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Photo-Assisted Green Synthesis with LED(590nm) of Silver Nanoparticles and Evaluation of Their Antibacterial Activity in Specific Water Based Coatings

ABSTRACT

This study investigates a light-driven green synthesis strategy with light emitting diodes at 590 nm for the production of silver nanoparticles using Mentha spicata extract as a bioactive reducing and capping agent. Unlike conventional sunlight-assisted approaches, a controlled LED irradiation system was employed to promote nanoparticle formation under reproducible conditions. The influence of the plant-derived compounds on the nucleation and stabilization of silver nanoparticles was evaluated through UV–Vis spectroscopy and Transmission Electron Microscopy (TEM). The obtained silver nanoparticles were subsequently incorporated into a specific water-based varnish matrix designed for paperboard packaging coating applications. The antimicrobial performance of the developed nanocomposite coatings was assessed against Escherichia coli K12 and Bacillus subtilis 3562, demonstrating effective antibacterial activity after nanoparticle incorporation. The results highlight the potential of LED-assisted biosynthesis as a controllable and sustainable route for the preparation of silver nanoparticle-based coatings with antimicrobial functionality, offering promising perspectives for advanced packaging applications in the food and pharmaceutical sectors.

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KEY WORDS
LED (590nm) photo-assisted green synthesis of silver nanoparticles, silver nanoparticles coatings, antibacterial coatings, packaging

1. Introduction

Among the most extensively studied nanomaterials are silver nanoparticles (AgNPs), which have attracted considerable attention because of their diverse physicochemical and biological characteristics. In particular, AgNPs exhibit notable antimicrobial and antioxidant activity, as well as distinctive electrical and optical properties, making them valuable for a wide range of technological and biomedical applications.

In recent years, increasing attention has been directed toward environmentally sustainable approaches for the synthesis of AgNPs. Green synthesis methods have emerged as promising alternatives to conventional chemical and physical techniques because they can reduce the use of hazardous reagents and energy-intensive processes. Depending on the biological source and reaction conditions, these approaches can facilitate the formation of nanoparticles with controlled physicochemical characteristics, good colloidal stability, and significant biological activity. Consequently, green-synthesized AgNPs have become an important area of research for applications where both nanoparticle performance and environmental compatibility are important considerations (Fahim et al., 2024; Zehra et al., 2025).

A considerable number of recent studies have focused on biological approaches for the synthesis of nanoparticles, primarily because these methods offer an environmentally sustainable alternative to conventional synthesis techniques. Although traditional physical and chemical

approaches can provide effective nanoparticle production, they may require substantial energy and material inputs and can involve chemical reagents with potential environmental and toxicological concerns. These limitations have contributed to the increasing interest in greener and more sustainable synthesis strategies. Plant-derived extracts represent one of the most widely investigated biological systems for the synthesis of silver nanoparticles (AgNPs). Extracts obtained from different plant parts, including leaves, stems, bark, flowers, fruits, roots, and seeds, contain a diverse range of biologically active compounds. These include polyphenols, flavonoids, alkaloids, quinones, terpenoids, enzymes, essential oils, and other secondary metabolites. Such phytochemicals can participate in the reduction of silver ions (Ag^{+}) to elemental silver (Ag^{0}) and can also contribute to the stabilization of the newly formed nanoparticles. The adsorption of these compounds onto the nanoparticle surface can reduce particle aggregation and consequently improve the stability of the resulting colloidal system. Numerous plant species have been investigated as biological sources for the production of AgNPs, with successful examples including Aloe vera (Anju et al., 2021), Lawsonia inermis (Alhomaidi et al., 2022), and Magnolia alba (De Mel et al., 2025). These studies demonstrate the potential of plant-mediated synthesis as an environmentally compatible approach for producing silver nanoparticles while utilizing naturally occurring compounds as reducing and stabilizing agents. Nevertheless, the properties of the synthesized AgNPs can vary considerably depending on the plant species, the composition and concentration of the extract, and the specific physicochemical conditions employed during synthesis.

