Article

BIOSYNTHESIZED GOLD NANOPARTICLES FROM *HEDERA HELIX* BIOMASS WASTE

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Abstract: *Hedera helix is an evergreen perennial vine plant often pruned for ornamental or ecological purposes. This process generates a significant amount of biomass waste. Therefore, this study aimed to use this biomass to obtain extracts rich in phenolic compounds and as a mediator for the biosynthesis of gold nanoparticles (AuNPs). Biochemical assays (DPPH, FRAP, Folin–Ciocalteu, and colorimetric determination of total flavonoid content) revealed that the extracts have significant radical scavenging activity and moderate phenolic and flavonoid compound levels. These findings were further supported by UHPLC analysis, providing insight into the chemical composition of these extracts. Only the most efficient extract (water infusion) was further used for AuNPs synthesis in three different synthesis medium concentrations of $HAuCl_4$ (0.5, 1, and 2 mM). Visual and UV-Vis spectroscopy analyses revealed the successful formation of AuNPs, while SEM-EDS analysis confirmed the morphology (spherical and polydisperse nanoparticles) and elemental composition. The antimicrobial activity was evaluated through the broth microdilution method against five microbial strains (Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, and Candida albicans) and two antibiotics (penicillin and gentamicin). The resulting bacterial inhibition rate (BIR%) revealed a low inhibitory activity for AuNPs, significantly lower than for the tested antibiotics. It suggests that further AuNPs functionalizations may be required to increase their biological activity.*

Keywords: antimicrobial activity, gold nanoparticles, green synthesis, *Hedera helix*, phytochemical characterization.

INTRODUCTION

Throughout history, plants have played a crucial role in overcoming human ailments and diseases due to the biocompatibility of their secondary metabolites. These metabolites act as antioxidants, anticancer agents (Belmehdi et al., 2023; Mungwari et al., 2025), and antimicrobial substances with antibacterial and antifungal properties (Bezruk et al., 2020; Shokry et al., 2022; Vaou et al., 2021). However**,** unlike synthetic drugs, typically composed of single molecules, plant-based natural compounds are usually complex mixtures. These mixtures contain a vast array of chemicals (phenolic compounds, flavonoids, proteins, alkaloids, essential oils, saponins) (Belmehdi et al., 2023; Gavrila et al., 2023; Mungwari et al., 2025), some of which may have health benefits that haven't even been explored yet. Natural compounds' most significant advantages are that they often cause fewer side effects and work better within the organism, being highly biocompatible (Belmehdi et al., 2023; Shokry et al., 2022; Vaou et al., 2021).

The need to use ecologically derived plant compounds in green synthesis has recently opened new directions in nanotechnology (Sorbiun et al., 2018; Thipe et al., 2022). Using biological methods for synthesizing metal nanoparticles has the advantage of being low-cost and eco-friendly, and several plant-based compounds (alkaloids, terpenes, phenols, saponins, and proteins) can act as both reducing and stabilizing agents (Csakvari et al., 2021; Saravanan et al., 2021), making them the perfect candidates for green synthesis while lowering their overall toxicity and environmental impact (Thipe et al., 2022).

These resulting nanoparticles can often have therapeutic properties as antimicrobial agents (Belete, 2019), or they can further be used in obtaining biosensors or for several other applications in pharmacology and diagnostics (Rónavári et al., 2021; Sorbiun et al., 2018).

Hedera helix L., a member of the *Araliaceae* family, is a well-known climbing evergreen perennial vine plant native to a wide geographic range spanning western, central, and southern Europe, extending into northern Africa (Bezruk et al., 2020; Sen et al., 2023; Suica-Bunghez et al., 2020; Wyka et al., 2023). Documented records present an approximate length of over 30 meters, with robust shoots having a diameter of up to 25 centimeters forming a small trunk. The stems connect with the surrounding substrate through multiple leaf node roots, adhering to vertical surfaces like trees, on rocky cliffs similar to lianas, or creeping on the ground (Al-Snafi, 2018; Baharara et al., 2021; Suica-Bunghez et al., 2020). The leaves are colored in vibrant green shades, usually smaller than 8 cm, and present a particular smell when crushed. The flowers are small and organized in clusters of yellow-green color that lead to small drupe-like fruits containing between 2-5 seeds (Al-Snafi, 2018; Baharara et al., 2021).

