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Effect of Reaction Conditions on the Green Synthesis of Silver Nanoparticles Using Aqueous Extract of Rosmarinus officinalis L. and Evaluation of Antimicrobial Activity.

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Index Terms

Green synthesis, Silver nanoparticles, Plant-mediated synthesis, Rosmarinus officinalis, Antimicrobial activity

Abstract

Green synthesis offers an environmentally friendly route to silver nanoparticles (AgNPs). In the present study, AgNPs were synthesized using an aqueous extract of rosemary (Rosmarinus officinalis L.) collected from Al-Marj, eastern Libya, in August 2019. The effects of $AgNO_3$ concentration, extract concentration, pH, reaction time and temperature were examined, using the intensity of the surface plasmon resonance (SPR) band (λ_{max} 430 nm) as the response. Among the values tested, the highest SPR absorbance was obtained at 2.5 mM $AgNO_3$, an extract-to-water ratio of 1:1, pH 7 and 210 min; SPR intensity increased with temperature over 25–55 °C. FT-IR spectra of the extract and the AgNPs showed shifts of the O–H (3294 → 3175 cm^{-1}), aromatic C=C (1590 → 1583 cm^{-1}) and C–O (1030 → 1012 cm^{-1}) bands, suggesting the involvement of phenolic and other oxygen-containing groups in reduction and capping. Antimicrobial activity was assessed by disc diffusion (n = 3). The extract showed no inhibition zone against Escherichia coli or Candida albicans, whereas the AgNPs produced zones of 13.8 ± 0.81 mm and 13.5 ± 0.75 mm, respectively. The highest activity against E. coli and Candida albicans was at 9400 mg/L. The study is limited by characterization with UV–Vis and FT-IR only, so particle size, shape, crystallinity and stability were not determined; by the study design, which does not assess interactions between parameters; and by the use of one bacterial and one fungal strain without MIC/MBC determination.

تأثير ظروف التفاعل على التخليق الأخضر لجسيمات الفضة النانوية باستخدام المستخلص المائي لنبات إكليل الجبل وتقييم النشاط المضاد للميكروبات

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الكلمات المفتاحية

التخليق الأخضر؛ جسيمات الفضة النانوية؛ التخليق بوساطة النباتات؛ إكليل الجبل؛ النشاط المضاد للميكروبات.

الملخص

يُعدّ التخليق الأخضر طريقة صديقة للبيئة لإنتاج جسيمات الفضة النانوية (AgNPs). خُلِّقت الجسيمات في هذه الدراسة باستخدام المستخلص المائي لنبات إكليل الجبل (Rosmarinus officinalis L.) المجموع من مدينة المرج شرق ليبيا في أغسطس 2019. دُرس تأثير تركيز $AgNO_3$ وتركيز المستخلص ودرجة الحموضة وزمن التفاعل ودرجة الحرارة بطريقة تغيير عامل واحد في كل مرة، باعتماد شدة نطاق رنين البلازمون السطحي (SPR) مؤشراً (λ_{max} 430 نانومتر). وبين القيم المختبرة، سُجّلت أعلى امتصاصية عند تركيز 2.5 mM من $AgNO_3$ ونسبة مستخلص إلى ماء 1:1 وpH 7 وزمن 210 دقيقة، بينما ازدادت شدة SPR مع ارتفاع الحرارة ضمن المدى 25–55 °م. أظهرت أطياف FT-IR للمستخلص وللجسيمات انزياح نطاقات O–H (من 3294 إلى 3175 cm^{-1}) وC=C العطري (من 1590 إلى 1583 cm^{-1}) وC–O (من 1030 إلى 1012 cm^{-1})، مما يشير إلى مشاركة المجموعات الفينولية والمجموعات الأخرى الحاوية على الأكسجين في الاختزال والتغليف. وقُيّم النشاط المضاد للميكروبات بطريقة الانتشار في الأقراص (3 مكررات). لم يُظهر المستخلص أي منطقة تثبيط ضد Escherichia coli وCandida albicans، في حين أعطت AgNPs مناطق تثبيط قدرها 13.8 ± 0.81 مم و 13.5 ± 0.75 مم على التوالي. كان أعلى نشاط ضد E. coli و Candida albicans عند 9400 ملجم/لتر. من حدود الدراسة اقتصار التوصيف على UV–Vis وFT-IR، فلم يُحدَّد حجم الجسيمات وشكلها وبلوريتها وثباتها؛ وتصميم الدراسة الذي لا يقيّم التفاعل بين العوامل؛ والاقتصار على سلالة بكتيرية وأخرى فطرية دون تحديد MIC/MBC.

1. Introduction

Nanotechnology has emerged as one of the fastest-growing fields in modern science, with far-reaching implications for medicine, agriculture, environmental science, and materials engineering. Nanotechnology is fundamentally based on manipulating matter at the nanoscale (1–100 nm), where materials exhibit unique physical and chemical properties that differ significantly from their larger counterparts. Among the various nanomaterials studied to date, metallic nanoparticles have garnered considerable scientific attention due to their excellent optical, electronic, catalytic, and biological properties (Aminzai et al., 2024; Hada et al., 2025). In particular, silver nanoparticles (AgNPs) have been the subject of intensive research due to their broad antimicrobial activity, low toxicity, and versatility in biomedical and industrial applications. Silver nanoparticles are currently used in many different sectors, including wound dressings, medical devices, water purification systems, textiles, food packaging, and personal care products (Almatroudi, 2024).

Traditional methods for manufacturing silver nanoparticles (AgNPs) can be broadly categorized into two main approaches: physical and chemical. Physical methods, such as laser ablation, evaporation, condensation, and ball milling, produce nanoparticles with precisely defined shapes; however, they are energy-intensive, require sophisticated equipment, and have limited scalability (Bamal et al., 2021; Sati et al., 2025). Chemical reduction methods, while widely used due to their relative simplicity and high productivity, rely on hazardous reducing and stabilizing agents—including sodium borohydride, hydrazine, and various organic solvents—posing significant risks to human health and the environment. Furthermore, the use of these reagents can negatively impact the biocompatibility of the resulting nanoparticles, limiting their application in medical settings (Szczyglewska et al., 2023). These limitations have prompted the scientific community to seek more sustainable and environmentally friendly alternatives for nanoparticle manufacturing (Naganthran et al., 2022).

