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PHYTOCHEMICAL STUDY OF C. OFFICINALIS L. EXTRACT AND ITS EFFECT ON SKIN MICROBIOTES

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Introduction. Chemical compounds extracted from *Calendula officinalis* have been used for thousands of years, worldwide, as prevention of diseases or as adjuvants in their treatment. Phytochemicals are enhancing tissue reparation when applied on wounds and acting as pro-angiogenic compounds for wound healing. *C. officinalis* is known for it's reparative, antiinflammatory, antibacterial, antioxidant properties and it's ability to improve process of wound healing [1, 4].

The results of the systematic review provide some evidence for the efficacy of *C. officinalis* extract in wound healing. While the use of *C. officinalis* extract in wound care has traditionally been used within complementary and alternative medicine, the review collects the current evidence for the use of *C. officinalis* extract in management of in vivo wound care. The role of *C. officinalis* in acute wound healing was mainly explored in the in vivo animal models studies, with only one clinical trial assessing acute wounds. All animal models used were rodents, providing homogeneity of study design. The animal studies provide moderate evidence for improved recovery from the inflammation phase and increased production of granulation tissue in Calendula extract treatment groups. This finding may be explained by the anti-inflammatory and enhanced fibroblasts activation and migration properties of Calendula, as observed in the in vitro studies. A combination of these effects may allow for improved wound healing [1]. Due to the limited number of clinical trials and their methodological differences, there is a need for large prospective well-designed randomized control trials with validated and consistent outcome measures to establish the efficacy of Calendula extract on wound healing.

Aim. Evaluation of chemical composition of *C. officinalis* plant raw material and identification of metabolites from skin microbiota.

Materials and methods. Extraction. 10 mg of each raw extract was weighted on analytical balances and then dissolved in 1 ml of 50 % methanol + 0,1% formic acid solution (mobile phase). Then prepared solution was centrifuged at ± 8000 revolutions per minute and filtrated in vials for further HPLC-DAD-MS – analysis (high-performance liquid chromatography-diode array detection - mass spectrometry - analysis). Analysis. The HPLC-DAD-MS analysis of investigated extracts were performed using Ultimate 3000 RS system (Dionex, Sunnyvale, CA, USA) coupled with ion trap mass spectrometer Amazon SL (Bruker Daltonik, Bremen, Germany). The separation was carried on Kinetex XB-C18 column (150 mm × 2.1 mm × 1.7 μm, Torrance, CA, USA). The column was eluted with 0.1% formic acid in deionized water (A) and 0.1% formic acid in acetonitrile (B). The gradient program was used 0 min – 4 % B, 45 min – 26 %, 50min – 100%, 60 min – 100%B. The flow rate was 0.2 ml/min and column temperature was kept at 25 °C. The eluate was introduced directly to the ESI source of the mass spectrometer. The ESI source parameters were:

nebulizer pressure 40 psi; dry gas flow 9 l/min; dry temperature 300 °C; and capillary voltage 4.5 kV. Compounds were analyzed in the negative and positive ion modes. The MS/MS mode was active and the most abundant ion in the recorded spectrum was subjected to the fragmentation. Signals obtained in MS/MS spectrum were used for further fragmentation whenever possible with Smart Frag mode. Using DAD device, the UV-Vis spectrum of detected compounds were monitored from 190 to 450 nm [2, 3]. Skin microbiota analysis. For experiment 2 donors and *C. officinalis* raw extract were used. *C. officinalis* flowers extract was transferred to the plate at different concentrations. The samples of skin microbiota were mixed with 10 ml of brain heart infusion and solution was incubated for 24 h at 37 °C and aerobic conditions. As control of bacteria and fungi growth was used BHI [5].

Results and discussion. After UHPLC-DAD-MS method of analysis some changes had been seen on chromatogram at 254 nm. At chromatogram of skin microbiota of Donor 2 at all concentrations appeared peaks at 2.5 min; 5.3 min; 8.8 min and disappeared at 6.6 min; 20.7 min; 22.7 min. As control were used mixture of *C. officinalis* flower extract with 300 µL of BHI (brain-heart infusion). At chromatogram of skin microbiota of Donor 1 at concentrations 2 mg/ml and 1 mg/ml appeared peaks 26.6 min. The appearance of this peaks on chromatogram means that new compounds from *C. officinalis* have been metabolized by skin microbiota.

