NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF MEDICAL GENETICS

MODELLING HUMAN MESENCHYMAL STEM CELLS IN CANCER THERAPEUTIC APPROACHES:

Response of Human Mesenchymal Stem Cells towards *Thymus capitatus, Origanum dubium* Essential Oils and Their Key Components

PhD THESIS

Merdiye MAVIS

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Approval

We certify that we have read the thesis submitted by Merdiye Mavis titled **"Modelling human mesenchymal stem cells in cancer therapeutic approaches"** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of PhD of Medical Genetics.

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Declaration

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Merdiye Mavis

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Merdiye Mavis

Abstract

Modelling Human Mesenchymal Stem Cells in Cancer Therapeutic Approaches: Response of Human Mesenchymal Stem Cells towards *Thymus capitatus*, *Origanum dubium* Essential Oils and Their Key Components

Mavis, Merdiye

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Origanum dubium Boiss. and *Thymus capitatus* (L.) Hoffm. & Link are native species in Mediterranean region that belongs to genus of Lamiaceae. In current study the cytotoxic actions of essential oils derived from these species and of their primary constituents, thymol for *Thymus capitatus* and carvacrol for *Origanum dubium*, on normal and tumorigenic hMSC-telo1cells were studied to assess their potential use in cancer therapies. Viability of hMSC-telo1 cells and their tumorigenic counterparts were evaluated by treating cells with varying *O. dubium* and *T. capitatus* essential oil, carvacrol and thymol concentrations and conducting MTT assay. TUNEL assay was conducted to identify apoptotic cells.

T. capitatus essential oil at 5×10^{-3} v/v % concentration and thymol at 5×10^{-3} v/v % and 5×10^{-2} v/v % concentrations preserved hMSC-telo1 cells from the cytotoxic action of chemotherapeutic agent; Deferasirox and the viability and the proliferation rate of the cells have also been conserved. Combining 5×10^{-3} v/v % of *T. capitatus*, 5×10^{-3} v/v % and 5×10^{-2} v/v % of thymol with Deferasirox had also been cytotoxic towards the tumorigenic hMSC-telo1 cells. These suggest that *T. capitatus* essential oil and thymol when combined together with conventional chemotherapeutic agents might enhance the action of the agent on tumor cells while preserving the normal cells. *O. dubium* essential oil and carcavrol were highly cytotoxic towards both normal and tumorigenic cells at all tested concetrations. To conclude *T. capitatus* essential oil and thymol can be regarded as promising candidates for cancer therapies.

Keywords: human mesenchymal stem cells, tumorigenic, essential oil, cancer therapeutics

Özet

Kanser Tedavi Yaklaşımlarında İnsan Mezenkimal Kök Hücrelerinin Modellenmesi: İnsan Mezenkimal Kök Hücrelerinin *Thymus capitatus, Origanum dubium* Esansiyel Yağları ve Ana Bileşenlerine Karşı Tepkisi

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Origanum dubium Boiss. ve *Thymus capitatus* (L.) Hoffm. & Link Lamiaceae cinsine ait Akdeniz bölgesinin yerli türleridir. Bu çalışmada bu türlerden elde edilen esansiyel yağların ve *Thymus capitatus*'un ana bileşeni olan timol ve *Origanum dubium'*un ana bileşeni olan karvakrol'un normal ve tümörijenik hMSC-telo1 hücreleri üzerindeki sitotoksik aktivitelerini inceleyerek, kanser tedavilerinde potansiyel kullanımlarını değerlendirmek amaçlanmıştır. Normal ve tümörijenik hMSC-telo1 hücrelerinin canlılığı, hücrelere değişen konsantrasyonlarda *Thymus capitatus* ve *Origanum dubium* esansiyel yağı, timol ve karvakrol uygulanması ile ve MTT testi yapılarak değerlendirildi. Apoptoz nedeniyle hücrelerde DNA parçalanmasını tanımlamak için TUNEL testi kullanıldı.

T. capitatus esansiyel yağı 5×10^{-3} v/v % konsantrasyonunda ve timol 5×10^{-3} v/v % ve 5×10^{-2} v/v % konsantrasyonlarında normal hMSC-telo1 hücrelerini kemoterapötik ajan olan Deferasirox'un sitotoksisitesinden korudu ve hücrelerin canlılık ve proliferasyon oranı da korunmuştur. 5×10^{-3} v/v % *T. capitatus* ve 5×10^{-3} v/v % ve 5×10^{-2} v/v % timol'ün Deferasirox ile birlikte uygulanmasının ise tümörijenik hMSC-telo1 hücrelerine karşı sitotoksik etkisi olduğu görülmüştür. Bunlar *T. capitatus* esansiyel yağının ve timolun standard kemoterapötik ajanlarla birlikte uygulandığında, ajanın tümör hücreleri üzerindeki etkisini artırabilirdiğini ve normal hücreleri koruduğunu önerebilir. *O. dubium* esansiyel yağı ve karvakrol test edilen tüm konsantrasyonlarda hem normal hem de tümörijenik hücrelere karşı yüksek seviyede

sitotoksisite gösterdi. Sonuç olarak *T. capitatus* esansiyel yağı ve timol, kanser tedavileri için umut verici adaylar olarak kabul edilebilir.