Biosynthesis, also known as green synthesis, represents a sustainable model for producing nanoparticles using living organisms such as plant extracts, fungi, bacteria, and algae. Among these methods, phytosynthesis has received the most attention due to its simplicity, cost-effectiveness, and ability to produce nanoparticles naturally coated with plant-based chemical components, thus enhancing their biocompatibility and stability (Villagrán et al., 2024). Plant extracts are rich in secondary metabolites—including polyphenols, flavonoids, terpenoids, alkaloids, and organic acids—which simultaneously act as reducing agents, converting silver ions (Ag^{+}) to silver atoms (Ag^{0}), and as coating agents that stabilize the resulting nanoparticles and prevent them from clumping (Ullah et al., 2023; Najah & Elsherif, 2016). A growing body of studies has demonstrated the successful biosynthesis of silver nanoparticles using extracts derived from various plant species, including aloe vera, holy basil, neem, aegle marmelos, citrus limon, and others, with the resulting nanoparticles exhibiting antimicrobial, antioxidant, and anticancer properties (Ghatage et al., 2023; Dutt et al., 2023; Rama et al., 2023; Khane et al., 2022).

Rosemary (Rosmarinus officinalis L.), a widely distributed aromatic herb belonging to the mint family (Lamiaceae), is native to the Mediterranean region and is widely cultivated for its nutritional, cosmetic, and medicinal properties (Atiya et al., 2024; Elsherif et al., 2023a). This plant is known to be a rich source of bioactive phytochemicals, including rosmarinic acid, carnosic acid, carnosol, ursolic acid, caffeic acid, and numerous flavonoids (Pomi et al., 2023). These compounds possess well-documented antioxidant, anti-inflammatory, antimicrobial, and neuroprotective properties, making rosemary a particularly attractive candidate for the green synthesis of metal nanoparticles. Phytochemical studies have confirmed that the aqueous extract of rosemary contains abundant hydroxyl-bearing phenolic compounds capable of donating electrons to reduce metal ions under mild reaction conditions (Lešnik et al., 2021). Furthermore, the natural polyphenolic matrix provided by the extract can act as an effective stabilizing coating around the synthesized nanoparticles, giving them colloidal stability under physiological and environmental conditions (Lithi et al., 2025).

Green synthesis of silver nanoparticles (AgNPs) mediated by Rosmarinus officinalis (rosemary) has garnered substantial scientific interest as an eco-friendly alternative to conventional physical and chemical techniques (Elmehalawy et al., 2025; Ghaedi et al., 2015; Sganzerla et al., 2023). The plant's dual utility as an effective bio-reductant and stabilizing capping agent is primarily attributed to its rich pool of secondary metabolites, including polyphenols, flavonoids, and diterpenes such as rosmarinic acid, carnosic acid, and carnosol (Elmehalawy et al., 2025; Ghaedi et al., 2015). In an early investigation, Das and Velusamy (2014)

developed a rapid green approach yielding spherical, face-centered cubic (FCC) AgNPs (14.20–42.42 nm) within 15 min at 60 °C, confirming that flavonoid and terpenoid functional groups were responsible for stabilizing the nanoparticles and conferring strong bactericidal efficacy against *Pseudomonas aeruginosa* and *Staphylococcus aureus*6unfold. Ghaedi et al. (2015) successfully synthesized spherical AgNPs (10–33 nm, surface plasmon resonance [SPR] centered at 450 nm) at room temperature via phenolic reduction, demonstrating significant broad-spectrum antibacterial and antifungal activities. Beyond microbial pathogens, Farghaly and Nafady (2015) biosynthesized crystalline FCC AgNPs (~17 nm, SPR at 427 nm) and characterized their agro-physiological impacts on tomato and wheat seedlings, noting concentration-dependent changes in biomass, chlorophyll retention, lipid peroxidation (malondialdehyde), and antioxidant enzymes. Furthermore, Daghestani et al. (2020) utilized aqueous leaf extracts of desert-cultivated rosemary to synthesize spherical nanosilver (12–22 nm, SPR at 400 nm), confirming potent bactericidal effects against eight human pathogens alongside pronounced in vitro cytotoxicity against human breast cancer cells (MDA-MB-231). Expanding beyond leaf infusions, Sganzerla et al. (2023) employed rosemary essential oil—predominated by α-pinene, 1,8-cineole, and bornyl acetate—to biosynthesize highly stable spherical AgNPs (<50 nm, zeta potential of approximately −30 mV), successfully incorporating them into biopolymer nanocomposite films for active antimicrobial food preservation. More recently, Elmehalawy et al. (2025) confirmed the multifunctional biological activities of rosemary-synthesized AgNPs (average diameter 60.5 nm, SPR at 425 nm), documenting marked inhibitory activity against multidrug-resistant (MDR) clinical strains, potent DPPH radical scavenging (EC_{50} = 7.81 g/mL), antidiabetic α-amylase and α-glucosidase inhibition, and selective cytotoxicity toward malignant cells

The physicochemical properties, yield, and colloidal stability of green-synthesized silver nanoparticles are exceptionally sensitive to the underlying reaction conditions (Velgosova et al., 2019). Essential reaction parameters—namely precursor salt concentration, plant extract ratio, solution pH, reaction temperature, and contact time—fundamentally regulate reduction kinetics, the ionization behavior of reducing phytochemicals, nucleation dynamics, and the electrostatic repulsion of the capping layer (Ghasemi et al., 2024; Liaqat et al., 2022). Velgosova et al. (2025) demonstrated that reaction pH plays a decisive role during synthesis; a slightly alkaline medium (pH 8) optimized localized surface plasmon resonance intensity, suppressed polydispersity (~17.5 nm), and maintained colloidal stability for over 30 days, whereas extreme acidic (pH ≤ 3) or strongly alkaline (pH ≥ 13) regimes triggered rapid agglomeration and precipitation. Furthermore, temperature modulates reaction velocity and reduction efficiency; while moderate heating accelerates synthesis without altering particle morphology, excessive thermal exposure can induce agglomeration or destabilize thermal-sensitive phytomolecules (Velgosova et al., 2025). Multi-parameter evaluations by Liaqat et al. (2022) corroborated that systematically adjusting silver nitrate concentration (optimum 1 mM), extract volume, reaction time, and temperature (optimum 75 °C) is vital to control particle size, achieve high negative zeta potential values that prevent coalescence, and minimize cytotoxicity. In addition, statistical modeling utilizing Response Surface Methodology (RSM) with Central Composite Design (CCD) conducted by Ghasemi et al. (2024) revealed that reaction variables do not act independently; rather, strong cooperative interaction effects—particularly between metal precursor concentration and extract percentage—govern nanostructure formation and yield. Likewise, preliminary parameter evaluations by Elmehalawy et al. (2025) confirmed that variations in pH (peaking at pH 9), extract-to-water ratio (9:1), and mixing duration (3 h) directly determined the extent of Ag^{+} reduction and protected the resulting nanocolloids against aggregation.

