [4–6]

Nanotechnology has led to the development of materials like silver nanoparticles (AgNPs), which show promising antimicrobial properties. These nanoparticles are explored as alternatives to combat healthcare-associated infections and antibiotic resistance. Several methods for synthesizing AgNPs exist, with chemical reduction being the most widely used. However, challenges like colloidal instability arise due to AgNPs' tendency to cluster.

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Stabilizing agents are used to manage size, shape, and stability, as these characteristics significantly influence the antimicrobial activity of the nanoparticles.[7-10]

Graphene oxide (GO), a chemically oxidized form of graphene, has gained attention for its ability to disperse and stabilize silver nanoparticles (AgNPs). Due to its large surface area and abundant oxygenated functional groups, GO enhances AgNP stability and acts as a platform for their controlled growth, preventing agglomeration. The synthesis of GO-AgNP hybrids involves various methods and reducing agents such as hydroquinone, sodium borohydride ($NaBH_4$), and glucose, often combined with stabilizers like trisodium citrate or polyethylene mine to optimize the process.

GO-AgNP hybrids combine the unique properties of both materials, leading to enhanced antimicrobial activity, improved catalytic performance, and better thermal conductivity. These composites are being explored for diverse applications, including electronics, catalysis, drug delivery, and as antimicrobial agents. Their antimicrobial properties, in particular, show great promise in combating microbial infections, presenting an alternative to traditional antibiotics.

Moreover, graphene and AgNPs have been integrated into polymer matrices to create antimicrobial materials. Techniques like electro spinning are used to fabricate polymer nanofibers, which can be applied in biomedical devices and food packaging to reduce microbial contamination and prevent pathogen transmission. Antimicrobial resistance (AMR) is a growing concern, and we need new ways to fight it. Antimicrobial nanomaterials show promise, offering innovative ways to target resistant bacteria. These materials could be crucial in developing effective treatments to tackle AMR.. [10]

The MTT assay is a colorimetric test used to measure cell growth and toxicity. It works by converting a yellow dye (MTT) into purple formazan crystals through the activity of live cell enzymes. The intensity of the purple color, which can be measured at 570nm, reflects the number of viable cells. The more cells present, the stronger the color. (Alley, M. C et al., 1986, Mossman et al., 1983).

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2. Experimental Procedure

2.1 Sample preparation

2.1.1 Black seed solution

To prepare black seed extract, add 5 grams of dried black seeds (Nigella sativa) to 100 mL of distilled water and let it steep to extract bioactive compounds like thymoquinone. After steeping, filter the mixture to remove seed particles, then gently heat the filtrate to concentrate the extract without overheating. The concentrated extract can be used for nanoparticle synthesis or medicinal purposes.

To prepare a silver nitrate solution, weigh 0.691 grams of silver nitrate ($AgNO_3$) and add it to a 100 mL beaker. Slowly add 100 mL of distilled water and stir until fully dissolved. The resulting clear solution is ready for use in various experiments or nanoparticle synthesis.

1.1

1.1.1 Silver Nitrate solution

To prepare a silver nitrate solution, weigh 0.691 grams of silver nitrate ($AgNO_3$) and add it to a 100 mL beaker. Slowly add 100 mL of distilled water and stir until fully dissolved. The resulting clear solution is ready for use in various experiments or nanoparticle synthesis.

1.2 Biosynthesis of silver nanoparticles

1.2.1 Natural synthesis

In this experiment, 100 ml of black seed solution was p To help create silver nanoparticles, specific processes are used to guide their formation mixed with 100 ml of silver nitrate solution to facilitate the synthesis of silver nanoparticles. The black seed solution acts as a reducing and stabilizing agent, while silver nitrate provides the silver ions necessary for nanoparticle formation. After thoroughly mixing the two solutions, the mixture was placed

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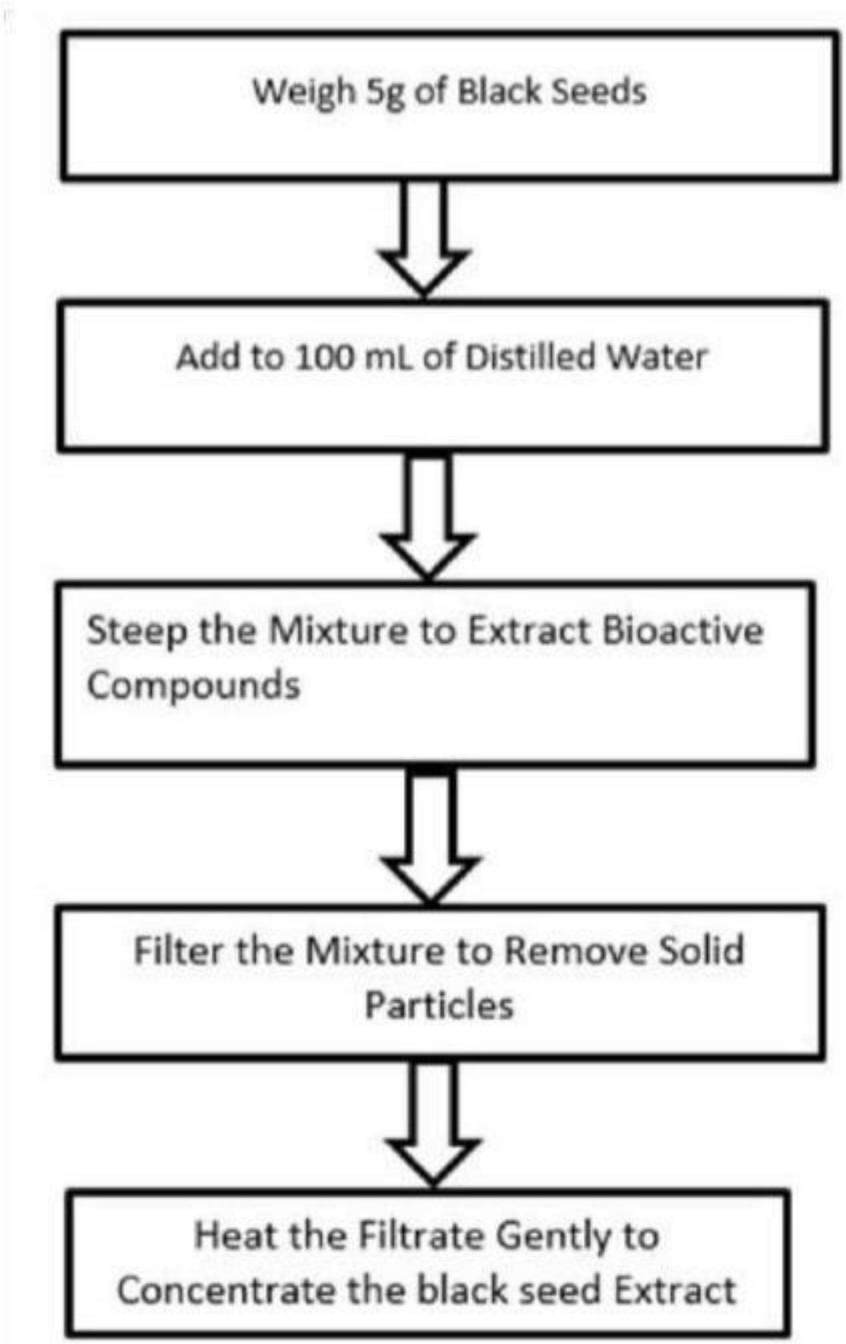

