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Exploring the Antibacterial Efficacy of Silver Nanoparticles Synthesized through Abiotic Stress-Induced Germinated Seeds of *Vigna radiata*: A Comparative Analysis

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Abstract: The novel microwave-assisted green synthesis of silver nanoparticles (AgNPs) from stress-induced germinated seeds of *Vigna radiata* (VR) is explored in this research. AgNPs were successfully synthesized using abiotic stress-induced germinated seeds of VR, induced by salinity, drought, and heavy metals such as sodium chloride (NaCl), polyethylene glycol (PEG), and a chromium solution, respectively. The characterization of the synthesized AgNPs was performed using various techniques, including UV-visible spectrophotometer, dynamic light scattering (DLS), zeta potential, XRD, FT-IR, and FE-SEM. The concentration of AgNPs synthesized from Vr-NaCl, Vr-Cr, Vr-PEG, and Vr-DW followed the order Ag/Vr-DW > Ag/Vr-NaCl > Ag/Vr-PEG > Ag/Vr-Cr. Notably, the synthesized AgNPs exhibited significant antibacterial activity against *Staphylococcus aureus* bacteria. A comparative analysis of the antibacterial efficacy of AgNPs synthesized using different stress-induced VR seed extracts revealed that AgNPs from PEG stress-induced germinated seeds of VR displayed excellent antibacterial activity. These findings underscore the potential of stress-induced germinated seeds of VR as a promising resource for producing AgNPs with exceptional antibacterial properties, thereby opening avenues for the development of innovative antimicrobial agents.

Keywords: Silver nanoparticles, *Vigna radiate*, Microwave, Green synthesis, Stress-induced germination, Antibacterial.

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1. INTRODUCTION

Silver nanoparticles (AgNPs) have gained significant attention in recent years due to their unique physical and chemical properties, including excellent antibacterial activity against a broad range of microorganisms (1,2). The antibacterial properties of AgNPs have been widely utilized in various fields, including medicine, food packaging, water treatment, and cosmetics (3). The use of AgNPs in these fields has been facilitated by their small size, high surface area-to-volume ratio, and ability to penetrate bacterial cell walls and membranes, leading to the disruption of cell functions and eventual cell death (4,5).

For the production of AgNPs, a number of techniques have been devised, including chemical, physical, and biological methods (6,7). Chemical methods are widely used due to their simplicity and reproducibility (8). However, they frequently include the usage of hazardous substances, which can be harmful to both the environment and human health. Physical methods, such as laser ablation, have also been used for the synthesis of AgNPs. However, they are often expensive, time-consuming, and require specialized equipment (9). Biological methods, including plant extracts and microorganisms, have emerged as a green and sustainable approach for synthesizing AgNPs, avoiding the use of toxic chemicals and reducing the environmental impact of AgNP synthesis (10-12). Among various green synthesis strategies, microwave-assisted synthesis offers a rapid and efficient method for producing AgNPs with precise control over their size and morphology (11,12). The use of microwave irradiation allows for a faster reaction rate and reduced synthesis time compared to conventional methods, enabling high throughput production of AgNPs (15,16). Microwave-assisted synthesis also promotes uniform heating and improved particle size distribution, leading to enhanced stability and performance of AgNPs in various applications such as catalysis, sensing, and biomedical fields (17,18).

Using plant extracts to synthesize AgNPs has several advantages, including low cost, easy availability, and a wide range of phytochemicals that can act as reducing agents, capping agents, and stabilizers (19-22). Among various plant species, *Vigna radiata* (VR), also known as mung bean or green gram, has been widely used to synthesize AgNPs (23,24). Several studies have reported the synthesis of AgNPs using VR extracts, including stem, leaf, and seed extracts (23-26). Some studies showed the effect of AgNPs on the seed germination stages of VR (27,28). The phytochemicals present in VR are flavonoids, saponins, tannins, phytic acid, carotenoids, starch, proteins, fibre, and minerals (29). However, the use of stress-induced germinated seeds of VR for synthesizing AgNPs has not been explored in depth.

Stress-induced germination can occur through the activation of abiotic stress response pathways in the seeds, leading to changes in gene expression and metabolic activity (30-32). For example, exposure to high salinity levels can activate signalling pathways that regulate the release of seed dormancy and promote germination (33). Under stress conditions, the seeds release certain chemicals and hormones that initiate the growth of the embryonic axis and the shoot (34,35). Stress-induced germination process can enhance the synthesis of secondary metabolites, including phenolic compounds and flavonoids, which can act as reducing agents and stabilize the synthesized AgNPs (32). Therefore, stress-induced seed germination of VR can be a potential approach for enhancing the synthesis of AgNPs with improved antibacterial activity.

In this study, we aimed to explore the antibacterial efficacy of AgNPs synthesized from stress-induced germinated seeds of VR. The seeds were germinated in various extract media, including NaCl, PEG, distilled water, and chromium solution. The seed extract prepared from germinated seeds in distilled water was taken as the control. The AgNPs were synthesized using a green approach of microwave irradiation, which is a simple and efficient method for synthesizing AgNPs. The size, shape, and distribution of the AgNPs can be determined using various techniques such as field emission scanning electron microscopy (FE-SEM), UV-visible spectrophotometer, dynamic light scattering (DLS), zeta potential, and Fourier transform infrared spectroscopy (FT-IR). The antibacterial activity of the prepared AgNPs was evaluated against *Staphylococcus aureus (S. aureus)*. A comparative analysis of the antibacterial efficacy of AgNPs synthesized using different seed extracts was also performed.

The environmental significance of the present work lies in the utilization of stress-induced germinated seeds of VR for the synthesis of AgNPs through a green approach (36). This approach offers the following environmental benefits: (a) Sustainable resource utilization: VR seeds are commonly available and easily cultivated. By utilizing germinated seeds under stressful conditions, the research taps into a readily accessible and renewable resource. This reduces the reliance on scarce or non-renewable materials for AgNPs synthesis, making it more sustainable (9). (b) Eco-friendly synthesis: The green approach of microwave irradiation and the use of plant extracts as the reaction medium reduce the dependence on hazardous chemicals and solvents typically employed in conventional nanoparticle synthesis methods. This eco-friendly process minimizes the generation of toxic by-products and waste, contributing to the reduction of environmental pollution (37). (c) Reduced energy consumption: microwave irradiation is a rapid and energy-efficient method for AgNPs synthesis. Compared to traditional heating methods, it requires less time and energy to complete the reaction (38). This energy-saving aspect aligns with efforts to reduce the carbon footprint associated with scientific research and industrial processes. (d) Potential alternative to conventional antimicrobial agents: The synthesized AgNPs demonstrated significant antibacterial activity against *Staphylococcus aureus* bacteria (39). If further developed and optimized, these AgNPs derived from VR seeds could offer an environmentally friendly alternative to conventional antimicrobial agents. This could potentially contribute to reducing the widespread use of chemical-based antibiotics and antimicrobials, which can have negative impacts on ecosystems and promote the development of antibiotic-resistant bacteria.

