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Antimicrobial effects produced by gold nanoparticles obtained with extracts of *Allium sativum* and *Allium ursinum*

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Abstract. Gold nanoparticles (AuNPs) obtained by green synthesis using plant extracts from the genus *Allium* have attracted significant scientific interest due to their potential applications as antimicrobial agents in the biomedical field. This study investigates the antimicrobial potential of AuNPs obtained by green synthesis using extracts of *Allium sativum* and *Allium ursinum*. These plant extracts are rich in sulfur compounds (allicin), flavonoids and polyphenols, which not only facilitate the formation of nanoparticles, but also confer them increased antimicrobial properties. The nanoparticles thus obtained were characterized by spectroscopic methods (UV-Vis) and were tested for antimicrobial activity. Microbiological tests performed *in vitro* demonstrated an antimicrobial activity of the nanoparticles against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria but also against *Candida albicans* and *Candida parapsilosis*. The results support the idea that gold nanoparticles functionalized with *Allium* extracts may constitute a promising alternative in the development of natural antimicrobial compounds with applications in medicine, the food industry and the pharmaceutical field.

Keywords: *Allium sativum, Allium ursinum,* antimicrobial, nanoparticles

Introduction

The synthesis of metal nanoparticles (NPs) often requires the use of toxic materials and high costs, limiting their clinical use. Nanoparticles of various multifunctional metals and metal oxides have been produced, such as gold, silver, platinum, zinc oxide, nickel oxide, iron oxide, cesium oxide, magnesium, and several others (Ezhuthupurakkal *et al.,* 2017; Cui *et al.,* 2021; Uddin *et al.,* 2021; Chowdhury *et al.,* 2025). Recently, green synthesis methods have helped to overcome these problems. Extracts from plants, algae, yeast, actinomycetes, fungi and even bacteria are used in green synthesis methods (Abdelsattar *et al.,* 2024; El-Gebalya *et al.,* 2024; Bogale *et al*., 2025). The use of metal oxide nanoparticle materials that possess antimicrobial characteristics presents a significant benefit: a better equilibrium between therapeutic benefits and adverse effects when compared to traditional antibiotics (Ma *et al.,* 2024). Plants contain a variety of therapeutically beneficial bioactive compounds or phytochemicals, such as alkaloids, flavonoids, terpenoids, saponins, amino acids, tannins, steroids, glycosides and polyphenols, vitamins, and minerals providing routes for the synthesis of easier and non-toxic nanoparticles (Tan *et al.,* 2023; Chowdhury *et al.,* 2025). Nanoparticles derived from noble metals like gold, silver, and platinum have been widely applied in various fields, including medicine, cosmetics, biological sensors, and catalysis (Yulizar *et al.,* 2017). Currently, nanoparticles of gold, iron, zinc, cobalt, and silver are widely utilized in the early diagnosis and treatment of various diseases caused by pathogens, viruses, and parasites. They also show significant potential in addressing refractory conditions such as cancer (Rabiee *et al.,* 2020; Baran *et al.,* 2023).

In plant-based synthesis methods, phytochemicals extracted from different plant parts—such as fruits, leaves, flowers, or roots—using either hot or cold extraction serve as reducing agents for metal ions while also stabilizing the metallic core. The synthesis process can be controlled by adjusting the ratio of reducing/stabilizing agents to metal salts, as well as modifying ambient conditions like temperature and pH. This allows the precise control of nanoparticle size and shape (Tan *et al.,* 2023).

Garlic (*Allium sativum*) has been valued since ancient times for its culinary, seasoning, medicinal, nutraceutical, and insecticidal properties. Traditional remedies utilizing garlic for various health conditions are well-documented across different cultures and historical texts. The health advantages of garlic arise from the combined effects of its complex chemical components. It is a rich source of essential minerals, including potassium, phosphorus, sulfur, zinc, selenium, and germanium, along with major amino acids and moderate amounts of vitamins A and C (Suleria *et al.,* 2015, Subbanna *et al.,* 2020). Garlic is a rich source of bioactive compounds, particularly organosulfur compounds and thiols, which constitute up to 2.3% of its total nutritional composition. Allicin (allyl 2-propenethiosulfinate, diallyl thiosulfinate, or S-allyl cysteine sulfoxide) is the most abundant thiosulfinate in fresh garlic, making up approximately 70% (w/w). It is an unstable, volatile molecule formed through an enzymatic reaction catalyzed by alliinase, which converts its precursor amino acid into allicin (Suleria *et al.,* 2015).