Figure 1: 1.1: Preparation of Black seed solution

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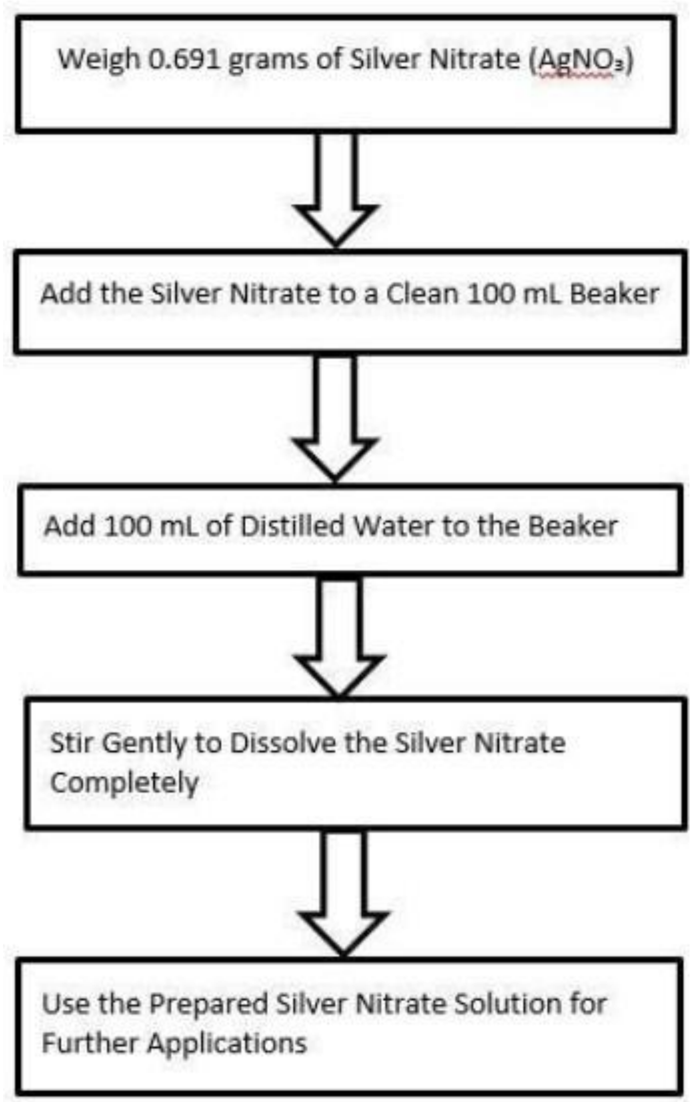

Figure 2: 2.1: Preparation of silver nitrate solution

in an incubator under controlled temperature conditions to ensure an optimal environment for the reaction. The incubation allows for the reduction of silver ions.

1.2.2 Heat assisted synthesis

In this procedure, 100 ml of black seed extract was combined with 100 ml of a pre synthesized silver nanoparticle solution to study their interaction or to functionalize the nanoparticles. The mixture was transferred to a reaction vessel and subjected to control heating at a specific temperature to enhance the interaction between the black seed components and

