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Valorization of *Vaccinium corymbosum* Leaf Waste for Silver Nanoparticles: Characterization and In Vitro Biological Dynamics

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Abstract

This study explores the sustainable valorization of *Vaccinium corymbosum* L. (blueberry) leaf waste as a potent bio-reductant and stabilizing agent for the phyto-mediated synthesis of silver nanoparticles (AgNPs). The phytochemical profiling of the leaf extract confirmed a terpene- and phenolic-rich composition, facilitating the biogenic reduction of Ag⁺ ions. Successful formation of AgNPs was validated by UV–Vis spectroscopy, exhibiting a characteristic surface plasmon resonance (SPR) peak at 420–430 nm. Electron microscopy (SEM and TEM) revealed predominantly spherical nanostructures with a narrow size distribution (10–35 nm), while XRD analysis confirmed a high-purity face-centered cubic (FCC) crystalline lattice. FT-IR spectra highlighted the pivotal role of hydroxyl, carbonyl, and C–O functional groups in the capping and stabilization process. The synthesized AgNPs exhibited significant broad-spectrum antimicrobial activity, with enhanced efficacy against Gram-positive pathogens. Mechanistic evaluations via growth kinetics demonstrated that the antibacterial action is driven by membrane disruption, intracellular leakage, and oxidative stress. Furthermore, the NPs displayed dose-dependent antibiofilm activity, complemented by the intrinsic antioxidant capacity of the leaf extract. These findings demonstrate that *V. corymbosum* leaf waste can be effectively transformed into high-value biofunctional nanomaterials. This eco-friendly and low-energy synthesis route aligns with circular economy principles and provides a promising platform for sustainable applications in food safety and nanobiotechnology.

Keywords: Bioactive compounds; Biomass valorization; Circular bioeconomy; Green nanotechnology; Polyphenols; *Vaccinium corymbosum* L.

1 Introduction

The large-scale global production of cereals, legumes, fruits, and vegetables generates a substantial volume of agro-industrial residues, such as stalks, straw, hulls, bran, and stubble. Derived from widely cultivated crops like wheat, rice, maize, and lentils, these biomass wastes are currently underutilized and commonly incinerated or discarded in fields. However, biomass incineration directly accelerates global warming by releasing significant carbon dioxide (CO₂), nitrogenous compounds, and particulate matter; for instance, uncontrolled burning of cereal residues in Spain alone emits 11 Tg of CO₂, 23 Gg of nitrogen compounds, and 80 Gg of particulate matter annually [1]. Conversely, the circular economy model shifts the perception of agro-food by-products from "waste" to valuable "resources" by extending lifespans and encouraging recycling. Within sustainable development frameworks, these low-cost, accessible agro-wastes serve as excellent biological matrices for renewable raw materials due to their rich polysaccharide, protein, and polyphenol content. Therefore,

valorizing these residues into high-value technological products via green technologies holds strategic importance for both mitigating the climate crisis and establishing a sustainable circular market [2].

Vaccinium corymbosum L. (blueberry), are flowering plants belonging to the large genus of *Vaccinium* [3]. Blueberries are recommended for consumption, both by healthy people and by those with various conditions, especially diabetics. Blueberries are considered the most powerful antioxidants, due to their high content in flavonoids and anthocyanins [4]. *V. corymbosum* leaves are primary plant parts that have been used medicinally for generations [5]. The phenolic compounds, flavonols, and anthocyanins of blueberries stand out for their antiproliferative effects against cancer cells and their cardioprotective benefits. Meanwhile, its constituent phenolic acids and flavonoids attribute potent antimicrobial properties and radical scavenging capabilities to this biological matrix [6]. According to FAO [7] data, this medically highly valuable agricultural commodity was cultivated across 186,414 hectares in 35 countries globally in 2023, yielding a total production of 1,220,666 tons. In the same year, Turkey ranked 15th with a production volume of 5,274 tons obtained from a cultivation area of approximately 842 hectares. Turkey exhibits a rapid upward trend in terms of both cultivation area and production volume. Indeed, in 2024, the cultivation area expanded to approximately 1,017 hectares, while the production volume rose to 6,620 tons. Consequently, this continuous growth inevitably brings about a proportional increase in the volume of leaves and pomace generated from this high-economic-value agricultural product, which are conventionally discarded as waste [8].

