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Bioengineered silver nanoparticles using *Brassica oleracea* sub sp. *botrytis* (L.) for enhanced antibacterial activity

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The green methodologies of nanoparticles with plant extracts have received an increase in interest. Silver nanoparticles (Ag NPs) have been utilized in a many of applications in the last few decades. The current study presents the synthesis of Ag NPs with aqueous extract of *Brassica oleracea* sub sp. *botrytis* (L.) as a stabilizing agent. UV-visible spectroscopy, FTIR, XRD, SEM, TEM, and EDAX analysis were performed to study the synthesized Ag NPs. The synthesized Ag NPs have been measured with dynamic light scattering (DLS), average size and charge were discovered to be 81.62 ± 1.14 nm and -11.3 ± 2.51 mV, respectively. Furthermore, as-formed AgNPs showed strong antibacterial activity against the Gram-positive bacteria (*Staphylococcus aureus*). According to the results of this investigation, green synthesized Ag NPs with *Brassica oleracea* sub sp. *botrytis* (L.) may be used in biomedicine as a replacement agent for biological applications.

Keywords: *Brassica oleracea* leaves, plant metabolites, silver nanoparticles, TEM with SAED, antibacterial activity.

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Introduction

Green chemical processes are gaining popularity and are becoming increasingly necessary to reduce environmental concerns. Green Nanotechnology is an example of a move that can assist in achieving the Sustainable Development Goals. Due to their specific properties, the use of various metals and their oxides in the nanometer range is growing. Silver nanoparticles are one of the most commercialized nanoparticles, and their use as biosensors, catalysis, antimicrobial, anti-inflammatory activities, and other applications in fields such as agriculture, industry, medicine, and the environment is growing in the present era. The synthesis process determines the characteristics of metal nanoparticles. Bottom-up approaches are used to classify biogenic silver nanoparticle syntheses [1]. Silver nanoparticles are currently manufactured using a variety of physical and chemical methods such as electrical irradiation, lithography chemical reduction, sol-gel, and so on. However, these synthetic methods have several disadvantages that could endanger human health and the environment. The synthesis process using biological organisms can help to overcome the drawbacks of other synthetic methods to some extent [2-4].

Green synthesis of AgNPs utilizing microorganisms and plants is better than different strategies since it is a one-venture measure that is savvy, earth-reasonable, and creates more steady materials [5]. Looking at changed organic strategies, the pace of nanoparticle synthesis through plant extract is quicker than those including microorganisms [6]. The metal ions are diminished to their nano size followed by stabilization through different biochemical components like alkaloids, phenols, flavonoids, etc. present in plant leaves. The variety in composition and amount of these phytoconstituents alongside their connection with watery metal ions impact the components of orchestrated nanoparticles [7].

Metal nanoparticle biosynthesis in plants is isolated into three phases: activation, development, and termination. The activation phase incorporates the bio-reduction of metal particles followed by its nucleation; in the subsequent stage, little nearby nanoparticles go through the Ostwald ripening process precipitously to blend into a bigger size, joined by a thermodynamic expansion of nanoparticles. Lastly, the termination phase characterizes the nanoparticles' final shape, and they receive the most enthusiastically good adaptation, which is intensely influenced by plant concentrate's capacity to settle metal nanoparticles [8].

Agricultural residues, which are rich in bioactive compounds, are being tested as a biological source for nanoparticle synthesis, considering the efficacy of plant-mediated nanoparticle synthesis [9]. The use of these wastes for nanoparticle synthesis may be a long-term solution, as it can minimize production costs while also reducing emissions. *Brassica oleracea* sub sp. *botrytis* (L.) (generally known as Cauliflower) is a popular vegetable with high demand. Its leaves account for around half of the weight of cauliflower, and since it is inedible, it is often discarded in large quantities from vegetable markets. As a result, it is classified as a waste product with a higher waste index [10-11]. Cauliflower leaves also contain alkaloids, hormones, flavonoids, anthraquinone, terpenoids, phenols, proteins, quinine, and carbohydrates among other phytoconstituents [12]. These phytoconstituents can aid in the reduction of silver ions to their nano form. Biosynthesis of nanoparticles of oxides of iron, copper, zinc, and lead [7] as well as environmental remediation of metal ions from industrial effluents by biosorption process [11], are just a few of the many applications of these leaves that have already been recorded. These applications can lead to the management of agro-waste.