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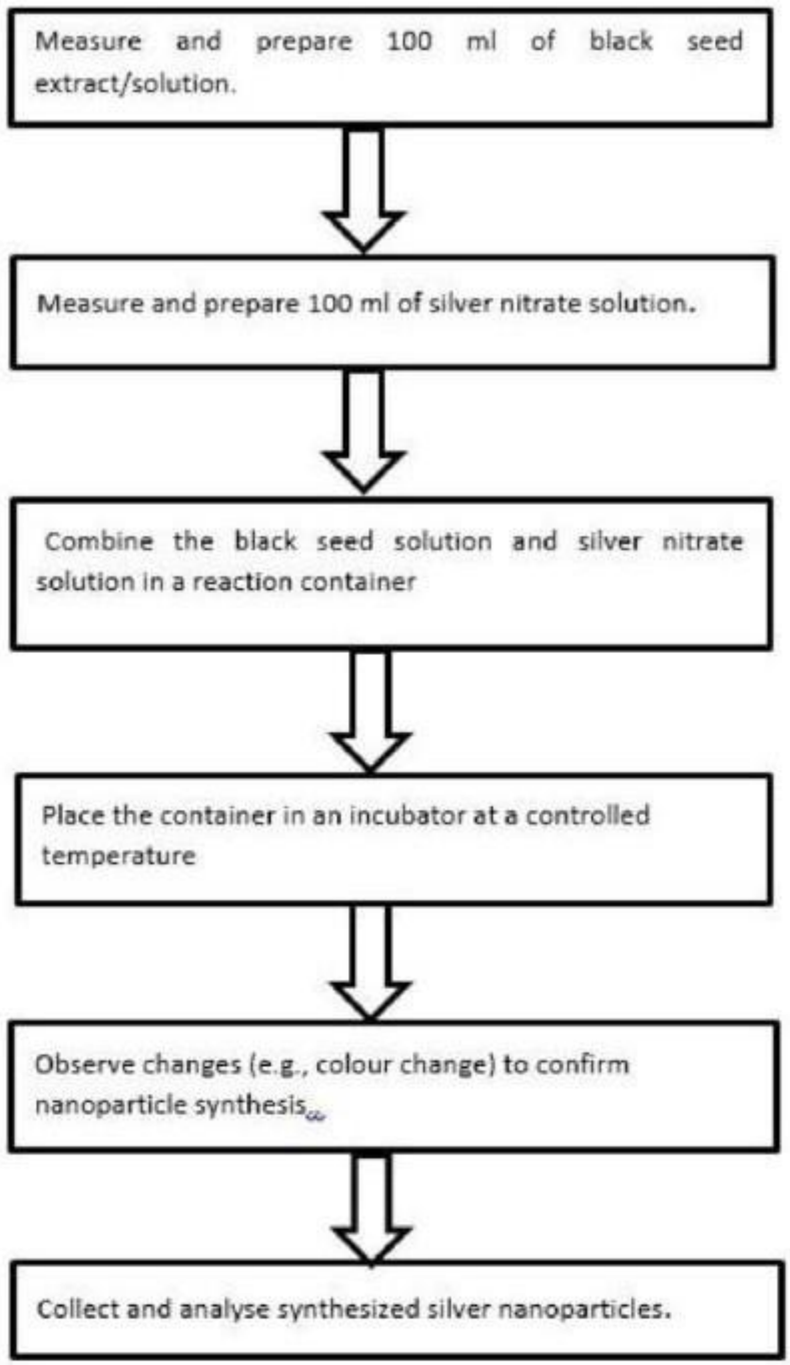

Figure 3: 1.1: Preparation by natural synthesis method

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the silver nanoparticles. The heating process facilitates bonding, stabilization, or coating of nanoparticles with bioactive compounds from the black seed extract. Throughout the process, the reaction is

monitored for any visible changes, such as color shifts or precipitation, indicating a successful reaction or modification of the nanoparticles.

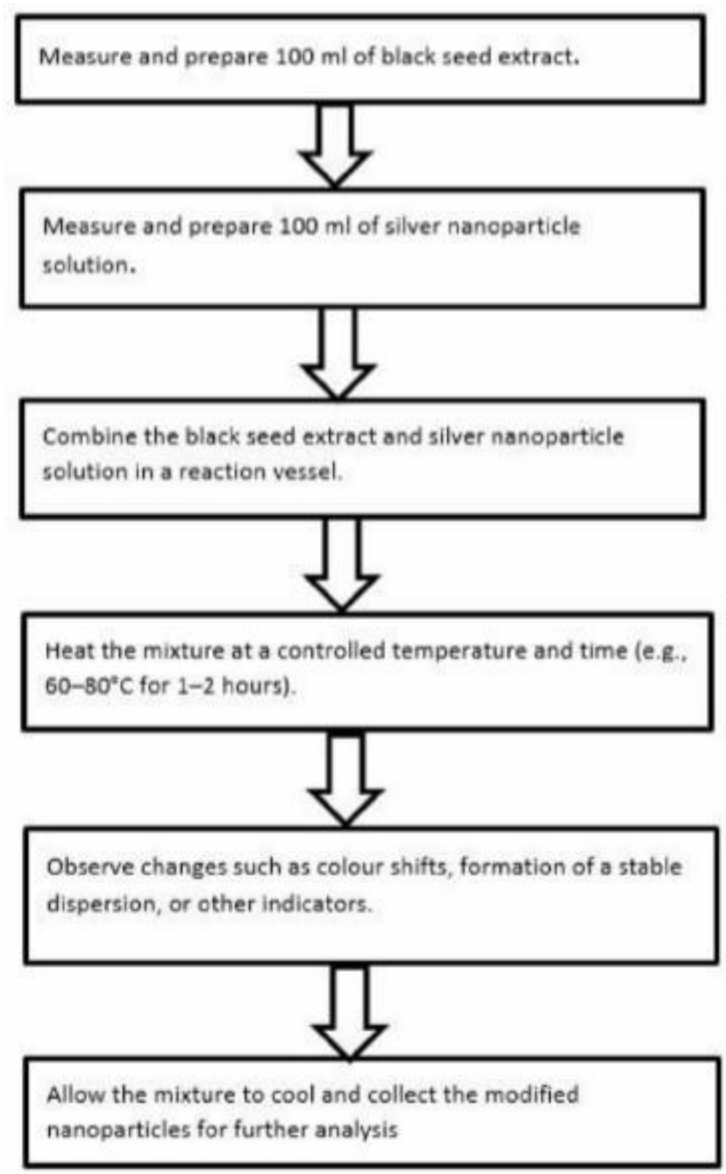

Figure 4: 1.2: Preparation of heat Assisted Synthesis

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1.2.3 Characterization

UV-Visible Spectrophotometer – Systronic UV-Vis spectrophotometry plays a crucial role in confirming the synthesis and characterizing the properties of silver nanoparticles (AgNPs), especially by observing the surface plasmon resonance (SPR) peak in the visible range, typically between 400 and 450 nm. This peak provides important information about the size, shape, uniformity, and stability of the nanoparticles. In this study, the natural synthesis of AgNPs using black seed (Nigella sativa) extract was analyzed through UV-Vis spectroscopy. The extract was prepared by boiling powdered black seeds in distilled water, with two versions: one filtered to remove solid particles and one unfiltered, which retained all components. Both extracts were mixed with

silver nitrate solution, leading to a visible color change that signified nanoparticle formation. UV-Vis spectra revealed distinct differences between the filtered and unfiltered samples. The filtered sample showed a sharp SPR peak around 420–430 nm, indicating a uniform nanoparticle size distribution and better stability. On the other hand, the unfiltered sample had a broader, less defined SPR peak, suggesting a more heterogeneous nanoparticle size distribution, likely influenced by the solid particles that affected nucleation and growth. This study demonstrates that while black seed extract is effective for eco-friendly AgNP synthesis, filtration plays a crucial role in optimizing nanoparticle quality, uniformity, and stability. The bioactive compounds in the extract, such as flavonoids and phenolic, also contribute to the reduction and stabilization of AgNPs, supporting green chemistry principle.

