

# Extraction and Biosynthesis of Silver Nanoparticles from *Allium sativum* Essential Oil

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**Abstract:** Garlic was used as a natural reducing and stabilizing agent for the creation of nanoparticles since it is a rich source of organosulfur compounds. 29.58% of the essential oil was extracted using Soxhlet extraction with ethanol as a solvent, demonstrating effective extraction under ideal circumstances. Under carefully regulated pH and temperature settings, diluted garlic oil was reacted with an aqueous silver nitrate (AgNO<sub>3</sub>) solution to produce AgNPs. The creation of spherical silver nanoparticles was confirmed by characterization using UV–visible spectroscopy, which showed a clear absorption peak at 420 nm. The limited peak range suggested that the nanoparticles were modestly distributed and relatively stable. Furthermore, Gram staining and microbial isolation verified that soil samples included Gram-positive bacteria, which were utilized for additional antibacterial assessment. The study shows that the manufacture of silver nanoparticles using garlic essential oil is an efficient, economical and environmentally friendly method. This study emphasizes the importance of green nanotechnology derived from plants as a sustainable substitute for traditional chemical synthesis techniques.

**Keywords:** Garlic Essential oil, *Allium sativum*, Silver Nanoparticles, Green Synthesis, UV–Vis Spectroscopy, Antimicrobial Activity, Surface plasmon Resonance, etc.

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## I. INTRODUCTION

Garlic (*Allium sativum* L) is an Alliaceae plant native to Central Asia. *Allium sativum*, the scientific name for garlic belongs to the Amaryllidaceae family. In traditional medicine this herb is used to treat infectious disorders (Sasi et al., 2021). The enormous abundance of phytochemicals from many medicinal plants still holds many answers to human health issues (Chen et al., 2016). Garlic *Allium sativum* is one of the most widely used medicinal herbs in the world. Its bulbs have long been utilized as a flavoring ingredient in many international dishes (Dehariya et al., 2021). Garlic has a varied chemical composition and a higher concentration of physiologically active chemicals. Fiber, water (65%), adenosine, sulfur compounds, pectin, carbs, fructan, fatty acids, nicotinic acid, essential amino acids, phospholipids, lectins, prostaglandins, vitamins, minerals and enzymes are all present (Marchese et al., 2016). Diallyl disulfide and dilly trisulfide are two of the many sulfides found in garlic oil, which is rich in sulfureted organic molecules (Satyal et al., 2017).

Garlic cloves release an organic sulfur chemical called alliin when they are crushed, which gives them their distinctive flavor and fragrance. The food sector uses fresh garlic and its essential oil as natural antioxidants, flavor enhancers and antibacterial agents, especially for processed meat and chicken products (Yasin et al., 2022). Because of its biological qualities, garlic (*Allium sativum* L.) is employed extensively in both scientific studies and human diets. The quantity and kind of bioactive chemicals derived from garlic extracts are influenced by a number of variables, including temperature, pressure, extraction technique, solvent type, garlic size and geographic origin. The most common organosulfur compounds found in garlic essential oil are diallyl disulfides (DADS) and diallyl trisulfides (DATS); these compounds have drawn a lot of attention in the sectors of medicine, food and agriculture because of their numerous biological functions (Bar et al., 2022).

In addition to being used in toothpaste to prevent dental caries, garlic pastes and lime have been used to heal sore throats and mouths (Ashfaq et al., 2021). According to Khubber et al. (2020), garlic has been found as a potentially

unique therapeutic food that can help prevent and treat coronavirus disease (COVID-19).

Garlic essential oil (GEO) can assist in the green synthesis of silver nanoparticles (AgNPs), where it functions as both a reducing and capping agent (Althumairy, 2025). The nanoparticles produced through this method exhibit enhanced antimicrobial and antibiofilm activity against a wide range of pathogenic microorganisms including multidrug-resistant strains. Research indicates that their antimicrobial action involves several mechanisms such as disruption of the bacterial cell membrane induction of oxidative stress through depletion of cellular antioxidants like glutathione and leakage of intracellular components, ultimately leading to bacterial cell lysis (Moradi et al., 2023).