Because of its expansive action against many microorganisms, silver nanoparticles have been discovered to be effective as antibacterial specialists. These nanoparticles have previously appeared to enter the cytoplasm and denature the ribosome. This may bring about the inactivation of a few bacterial catalysts and proteins keeping them from playing out their metabolic capacities and causing bacterial cell demise. Cell wall synthesis obstruction, concealment during protein biosynthesis, transcription process interference, and interruption of important metabolic pathways are four potential components of silver nanoparticles' bactericidal action that have been proposed [13-14]. Silver nanoparticles are utilized as an antibacterial specialist in an assortment of utilizations including cleaning clinical gear, domestic devices, water treatment, the wellbeing industry, food stockpiling, material coatings, and various natural applications [15]. The human microbe *Staphylococcus aureus* is notable. It's a typical reason for boils, abscesses, and other skin diseases [16]. Anti-microbial bound wraps (bandages) are regularly used to treat an assortment of shallow skin wounds and diseases. The current examination intended to explore the antimicrobial action of silver nanoparticles created through *Brassica oleracea* leaves in a harmless to the ecosystem way. Moreover, silver nanoparticles were utilized to improve the antibacterial adequacy of antibiotic-covered wraps against *Staphylococcus aureus*.

I. Materials and Methods

1.1 Materials

The waste leaves of *Brassica oleracea* were picked manually from a local grocery market in Mumbai (MS) and were authenticated at Agharkar Research Institute (ARI) in Pune (MS). As a source of silver ions, analytical grade $AgNO_3$ (silver nitrate) was used. By dissolving the metal salt in DW, a stock solution of $AgNO_3$ (1 M) was formed. Metal ions in high concentrations may cause freshly formed nanoparticles to aggregate and precipitate [17].

1.2 Preparation of the leaf extract

Collected leaves were properly washed under tap water before being rinsed with DW (deionized water) to prepare the extract. The leaves samples were then allowed to oven-dry (60 °C) to free them from moisture. They were also ground into a fine powder and stored at room temperature (RT) in an airtight polythene bag until required. 2 g of leaves powder was added to 0.1 liter deionized water for silver nanoparticle synthesis, and the solution was boiled for 5-10 minutes. Filtration of the solution with Whatman filter paper grade 1 yielded the extract.

1.3 Bioengineered silver nanoparticles

As a result, for nanoparticle synthesis, a typical solution of 5 mM $AgNO_3$ was used. The ratio of the plant extract (warm) to the metal salt solution used was 3:1. The solution was sonicated for 20 minutes at 30°C, then incubated for 2 hours at RT (28 °C $\pm$ 2 °C). It was noted that the color of the colloidal solution was changed. The following characterization of synthesized nanoparticles was done as mentioned in [7].

1.4 Characterization

The colloidal solution was spectroscopically scanned on a Systronics Double Beam Spectrophotometer 2203 between frequencies 200 and 600 nm having a resolution of 1 nm and 10 mm optical path length of quartz cuvettes. The absorption spectrum of the colloidal solution was recorded after it was diluted with an equivalent amount of DW. The spectrophotometer's standard was revised utilizing a clear reference. Atomic Force Microscope (AFM) was utilized to explore the surface topography of silver nanoparticles. The colloidal solution was sonicated for 20 minutes at 30°C and its dainty film was shaped on a spotless oil-free quartz chip followed by air-drying and the film was seen under AFM (Nanosurf, NaioAFM). Dynamic Light Scattering (DLS) was likewise used to gauge the molecule size and surface charge of silver nanoparticles.

