



Investigation of the antiproliferative effect of essential oil of the plant *Lavandula angustifolia* against colon cancer cells

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INTRODUCTION

Essential oils have been used in pharmaceutical sciences as natural products for many years¹. *Lavandula angustifolia* is a plant cultivated in Greece and the essential oil derived from it is used as a medicine to relieve stress and anxiety². Colorectal cancer is the third most common cancer in the Western hemisphere and the incidence increases with increasing age³.

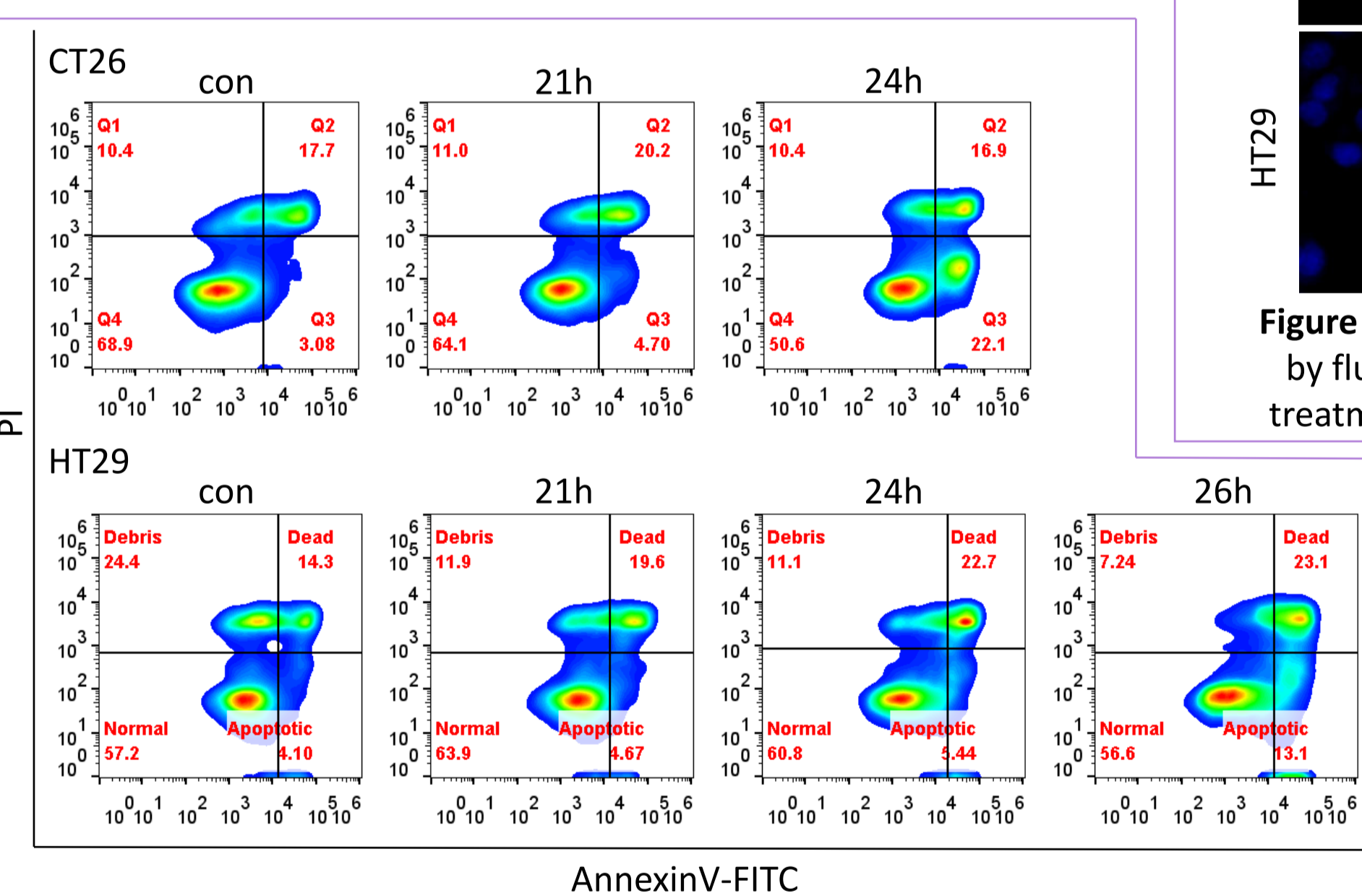
The aim of this study was (a) to characterize the chemical profile of the Lavender Essential Oil (LEO) and (b) to investigate the potential antiproliferative effect of LEO against colon cancer cells.