Anahtar Kelimeler: insan mezenkimal kök hücreleri, tümörijenik, esansiyel yağ, kanser tedavileri

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List of Abbreviations

EOs:	Essential Oils
OD:	Origanum dubium
TC:	Thymus capitatus
CV:	Carvacrol
TOH:	Thymol
hMSC:	Human Mesenchymal Stem Cell
DFX:	Deferasirox
WHO:	World Health Organization
UV	Ultraviolet
HBV	Hepatitis B virus
HPV	Human papilloma virus
ROS:	Reactive Oxygen Species
RNS:	Reactive Nitrogen Species
NF-κB:	Nuclear factor kappa B
Akt:	Protein kinase B
mTOR:	Mammalian target of rapamycin
MAPKs:	Mitogen-activated protein kinases
Bcl-2:	B-cell lymphoma 2
PARP:	Poly (ADP-ribose) polymerase
RRI:	Relative retention indices
Tr:	Trace
ILs:	Interleukins
TNF-α:	Tumor necrosis factor alpha
BAX:	Bcl-2-associated X protein
VEGF:	Vascular endothelial growth factor
hTERT:	Human telomerase reverse transcriptase
GC/MS:	Gas Chromatography/ Mass Spectrometry
DMEM:	Dulbecco’s Modified Eagle’s Medium
FBS:	Fetal bovine serum

DMSO:	Dimethyl sulfoxide
PBS:	Phosphate Buffer Saline
EDTA:	Ethylenediaminetetraacetic acid
MTT:	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
TUNEL:	TdT-mediated dUTP-biotin nick end labeling
MSC:	Mesenchymal stem cell
ERK5:	Extracellular signal-regulated kinase 5
PBMC:	Peripheral blood mononuclear cell
MMP:	Matrix metalloproteinases
EMT:	Epithelial–mesenchymal transition
MDA:	Malondialdehyde
8-OHdG:	Hydroxy-2′-deoxyguanosine
DMBA:	7,12-Dimethylbenz-[α]-anthracene

CHAPTER I

Introduction

1. Statement of the Problem

Cancer is globally causing significant amounts of death and while it is complex, its treatment is also a complex aspect (Debela et al., 2021). For cancer there are conventional/standard treatment strategies as well as advanced treatment options such as targeted therapies, stem cell based therapies, gene therapy or therapies using nanoparticles (Debela et al., 2021). Conventional treatment options can be given as surgery, chemotherapy or radiotherapy. Although these treatment approaches are being used widely in clinics for a long time, they have major limitation that as well as targeting tumorigenic cells, they also target and damage normal and healthy cells. In the case of chemotherapy, rapidly dividing and growing cells are being negatively affected, leading to adverse effects. Therefore in the field of cancer therapies there is a seek for new therapy approaches and improvement in the utilisation of existing ones as well as need for novel chemical entities that can have promising potential to be used in cancer treatment (Gautam et al., 2014).

2. Purpose of the Study

To identify potential chemical entities that can be used to improve existing cancer therapies, one of the perspectives is to focus on alternative traditional approaches (Gautam et al., 2014). Several *in vitro* and *in vivo* investigations suggested that metabolites originated from plants have anticancer actions (Gautam et al., 2014). One of the plant derived product that showed anticancer activity is essential oils (EOs) from aromatic plants and they or their constituents can be investigated to identify novel anticancer agents (Gautam et al., 2014).

It has also foreseen that the products derived from plants may possess fewer adverse effects compared to synthetic drugs and EOs might be used together with standard cancer treatments to diminish their adverse effects (Gautam et al., 2014).

Therefore in current study we meant to determine whether *Origanum dubium* Boiss. (OD) and *Thymus capitatus* (L.) Hoffm. & Link (TC) EOs and their key

constituents; carvacrol (CV) and thymol (TOH) can be used as complementary agents for classic cancer therapy approaches and to investigate the response of telomerase-immortalized human mesenchymal stem cells (hMSC-telo1) and their irradiated counterparts towards an anti-cancer agent, Deferasirox (DFX) in combination with TC or TOH.

3. Research Questions / Hypotheses

1) Do TC, OD EOs and their major constituent's, TOH and CV have anticancer potential? Will these agents lead to the death of irradiated hMSC-telo1 cells with tumour forming potential while preserving the viability of the normal cells?
2) When TC and TOH used in combination with an anticancer agent, they will spare its adverse effects on normal cells and affect the viability of tumorigenic cells negatively. Thus TC, OD EOs and their main constituent's TOH and CV will be proposed to be used as complementary agents for standard chemotherapeutic agents.
3) What will be the reaction of hMSC-telo1 and their tumorigenic counterparts following being exposed to TC, OD EOs, TOH and CV in regards to viability?

4. Significance of the Study

This study has several significances;

1) This study is providing a unique perspective for our understanding on possible use of EOs; TC and OD EOs and their main constituents; TOH and CV in cancer therapy.
2) In this study TC, OD EOs and their main constituents; TOH and CV have examined for their cytotoxicity towards well characterized cell line hMSC-telo1 cells as well as their tumorigenic counterpart. These agents have been tested on hMSC-telo1 cells for the first time. There are studies testing other EOs on mesenchymal stem cells or on other cancer cell lines, however this

study is foremost investigation in which TOH and CV have tested on hMSC-telo1 cells.

3) Using EOs as complementary agents for classic cancer therapeutic approaches has been the major novelty of the study. TC and TOH has been tested in combination with an anti-cancer agent to investigate if they can eliminate the side effects of chemotherapeutics; sparing normal cells from those effects and preserving their viability, while targeting tumorigenic cells.
4) Varying concentrations of TC, OD EOs, TOH and CV have used to investigate if any can preserve the viability of healthy cells and their capability to be expanded *in vitro* against classic therapeutic agents.
5) Assessment of apoptotic death of hMSC-telo1cells with the exposure of OD EO and CV.