In recent years, increasing scientific attention has been directed toward the use of LED-assisted green photochemical synthesis as an external physical factor for regulating the formation of silver nanoparticles (Anjum et al., 2022). LED sources offer several advantages, including relatively low energy consumption, precise control over the emitted wavelength, and broad technological accessibility. The application of LED irradiation can influence the kinetics of nanoparticle formation and may also affect important physicochemical characteristics, such as particle size and morphology, as well as their resulting functional properties. The selection of an appropriate wavelength or spectral range can therefore provide an additional means of controlling the synthesis process and tailoring the properties of the obtained AgNPs. Previous studies have demonstrated that light-assisted synthesis can contribute to the production of silver nanostructures with enhanced catalytic and antimicrobial performance (Anh et al., 2020; Gurung et al., 2024; Mai-Ngoc et al., 2025).

Coatings are applied to printed products and packaging to enhance their optical, physical, mechanical, barrier and antibacterial properties (Bozhkova et al., 2015; Bozhkova et al., 2017; Spiridonov et al., 2024). In the present study, a single type of water-based coating was selected. This coating is formulated for in-line application on printing presses using anilox cylinders with different transfer volumes, selected according to the specific processing conditions, substrate characteristics, and quality requirements. The experimental work aimed to enhance the antibacterial performance of the coating while also improving its optical, mechanical and barrier properties.

2. Methods

2.1. Silver Nanoparticles Synthesis and incorporation in water-based coating

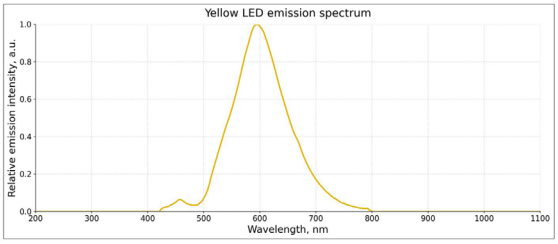
Aqueous extracts of Mentha spicata were prepared by mixing 5 g of dried plant material with 200 mL of distilled water. The suspension was placed on a heated magnetic stirrer and maintained at 500 rpm. When the temperature of the mixture reached approximately 70 °C, stirring was continued for a further 10 min to facilitate the extraction of water-soluble phytochemicals. The resulting extract was subsequently allowed to cool to room temperature and was clarified by vacuum filtration. Silver nitrate solutions with accurately defined concentrations were prepared by dissolving $AgNO_3$ (purity >99.8%, Sigma-Aldrich) in distilled water. The solutions were mixed vigorously at room temperature using a magnetic stirrer (Boeco MMS-3000, Germany). The concentrations used for the four $AgNO_3$ samples are presented in Table 1.

For the nanoparticle synthesis, 500 µL of the prepared Mentha spicata extract was introduced dropwise into 20 mL of the respective aqueous $AgNO_3$ solution under continuous vigorous stirring. The photo of light-emitting-diode-assisted configuration for silver nanoparticle synthesis is shown on Figure 1.

Figure 2 shows the relative emission intensity of used in this experiment yellow light emitting diodes with peak



» **Figure 1:** *Light-emitting-diode assisted configuration for silver nanoparticle synthesis*



» **Figure 2:** *Relative emission intensity of used in this experiment yellow light emitting diodes*

at 590nm. The reaction mixture was simultaneously irradiated with yellow LED light, and stirring was maintained for 3 min. Following synthesis, the silver nanoparticles were evaluated using a UV–Vis spectrophotometer (T60, PG Instruments Ltd., U.K.).

The AgNP dispersions obtained from all four $AgNO_3$ concentrations were subsequently incorporated into DRUCKFARBEN NAB109L0000Fvarnish at a concentration of 30% (v/v). For each formulation, the final mixture volume was adjusted to 40 mL. Following the addition of the nanoparticle dispersion, each formulation was thoroughly homogenized under continuous stirring to ensure uniform distribution of the AgNPs throughout the varnish matrix.

Table 1
$AgNO_3$ concentration

Name	$AgNO_3$ concentration, (mmol/L)
Sample 1	5
Sample 2	7
Sample 3	10
Sample 4	20

2.2. Antibacterial experiments

The antibacterial activity of the samples was assessed using the agar disc diffusion method against facultatively anaerobic Gram-negative *Escherichia coli* K12 NBIMCC 407 and aerobic Gram-positive *Bacillus subtilis* NBIMCC 3562. Both strains were obtained from the Bulgarian National Bank of Industrial Microorganisms and Cell Culture. Liquid and solid cultivation media were prepared using Luria–Bertani (LB) medium for *E. coli* and Nutrient Broth (NB) and Nutrient Agar (NA) for *B. subtilis*. All media were supplied by HiMedia Laboratories. The strains were cultivated, subcultured, and maintained on their respective media, with stock cultures stored at 4 °C. Culture incubation was performed in an ES-20/60 shaker.