Scanning electron microscope (SEM - JEOL The working principle of the JEOL SEM involves directing a focused electron beam onto a sample, then detecting the signals generated from the interaction between the electrons and the sample. The resulting data provides detailed surface images and information about the sample composition, structure, and properties at the microscopic or nanoscopic level. This makes the JEOL SEM an essential tool in fields such as materials science, nanotechnology, biology, and semiconductor research,

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where high-resolution imaging and elemental analysis are critical.

Scanning Electron Microscopy (SEM) was utilized to further characterize the silver nanoparticles (AgNPs) synthesized from black seed (Nigella sativa) extract, offering valuable insights into their surface morphology, size, and structural properties. SEM is a sophisticated imaging technique that enables high-resolution visualization of nanoparticles, which is essential for assessing their potential applications, including antimicrobial, antifungal, and antioxidant activities.

In this study, AgNPs synthesized from both filtered and unfiltered black seed extracts were examined using SEM. The samples were prepared by drop-casting a small amount of the nanoparticle solution onto a conductive substrate and allowing it to dry, ensuring clear imaging and minimizing nanoparticle aggregation. SEM analysis revealed distinct differences between the two samples. Nanoparticles from the filtered extract exhibited a predominantly spherical shape with a uniform size distribution ranging from 10 to 50 nm. The absence of large agglomerates suggested that the bioactive compounds in the black seed extract effectively stabilized the nanoparticles.

Transmission Electron Microscopy (TEM Transmission Electron Microscopy (TEM) was employed to provide a detailed analysis of

the silver nanoparticles (AgNPs) synthesized from black seed (Nigella sativa) extracts, focusing on their size, shape, and structural characteristics at the manometer scale. TEM is a highly effective imaging technique that provides high-resolution insights into the morphology and crystallinity of nanoparticles, which are essential for assessing their biological and functional properties, especially in antimicrobial, antifungal, and antioxidant applications.

For the TEM analysis, AgNPs synthesized from both filtered and unfiltered extracts were prepared by drop-casting a diluted nanoparticle suspension onto a carbon-coated copper grid and allowing it to dry at room temperature. This preparation ensured a uniform distribution of nanoparticles, facilitating clear visualization. The TEM images revealed

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distinct differences between the two samples. The filtered extract produced nanoparticles with a consistent spherical shape and a narrow size distribution, typically ranging from 10 to 20 nm. These nanoparticles were well-dispersed, indicating effective stabilization by the bioactive compounds in the black seed extract, which acted as natural capping agents. The high resolution TEM images also revealed clear lattice fringes, confirming the crystalline nature of the nanoparticles, which is critical for their bioactivity.

1.3 Antimicrobial studies (Zone of Inhibition

The zone of inhibition is a circular area surrounding the antibiotic disk where bacterial Colonies are unable to grow. This zone serves as an indicator of the bacteria susceptibility to the antibiotic. The measurement of the diameter of the zone of inhibition can be streamlined and automated using image processing techniques. The zone of inhibition is a crucial metric in pre-diagnostic laboratory tests. Determining whether a specific antibiotic is effective against the bacteria present in a patient serves as a foundational step before initiating any treatment. The zone of inhibition, characterized by the absence of bacterial colonies, appears distinctly different in color compared to the surrounding areas of bacterial growth, making it easily noticeable to the naked eye. Measuring the diameter of this zone confirms the antibiotics effectiveness in treating the patient. A larger diameter indicates greater antibiotic potency [15- 20].

1.4 Anticancer study Assay controls:

In the experiment, three controls were used: (i) Medium control, which contained only the medium without cells, (ii) Negative control, which had cells but no experimental drug or compound, and (iii) Positive controls, which included cells and approximately 7.99 μg/ml of Doxorubicin. It's important to note that extracellular reducing components like ascorbic acid, cholesterol, alpha-tocopherol, and dithiothreitol in the culture media can reduce MTT to formazan. To account for this, the same medium must be used in both control and test

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wells.

The procedure begins by seeding 200 µl of cell suspension in a 96-well plate at the required cell density, without the test agent, and allowing the cells to grow overnight. After this, appropriate concentrations of the test agent are added, and the plate is incubated for 48 hours at 37°C in a 5% CO_2 atmosphere. Following incubation, the spent media is removed for adherent cell lines, and MTT reagent is added to a final concentration of 0.5 mg/mL. The plate is then wrapped in aluminum foil to avoid light exposure and returned to the incubator for another 3-hour incubation (note: the incubation time may vary for different cell lines but should remain constant within an experiment for accurate comparison).

Next, 100 µl of DMSO is added, and gentle stirring using a gyratory shaker helps dissolve the formazan crystals. For dense cultures, pipetting up and down may be necessary to fully dissolve the crystals. Finally, the absorbance is measured at 570 nm using a spectrophotometer or ELISA reader. The IC50 value is calculated using a logarithmic equation, with Y = 50 and M and C values derived from the viability graph.