The results of this study can provide insights into the potential use of stress-induced germinated seeds of VR as a novel and eco-friendly approach for synthesizing AgNPs with enhanced antibacterial activity. The utilization of stress-induced germinated seeds of VR for AgNP synthesis presents an environmentally significant approach that aligns with the principles of sustainability, eco-friendliness, and reduced energy consumption. By exploring greener alternatives, this research contributes to the development of environmentally benign strategies for nanoparticle synthesis and antimicrobial applications, which can have positive implications for both human health and the environment.

2. EXPERIMENTAL

2.1. Materials

VR seeds were obtained from the local market in Kottayam, India. Analytical grade chemicals such as $AgNO_3$, NaCl, PEG 6000, and $K_2Cr_2O_7$ were purchased from Merck, India. All the solutions were prepared in double distilled water.

2.2. Stress-induced Seed Germination of VR

Stress-induced germination of VR refers to the process of inducing germination in the seeds of this plant species under stress conditions such as high salt, drought, temperature fluctuations, or exposure to chemicals. Here we opted for exposure to chemicals such as salt, heavy metal ion solution, and PEG, keeping distilled water as control. For the culturing of VR under different stress conditions, initially wash the VR seeds thoroughly with distilled water and dry them. Then place 50 seeds in four petri dishes and add 50 mL of stress-inducing 0.5 ppm solutions of NaCl, PEG, and chromate solution to three separate petri dishes and 50 mL of distilled water to the fourth petri dish. Wet a filter paper with distilled water and place it on top of the seeds in each petri dish. Close the petri dishes and incubate them in a dark place at room temperature for 48 hours. After 48 hours, carefully remove the filter paper using forceps and examine the germination rate of the seeds in each petri dish. Record the number of germinated seeds in each dish.

2.3. Extraction Procedure

Collect the stress-induced germinated seeds of VR in NaCl, PEG, distilled water, and chromium solution. Wash the seeds thoroughly with distilled water to remove any residual salt or chemicals. Dry the seeds separately in an oven at 50 °C. Place 6 g of dried seeds in RB flask with 50 mL of distilled water and reflux it for 30 minutes at 80 °C. The resultant extract in each case was filtered through Whatman 41 filter paper. The extracts obtained from NaCl, PEG, chromium solution, and distilled water are designated as Vr-NaCl, Vr-PEG, Vr-Cr, and Vr-DW, respectively.

2.4. Synthesis of Silver Nanoparticles

To reduce Ag (I) ions to Ag (0), the four extracts obtained from VR were used. To achieve this, 10 mL of the extract was mixed with 90 mL of a 1 mM aqueous silver nitrate solution, and the resulting mixture was exposed to microwave irradiation for 4 minutes in a domestic microwave oven [Sharp R219T (W)] operating at 800 W power and 2450 MHz frequency. The formation of AgNPs was monitored at 30-second intervals using a UV-vis. spectrophotometer. The AgNPs obtained from Vr-NaCl, Vr-PEG, Vr-Cr, and Vr-DW were designated as Ag/Vr-NaCl, Ag/Vr-PEG, Ag/Vr-Cr, and Ag/Vr-DW, respectively.

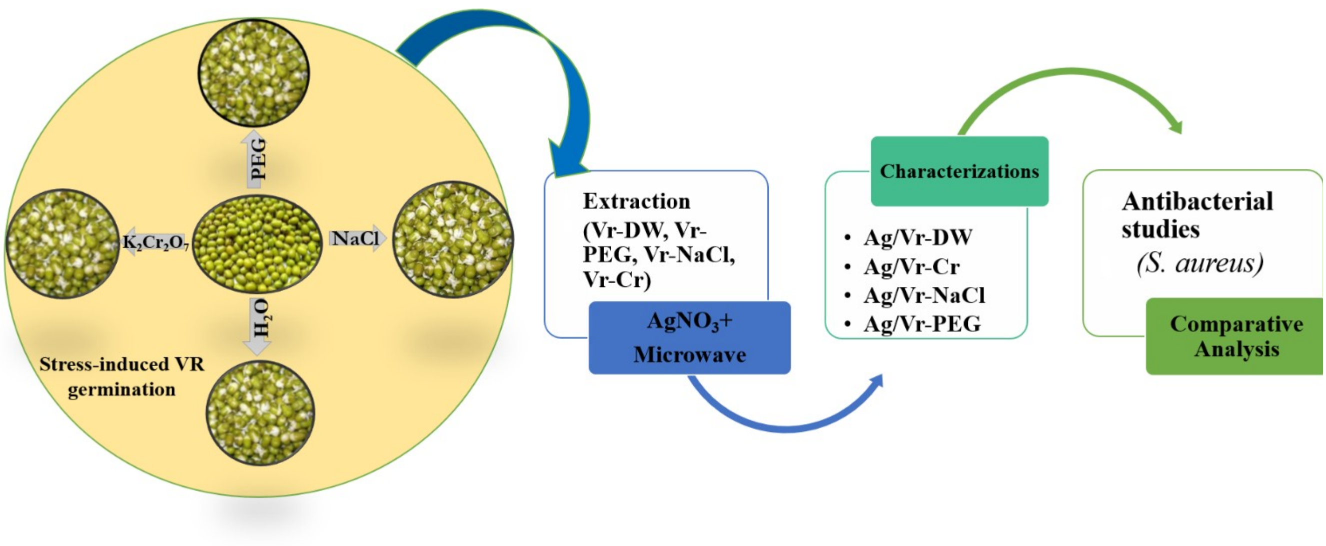
2.5. Characterization of Silver Nanoparticles

The surface plasmon resonance of synthesized AgNPs has been analyzed by UV-vis spectrophotometer. The AgNPs colloid was diluted with distilled water and loaded in a quartz cuvette. The UV-vis spectral range was set between 200-600 nm. The study was performed with the Shimadzu UV-2450 Spectrophotometer. FT-IR spectra of synthesized AgNPs were analyzed using the Perkin Elmer-400 spectrometer with ATR attachment. *VR* extracts and nanoparticles synthesized from different extracts of VR were scanned, and the scans were collected with resolution and 500-3500 cm^{-1} wavenumber.