Within the ivy plant, many phytochemicals are present, some of which are used in traditional medicine across various cultures. These compounds include saponins: α-hederin (Belmehdi et al., 2023; Gavrila et al., 2023; Shokry et al., 2022), hederacoside E and F, hederasaponin-C, flavonoids: quercetin, rutin, kaempferol, polyacetylenes, and phenolic compounds: caffeic acid, rosmarinic acid, *p*-coumaric acid, as well as amino acids, steroids, vitamins, and volatile and fixed oils (Al-Snafi, 2018; Suica-Bunghez et al., 2020). One of the traditional medicinal applications of *Hedera helix* was the preparation of a decoction from its leaves and fruits, used to treat coughs and other respiratory disorders (Al-Snafi, 2018; Baharara et al., 2021). In most of Europe's territories, ivy leaf extracts are still used in traditional treatments for most acute and chronic respiratory tract problems and inflammatory conditions (Shokry et al., 2022).

From a landscape perspective, common ivy is often integrated into vertical greenery systems and, due to its high biomass productivity, requires regular trimming, which results in the generation of substantial biomass waste (Vercruysse et al., 2024). The generated biomass trimmings have a rich chemical profile, containing various phytochemicals such as flavonoids, saponins, and essential oils, representing a promising and untapped source of natural capping and reducing agents essential for green nanoparticle synthesis (Al-Snafi, 2018; Vercruysse et al., 2024).

The objectives of this study were: (i) to recover and utilize the bioactive compounds from *Hedera helix* biomass; and (ii) to synthesize and characterize AuNPs from these bioactive compounds using a green, plant-mediated approach, and (iii) to evaluate the antioxidant and antimicrobial potential of both the extracts and the synthesized AuNPs.

MATERIALS AND METHODS

All utilized chemicals and reagents were of analytical grade and procured from Sigma-Aldrich and Merck.

Plant-based materials

Hedera helix L. leaves were gathered locally from Arad city, Romania (46°10′36″N 21°18′4″E). Until required, they were air-dried and then placed in paper bags for storage.

Preparing the plant extracts

The dry plant leaves were ground to a fine powder using an electric milling machine. Several extracts were prepared by 7 days aqueous maceration (HH1) and hydroalcoholic maceration (HH2) or a 30-minute aqueous infusion (HH3), using a 1:10 w/v plant biomass to solvent. The resulting extracts were filtered and further centrifuged at 8000 rpm for 15 minutes, and the upper layer was collected for AuNPs synthesis and biochemical analysis. For the UHPLC analysis, the extracts were filtered using a 15 mm, 0.45 μm PVDF syringe filter.

Biochemical Assays

Total Phenolic Content

Phenolic quantification was assessed using the Folin-Ciocalteu (FC) method, and the results were expressed as gallic acid equivalents (GAE/L) following the procedure described by Moisa et al. (2018). Briefly, diluted extract (1:25) was mixed and reacted with FC reagent, 20% sodium carbonate, and water, and the mixture was incubated for 90 minutes at room temperature in the dark. The absorbance values were read at a wavelength of 765 nm.

Antioxidant Activity

DPPH Assay

The DPPH• (1,1-diphenyl-2-picrylhydrazyl) free radical assay was performed following the method previously described by Csakvari et al. (2021). Briefly, 100 µL sample was mixed with 3 mL DPPH• solution and incubated in the dark for 1 hour. The absorbance was measured at 517 nm, and the results were expressed as a percentage of inhibition and mg GAE/L.

