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Evaluation of the anticancer and antibiofilm properties of *Agrimonia eupatoria* L. silver nanoparticles

Katarina G. Marković [1*], Mirjana Ž. Grujović [1], Ana Kesić[1], Milica Paunović[2], Katarina Ćirković[2], Ivana Radojević [2], Nevena H. Djukić[2]

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Abstract: This study evaluates the cytotoxic and antibiofilm effects of silver nanoparticles (AgNPs) synthesised with aqueous and acetone extracts of *Agrimonia eupatoria* L. AgNPs significantly reduced HCT-116 colorectal cancer cell viability after 72 h, with AgNPs-acetone showing stronger effects but AgNPs-H_2O being more effective at lower doses. AgNPs-H_2O also inhibited biofilm formation by clinically relevant bacteria, with a wastewater isolate of *Pseudomonas aeruginosa* being the most sensitive. The results confirm the strain- and medium-specific activity of these AgNPs and support their potential as multifunctional anticancer and antibiofilm agents. Further studies are needed to assess their selectivity, safety, and mechanisms of action.

Keywords: silver nanoparticles, cell line, bacterial biofilm, antibiofilm activity.

1. Introduction

Limited effects and side effects of conventional cytostatics have driven research towards natural and novel synthetic anticancer agents. Nanotechnology, especially green-synthesised silver nanoparticles, shows strong potential against various cancer cell lines [1–4]. AgNPs also affect bacterial biofilms, which are highly resistant to antibiotics. Smaller particles (<10 nm) penetrate biofilms like those of *S. aureus* and *P. aeruginosa* more effectively due to higher surface area and better ion release [5]. This study investigated the cytotoxicity of AgNPs synthesised with aqueous and acetone extracts of *A. eupatoria* and their antibiofilm activity, aiming to demonstrate their pharmaceutical potential.

2. Materials and Methods

Silver nanoparticles (AgNPs-H_2O and AgNPs-acetone) were prepared as described by Marković et al. [7]. HCT-116 cells were grown in DMEM supplemented with 10% FBS at 37 °C and 5% CO_2. Cells (10^4/well) were seeded in 96-well plates and treated with different concentrations of AgNPs (1–150 µg/mL) for 72 h, while untreated cells served as controls. Cell viability was determined by the MTT assay: after 72 h, MTT was added for 4 h, the resulting formazan were dissolved in DMSO, and absorbance was measured at 550 nm to calculate the IC_{50}. The antibiofilm activity of AgNPs-H_2O was assessed by crystal violet staining [6]; after 24 h incubation, the biofilms were fixed, stained, and quantified at 630 nm.

2.1. Statistics

Data (mean ± SEM, n=3) were analyzed by one-way ANOVA with Bonferroni/Dunnett's test ($p < 0.05$). IC_{50} was calculated in CalcuSyn (BIOSOFT, UK).

3. Results and Discussion

The cytotoxic effects of AgNPs-H_2O and AgNPs-acetone on HCT-116 cells after 72 h are shown in Figures 1 and 2. Both were tested at 1–150 µg/mL, and IC_{50} values were determined. Both treatments significantly reduced cell viability in a dose-dependent manner. AgNPs-acetone showed stronger overall cytotoxicity (lower IC_{50}), but at 10 µg/mL, AgNPs-H_2O was more effective (22% viable vs. 65%), suggesting its potential for further testing.