Despite the growing number of reports on the biosynthesis of AgNPs using Rosmarinus officinalis, the influence of reaction conditions on the process has been examined only partially. Many rosemary studies focused on the antimicrobial, cytotoxic or agricultural effects of nanoparticles prepared under a single, fixed set of conditions (Farghaly & Nafady, 2015; Ghaedi et al., 2015; Sganzerla et al., 2023). Where reaction parameters were investigated, only a few variables were covered, for example pH and temperature (Velgosova et al., 2025), or pH, extract-to-water ratio and mixing time (Elmehalawy et al., 2025). In other plant systems, multivariate designs such as response surface methodology have shown that variables such as precursor concentration and extract percentage interact (Ghasemi et al., 2024), but comparable data are not available for rosemary. Moreover, the optimum values reported for green AgNP synthesis vary widely, with pH values from 7 to 9 and temperatures from ambient to 75 °C (Elmehalawy et al., 2025; Liaqat et al., 2022; Velgosova et al., 2025), which makes protocols

difficult to compare and reproduce. To the best of our knowledge, a side-by-side evaluation of extract concentration, $AgNO_3$ concentration, pH, reaction time and temperature for aqueous rosemary extract, combined with a functional antimicrobial test of the product, is still lacking. A one-factor-at-a-time evaluation defines the working range of each variable and provides the baseline required for subsequent multivariate optimization.

This study aimed to synthesize silver nanoparticles using an aqueous extract of rosemary (Rosmarinus officinalis L.) as a reducing and stabilizing agent, and to examine how reaction conditions affect their formation. The effects of extract concentration, $AgNO_3$ concentration, pH, reaction time and temperature were evaluated one factor at a time using UV–Vis spectroscopy, and the functional groups involved in reduction and capping were identified by FT-IR spectroscopy. The antimicrobial activity of the extract and of the biosynthesized AgNPs was then evaluated by the disc diffusion method against Escherichia coli, a Gram-negative bacterium, and Candida albicans, a pathogenic yeast, both obtained from clinical samples. These two organisms were selected as clinically relevant representatives of a bacterium and a fungus, to confirm the bioactivity of the synthesized product. The results provide a baseline for future multivariate optimization.

2. Materials and methods

2.1 Sample collection and preparation

Fresh leaves of Rosmarinus officinalis L. were collected from Al-Marj city, eastern Libya, in August 2019. The plant was identified at the Department of Botany, Faculty of Science, Misurata University. The leaves were washed with tap water, rinsed with deionized water, air-dried in the shade for 15 days, and ground to a fine powder, which was stored in airtight, opaque glass bottles in a dry place until use (Villagrán et al., 2024; Elsherif et al., 2023b).

2.2 Preparation of aqueous plant extract

5.0 g of plant powder were mixed with 100 mL of ion-free distilled water and boiled for 10 min until the color changed from green to yellowish-green. The solution was cooled, filtered, and centrifuged at 3500 rpm for 5 minutes to remove heavy biological particles. The supernatant was further filtered through Millipore filter paper to obtain the final extract used for nanoparticle synthesis (Alkherraz et al., 2026).

2.3 Biosynthesis of silver nanoparticles (AgNPs)

Silver nanoparticles were synthesized by adding 50 mL of silver nitrate solution at various concentrations to 5 mL of the plant extract. Color changes observed after one hour indicated nanoparticle formation. This was confirmed by measuring the UV-Vis absorption spectrum in the range of 300–600 nm (Farhan et al., 2017).

2.4 Factors affecting AgNPs formation

The effect of each parameter was evaluated independently using a one-factor-at-a-time (OFAT) approach around a common baseline condition, in which only the parameter under study was varied. The values found for one parameter were therefore not carried over to the other experiments. All experiments were performed in triplicate (n = 3).

2.4.1 Effect of extract concentration

Different concentrations of aqueous plant extracts were prepared in ratios of 1:1, 1:2, 1:3, and 1:3 (plant extract : ion-free water). For each concentration, 2.5 mL of extract solution was mixed with 25 mL of 1 mM silver nitrate solution. The mixtures were incubated for one hour, and their UV-Vis absorption spectra were recorded (Omar et al., 2018).

2.4.2 Effect of silver nitrate concentration

To study the effect of silver nitrate concentration, 2.5 mL of plant extract was added to 25 mL of silver nitrate solutions at concentrations of 0.075, 0.75, 1.0, 2.5, and 5 mM. The mixtures were incubated at room temperature for one hour, followed by UV-Vis spectral analysis (Ghasemi et al., 2024).

2.4.3 Effect of temperature

The influence of temperature on AgNPs synthesis was evaluated by incubating mixtures of 2.5 mL plant extract and 25 mL of 1 mM silver nitrate at different temperatures (25 – 55 ºC) for 60 min. The solutions were placed in sealed flasks to prevent evaporation and heated in a water bath to the desired temperatures. UV-Vis absorption measurements were taken to monitor nanoparticle formation (Alkherraz et al., 2026).

2.4.4 Effect of reaction time

Three solutions were prepared by mixing 2.5 mL of plant extract with 25 mL of 1 mM silver nitrate. UV-Vis absorption spectra were recorded every 30 min using a UV spectrophotometer to determine the optimal reaction time for nanoparticle synthesis (Khleifat et al., 2022).