Garlic-mediated AgNPs generally display spherical morphology with particle sizes around 15–20 nm and their formation is commonly confirmed using analytical techniques such as UV–Vis spectroscopy, FTIR, TEM and XRD. These nanoparticles often show strong zones of inhibition and low minimum inhibitory concentration (MIC) values (Bakhshi et al., 2022). In addition, garlic extract has also been used in the biosynthesis of other nanoparticles such as zinc oxide and sulfur nanoparticles sometimes in combination with plant extracts like ginger, producing synergistic antibacterial effects. For example, nanoparticles synthesized using ultrasonicated garlic extract have demonstrated strong activity against oral pathogens such as *Streptococcus mutans* and *Porphyromonas gingivalis*, mainly due to the presence of organosulfur compounds. Similarly, nano formulations such as zein nanoparticles incorporated with eugenol and garlic oil have shown effective inhibition of fish pathogens. Allicin-capped silver nanoparticles have also been reported to possess notable antioxidant and biomedical potential. Furthermore, garlic oil nano emulsions with particle sizes around 36 nm have been found to enhance antibacterial activity particularly against *Staphylococcus aureus* compared to *Escherichia coli*, although their effectiveness may depend on factors such as particle size, zeta potential and concentration. Overall nanoparticles synthesized through garlic-based green methods often show improved stability and more targeted antimicrobial delivery compared to conventional extracts, making them promising candidates for combating antibiotic-resistant infections (Sarangi et al., 2024).

## II. MATERIALS & METHODOLOGY

### ➤ Materials

- Biological Materials: Garlic bulbs (*Allium sativum*), fresh soil samples collected from different nearby locations and pure bacterial cultures including Gram-positive and Gram-negative bacteria were used as biological materials.
- Glassware: The glassware used during the experimental work included conical flasks (250 mL and 500 mL), beakers, test tubes, Petri plates, measuring cylinders, glass rods, pipettes with micropipette tips, watch glasses, funnels and glass slides with cover slips.

- Instruments & Equipment: Various laboratory instruments such as an autoclave, hot air oven, laminar airflow chamber, incubator, colony counter, centrifuge, Soxhlet extraction apparatus, rotary evaporator (if available), analytical balance, pH meter, refrigerator (4°C) and microscope were used to carry out the experimental procedures.
- Chemicals and Reagents: The chemicals used in this work included nutrient agar, nutrient broth, distilled water, 70% ethanol, methanol or hexane for oil extraction, dimethyl sulfoxide (DMSO), and gram staining reagents such as crystal violet, Gram's iodine, alcohol as decolorizer and safranin. Normal saline solution and garlic oil extract were also used.
- Microbiological Tools: Microbiological tools such as an inoculation loop, L-shaped spreader (L-rod), sterile cotton swabs, forceps, corkborer for well diffusion method and sterile filter paper discs were used for microbial handling and antibacterial testing.
- Culture Media Preparation Materials: For the preparation of culture media, agar powder, peptone, beef extract and sodium chloride were used.
- Personal Protective Equipment (PPE): During the laboratory work, safety measures were followed by using a lab coat, gloves, face mask and safety goggles.
- Antibacterial Assay Materials: Mueller-Hinton agar plates, sterile discs or wells and a standard antibiotic were used for performing the antibacterial activity assay.

### ➤ Methodology

- Collection of Soil Samples- Soil samples were collected from different nearby locations using sterile spatulas and transferred into sterile polythene bags. The samples were properly labeled and transported to the laboratory for microbial isolation. The collected soil was stored at room temperature until further analysis (Nweke et al., 2017).
- Isolation of Bacteria from Soil- One gram of soil sample was suspended in 9 ml of sterile distilled water to prepare serial dilutions. Different dilution levels were prepared up to  $10^{-6}$ . From suitable dilutions, 0.1 ml suspension was spread on Nutrient Agar plates using a sterile spreader. The plates were incubated at 37°C for 24 hours to obtain bacterial colonies (Cappuccino et al., 2017).
- Purification of Bacterial Cultures- Distinct bacterial colonies showing different morphology were selected and sub-cultured on fresh Nutrient Agar plates using streak plate technique. Pure cultures were obtained after incubation and maintained on agar slants at 4°C for further experiments (Tripathi et al., 2025).
- Identification of Bacterial Isolates- Bacterial isolates were identified on the basis of colony morphology and Gram staining technique. Smears were prepared on clean glass slides, heat fixed, and stained using crystal violet, Gram's iodine, alcohol, and safranin. The stained slides were observed under microscope to differentiate Gram-positive and Gram-negative bacteria (Tripathi et al., 2025).
- Gram Staining: Bacterial sample was placed on glass slide with the help of micropipette. A smear of bacterial sample was made at an angle 45 degree with the help of another

glass slide. Bacterial smear was air dried and then 2-3 drops add primary stain (crystal violet) was placed on smear and left 90 seconds. Then the slide was washed with distilled water to remove excess dye. Now, add 0.5% iodine solution as a mordant on above slide which will form complex. After that wash the slide with decolourizer (alcohol). Then safranin (secondary stain) was added to counterstain the decolourized bacteria. Wash the above slide with distilled water to remove excess stain and then add glycerine on slide and place coverslip with the help of needle to avoid air bubble and clean the slide with filter paper before observing under microscope (Smith et al., 2018).