FE-SEM is used to determine the particle size distribution and average size of particles in nanometer scale of the synthesized nanoparticles. For FE-SEM analysis, MAIA3 XMH FE-SEM was used. The hydrodynamic size of synthesized nanoparticles was measured with a dynamic light scattering detector (DLS). The surface charge and stability were also examined. The overall experimental procedure is depicted in Scheme 1.

2.6. Antibacterial Study

Antibacterial activity was determined using the standard agar well diffusion method against the human pathogenic bacteria *Staphylococcus aureus* (40-42). The cultures were swabbed on nutrient agar plate,s and wells were prepared on each plate using sterile Cork borer. 100 µL of samples were loaded into respective wells, and six replications were maintained for each sample using the respective VR extracts as control (C). The plates were incubated at 37 °C for 48 hours. After incubation, the zone of inhibition around the well was measured.



Scheme 1: Schematic representation of the synthesis and analysis.

3. RESULTS AND DISCUSSION

3.1. Germination Percentage

The germination percentage of VR under different chemical stress was recorded at 48 hours after incubating it in the respective solutions of NaCl, PEG, chromium solution, and distilled water. The percentage of germination was determined using the equation (33) given below.

$$Germination\,\% = \frac{Number\ of\ germinated\ seed}{Number\ of\ seeds\ kept\ for\ germina} \times 100$$

The percentages of germination of VR in NaCl, PEG, chromium solution, and distilled water are found to be 96%, 100%, 90%, and 98%, respectively. Stress-induced germination in VR is a complex process involving multiple physiological, biochemical, and molecular changes such as water uptake, enzyme activation, respiration, oxidative stress, hormone levels, and gene expression. These changes enable the seed to cope with adverse conditions and promote germination.

Seed germination of VR can be affected by the presence of sodium chloride, chromium solution, and polyethylene glycol in the growth medium. The effects of VR seed germination in the presence of these solutions depend on the concentration of chemicals used and the duration of exposure (31,32). High concentrations and prolonged exposure can have detrimental effects on seed germination and seedling growth, while moderate stress can stimulate the synthesis of protective compounds and improve stress tolerance (31,43). NaCl is known to cause osmotic stress in plants, which can lead to various physiological and biochemical changes such as delayed germination, reduced growth, accumulation of proline, increased antioxidant activity, and changes in gene expression (30,34,43,44). PEG (polyethylene glycol) is a water-soluble polymer that is often used to induce osmotic stress in plants during germination studies. When seeds of VR are germinated in the presence of PEG solution, it can affect their growth and development in various ways, such as delayed germination, reduced seedling growth, increased accumulation of osmoprotectants, and changes in gene expression (45). Chromium is a heavy metal that can have toxic effects on plant growth and development, such as inhibition of seed germination, reduction in seedling growth, chlorosis and leaf necrosis, accumulation of chromium in plant tissues, and activation of defense mechanism (46-48). Even if we are applying different kinds of stress, the plant has various adaptive mechanisms that help it to cope with the stress and continue to grow and develop under adverse conditions (30). Some of the secondary metabolites that have been identified in *VR* are flavonoids, alkaloids, tannins, saponins, and phenolic acids (49-50).

3.2. Synthesis of Silver Nanoparticles

AgNPs were produced through the application of microwave radiation to a mixture comprising 90 mL of a 1 mM silver nitrate aqueous solution and 10 mL of germinated VR extracts. During the process of microwave irradiation, the colourless reaction mixture gradually transformed into a yellowish-brown hue. Over time, the colour continued to evolve towards a consistent yellowish-brown shade, even with prolonged microwave irradiation, suggesting the successful formation of AgNPs. This colour change can be attributed to the presence of a metal surface containing free electrons in the conduction band, as well as positively charged nuclei, which collectively contribute to the observed coloration of the reaction mixture. The images of extracts and the respective AgNPs are depicted in Figure 1.

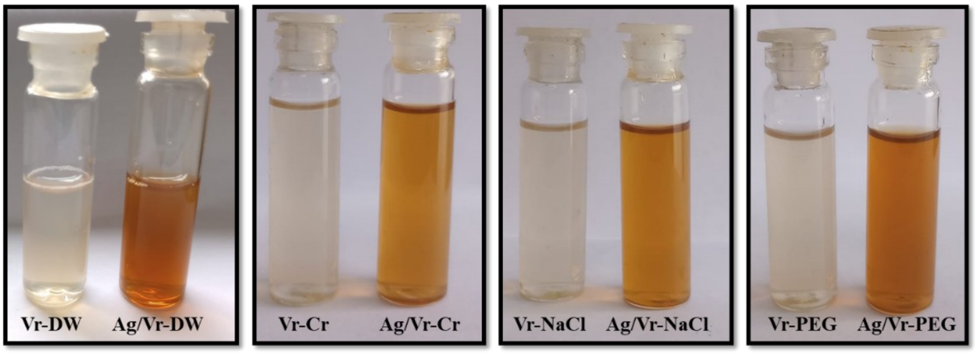


Figure 1: Images of VR-extracts and the respective AgNPs.

3.3. Characterization of silver nanoparticles

3.3.1. UV-visible spectrophotometer analysis

UV-vis. spectroscopy is a common analytical technique used to determine the optical properties of nanoparticles, including the size, shape, and concentration of the particles. The technique involves measuring the absorption of light by the nanoparticles at specific wavelengths in the UV-vis. range. Here, the four different AgNPs samples, Ag/Vr-DW, Ag/Vr-PEG, Ag/Vr-NaCl, and Ag/Vr-Cr, with 4 minute microwave irradiation were analyzed using UV-vis spectroscopy, and the obtained absorption peaks are depicted in Figure 2.