CHAPTER II

Literature Review

1. Cancer and Tumorigenesis

Cancer is a complex disease with unrestrained proliferation of the cells due to accumulation of genetic and epigenetic mutations that cells acquire. There are several factors contributing to the accumulation of these mutations such as aging, environmental factors, lifestyle, physical and chemical factors (Sharma et al., 2022).

Cancer is one of the primary reasons of mortality, leading one in six deaths worldwide (Debela et al., 2021). It is given that in 2020 new cases of cancer worldwide was 19.3 million and there were around 10 million deaths caused by cancer (Debela et al., 2021). As it is given in report of the WHO (World Health Organization), approximately 20% people globally have few malignant neoplasm and it is expected to rise exponentially by 2040 (World Health Organization, 2020).

Cancer development can be explained by three stages; initiation, promotion and progression. At initiation stage DNA repair mechanisms fail to correct the DNA damage caused by carcinogens, at promotion stage transformed cells are triggered to proliferate and at the stage of progression pre-neoplastic cells transform into neoplastic cells (Sharma et al., 2022). Preventing these stages or related signal transduction pathways might have a destructive impact on the carcinogenesis (Fresco et al., 2006).

The genomic instability originated due to DNA damage is the major source of the cancer development (Sharma et al., 2022). There are several chemical and physical carcinogens like UV and ionizing radiation, tobacco. In addition to these HBV and HPV infections, oxygen free radicals or reactive oxygen species (ROS) can also be considered as causes of cancer (Bhalla et al., 2013).

2. Treatment Approaches for Cancer

For several decades cancer treatment approaches were offered as surgery, radiation therapy and chemotherapy and they were classified as conventional (traditional) approaches. However in recent years there have been several advances such as adopting novel strategies such as immune mediated therapies, stem cell based

therapies or biological molecules (Debela et al., 2021). In addition to that there have been combinatorial approaches such as using various targeted therapies together or with traditional chemotherapeutics.

Although conventional treatment options are widely used in clinics and have positive effect on survival rate, they can have some side effects or limitations and can have negative impact on quality of life (F. Khan et al., 2023). Surgery followed by radiotherapy, chemotherapy or immunotherapy is one of the traditional approaches which provide better outcomes at early stages of the disease and enhances the chances of remission (Arruebo et al., 2011). Radiotherapy might have negative effects on healthy cells, organs and tissues. Chemotherapy is suggested to be the best treatment strategy in the case of its effectiveness and has been standard therapy that is extensively used (Debela et al., 2021). However all chemotherapeutic agents target and negatively affect healthy normal cells as well as tumorigenic ones (Moses et al., 2003). They mostly damage rapidly dividing and growing cells. There is also other factors lowering the efficacy of chemotherapeutic drugs such as drug resistance, alterations on chromosomes, changes in signal transduction pathways that initiates the actions of drugs, reduced drug buildup, suppression of apoptosis, reversed DNA damage that is triggered by drugs due to DNA repair, alterations in DNA due to epigenetic mechnanisms (Housman et al., 2014).

Overall, there is seek for alternative, promising advancements in the cancer treatment which are safe and effective, especially by using conventional chemotherapy agents together with other agents as complementary therapy to lessen the side effects of chemotherapy on healthy cells and promoting cancer cell specific cytotoxicity.

Traditional medicine can form an alternative for identifying novel molecules that can be used in cancer therapies (Gautam et al., 2014). Currently there are several agents that have natural origin and are being applied in cancer treatments and also majority of the approved medications for treating cancer are naturally derived (Bhalla et al., 2013). However, in cancer biology, there is seek for further studies on medicinal plants and on figuring out the actions of phytocompounds that may be adopted as more potent anticancer agent with a lesser amount of side effects (M. I. Khan et al., 2022).

3. Natural Products in Cancer Treatment

Traditional medicinal herbs have adopted widely in East Asia in pharmaceutical perspective for various diseases (Bhalla et al., 2013). More than 5000 phytochemicals with considerable structural differences are being present in fruits, vegetables, grains and other plants and within these, EOs have been extensively focused due to their considerable range of bioactivities (Bhalla et al., 2013).

For alternative cancer therapy strategies, alkaloids, saponins, triterpenes, glycosides, and polyphenols have revealed cancer preventative activity both *in vitro* and *in vivo* (Gautam et al., 2014). Significant number of plants have extensive anticancer properties and secondary metabolites derived from these plants such as vincristine, colchicine, paclitaxel, etc. are widely being used in cancer treatment by targeting signaling pathways in cancer (Cragg & Newman, 2005)(F. Khan et al., 2023).

For the search of novel and natural agents, EOs can be regarded as key agents in cancer therapies due to their pharmacological activities (Sharma et al., 2022).

3.1 Essential Oils

In the history of health care natural plant originated products have important role and their use in combination with synthetic drugs had critical role in several clinical conditions (Blowman et al., 2018)(Atanasov et al., 2021). Thus the extraction of secondary products such as EOs from aromatic plants by using steam or hydro-distillation has improved over the years (Sharma et al., 2022). EOs have multiple biological and medicinal activities and in addition to these they have been used in several areas such as cosmetics, food industry due to their aromatic characteristics (Sharmeen et al., 2021) (Thomas et al., 2016).