2 RESULTS AND DISCUSSION

2.1 Results from UV spectroscopy

On Day 3, the solution of black seed extract and silver nitrate was again placed in the shaker, which ensured consistent mixing and enhanced the interaction between the reactants. The shaker continuous agitation promotes the uniform reduction of silver ions, aiding in the formation of silver nanoparticles. The solution underwent a noticeable color change, deepening from pale yellow to a darker brown, signaling the successful synthesis of the nanoparticles. UV-Vis spectroscopy was subsequently employed to assess the optical properties of the

nanoparticles, revealing distinct absorbance peaks that provided valuable information about their size and surface Plasmon resonance behavior.

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1: UV- Spectroscopy readings taken on day 3

WAVLENGTH (nm)	OD (Absorbance)
360	1.148
370	0.912
380	0.71
390	0.583
400	0.625
410	1.088
420	2.258
430	0.813
440	0.716
450	0.64
460	0.555
470	0.484

Figure 5:

The X-axis, ranging from 360 to 500, likely represents a variable such as wavelength, temperature, or time, while the Y-axis, ranging from 0 to 2.5, measures the response, such as absorbance, intensity, or concentration. The graph shows an initial response of 1.148 at 360, which decreases to a minimum of 0.589 around 390. A gradual rise follows, reaching a sharp peak at 420 with a maximum value of 2.258. Beyond this point, the response declines significantly to 0.813 at 430 and continues decreasing steadily, stabilizing at lower values around 0.267 by 500. The sharp peak at 420 indicates optimal conditions for the measured response, possibly due to enhanced reaction rates or efficiency under heat assisted conditions. The decline and stabilization after the peak suggest reduced efficiency or a baseline state. A

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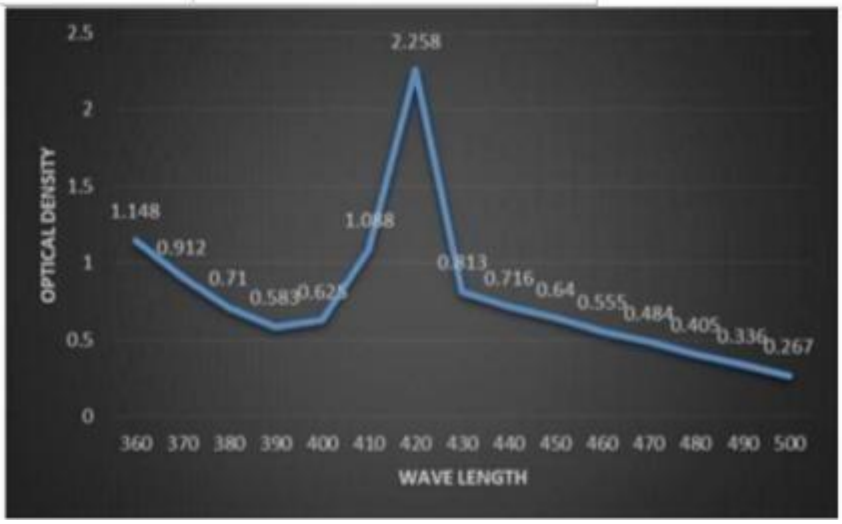

Figure 6: 2: Graphical representation of UV-Spectroscopy readings of day 3

study has proved the UV-visible spectroscopy analysis demonstrated plasmon the presence of a surface resonance (SPR) peak in the 417–430 nm range. This peak is characteristic of silver nanoparticles (AgNPs) formation [66]. The observation confirms the successful synthesis of AgNPs. These results highlight the effectiveness of the green synthesis approach this trend highlights the critical role of heat in influencing the system's behavior, with 420 being the optimal condition for maximum response.

2.2 Results from Scanning Electron Microscope

The SEM analysis of t Electron Microscope he synthesized silver nanoparticles revealed a size range of 30–70 (AgNPs) using black seed extract nm with predominantly spherical shapes and uniform distribution. These findings align with earlier studies while exhibiting slight variations due to differences in synthesis methods and biological reducing agents. The size and shape of

the silver nanoparticles synthesized in this study fall within the typical range reported in the literature but are influenced by the unique properties of black seed extract. The uniform distribution and spherical morphology suggest effective capping and stabilization, making these nanoparticles promising candidates for various biomedical and antimicrobial

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applications.