The absorption peak suggests that all four samples showed characteristic absorption peaks of AgNPs. AgNPs exhibit a phenomenon called localized surface plasmon resonance (LSPR), which occurs due to the collective oscillation of conduction electrons in response to incident light (13,14,25). This results in a characteristic absorption peak in the UV-vis. spectrum. The position of this peak depends on the size, shape, and composition of the nanoparticles. Generally, spherical AgNPs exhibit a plasmon peak around 400-450 nm (visible range) (51,52). The intensity of the plasmon resonance peak is related to the concentration or density of AgNPs in the sample. Higher intensities indicate a greater number or higher concentration of nanoparticles. The intensity of the peak varied among the samples, with the highest peak observed in Ag/Vr-DW and the lowest peak observed in Ag/Vr-

Cr. The change in peak intensity implies the varying concentration of AgNPs formation. The shape of the peak can also provide information about the size distribution and uniformity of the nanoparticles. Here, a narrow, symmetric peak suggests a monodisperse population of nanoparticles (40,43,54). In addition to the plasmon resonance peak, AgNPs may exhibit additional absorption bands in the UV or visible region. These bands can arise from other electronic transitions or higher order plasmon modes.

The phytochemicals present in VR are responsible for the reduction process and stabilization of AgNPs (23,55). The stress-induced germination affected the formation of AgNPs compared to the control, Ag/Vr-DW. Chemical stress such as salinity, heavy metals, and PEG delayed the germination of seeds and, hence, the production of primary metabolites. Stress-induced process promote the synthesis of secondary metabolites in plants that could help the formation of AgNPs (30).

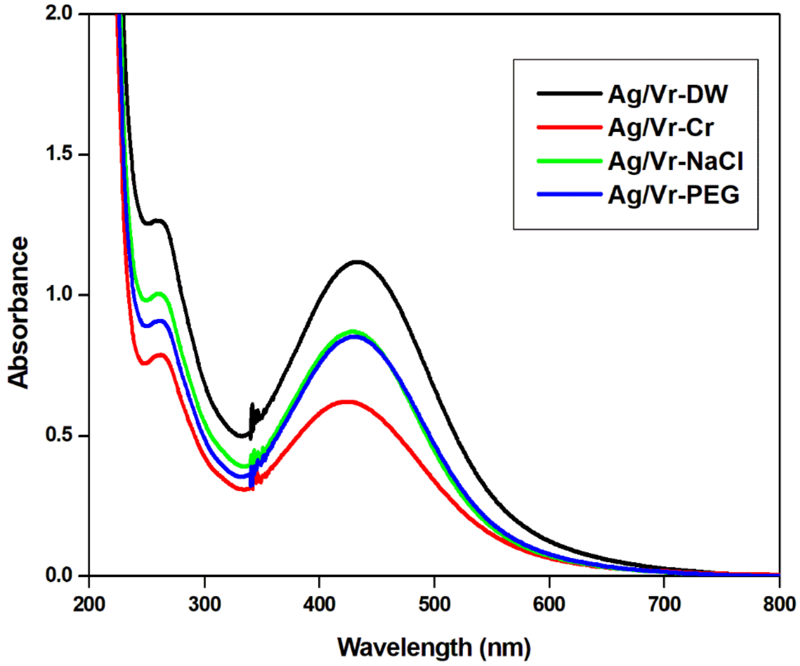


Figure 2: UV-vis. absorption spectrum of Ag/Vr-DW, Ag/Vr-Cr, Ag/Vr-NaCl and Ag/Vr-PEG.

3.3.2. FT-IR analysis

The method of using FT-IR (Fourier transform infrared) spectroscopy to pinpoint the functional groups present in a given sample. The vibrations of various functional groups in the sample are represented by the peaks seen in the spectrum. FT-IR spectra of Ag/Vr-DW, Ag/Vr-Cr, Ag/Vr-NaCl, and Ag/Vr-PEG and their respective extracts show four characteristic vibrational peaks at 3311cm^{-1}, 2074cm^{-1}, 1635 cm^{-1}, and 576cm^{-1} (Figure 3). The peaks observed at 3311cm^{-1} and 1635cm^{-1} attributed to the stretching vibrations of O-H and N-H functional groups, respectively (56). The peak observed at 2074cm^{-1} is likely due to the presence of a C≡N triple bond, which is indicative of a nitrile functional group. The vibrational peak at 576cm^{-1} indicates N-H bending vibrations (57). The extracts of VR contain various biomolecules such as carbohydrates, proteins, lipids, nucleic acids, and secondary metabolites. These peaks suggest that there may be some organic molecules present in the sample that are interacting with the AgNPs. The presence of the same peaks in both the extract and AgNPs samples indicates that the nanoparticles are likely coated or capped with the same organic molecules that are present in the extract. These organic molecules may act as stabilizing agents for the nanoparticles, preventing them from agglomerating or undergoing further chemical reactions. This suggests that the extract may be a potential source of natural capping agents for the synthesis of AgNPs.

3.3.3. FE-SEM analysis

FE-SEM analysis is a type of electron microscopy that uses a focused beam of electrons to generate high-resolution images of the surface of a sample. The FE-SEM images of Ag/Vr-DW, Ag/Vr-Cr, Ag/Vr-NaCl, and Ag/Vr-PEG are depicted in Figure 4. All the FE-SEM images of the synthesized nanoparticles show a spherical shape. The spherical shape of the nanoparticles is a significant characteristic of AgNPs synthesized using plant extracts (58,59). The uniformity in shape suggests that the synthesis process is well controlled and reproducible (53). The average size of the nanoparticles can also be determined from the FE-SEM images by measuring the diameter of the spheres using ImageJ software. The mean areas of the AgNPs in Ag/Vr-DW, Ag/Vr-Cr, Ag/Vr-NaCl, and Ag/Vr-PEG are 6.7nm, 5.5nm, 924nm and 4.7nm, respectively.

