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P013 - Helix aspersa muller mucus (Helixcomplex®) p41 protein prevents Pseudomonas aeruginosa growth and promotes mammalian bronchial epithelial cell proliferation

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Introduction: Snail mucus is a mixture of active substances commonly thought to have healthy properties for the treatment of skin disorders. Although snail mucus is an ingredient of various cosmetic and para-pharmaceutic products, the chemical composition and the biological effects are still unknown.

Materials and Methods: Crude extract from *Helix aspersa muller* mucus (HelixComplex®) was obtained (Patent N: 102017000117547) and, after chemical and molecular characterization, tested on *in vitro* experimental models of microbial infections and bronchial epithelial cell proliferation.

Results: Differently from what expected, HelixComplex® was characterized by the presence of small amounts of glycolic acid and allantoin. In size separation experiments, we observed a peculiar protein band at 30-40 kDa. Protein band was extracted and analyzed by ESI-Q-TOF-HPLC-MS instrument: the protein band in the range of 40-30kDa was composed by a single protein of 41kDa (p41). The product obtained from size exclusion centrifugation at 41 kDa showed anti-bacterial activities. In particular, it presents a strong bactericidal effect on *P. aeruginosa*, with an IC50 dose of 16ug/ul, as early as 15 minutes after treatment, on both laboratory strain PAO1 and *P. aeruginosa* clinical strains. This bactericidal effect was evident on both planktonic and biofilm-forming cultures of clinical *P. aeruginosa* strains. By using different *in vitro* assays on epithelial bronchial cell cultures, we found that HelixComplex® lacked of cytotoxicity, and, importantly, that it was able to significantly induce cell proliferation.

Conclusions: These results identify the *H. aspersa* snail mucus p41 as a suitable antimicrobial compound for the treatment of *P. aeruginosa* associated infections, where we need to: i) block *P. aeruginosa* proliferation and biofilm formation; ii) ensure a regenerative efficacy on bronchial epithelial cells. Interestingly, we obtained efficacy results only in the presence of a protein complex purified by size exclusion centrifugation. This procedure guarantees the presence of the p41, but also of other actives present in the *H. aspersa* mucus, confirming the power and uniqueness of the actives present inside this natural product.