Supporting Information

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Evaluation of Therapeutic Role of *Thymus capitatus* (L.) Hoffm. & Link, *Origanum dubium* Boiss. Essential Oils and their Major Constituents as Enhancers in Cancer Therapy

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S.1: Essential Oil Isolation and GC/MS Analysis

The plant materials, isolation of the essential oils and GC/MS analysis in this study are in parallel with those used in previous studies to obtain the essential oils [1, 2]. The essential oils utilized in this study have been obtained and characterized in previous studies by the authors [1, 2]. Therefore, the steps in the isolation and characterization of these essential oils shall not be discussed in the present study.

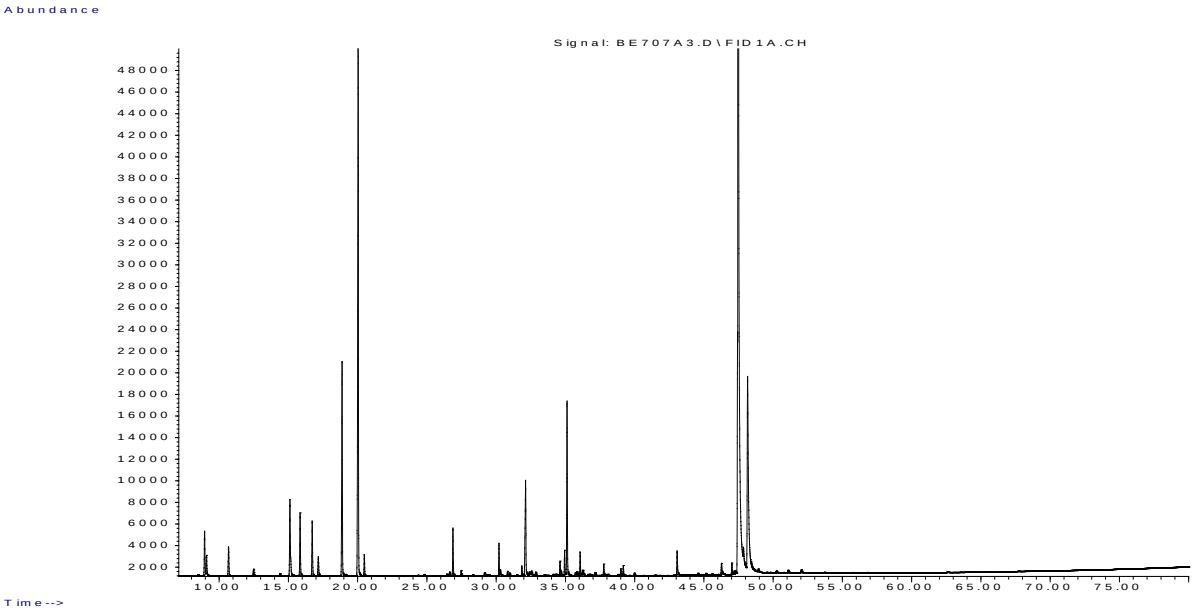

Figure S1: GC Chromatogram of *Thymus capitatus* essential oil

RRI	Compound	Relative %
1032	α-Pinene	1.0
1035	α-Thujene	0.6
1076	Camphene	0.4
1174	Myrcene	2.2
1176	α-Phellandrene	0.2
1188	α-Terpinene	1.6
1203	Limonene	1.2
1218	β-Phellandrene	0.1
1255	γ-Terpinene	5.1
1280	*p*-Cymene	10.9
1290	Terpinolene	0.5
1553	Linalool	0.6
1604	Thymol methyl ether (=*Methyl thymol*)	0.4
1611	Terpinen-4-ol	1.2
1612	β-Caryophyllene	1.0
1694	Neral	t
1706	α-Terpineol	t
1719	Borneol	1.6
1740	Geranial	0.5
2198	Thymol	62.3
2226	Methyl hexadecanoate (=*methyl palmitate*)	0.7
2239	Carvacrol	6.7
2456	(*Z*)-9-Methyl octadecanoate (=*Methyl oleate*)	1.0
	Total	**99.8**

Figure S2: Essential oil composition of *Thymus capitatus* (t :trace)