- Collection of garlic: Fresh garlic of the local variety was purchased at Tech Market in Mandhana, Kanpur and all samples were kept in our lab under the proper conditions (dark, 27°C) throughout the trials. The garlic cloves were carefully chopped into two equal-sized pieces using a stainless-steel knife. They were then placed in an oven set at 60°C to dry for 48 hours before being ground into a powder using mechanical tools.
- Extraction of Garlic Essential Oil: Garlic oil was extracted using solvent extraction method. Crushed garlic paste was mixed with suitable organic solvent such as ethanol or hexane and kept for extraction. The mixture was filtered and solvent was evaporated using rotary evaporator or water bath to obtain concentrated garlic oil extract. The extracted oil was stored at 4°C until use (Bar et al., 2022).
- Determination of Oil Yield: The yield of garlic oil was evaluated using the Soxhlet extraction method, in which garlic powder was extracted with ethanol (1:20, w/v) and the solvent was then evaporated under decreased pressure using a rotary evaporator (El-Saber Batiha et al., 2020). The percentage yield of garlic oil was estimated as follows:

$$\text{Yield (\%)} = \frac{\text{Mass of concentrated garlic oil (g)}}{\text{Initial mass of dried garlic powder (g)}} \times 100$$

This standard method measures the effectiveness of extracting essential oils and lipids from plant matrices. In this work, approximately 50 g of garlic powder was used every batch, allowing for consistent yield estimation across multiple extraction conditions (Gupta et al., 2024).

Several parameters influence extraction yield, including solvent type, solvent-powder ratio, extraction time, and temperature. These factors affect the diffusion and solubilization of lipid-soluble garlic elements such as organosulfur compounds and sterols. Higher temperatures and longer extraction times often boost oil recovery by improving solvent penetration and mass transfer from the plant matrix to the extracting phase. Excessive heating can partially degrade thermolabile bioactive chemicals, including allicin-derived sulfides, reducing the functional quality and biological activity of the finished oil. To increase yield and preserve important bioactive elements for future research and nano-formulation, an optimal extraction condition was used (Jain et al., 2025).

- *Synthesis of Silver Nanoparticles (AgNPs) Using Garlic Essential Oil as a Reducing Agent:*

Garlic essential oil (GEO) produced from *Allium sativum* can be used as a reducing and stabilizing ingredient to create silver nanoparticles (AgNPs) through green chemistry. This strategy aligns your experimental plan with conventional scientific language and references used in green synthesis protocols (Sarangi et al., 2024).

In the experimental (test) setting, garlic essential oil (GEO) was initially diluted in acetone at a volume ratio of 1:170 (v/v) to increase its solubility and promote homogeneous dispersion in the aqueous phase. The diluted GEO was added dropwise to 200 mL of freshly made 10 mM aqueous silver nitrate (AgNO<sub>3</sub>) solution while continuously magnetically stirring. To reduce Ag<sup>+</sup> ions and stabilize the nanoparticles, the reaction mixture's pH was lowered to around 8 with a buffer or dilutes NaOH/HCl. In the control sample, an identical amount (200 mL) of 10 mM AgNO<sub>3</sub> solution was made without adding garlic essential oil. This control was kept at the same temperature, pH, and stirring rate to isolate the influence of GEO on nanoparticle production (Bouqellah et al., 2019).

The experimental and control mixtures were incubated at room temperature for 24 hours with constant stirring to ensure uniform mixing and interaction of the reagents. During this period, bioactive organosulfur compounds in GEO, such as diallyl trisulfide and other allyl sulfides, function as electron donors, reducing silver ions (Ag<sup>+</sup>) to metallic silver (Ag<sup>0</sup>), resulting in the nucleation and development of AgNPs (Mohamed et al., 2021).

The experimental sample showed a perceptible color change from colorless to yellowish-brown, indicating successful production of spherical silver nanoparticles via surface plasmon resonance (SPR). The control sample remained mostly unaffected, indicating that the reduction and stability of Ag<sup>+</sup> to Ag<sup>0</sup> is due to the phytochemical elements of GEO rather than spontaneous reduction of AgNO<sub>3</sub> under these conditions. After 24 hours of incubation, both reaction solutions were filtered through Whatman filter paper (grade 1 or 42) to separate solid nanoparticles from liquid phase. The residue left on the filter paper was carefully collected, transferred to sterile test tubes and rinsed with distilled or deionized water to remove any remaining salts or organic residues. The washed nanoparticles were dried (e.g., in a hot air oven or under vacuum) and stored in a desiccated environment for further physicochemical analysis, including UV-Vis spectroscopy (Sarangi et al., 2024).

- *UV-VIS Absorption Spectroscopy:*

UV-Visible spectrophotometry confirms the creation of silver nanoparticles (AgNPs) by detecting the localized surface plasmon resonance (LSPR) band, which appears as a strong absorption peak at 400–450 nm for spherical AgNPs with a diameter of <50 nm. Following synthesis, the filtered garlic essential oil-derived AgNP suspension is diluted (1:10-1:50 v/v) in ultrapure water and scanned (300–800 nm, 1 nm resolution) in a quartz cuvette with a dual-beam spectrophotometer (e.g., Shimadzu UV-1650), with the

blank-matched baseline accounting for solvent and biogenic background absorbance (Von White et al., 2012).