In recent years, the green synthesis of nanoparticles (NPs) has emerged as a cornerstone of nanotechnology due to its eco-friendly, cost-effective, and sustainable nature. Among various metallic nanostructures, silver nanoparticles (AgNPs) have regained significant attention, primarily driven by their potent broad-spectrum antimicrobial, anti-inflammatory, antioxidant etc. properties [9]. The “green synthesis” paradigm prioritizes the use of biological entities, such as plants, to minimize the environmental footprint associated with nanoparticle production [10]. Bioactive secondary metabolites present in plant extracts—including alkaloids, phenolics, and terpenes—act as both reducing and stabilizing (capping) agents [11]. *V. corymbosum* is a high-value horticultural crop renowned for its rich antioxidant and bioactive profile. While the fruits are extensively processed, the associated leaf biomass is frequently discarded as agricultural waste. However, these leaves possess significant phytochemical potential, making them an ideal candidate for waste valorization within the framework of a circular economy [12].

Although literature features various green synthesis studies using different plant parts, research on the phytochemical profile of *V. corymbosum* leaf residues and their utilization in nanomaterial conversion remains strictly limited. Therefore, this study fundamentally aims to elucidate the silver ion reduction potential of the rich polyphenol library present in these leaf wastes and to comprehensively clarify the biological efficacy mechanisms of the resulting nanostructures.

This study aimed to perform the green synthesis of silver nanoparticles (YM-AgNP) using *V. corymbosum* leaf residues as a sustainable bioresource. The synthesized nanoparticles were comprehensively characterized via UV-Vis, Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), and Zeta potential, while the phytochemical profile of the leaf matrix was mapped using GC-MS. Furthermore, the antioxidant capacity and antimicrobial kinetics (including disk diffusion, MIC, antibiofilm, and time-kill assays) of these green nanomaterials were evaluated against diverse

pathogenic bacteria and yeasts. This study provides an original know-how for converting agro-industrial by-products into high-value advanced technological assets, thereby contributing directly to the circular bioeconomy.

2 Materials and methods

2.1 Plant material and Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The bioactive profile of the aqueous extract obtained by boiling room-dried *V. corymbosum* leaf biomass in Milli-Q water was characterized. The *V. corymbosum* leaf extract (VLE), maintained at +4 °C for biogenic synthesis and subsequent analyses, was analyzed via GC-MS to identify its volatile and semi-volatile constituents.

2.2 Synthesis and Characterization of AgNPs

AgNPs were synthesized by reacting the leaf extract with 1 mM AgNO₃ (1:9 v/v) at room temperature for 30 min, followed by isolation via centrifugation at 10,000 rpm, washing, and drying at 65 °C for 48 h [13]. The synthesized AgNPs underwent comprehensive characterization, where morphology and size distribution were evaluated using TEM and SEM with gold-coated samples, and optical properties were recorded via UV–Vis spectroscopy in the 200–875 nm range. Furthermore, surface functional groups were identified using FTIR within 50–4000 cm⁻¹, the crystalline structure was verified by XRD analysis scanning between 10°–90° (2θ) with a 0.02° step size, and colloidal stability was assessed through Zeta potential measurements. It was also carried out as service procurement by DAYTAM, such as TEM and SEM analyses.

2.3 Screening of Antimicrobial activities of VLE and AgNPs

To determine the antimicrobial profiles of VLE and AgNPs, both the Kirby-Bauer disk diffusion method [14] and broth microdilution (MIC) assays were performed following the protocols established by the Clinical and Laboratory Standards Institute [15]. The test panel included *Escherichia coli* NRRL B-3704, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Staphylococcus haemolyticus* ATCC 43252, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Acinetobacter baumannii* ATCC 19066, and *Candida albicans* ATCC 10231. Penicillin (P10), streptomycin (S10), and nystatin (NY100) served as positive controls.

2.4 Growth Kinetics Analysis

The influence of AgNPs on the growth kinetics of *S. aureus* was monitored at 540 nm. Measurements were performed at specific time intervals (2, 6, 10, and 14 h) to construct growth curves representing the bacterial proliferation stages [16].

2.5 Antibiofilm Activity Assay

The antibiofilm potential was determined using the crystal violet staining method. Bacterial cultures in Mueller–Hinton Broth (MHB) were treated with varying concentrations of AgNPs (6.25–100 µg/mL). Following incubation, plates were washed with PBS, stained with 0.1% crystal violet, and solubilized in 95% ethanol. Absorbance was measured at 620 nm. The percentage of biofilm inhibition was calculated as follows:

$$\% \text{ Biofilm Inhibition} = [1 - (\text{OD}_{620} \text{ AgNP} / \text{OD}_{620} \text{ Control})] \times 100$$

2.6 Antioxidant Capacity Assays

DPPH Radical Scavenging: The scavenging activity was determined using the DPPH method [17]. Samples were mixed with DPPH solution, and the absorbance decrease at 517 nm was recorded after dark incubation. BHT was used as a reference standard.