FRAP Assay (Ferric Reducing Antioxidant Power)

The antioxidant activity of the *Hedera helix* extracts was evaluated by measuring their ability to reduce the Fe^{3+}-TPTZ complex to its Fe^{2+}-TPTZ form, resulting in a blue-colored solution with a maximum absorbance at 593 nm. For this, FRAP reagent was freshly prepared by mixing acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM in 40 mM HCl), and $FeCl_3$ solution (20 mM) in a 10:1:1 (v/v/v) ratio, as described by Mot et al. (2022). For each assay, 0.2 mL of extract was mixed with 1.5 mL of FRAP reagent, incubated in the dark for 20 minutes, and the absorbance was recorded at 593 nm, using water as a blank.

Total Flavonoid Content

The previously obtained *Hedera helix* extracts were used to determine the flavonoid content following the method described by Copolovici et al. (2021). For this, 250 µL of the extract was mixed with 1250 µL of sodium acetate solution (100 g/L), 750 µL of aluminum chloride solution (25 g/L), and 250 µL of deionized water. The mixture was incubated in the dark for 15 minutes, and absorbance was measured at 434 nm against a reference sample prepared under the same conditions.

Statistical Analysis

Statistical analysis of the biochemical assay results was performed using two-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test in order to determine significant differences between samples. Values presenting different superscript letters are significantly different ($p < 0.05$); values sharing the same letters are not significantly different ($p > 0.05$).

Chemical Composition Determined by UHPLC-DAD

The extracts were evaluated using an ultra-high-performance liquid chromatograph (UHPLC Nexera X2, Shimadzu, Tokyo, Japan) equipped with a reversed-phase Nucleosil C18 column (Macherey-Nagel, Düren, Germany), following the method described by Csakvari et al. (2021). Compounds were identified and quantified by comparing retention times and UV spectra with reference standards analysed under identical conditions.

Synthesis of Gold Nanoparticles

Gold nanoparticles were synthesized using a procedure adapted from Chiravoot et al. (2021), with several modifications. Therefore, 27 mL of 0.5, 1, and 2 mM $HAuCl_4 \cdot 3H_2O$ solution was added at 30 ˚C to 4 mL plant extract at room temperature under constant mixing. After 1 h of reaction time, the mixture was centrifuged at 8000 rpm for 30 minutes and then the supernatant was discarded. The resulting AuNPs were washed three times with deionized water and resuspended in a small volume to obtain a concentrated suspension, which was used for further analysis.

UV-Vis Characterization of AuNPs

UV–Vis spectroscopy analysis using a Specord 200 spectrophotometer (Analytik Jena AG, Germany), with the absorbance recorded in the range of 400–700 nm revealed a characteristic surface plasmon resonance (SPR) peak at

approximately 550 nm, confirming the presence of AuNPs.

SEM–EDS Characterization of AuNPs

The morphology, size, and distribution of the synthesized AuNPs from *Hedera helix* extracts were examined using a scanning electron microscope (SEM, LYRA 3 XMU, Tescan, Czech Republic) at 50 kx magnification. Elemental composition was assessed through Energy-Dispersive X-ray spectroscopy (EDS) (EDAX Inc., Mahwah, NJ, USA).

Antimicrobial Activity – MIC Assay

The antimicrobial potential of the concentrated AuNPs suspension was assessed through a broth microdilution method using a 96-well plate. The microbial strains used in this study (*E. coli* ATCC 25922, *S. typhimurium* ATCC 14028, *S. aureus* ATCC 25923, *C. albicans* ATCC 10231, *P. aeruginosa* ATCC 27853) were obtained from the in-house microbial collection of the "Aurel Vlaicu" University of Arad.