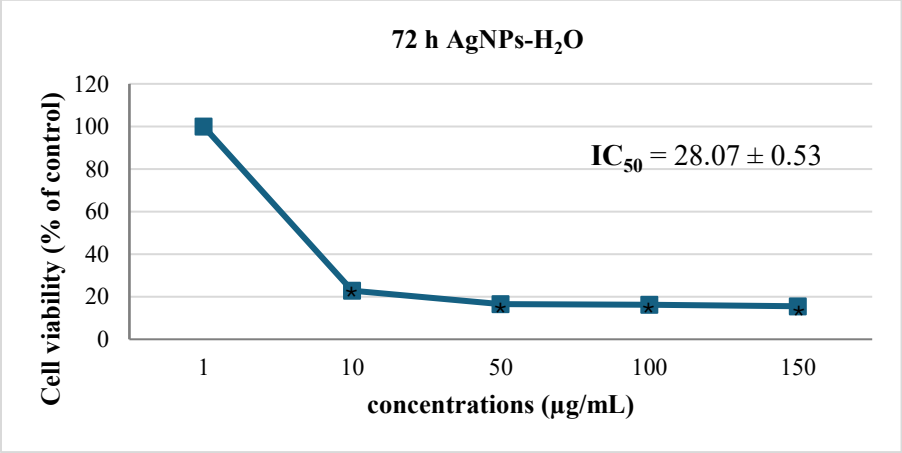

Figure 1. Effect of AgNPs-H_2O on HCT-116 cells viability after 72 h of treatment.

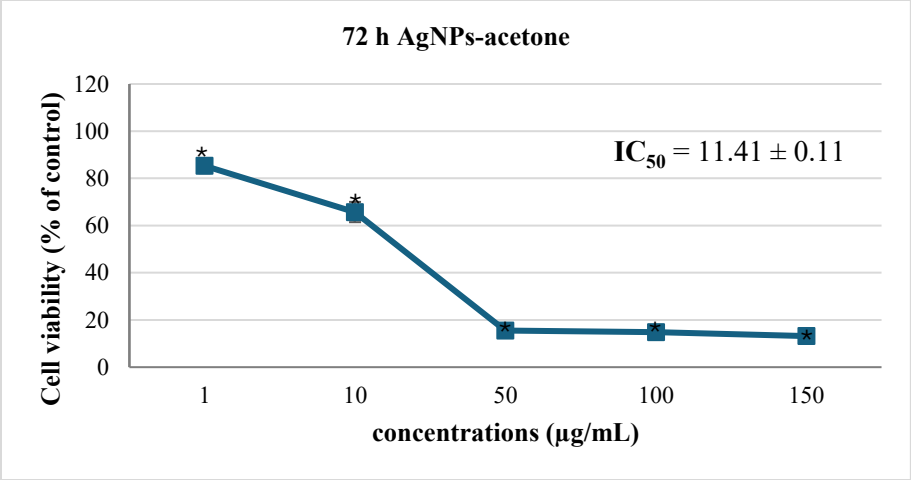

Figure 2. Effect of AgNPs-acetone on HCT-116 cells viability after 72 h of treatment.

The synthesis, characterisation, and biological effects (anticancer, antimicrobial, hemolytic, genotoxic) of these nanoparticles have been reported previously [7]. In that study, AgNPs showed strong anticancer activity against SW-480 cells, especially at higher doses (50–150 µg/mL), which also affected healthy cells [7]. Compared to the previous data, the present study confirms that AgNPs-H_2O had the strongest effect on HCT-116 cells, with both AgNPs-H_2O and AgNPs-acetone significantly reducing viability and showing low IC_{50} values, highlighting their strong cytotoxic potential. Marković et al. (2024) also showed antimicrobial effects on planktonic bacteria. Here, antibiofilm activity was confirmed as well, which is relevant since bacteria predominantly exist in biofilms [8]. The wastewater *P. aeruginosa* isolate was most sensitive (BIC_{50} 0.094 mg/mL). Other strains showed varying susceptibility depending on strain and medium. Results are summarized in Table 1. Overall, AgNPs-H_2O proved effective against both Gram-positive and Gram-negative biofilms, with efficacy influenced by bacterial species and conditions [9–11]. These results support further research on AgNPs-H_2O as a broad-spectrum antibiofilm agent.