CUPRAC Assay: The Cupric Reducing Antioxidant Capacity (CUPRAC) was measured according to the method of Apak et al. [18]. The reduction of the Cu(II)-neocuproine complex to Cu(I)-neocuproine was monitored at 450 nm.

3 Results

3.1 Bioactive compounds of VLE

GC–MS analysis of the VLE revealed a rich phytochemical profile comprising 15 major compounds, with terpenes identified as the predominant chemical class (Table 1). The most dominant component of the extract was identified as 1,8-cineole (36.35%), followed by δ -3-carene (25.59%) and D-limonene (15.39%). In addition, monoterpene hydrocarbons such as β -pinene, β -myrcene, α -terpinene, γ -terpinene and α -terpinolene were detected, alongside oxygenated monoterpene derivatives including linalool (3.10%), α -terpineol (3.12%), and linalyl acetate (1.76%). Furthermore, trans- β -caryophyllene and α -humulene were identified in minor proportions.

Table 1 Phytochemical constituent of VLE

Name of compounds (molecular formula)	Retention time, min	%
Isopropyl Alcohol	1,067	0,59
Ethanol	1,147	0,58
δ -3-Carene	2,731	25,59
β -Pinene	5,012	0,4
β -Myrcene	7,164	0,76
α -Terpinene	7,512	0,4
D-Limonene	8,198	15,39
1,8-Cineole	8,638	36,35
γ -Terpinene	9,82	0,68
α -Terpinolene	11,094	0,54
Linalool	20,096	3,1
Linalyl Acetate	20,188	1,76
Trans- β -Caryophyllene	21,066	0,46
α -Humulene	23,167	0,22
α -Terpineol	24,348	3,12

These findings indicate that the extract is particularly rich in monoterpenes and oxygenated terpene compounds. The presence of these constituents suggests the availability of functional groups that may play a crucial role in the reduction of silver ions and the subsequent stabilization of the synthesized nanoparticles. The terpenoid- and phenolic-rich profile identified in the GC–MS analysis is consistent with the volatile and polyphenolic compositions reported for *Vaccinium* species in the literature [19, 20].

3.2 Characterization analysis of AgNPs

3.2.1 Visual observation and UV-Vis spectra of VLE and AgNP

The primary visual indicator of NP formation was a distinct color transition in the reaction mixture. Specifically, the shift of the VLE from a light yellow hue to a deep brown tone, driven by the reduction of Ag^+ ions, confirmed the successful synthesis of AgNPs (Fig 1).

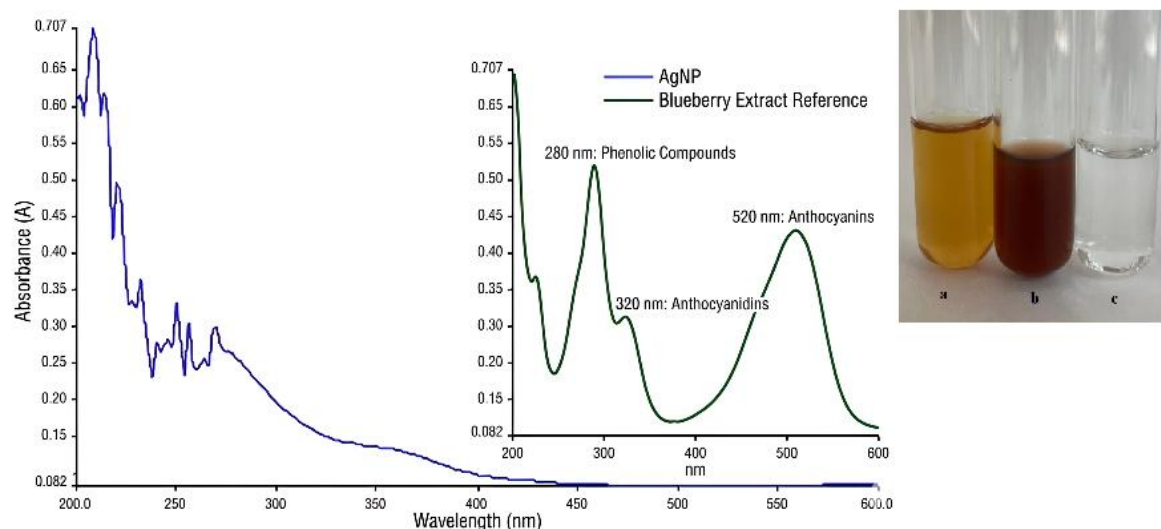


Fig. 1 The UV-Vis spectra of VLE and AgNP; a: VLE, (b) AgNO_3 mixture with VLE indicating AgNP formation, and (c) AgNO_3 solution

UV–Vis spectroscopic analysis was performed to evaluate the formation of AgNPs. In the resulting spectrum, a characteristic Surface Plasmon Resonance (SPR) band for AgNPs was observed in the 420–430 nm range (Fig 1). This absorption band indicates the reduction of Ag^+ ions and the subsequent formation of nanoparticles. Significant absorption bands were also detected in the 200–300 nm regions, which are attributed to phenolic compounds and other biomolecules present in the plant extract. The relatively broad nature of the SPR band suggests that the NPs possess a certain size distribution.

