C20. **Evaluation by microbiological and flow cytometry methods of antifungal activity of cinnamon essential oil incorporated in alginate**

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Introduction: Due to the numerous active biological properties, including antimicrobial activity, Cinnamon essential oil (SCO) has many uses in the pharmaceutical, medical and food industry. However, practical applications of SCO are limited due to the volatility of the active components. To overcome this limitation, we developed SCO embedded in alginate beads. This system represents both a way of protection and the controlled release of active the principles. Alginates (Alg) are natural polymers, with hydrophilic and biocompatible properties, and because of their ability to form hydrogels (by reaction with polyvalent metal cations) they are very often used as controlled release systems for therapeutic agents. The purpose of this study was to obtain alginate beads embedded with SCO and to demonstrate antimicrobial activity of the obtained system by microbiological and flow cytometry classical methods.

Material and methods: Chemical composition of the cinnamon essential oil (*Cinamomum cassia L.*) used in the experiment (provided by COZAC PLANT SRL, Bucharest, Romania) was analyzed by GC- MS. Antimicrobial properties of SCO were evaluated against Gram-positive, Gram-negative bacteria and microfungi by disk diffusion and the successive binary microdilution methods. Antimicrobial activity results obtained for *Candida albicans* ATCC 10231 strain have been correlated with results obtained from flow cytometry method (assessed at 3h, 6h and 24h of incubation), using the parameters of cell membrane integrity (viable cells) and permeability (cell death). The cytotoxic effect of cinnamon essential oil was evaluated using HEp-2 cell line, by CellTiter (cell viability) and flow cytometry (cell cycle quantification) methods.

Manufacturing of beads (AlgSCO) was achieved by emulsion extrusion method. Different volumes of SCO (10, 20, 50, 100 and 200 µL) were embedded in the sodium alginate solution (3%) and the obtained emulsions were homogenized by magnetic stirring (for 15 min. at 40°C and 300 rpm). Beads formation was possible by cross-linking emulsion in $CaCl_2$ solution (5%). Quantification of the antifungal activity against *Candida albicans* ATCC 10231 was performed by three methods, spectrometry, determination of colony forming units (CFU) and flow cytometry.

Results: GC-MS analysis revealed that SCO contains cinnamaldehyde (86.27%), cinnamic acid (1.93%), o-methoxycinnamic aldehyde (2.57%) and other compounds in concentrations of below 1%. All tested strains were susceptible to SCO, correlating the results obtained for antifungal activity by classical microbiological methods with the results obtained by flow cytometry we observed changes of membrane integrity, occurring after 24 h of incubation in the presence of SCO. SCO toxicity at dilutions higher of 1:3,000 is reduced but the results showed a a noticeable change in the G2 phase of the cell cycle. Among all beads variants obtained, only AlgSCO-100 µL and AlgSCO-200 µL variants demonstrated antifungal activity, confirmed by all three methods used. Antifungal activity quantification by flow cytometry proved the advantage of reduced analysis time and the possibility to make certain determinations in temporal dynamics.

Conclusions: The SCO has a broad spectrum antimicrobial action and low toxicity at decreased concentrations. The inhibitory activity of the SCO can be attributed to membrane changes, according to analysis by flow cytometry. Alg beads with higher concentration of SCO show great antifungal activity, demonstrating the possibility of subsequent use for the development of new biomaterials with antifungal properties.