Following a microdilution protocol described by Hilpert et al. (2008), each well in columns 1 to 10 was initially filled with 100 µL of Mueller-Hinton Broth (MHB, VWR, BDH®). Afterward, a volume of 100 µL of the AuNPs stock suspension was added to the first well (column 1), mixed thoroughly, and 100 µL was transferred to the next well (column 2). This serial dilution continued until column 10, discarding the final 100 µL to maintain equal volumes.

Following the serial dilution, 100 µL of microbial inoculum adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) was added to wells in columns 1 to 10. Column 11 was the positive control (200 µL of microbial inoculum in MHB), while column 12 was the negative control (200 µL of sterile MHB, without microorganisms).

Plates were incubated at 37 °C for 24 hours, and optical density (OD) was measured at two wavelengths, 600 nm and 620 nm, using a Tecan Spark multimode microplate reader (Tecan Group Ltd., Switzerland).

RESULTS AND DISCUSSIONS

Chemical Composition of the Hedera helix Extracts

All the extracts obtained were evaluated for their content of phenolic compounds and flavonoids, and their antioxidant activity was assessed.

The biochemical analyses indicated that the HH3 extract presented the highest total phenolic content (714.36 ± 63.48^a mg GAE/L), followed by the HH1 extract (515.13 ± 8.00^b mg GAE/L) and lastly, the HH2 extract (508.78 ± 8.18^b mg GAE/L), values sharing different superscript letters are significantly different ($p < 0.05$); values sharing the same letters are not.

The antioxidant activity measured using the DPPH method presented a comparable trend, with the HH3 extract showing a higher activity (58.76% inhibition; 28.45 ± 0.23 mg^a GAE/L), followed by the HH2 extract (43.74%; 20.46 ± 0.60^b mg GAE/L) and lastly the HH1 extract (17.96%; 6.74 ± 0.09^c mg GAE/L).

Flavonoid content and FRAP values were similar for all the extracts. For the flavonoid content, the results ranged from 0.24 ± 0.01^c meq rutin/mL in the HH1 extract to 0.32 ± 0.01^b and 0.38 ± 0.01^a meq rutin/mL in the HH3 and HH2 extracts, while for FRAP assay the results ranged from 0.52 ± 0.01^b to 0.55 mM $\pm 0.01^a$ Trolox/L.

Considering these results, only the infusion extract (HH3) was selected for nanoparticle synthesis due to its higher phenolic and antioxidant levels, quick preparation time, and avoidance of organic solvents.

Within the UHPLC analysis, several phenolic and antioxidant compounds in the *Hedera helix* extracts were confirmed (Figure 1). The highest concentration of rutin was observed in the HH3 extract (467.91 mg/L) and the HH2 extract (430.19 mg/L), while the HH1 extract contained the lowest amount (253.13 mg/L). Vanillic acid was also in a slightly higher concentration within the HH3 extract (299.61 mg/L), compared to the HH2 extract (281.58 mg/L), and was not detected in the HH1 extract. Caffeic acid followed a similar pattern, with 185.14 mg/L in HH3 and

44.06 mg/L in HH2, and was not detected in the HH1 samples.

However, ascorbic acid was only found in the macerates 90.65 mg/L in HH1 extract and 190.62 mg/L in the HH2 extract but was not detected in the HH3.

Table 1. UHPLC analysis of extracts

Compound mg/L	RT	HH1	HH2	HH3
Ascorbic acid	3.064	90.65	190.62	-
Vanillic acid	9.098	253.13	430.19	467.91
Rutin	9.397	-	281.58	299.61
Caffeic acid	9.676	-	44.06	185.14

These results are similar to those reported in existing literature for the extracts of *Hedera helix* leaves (Ahchouch et al., 2024; Shawky and El Sohafy, 2020).

The resulting data further support the biochemical assay results and justify the selection of the HH3 extract for nanoparticle synthesis due to its higher composition in phenolic compounds and the potential use of the reaction in the absence of organic solvents.