The functional groups present on the silver nanoparticles were investigated by using a Fourier Transform Infrared Spectrophotometer (FT-IR) (Model: Jasco FT/IR-4100 type A C208161016). Using a manual dye press system, the silver nanoparticles powder was thoroughly mixed with potassium bromide in a 1:10 ratio and forced into a disc to form a pellet. The spectrum was generated with wavenumbers ranging from 4000 to 400 cm^{-1}. Cryo FE-SEM was used to examine the surface morphology of a thin film of silver nanoparticles coated on carbon tape. On a JEM-2100F JEOL Field Emission Electron Microscope, the shape and size of AgNPs were examined using a FE-TEM. AgNPs were suspended in deionized water and sonicated for 30 minutes. Before the inspection, one drop of the suspension was spotted on a double copper-coated carbon grid and dried using IR light under ambient conditions. The X-ray diffraction pattern of the sample was taken by using graphite-monochromated Cu/Kα radiation (λ=1.5406 Å) having 10 × 10 mm beam size at 30 kV and 15 mA. The 2θ range was 10° to 80° and scan speed was kept was 5.0 deg./min.

1.5 Antibacterial assay

The antibacterial activity of the AgNPs was determined using the agar diffusion assay method. As a research organism, *Staphylococcus aureus* was used. Agar plates were seeded with bacteria using the pour plate technique. Three wells were drilled with a cork borer (9 mm diameter) at equivalent distances and filled with 100 µL of synthesized AgNP solution, $AgNO_3$ salt solution, and plant extract, followed by incubation. For the bandage analysis, the disc method was used. The bandage was purchased from a local medical store and the central active part was cut into two halves and autoclaved. One half was immersed into silver nanoparticle suspension, dried, and placed onto the sterile nutrient agar plate already spread with *S. aureus*. Half of the bandage (untreated) was placed as the control similarly. The experiments were carried out three times, with plates incubated at 37°C for 24 hours. The antibacterial activity represented by the zone of inhibition around the well/bandage suggested that bacterial growth was limited.

II. Results and Discussion

2.1 Optical analysis

The waste leaves were identified as *Brassica oleracea* sub sp. *botrytis* (L.) of Metzg. (Family-Brassicaceae Burnett) after authentication. The color change of colloidal solution is the preliminary indication of metal nanoparticle synthesis [18]. After incubation with silver nitrate solution, the leaf extract changed color from yellow to dark brown, as depicted in Fig 1. This suggests that AgNPs synthesis may have taken place. By using a UV-visible Spectrophotometer, the absorbance of light passing through the colloidal solution was determined. The maximum absorption was obtained at 410 nm. The UV-visible spectrum of AgNPs synthesized using *Pedalium murex* leaf extract showed maximum absorption at 430 nm in a related study [19]. The surface plasmon resonance causes a metal's conduction electrons to collectively excite, resulting in maximum absorption [12]. Nanoparticles' optical properties are determined by their form, scale, agglomeration, and concentration. The particle size of nanoparticles in a mono-disperse colloid is known by UV-visible spectroscopy. However, it cannot reveal the particle size of poly-disperse colloid particles [20].

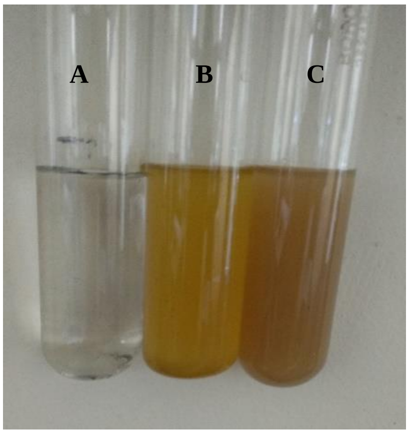
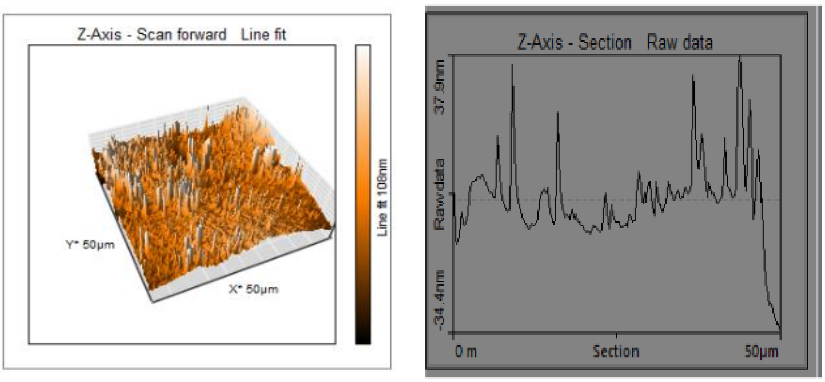


Fig. 1. Change in the colour of the colloidal solution after incubation with 5mM $AgNO_3$ solution A – $AgNO_3$ solution, B – Plant extract, C – Silver nanoparticles solution.