RESULTS

- UPLC MS/MS analysis identified that LEO contains high concentrations of flavonoids and phenolic compounds (Table 1).
- LEO demonstrated a remarkable inhibitory effect on the cell growth of both CT26 and HT29 cancer cell lines (Figure 1). As demonstrated in the diagrams, oil concentrations greater than 0,025% v/v seem to significantly inhibit the proliferation of the cancer cells. The concentration chosen for use in further studies was 0,035% v/v.
- Flow cytometry (Figure 2) and fluorescence microscopy (Figure 3) were used to uncover the ability of LEO to induce apoptotic cell death. As seen in Fig. 2, LEO induces apoptotic cell death in both CT26 and HT29 cells. In the diagram, after a 24h exposure to an oil concentration of 0,035% v/v, 22% of the CT26 cells were undergoing apoptosis. On the other hand, in HT29 the percentage of the apoptotic cells was significantly lower. Fluorescence microscopy was used to observe the characteristic lesions of the nucleus of apoptotic cells, as seen in Fig. 3.
- RT-PCR was used to study the expression of different genes that participate in the apoptosis pathway (Figure 4). Results showed that in CT26 cells pro-apoptotic genes are overexpressed, contrary to the downregulation of the anti-apoptotic gene *Bcl-2*.

Table 1: Chemical characterization of the Lavender Essential Oil.

Contents of LEO	Concentration
Total Phenolic Content (μg of gallic acid eq / g of dry extract)	194.90±3.69
Total Flavonoid Content (μg of catechin eq / g of dry extract)	165.10±6.32
Total Soluble Protein Content (mg of BSA eq / g of dry extract)	n.d.
Total Soluble Sugar Content (nM/ of mannose eq / g of dry extract)	194.18±9.81
Chlorophyll-a (μg of chlorophyll-a / g of dry extract)	0.07±0.001
Chlorophyll-b (μg of chlorophyll-b / g of dry extract)	3.13±0.10
Lycopene (μg of lycopene / g of dry extract)	0.01±0.001
β-carotene (μg of β-carotene / g of dry extract)	n.d.