The  $\lambda_{max}$  position and full width at half maximum (FWHM) is important metrics. A short FWHM (<50 nm) suggests monodispersity, while a wider FWHM shows signal aggregation, as predicted by Mie scattering theory for dielectric-metal surfaces. Garlic organosulfur compounds, such as allicin, stabilize the LSPR by redshifting the peak to around 420-430 nm, allowing for preliminary size assessment without electron microscopy (Otunola et al., 2017).

This spectrum demonstrates the characteristic AgNP absorbance and synthesis optimization, indicating efficient bioreduction of  $Ag^+$  ions (Abdelkhalig et al., 2025).

### III. RESULT AND DISCUSSION

#### ➤ Isolation of Microbial Strains from Different Species

- Collection of Soil Samples- Soil samples were collected from different nearby locations of Rama University i.e. (near V. C Building Garden area) using sterile spatulas and transferred into sterile polythene bags as shown in Fig. 1. The samples were properly diluted using distilled water and labeled for microbial isolation.

In order to prevent exogenous microbial infiltration, soil samples were aseptically collected from proximal areas using sterile stainless-steel spatulas. The samples were then decanted into polyethylene containment vessels that had been autoclave-sterilized and exposed to gamma radiation. To guarantee traceability and avoid cross-contamination artifacts during subsequent metagenomic or culturomics operations, each aliquot received exact alphanumeric labeling in accordance with defined nomenclature protocols (e.g., site coordinates, edaphic depth, collection timestamp). Following acquisition, specimens were quickly transported to a biosafety level 2 (BSL-2) microbiology facility at room temperature (20–25°C) to maintain the viability of autochthonous microbial consortia, prevent cryogenic shock

and limit proliferative shifts before isolation using serial dilution plating or molecular enrichment (Gaete et al., 2020).



Fig 1 Aseptically Collected Soil Sample for Microbial Analysis

- Isolation of Bacteria- Nearby collected soil sample was serially diluted to obtain pure culture. The proximal edaphic matrix was serially decimal diluted in sterile phosphate-buffered saline (PBS, pH 7.2) to reduce microbial density and enhance discrete colony genesis, which culminated in axenic clonal proliferation. Discrete monoclonic bacterial propagules, identified by colonial morphology on primary isolation plates, were then subcultured onto solidified nutrient agar (peptone 5 g/L, beef extract 3 g/L, NaCl 5 g/L, agar 15 g/L; autoclaved at 121°C, 15 psi for 20 min) to yield pure cultures amenable to phenotypic and genotypic characterization (Bonnet et al., 2020), as shown in Fig. 2.

Nutrient agar is a non-selective, general-purpose basal medium that promotes the growth of non-fastidious, heterotrophic soil bacterioplankton by providing the necessary peptic hydrolysates, osmotic equilibrium, and gelling matrix for spatial clonal development. This paradigm is consistent with conventional pour-plate or streak-plate methods for extracting cultivable species from rhizospheric consortia, preventing overgrowth by dominant opportunists (Nguyen et al., 2018).

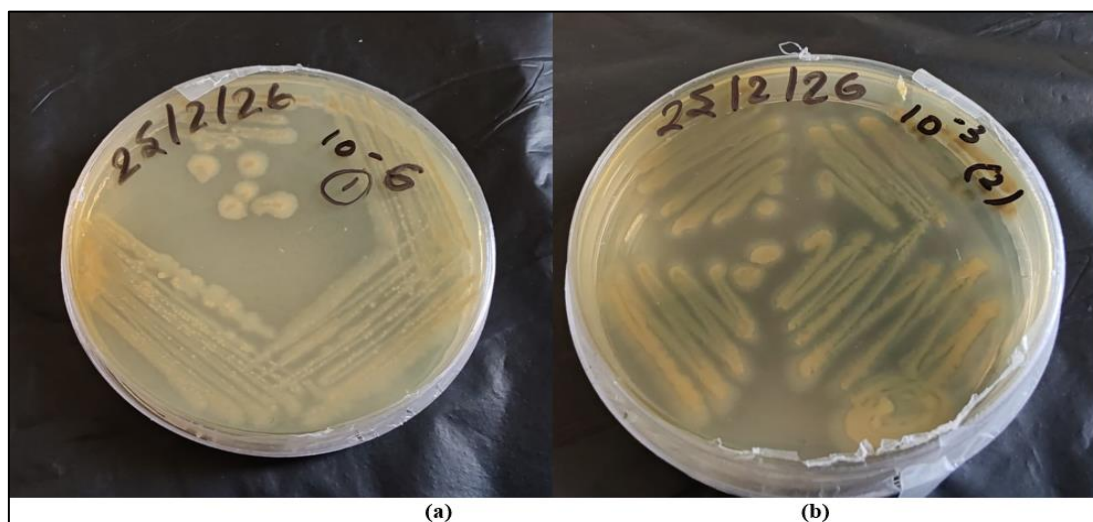


Fig 2 Bacterial Isolates from Different Dilution of Soil Sample (a)  $10^{-6}$  (b)  $10^{-3}$