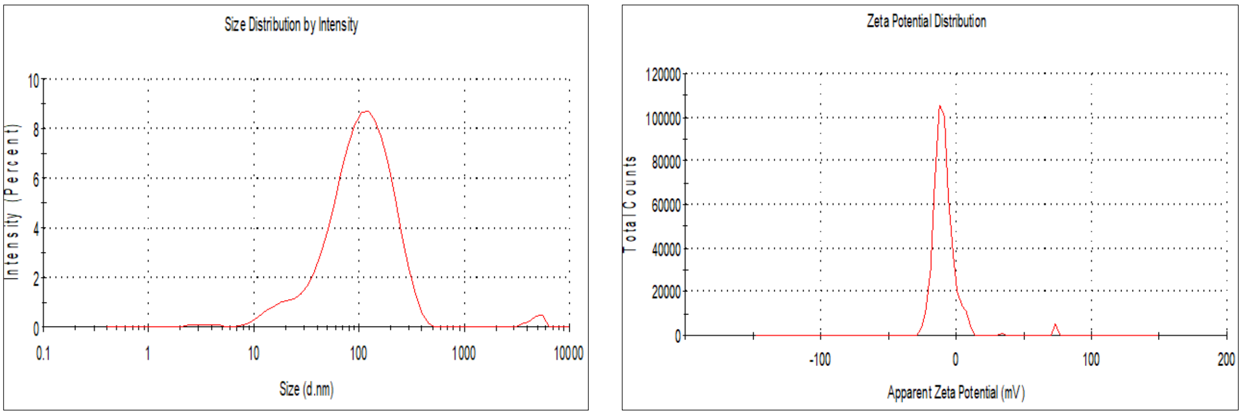
2.2 Morphological analysis

Using AFM, Fig 2a depicts the three-dimensional surface topography of synthesized nanoparticles. The deflection pattern suggested that the nanoparticles are less than 100 nm in size (Fig 2b). Using DLS and FEG-TEM, this finding was further tested. DLS [21] can be used to calculate the size distribution as well as the surface charge of the silver nanoparticles. Fig 3a and b rectify that the size (average) and charge of silver nanoparticles were



a) b)

Fig. 2. AFM image (a) Topography and (b) Deflection pattern of bioengineered Ag NPs.



a) b)

Fig. 3. DLS image (a) Size distribution (b) Zeta potential of bioengineered Ag NPs.

81.62 ± 1.14 nm and -11.3 ± 2.51 mV, respectively. The particle size and zeta potential of silver nanoparticles contribute to their suitability for biomedical research. The smaller the particle, the easier it will be to move it inside the cell. Particles with a diameter of fewer than 100 nm have many applications in drug delivery and biosensors [22]. The silver nanoparticles' surface charge is critical for interacting with various biomolecules and biochemical pathways within the cell [23].

When observed under FE-SEM, the surface morphology of silver nanoparticles (as shown in Fig 4) revealed different shapes with agglomeration. TEM images of AgNPs in Fig. 5a-c display spherical shapes with no agglomeration. Most of the nanoparticles had average sizes of less than 100 nm. The crystalline nature of a single particle was further investigated via high-resolution (HRTEM) images, which showed the presence of lattice fringes with d-spacing values. As shown in Fig. 5d, one group of lattice fringe is resolved, and the lattice distance is 0.24 nm, indexing to the (111) crystal planes of Ag, respectively Fig. 5e showed the SAED pattern of AgNPs having bright spots arranged in a ring, indicating that Ag NPs are polycrystalline.

