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Połączenie nanocząstek srebra i metabolitów wtórnych produkowanych w tkankach roślin owadożernych w walce z bakteryjnymi patogenami ludzkimi
Combining silver nanoparticles and secondary metabolites from tissues of carnivorous plants as a strategy to combat human pathogenic bacteria

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Summary

The drug resistance phenomenon of microbes is a fascinating feature of microbial cells. Nevertheless, the resistance to antibiotics and chemotherapeutics comprises of both clinically and economically relevant problems. Thus, there is a growing demand for new antimicrobials and the development of anti-infectious approaches towards drug-resistant pathogens. In the presented work, the antibacterial potential of secondary metabolites from carnivorous plants combined with silver nanoparticles was investigated against antibiotic-resistant bacterial pathogens.

Firstly, the bactericidal potential of plant extracts prepared with selected methods and silver nanoparticles used simultaneously was determined. The obtained results of phytochemical and microbiological analyses allowed to have identified the secondary metabolites of the naphthoquinone group to have been responsible for the synergistic, bactericidal effect caused by the plant tissue extracts and the silver nanoparticles combination. What is more, as a consequence of the synergy, the bactericidal concentrations of naphthoquinones were reduced by 97 and 98.5% when they were used in combination with silver nanoparticles against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively.

The objective of the study was to assess the significance of nanoparticle's parameters and a chemical structure of the naphthoquinone for the phenomenon of synergy. For this purpose, nanoparticles stabilized with polyvinylpyrrolidone and alkanethiols varied in length of carbon chain and functional groups (carboxylate, amine and trimethylamine) and selected naphthoquinones (plumbagin, 3-chloroplumbagin, ramentaceone, droserone, juglone, dichlone, lawsone and lapachol) were used. The results clearly depict that ligands coating the surface of a nanoparticle play a crucial role in their synergistic, bactericidal activity together with naphthoquinones against *S. aureus* cells. Furthermore, among all tested naphthoquinones, only plumbagin, 3-chloroplumbagin, ramentaceon, juglone and dichlone had strong synergistic, bactericidal potential when combined with silver nanoparticles. Since the bactericidal potential of naphthoquinones is considered to be related to their prooxidative activity, the level of

reactive oxygen species in bacterial cells treated with those secondary metabolites was determined. Although the relationship of the oxidative stress generation and the strong bactericidal activity of naphthoquinones were observed, their prooxidative activity did not determine the synergistic interaction with silver nanoparticles.

The objective of the study was also to establish the cytotoxicity of nanoparticles, naphthoquinones and their combinations towards eukaryotic cells cultures. It was observed that, as a result of synergistic interactions with the silver nanoparticles, the effective bactericidal concentration of naphthoquinones can be reduced to a level which exhibits a marginally toxic or a non-toxic effect to eukaryotic cells. It is worth emphasizing that, unlike silver nitrate, bactericidal concentrations of silver nanoparticles used in this study were non-toxic towards eukaryotic cells. Further, silver nanoparticles added to naphthoquinones did not enhance the cytotoxicity of the latter ones. The results obtained during the study confirmed a synergistic or at least additive bactericidal potential of silver nanoparticles and naphthoquinones towards other antibiotic resistant pathogens – *Klebsiella pneumoniae*, *Escherichia coli* and *Acinetobacter baumannii*.

Further research concerned the attempt to elucidate the mechanism by which silver nanoparticles enhance the antimicrobial activity of naphthoquinones. Results highlighted the complexity of the phenomenon of synergy observed for the tested antimicrobials and emphasized the role of the bactericidal co-action between silver ions and naphthoquinones, as well as a direct interaction of silver nanoparticles with molecules of naphthoquinone and their role in the disruption of bacterial cell membranes. The last aim of the study was to evaluate the anti-infectious potential of combined secondary metabolites and nanostructures on *Caenorhabditis elegans* as an infection model. Simultaneously used silver nanoparticles and two selected naphthoquinones (plumbagin and 3-chloroplumbagin) improved the survival rate of nematodes infected with *P. aeruginosa* after 4 days by 47.19 - 67.86% in comparison to the untreated control where all worms died.

Results described in this thesis confirm the high antimicrobial potential of synergistic combinations of secondary metabolites from carnivorous plants and silver nanoparticles. Moreover, they are the starting point for further research on the molecular basis of the synergistic interactions of nanoparticles and naphthoquinones and their application to bacterial infections.