2.4.5 Effect of pH

Six solutions were prepared by mixing 2.5 mL of plant extract with 25 mL of 1 mM silver nitrate. The

pH of the solutions was adjusted to 3, 4, 6, 7, 9, and 11 using 0.1 M nitric acid and 0.1 M sodium hydroxide. The solutions were incubated for 60 min, and UV-Vis absorption was recorded to evaluate the impact of pH on nanoparticle synthesis (Alfaid et al., 2024).

2.5 Separation and purification of nanoparticles

The synthesized silver nanoparticles were separated by centrifugation at 15,000 rpm for 30 minutes. The supernatant was discarded, and the pellet was washed multiple times, dried at 60 ºC, and collected in tubes for further use (Farhan et al., 2017).

2.6 Characterization of plant extracts and silver nanoparticles using UV-Vis Spectroscopy

The formation of silver nanoparticles was confirmed by measuring the absorption spectra of plant extracts before and after silver nitrate addition using Agilent Technologies Cary 60 UV-Vis Spectrophotometer over approximately 4 hours to monitor the development of AgNPs.

2.7 Characterization of plant extracts and silver nanoparticles using Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR spectra were recorded on a PerkinElmer spectrometer equipped with a Universal ATR accessory over 4000–650 cm^{-1} at a resolution of 0.5 cm^{-1}. The extract was analysed in its dried form.

2.8 Preparation of concentrations for biological activity assays

Silver nanoparticles synthesized from plant extract was weighed after centrifugation to determine their concentration in mg/L. The concentrations were calculated based on the nanoparticle weight and volume. Corresponding concentrations of plant extracts were prepared to match the nanoparticle concentrations for comparative biological testing.

2.9 Biological activity evaluation

The antimicrobial effects of the plant extract and its corresponding silver nanoparticles were evaluated against one bacterium, Escherichia coli, and one yeast, Candida albicans. Both microorganisms were obtained, already identified, from the Misurata Central Laboratory; the E. coli isolate originated from a urine sample of a patient with a urinary tract infection. The bacterial isolate was activated by repeated subculturing in nutrient broth and on blood agar, and the yeast was grown in 4 mL of Sabouraud dextrose broth at 37 °C for 24 h (Alkherraz et al., 2026).

2.9.1 Preparation of culture media

Mueller–Hinton (MH) agar (38 g/L) was used for E. coli and Sabouraud dextrose agar (SDA) for C. albicans. Both media were sterilized by autoclaving at 121 °C (15 psi) for 15 min, poured into sterile 9-cm Petri dishes under aseptic conditions, and stored in a refrigerator until use (Lithi et al., 2025).

2.9.2 Preparation of inocula

E. coli was subcultured on nutrient agar at 37 °C for 24 h to obtain fresh colonies. Colonies of each microorganism were suspended in sterile physiological saline, vortexed, and the turbidity was adjusted to the 0.5 McFarland standard. A sterile cotton swab was dipped into each suspension and used to streak the surface of MH agar (E. coli) or SDA (C. albicans) in overlapping lines, repeated three times with rotation of the plate. After inoculation, the plates were left to dry for 15 min before the discs were applied (CLSI, 2006; CLSI, 2017; Liaqat et al., 2022).

2.9.3 Disc Diffusion Method

Sterile Whatman filter-paper discs (5 mm in diameter) were impregnated with 20 µL of the AgNP suspension (dispersed in distilled water) or the plant extract at the stated concentrations. Each plate carried three discs: AgNPs, plant extract, and a negative control disc soaked in sterile ion-free water. The discs were placed on the inoculated plates under aseptic conditions, and the plates were incubated inverted at 37 °C for 24 h. Inhibition-zone diameters were measured manually in mm. Each treatment was tested in triplicate (Liaqat et al., 2022).

3. Results and Discussion

3.1. Effect of Reaction Conditions on the Synthesis of Silver Nanoparticles Using Rosmarinus officinalis Aqueous Extract

Silver nanoparticles (AgNPs) were synthesised via a green approach using the aqueous extract of Rosmarinus officinalis L. as both a reducing and stabilising agent. The formation of AgNPs was initially indicated by the gradual colour change of the reaction mixture from colourless (or pale yellow) to yellowish-brown and finally dark brown, reflecting the reduction of Ag^{+} ions into metallic silver (Ag^{0}). This colour development is attributed to the excitation of surface plasmon resonance (SPR), a characteristic optical phenomenon exhibited by silver nanoparticles (Ghatage et al., 2023).

The formation of silver nanoparticles (AgNPs) was monitored using UV–Visible spectroscopy by

examining the characteristic surface plasmon resonance (SPR) absorption band. AgNPs typically exhibit an SPR band within the wavelength range of approximately 400–450 nm, although its position, intensity, and width may vary depending on particle size, morphology, aggregation state, and the surrounding dielectric environment (Anees Ahmad et al., 2020). In this study, the SPR band, with a maximum wavelength (λ_{max}) of approximately 430 nm, was used to monitor nanoparticle formation and evaluate the effects of $AgNO_3$ concentration, extract concentration, pH, reaction time, and temperature. The spectral response was assessed primarily in terms of SPR absorbance intensity under the tested experimental conditions.

3.1.1. Effect of Silver Nitrate Concentration

The concentration of silver nitrate ($AgNO_3$) plays a crucial role in the green synthesis of silver nanoparticles (AgNPs), as it determines the availability of Ag^+ ions for reduction by the phytochemicals present in the plant extract. To investigate this effect, $AgNO_3$ solutions of different concentrations (0.075, 0.75, 1.0, 2.5, and 5.0 mM) were reacted with a fixed volume of Rosmarinus officinalis aqueous extract.

As shown in Figure 1, the intensity of the SPR band increased with increasing $AgNO_3$ concentration up to 2.5 mM, indicating enhanced formation of AgNPs due to the greater availability of silver ions for reduction. Among the concentrations examined, 2.5 mM $AgNO_3$ produced the highest SPR absorbance, suggesting that this concentration provided the optimum balance between the amount of Ag^+ ions and the reducing capacity of the plant extract. In contrast, when the $AgNO_3$ concentration was increased to 5.0 mM, no distinct SPR peak was observed. Instead, the UV–Visible spectrum exhibited broad and overlapping absorption features, accompanied by a very dark coloration of the reaction mixture. These observations suggest that increasing the $AgNO_3$ concentration beyond 2.5 mM altered the optical response of the reaction system and did not result in a well-defined SPR band. Possible explanations include aggregation or agglomeration of the nanoparticles, changes in their size distribution, and/or the formation of other silver-containing phases. Previous studies have attributed similar observations under certain synthesis conditions to the possible formation of silver oxide (Ag_2O) (Khane et al., 2022; Ansari et al., 2023).