The characteristic SPR peak observed in the UV-Vis spectrum and the fcc crystal structure confirmed by XRD analysis support the formation of metallic silver and high crystallinity. It has previously been reported that small crystallite size is associated with biological activity [21], and it is suggested that the strong antimicrobial effect (Table 2) observed in our study may be linked to these structural characteristics.

3.2.2 FT-IR Analysis

The surface functional groups of synthesized AgNPs were characterized using FT-IR spectroscopy (Fig 2). The distinct absorption band at 1740 cm^{-1} is assigned to C=O stretching vibrations, signifying phenolic acids and flavonoids. Bands in the $1360\text{--}1400\text{ cm}^{-1}$ region originate from phenolic O-H bending, while peaks at 1200 cm^{-1} are related to C-O-C and C-O stretching, indicative of carbohydrate-like moieties. Additionally, C-H and O-H stretching vibrations were observed in the $2900\text{--}3000\text{ cm}^{-1}$ and $3200\text{--}3400\text{ cm}^{-1}$ range. Overall, the results confirm the successful coating of NPs by plant-derived oxygenated functional groups.

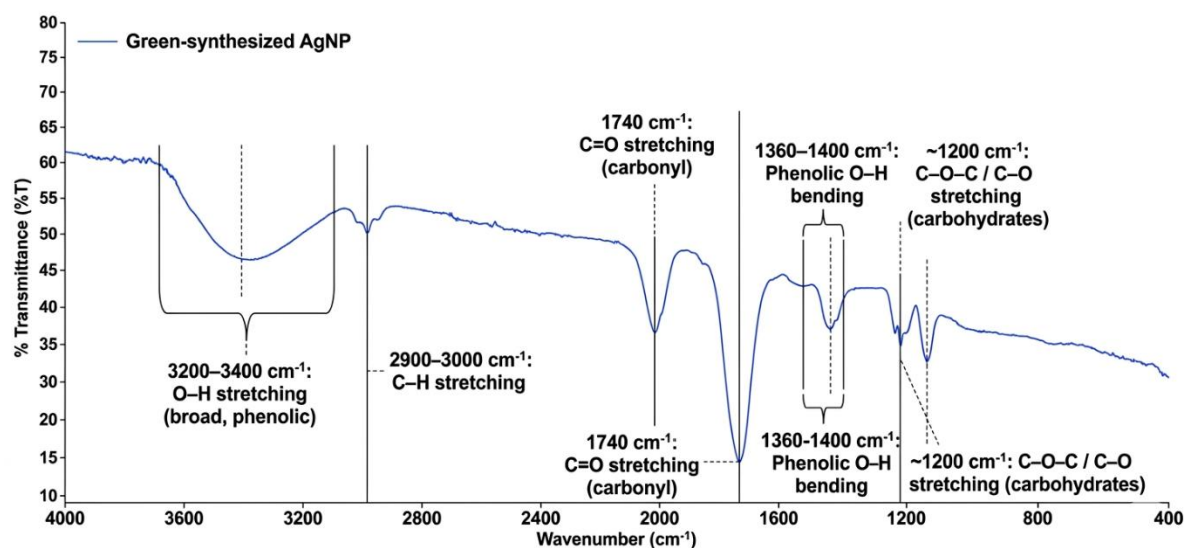


Fig. 2 FT-IR spectrum of AgNPs with identified functional group bands

The phytochemical structure identified via GC-MS analysis appears to play a dual role in the reduction of Ag^+ ions and the stabilization of the nanoparticle surface. Indeed, the carbonyl and hydroxyl groups detected in the FT-IR analysis support the involvement of polyphenols in both the reduction and capping mechanisms [22]. The negative zeta potential and narrow size distribution confirm the effectiveness of the biomolecular stabilization.

3.2.3 Zeta Potential and Particle Stability Analysis

Zeta potential analysis was performed to determine the surface charge and colloidal stability of the synthesized AgNPs. Based on triplicate measurements, the average zeta potential value was determined to be -10.9 mV (Fig 3). The obtained negative value indicates the presence of anionic functional groups on the nanoparticle surface. A zeta potential of -10.9 mV suggests that the system possesses moderate electrostatic stability.