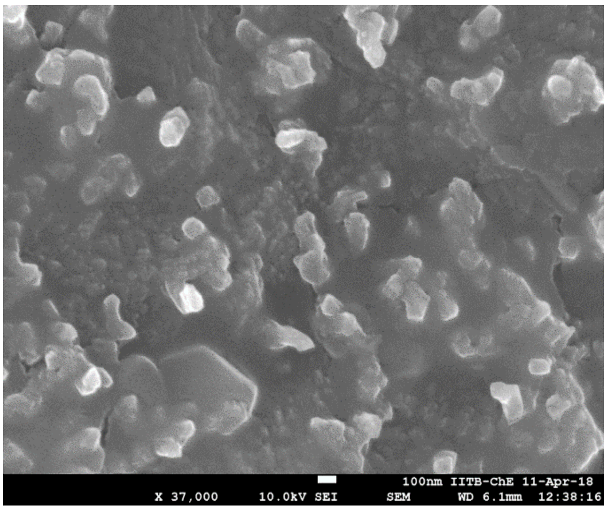


Fig. 4. FE-SEM image of bioengineered Ag NPs.

2.3 FTIR analysis

Fig 6 shows the spectrum of synthesized AgNPs obtained from FT-IR. The –OH group could be identified by the existence of a strong and wide absorption band at 3190.28 cm^{-1}. Aldehydic (C-H) stretch has peaked at 2924.95 and 2855.06 cm^{-1}, (C=O) stretch has peaks between 1735.34 and 1377.20 cm^{-1}, (C-O) stretch has peaked at 1176.9 cm^{-1}, and (C-H) stretch has a peak at 790.671 cm^{-1}. The peak at 629.85 cm^{-1} may be assigned to -CH out of plane bending vibrations of ethylene systems -C=H- (cis) [24].

2.4 XRD analysis

The XRD pattern, which showed some sharp peaks, also confirmed the crystallinity of the silver nanoparticles (Fig. 7). These data are consistent with the values for a face-centered cubic (fcc) crystal structure of silver. The peaks at 38.28°, 44.38°, 64.56°, and 77.62° correspond to crystal planes (111), (200), (220), and (311), respectively. No other peaks for impurities were detected. The crystallite size of the silver nanoparticles calculated by XRD analysis (using the Scherrer equation with (111) as the most intense plane) was 18 nm, slightly smaller than that determined by TEM. For smaller nanoparticles ($\leq$ 10 nm), the crystallite size largely agreed with the corresponding particle size determined in the TEM. This confirmed our earlier finding that the smallest nanoparticles appeared mainly in their polycrystalline form. Thanks to the rapid synthesis procedure, the uniform silver nanoparticles were easily synthesized green.

2.5 Antibacterial activity

S. aureus, a Gram-positive bacterium, was used to find out the antibacterial properties of silver nanoparticles synthesized in the present work. In the well-containing plant leaf extract, no antibacterial activity was detected. Silver nanoparticles had the largest inhibition zone (23 mm) relative to silver nitrate solution (11 mm) (Fig. 8a). Furthermore, in a bandage experiment, a bandage containing an antibiotic had a smaller zone of inhibition (16 mm) than a comparable bandage coated with silver nanoparticles, which had a zone of inhibition of 22 mm (Fig 8b). Hence, antibiotics coated with silver nanoparticles in bandages can be a promising alternative for healing skin infections subject to their reactivity with human skin cells. Thus, in conclusion, AgNPs are showing good antibacterial activity and can be considered commercially for medical applications.

Antimicrobial activity is inclined by limited basic factors like size, surface area, optical absorption, and morphology and separation performance of photo-generated charge carriers [25, 26].