Garlic extract and its isolated bioactive compounds serve as excellent reducing and capping agents in the synthesis of various metal and metal oxide-based nanoparticles. This effectiveness stems from garlic's rich composition of phytochemicals, particularly organosulfur compounds, which offer superior chemical interactions with metal and metal oxide components compared to other plant-based bioactives. A key advantage of nano-based formulations is their ability to release active ingredients gradually and in a controlled manner at the targeted site. The antimicrobial properties of garlic extract are primarily attributed to allicin, a highly abundant dithiosulfinate. Allicin modifies sulfhydryl groups, inhibiting essential sulfhydryl-containing enzymes in bacterial cells, ultimately leading to cell death (Subbanna *et al.,* 2020).

In addition to *A. sativum* extract, *A. ursinum* is also noteworthy. The allicin component of *A. ursinum* extract is comparable to that of garlic extract, giving it antimicrobial properties (Barbu *et al.,* 2023). Due to the proven antimicrobial properties of these extracts, but also to the numerous existing studies in which gold nanoparticles were synthesized using *A. sativum* extracts, this study aims the synthesis and testing gold nanoparticles using both extracts. To our knowledge, gold nanoparticles using hydroalcoholic extract of *A. ursinum* have not been tested before.

Materials and methods

Extracts preparation

Bulbs of *Allium sativum* were collected from a private garden, while leaves of *Allium ursinum* were gathered from the "Alexandru Borza" Botanical Garden in Cluj-Napoca. In both cases, samples were obtained from mature plants cultivated under natural conditions without any special treatment. The preparation of the extracts followed protocols previously described in the literature (Barbu *et al.,* 2023).

Au-Nanoparticles biosynthesis and characterization

For the gold nanoparticles synthesis, a protocol by Coman *et al*. (2013) was followed. 25 ml of a 0.2 mM $HAuCl_4x4H_2O$ solution was brought to a boil and 50 µl of *A. sativum* extract were added to the boiling solution under vigorous stirring. A rapid colour change could be observed in about 30s. The mixture was left to boil for another 5 minutes. In the case of *A. ursinum*, because of the difference in extract concentrations, 120 µl were added to the tetrachloroaurate solution and the mixture was left to boil for 15 minutes. The amounts were chosen based on author recommendations as well as several incremental tries starting at 30 µl for *A. sativum* and at 60 µl for *A. ursinum*, all these solutions having a blue colour and being deemed unstable (Coman *et al.,* 2013).

The *Allium* AuNPs were characterised through UV-Vis spectroscopy. The UV-Vis absoption spectra were recorded on a Jasco V-630 spectrophotometer. The measurements were performed in the 400-1000 nm wavelength range, with a spectral resolution of 1 nm, using quartz cuvettes with 1 cm optical path.

Antimicrobial activity

The antimicrobial activity of the nanoparticles was evaluated by disk-diffusion method against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and two *Candida* species: *Candida albicans* ATCC 10231 and *Candida parapsilosis* ATCC 22019 on Mueller–Hinton agar (MH) in which 5 mm wells were made using a sterile cut pipette tip. A 0.5 McFarland standard suspension of each pure microbial culture was evenly inoculated onto the agar surface using a sterile swab. Sterile cotton discs, 5 mm in diameter, were placed in the wells, and 150 µL of each AuNP suspension, along with 150 µL of 30% alcohol as a control, was pipetted onto the discs. Sulfamethoxazole, cefuroxime and voriconazole were used as controls. The plates were incubated at 37 °C for 24 hours, after which the zones of inhibition were measured. All tests were conducted in triplicate, and results are reported as the mean ± standard error (Barbu *et al.,* 2023).

Results

Characterization of AuNPs by UV-Vis spectrometry

The *A. sativum* AuNPs have a maximum peak at 539 nm, while the *A. ursinum* have the plasmonic band at 571 nm as shown in Figure 1. Both peaks are in accordance with the ones indicated in literature for gold nanoparticles, the red-shift indicating larger nanoparticles were formed with *A. ursinum* extract (Huang and El-Sayed, 2010). The *A. sativum* nanoparticles have a much sharper peak, indicating less polydispersity than the *A. ursinum* ones, which have a broader band. The *A. ursinum* ones display a double colour in solution, indicating the possibility of existence of at least two different shapes that interact differently with light.