EOs are categorized as terpenes and terpenoids, phenolics, and aliphatic compounds (Sharma et al., 2022). EOs consist of monoterpenes that are hydrophobic and they have several biological activities (Özkan & Erdoğan, 2011). Containing diverse chemical constituents and this variation make them to have numerous biological properties (Gautam et al., 2014) (Figueiredo, 2017). EOs have stronger effect compared to their constituents as they have synergistic and more selective impact (Özkan & Erdoğan, 2011).

Analyzing the primary components of EO, determining their safety and therapeutic efficacy for diseases have key importance for the drug development. EOs are lipophilic and they have the capability to pass through the plasma membrane and act on the intracellular and/or intraorganelle sites (Özkan & Erdoğan, 2011).

These oils are promising therapeutic agents for clinical conditions such as hypertension and atherosclerosis (Ji et al., 2009)(Raut & Karuppayil, 2014) and can have anti-inflammatory properties (Shayganni et al., 2016) (De Andrade et al., 2017). Moreover, they have antimutagenic, antiproliferative, antioxidant and detoxifying activities which make them promising candidates for cancer research (Blowman et al., 2018). Both EOs and their constituents have showed anticancer activities (Bhalla et al., 2013) (Gautam et al., 2014).

Conventional chemotherapy agents target dividing cells so they are cytotoxic towards cancer cells and also towards healthy cells. This affects the recovery of the patient and can also be life-threatening. Thus cancer cell specific treatment options or an expansion in the therapeutic window across healthy and cancer cells are required (Blowman et al., 2018). Recent strategies have considerable progress but still there are problems with specificity. Monoclonal antibodies are selective but they do not offer enough cytotoxicity (Blowman et al., 2018). EOs' extracts have tested in several *in vitro* studies and they specifically targeted cancer cells while not affecting healthy cells or being less cytotoxic toward healthy cells (Blowman et al., 2018). In order to maintain target specific delivery of the EOs, they can be adopted into nanoemulsions, niosomes, liposomes, etc. and this will enhance the possible use of EOs in anticancer treatment approaches (Sharma et al., 2022). As EOs are insoluble in water and as they are volatile and instable so their pharmacological use is restricted (Trinetta et al., 2017). Their efficacy can be influenced by a number of aspects such as oxidation, degradation by digestion, alterations caused by enzymes, inefficient absorption and removal by excretion (Sharma et al., 2022). To lessen the effect of these factors and to manage effective transport of EOs to the target cells microcapsules/nanocapsules, different formulations of lipid carriers, etc. can be used (Sharma et al., 2022). Enclosing EOs in nanoparticles can reduce the volatility, toxic action and can provide more stable formulation and augment their biological properties (Sharma et al., 2022).

3.2 Method of Functioning of Essential Oils

Standard cancer treatment strategies aims to trigger apoptosis or cell cycle arrest. Thus, natural products with these capabilities can serve as important agents in cancer prevention. There are several mechanisms that play part in the antiproliferative action of the EOs and EOs are also effective *in vitro* studies by reducing tumor size (Gautam et al., 2014). Anticancer strategies provided by EOs are triggering cell cycle arrest, apoptosis, and DNA repair mechanisms. EOs lead to decrease in the tumor cell proliferation, avoidance of metastasis, and resistance towards several drugs (Gautam et al., 2014).

EOs and their constituents can have an influence on several molecular cascades that have roles in cancerous cells. EOs can cross the cell membrane and target multiple proteins in numerous cancer related pathways. They elevate ROS and Reactive Nitrogen Species (RNS) levels and causes changes in the Nuclear factor kappa B (NF-κB) and these lead to apoptosis (Gautam et al., 2014). Protein kinase B (Akt), mammalian target of rapamycin (mTOR), and mitogen-activated protein kinases (MAPKs) pathways are inhibited by EOs and this increases or decreases level of important biomolecules (Gautam et al., 2014). Akt is dephosphorylated by EOs and this increase the levels of p21 which triggers apoptosis (Gautam et al., 2014). EOs also trigger mitochondrial stress and this activates B-cell lymphoma 2 (Bcl-2) and membrane depolarization that leads into apoptosis (Gautam et al., 2014). They also inhibit DNA polymerase which affect the DNA repair mechanisms and causes Poly (ADP-ribose) polymerase (PARP) cleavage that also induces apoptosis (Gautam et al., 2014).

3.3 Anticancer Activities of Essential Oils

3.3.1 Antioxidant Activity

Oxidation harms several biological elements and can cause diseases like cancer, aging, Alzheimer's disease, inflammation, diabetes (Bhalla et al., 2013) and antioxidants are compounds that have the ability to obstruct the free radical oxidation and oxidative damage (Amorati et al., 2013). There has been several research studying the antioxidant activity of numerous EOs to discover natural antioxidants and it was revealed that EOs are excellent suppliers of antioxidants (Stadler et al., 1995). Antioxidant activity of EOs

make them to manage oxidative stress (Amorati et al., 2013). When eukaryotes are susceptible to oxidation, the mitochondrial DNA damage caused by ROS restrains the electron transport chain, being responsible for accumulation of ROS. In the presence of EO, ROS comes together with EO and reactive phenoxy radicals are being arisen. Reactive phenoxy radicals come together with ROS to halt extra oxidative damage (Stadler et al., 1995). EOs also upregulate the antioxidant enzymes and non – antioxidants (Manjamalai & Grace, 2013). In one of the studies it has been shown that thyme EO has the greatest antioxidant activity (Wei & Shibamoto, 2010). EOs such as clover leaf, basil, eucalyptus, chamomile, etc. have antioxidant activity (Bhalla et al., 2013). It was also reported that *Thymus spathulifolius* has antioxidant properties due to its high thymol (36.5%) and carvacrol (29.8%) content (Bhalla et al., 2013). Another EO that has antioxidant activity with high thymol (20.5%) and carvacrol (58.1%) content is Egyptian corn silk (Bhalla et al., 2013).