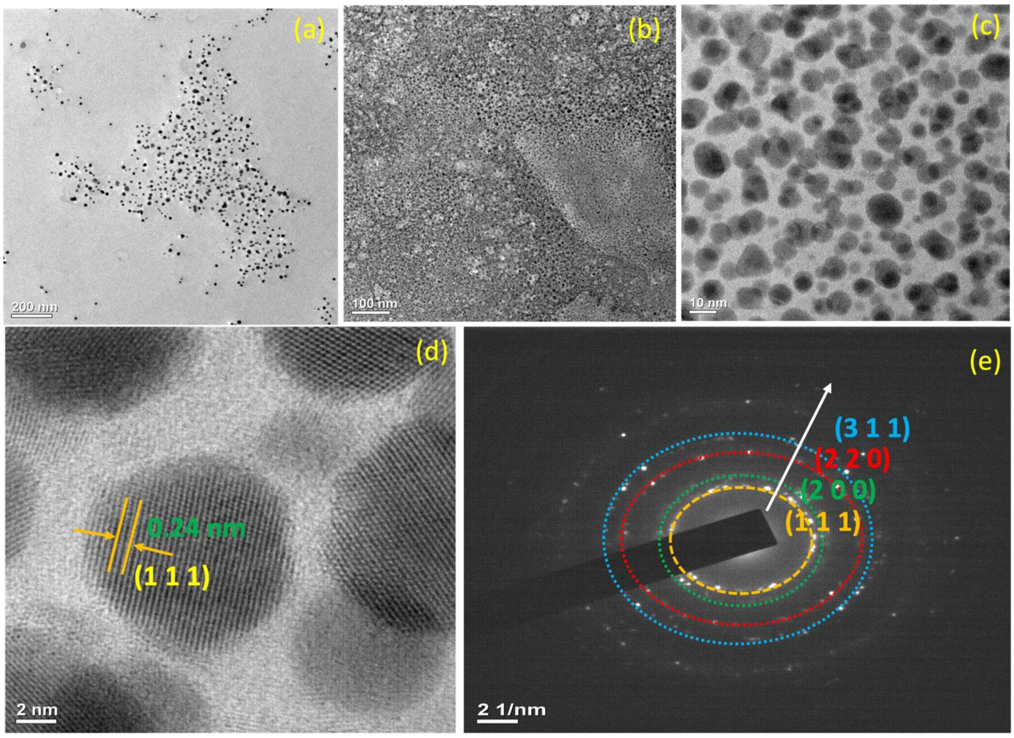


Fig. 5 (a-c). TEM Images with different magnifications (d) d-spacing image, (e) SAED pattern of the prepared bioengineered Ag NPs.

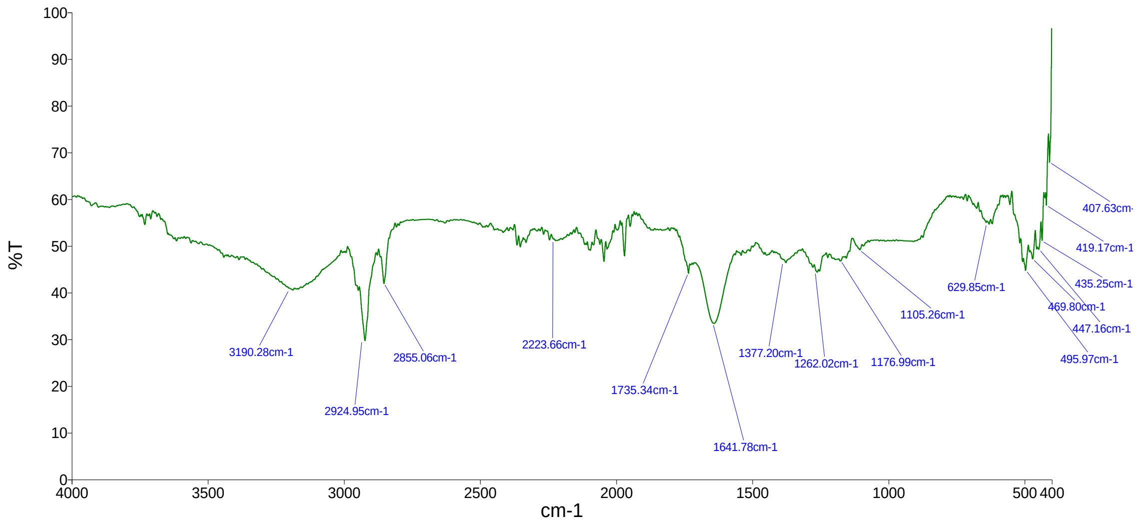


Fig. 6. FT-IR spectrum of bioengineered Ag NPs.