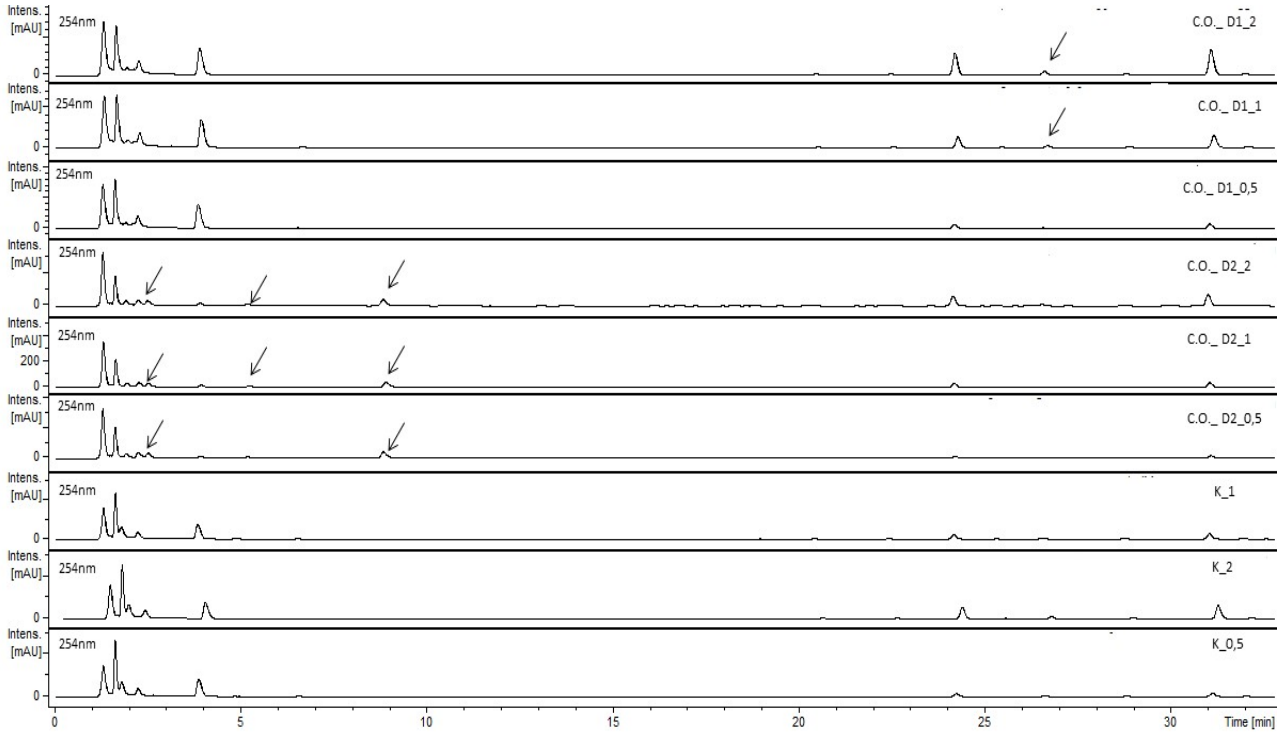

Figure 3. The HPLC-DAD-MS analysis of Calendula extract with brain heart infusion and skin microbiota at UV 254 nm. C.O._ D1_2 - 2 mg/ml *C. officinalis* extract with BHI and Donor 1 skin microbiota; C.O._ D1_1 - 1 mg/ml *C. officinalis* extract with BHI and Donor 1 skin microbiota; C.O._ D1_0,5 – 0,5 mg/ml *C. officinalis* extract with BHI and Donor 1 skin microbiota; C.O._ D2_2 - 2 mg/ml *C. officinalis* extract with BHI and Donor 2 skin microbiota; C.O._ D2_1 - 1 mg/ml *C. officinalis* extract with BHI and Donor 2 skin microbiota; C.O._ D2_0.5 – 0.5 mg/ml *C. officinalis* extract with BHI and Donor 2 skin microbiota; K_1 – control with 1mg/ml *C.*

officinalis extract and 300μL of BHI; K_2 - control with 2 mg/ml *C. officinalis* extract and 300 μL of BHI; K_0.5 - control with 0,5 mg/ml *C. officinalis* extract and 300 μL of BHI

Conclusion. Thus, we have studied influence of *Calendula officinalis* flowers extract on skin microbiota and identified phytochemical compounds in *C. officinalis* flowers extract by HPLS-MS-DAD.

References

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