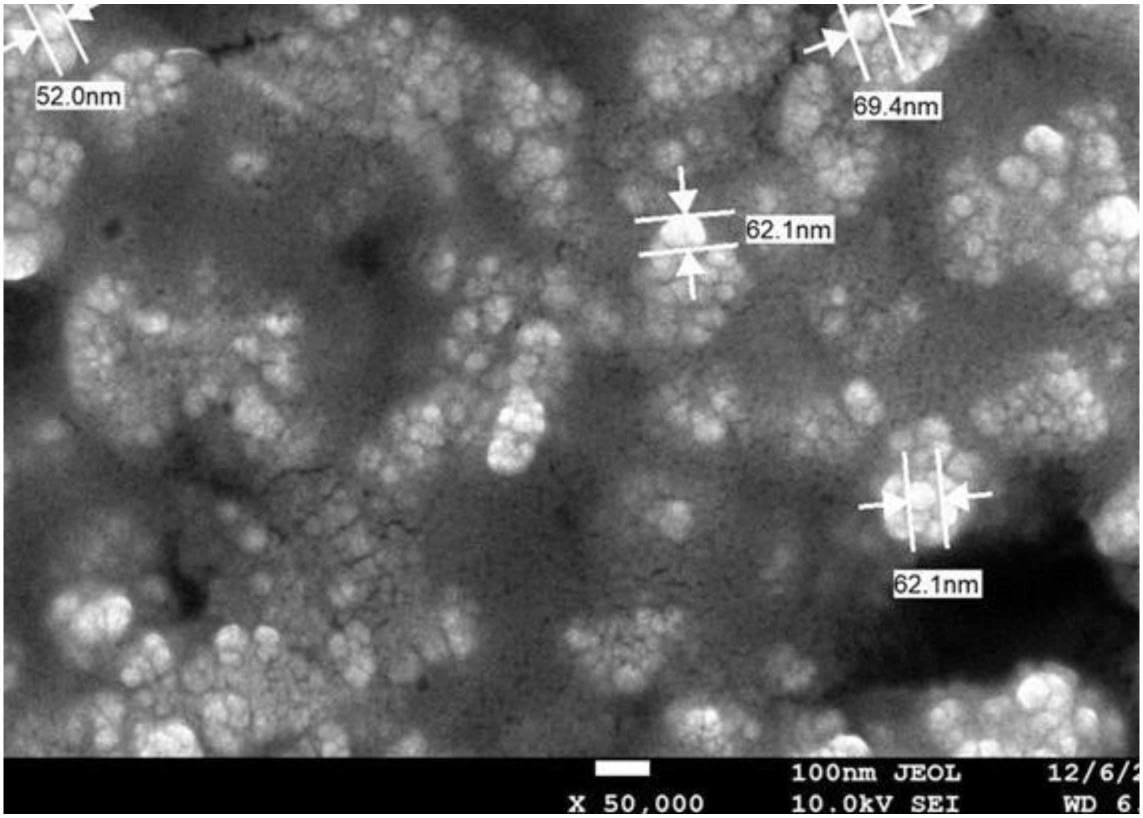

Figure 7: 1: SEM image clearly showing the presence silver nanoparticles of range 50- 70nm

2.3 Results from TEM

The Transmission Electron Microscope (TEM) analysis reveals that the silver nanoparticles synthesized in this study are predominantly spherical in shape, with particle sizes ranging between 40 and 70 nm. This morphology is typical of well-formed silver nanoparticles and aligns with findings from various studies in the literature. The consistent shape and size of the nanoparticles suggest a successful synthesis process, which is important for their effectiveness in applications like antimicrobial and anticancer research. TEM imaging plays a crucial role in confirming the nanoparticles' Nano scale dimensions, providing detailed information about their structural characteristics.

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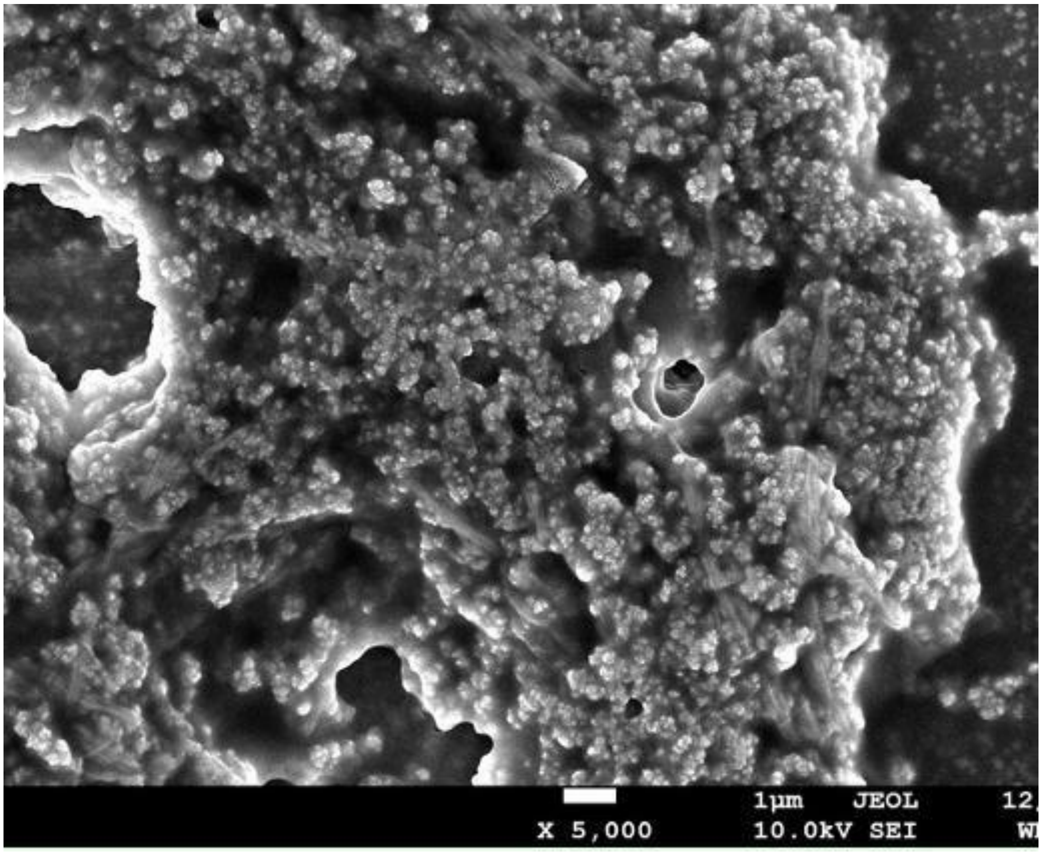