On the other hand, no characteristic SPR peak was detected at the lowest $AgNO_3$ concentration (0.075 mM), and no noticeable colour change was observed in the reaction mixture. This behaviour may be attributed to the insufficient availability of Ag^+ ions for reduction to metallic silver (Ag^0), resulting in limited nanoparticle formation (Khane et al., 2022).

These findings are consistent with previous studies demonstrating that metal-ion concentration is an important factor governing nanoparticle synthesis. At very low Ag^+ concentrations, the limited availability of silver ions may restrict nanoparticle nucleation and growth, resulting in weak or undetectable SPR absorption. Conversely, excessively high Ag^+ concentrations may alter nanoparticle growth and dispersion or favour the formation of other silver-containing species, potentially producing broad or poorly defined absorption spectra. Therefore, an $AgNO_3$ concentration of 2.5 mM exhibited the highest SPR absorbance among the concentrations tested in the present study (Balčiūnaitienė et al., 2025; Villagrán et al., 2024).

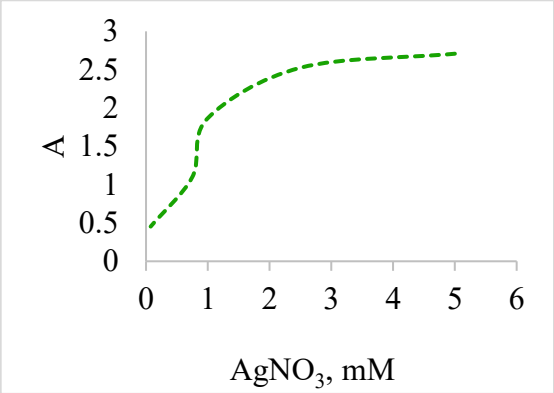


Figure 1. Effect of silver nitrate (AgNO3) concentration on the biosynthesis of silver nanoparticles (AgNPs)

3.1.2 Effect of Reaction Time

Reaction time is one of the key parameters influencing the reduction of silver ions and the subsequent growth of silver nanoparticles (AgNPs) (Alkherraz et al., 2026). To evaluate its effect, the reaction mixture was monitored at different time intervals ranging from 0 to 240 min using UV–Visible spectroscopy. As illustrated in Figure 2, increasing the reaction time resulted in a progressive increase in the intensity of the surface plasmon resonance (SPR) absorption band, which also became sharper with time. Simultaneously, the reaction mixture gradually changed in colour from pale yellow to dark brown, confirming the continuous reduction of Ag^+ ions into metallic silver (Ag^o) and the formation of AgNPs. No characteristic SPR peak was observed at the beginning of the reaction (0 min), indicating that nanoparticle formation had not yet occurred. The onset of AgNP formation was first detected after approximately 30

min, as evidenced by the appearance of the SPR absorption band together with a noticeable colour change. Thereafter, the SPR absorbance increased steadily with increasing reaction time, indicating continuous nucleation and growth of the nanoparticles (Ullah et al., 2023). The absorbance reached its maximum at approximately 210 min, after which no significant increase was observed up to 240 min, suggesting that the reduction of Ag^+ ions had reached completion and that the synthesis reaction had attained equilibrium (Naganthran et al., 2022). Consequently, a reaction time of 210 min was considered the most favourable condition for AgNP biosynthesis among the reaction times investigated in the present study. The influence of reaction time on AgNP formation has also been reported in previous studies, although the observed trends vary depending on the plant extract and synthesis conditions. Kaur et al. (2021) reported that the characteristic SPR band became sharper with increasing reaction time and that no further spectral changes were observed after approximately 30 min, suggesting that the reaction had reached a plateau under their experimental conditions. In contrast, Ansari et al. (2023) found that the reaction time associated with the highest absorbance varied among extracts prepared using different solvents and extraction methods, with maximum absorbance generally observed after 2–4 h in the systems investigated. These findings indicate that the time required to achieve the highest SPR response is system-dependent and is influenced by the composition of the plant extract and the synthesis conditions. In the present study, the highest SPR absorbance was observed at 210 min, identifying this as the most favourable reaction time among those tested.

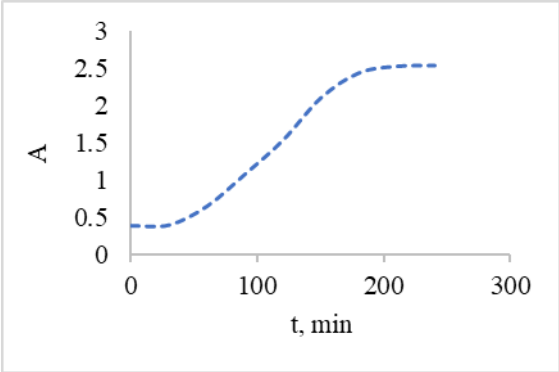


Figure 2. Effect of reaction time on the biosynthesis of silver nanoparticles (AgNPs)

3.1.3 Effect of pH

The pH of the reaction medium is an important factor affecting the reduction of silver ions, the nucleation process, and the stability of biosynthesised silver nanoparticles (AgNPs) (Alkherraz et al., 2026). Therefore, the synthesis was investigated over a pH range of 3–11 while maintaining all other reaction parameters constant.

As shown in Figure 3, the colour of the reaction mixture became progressively darker as the pH increased from acidic to neutral conditions, indicating enhanced formation of AgNPs. The corresponding UV–Visible spectra showed an increase in the intensity of the characteristic surface plasmon resonance (SPR) band with increasing pH up to pH 7. The highest SPR absorbance was recorded at pH 7, where the absorption band was also distinct and well-defined. At pH values of 7 and above, no further noticeable increase in SPR absorbance was observed, as evident from Figure 3. Therefore, pH 7 was considered the most favourable condition among the pH values tested.