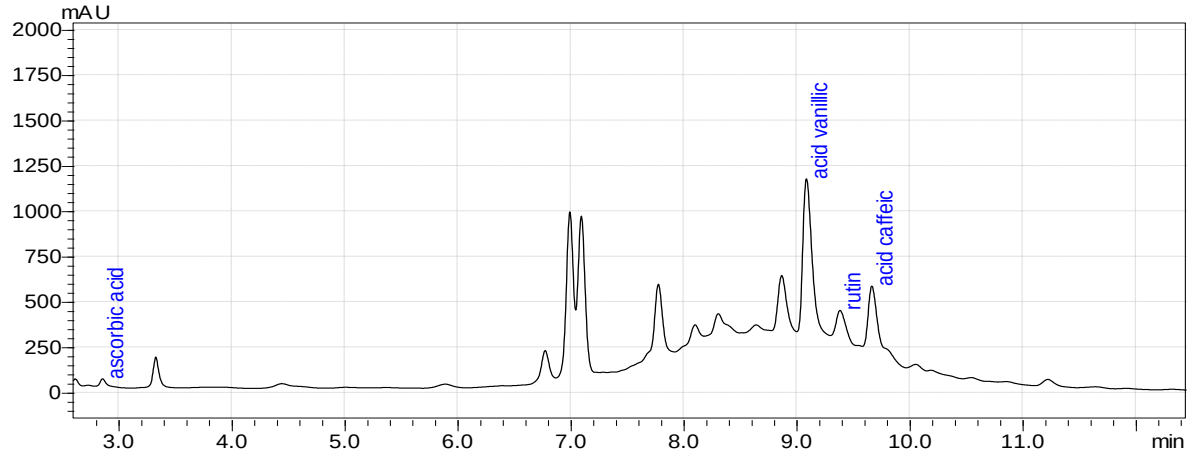

Figure 1. UHPLC chromatogram of *Hedera helix* extract

Synthesis and Optical Properties of AuNPs

The reaction between *HH3* extract and $HAuCl_4$ solutions revealed the synthesis of gold nanoparticles, which was visually observed by the color shift of the reaction mixtures from pale yellow to dark pink. UV corroborated the visual observation–Vis spectroscopy analysis, which presented distinctive surface plasmon resonance (SPR) peaks ranging from 540 to 550 nm (Figure 2) for all samples, as was also reported by Yi et al. (2013).

The intensity of the SPR peak increased with the concentrations of $HAuCl_4$ (0.5 mM < 1 mM < 2 mM), indicating a rapid nanoparticle synthesis. The higher intensity peak recorded for using the 2 mM gold solution could possibly indicate the development of a highly concentrated and perhaps monodisperse colloidal suspension.

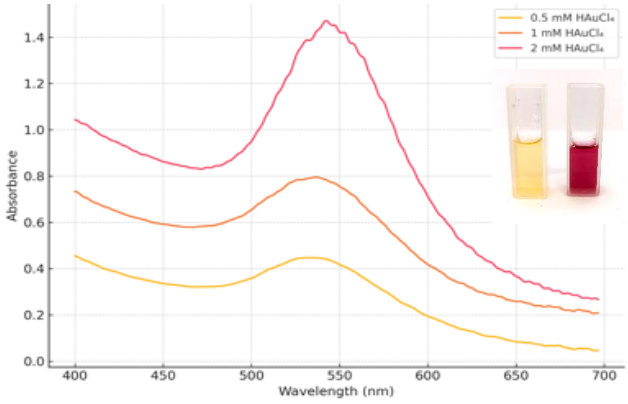

Figure 2. UV-Vis spectra after synthesis prior to purification of AuNPs.

The presence of a higher concentration of phenolic compounds, including rutin and caffeic acid, in the extract could have influenced the reduction and stability of the

nanoparticles, acting as reducing and capping agents.