3.3.2 Anti-mutagenic Activity

EOs have anti-mutagenic activity and have a key role in preventing cancer. Their anti-mutagenic activity is based on various mechanisms that are inhibiting infiltration of the mutagens through the cell membrane, deactivating the mutagens through direct scavenging, picking up the free radicals created by mutagens, preventing the change of promutagens to mutagens by cytochrome P450 enzyme, detoxification of mutagens, and efficient accurate DNA repair (Bhalla et al., 2013).

3.3.3 Anti-proliferative Activity

EOs have preventative action on proliferation which has been exerted with several mechanisms that are disruption of cell membrane or triggering apoptosis (Russo et al., 2015). There have been several studies showing that EOs are inhibiting the proliferation and growth of numerous cancer cell lines (Seal et al., 2012).

3.4 Thymus capitatus (L.) Hoffm. & Link

Thymus capitatus belongs to the genus *Thymus* (Lamiaceae) and genus *Thymus* characterized by around 350 species around the world. *T. capitatus* is commonly found

in Mediterranean region (D. Ö. Yavuz et al., 2021). In Cyprus there are two species that are growing wild and these are *T. integer* and *T. capitatus* (Hanoglu et al., 2017).

Essential oils isolated from genus *Thymus* mainly contains thymol, carvacrol, γ-terpinene and p- cymene and these compounds provide pharmacological properties of essential oils. If the major constituent is forming > 50% of the total composition, this is named as "pure chemotype". If there are two or more constituents that are less than 50% of the total composition, this is "mixed chemotype". For genus *Thymus*, there are thymol or carvarol chemotype or thymol/carvacrol chemotype.

Volatile oils have great variety of pharmacological activities and chemical changes of volatile compounds provide us information about pharmacological activities of EOs (Hanoglu et al., 2017). These chemical changes are arising due several factors such as ecology, climate, geography, genetic factors (Koutsaviti et al., 2013). The constituents of EOs derived from TC are given in Table 1 and 62.3% of the EO is thymol. Constituents provide EOs with several pharmacological activities (Hanoglu et al., 2017). Hanoglu et al. reported that TC growing wild is thymol chemotype and this is uncommon. Especially the TC collected from Yedidalga is a possible source of thymol (Hanoglu et al., 2017).

EOs and compounds derived from them have widely used for medicinal, agricultural, industrial applications etc. (Hammer et al., 1999)(Rota et al., 2008)(Bakkali et al., 2008). TC has significant importance in cosmetic and fragrance industries. It is also applied in traditional treatment approaches against gastro-intestinal disorders

Table 1.

Composition of TC EO (Hanoglu et al., 2017)

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	tr
1706	α-Terpineol	tr
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

3.5 Origanum dubium Boiss.

Origanum species are commonly originate in the Mediterranean, North Africa, and Siberia (Sharifi-Rad et al., 2021). Plants that are classified under genus are highly abundant in EOs and the primary components are terpenoids, that generally consist of carvacrol, thymol, carbamene, and terpilenol (L. Zhou et al., 2021). There are 42 species in *Origanum* genus and they are utilized in the treatment of bronchitis, diabetes,

cholesterol, hypertension, etc. (Sharifi-Rad et al., 2021). When *Origanum* species werestudied in regards to determine their phytochemical and biological properties, it was shown that they can supply compounds with insecticidal, antibacterial, antifungal, antioxidant, and anti-carcinogenic properties (Karadağlıoğlu et al., 2019) (Burt, 2004). Moreover, EOs and compounds obtained from *Origanum* species have anticancer, antiproliferative and apoptotic activities towards cancer cell lines like leukemic cell, platelets and breast adenocarcinomas (Al-Kalaldeh et al., 2010) (I. Khan et al., 2018).

O. dubium (Lamiaceae) grows wild in the Mediterranean region. Previous studies determined the composition of OD as given in Table 2 and carvacrol forms the 88.3% of the EO (Karadağlıoğlu et al., 2019). The research stated that the antimicrobial and antioxidant action of OD is linked to its abundant carvacrol content (A. Karioti et al., 2006).

Table 2.

C*omposition of OD EO* (Karadağlıoğlu et al., 2019)

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	tr
1718	α-Terpineol	0.5
1728	Borneol	0.1
1771	Carvone	tr
2108	Elemol	0.1
2159	Spathunelol	0.1
2210	Thymol	0.2
2243	Carvacrol	88.3
2273	β-Eudesmol	0.1
	Total	100.0

3.6 Thymol

Thymol is a natural monoterpene phenol derivative of *p*-cymene and an isomer of carvacrol (Özkan & Erdoğan, 2011). It is the key constituent of EOs acquired from various species that belongs to Lamiaceae and Verbenaceae families (Sampaio et al., 2021). It is also a main constituent of the TC EO from Northern Cyprus (Hanoglu et al., 2017). Thymol is hydrophobic and so can have a reaction with the lipids forming the cell membrane and mitochondria, this makes cell membrane and mitochondria permeable and content of the cell escape outside (Gündogan & Nath, 2021).