➤ *Identification of Isolated Microbes*

• *Identification of Bacteria*

The recovered bacterial propagules appeared as opaque, alabaster-colored colonies on nutrient agar, demonstrating strong proliferative capacity under mesophilic conditions (37°C, 24-48 h). After purification, Gram-positive affirmation was performed using a differential staining protocol that included primary crystal violet impregnation (1% w/v, 60 s), iodine mordant fixation (1:2 Lugol's, 60 s), acetone-alcohol decolorization (95% ethanol, 10-20 s) and safranin counterstain (0.5% w/v, 45 s), which resulted in consistent retention of the violet chromophore due to the

multilayered peptidoglycan lattice (20-80 nm thick, 50-90% cell wall dry mass). Microscopic examination (1000× oil immersion) revealed elongated, cylindrical rods (0.5-1.2 × 3-5 μm) as typical Gram-positive bacilli (Smith et al., 2018), depicted as amethyst clusters in Fig. 3.

This phenotypic constellation is consistent with ubiquitous soil saprophytes (e.g., *Bacillus spp.*), in which teichoic acid anchors and cross-linked glycan strands protect structural integrity from osmotic lysis and phagocytic intrusion. Colonial opacity is caused by internal poly-β-hydroxybutyrate accumulation and extracellular matrix deposition, distinguishing it from Gram-negative translucence (Lu et al., 2018).

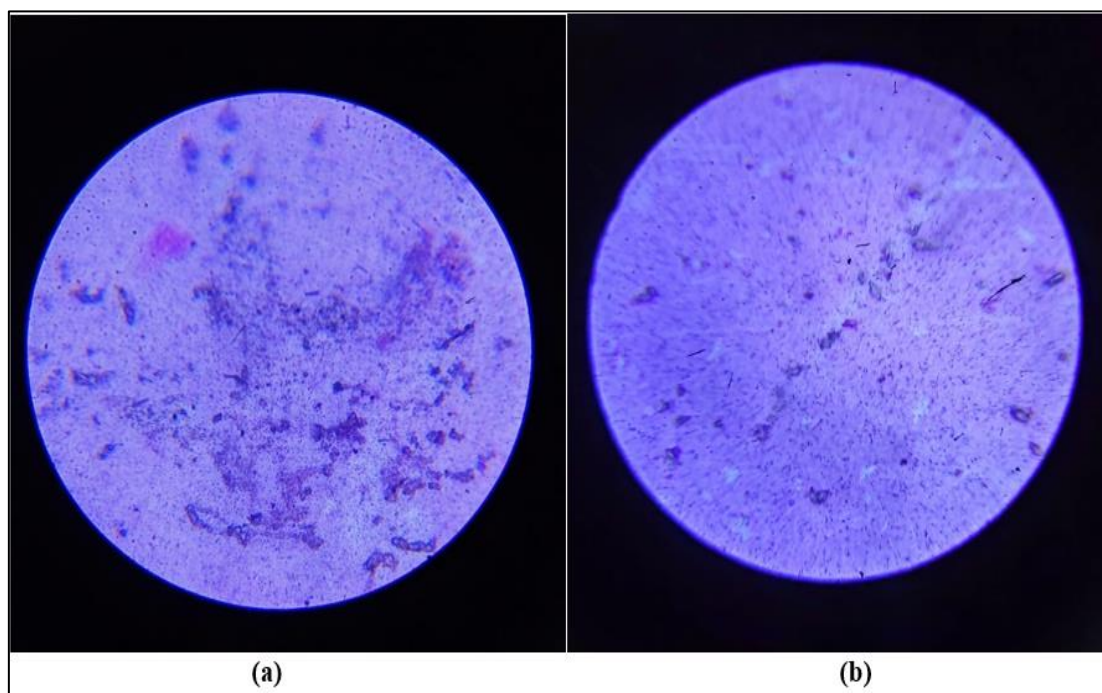


Fig 3 Purple Bacterial Cells were Observed Microscopically After Gram Staining (a)  $10^{-6}$  (b)  $10^{-3}$

➤ *Extraction of Essential Oil*

Using a Soxhlet extractor and commercial ethanol, the solvent extraction procedure was carried out for two, three, and four hours at various temperatures of 50, 60, and 70°C. A preliminary series of trials determined the temperature and time combination. To lower the error, three replicates were performed for each of the nine treatments. 50 g of garlic powder were used at a sample to solvent ratio of 1:20 Fig 3. After extraction the solvent was removed using a rotary evaporator equipped with a condenser and a rotating round-bottom flask under reduced pressure. The evaporation was carried out at a controlled water bath temperature of approximately 55°C and a rotation speed of around 45 rpm. This setup facilitated efficient removal of ethanol at lower temperatures thereby preserving the heat-sensitive bioactive compounds present in garlic oil. The concentrated extract obtained was further stored at 4°C for subsequent analysis (Dehariya et al., 2021) as depicted in Fig. 4 and Fig. 5.