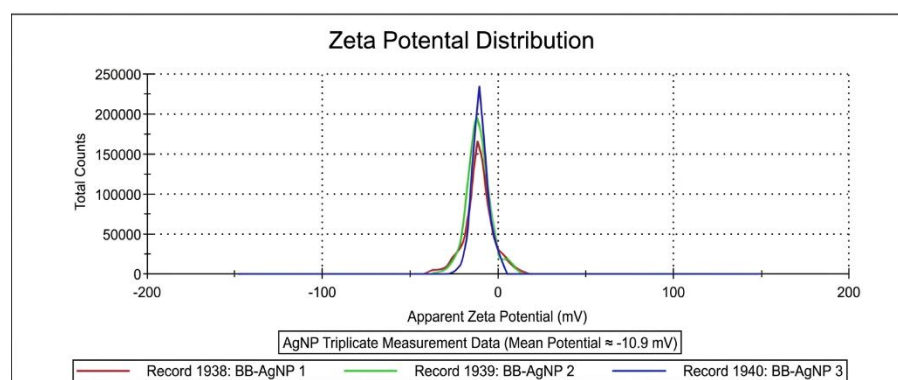


Fig. 3 Zeta potential profiles of AgNPs obtained from triplicate measurements

3.2.4 EDX Analysis and Elemental Composition

The elemental profile of the AgNPs was characterized via EDX analysis (Fig 4). The spectrum exhibited a prominent signal at approximately 3 keV, corresponding to the characteristic energy levels of silver. This confirms the metallic nature of the synthesized particles. Minor peaks representing other elements were also identified, likely originating from the biomolecular constituents of the plant extract used during the synthesis.

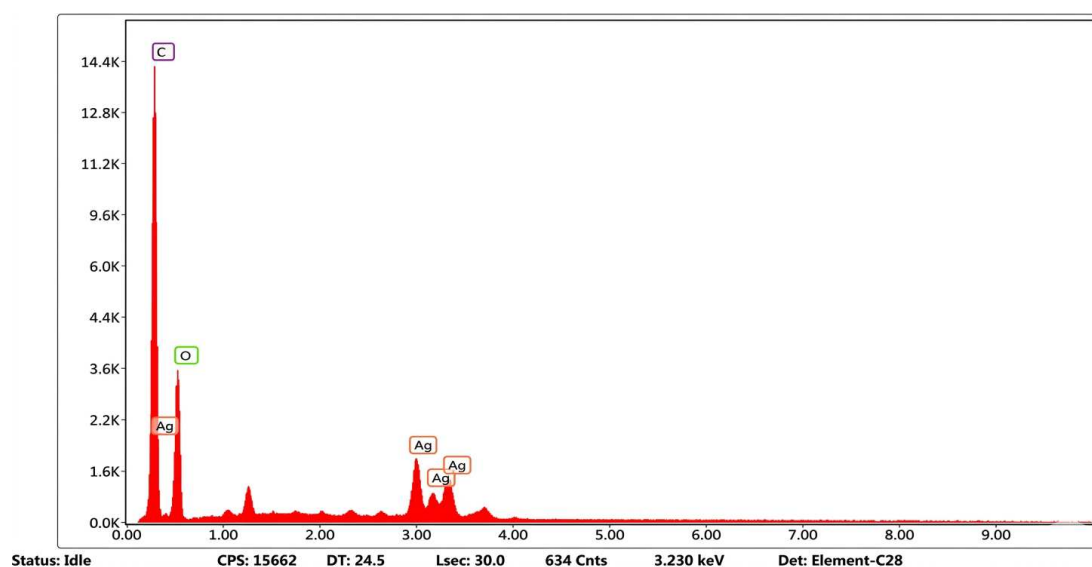


Fig 4. EDX spectrum of the synthesized AgNPs

3.2.5 XRD Analysis

XRD patterns (Fig. 5) revealed four distinct diffraction reflections at 38.15° (111), 44.27° (200), 64.51° (220), and 77.46° (311), consistent with the metallic silver fcc structure (JCPDS No: 04-0783). The well-defined peaks signify a high degree of crystallinity. Utilizing the Debye–Scherrer equation on the most intense (111) peak, the average crystallite size was estimated to be 15.65 nm.