At pH values of 4 and below, no noticeable colour change or characteristic SPR band was observed, suggesting that AgNP formation was limited under strongly acidic conditions. This behaviour may be attributed to the protonation of functional groups, particularly hydroxyl (–OH) groups in polyphenols and other phytochemicals present in the Rosmarinus officinalis extract (Melkamu & Bitew, 2021). Protonation can reduce the electron-donating ability of these compounds, thereby limiting the reduction of Ag^+ ions to metallic silver (Ag^o) and consequently hindering nanoparticle nucleation and growth (Khane et al., 2022).

These findings agree well with previous studies, which have demonstrated that the pH of the reaction medium strongly influences the green synthesis of AgNPs. Neutral to mildly alkaline conditions generally promote faster reduction of silver ions and improve nanoparticle stability, whereas strongly acidic media suppress nanoparticle formation because of reduced reducing activity of the phytochemicals (Farhan et al., 2017; Omar et al., 2018). The enhanced synthesis observed at pH 7 in the present study is therefore consistent with the role of pH in regulating both the reduction kinetics and the stabilisation of biosynthesised AgNPs.

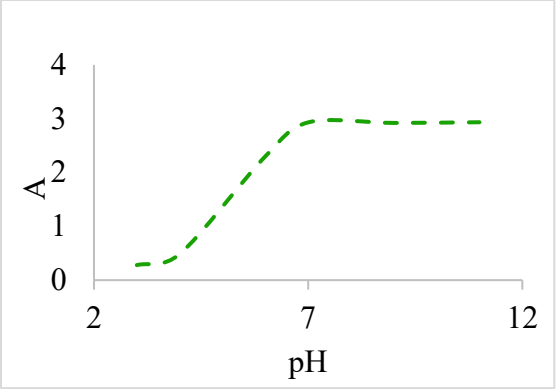


Figure 3. Effect of pH on the biosynthesis of silver nanoparticles (AgNPs)

3.1.4 Effect of extract concentration

The concentration of the Rosmarinus officinalis aqueous extract is a critical parameter in the green synthesis of silver nanoparticles (AgNPs), as it determines the amount of phytochemicals available to act as reducing and stabilising agents (Bamal et al., 2021). To investigate this effect, four different extract concentrations were prepared by diluting the extract with distilled water at different extract-to-water ratios, while maintaining the volume and concentration of the $AgNO_3$ solution constant.

As shown in Figure 4, increasing the extract concentration significantly enhanced the formation of AgNPs, as evidenced by the increased intensity and improved definition of the characteristic surface plasmon resonance (SPR) absorption band. The extract concentration (1:1) produced the highest SPR absorbance and the most distinct absorption peak, indicating the most efficient nanoparticle synthesis. In contrast, progressive dilution of the extract (1:2, 1:3, and 1:4) resulted in lower SPR absorbance, reflecting reduced nanoparticle formation. The enhanced nanoparticle synthesis observed at higher extract concentrations can be attributed to the greater abundance of bioactive phytochemicals, including polyphenols, flavonoids, and other antioxidant compounds, which provide the electrons required for the reduction of Ag^+ ions to metallic silver (Ag^o) (Alfaid et al., 2024). In addition to facilitating reduction, these phytochemicals adsorb onto the nanoparticle surface, acting as natural capping agents that improve colloidal stability and prevent particle aggregation (Khane et al., 2022; Ansari et al., 2023). Consequently, decreasing the extract concentration reduces the availability of these active constituents, thereby lowering both the reduction efficiency and the stabilisation capacity of the reaction medium.

The present findings are in good agreement with previous studies (Lithi et al., 2025; Vanlalveni et al., 2021), which have demonstrated that the concentration of plant extract strongly influences the biosynthesis of AgNPs owing to the concentration-dependent availability of reducing and capping biomolecules. Similar trends have been reported for the green synthesis of silver nanoparticles using various medicinal plant extracts, where higher extract concentrations resulted in enhanced SPR intensity and increased nanoparticle yield.

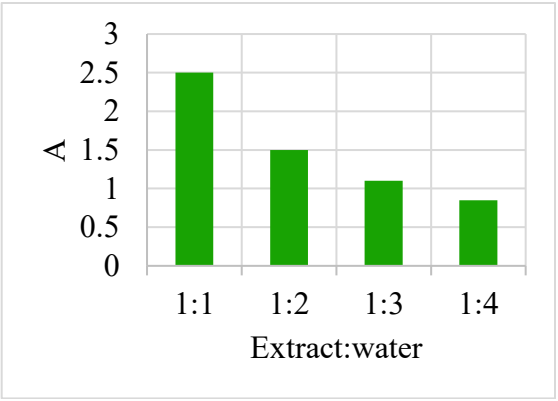


Figure 4. Effect of extract concentration on the biosynthesis of silver nanoparticles (AgNPs)

3.1.5 Effect of temperature

Reaction temperature is one of the most influential parameters affecting the rate of silver ion reduction, nanoparticle nucleation, and crystal growth during the green synthesis of silver nanoparticles (AgNPs) (Villagrán et al., 2024). The effect of temperature on AgNP formation was therefore investigated under the baseline conditions.

As shown in Figure 5, increasing the reaction temperature from 25 to 50 °C resulted in a progressive increase in the intensity of the characteristic surface plasmon resonance (SPR) absorption band. At temperatures of 50 °C and above, the SPR absorbance showed no further noticeable increase, indicating that the optical response approached a plateau within the investigated temperature range. Based on these observations, 50 °C was considered the most favourable reaction temperature among the conditions tested in this study. The reaction mixture also exhibited a more pronounced colour change as the temperature increased, consistent with changes in the formation of silver nanoparticles (AgNPs). The increased SPR intensity up to 50 °C may be associated with accelerated reduction of Ag^+ ions by phytochemicals present in the Rosmarinus officinalis extract, potentially promoting nanoparticle nucleation and formation (Alkherraz et al., 2026). Increasing temperature can enhance molecular kinetic energy and collision frequency

between reactants, thereby accelerating the reduction process (Naganthran et al., 2022).

The present observations are broadly consistent with previous studies reporting that temperature can influence the biosynthesis and optical properties of AgNPs (Ghatage et al., 2023; Dutt et al., 2023; Rama et al., 2023; Khane et al., 2022; Alfaid et al., 2024). Nevertheless, the effect of temperature depends on the synthesis system and experimental conditions, and excessively high temperatures may alter nucleation, particle growth, or aggregation. For example, Omar et al. (2018) reported changes in particle growth at temperatures above approximately 75 °C. Accordingly, 50 °C represents the most favourable temperature based on the SPR absorbance observed within the tested range of 25–55 °C.

