

Supplementary Materials

Comparative Studies on the Anti-Inflammatory and Apoptotic Activities of Four Greek Essential Oils: Involvement in the Regulation of NF- κ B and Steroid Receptor Signaling

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Supplementary Data S1. Chemical composition of the essential oils from Chios Mastic, *Melissa officinalis*, Oregano, and Lavender

Supplementary Figure S1. Effect of essential oils on ERs transcriptional activation

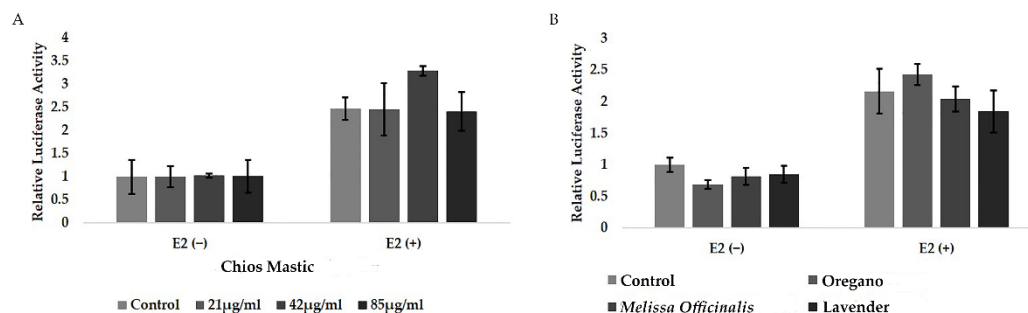


Figure S1. A. Chios Mastic essential oil caused no effect on the ER β transcriptional activation. Oregano, *Melissa officinalis* and Lavender showed no effect on the ER α transcriptional activation. Cells grown in hormone-free medium for 48 h were transiently co-transfected with a ERE dependent Luciferase reporter gene construct, a PEGFPC2ER β (A) or PEGFPC2ER α (B) and a β -galactosidase reporter construct. Subsequently, cells were treated with the indicated amounts of the essential oils, in the presence or absence of E2, for 6 h. Then, cells were harvested and lysed. Assessment of the luciferase and β -galactosidase activity was followed in cells extracts. Relative luciferase activity was expressed as normalized against the β -galactosidase activity. Relative luciferase activity in control cells was set as 1. Data were analyzed by two-way ANOVA and are expressed as mean \pm SD, (n = 6).

Supplementary Figure S2. Cytotoxic effects of essential oils on HEK293 cells, upon 24h incubation.

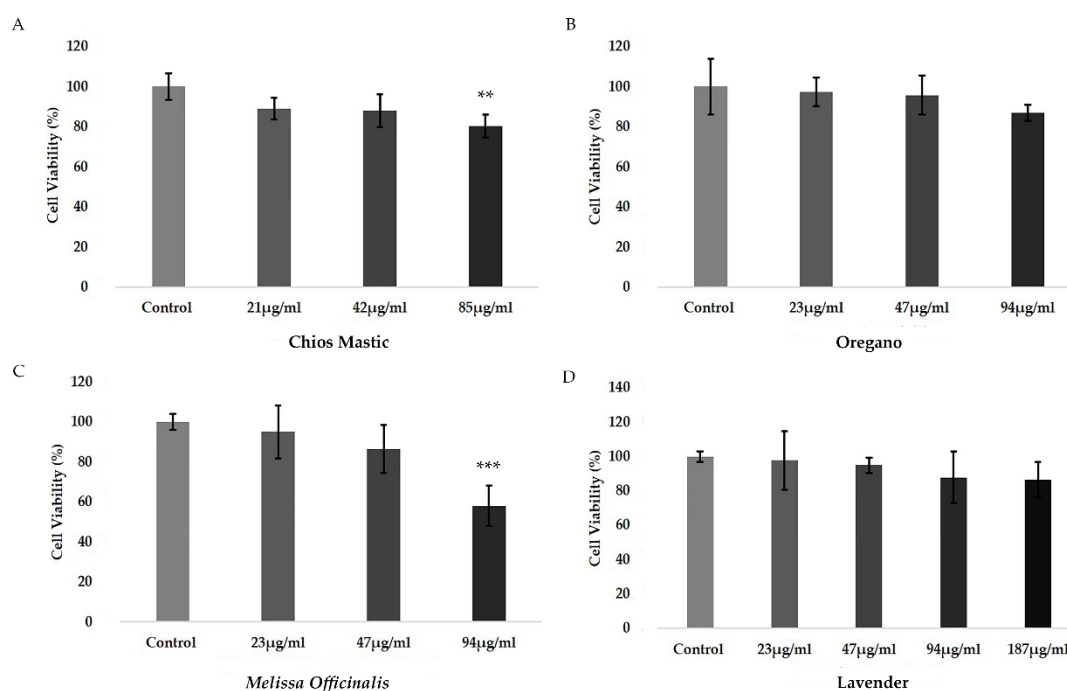


Figure S2. Evaluation of the cytotoxic effects of the essential oils from Chios Mastic, Oregano, *Melissa officinalis* and Lavender on HEK-293 cells. Cytotoxicity was assessed by applying MTT assay in HEK-293 cells subjected to treatment with essential oils for 24h. Viability of control cells was considered as 100%. Relative cell viability (Cell viability, %) is expressed as the viability of cells treated with various

concentrations of the respective essential oil, compared to the viability of the control cells. Data are expressed as mean \pm SD (n=5), ** p<0.01, *** p<0.001.

Supplementary Figure S3. Assessment of the anti-inflammatory action of Lavender

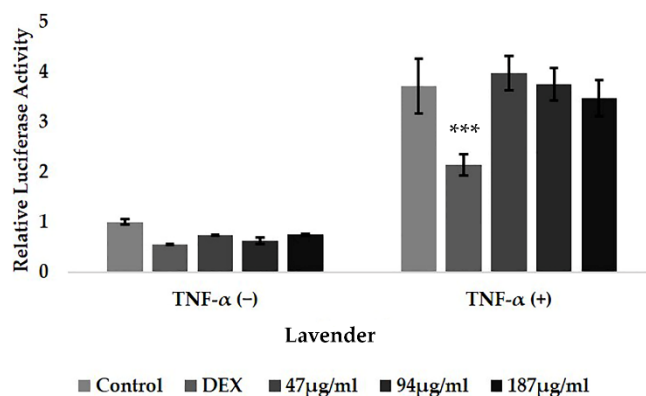


Figure S3. No anti-inflammatory activity was observed by the Lavender essential oil at a concentration range of 47 to 187 μ g/ml. HEK-293 cells were co-transfected with the NF- κ B reporter luciferase and the β -galactosidase reporter constructs and subsequently were treated with the indicated concentrations of the Lavender essential oil, or with 1 μ M DEX, for 6h, in the presence or absence of 20ng/ml TNF- α , at hormone-free medium. Assessment of the luciferase and the β -galactosidase activity was followed. Results were expressed as relative luciferase activity normalized against β -galactosidase activity. Relative luciferase activity in control vehicle-treated cells was set as 1. Data are expressed as mean \pm SD, (n=6), *** p<0.001.