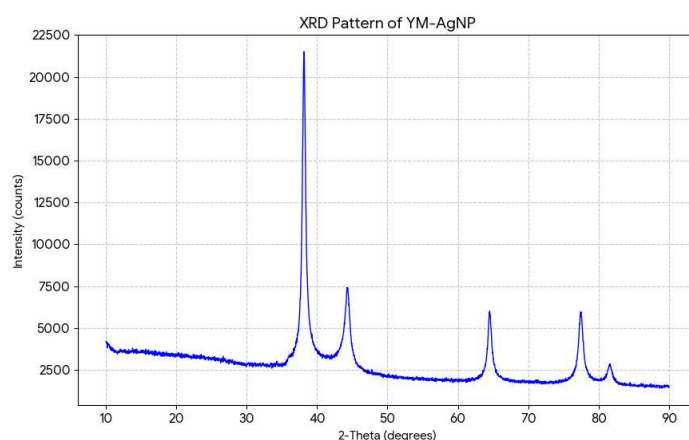


Fig. 5 XRD analysis of AgNP

The characteristic SPR peak observed in the UV-Vis spectrum and the fcc crystal structure confirmed by XRD analysis support the formation of metallic silver and high crystallinity. It has been previously reported that small crystallite size is associated with biological activity [21]; thus, the potent antimicrobial effect observed in our study is thought to be linked to these structural characteristics

The dual functionality of *V. corymbosum* extract in Ag⁺ reduction and nanoparticle surface stabilization is attributed to its complex phytochemical composition elucidated by GC-MS. The involvement of polyphenols in these dual mechanisms is corroborated by FT-IR data showing prominent carbonyl and hydroxyl groups [22]. These findings, coupled with the negative zeta potential and narrow polydispersity, confirm a robust biomolecule-mediated stabilization.

3.2.6 SEM and TEM Analysis

As illustrated in Figure 6, SEM micrographs at 80,000× and 100,000× magnifications revealed predominantly spherical and granular AgNPs with primary particle sizes in the 20–50 nm range. The presence of occasional agglomerates is ascribed to the high surface energy characteristic of the NPs.

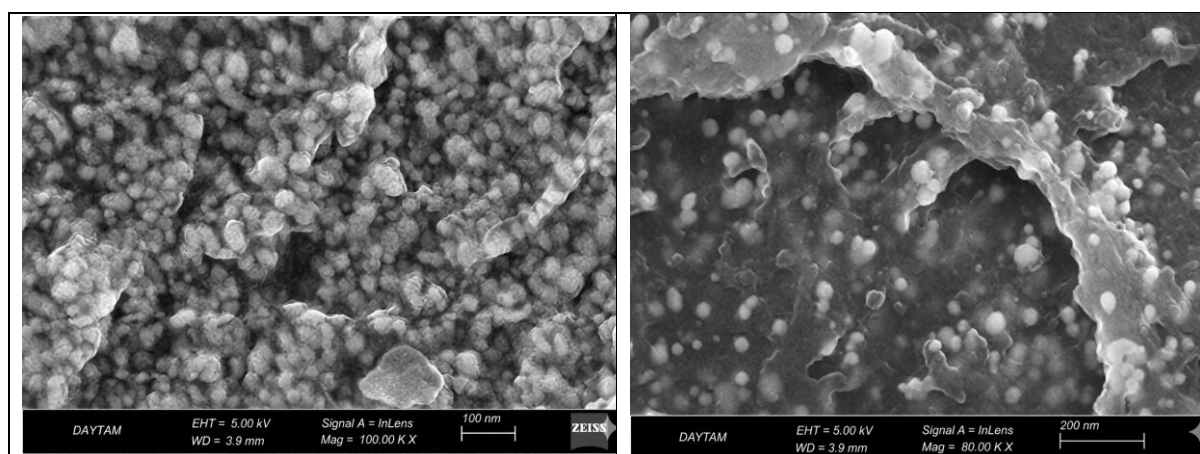


Fig. 6 SEM micrographs of AgNPs

The predominantly spherical and partially ellipsoidal nature of the particles was confirmed by TEM analysis (Fig 7). A narrow size distribution of 10–35 nm was determined through measurements at different magnifications. Moreover, the observation of a thin contrast layer surrounding the particles implies that biomolecules originating from the plant extract act as a capping agent on the nanoparticle surface.

