# Study of the synergy between silver nanoparticles and selected antimicrobial agents against human bacterial pathogens.

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The main goal of the project is to determine if the combination of silver nanoparticles (AgNPs) obtained by green chemistry from extracts from carnivorous plants and commercially available antibiotics used in the treatment of burn wounds can be used to control bacterial infections caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Generating antibiotic (AB)-conjugated AgNPs (AB-AgNPs) will allow us to explore a new potential use of *in vitro* plant cultures for the green synthesis of AgNPs which, in combination with antibiotics, could combat antibiotic-resistant bacteria.

The main research hypothesis presented in this project is that exploiting the antioxidant potential of secondary metabolites contained in the tissues of carnivorous plants of the genus *Drosera*, subjected to elicitation in *in vitro* cultures, will allow to obtain biologically active silver nanoparticles. The combination of these AgNPs with antibiotics will be used to study their potential synergistic effect in eradication of dangerous human pathogens - *S. aureus* and *P. aeruginosa*, as well as their safety for eukaryotic cells.

The project plans to breed 20 different species of carnivorous plants of the genus *Drosera* in *in vitro* cultures and use elicitation to increase the level of secondary metabolites contained in their tissues. The level of metabolites will be determined using HPLC, and the antioxidant activity of extracts from carnivorous plants will be measured using the DPPH test. Obtained AgNPs will be analyzed using X-ray photoelectron spectrometry, dynamic light scattering and Fourier's spectroscopy. The characteristics of AgNPs will also be complemented with transmission electron microscopy and scanning electron microscopy. The antimicrobial potential of AgNPs will be evaluated using microdilution method. The established minimum bacterial growth inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) values will then be used to study the interaction between AgNPs and antibiotics using the Checkerboard Titration Technique. The next step will be generating AB-AgNPs and studying their effectiveness in eradicating *S. aureus* and *P. aeruginosa* biofilms using MBEC ™ High-throughput (HTP) Assay. In order to clarify the mechanism of AB-AgNPs activity, the analysis of bacterial cell integrity changes under the influence of AB-AgNPs will be carried using a method based on two fluorescent dyes. Additionally, the intracellular level of oxidative stress in bacterial cells will be determined using dihydroetidine. Transmission electron microscopy will also be used to show localization of AB-AgNPs in *S. aureus* and *P. aeruginosa* cells. Using the radioactive precursors of [$^3$H] thymine, [$^3$H] uridine and [$^3$H] leucine, the effect of AB-AgNPs and their combinations with antibiotics on DNA kinetics, RNA transcription and protein translation in pathogenic bacteria cells will be demonstrated. The next stage of research will be to examine the effect of AB-AgNPs on human fibroblasts and the nematode *Caenorhabditis elegans*. Studies using *C. elegans* model show a strong correlation of $LD_{50}$ values (Lethal Dose 50) with those of higher organisms such as rats and mice. This will allow us to study the biological effects of AB-AgNPs on eukaryotic cells.

The results of our research will facilitate the deeper understanding of the interaction between silver-based antibiotics and nanoparticles in action on human pathogens.