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Short communication

Formulation and characterization of snail slime-chitosan microparticles with herbal extracts intended for treatment of chronic wounds

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Introduction

Wound healing is a highly regulated process that is critical in maintaining the barrier function of the skin. The cascade of events in wound healing can be affected by a number of diseases which can result in chronic, non-healing wounds. In chronic wounds, the antioxidative defenses that are usually present in acute wounds are hindered and are unable to subside the oxidative stress, causing the wound to be stagnant in the inflammatory phase of wound healing (Moseley et al., 2004).

Chitosan is a biocompatible and biodegradable polysaccharide that comes from chitin, and is commonly used in mucoadhesive dosage forms because of its adhesive properties. Garden snail's mucus contains vitamin E, polyunsaturated fatty acids and steroids that help in the regeneration of epidermal cells (Dhiman & Pant, 2021). *Hypericum perforatum* herbs (HP), *Calendula officinalis* flowers (CO) and CBD oil have antioxidative and anti-inflammatory properties that can help in the treatment of chronic wounds (Borges et al., 2013; Butnariu et al., 2012; Silva et al., 2005). Chitosan based microparticles containing these ingredients would theoretically be able to swell in the wound exudate, which would allow a sustained release of the active ingredients and stimulate the repair process, thus, providing a prolonged therapeutic effect (Pagano et al., 2020).

The aim of this study was to perform proper physicochemical characterization of slime-chitosan microparticles (SCMPs) containing selected plant extracts, and to investigate there *in vitro* potential in the treatment of chronic wounds.

Materials and methods

Plant material extraction - The extraction from dried *HP* herbs and *CO flowers* was carried out in 40% and 75% ethanol (*v/v%*) for 24 hours, respectively. Afterwards, the ethanolic extracts were spray-dried at 105 °C (5 mL/min, Buchi Mini Spray dryer B-290, Switzerland).

Preparation of SCMPs - Three different formulations of SCMPs (samples *F1, F2, F3*) containing 2 g of previously spray-dried snail slime (5 mL/min, 105 °C; Patent application MK/P/2021/000853) and different amounts of spray-dried HP, CO extracts and CBD oil (European patent application EP22188230) were prepared. Firstly, the plant extracts and slime were dissolved in water, while the chitosan (0.5 g) was left in 1% glacial acetic acid for 20 hours. Afterwards, 1.5 g of TPP was added to the extract-slime mixture and the solutions were spray-dried using a two-fluid nozzle (6 mL/min, 150 °C, Buchi Mini Spray dryer B-290, Switzerland).

Characterization of SCMPs - Particle size (D50) and particle size distribution (SPAN factor) were determined by laser diffractometry using ethanol as a solvent (RI 1.520, absorption 0.01; Mastersizer 2000 equipped with Hydro 2000S, Malvern Instruments, UK). Total phenolic content of 3 mg/mL SCMPs dissolved in phosphate buffer pH 7.4:methanol mixture (1:1, *v/v%*), was investigated using a slightly modified Folin-Ciocalteu assay (Singleton et al., 1999). The antioxidant potential of the prepared SCMPs formulations (0.01-1 mg/mL) during 120 min (expressed as % of the initial fluorescence at 0 min for each formulation), were evaluated by The Oxygen Radical

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Absorbance Capacity (ORAC) assay (37 °C, excitation at 492 nm and emission at 535 nm; VICTOR Perkin Elmer, USA) (Shalabalija et al., 2021). The neutralization of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay was done in accordance with Gyamfi et al. (1999), with slight modifications, where SCMPs were dissolved in a mixture of water:methanol (1:1, *v/v%*) in final conc. of 0.1-2 mg/mL. MTT test was conducted on a keratinocyte cell culture line (HaCaT, CLS Cell Lines Service GmbH) in order to investigate the cytotoxic activity of the SCMPs (0.05-0.10 mg/mL) after 48 and 72 h of incubation (570 nm, microplate reader VICTOR Perkin Elmer, USA) (Pagano et al., 2020). The cell culture maintenance was in accordance with the guidelines of the manufacturer.

Results and discussion

D50 of SCMPs containing selected plant extracts was 5.87±0.1 to 5.96±0.13 for all three prepared formulations, while the SPAN values of ~1.5 indicated that all samples followed uniform and narrow particle distribution.

The phenolic content, expressed as mg of plant extracts in 100 mg of SCMPs was 1.134±0.21, 1.514±0.1 and 2.197±0.09 mg for *F1, F2* and *F3*, respectively, which shows a successive increase in the encapsulation efficacy of the formulations by incorporating higher amount of the used extracts.

Results from the ORAC test suggested a concentration and time dependent increase of SCMPs antioxidant capacity (AC). AC for the concentration range of 0.01-1 mg/mL was 5.52-98.41% for *F1*, 15.58-91.34%) for *F2*, and 5.15-99.42% for *F3*, over a period of 120 min. The decline of IC_{50} values obtained from DPPH test (1.802 mg/mL, 0.953 mg/mL and 0.764 mg/mL for *F1, F2* and *F3*, respectively) confirmed the positive influence of the quantity of incorporated herbal components on the antioxidant potential of SCMPs. Additionally, blank SCMPs (without HP, CO and CBD oil) did not show any antioxidant activity.

Cell viability of HaCaT cell culture line was 78.58-87.00% after 48 h and 65.81-83.56% after 72 h incubation with for all 3 SCMPs samples in their highest concentration tested (0.1 mg/mL)

Conclusion

SCMPs show substantial antioxidant properties and low cytotoxicity after 48 h incubation, which indicates on their successful potential in the treatment of chronic wounds.

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