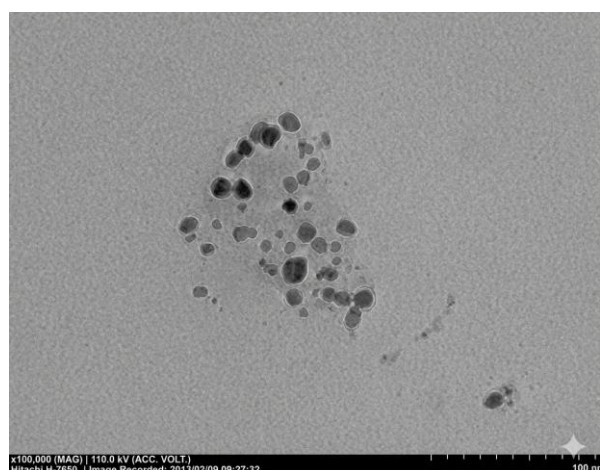


Fig. 7 TEM micrograph of the AgNPs

Both SEM and TEM analyses demonstrated the synthesis of nanoparticles exhibiting spherical morphology and distribution within the nanometric range. These observations are consistent with literature reports regarding the green synthesis of AgNPs using VLE [23, 24].

3.3 Biological Analysis of VLE and AgNP

In the disk diffusion assay, the highest susceptibility among Gram-positive bacteria was recorded for *B. subtilis* with a zone of inhibition of 20.00 mm. Regarding Gram-negative strains, AgNPs exhibited moderate activity against *E. coli* and *A. baumannii*, both yielding 10.00 mm zones. The MIC analysis further quantified these effects, highlighting a potent inhibition of *B. subtilis* at a concentration of 25 $\mu\text{g mL}^{-1}$ for both VLE and AgNPs. Notably, VLE demonstrated superior efficiency against *S. aureus* (100 $\mu\text{g mL}^{-1}$) compared to AgNPs (200 $\mu\text{g mL}^{-1}$). While Gram-negative pathogens showed higher resistance with MIC values of 200 $\mu\text{g mL}^{-1}$, the overall findings suggest that the AgNPs possess significant broad-spectrum antimicrobial efficacy, particularly effective against specific Gram-positive reflections (Table 2). VLE demonstrated a pronounced effect, particularly against Gram-positive bacteria; this finding aligns with antibacterial and membrane-disrupting mechanisms reported in the literature [3].

Table 2 Antimicrobial and antibiofilm activities of VLE and AgNP extracts

Test microorganism s	Test Methods									
	Disc Diffusion (mm)				MIC ($\mu\text{g mL}^{-1}$)				Antibiofilm (% inhibition rates)	
	Extracts		Control		Extracts		Control		Extracts	
	VLE	AgNP	P10	NY100	VLE	AgNP	ST10	NY100	VLE	AgNP
<i>E. coli</i> NRRL B-3704	10.00	10.00	12.00	D	200	200	4.0	NT	%25.00	%32.18
<i>Paeruginosa</i> ATCC 27853	6.00	9.00	14.00	D	200	200	1.5	NT	NT	NT
<i>P.vulgaris</i> ATCC 13315	6.00	7.00	16.00	D	200	200	4.0	NT	%0	%11.33
<i>A.baumannii</i> ATCC 19606	9.00	10.00	8.00	D	200	200	2.0	NT	NT	NT
<i>B.subtilis</i> ATCC 6633	20.00	15.00	15.00	D	25	25	4.0	NT	%0	%36.81
<i>S.aureus</i> ATCC 6538P	11.00	9.00	16.00	D	100	200	4.0	NT	NT	NT
<i>S.haemolyticus</i> ATCC 43252	10.00	6.00	16.00	D	100	200	5.0	NT	%0	%11.76

<i>C.albicans</i> ATCC 10231	11.00	6.00	NT	9.00	200	200	NT	5.0	%0	%3.70
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P10 = penicillin (10 ug/disc); ST: Streptomycin (10 ug/disc); NY100: Nystatin (100 ug/disc), Nt: not tested.

As illustrated by the growth curves (Fig 8), AgNP administration exhibited a significant time- and dose-dependent inhibition of *S. aureus* proliferation. The data indicated that the presence of AgNPs effectively decreased the bacterial growth rate and extended the lag phase. These findings are consistent with the results reported in the literature by Chatterjee et al. [25] and demonstrate that AgNPs alter the growth of *S. aureus* and suppress its proliferation. The obtained data indicate that AgNPs exert a significant inhibitory effect, particularly during the early growth phase.