Figure 8: 2: SEM image of silver nanoparticle at 1micrometer

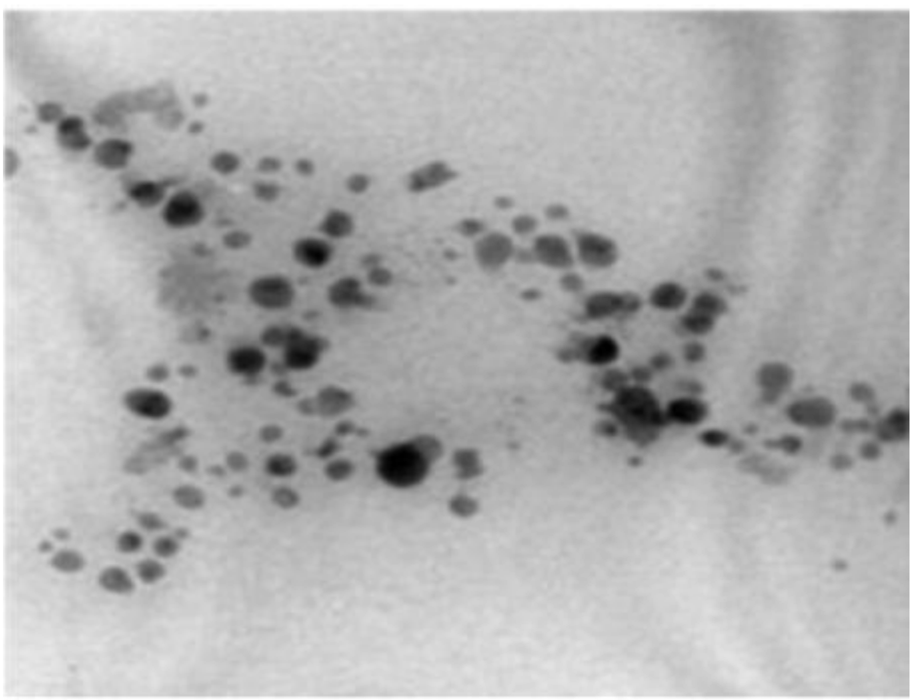

Figure 9: 1 : TEM image showing presence of spherical silver nanoparticle

2.4 Results from Zone of Inhibition tests

In this study, silver nanoparticles were biosynthesized using Nigella sativa (commonly known as black seed) extract, and their antimicrobial properties were tested against four microorganisms: Candida auris, Aspergillus niger, Escherichia coli, and Streptococcus sp. To determine the zone of inhibition for each microorganism, providing insights into the nanoparticles' effectiveness in combating both bacterial and fungal pathogens.

Figure 10: 1: Zone of inhibition test for Candida auris

For Candida auris, the zones of inhibition on potato dextrose agar (PDA) plates were clearly visible. This demonstrated the nanoparticles' antifungal activity against this multidrug-resistant yeast. Given the clinical challenge posed by Candida auris, which is known to resist conventional antifungal treatments, the observed inhibition highlights the potential of silver nanoparticles as alternative antifungal agents. The nanoparticles likely disrupted the fungal cell membrane or interfered with cellular processes, leading to the observed inhibition.

The antifungal activity was further confirmed against Aspergillus niger, where distinct zones of inhibition were also present on PDA plates. The biosynthesized nanoparticles

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Figure 11: 2: Zone of inhibition test for Aspergillus niger

appeared to be effective in halting the growth of this mold, suggesting their utility in preventing or treating fungal infections caused by filamentous fungi. The inhibitory effect is likely attributable to the nanoparticles' ability to generate reactive oxygen Species (ROS) and interfere with fungal metabolic pathways.

For bacterial pathogens, the nanoparticles showed strong antibacterial activity against Escherichia coli. On nutrient agar (NA) plates, clear inhibition zones were observed, indicating the nanoparticles' efficacy in targeting gram-negative bacteria. The mechanism of action may involve interaction with the bacterial cell wall and membrane, leading to increased

permeability, leakage of intracellular contents, and eventual cell death. This finding is particularly promising as gram-negative bacteria are often harder to treat due to their outer

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membrane, which acts as a barrier to many conventional antibiotics.

Figure 12: 3: Zone of inhibition test for Escherichia coli

Unlike the positive results observed for other tested microorganisms, no zone of inhibition was detected around the wells containing the nanoparticles. This indicates that the silver nanoparticles did not exhibit antibacterial activity against Streptococcus sp. under the experimental conditions. The lack of inhibition may be attributed to several factors such as environmental factors may also have played a role. Variations in the pH, temperature, or nutrient composition of the agar medium could influence the efficacy of the nanoparticles. For instance, nutrient-rich conditions might enhance bacterial resilience, reducing the nanoparticles' impact This result emphasizes the importance of exploring further how biosynthesized silver nanoparticles interact with gram-positive bacteria, such as Streptococcus sp.

These results suggest that the silver nanoparticles synthesized from natural sources have some degree of antimicrobial activity.

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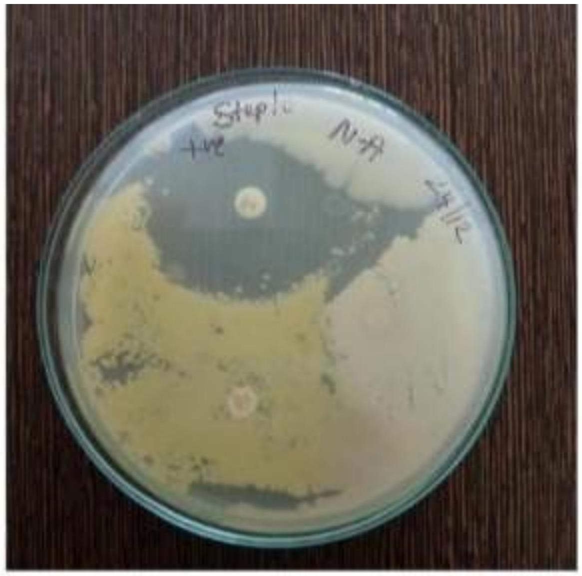

Figure 13: 3: Zone of inhibition test for Streptococcus sp.

2.5 Anticancer study

In this study, given test compound is evaluated to analyse the cytotoxicity effect onHepG2 cell line. The concentrations of the test compound used to treat the cells are as follows:

1 : Details of test compound concentrations

1 : Mean %cell viability of HepG2 cell line after exposing to test compound

3 CONCLUSION

The study successfully produced silver nanoparticles, demonstrating the effective synthesis of these particles for further analysis and potential applications (Nigella sativa) as a green and sustainable method, highlighting the growing potential of plant- based synthesis in nanotechnology. This approach avoids the use of hazardous chemicals, aligning with the principles of green chemistry while ensuring cost-effectiveness and environmental compatibility. The synthesized silver nanoparticles were first analyzed to understand their properties and characteristics. UV spectrophotometry, which confirmed their formation,

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Antibiotics	Gram positive *Streptococcus sp* (zone in mm)	Gram negative *Escherichia coli* (zone in mm)	Fungal organism *Candida auris*	Fungal organism *Aspergillus niger* (zone in mm)
			(zone in mm)	
Amphilicin	No clear zone	No clear zone	No clear zone	No clear zone
Kanamycin	33	35	25	23
Synthesized silver nanoparticles	No clear zone	30	10	20

Figure 14:

followed by high-resolution field emission scanning electron microscopy (HR-FESEM), which provided detailed morphological insights. These analyses revealed that the nanoparticles were uniformly distributed, with a size range of 50-70 nm, and displayed a consistent spherical morphology. Such uniformity and spherical nature are considered ideal in nanotechnology, as they significantly enhance the surface-to-volume ratio a crucial factor influencing the efficiency and reactivity of nanoparticles in various applications.