TOH and EOs with high proportion of thymol have promising benefits in various areas such as medicine, agriculture and pest control (Hanoglu et al., 2017). The

anticancer measure of thymol has detected *in vivo* and *in vitro* studies, whereas the antioxidant, anti-inflammatory/immunomodulatory, anti-genotoxicity and antimicrobial features have also revealed (Islam et al., 2019) (Vassiliou et al., 2023).

By several experimental model investigations, TOH has shown to have anticancer actions by numerous means such as triggering depolarizing mitochondrial membrane potential, and stimulating the pro-apoptotic caspase proteins (Figure 7) (Chapa et al., 2018)(Deb et al., 2011)(Llana-Ruiz-Cabello et al., 2014).

3.7 Carvacrol

Carvacrol is a monoterpenoid phenol that is the key element of EOs derived from members of Lamiaceae and Verbenaceae families like OD (Sampaio et al., 2021). It is also one of the main constituents of *Origanum vulgare, Lippia gracilis,* and *Thymus vulgaris* EOs (Camilo et al., 2022)(Lombrea et al., 2020) (Galovičová et al., 2021)(Khazraei et al., 2022)(Sampaio et al., 2021). Similar to TOH, CV is also hydrophobic and can have a reaction with the lipids of the cell membrane and mitochondria.

Studies have showed that CV has several biological effects like antimicrobial, insecticidal, anti-angiogenic (Abdel-Massih et al., 2010), antiviral (Wani et al., 2021), antibacterial (de Almeida de Souza et al., 2021), antifungal (Rashed et al., 2021), antioxidant (Özkan & Erdoğan, 2011) (A. A. A. Khalaf et al., 2021) and anticarcinogenic (Özkan & Erdoğan, 2011) (Arunasree, 2010) activities. CV has also been used in food industry as it has antimicrobial and flavoring properties (F. Khan et al., 2023).

Studies showed that CV has a potential to halt carcinogenesis by regulating numerous cell signalling pathways disrupted in cancer cells and which are linked with apoptosis, autophagy, inflammation and angiogenesis (F. Khan et al., 2023). It has affected intracellular signalling molecules such as interleukins (ILs), tumor necrosis factor alpha (TNF-α), Bcl-2-associated X protein (BAX), vascular endothelial growth factor (VEGF), Beclin, caspases, and Bcl-2 (A. A. A. Khalaf et al., 2021).

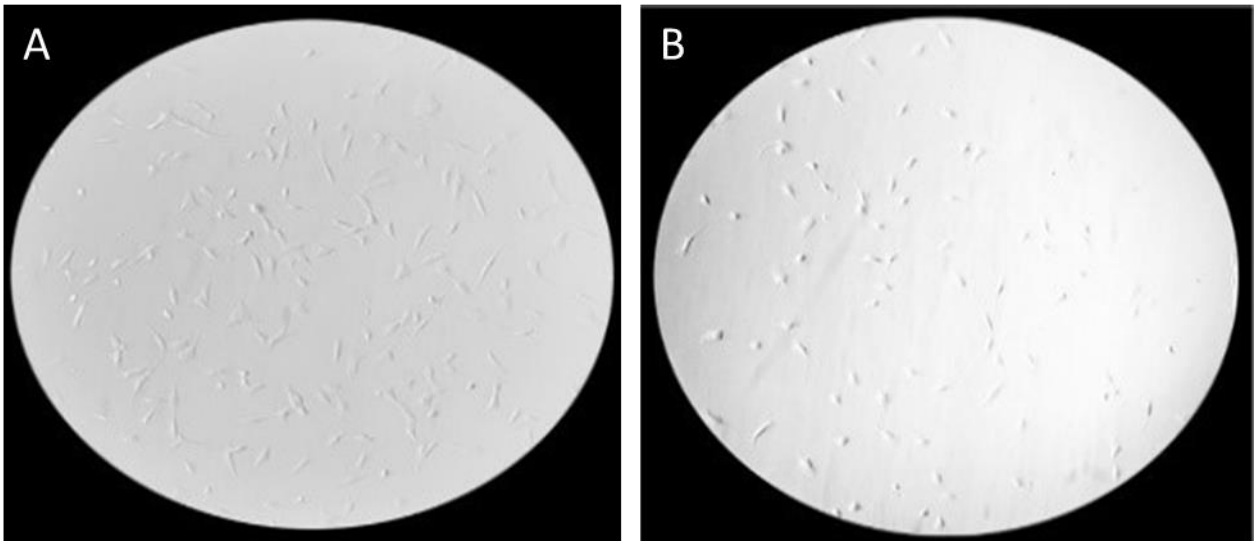
3.8 Human Mesenchymal Stem Cells

Human mesenchymal stem cells (hMSCs) can generate range of differentiated tissue and several tissues that originated from hMSCs can transform into cancerous tissues. Thus, hMSCs have been selected to study the anticancer potential of EOs, TOH and CV. Telomerase-immortalized hMSCs (hMSC-telo1) with expanded life span are previously established by transducing hMSCs with human telomerase reverse transcriptase (hTERT) domain of telomerase. hMSC-telo1 cells have elevated telomerase actions and their telomeres are longer in contrast to o hMSC, this gives them extensively prolonged lifespan (Christensen et al., 2008).

Tumorigenic hMSC-telo1cells were previously established by irradiating hMSC-telo1 cells with 2.5 Gy of γ-rays to get malignant transformation. Irradiated hMSC-telo1 cells were able to form tumors prior to engraftment of cells to severe combined immunodeficiency mice (Christensen et al., 2008).