Fig 4 Soxhlet Extractor Setup for Garlic Oil Extraction



Fig 5 Rotary Evaporator for Solvent Removal

➤ *Yield of Garlic Oil*

The yield of garlic oil obtained in this study was assessed using a standardized Soxhlet extraction process in which garlic powder was exhaustively extracted with ethanol (1:20, w/v) and the solvent was then evaporated under decreased pressure using a rotary evaporator. The percentage yield of garlic oil was measured as the ratio of the final concentrated oil to the original mass of dried garlic powder, given as a percentage. This metric is commonly used to evaluate the effectiveness of lipid and essential oil extraction from plant matrices. In this study, about 50 g of garlic powder was utilized per batch, allowing for a consistent calculation of yield across varied extraction conditions (El-Saber Batiha et al., 2020). Soxhlet extraction efficiency is determined by a number of physicochemical and operational parameters, including extraction duration, temperature, solvent type, solvent-powder ratio and the starting material's physical qualities (particle size and moisture content). This study found that higher extraction temperatures and longer durations improve oil recovery by increasing solvent penetration and diffusion of lipid-soluble chemicals from the plant matrix into the extracting phase. Excessive heating can degrade, oxidize, or isomerize heat-sensitive organosulfur constituents, such as alliin-derived sulfides. This reduces the concentration of bioactive components and may alter the oil's odor, color and biological activity (Tudu et al., 2022) as shown in Fig. 6.

$$\text{Yield (\%)} = \frac{\text{Mass of concentrated garlic oil (g)}}{\text{Initial mass of dried garlic powder (g)}} \times 100$$

$$\text{Yield (\%)} = \frac{14.793 \text{ g}}{50 \text{ g}} \times 100$$

$$\text{Yield (\%)} = 29.58\%$$

To balance production and quality, an optimal extraction methodology was adopted, based on early studies that tracked oil yield and selected marker compounds across several temperature-time combinations. Soxhlet extraction with ethanol is a reliable and scalable method for isolating lipid-rich garlic fractions, especially for obtaining concentrated oil for downstream applications like nano-formulation or functional-food enrichment. The recovered oil was kept at 4°C in dark, airtight containers to prevent oxidative and thermal deterioration and preserve its chemical integrity for future physicochemical research and nanoparticle formation studies (Gupta et al., 2024).



Fig 6 Extracted Garlic Oil Sample After Soxhlet Extraction

➤ *Physicochemical Properties of Garlic Oil*

In this study, garlic oil extracted from *Allium sativum* appeared as a dark brown to nearly black viscous liquid, indicating a high concentration of lipid-soluble constituents and the presence of co-extracted phenolic or Maillard-type products, which may contribute to the intensity of coloration. The sample had a pungent odor similar to garlic-derived essential oils, suggesting the presence of volatile organosulfur compounds like alliin and its breakdown products e.g., diallyl disulfide (Bhatwalkar et al., 2021) as shown in Fig. 7.

The oil was found to be hydrophobic, insoluble in water but miscible in organic solvents like ethanol, chloroform, and hexane, indicating it is a non-polar lipid-rich phase. The oil's density ranged between 0.89 and 0.92 g/cm<sup>3</sup>, somewhat lower than water and commensurate with previously reported values for garlic-derived oils. The dark hue of the sample may indicate partial oxidation or mild thermal degradation during extraction or storage, a common occurrence for heat-sensitive garlic-derived compounds also shown in Fig. 8 (Lemma et al., 2022).

The oil had a refractive index of 1.56-1.58, which is within the typical range reported for garlic essential oils and indicates the presence of terpenoids, alkenyl sulfides and unsaturated fatty acid components. The physicochemical profile of garlic oil, including viscosity, color, density, solubility behavior, and refractive index, supports its

potential suitability as a reducing and stabilizing agent in green-synthesis-based nanoparticle formulations. Sulfur-containing functional groups can participate in metal ion reduction and nanoparticle capping (Sarangi et al., 2024).



