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Short communication

Evaluation of antioxidant properties of herbal mixture and CBD oil for the treatment of chronic wound

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Introduction

Non-healing wounds could result in significant discomfort and distress for the patient while draining the medical system of its resources (Han et al., 2017). Wound healing has three overlapping phases: homeostasis/ inflammation, proliferation and remodeling. During the inflammatory phase of wound healing process, there is a temporary increase in the level of oxidants, which prompts the activation of the antioxidant defense mechanisms based on gradual detoxification of oxidants and on a gradual return of cells to the state of redox homeostasis. If this detoxification process is hindered in any way, the result will be a shift in the homeostasis, leaving the chronic wounds stagnant in the inflammatory phase (Moseley et al., 2004).

Hypericum perforatum (HP) has a few mechanisms with relevance to wound healing, such as antimicrobial and antioxidative activity, anti-inflammatory effects and promoting keratinocyte differentiation. *Calendula officinalis* (CO) flowers contain phenolic and flavonoidic substances which have antioxidative and antimicrobial properties, both crucial factors in the wound healing process (Butnariu et al., 2012). Cannabidiol (CBD) is one of the main pharmacologically active phytocannabinoids that has anti-inflammatory and antioxidant effects, as well as anxiolytic, antipsychotic and antidepressant properties. CBD interrupts free radical chain reactions, capturing free radicals and transforming them into less active forms (Borges et. al., 2013).

Therefore, the aim of this study was to evaluate the antioxidant properties of the aforementioned materials, and to show their potential use in treatment of chronic wounds.

Materials and methods

Preparation of herbal extracts

Dried herbs from HP and flowers from CO were powdered with an electric grinder (Iskra Coffee Grinder Girmi 80s, Slovenia) and were added to 45% and 70% (*v/v*) ethanol, respectively, followed by 24 hours' extraction period. Afterwards, the ethanolic extracts were spray dried (Buchi Mini Spray dryer B-290, Switzerland; at 105 °C).

ORAC assay

The modified Oxygen Radical Absorbance Capacity (ORAC) assay was used to examine the antioxidant capacity of the extracts, according to the method described in Shalabalija et al. (2021). Namely, the test was performed at 37 °C in darkness. The measuring was done at 492/535 nm (excitation/emission) using plate reader (VICTOR Perkin Elmer, USA) at the following time intervals: 0, 30, 60, 90 and 120 minutes.

DPPH assay

The neutralization of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay was done in accordance with Gyamfi et al., with slight modifications. Namely, 200 µL of the prepared samples in concentration range between 0.1 and 2 mg/mL, were placed in a test tube to which 4 mL of 100 µm ethanol solution of DPPH radical was added. The mixture was stirred for 1 minute and left at room temperature in darkness for 10 minutes. The reduction of the DPPH radical was spectrophotometrically determined at 517 nm (Agilent 8453 UV-Vis spectrophotometer, Agilent Technologies, USA). Methanol was used as a control. Quercetin, ascorbic acid and BHA were used as standards. The antioxidant capacity

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was presented as a concentration needed to decrease the initial DPPH concentration by 50 % (IC_{50}) (mg/mL). The inhibition percent was calculated with the following formula:

$$\% \text{ inhibition} = \frac{(Ac-As)}{Ac} * 100 \qquad \text{Eq. 1}$$

where Ac is the absorbance of the control, and As is the absorbance of the sample.

Total phenolic content

The total phenolic content of the prepared extracts and CBD oil was evaluated using a modified Folin-Ciocalteu assay, as proposed by Singleton et al. (1999). Namely, 1 mL of the prepared samples and 0.5 mL of Folin-Ciocalteu reagent (1:10 *v/v* dilution with deionized water) were placed in a 10 mL flask, while the solution turns green, they were mixed for 5 minutes at room temperature, after which 0.4 mL of 7.5% sodium carbonate were added and the flask was filled to 10 mL with distilled water. The total phenolic content was expressed as miliequvalents of gallic acid per gram of dried plant material.

Results and discussion

The antioxidant capacity of the prepared extracts and CBD oil was evaluated using two different assays (ORAC and DPPH assays). The results from the ORAC assay, obtained at the aforementioned time intervals showed that in the concentration range between 0.01 – 1 mg/mL after 120 minutes incubation time, the antioxidant effect was between 89.54% – 116.88% and 93.26% - 106.94% for CO extract and CBD oil, respectively. On the other hand, the obtained results from HP extract showed that the antioxidant effect was ≥100 % for all tested concentrations. The results from ORAC assay indicated that, at the examined concentration range, both extracts and CBD oil exhibit high antioxidant capacity and great potency to protect against oxidative damage.

The results from the DPPH assay, expressed as IC_{50} values, showed that prepared HP extract has higher antioxidant capacity (0.463 mg/mL) compared to CO extract (2.829 mg/mL) and CBD oil (3.261 mg/mL), respectively.

The total phenolic content showed that HP extract has 18.86 mgG AE/g in the concentration range of 0.1 - 0.5 mg/mL; CO extract has 6.997 mg GAE/g in the concentration range of the samples between 0.1 - 5 mg/mL; CBD oil showed 0.829 mg GAE/g in concentration ranges of the samples between 0.05 - 1 mg/mL.

Conclusion

The prepared extracts from HP and CO as well as the CBD oil showed substantial antioxidative capacity. As such, they could prove useful in the treatment of chronic wounds and should be the subject of further testing.

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