Figure 1

Phase contrast microscopy images of (A) hMSC-telo1 cells and (B) irradiated hMSC-telo1 cells with tumor forming ability at ×100 magnification.



CHAPTER III

Methodology

1. Research Design

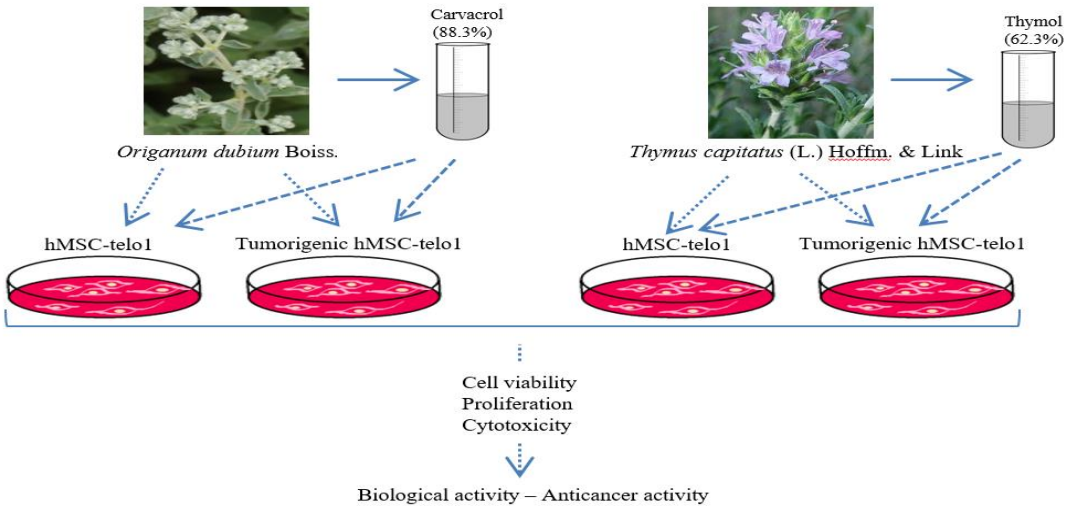
a) Both hMSC-telo1 cells and their tumorigenic counterparts were plated into flat-bottomed 96- well plates and were treated with 1×10^{-3} v/v %, 2.5×10^{-3} v/v %, 5×10^{-3} v/v %, 2.5×10^{-2} v/v %, and 5×10^{-2} v/v % TC and OD EOs, TOH and CV for 24 h. Then then to evaluate the viable cell proportion MTT assay was done.

b) For 1×10^{-3} v/v %, 2.5×10^{-3} v/v %, 5×10^{-3} v/v %, 2.5×10^{-2} v/v %, and 5×10^{-2} v/v % OD EO and CV TUNEL assay was preformed to assess the apoptotic cell death.

c) 5×10^{-2} v/v % and 5×10^{-3} v/v % TC EO and TOH were used in combination treatment with DFX and then to evaluate the viable cell proportion MTT assay was done.

Figure 2

Research design. TC EO, OD EO, CV and TOH were applied on both normal hMSC-telo1 and their tumorigenic counterpart at various concentrations and cells were incubated for 24h. Then cell viability and apoptosis were assessed.



2. Isolation of the EOs and Content Analysis of EOs through Gas Chromatography/ Mass Spectrometry Analysis

Essential oils used in the study were TC and OD EO and they were generously made available by Prof. Dr. Dudu Özkum Yavuz in agreement with Prof. Dr. Hüsnü Can Başer.

Aerial parts of TC and OD that are growing naturally in Northern Cyprus were gathered in the course of the post- flowering stages. TC was obtained from Yedidalga- 35°8’36.03"N 32°48’ 11.37"E and OD was obtained from Yesilırmak- 35°10’1"N 32°44’ 01.2"E. EO isolated from TC and OD according to the former studies by Hanoglu et al. and Karadağlıoğlu et al. and for the content analysis of EOs gas chromatography/ mass spectrometry (GC/MS) analysis were carried out regarding to the same studies (Hanoglu et al., 2017)(Karadağlıoğlu et al., 2019). EOs used in this investigation have been acquired and their properties have determined in prior investigations (Hanoglu et al., 2017)(Karadağlıoğlu et al., 2019). Thus, the isolation and characterization methodologies of TC and OD EOs will not be debated in this study.

The voucher samples were located in Herbarium of the Near East University.

3. Cell Culture

Cell lines used in the study were hMSC-telo1 and irradiated hMSC-telo1 cells that have tumour forming capability. hMSC-telo1 cells used in the study were previously established by Serakinci et al. (Serakinci et al., 2007). Both cell lines were at passage 43 at the beginning of the study.

In order to establish hMSC-telo1 cells primary hMSCs were obtained from the bone marrow and were transduced with *hTERT* gene by using retroviruses as transduction vectors (Serakinci et al., 2007). Thus they were immortalized by the *hTERT* gene transduction. Tumorigenic counterpart of hMSC-telo1 cells were generated by exposing hMSC-telo1 cells to 2.5 Gy irradiation (Christensen et al., 2008). Both hMSC-telo1 and tumorigenic hMSC-telo1cells were kept in Dulbecco’s Modified Eagle’s Medium (DMEM, Sigma-Aldrich) enhanced with 10% fetal bovine serum (FBS, Sigma-Aldrich), penicillin (64 μg/ml), streptomycin (0.1 mg/ml) and L-glutamine. Cells were kept in the incubator that maintain 5% CO^2 concentration and 37°C temperature.