For each experiment, a single colony of each strain was inoculated into 50 mL of the corresponding broth: LB for *E. coli* K12 and NB for *B. subtilis* NBIMCC 3562. The cultures were incubated for 24 h at 37 °C and 30 °C, respectively, with agitation at 220 rpm. Subsequently, 100 µL aliquots of bacterial suspensions adjusted to a turbidity of 0.5 McFarland, corresponding to approximately 1.5×10^8 CFU/mL, were distributed uniformly over the surfaces of the respective agar plates. Sterile filter paper discs supplied by HiMedia Laboratories, each measuring 6 mm in diameter, were impregnated with 6 µL of the test sample and placed on the agar surface.

Varnish formulations containing different concentrations of silver nanoparticles were examined to determine the antibacterial activity of the resulting nanocomposites. Varnish without silver nanoparticles served as a matrix control, allowing assessment of the intrinsic antibacterial activity of the varnish itself. A disc impregnated with an equivalent volume of distilled water was included as a negative control. The formation of a clear zone of inhibited bacterial growth surrounding a disc was interpreted as evidence of antibacterial activity.

Following overnight incubation at the temperature appropriate for each strain, inhibition zone diameters were measured from edge to edge through the centre of the disc in accordance with the Kirby–Bauer Disk Diffusion Susceptibility Test Protocol (Hudzicki, 2009). All experiments were conducted in triplicate, and mean inhibition zone diameters were calculated.

3. Results

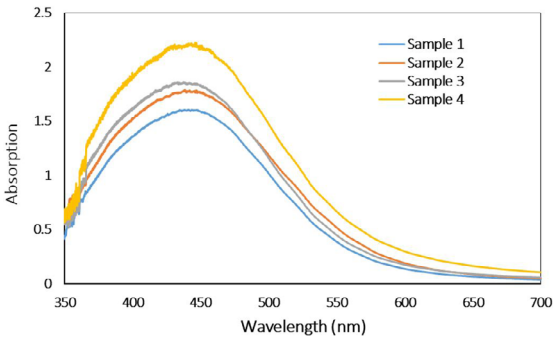
Following the addition of the Mentha spicata extract to the aqueous $AgNO_3$ solutions, the reaction mixtures were subjected to continuous yellow LED irradiation for 3 min under vigorous stirring. The formation of silver nanoparticles was accompanied by a visible change in the appearance of the reaction mixtures, indicating the reduction of Ag^+ ions and the formation of metallic silver nanoparticles. The synthesized AgNPs were subsequently characterized by UV–Vis spectroscopy, and the corresponding absorption spectra are presented in Figure 3. A clear dependence of the absorption intensity on the initial $AgNO_3$ concentration was observed. In general, increasing the concentration of silver nitrate resulted in a higher UV–Vis absorbance, which can be associated with a greater amount of silver nanoparticles formed in the reaction medium. The observed peaks, with maxima in interval of 443-447 nm, correspond to the characteristic surface plasmon resonance of spherical silver nanoparticles.

Several samples were prepared by incorporating different volumes of the AgNP suspension into the paper varnish, according to Table 1. The antibacterial activity was evaluated against B. subtilis 3562 and E. coli K12 using the disk diffusion method. No inhibition zones were observed for the varnish samples without silver nanoparticles or for the negative control. The antibacterial activity of the varnish formulations (Figure 4)

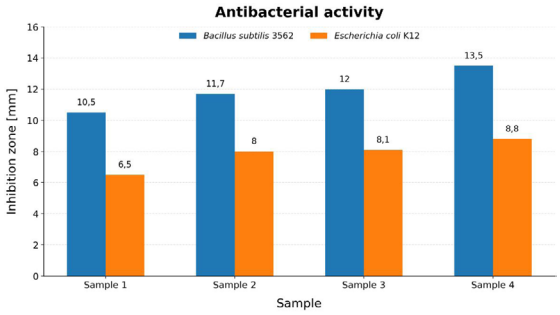
increased with increasing volume of the AgNP suspension incorporated into the varnish, with B. subtilis 3562 exhibiting greater susceptibility than E. coli K12, consistent with our previous findings (Spiridonov et al., 2024).