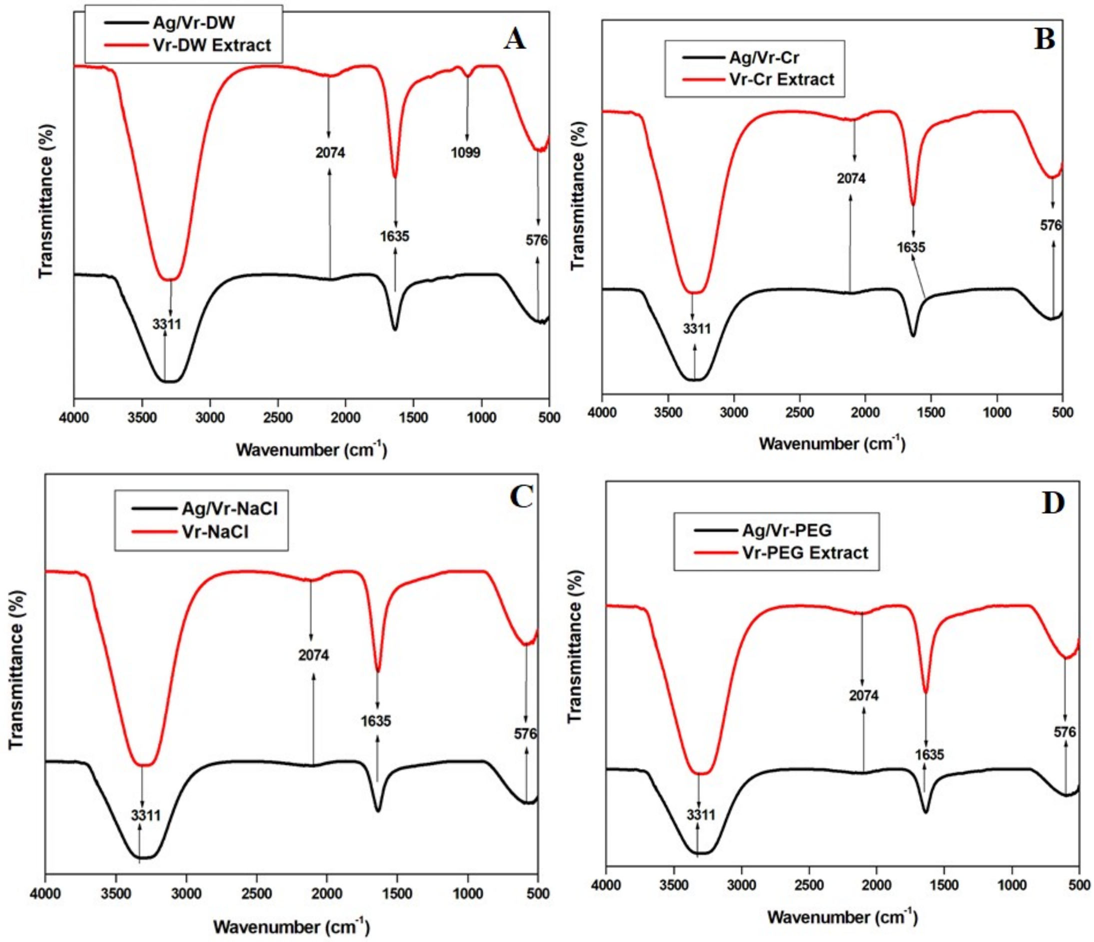


Figure 3: FT-IR spectra of (A) Ag/Vr-DW, (B) Ag/Vr-Cr, (C) Ag/Vr-NaCl and (D) Ag/Vr-PEG.

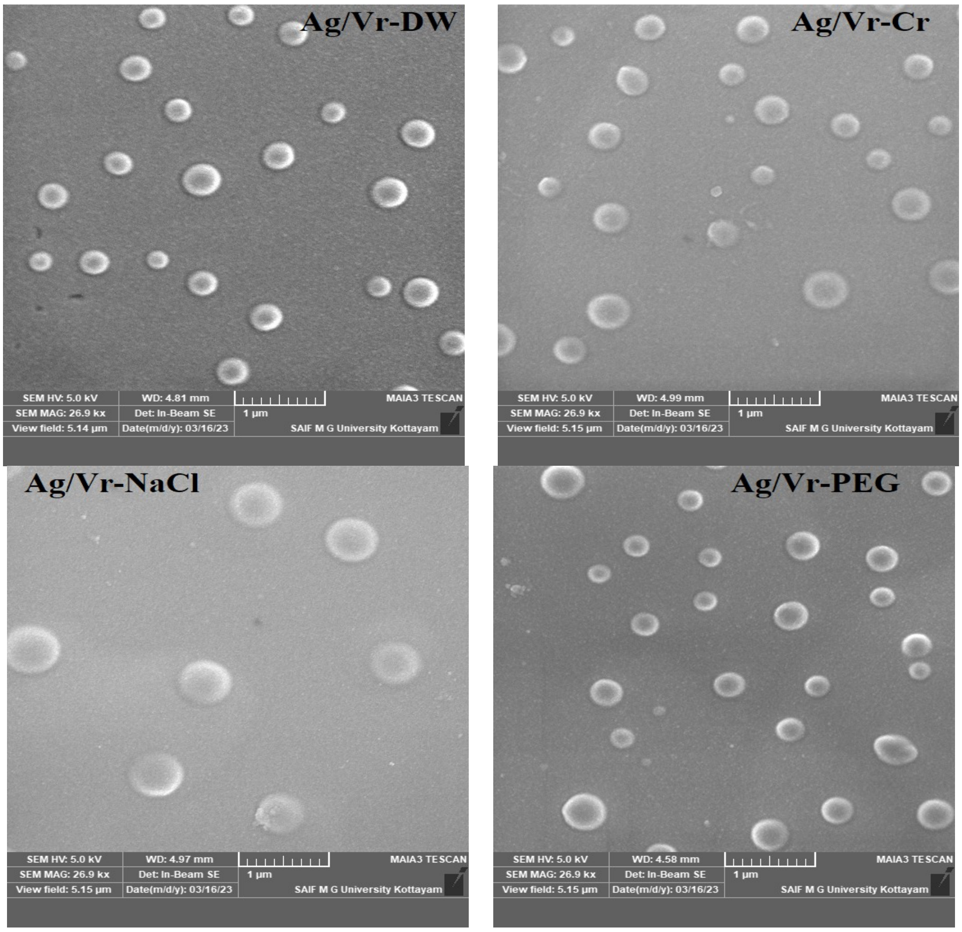


Figure 4: FE-SEM images of Ag/Vr-DW, Ag/Vr-Cr, Ag/Vr-NaCl and Ag/Vr-PEG.

3.3.4. Dynamic light scattering and zeta potential analysis

Dynamic light scattering (DLS) and zeta potential analysis are commonly used techniques to characterize the size distribution and surface charge of nanoparticles (60). Here, the DLS and zeta potential analyses were performed on four different types of AgNPs synthesized from stress-induced germinated seeds of VR, with different chemical stresses applied: Ag/Vr-DW, Ag/Vr-Cr, Ag/Vr-NaCl, and Ag/Vr-PEG. The obtained data are depicted in Table 1 and the respective graphs are plotted in Figure 5.