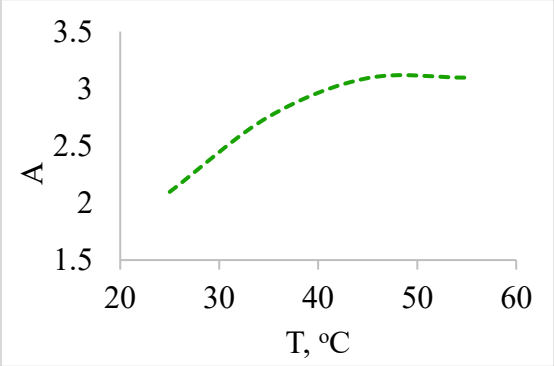


Figure 5. Effect of reaction temperature on the biosynthesis of silver nanoparticles (AgNPs)

3.2. FTIR Analysis of Rosmarinus officinalis Extract and Biosynthesized Silver Nanoparticles

Fourier-transform infrared (FTIR) spectroscopy was employed to identify the major functional groups present in the aqueous extract of Rosmarinus officinalis and to investigate their involvement in the biosynthesis of silver nanoparticles (AgNPs). FTIR is a valuable analytical technique for identifying the biomolecules responsible for the reduction of Ag^{+} ions to metallic silver (Ag^{o}) as well as for the stabilization of the synthesized nanoparticles.

The FTIR spectra of the aqueous rosemary extract and the corresponding AgNPs are presented in Figure 6. Both spectra exhibited several characteristic absorption bands, indicating that the phytochemicals present in the extract remained associated with the nanoparticle surface after synthesis. However, noticeable shifts in peak positions and changes in band intensity were observed after nanoparticle formation, suggesting the participation of these functional groups in the reduction and capping processes (Farhan et al., 2017; Omar et al., 2018).

For the aqueous extract, a broad absorption band at approximately 3294 cm^{-1} was assigned to the stretching vibration of hydroxyl (O–H) groups of phenolic compounds and alcohols. After AgNP formation, this band shifted to approximately 3175 cm-1, indicating the involvement of hydroxyl-containing phytochemicals in the reduction of silver ions. The absorption band observed at 1590 cm-1 in the extract, corresponding to C=C stretching vibrations of aromatic rings, shifted slightly to 1583 cm-1 in the AgNP spectrum. This shift suggests interaction between aromatic polyphenolic compounds and the nanoparticle surface. Another characteristic band appeared at approximately 1030 cm^{-1}, which can be attributed to C–O stretching vibrations of alcohols, phenols, and ether groups, with a possible contribution from C–N stretching vibrations of aliphatic amines. Following nanoparticle synthesis, this band shifted to approximately 1012 cm^{-1}, further supporting the participation of oxygen-containing phytochemicals in the reduction and stabilization of AgNPs (Lithi et al., 2025; Liaqat et al., 2022; Omar et al., 2018).

The observed peak shifts, together with the preservation of the main functional groups after synthesis, indicate that phenolic compounds, flavonoids, and other oxygen-containing biomolecules present in the rosemary extract acted as both reducing agents and natural capping agents during the green synthesis of AgNPs. Similar observations have been reported in previous studies employing plant extracts for the biosynthesis of silver nanoparticles (Omar et al., 2018; Alfaid et al., 2024). The principal FT-IR absorption bands identified in the aqueous rosemary extract and the corresponding AgNP sample, together with their tentative functional-group assignments, are summarized in Table 1.

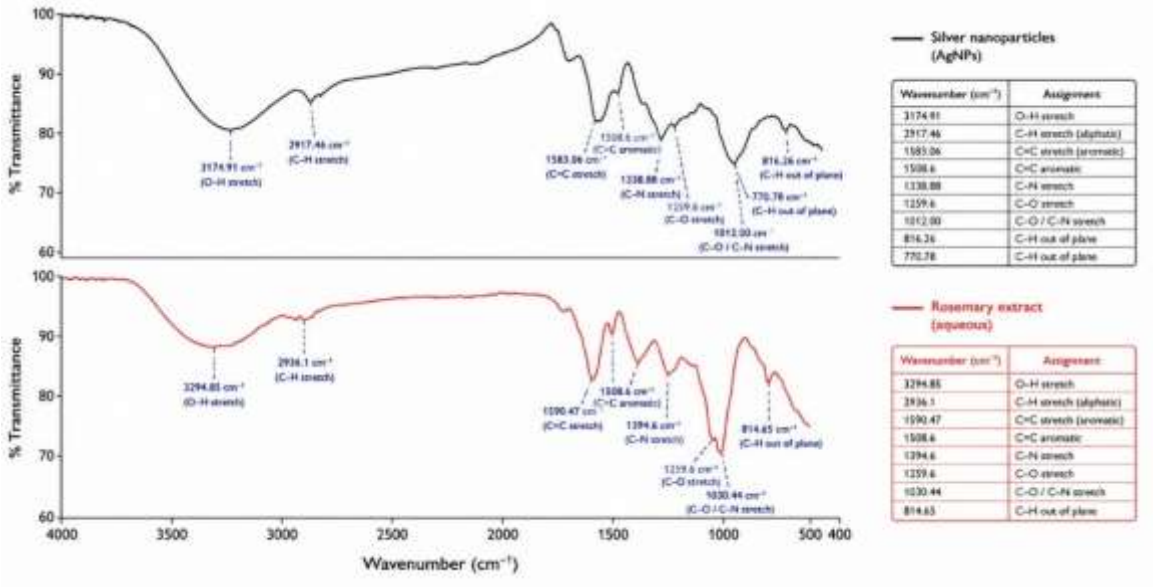


Figure 6. FTIR spectra of the aqueous extract of Rosmarinus officinalis (red) and the biosynthesized silver nanoparticles (black)

Table 1. FT-IR absorption bands of the aqueous rosemary extract before and after AgNP synthesis

Functional group	Aqueous rosemary extract (cm^{-1})	Rosemary-mediated AgNPs (cm^{-1})
O–H stretching	3294.85	3174.91
Aromatic C=C stretching	1590.47	1583.06
C–O and possible C–N stretching	1030.44	1012.00

3.3 Antibacterial activity against Escherichia coli

The antibacterial activity of the aqueous extract of Rosmarinus officinalis and the silver nanoparticles (AgNPs) biosynthesized from the extract was evaluated against Escherichia coli. The results, presented in Figure 7, demonstrated that the biosynthesized AgNPs exhibited substantially greater antibacterial activity than the corresponding aqueous plant extract when tested at identical concentrations.