Table 1. Inhibition of biofilm formation by AgNPs-H_2O in different medium expressed

BIC values (mg/mL) - means biofilm inhibitory concentration

Microorganisms	Mueller-Hinton Broth		Tryptic Soy Broth	
	BIC_{50}	BIC_{90}	BIC_{50}	BIC_{90}
S. aureus	/	/	0.83	1.18
B. subtilis	/	/	/	/
P. mirabilis	0.33	0.43	/	/
P. aeruginosa (clinical isolate)	0.24	0.30	0.72	1.18
P. aeruginosa (wastewater isolate)	0.19	0.30	0.094	1.19

4. Conclusions

This study shows that silver nanoparticles (AgNPs-H_2O and AgNPs-acetone) have strong dose-dependent cytotoxic effects on HCT-116 cells and notable antibiofilm activity against various bacteria. Their low IC_{50} values and broad-spectrum action suggest promising anticancer and antimicrobial potential, supporting further biomedical research.

Acknowledgment

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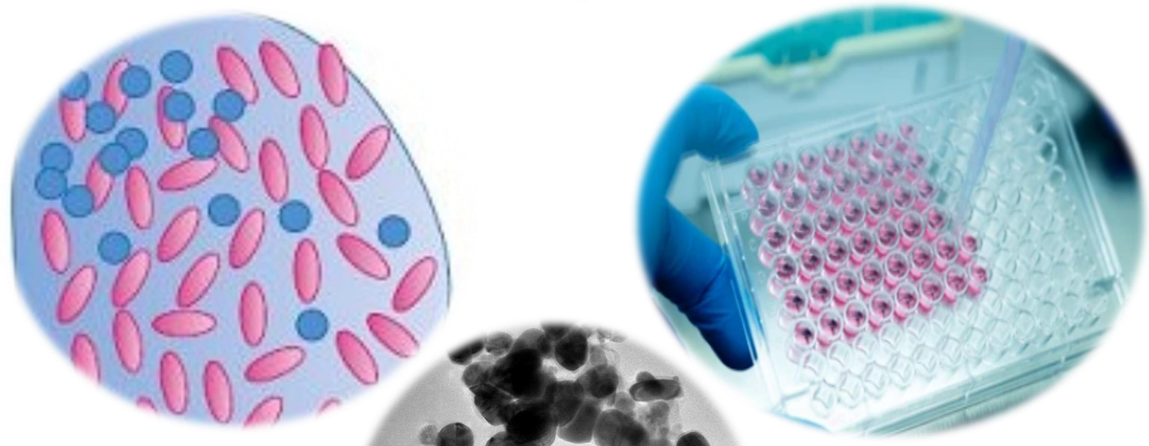
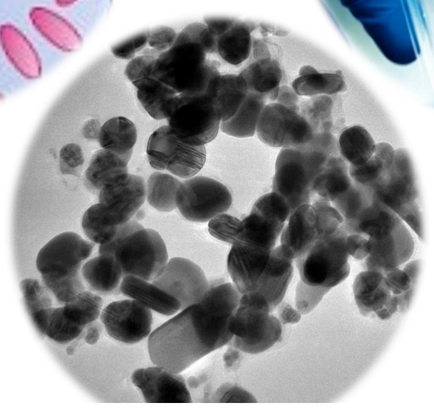