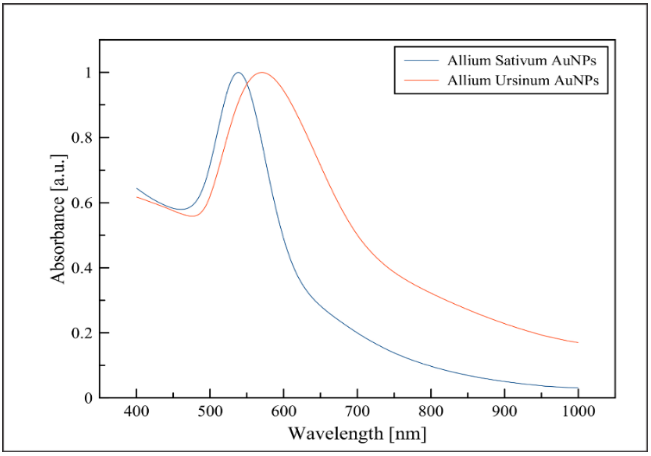

Figure 1. UV-Vis spectra of AuNPs biosynthesized using a bulb extract of *A. sativum* and leaves extract of *A. ursinum*

Stability of nanoparticles in time was assessed by comparing the UV-Vis spectra at day 0 and day 20. The broadening of the bands indicate the aggregation of the nanoparticles, and thus a lack of stability in time. The AuNPs from *Allium sativum* perform more effectively than those from *Allium ursinum*, which have almost completely dissolved within 20 days.

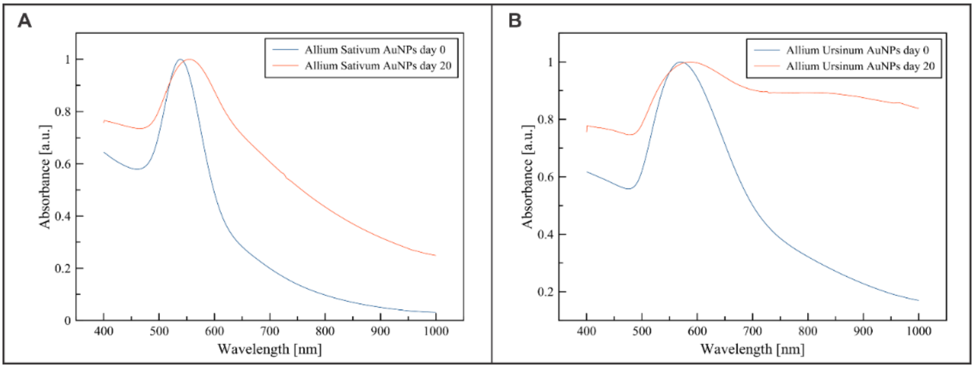

Figure 2. A. UV-Vis spectra of *A. sativum* AuNPs on day 0 and day 20; **B.** UV-Vis spectra of *A. ursinum* on day 0 and day 20.

Antimicrobial activity

Gold nanoparticles showed better effects on the two tested bacterial species than on the *Candida* species, as illustrated in Figure 3. The nanoparticles synthesized using *A. sativum* extract had similar effects on both bacterial species, with inhibition zones of 14 mm for *E. coli* and 13 mm for *S. aureus*. The effects on *E. coli* are even comparable to those of cefuroxime, used as a control. Nanoparticles obtained using *A. ursinum* extract showed weaker effects on S. aureus compared to sulfamethoxazole, used as control, with inhibition zones of 11 mm and 20 mm, respectively. For the two tested *Candida* species, the effects of the gold nanoparticles synthesized with both extracts were lower compared to voriconazole, used as control, showing inhibition zones of 7–8 mm versus 25–35 mm.

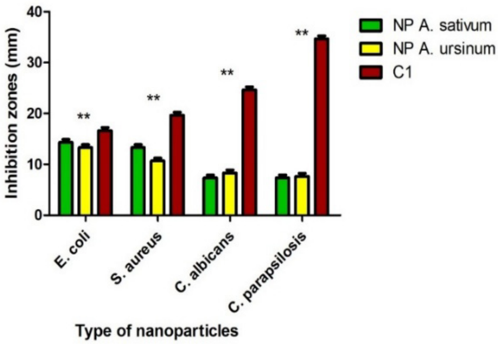

Figure 3. The antimicrobial effect of AuNPs obtained with *Allium sativum* and *A. ursinum* extracts on *S. aureus* (C1 – sulfamethoxazole), *E. coli* (C1 – cefuroxime), *C. albicans* and *C. parapsilosis* (C1 – voriconazole). The values represent the mean of three measurements ± standard deviation; ** $p < 0.005$, according to one-way ANOVA.