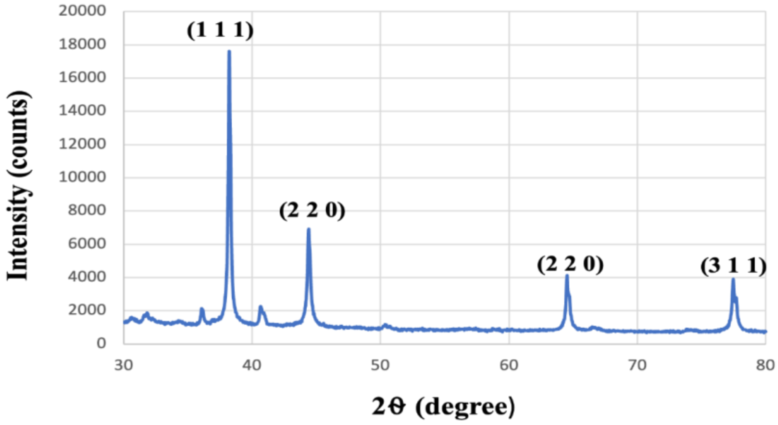


Fig. 7. XRD pattern of bioengineered Ag NPs.

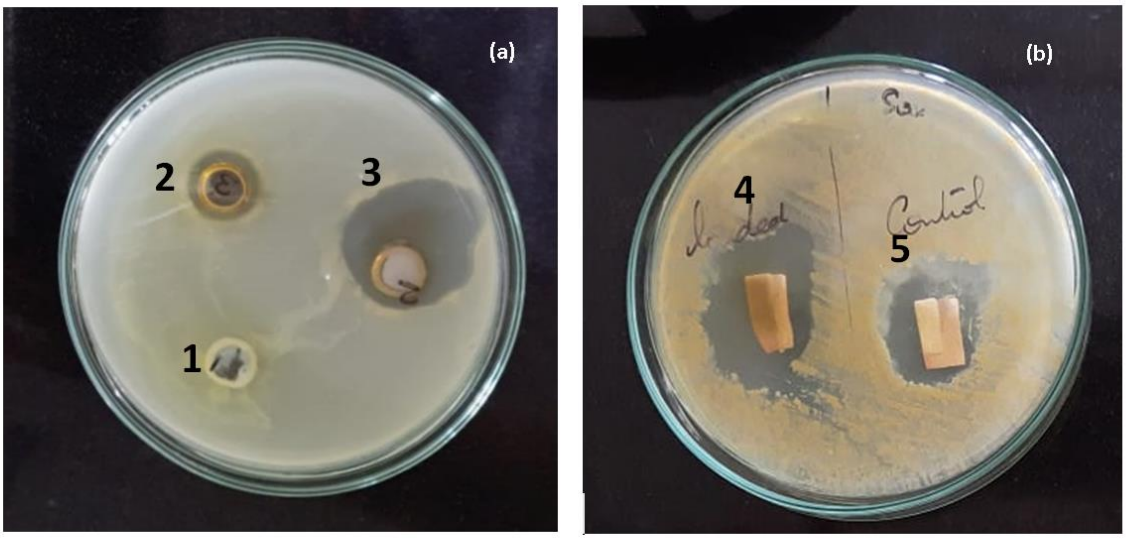


Fig. 8. Antibacterial activity of (a) bioengineered Ag NPs, (b) Bandage coated with silver nanoparticles against *S. aureus*; 1- plant extract, 2-$AgNO_3$ solution. 3-Ag NPs solution, 4-Bandage coated with Ag NPs, 5- Normal bandage.