AuNPs Morphological and Elemental Characterization

The biosynthesized AuNPs morphology was determined using a Scanning Electron Microscope (SEM) at 50 kx magnification for all samples. As depicted in Figure 3, the AuNPs synthesized from *HH3* extract and varying $HAuCl_4$ concentrations (0.5, 1, and 2 mM) presented predominantly spherical shapes, with an estimated diameter varying between 20 and 80 nm (Figure 3), with similar results reported by Xia et al. (2013).

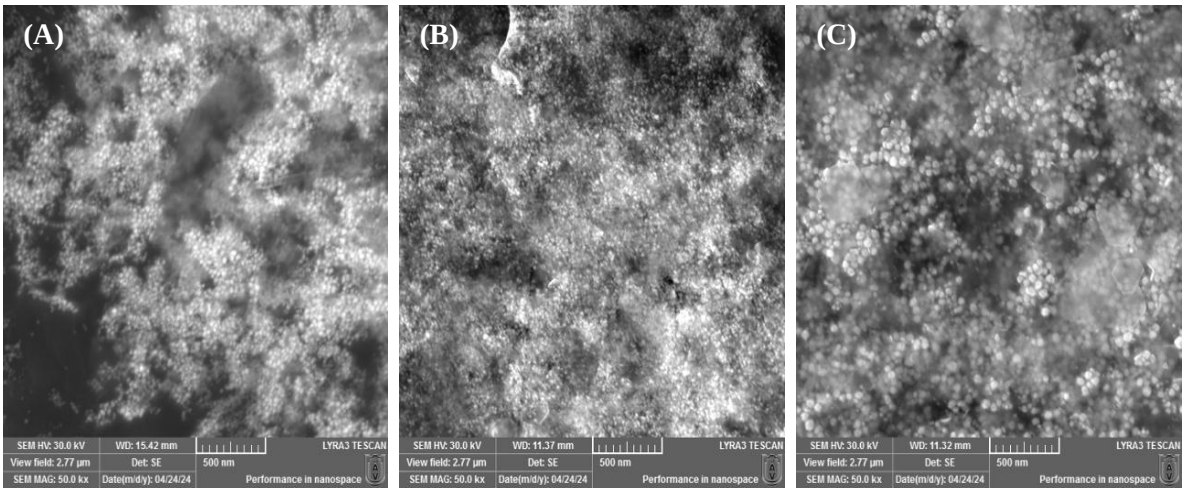

Figure 3. SEM micrographs of gold nanoparticles synthesized using HH3 extract and three different concentrations of $HAuCl_4$: (A) 0.5 mM, (B) 1 mM, and (C) 2 mM. All images were recorded at 50 kx magnification without prior sample coating.

More homogenous particle size and distribution were observed for the sample at 1 mM $HAuCl_4$, with reduced aggregation compared to 0.5 and 2 mM $HAuCl_4$ solution samples.

At the higher concentration (2 mM $HAuCl_4$), an increased particle density, clustering, and non-spherical structures were observed. These non-spherical structures presented truncated or irregular triangular shapes, suggesting a possible shift toward anisotropic growth specific for higher gold ion concentrations (Yi et al., 2013).

Therefore, due to their higher surface-to-volume ratio, which is characteristic of smaller and spherical nanoparticles, their ability to interact with microbial cell membranes, viruses and fungi, represents a crucial aspect for biomedical applications and the overall toxicity of metal NPs. (Osonga et al., 2020; Saed et al., 2024).

As was reported by Azad et al. (2023) morphological diversity can be caused by slight variation of the phytochemical composition of plant extracts and higher metal concentrations in the synthesis medium.

Energy Dispersive X-ray spectroscopy (EDS) analysis was used to determine the elemental composition of the biosynthesized AuNPs. In all samples, elemental gold (Au) was the main constituent, and strong distinctive peaks were observed at around 2.1 keV. Low-intensity signals were also detected and attributed to carbon (C), most probably from the small quantity of biomolecules from the extracts that may be present in the sample.