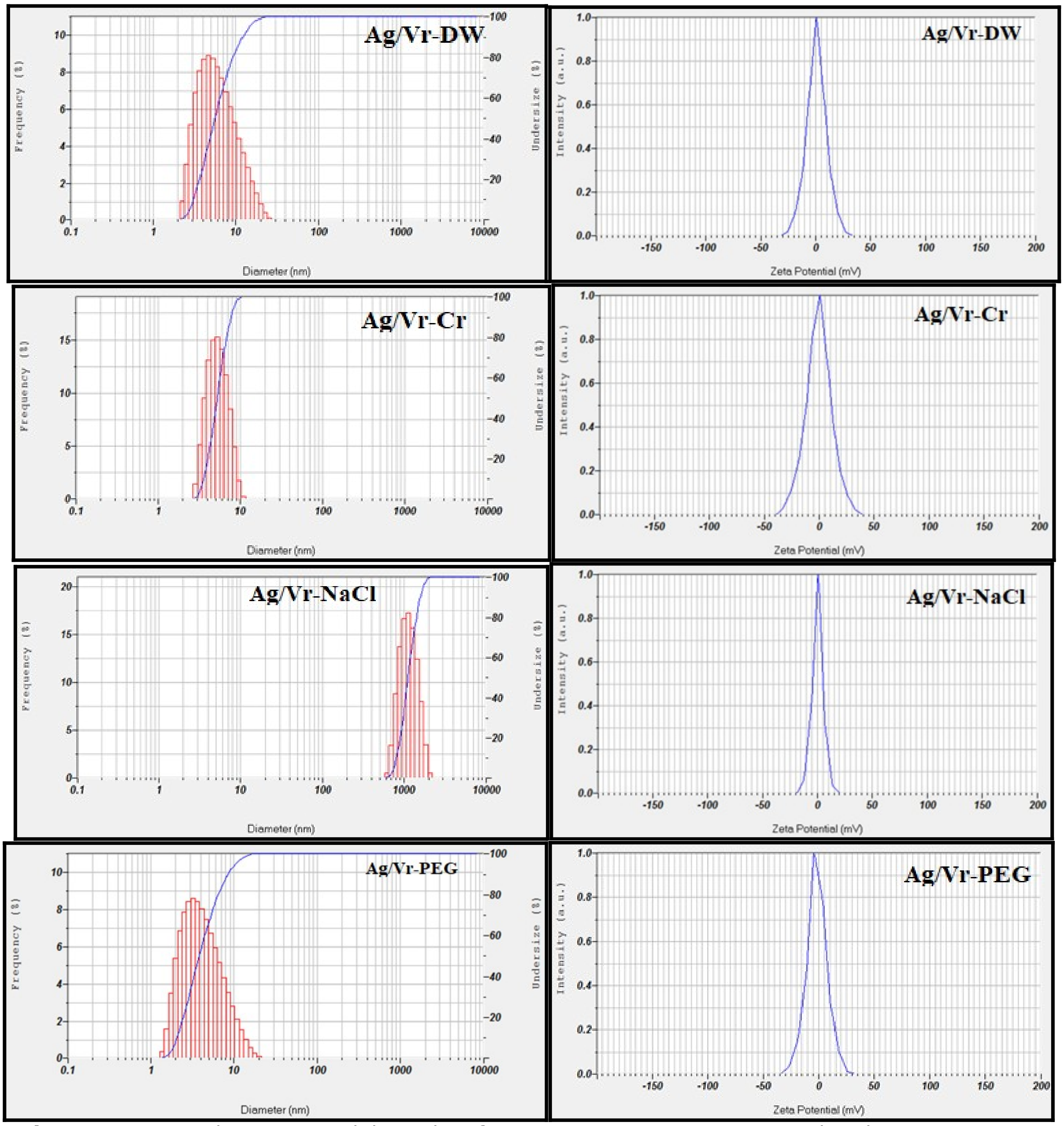


Figure 5: DLS and Zeta potential graphs of Ag/Vr-DW, Ag/Vr-Cr, Ag/Vr-NaCl and Ag/Vr-PEG.

The hydrodynamic particle size distribution of Ag/Vr-DW, Ag/Vr-Cr, Ag/Vr-NaCl, and Ag/Vr-PEG obtained from DLS analysis are 6.7nm, 5.5nm, 925nm, and 4.7nm, respectively. To minimize background scattering, the nanoparticle samples were diluted appropriately before conducting the analysis. The hydrodynamic size measurement of AgNPs takes into account the hydration layer present on the surface, resulting in a larger size compared to the size determined from FE-SEM images (61). The hydrodynamic size of AgNPs may be influenced by the presence of phytochemicals present in the VR extract.

The zeta potential value is a measure of the surface charge of the nanoparticles. A positive zeta potential value indicates that the surface of the nanoparticles is positively charged, whereas a negative value indicates a negative surface charge (62). In accordance with the results of the analysis, the zeta potential values for Ag/Vr-DW, Ag/Vr-Cr, Ag/Vr-NaCl, and Ag/Vr-PEG were 0.8, -0.5, 0.2, and -1.8, respectively. These values suggest that the surface of Ag/Vr-DW and Ag/Vr-NaCl nanoparticles is positively charged, whereas the surface of Ag/Vr-Cr and Ag/Vr-PEG nanoparticles is negatively charged. The zeta potential value is an essential factor that influences the stability of nanoparticles in suspension (40). When the surface of nanoparticles is charged, they repel each other, which prevents them from agglomerating or settling. A high absolute value indicates that the nanoparticles are highly stable.

Table 1: Mean size and zeta potential values of AgNPs.