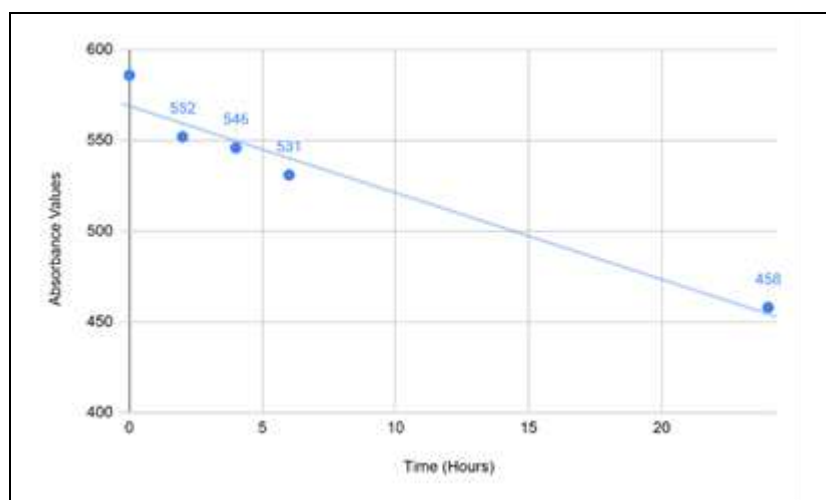


Fig. 8 Effect of AgNPs on the growth kinetics of *S. aureus*.

It has been determined that AgNPs exhibit higher antibiofilm activity compared to the VLE, particularly against *E. coli* and *B. subtilis* test bacteria (Table 2). The fact that anti-biofilm activity varies depending on the species indicates that factors such as NP concentration, surface charge, and the stage of biofilm maturation may be decisive. This suggests that AgNPs may exhibit stronger activity against planktonic cells, while showing more limited effects on established biofilm structures [26].

3.4 DPPH and CUPRAC activities of AgNPs

It demonstrates that AgNPs exhibit dose-dependent radical-scavenging activity, although it is lower than that of the BHT control (Table 3).

Table 3 Results of DPPH free radical scavenging activity of AgNPs and BHT

Concentration ($\mu\text{g}/\mu\text{L}$)	AgNP % Inhibition	BHT % Inhibition
0.01	21.20	81.21
0.04	23.49	83.21
0.08	29.79	84.11
0.1	61.17	84.81

According to this CUPRAC method, an increase in the measured absorbance values indicates that the samples possess a high potential to reduce copper (Cu^{2+}) ions and, consequently, a high antioxidant capacity (Fig 9).

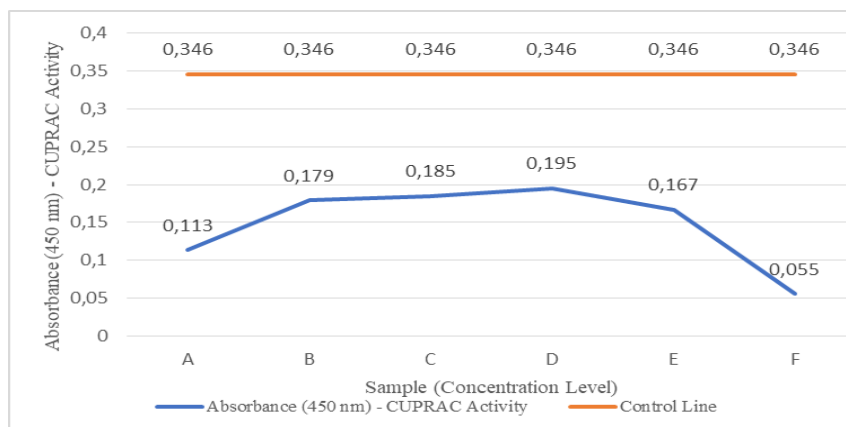


Fig. 9 CUPRAC antioxidant capacity of AgNPs.

The strong antioxidant capacity of VLE has been clearly demonstrated in the literature [3, 27], and the dose-dependent antioxidant activity observed in our study supports the functional contribution of its phenolic and anthocyanin content. These components contribute not only to biological activity but also to the nanoparticle synthesis process.

4 Conclusion

Overall, the use of *V. corymbosum* leaf waste as a reducing and stabilizing agent has enabled the production of structurally controlled AgNPs at room temperature without the need for additional toxic chemicals. The results demonstrate that converting agricultural waste into high-value-added nanomaterials offers a both biofunctional and sustainable approach. In this context, the developed system represents a promising approach for biomedical and antimicrobial applications under optimized conditions.

The findings demonstrate the feasibility of converting agricultural leaf waste into high-value-added nanomaterials, highlighting the potential of this approach for both sustainable production strategies and biomedical applications. When viewed through the lens of the circular economy, this scalable synthesis model—which is free from toxic chemicals and requires low energy—offers a viable platform for natural resource-based nanotechnological production. Future mechanistic and in vivo studies will more clearly elucidate the true potential of the developed system for clinical and industrial applications.

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Ethics Declaration

This study did not involve human participants, human data, or animals; therefore, ethical approval was not required. Ethics declaration: not applicable.

Disclosure of conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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