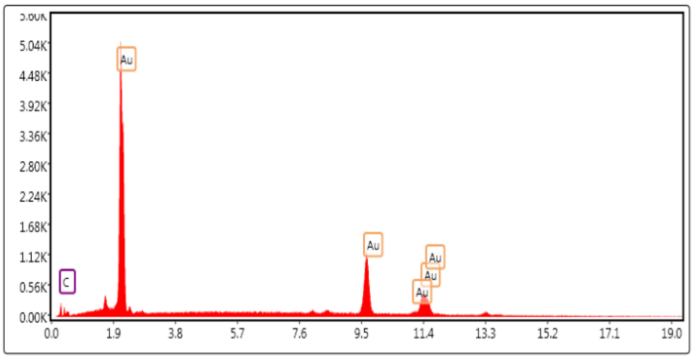

Figure 4. EDS spectrum of AuNPs synthesized using HH3 extract and 2 mM $HAuCl_4$.

As was presented in Figure 4, the gold content was overall high in all analyzed samples, with relative weight percentages of 87.9% (2 mM), 86.7% (0.5 mM), and 78.5% (1 mM), further supporting the successful biosynthesis of AuNPs using HH3 extract.

These results validate the efficiency of the green synthesis method and correlate with the SEM observations of nanoparticle size and distribution.

Antimicrobial Activity of AuNPs

Microbiological analyses were performed using the broth microdilution method to determine the antimicrobial potential of *Hedera helix*-derived gold nanoparticles biosynthesized at three different synthesis medium concentrations (0.5, 1, and 2 mM $HAuCl_4$) alongside two standard antibiotics. Fresh microbial cultures were prepared, and prior to inoculation, they were adjusted to 0.5 McFarland, and afterward, they were inoculated in 96-well microplates containing serial dilutions of the AuNPs suspensions.

The resulting BIR% values are indicating that the *Hedera helix*-mediated biosynthesized AuNPs did not exhibit inhibitory effects against the five tested microbial strains under the conditions employed. However, these results were affected due to substantial optical interference caused by the AuNPs. In contrast, both reference antibiotics demonstrated strong antimicrobial activity. A limitation of this study is the absence of an antifungal agent as a control and an antimicrobial protocol optimization is required. These findings also suggest that further adjustment of the synthesis protocol is required, either to enhance nanoparticle dispersion efficiency or to functionalize their surface with bioactive ligands, thereby improving the antimicrobial efficacy of the green-synthesized AuNPs.

CONCLUSIONS

The extracts obtained from *Hedera helix* biomass were characterized and presented remarkable antioxidant potential and moderate content of phenolic and flavonoid compounds. It represents a good source of bioactive compounds that can be successfully used to biosynthesize gold nanoparticles (AuNPs) of both spherical and polydisperse shapes, as confirmed by UV–Vis and SEM-EDS analyses, representing a sustainable approach for the valorization of plant-based biomass found in high amount in vertical greenery systems present in city landscapes.

However, the MIC antimicrobial assay against several microbial strains (bacteria and fungi) revealed that the biosynthesized nanoparticles presented low antimicrobial activity compared to standard antibiotics. These results suggest that, although AuNP synthesis was effective, further optimization of either the particle surface or their concentration is still necessary for enhancing antimicrobial properties, therefore, AuNPs with low antimicrobial activity could be functionalized and used as drug delivery carriers to enhance their antimicrobial potential.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS - UEFISCDI, project number PN-III-P4-PCE-2021-0639, within PNCDI III and by a grant from the Romanian Ministry of Education and Research, CNCS—UEFISCDI, project number PN-III-P1-1.1-PD-2019-0607, within PNCDI III.

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ISSN 1582-1021
e-ISSN 2668-4764
Edited by "Aurel Vlaicu" University of Arad Publishing House, Arad, Romania