MATERIALS AND METHODS

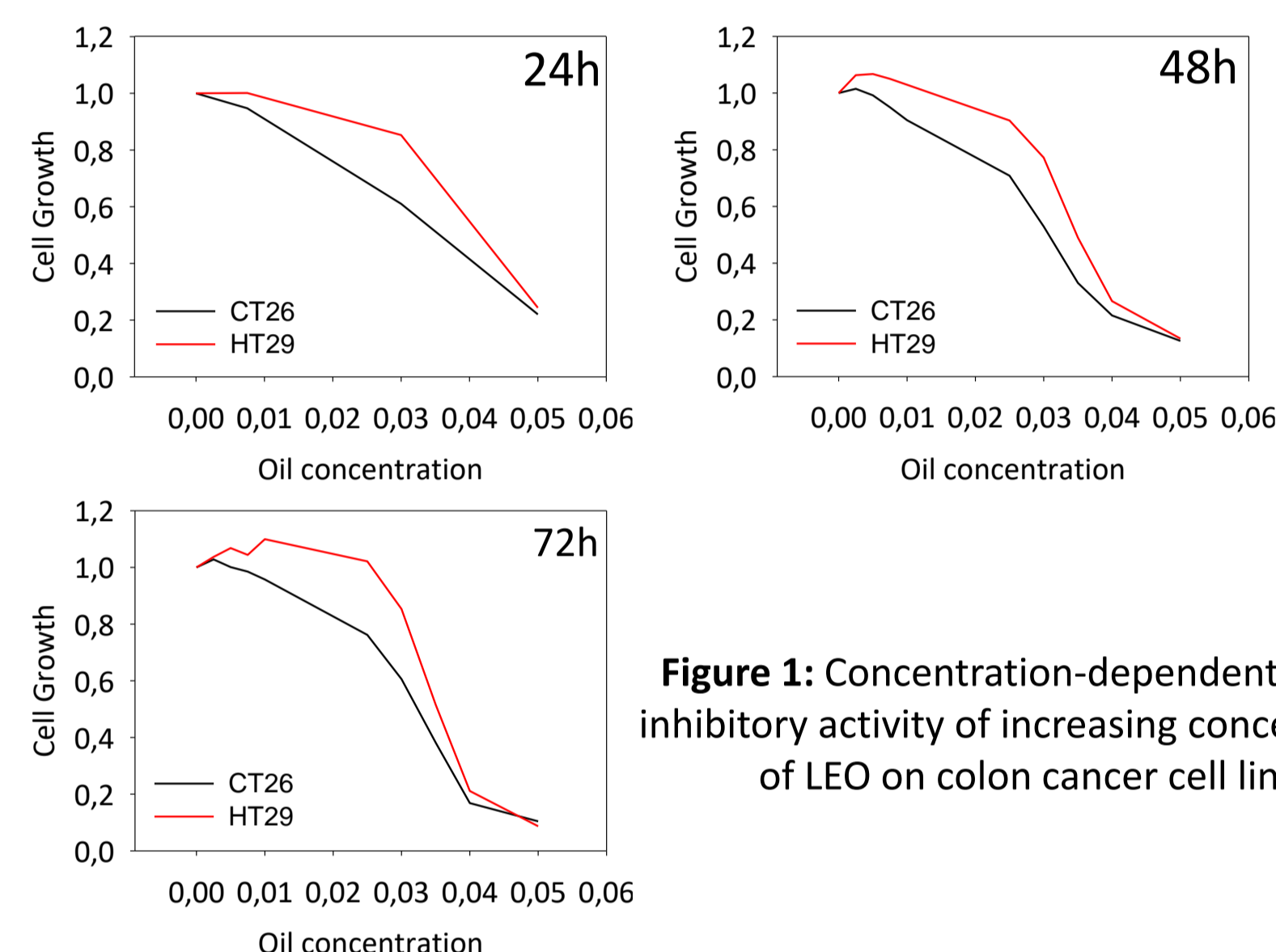
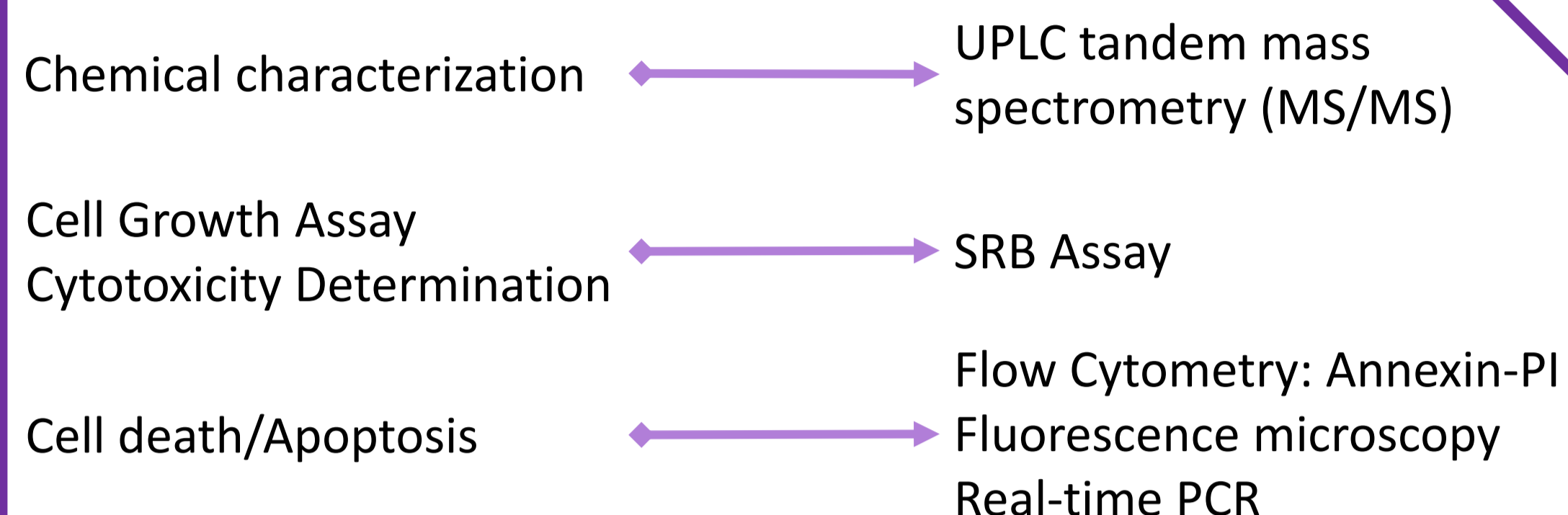


Figure 1: Concentration-dependent growth-inhibitory activity of increasing concentrations of LEO on colon cancer cell lines.