No inhibition zone was observed for the aqueous rosemary extract, indicating that the extract alone exhibited no detectable antibacterial activity against Escherichia coli under the experimental conditions employed. In contrast, the biosynthesized silver nanoparticles (AgNPs) exhibited antibacterial activity, with the highest activity observed at an AgNP concentration of 9400 mg/L, producing an inhibition-zone diameter of 13.8 ± 0.81 mm (mean ± SD, n = 3). These findings suggest that the green synthesis of AgNPs enhanced the antibacterial activity of the rosemary-based preparation under the conditions tested (Liaqat et al., 2022).

The enhanced antibacterial activity of AgNPs can be attributed to several complementary mechanisms. Owing to their small particle size and large surface-area-to-volume ratio, AgNPs readily interact with the bacterial cell membrane, causing structural damage and increased membrane permeability (Khane et al., 2022; Melkamu & Bitew, 2021). Several mechanisms have been proposed in previous studies to explain the antibacterial activity of silver nanoparticles (AgNPs). These may include the induction of reactive oxygen species (ROS), interference with cellular respiration, disruption of protein function through interactions with sulfhydryl (-SH) groups, and interactions with bacterial DNA that may interfere with DNA replication and cell division. However, these mechanisms were not directly investigated in the present study and are discussed here as possible explanations based on previous literature. The present findings are consistent with previous reports describing stronger antibacterial activity of biosynthesized AgNPs than that of their corresponding plant extracts under the conditions investigated (Ullah et al., 2023; Dua et al., 2023).

3.4 Antifungal activity against Candida albicans

The antifungal activity of the aqueous Rosmarinus officinalis extract and the corresponding biosynthesized silver nanoparticles (AgNPs) was evaluated against Candida albicans. As shown in Figure 7, the aqueous extract did not produce a detectable inhibition zone, indicating no measurable antifungal activity under the experimental conditions employed. In contrast, the biosynthesized AgNPs produced a measurable inhibition zone, with a mean diameter of 13.5 ± 0.75 mm (mean ± SD, n = 3) at an AgNP concentration of 9400 mg/L. These findings suggest that the biosynthesized AgNPs exhibited antifungal activity against C. albicans under the tested conditions.

The observed antifungal activity may be associated with several mechanisms reported for AgNPs, including interactions with the fungal cell wall and membrane, increased membrane permeability, leakage of intracellular components, oxidative stress induced by reactive oxygen species, and interference with mitochondrial function and essential cellular enzymes (Liaqat et al., 2022; Khane et al., 2022; Melkamu & Bitew, 2021).

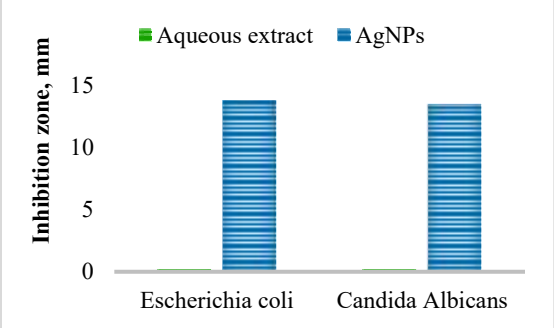


Figure 7. Antimicrobial activity of the aqueous Rosmarinus officinalis extract and the biosynthesized silver nanoparticles

Conclusion

The present study demonstrated the feasibility of using aqueous rosemary (Rosmarinus officinalis L.) extract as a natural and environmentally friendly reducing and stabilizing agent for the biosynthesis of silver nanoparticles. The formation of AgNPs was successfully monitored by UV–Vis spectroscopy through the development of the characteristic surface plasmon resonance (SPR) absorption band, while FT-IR spectroscopy provided evidence of the involvement of functional groups present in the plant extract in the nanoparticle synthesis and stabilization process.

The synthesis process was strongly influenced by the investigated reaction parameters. Increasing the $AgNO_3$ concentration enhanced the SPR band intensity up to 2.5 mM, while prolonged reaction time resulted in a progressively stronger and sharper SPR band. The highest absorbance was obtained at pH 7, and the 1:1 extract concentration produced the most pronounced SPR absorption. Similarly, increasing the reaction temperature enhanced the intensity of the characteristic SPR band, demonstrating the importance of reaction conditions in controlling AgNP formation.

The biological evaluation further demonstrated that the biosynthesized AgNPs possessed substantially greater antimicrobial activity than the aqueous rosemary extract. While the rosemary extract produced no detectable inhibition against either Escherichia coli or Candida albicans, the synthesized AgNPs produced clear inhibition zones against both microorganisms, indicating pronounced antibacterial and antifungal activities. The highest antibacterial activity against E. coli was observed at an AgNP concentration of 9400 mg/L.

The use of an aqueous Rosmarinus officinalis extract provides a plant-mediated approach to AgNP synthesis that may reduce reliance on conventional chemical reducing agents and makes use of a renewable biological resource. The biological activity observed in the present study further supports the potential of the synthesized AgNPs for applications requiring additional investigation. Nevertheless, the sustainability of the overall process remains to be established quantitatively, as nanoparticle yield, energy consumption, production costs, scalability, and the environmental management of silver-containing residues were not evaluated. Therefore, while the proposed method offers a promising green-chemistry approach, further quantitative environmental and techno-economic evaluations are needed to determine its overall sustainability and practical applicability.

Future studies should include further physicochemical characterization of the synthesized AgNPs using TEM, XRD, DLS, and zeta-potential measurements to determine their morphology, crystallinity, size distribution, and surface charge. The application of a structured experimental design, such as response surface methodology (RSM) or a Box–Behnken design, would help evaluate interactions among synthesis parameters and establish more reliable operating conditions. Further biological assessment should include MIC and MBC determination, testing against additional bacterial strains, including Gram-positive bacteria, and evaluating cytotoxicity and long-term colloidal stability.

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