MilkQua

Quality along the Dairy Chain for a Safe and Sustainable MILK
PRIMA S2 – 2018

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2. Revision history

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3. Dissemination level

PU	Public	no
CO	Confidential, only for members of the consortium	yes

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Content

Background	In previous tasks and deliverables of the project, plant species were selected and the corresponding essential oils (EOs) were obtained (**Table 1**). **Table 1** – Sample identity. In order to assure that only non-toxic concentrations were used subsequently, the present deliverable aims to elucidate the toxicity profile of the EOs selected earlier in the project.
Objectives	The main objective of this deliverable was to evaluate the toxicity of the selected EOs in human cells, thus establishing non-toxic concentrations for subsequent biological studies. To this end, toxicity across a concentration range was evaluated, after which the micronucleus assay was performed, in order to evaluate potential genotoxicity.
Methods	**Sample Identity & Handling** The EOs were kept in the freezer until use. For each sample, stock solutions were prepared solubilizing each EO in DMSO (1:1), with the exception of EO6 that was prepared in a proportion of (1:4) due to worse solubility. Then, several dilutions were prepared in cell culture medium and evaluated for their impact in the viability of HaCaT cells. **Cell culture** Human keratinocytes were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin, and were incubated in an incubator at 37 ºC, in a humidified atmosphere of 5% CO_2. **Viability assessment** Cells were seeded in 96-well plates (1.5×10^4 cells/well) and left to attach for 24h. After this period, cells were incubated with different concentrations of the EOs under study for another 24h. After this period, cell viability was evaluated based on the ability of metabolically active cells to convert MTT to formazan over the course of 2 hours. Absorbances were measured at 570 nm in a Multiskan GO plate reader (Thermo Fisher Scientific; Waltham, MA, USA) and results were expressed as percentage of the respective control

EO	Species
1	*Salvia officinalis*
2	*Laurus nobilis*
3	*Coriandrum sativum*
4	*Rosmarinus officinalis*
5	*Thymus capitatus*
6	*Nigella sativa*
7	*Juniperus oxycedrus*
8	*Pelargonium graveolens*
9	*Origanum vulgare*
10	*Artemesia herba alba*

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	and correspond to the mean ± standard error of the mean (SEM) of at least three independent experiments performed in triplicate. **Micronucleous assay** Cells were cultured as described above and, after incubation with either each of the EOs or the drug mitomycin C (positive control) for 24 hours, fixed for 20 minutes with formalin. After washing, cells were stained with DAPI (0.5 µg/mL) for 20 minutes and imaged in an inverted Eclipse Ts2R-FL (Nikon) equipped with a Retiga R1 camera and a S Plan Fluor ELWD 20x DIC N1 objective.
Results and implications	For each EO, several concentrations were prepared and assessed for their impact in the viability of the human cell line HaCaT (keratinocytes). As shown in **Figure 1**, the majority of the EOs (OE1, OE2, OE4, OE5, OE7-OE10) significantly affect the cellular viability of HaCaT cells in concentrations of 0.5 µL/mL (0.05 % of EO). Of note, the EO5 was the most cytotoxic among the EOs tested.

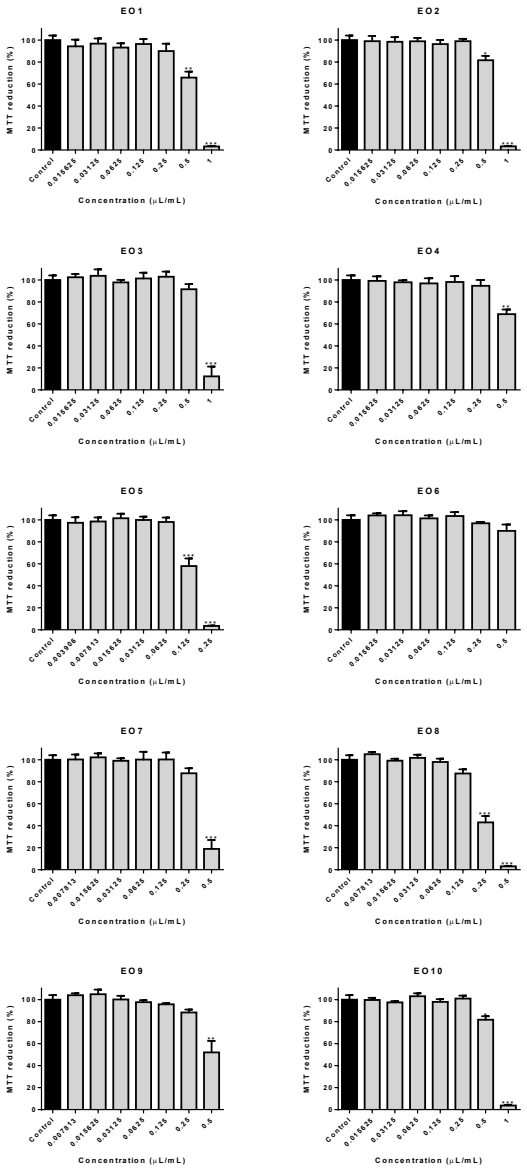

Figure 1 – Viability of HaCaT cells (keratinocytes) after incubation with the indicated EOs for 24 hours.

In order to assure that the EOs under study were not genotoxic, they subjected to the micronucleous assay at the highest non-toxic concentration. None of the samples tested positive for this assay.