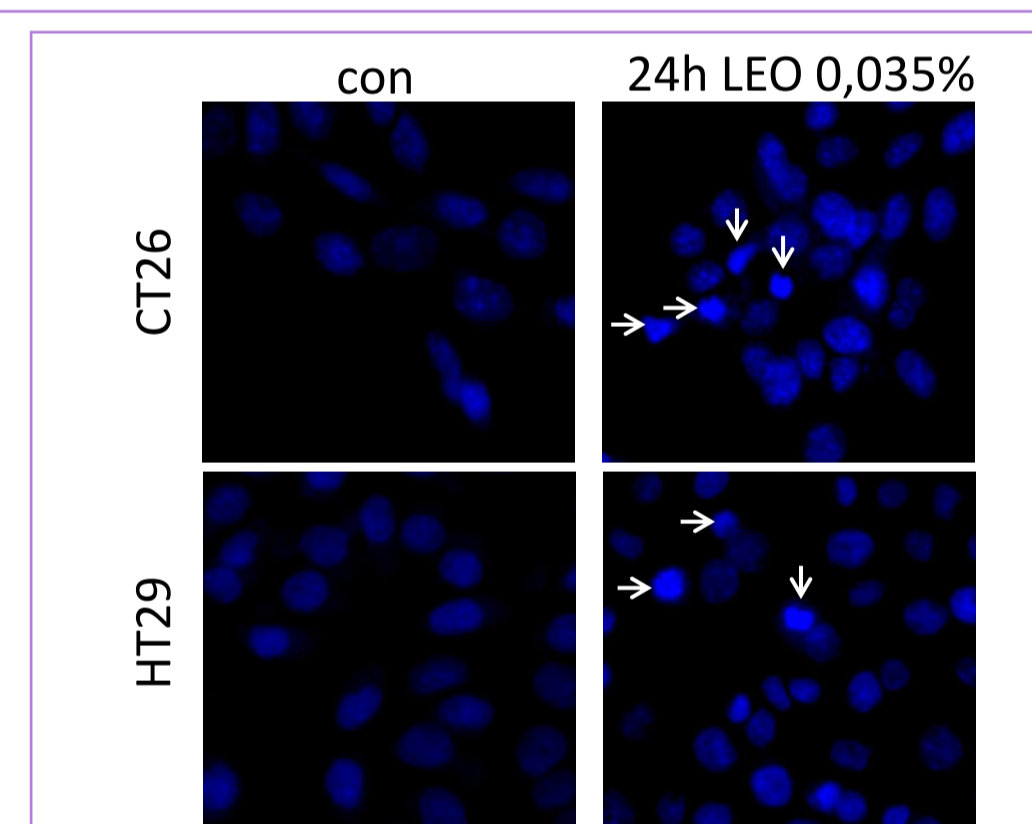


Figure 3: Observation of apoptotic nuclei by fluorescence microscopy following treatment with 0,035% v/v LEO for 24h.

Figure 2: Analysis of apoptotic cell death by flow cytometry following incubation of cells with 0,035% v/v LEO at different time-points.