Differences in susceptibility between Gram-positive and Gram-negative bacteria may be related to differences in cell envelope structure and composition. In particular, the outer membrane of Gram-negative bacteria, containing lipopolysaccharides, represents an additional permeability barrier that can influence interactions with antimicrobial agents (Delcour, 2009; Silhavy et al., 2010; Sun et al., 2022).

The higher susceptibility of B. subtilis 3562 observed in the present study is also consistent with the assay-dependent differences reported for AgNPs by Kim et al. (2011), who showed that the relative responses of E. coli and B. subtilis varied depending on the experimental method. In addition, Hsueh et al. (2015) demonstrated that Ag^+ ions released from AgNPs play an important role in their antibacterial activity against B. subtilis. Based on these findings, the enhanced inhibition observed for B. subtilis 3562 may be related to differences in bacterial cell envelope characteristics and susceptibility to AgNP-mediated



» **Figure 2:** *UV-Vis spectra of silver nanoparticles synthesized with 5, 7, 10, 20 mmol/l aqueous solution of silver nitrate and extract from Mentha spicata for 3 minutes under direct sunlight*



» **Figure 3:** *Antibacterial activity of silver nanoparticle-containing varnish formulations against B. subtilis 3562 and E. coli K12, determined by the disk diffusion method*

antibacterial effects. As the antibacterial activity was assessed using the disk diffusion method, the observed inhibition zones may also be influenced by the potential release of Ag^+ ions from the incorporated AgNPs and their subsequent diffusion through the agar (Hsueh et al., 2015), as well as by matrix–nanoparticle interactions and assay-dependent effects. Overall, the AgNP-containing varnish formulations demonstrated antibacterial activity against both tested strains, with a more pronounced inhibitory effect observed against B. subtilis 3562.

4. Conclusions

The developed LED-assisted method represents a rapid, energy-efficient, and environmentally compatible approach for nanoparticle synthesis, used for incorporation in water-based coatings. The combination of renewable plant-derived resources with low-energy LED irradiation enables nanoparticle formation under relatively mild conditions, while reducing reliance on harsh chemical reagents and energy-intensive processing. The possibility of controlling the irradiation wavelength and exposure conditions offers an important advantage for the systematic optimization of nanoparticle formation. The LED-assisted synthesis represents a promising development of the previously established by authors plant-mediated approach, providing greater control over the photochemical conditions while retaining the advantages of simple, energy-efficient and sustainable synthesis strategy. These features highlight the potential of LED-assisted synthesis as a sustainable alternative to conventional nanoparticle production methods and contribute to the development of more resource-efficient and environmentally responsible synthesis strategies implemented in water-based coatings, used in printing and packaging industry. Further studies could focus on optimizing the LED-assisted synthesis by investigation different LED configurations and irradiation wavelengths, irradiation conditions, as well as varying silver nitrate concentration.

This study leads to establishing reproducible LED-assisted protocols for the controlled production of silver nanoparticles with tailored characteristics and improved suitability for applications and implementation in water-based coatings with antibacterial characteristics.

The study provides a experiment for evaluating the antibacterial contribution of the LED assisted synthesis of silver nanoparticles (AgNPs) in the widely used in packaging and printing industry water based coating matrix. In addition to the negative control, a varnish formulation without AgNPs was included, allowing the intrinsic antibacterial activity of the varnish matrix to be distinguished from the effect of the incorporated nanoparticles. Furthermore, several formulations containing increasing amounts of AgNP suspension were evaluated, revealing a consistent increase in inhibition

zones for both B. subtilis 3562 and E. coli K12 (from 10.5 to 13.5 mm and from 6.5 to 8.8 mm, respectively). Thus, provides evidence for a concentration-dependent relationship between AgNP incorporation and antibacterial activity and offers a more systematic basis for optimizing the composition of the antibacterial varnish. The discussion also provides a comprehensive interpretation of the results by considering bacterial cell-envelope differences, Ag^+ ion release, diffusion through the agar, and matrix–nanoparticle interactions.

The obtained results clearly demonstrate significant antibacterial activity of the water-based coating with incorporated LED assisted synthesized AgNP, which intensifies with increasing nano particle concentration. The results could be directly applicable in practice and industry.

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