

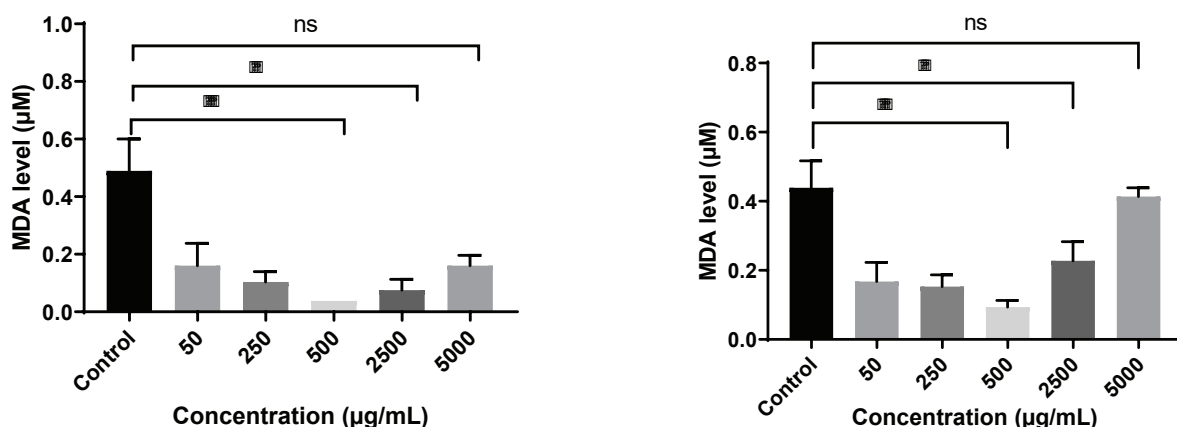
**Figure 1.** *Helichrysum arenarium* and *Lawsonia inermis* extracts (\*\*\*\* p < 0.0001).

*Helichrysum arenarium* was applied to ECV304 cells (Fig. 2A) and Ishikawa cells (Fig.2B). According to the results, 50-250-500-2500 µg/mL doses of *Helichrysum arenarium* applied to cancer cell line caused a significant inhibition in MDA levels. At the dose of 5000 µg/mL, a similar result was obtained with the MDA level of the control group.

Elaguel et al. (21) applied the antioxidant Lawsonia inermis (henna) essential oil to the human lymphatic cancer cell line (Raji) and analyzed the biological antioxidant levels with the TBARS assay method. They reported that henna essential oil inhibited the MDA level significantly (80%).

There have been found very few studies in the literature related with the effects of antioxidant substances on MDA levels in human cell lines. However, there are some studies showing that plants with antioxidant properties can inhibit MDA levels in other cancerous animal tissues (22, 23). Elaguel et al. (21) reported that when 50-5000 µg/mL doses of *Helichrysum arenarium* essential oil was applied to ECV304 cells and Ishikawa cells, it caused a significant inhibition in MDA levels. At the dose of 5000 µg/mL, a similar result was obtained with the MDA level of the control group.

In our study, 23 essential oil compositions were determined in the extract of *Helichrysum arenarium*. The most abundant compounds were 2-Palmitoylglycerol and palmitic acids. The detailed composition of the identified compounds is given in Table 1.



**Figure 2.** Changes of malondialdehyde levels in the presence of *Helichrysum arenarium* (A) and Ishikawa (B) (\*\*\*\* p < 0.0001).

**Table 1.** Chemical composition analysis of *Helichrysum arenarium* extract detected by GC-MS.

	Compound	<i>Helichrysum arenarium</i> (%)
1	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	18,79
2	Hexadecanoic acid (Palmitic acid)	16,75
3	Phenol	14,59
4	9-Octadecenoic acid (Oleic acid)	9,49
5	9,12-Octadecadienoic acid (Linoleic acid)	7,70
6	Octadecanoic acid (Stearic acid)	7,36
7	N,N-Dimethylpalmitamide	4,94
8	Nonadecanoic acid, 18-oxo-	3,63
9	Tridecanal	2,55
10	Borane, diethylmethyl-	2,32
11	Tetradecanoic acid (Myristic acid)	2,29
12	Palmitic acid, 2-(tetradecyloxy)ethyl ester	1,81
13	1-Octanol, 2-butyl-	1,22
14	Tetratetracontane	1,05
15	Triaccontane, 1-Bromo-	0,94
16	Dodecane	0,81
17	Tetratriacontane	0,81
18	1H-Purin-6-amine, [(2-fluorophenyl)methyl]-	0,73
19	Nonadecane	0,57
20	Hexadecane	0,54
21	Pentadecane	0,54
22	Docosane	0,28
23	Octadecane, 1-chloro-	0,27

Essential oils were reported very diverse in some studies (24, 25). It was reported that the major essential oil constituents were: decanoic acid (9.8%), dodecanoic acid (11.9%) and ester methyl palmitate (28.5%) (24), capric acid (19.8%) and methyl palmitate (28.5%) (26), limonene (11.4%), cyclosativene (11.9%),  $\alpha$ -ylangene (13.9%), and diepi- $\alpha$ -cedrene (17.9%) (27),  $\beta$ -caryophyllene (9.0–25.6%), heneicosane (3.0–32.1%) and  $\alpha$ -copaene (1.5–7.2%) (28),  $\alpha$ -humulene (15%), 1,8-cineole (16%) and  $\alpha$ -pinene (32%) (29) and 1,8-cineole (8.9%),  $\delta$ -cadinene (9.0%) and  $\beta$ -caryophyllene (5.8–36.2%) (27).

The differences in essential oil constituents could be explained by diversities in their origin and the climate. Also, esterification was used to increase the volatility of fatty acids in the sample preparation of our study.

Previously, Judzentiene et al showed that essential oils of *Helichrysum arenarium* contains palmitic, myristic and lauric acids, *n*-nonanal, and trans- $\beta$ -caryophyllene ( $\leq 6.5\%$ ) (30). In various studies it has been shown that phenolic compounds of *Helichrysum arenarium* had anti-atherosclerotic and antimicrobial activities (19,31). Additionally, Kucukoglu et al. suggested that *Helichrysum* extracts can inhibit

mammalian DNA topoisomerase I enzyme and these extracts may be used to prepare anticancer medications in the future (32). Since the potential functions of substances found in herbal ingredients can be investigated by using computational methods (33), extracts of the *Helichrysum arenarium* can be found out by using computational methods and possible signaling mechanisms.

#### 4. Conclusion

This study investigated the cytotoxic activities of *Helichrysum arenarium* on several types of human cells including ECV304 and Ishikawa cells. The results of our study have established the most significant extract dose. Furthermore, immortalized cells' essential oils were examined by using gas chromatography-mass spectrometry. The *Helichrysum arenarium* extract reduced both cell viability and MDA levels in Ishikawa and ECV304 cells at 500 µg/mL. We have found that the main essential oils in the DMSO extracts of *Helichrysum arenarium* were palmitic acids and palmitoylglycerol. In conclusion, *Helichrysum arenarium* has exhibited antioxidant activity.

#### Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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