Fig 7 Extracted Garlic Oil Showing Dark Brown Viscous Appearance



Fig 8 Solubility of Garlic Oil in Water and Ethanol

#### ➤ Synthesis of Nanoparticles Using Garlic Oil

Garlic essential oil (GEO) derived from *Allium sativum* was effectively used to create silver nanoparticles (AgNPs) utilizing green chemistry. Visual inspection and a comparison of experimental and control settings were used to assess the development of AgNPs.

After 24 hours of incubation at room temperature, the reaction combination of acetone, garlic essential oil (GEO) and 10 mM silver nitrate ( $\text{AgNO}_3$ ) gives a light yellow to yellowish-brown color as shown in Fig. 9. The color change from an initially colorless solution demonstrates the successful production of silver nanoparticles (AgNPs). The yellowish-brown appearance of AgNPs is caused by the reduction of silver ions ( $\text{Ag}^+$ ) into metallic silver ( $\text{Ag}^0$ ) and the activation of surface plasmon resonance (SPR), a unique optical property. The significantly clearer and more uniform color indicates improved nanoparticle formation and dispersion in the presence of acetone and GEO phytochemicals (Bouqellah et al., 2019).



Fig 9 AgNPs Synthesis with Garlic Oil and Acetone (Yellowish-Brown Color)

In contrast, the reaction mixture including garlic essential oil and  $\text{AgNO}_3$  without acetone appeared darker brown and more turbid as shown in Fig. 10. Although color development was seen, indicating that nanoparticle formation continued due to GEO's reducing activity, the solution was cloudy and uneven. This shows that nanoparticle production is less regulated and may aggregate in the absence of acetone. Garlic essential oil alone can reduce  $\text{Ag}^+$  ions, but adding acetone improves the reaction environment, resulting in better dispersion and stability of produced nanoparticles.

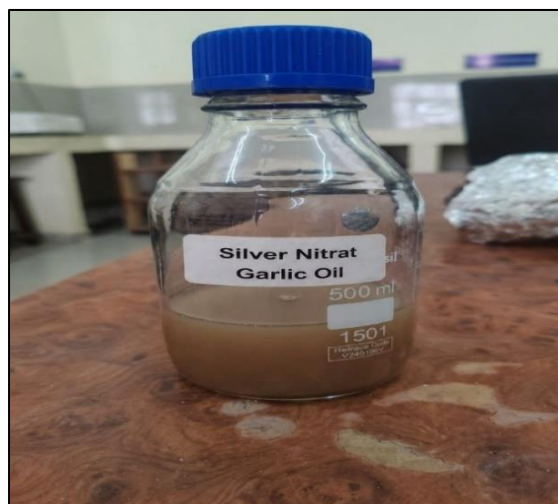


Fig 10 AgNPs Synthesis with Garlic Oil (Brown Color).

After incubation, both reaction mixtures were filtered through Whatman filter paper to separate solid particles from the liquid phase. A clearly visible residue was left on the filter paper in the experimental sample, indicating the presence of produced AgNPs and their associated organic matrix. This residue was carefully collected, transported to sterile test tubes and stored for further physicochemical analysis. In the control sample, there was little or no residue on the filter paper, corroborating the finding that nanoparticle formation was dependent on the presence of garlic essential oil as shown in Fig. 11.



Fig 11 Comparative Filtration of Silver Nanoparticles Synthesized Using Garlic Oil with and Without Acetone

Garlic essential oil acts as a bioreducing agent, transforming  $Ag^+$  ions into metallic  $Ag^0$  nanoparticles. Furthermore, the stability of the ensuing colloidal dispersion and the creation of a unique residue indicate that the phytochemical components of GEO help to stabilize the nanoparticles, most likely through adsorption onto the nanoparticle surface and inhibition of aggregation. Comparing experimental and control setups highlights the importance of plant-derived bioactive chemicals, namely organosulfur compounds like diallyl trisulfide, in the green production of silver nanoparticles as shown in Fig. 12 (Abdelkhalig et al., 2025).



Fig 12 Comparative Deposition of AgNPs Using Garlic Oil Extract (with or Without Acetone) (a) and (b)

➤ *Characterization of Nanoparticles*

The UV-Visible spectrophotometric examination of silver nanoparticles (AgNPs) generated using a green chemistry approach based on garlic oil revealed a specific absorption pattern, with a maximum absorbance peak observed at 420 nm within the 400-450 nm scan range as summarized in Table 1. This localized surface plasmon resonance (LSPR) peak is a defining feature of metallic nanostructures, caused by the collective oscillation of conduction electrons in silver nanoparticles when activated by incident electromagnetic radiation. The peak's particular placement at 420 nm strongly implies the creation of almost spherical silver nanoparticles, as deviations towards longer wavelengths are often associated with bigger particle sizes or considerable aggregation (Agustina et al., 2021).