Baterial biofilm

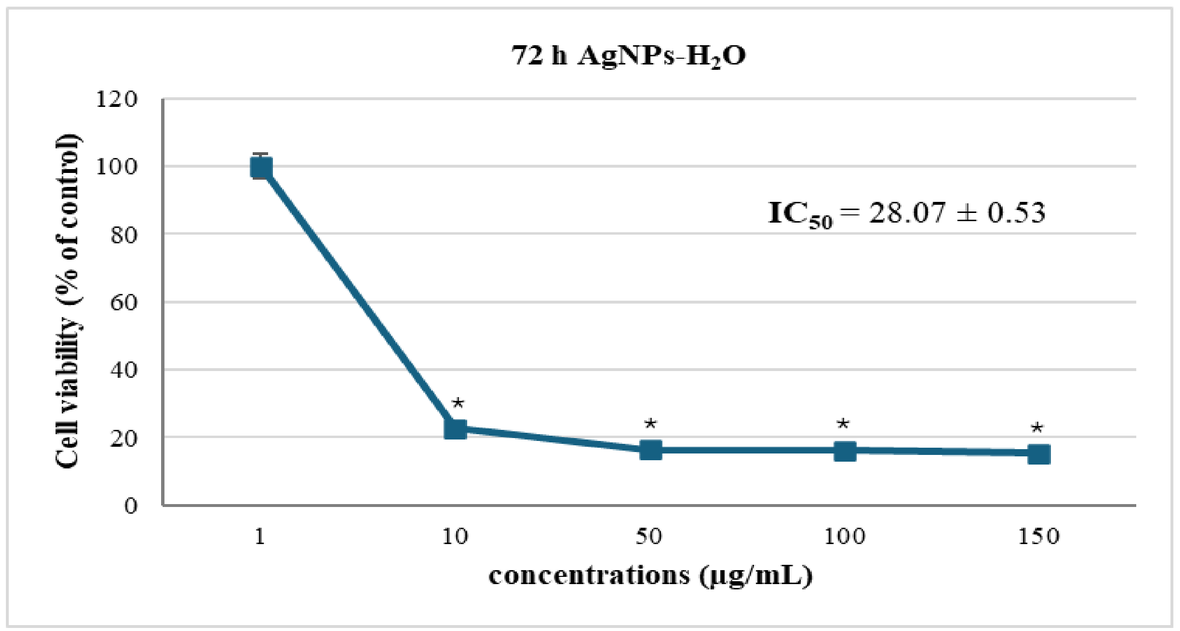


Silver nanoparticles

MMT assay

Agrimonia eupatoria

3. Results and Discussion



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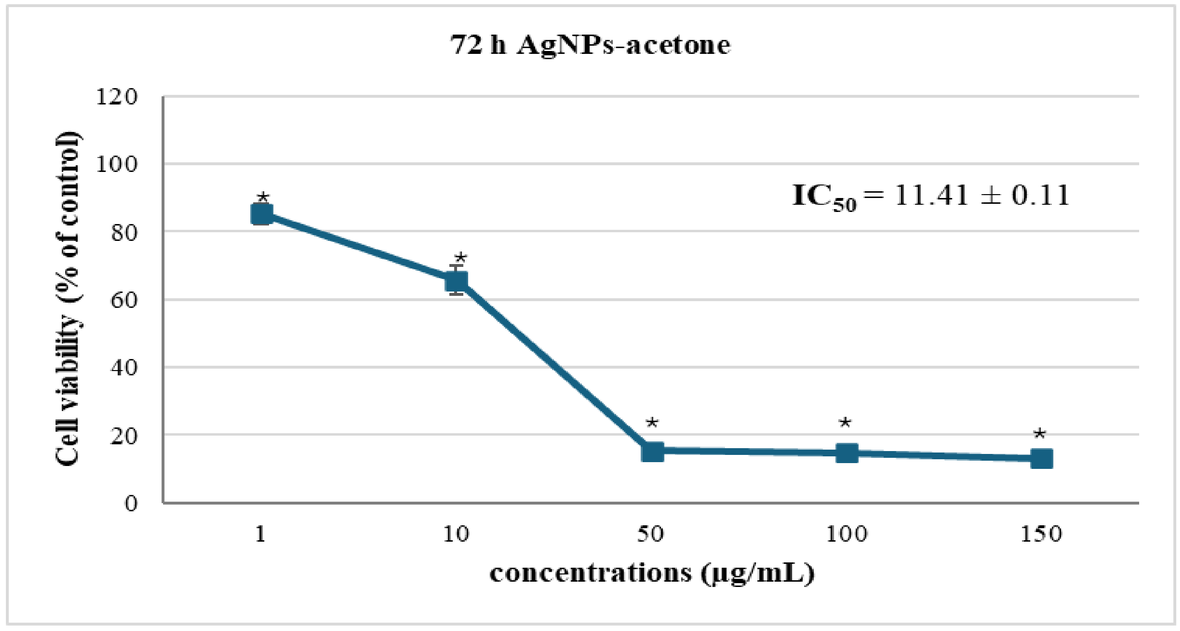

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