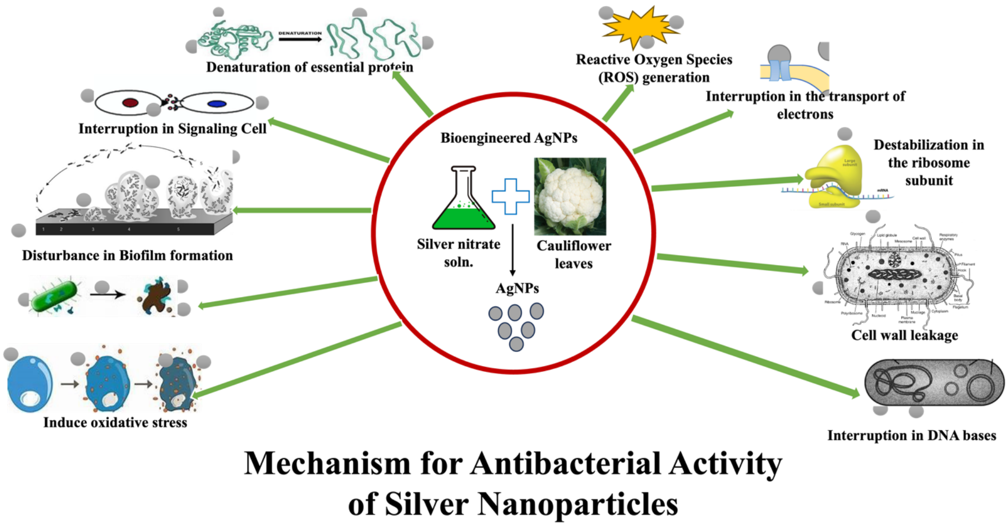


Fig. 9. Antibacterial activity mechanism of bioengineered Ag NPs.

The antibacterial activity is depending upon the reactive oxygen species (ROS), surface area, and size of the element [27-29]. Fig. 9 represents the antibacterial mechanism of biosynthesized Ag NPs. Nanoparticles can act as photocatalysts and produce ROS such as $^{\bullet}O_2^-$, $^{\bullet}OH$, and H_2O_2 in the existence of dissolved O_2 are important to extra free radical creation. The Ag NPs generate ROS thoroughly Fenton reaction leading to DNA damage, protein denaturation, and finally, it can reason for the death of the microorganism [30].

Conclusion

The plant-mediated biosynthesis of AgNPs is simple, cost-effective, and eco-friendly, and thus adheres to green chemistry principles. Plant extract-based silver nanoparticles with antibacterial activity may be a potential candidate for a variety of applications in the medical, textiles, cosmetics, and food industries. To provide better control over the dimensions and polydispersity of the nanoparticles, further research is required to understand the precise molecular mechanism resulting in the formation of silver nanoparticles through biological methods. At the same time, environmental monitoring of silver nanoparticles in the field is critical for determining their effects on the environment and human health.

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Declarations

Ethical Approval

This declaration is not applicable to the present work.

Funding

No funding was received from any funding agency for carrying out the research reported in this paper.

Availability of data and materials

This declaration is not applicable to the present work

Significance Statement:

The work focuses on the biosynthesis of AgNPs using agro-waste and its medical use. The use of Cauliflower leaves for AgNPs synthesis can be used commercially, thus sustainably contributing to solid waste management.

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Біоінженерія наночастинок срібла з використанням Brassica oleracea sub sp. *botrytis* (L.) для посилення антибактеріальної активності

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Інтерес до зелених методологій наночастинок із рослинними екстрактами постійно зростає. За останні кілька десятиліть наночастинки срібла (Ag NP) використовуються в багатьох сферах застосування. У поточному дослідженні представлено синтез НЧ Ag із водним екстрактом Brassica oleracea sub sp. botrytis (L.) як стабілізуючий засіб. Для вивчення синтезованих Ag NP використано методи УФ-видимої спектроскопії, FTIR, XRD, SEM, TEM та EDAX аналіз. Синтезовані наночастинки Ag досліджували за допомогою динамічного розсіювання світла (DLS), виявлено, що середній розмір і заряд становлять 81,62 ± 1,14 нм і -11,3 ± 2,51 мВ відповідно. Крім того, сформовані Ag NP показали сильну антибактеріальну активність проти грампозитивних бактерій (*Staphylococcus aureus*). За результатами цього дослідження зелені синтезовані НЧ Ag з *Brassica oleracea* sub sp. botrytis (L.) можуть використовуватися в біомедицині як замінники для біологічних застосувань.

Ключові слова: листки *Brassica oleracea*, метаболіти рослин, наночастинки срібла, ПЕМ з SAED, антибактеріальна активність.