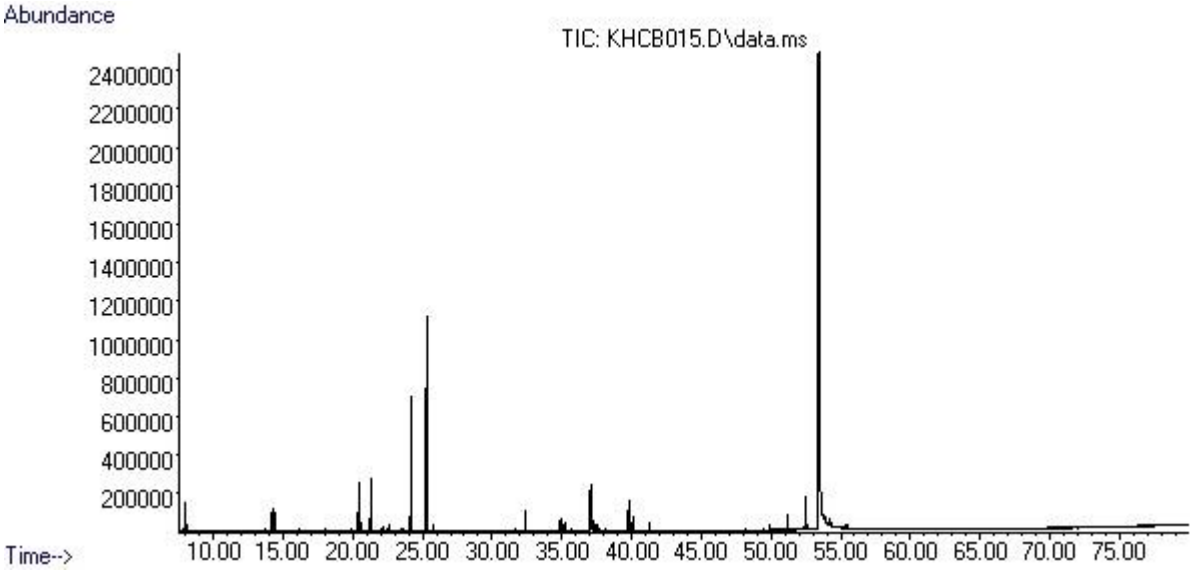

Figure S3: GC Chromatogram of *Origanum dubium* essential oil

RRI	Compound Name	Relative %
1020	α-Pinene	0.3
1024	α-Thujene	0.5
1172	Myrcene	0.4
1177	α-Phellandrene	0.2
1192	α-Terpinene	0.9
1211	Limonene	0.1
1223	β-Phellandrene	0.1
1260	γ-Terpinene	2.7
1288	*p*-Cymene	3.8
1299	Terpinolene	0.1
1478	trans- sabinene hydrate	0.4
1556	Linalool	0.1
1565	cis-sabinene hydrate	0.1
1625	Terpinene-4-ol	0.7
1629	β-Caryophyllene	0.1
1639	trans-dihydrocarvone	t
1718	α-Terpineol	0.5
1728	Borneol	0.1
1771	Carvone	t
2108	Elemol	0.1
2159	Spathunelol	0.1
2210	Thymol	0.2
2243	Carvacrol	88.3
2273	β-Eudesmol	0.1
	Total	100.0

Figure S4: Essential oil composition of *Origanum dubium* (t :trace)

S.2: Cell Culture

Telomerized-mesenchymal stem cells (hMSC-telo1) and their tumorigenic counterpart (tumorigenic hMSC-telo1) were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich), penicillin (64 µg/mL), streptomycin (0.1 mg/mL) and L-glutamine at 5% CO^2 and 37°C.

S.3: MTT Assay

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay was applied by using Cell proliferation Kit I (MTT) (Roche, Germany) to quantify cell viability and proliferation of hMSC-telo1 and tumorigenic hMSC-telo1 cells. MTT assay is a colorimetric assay where solution containing yellow tetrozolium compound is turning into purple formazan crystals as viable cells are metabolizing it. hMSC-telo1 and tumorigenic hMSC-telo1 cells were seeded into each well of a flat-bottomed 96- well plate at a concentration of 5×10^4 cells/mL in culture medium and incubated 24 h in 5% CO^2 and at 37°C. Cells were treated with different concentrations of *T. capitatus* and *O. dubium* essential oils, carvacrol and thymol for 24 h, 10 µl of MTT solution was added into each well, mixed gently and incubated at 37°C for 4 h. Following this incubation period, formazan crystals were dissolved by adding 100 µl Dimethylsulfoxide (DMSO) into each well. The absorbance was read at 570 nm using a microplate reader (VersaMax™). Untreated cells were considered as experimental control and experiments were conducted in triplicate. The absorbance from blank wells were read at 630 nm and subtracted from absorbance values of treated cells to eliminate background absorbance and so to obtain normalized absorbance values. The absorbance values are directly proportional to the number of live cells in culture. Thus the percentage cell viability was calculated by the following formula:

$$\text{Cell viability (\%)} = \frac{(\text{Absorbance of treated cells} - \text{Absorbance of blank})}{(\text{Absorbance of control} - \text{Absorbance of blank})} \times 100$$

S.4: TUNEL Assay

For the detection and quantification of apoptosis TUNEL assay was performed by using In Situ Cell Death Detection Kit, Fluorescein, Roche that is based on labelling DNA strand breaks. hMSC-telo1 and tumorigenic hMSC-telo1 cells were seeded into each well of a flat-bottomed 96- well plate at a concentration of 5×10^4 cells/mL in culture medium. Cells were treated with different concentrations of *O. dubium* EO and carvacrol for 24 h. After treatment cells were fixed and incubated for 1 h. Then incubated in permeabilization solution for 2 min on ice and incubated with TUNEL reaction mixture for 60 min at 37°C in the dark. Cells were analyzed under the fluorescence microscope and fluorescein-positive cells were counted as DNA-fragmented, apoptotic cells. DNase I recombinant has been used as a positive control.

References

[1] A. Hanoglu, D. Y. Hanoglu, B. Demirci and D. O. Yavuz. Chemical composition of essential oil of the aerial parts of wild growing *Thymus capitatus* (L.) Hoffm. & Link species collected from three different locations in Northern Cyprus, *J. Essent. Oil-Bear. Plants*. **20**, 546-551.

[2] O. I. Karadaglioglu, N. Ulusoy, K. H. C. Baser and A. Hanoglu (2019). Antibacterial Activities of Herbal Toothpastes Combined with Essential Oils against *Streptococcus mutans, Pathogens* **8**, 1-17.