C19. **Flow cytometry applications in antimicrobial and antipathogenic activities investigation of *Amorpha fruticosa* essential oil**

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Introduction. Desert false indigo (*Amorpha fruticosa* L.) is an invasive alien species, its fruits contain essential oil rich in compounds known for their antimicrobial properties. The aim of the study was to investigate the antimicrobial and antipathogenic activity of *A. fruticosa* essential oil through microbiological and flow cytometry methods.

Materials and Methods. The essential oil was obtained by steam distillation of water and its chemical composition was identified by GC -MS. For testing the antimicrobial activity were studied reference and clinical microbial strains belonging to the species *Bacillus subtilis, Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Candida albicans*. The screening of antimicrobial activity was made by adapted disk diffusion method and the values of MIC (minimum inhibitory concentrations) were determined by the binary microdilution method in liquid medium. In order to investigate the antimicrobial effect by flow cytometry were used as parameters the cellular membrane integrity (propidium iodide) and the efflux pump activity (Nile red). The antipathogenic properties of *A. fruticosa* essential oil were studied by determining the adhesion capacity of microbial strains to inert and cellular substrate (microtitration method, modified Cravioto method).

Results. *A. fruticosa* fruits contain 0.57 % essential oil rich in δ-cadinene (20,09 %), γ-muurolene (12,79 %), α-muurolene (12,54 %) and γ-cadinene (7,86 %). The volatile oil of *A. fruticosa* showed an antimicrobial activity against all microbial strains studied at concentrations ranging from 1.41-22.5 mg/mL, being most active against Gram-positive strains. The analysis on the essential oil influence on cellular membrane integrity by flow cytometry led to comparable results with those obtained by binary microdilution method in liquid medium and the efflux pumps' activity was disrupted in the presence of essential oil inhibitory concentrations. The *A. fruticosa* essential oil inhibited the microbial adhesion at concentrations ranging from 0.7-22.5 mg/mL, and at sub-inhibitory concentrations, the adhesion index decreased from 96.504 % to 36.932 %.

Conclusion. The antimicrobiological analysis concerning the investigation of the antimicrobial and antipathogenic activity of the *Amorpha fruticosa* essential oil showed microbial growth inhibition and the capacity of tested strains to colonize different substrates. The flow cytometry confirmed the antimicrobial effect of the essential oil to the proper concentration of the MIC value, indicating the disruption of the efflux pump activity as possible mechanism of action. In conclusion, the *A. fruticosa* essential oil may represent an alternative or aid to antibiotics therapy for infections caused by resistant and adhered microorganisms to various substrates and the flow cytometry can be a valuable tool in investigating these properties in real time.