The nanoparticles demonstrated remarkable antimicrobial properties, effectively inhibiting the growth of gram-positive and gram-negative bacteria, as well as fungi. This

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SL. No	Test Compounds	Cell Line	Concentration treated to cells
1	Untreated	HepG2	No treatment
2	Standard	HepG2	Doxorubicin - ~ 7.99 µg/mL
3	Blank	HepG2	Only Media without cells
4	Silver nanoparticle	HepG2	5 (10, 20, 30, 40 and 50 µg/ml)

Figure 15:

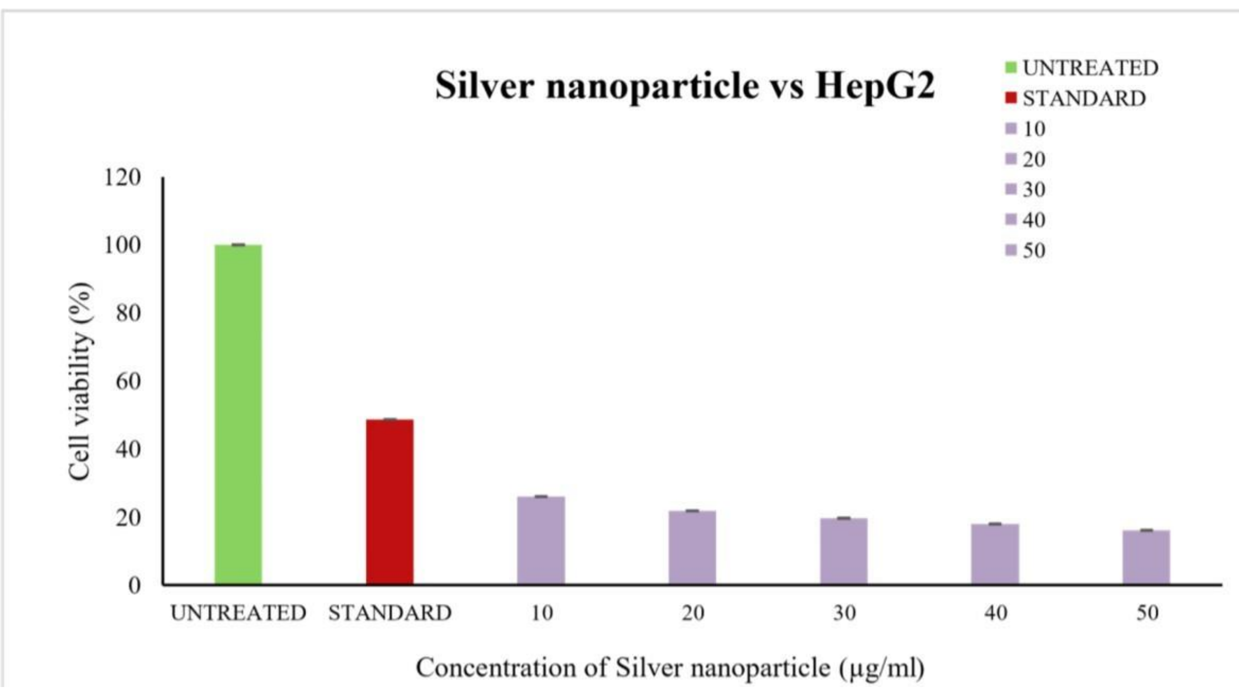

Figure 16:

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broad spectrum antimicrobial activity underscores their potential in combating microbial resistance, which is a pressing global health concern. The ability of silver nanoparticles to disrupt bacterial and fungal cell membranes and interfere with their metabolic processes makes them promising candidates for applications in medicine, food preservation, and agricultural sectors. These findings also suggest their potential to be developed into effective alternatives to conventional antibiotics, particularly in the era of rising antimicrobial resistance.

Furthermore, the uniform distribution and nanoscale properties of these particles open up possibilities for their utilization in diverse fields beyond antimicrobial applications, such as bio sensing, water purification, and drug delivery systems. The excellent properties of these nanoparticles, including stability and high reactivity, position them as multifunctional materials with versatile applications.

As an extension of this work, future studies will focus on exploring the anticancer potential of these silver nanoparticles. The high surface area and bioactive properties of silver

nanoparticles make them promising candidates for targeted cancer therapy. Studying their

interaction with cancer cells, mechanisms of apoptosis induction, and potential synergistic effects with existing therapies will help advance the field of Nano medicine. In this study, HepG2 cells were treated with silver nanoparticles at recommended concentrations, along with negative and positive controls, and incubated for 48 hours. The cytotoxicity of the test sample was assessed using an MTT assay. The results indicated a dose-dependent cytotoxic effect of the silver nanoparticles on the HepG2 cell line after 48 hours of incubation.

In conclusion, this research not only demonstrates the successful green synthesis of silver nanoparticles but also underscores their promising antimicrobial potential and paves the way for future studies on their applications in cancer treatment.. The combination of eco-friendly synthesis methods, excellent physicochemical properties, and broad-spectrum

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biological activities positions these nanoparticles as a valuable asset in various scientific and industrial fields. The study reinforces the need to further investigate and harness the potential of plant mediated nanoparticle synthesis for innovative solutions to global challenges in health and technology.

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