Discussion

Silver and gold nanoparticles, classified as noble metallic nanoparticles, have attracted significant attention due to their outstanding biological and physicochemical properties (Dauthal and Mukhopadhyay 2016; Saeed *et al.,* 2023).

In UV–Vis spectroscopy, the surface plasmon resonance (SPR) peak of gold nanoparticles typically lies between 510–580 nm, depending on size, shape, and surrounding medium (Huang and El-Sayed 2010). The red-shifted SPR band indicates larger or more irregularly shaped nanoparticles, consistent with findings from Sharma *et al.* (2009) and Jain *et al.* (2007) who showed that anisotropic AuNPs (e.g., rods, triangles) absorb light at higher wavelengths compared to spherical ones (Jain et al. 2007; Sharma, Yngard, and Lin 2009).

The *A. sativum*-AuNPs had a sharper and narrower peak, indicating a monodisperse or uniform size distribution. In contrast, the *A. ursinum* extract produced a broader, less defined peak, suggesting higher polydispersity. This finding aligns with previous results of Bastus *et al.* (2011), which demonstrated that monodisperse nanoparticles produce sharper SPR peaks, while heterogeneous mixtures result in broader bands. The phytochemical composition of *A. sativum* (especially the higher concentration of allicin and other sulfur-containing compounds) may have led to better capping and size control during nanoparticle formation (Bastús, Comenge, and Puntes 2011).

The *A. ursinum*-AuNP solution showed a dual color, suggesting a mixture of particle shapes. In literature, this is often linked to the presence of anisotropic nanoparticles, which can display multiple SPR modes. For instance, gold nanorods or triangular prisms can show both transverse and longitudinal absorption peaks, leading to solution colors ranging from violet to blue-green (Link and El-Sayed 2000). Mixed shapes can also arise when the reaction kinetics are slower or less controlled, possibly due to lower phytochemical concentrations or weaker reducing/stabilizing agents in *A. ursinum* (Huang & El-Sayed 2010).

The UV–Vis spectral analysis supports that both *Allium sativum* and *Allium ursinum* can mediate the green synthesis of gold nanoparticles, but with notable differences in nanoparticle characteristics. *A. sativum*-derived AuNPs exhibit smaller size, lower polydispersity, and greater homogeneity, likely due to more efficient capping and reducing action of its bioactive sulfur compounds. In contrast, *A. ursinum* results in larger, more diverse nanoparticle populations, possibly due to variations in phytochemical content or weaker stabilization capacity. These differences may influence the physicochemical behavior nanoparticles and biological activity, reinforcing the importance of extract composition in green nanoparticle synthesis.

The antimicrobial activity of gold nanoparticles (AuNPs) synthesized using *Allium* species extracts are similar to previous findings emphasizing previous studies emphasizing the broad-spectrum antibacterial potential of biogenic AuNPs, particularly against Gram-negative and Gram-positive bacteria. In this study, AuNPs showed greater efficacy against *E. coli* and *S. aureus* than against *Candida* species. Gabriel *et al.* (2022) tested different concentrations of nanoparticles with *Allium*, and their study showed that as the concentration increased, the inhibition zone also increased. At concentrations of 100 mg/mL, they obtained inhibition zones of 19 mm for *E. coli* and 17 mm for *S. aureus.* The results obtained in our study are comparable to those of Gabriel *et al.* (2022) at the concentration of 40 mg/mL (*S. aureus* 15 mm, *E. coli* 14 mm, and *S. aureus* 13 mm, *E. coli* 13 mm, respectively).

Meanwhile, AuNPs synthesized with *A. ursinum* extract exhibited a weaker antibacterial effect on *S. aureus* (11 mm inhibition zone), lower than that of sulfamethoxazole (20 mm). The lower efficacy may be linked to differences in nanoparticle characteristics, such as larger particle size or greater polydispersity, as inferred from the broader UV-Vis absorbance band observed in *A. ursinum*-derived AuNPs (Huang and El-Sayed 2010; Franci *et al.,* 2015). Larger or more polydisperse nanoparticles often show reduced antimicrobial potency due to decreased surface area-to-volume ratios and less efficient cellular interactions (Sondi and Salopek-Sondi 2004).

In contrast, both nanoparticle formulations demonstrated poor antifungal activity, with inhibition zones between 7–8 mm against *Candida* species, much lower than those observed with voriconazole (25–35 mm).

Conclusions

Our study confirms that biologically synthesized gold nanoparticles, especially those obtained with *A. sativum* extract, are promising antibacterial agents, but further optimization (e.g., size control, functionalization) may be necessary to enhance their antifungal and antibacterial effectiveness.

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