The intensity and profile of the absorption spectrum provide information about the efficiency of the synthesis process. The existence of a prominent, well-defined peak at 420 nm suggests that garlic oil acts as both a reducing agent, converting silver ions to their metallic state and a strong stabilizing ligand, preventing uncontrolled agglomeration. Furthermore, the progressive drop in absorbance beyond the 420 nm maxima indicates a relatively stable as depicted in Fig. 13, moderately dispersed colloidal solution, which is consistent with previous research demonstrating the efficiency of *Allium sativum*-derived extracts in creating stable, biocompatible nanoparticles (Ali et al., 2023).

Table 1 Spectral Absorbance Values of AgNPs at Different Wavelengths

S. No.	Wavelength ( $\lambda$ )	Absorbance (nm)
1.	400	1.687
2.	410	1.310
3.	420	1.753

4.	430	1.526
5.	440	1.379
6.	450	1.256

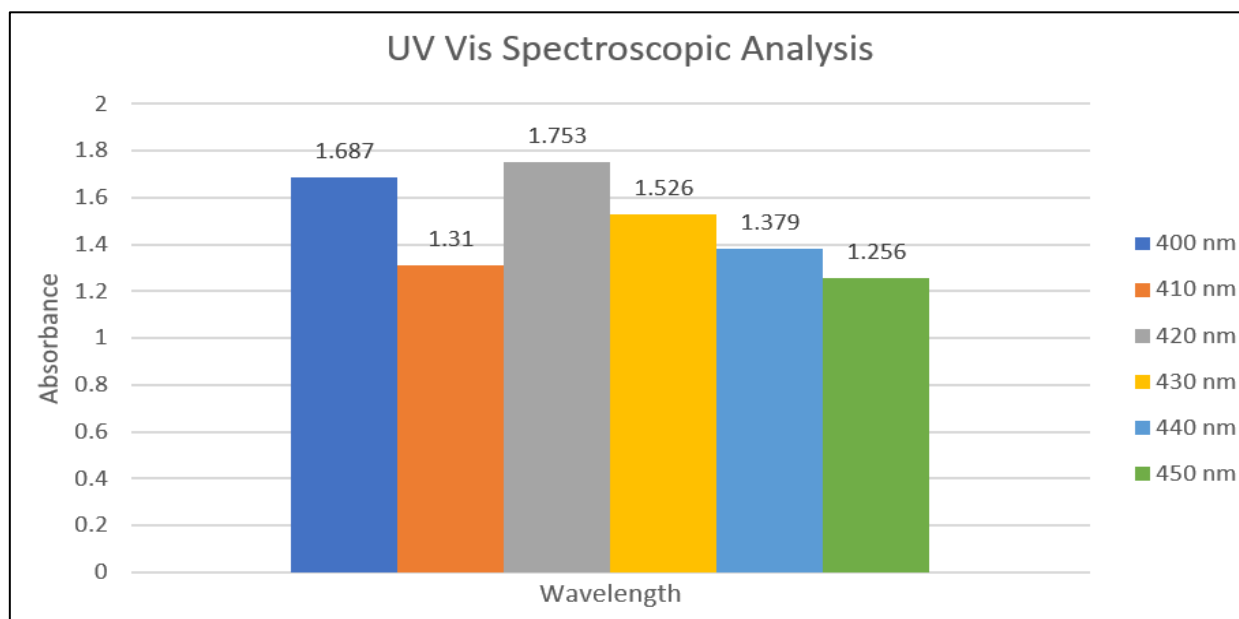


Fig 13 UV- Spectrum Showing Formation of Silver Nanoparticles Using Garlic Oil

**IV. CONCLUSION**

The extraction of garlic essential oil (GEO) from *Allium sativum* and its use in the environmentally friendly manufacture of silver nanoparticles (AgNPs) were successfully proven in this study. A notable oil yield of 29.58% was obtained utilizing the Soxhlet extraction method with ethanol, showing effective recovery of bioactive chemicals. The existence of organosulfur compounds like allicin and its derivatives was confirmed by the extracted oil's distinctive physicochemical characteristics, which included hydrophobicity, a dark brown hue, and a strong odor. A noticeable color shift from colorless to yellowish-brown, which is explained by surface plasmon resonance, verified the biogenesis of AgNPs. A clear absorption peak at 420 nm was shown by additional validation using UV-visible spectroscopy, suggesting the creation of stable and mostly spherical nanoparticles.

The study also demonstrated how reaction circumstances affect the creation of nanoparticles, showing that the presence of acetone enhanced stability and dispersion while its absence caused aggregation. Gram staining and microbiological isolation verified the existence of Gram-positive bacteria, indicating the possible antibacterial significance of the produced nanoparticles. Overall, this work shows that the synthesis of AgNPs via garlic essential oil is a sustainable, economical, and environmentally benign method. The produced nanoparticles contribute to the development of green nanotechnology by showing encouraging physicochemical properties and having potential uses in the pharmaceutical, biomedical and antibacterial industries.

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