Nanoparticles	Mean Size (nm)	Zeta Potential (mV)
Ag/Vr-DW	6.7	0.8
Ag/Vr-Cr	5.5	-0.5
Ag/Vr-NaCl	925	0.2
Ag/Vr-PEG	4.7	-1.8

3.4. Antibacterial Studies

The antibacterial activity of the synthesized AgNPs against *Staphylococcus aureus* indicates their potential application as antimicrobial agents. Several studies have reported the synthesis of AgNPs using *Vigna* radiata as a plant material and evaluated their antibacterial activity against different bacterial strains (23,24,63). The present study has tested four different types of AgNPs synthesized from stress-induced germinated seeds of VR, Ag/Vr-DW, Ag/Vr-Cr, Ag/Vr-NaCl, and Ag/Vr-PEG using the respective extracts as controls (C). The obtained results are depicted in Figures 6 and 7. According to the results, Ag/Vr-PEG exhibited the highest antibacterial activity against *S. aureus*, followed by Ag/Vr-NaCl, Ag/Vr-Cr, and Ag/Vr-DW, respectively. This trend is somewhat in the reverse order of the AgNPs concentration in the synthesized nanoparticles. The Ag/Vr-PEG exhibited higher antibacterial activity, likely due to the efficient reduction and stabilization facilitated by polyethylene glycol during synthesis. Moreover, the stress-induced germination process under PEG stress conditions may have led to the accumulation of bioactive compounds in the VR extract, enhancing the reduction efficiency of silver ions and promoting stronger interactions between AgNPs and bacterial cells.

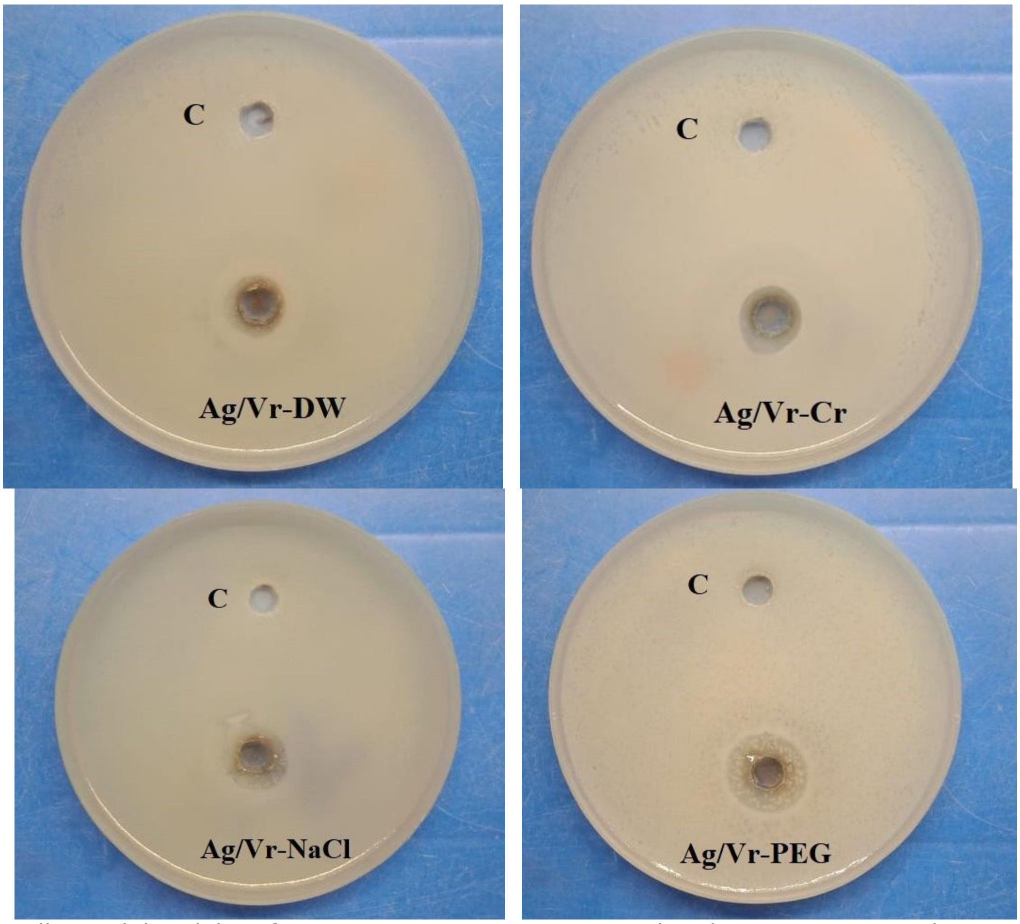


Figure 6: Antibacterial activity of Ag/Vr-DW, Ag/Vr-Cr, Ag/Vr-NaCl and Ag/Vr-PEG towards *Staphylococcus aureus.*

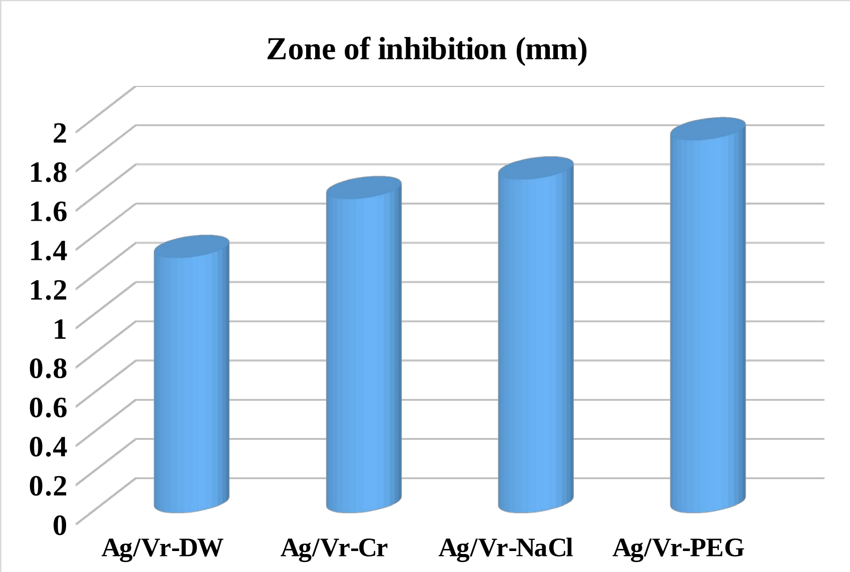


Figure 7: Zone of inhibition exhibited by different AgNPs.

The difference in antibacterial activity among the four types of synthesized nanoparticles could be due to variations in the size, morphology, or surface chemistry of the nanoparticles (64-66). For instance, the zeta potential analysis suggests that the surface charges of the nanoparticles differ, which can impact their interactions with bacterial cells. In addition, the chemical stresses applied during synthesis can also affect the antibacterial activity of the synthesized nanoparticles. For example, the presence of chromium ions may reduce the antibacterial activity of Ag/Vr-Cr nanoparticles, as chromium ions can interact with the silver ions and reduce their effectiveness.