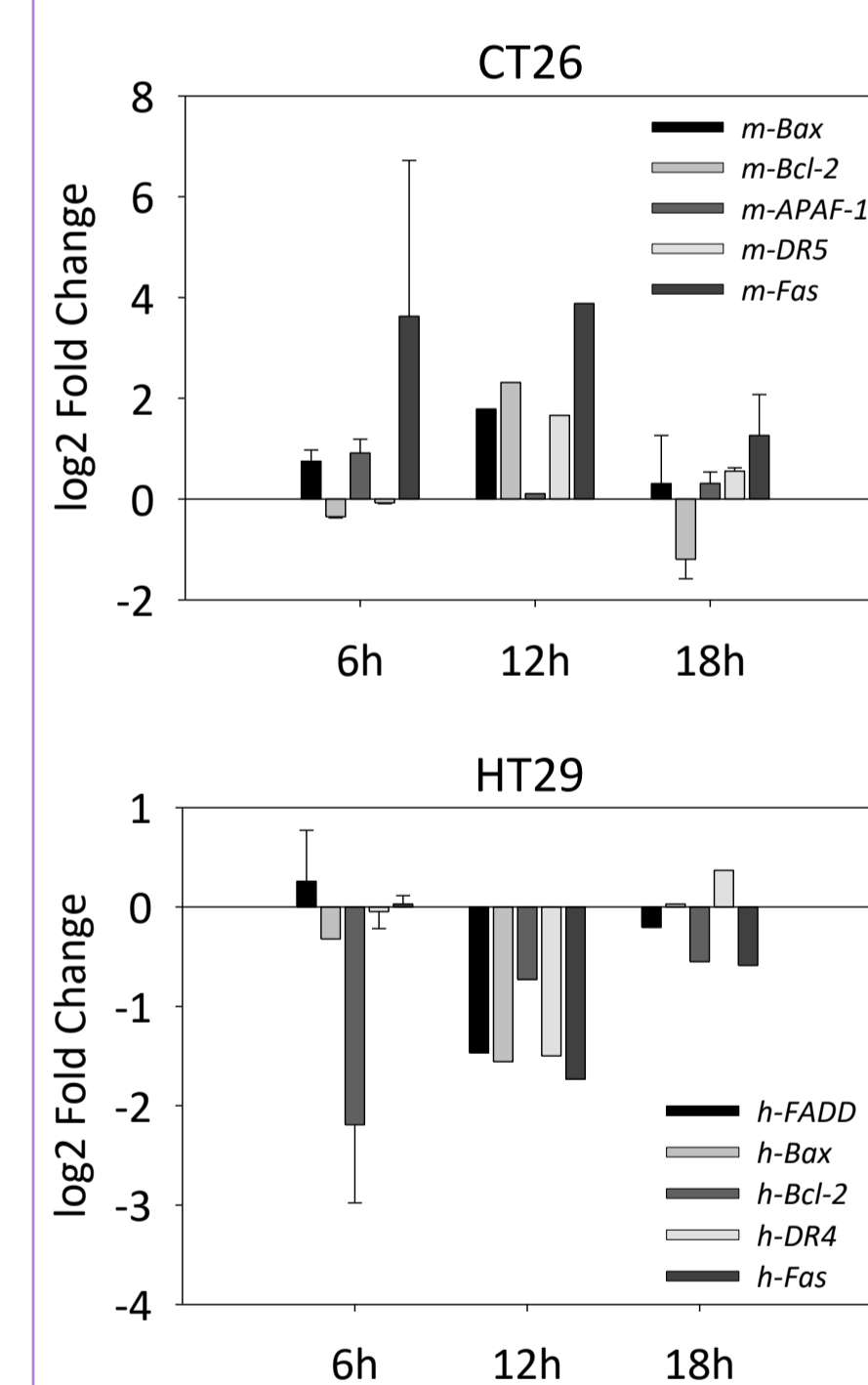


Figure 4: Gene expression of apoptosis-related genes by RT-PCR following incubation of cells with 0,035% v/v LEO.

CONCLUSIONS

Lavender Essential Oil:

- shows *in vitro* concentration-dependent anti-proliferative activity against the colon cancer cell lines CT26 and HT29, with CT26 cells being more susceptible.
- has the ability to induce apoptotic cell death

FUTURE WORK

- Investigation of further biological activities of Lavender Essential Oil (e.g. anti-inflammatory, anti-migratory, etc.).
- Investigation of Damage-Associated Molecular Patterns (DAMPs) release from tumor cells treated with Lavender Essential Oil and induction of immunogenic cell death.

ACKNOWLEDGEMENTS

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REFERENCES

- [1] Bakkali F, Averbeck S, Averbeck D, Waomar M (2008). Biological effects of essential oils-A review. *Food Chem Toxicol* 46, 446–475.
- [2] Setzer WN (2009). Essential oils and anxiolytic aromatherapy. *Nat Prod Commun* 4, 1305–1316.
- [3] Zhao Y, Chen R, Wang Y, et al. (2017). *In vitro* and *in vivo* efficacy studies of *Lavandula angustifolia* essential oil and its active constituents on the proliferation of human prostate cancer. *Integr Cancer Ther* 16, 215-226.