It is also worth noting that the higher concentration of AgNPs in Ag/Vr-DW nanoparticles does not necessarily correlate with higher antibacterial activity. The antibacterial activity of the nanoparticles is influenced by a range of factors, including the concentration of silver ions, the size and morphology of the nanoparticles, and their surface chemistry (67,68).

The diverse phytochemical compositions of the extracts can also influence the reduction kinetics and stabilization of AgNPs during synthesis, leading to variations in size, shape, and surface properties (69). Factors such as pH, ionic strength, and the presence of biomolecules within the extracts may also impact AgNP characteristics and their interactions with bacterial cells (11). Moreover, the inherent antimicrobial properties of specific plant extracts could synergistically enhance the antibacterial efficacy of the synthesized AgNPs. This comparative analysis highlighted the importance of understanding extract-mediated effects on AgNP properties to optimize their antimicrobial activity for various applications. Figure 8 depicts a potential mechanism for the formation of AgNPs and their antibacterial activity.

To enhance the antibacterial efficacy of synthesized AgNPs, optimization strategies encompass controlled synthesis parameters such as reaction time and precursor concentration to tailor nanoparticle size and surface characteristics (70). Surface functionalization techniques involving capping agents and ligands can enhance interactions with bacterial cells, while combination therapy with other antibacterial agents can exploit synergistic effects (71). Moreover, pH and ionic strength optimization, surface engineering for specificity, and biocompatibility enhancement are crucial considerations to improve AgNP efficacy and safety (67). Validation through rigorous in vitro and in vivo testing further ensures the efficacy and applicability of optimized AgNPs in various biomedical and environmental contexts.

4. CONCLUSION

The abiotic stress induced germinated VR seed through the microwave assisted method is a novel approach for the green synthesis of AgNPs. The abiotic stress was induced using sodium chloride, polyethylene glycol, distilled water, and chromium solution. The synthesized AgNPs were characterized using several techniques, including UV-vis, DLS, Zeta Potential, FT-IR, and FE-SEM. The AgNPs concentration synthesized from Vr-NaCl, Vr-Cr, Vr-PEG, and Vr-DW is of the order Ag/Vr-DW> Ag/Vr-NaCl> Ag/Vr-PEG> Ag/Vr-Cr. The study revealed that the prepared AgNPs exhibited a significant antibacterial activity against *Staphylococcus aureus* bacteria. Additionally, a comparative analysis of the antibacterial efficacy of AgNPs showed Ag/Vr-PEG> Ag/Vr-NaCl> Ag/Vr-Cr> Ag/Vr-DW. This research highlights the potential of stress-induced germinated seeds of VR as a sustainable and eco-friendly source for synthesizing AgNPs with excellent antibacterial properties. The findings contribute to the development of new antimicrobial agents that can be utilized in various applications, such as healthcare and biomedical fields, where combating bacterial infections is of utmost importance. Further investigations and optimizations can be followed to explore the full potential and applications of AgNPs derived from stress-induced germinated seeds of VR in antimicrobial research and development.

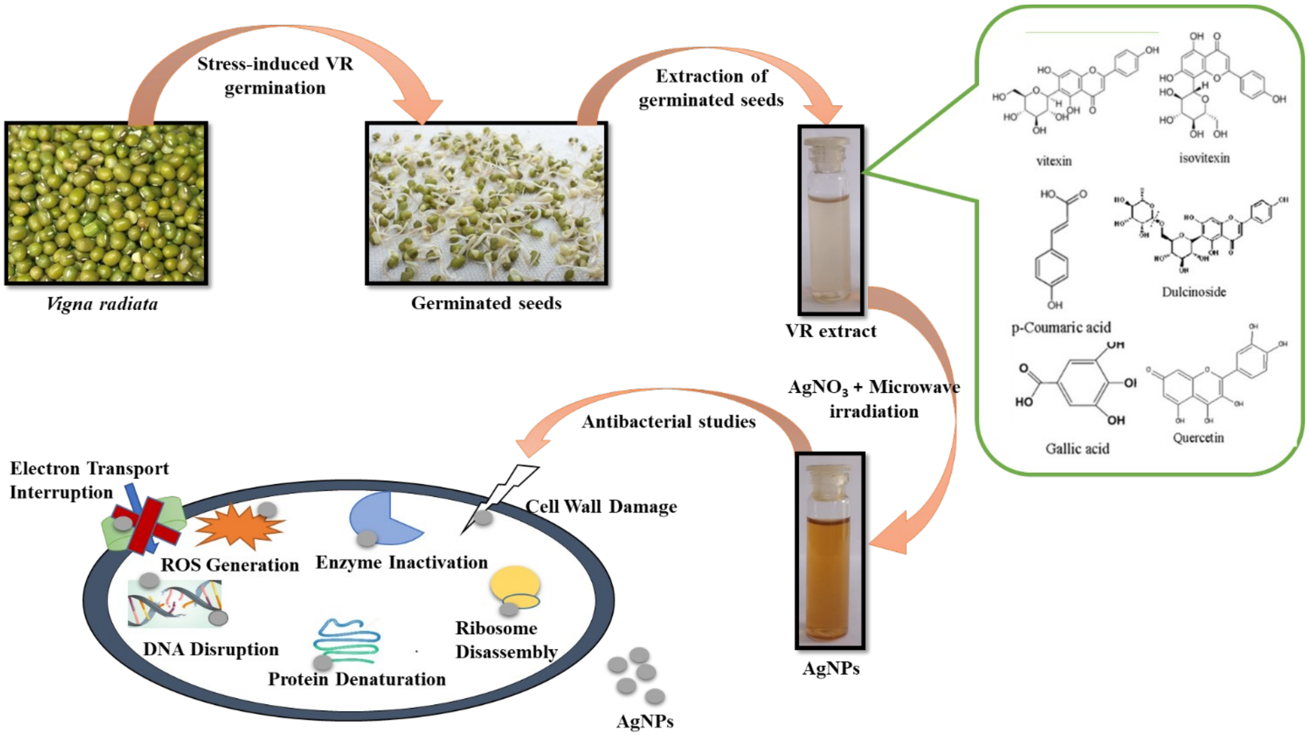


Figure 8: Potential mechanism for the formation of AgNPs and its antibacterial activity.

5. FUNDING

This research received no external funding.

6. CONFLICTS OF INTEREST

There are no conflicts to declare.

7. DATA AVAILABILITY STATEMENT

Data are available upon reasonable request.

8. ACKNOWLEDGEMENT

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