3.1 Thawing Cells

Ampoule of cells were kept in cryotubes within %5 Dimethyl sulfoxide (DMSO) in -140 °C. For thawing them they were put into water bath at 37 °C. Cells were then moved into centrifuge tubes containing 10 ml of pre-warmed DMEM enhanced with supplements as given in section 3. Centrifugation has conducted for the tubes at 1400 rpm for 3 min at 4°C. Following that, the supernatant was removed and the thawed cells were homogenised by resuspension and plated in T-25 flasks with growth medium. Cells were kept at 5% CO^2 and 37°C.

3.2 Passaging Adherent Cells

When cells were 80% confluent, cells were passage into new cell culture flasks. The spent media was thrown away and cells were washed with ice-cold Phosphate Buffer Saline (PBS) (DPBS, Invitrogen). Then PBS was thrown away and cells were exposed to 5 ml of trypsin/0.5 mM Ethylenediaminetetraacetic acid (EDTA) (0.25% Trypsin, Invitrogen) for 2 min. When cells were started to dissociate, 10 ml of DMEM with FBS was put into the flask to halt the trypsinisation. Cell suspension was poured into 15 ml tubes and centrifugation at 1100 × g for 4 min had performed. Following this, supernatant was removed. Cell pellets were resuspended in DMEM enhanced with supplements as given in section 3. Required volume of cell suspension is added into new culture flasks together with DMEM enhanced with supplements as given in section 3. Cells were kept at 5% CO^2 and 37°C.

3.3 Freezing cells

Cell pellets were collected as described in 3.2. Cell pellets were resuspended in freezing medium and 1 ml of cells suspensions are transferred into cryogenic storage vials. Cells were kept at -80°C overnight and following this they were transferred to and stored in -140°C.

4. MTT assay

MTT assay was conducted with Cell proliferation Kit I (MTT) (Roche, Germany) to measure the proportion of viable and proliferating normal and tumorigenic hMSC-telo1

cells. MTT assay has the basis that produce a colorimetric result in which viable cells break down the tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) into purple formazan crystals which then are dissolved by solubilisation solution. The absorbance is read from the obtained coloured solution by using spectrophotometer.

In this study hMSC-telo1 cells and their tumorigenic counterpart were plated into a flat-bottomed 96- well plate as 5×10^4 cells/ml per well and kept in 5% CO^2 and at 37°C for 24 h.

Removal of growth medium had performed and TC and OD EO, CV and TOH at doses given above in Research design section were applied on cells. Following 24 h of incubation, EOs, TOH and CV have removed from the wells and 10 µl of MTT solution was added per well, stirred mildly and kept at 37°C for 4 h. Subsequently crystals were dissolved by pippetting100 µl solubilisation solution, DMSO, into each well.

Absorbance values were recorded at 570 nm using a microplate reader (VersaMax™). As a negative control, cells which were not treated with any of the agents were used. The absorbance readings from blank wells were obtained at 630 nm and deducted from absorbance readings of treated cells to exclude the background reading, thus to get normalized absorbance readings. Absorbance values read in triplicate.

The absorbance values corresponds to the number of metabolically active cells in culture. The percent of viable cells was determined by the formulation given below:

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

5. TUNEL assay

TUNEL (TdT-mediated dUTP-biotin nick end labelling) assay was conducted with In Situ Cell Death Detection Kit, Fluorescein, Roche to detect apoptosis. The basis of the TUNEL assay lies on tagging DNA strand breaks.

In this study hMSC-telo1 and tumorigenic hMSC-telo1cells were plated into a flat-bottomed 96- well plate as 5×10^4 cells/ml per well and OD EO and CV at doses given above in Research design section were applied on them. After 24 h of treatment, OD EO and CV has been removed from the wells. Afterwards, cells were air dried and were immobilized with 100 µl 4% Paraformaldehyde in PBS, pH 7.4, for 1 h at 15-25°C. Following this cells were washed with PBS and were permeabilized by keeping them in 100 µl 0.1% Triton X-100 in 0.1% sodium citrate for 2 min on ice. Following this cells were rinsed two times by using PBS. Cells were air dried and kept with 50 µl TUNEL reaction mixture for 1 h at 37°C in the dark. PBS wash has been done for the cells, this action was repeated 3 times.

Cells were analysed by fluorescence microscope and cells with fluorescein-positive signals were regarded as cells that have DNA fragmentation showing the presence of apoptosis. Excitation wavelength should be 450-500 nm and detection should be 515-565 nm. For positive control fixed and permeabilized cells were incubated with 100 µl DNase I recombinant for 10 min at 15-25 °C to generate DNA strand breaks before labelling stage. Incubation with 50 µl Label solution as a substitute of TUNEL reaction mixture had done for negative control.

6. Statistical analysis

Data are given as the mean ± standard deviation (SD) of the three independent experiments. The results were interpreted by GraphPad Prism 9 software. The means among treatments were compared by one-way ANOVA followed Tukey's and Dunnett's tests. Tukey's test was conducted to associate the means among treatments, and Dunnett's test was conducted to associate the means between the treatments and the control group. Two-way ANOVA was conducted to associate the response between normal hMSC-telo1 and tumorigenic hMSC-telo1 cells. A p value of <0.05 was regarded as statistically significant.

CHAPTER IV

Findings

1. Assessment of hMSC-telo1 Cells and Their Tumorigenic Counterparts' Viability after Applying TC EO, OD EO, TOH and CV

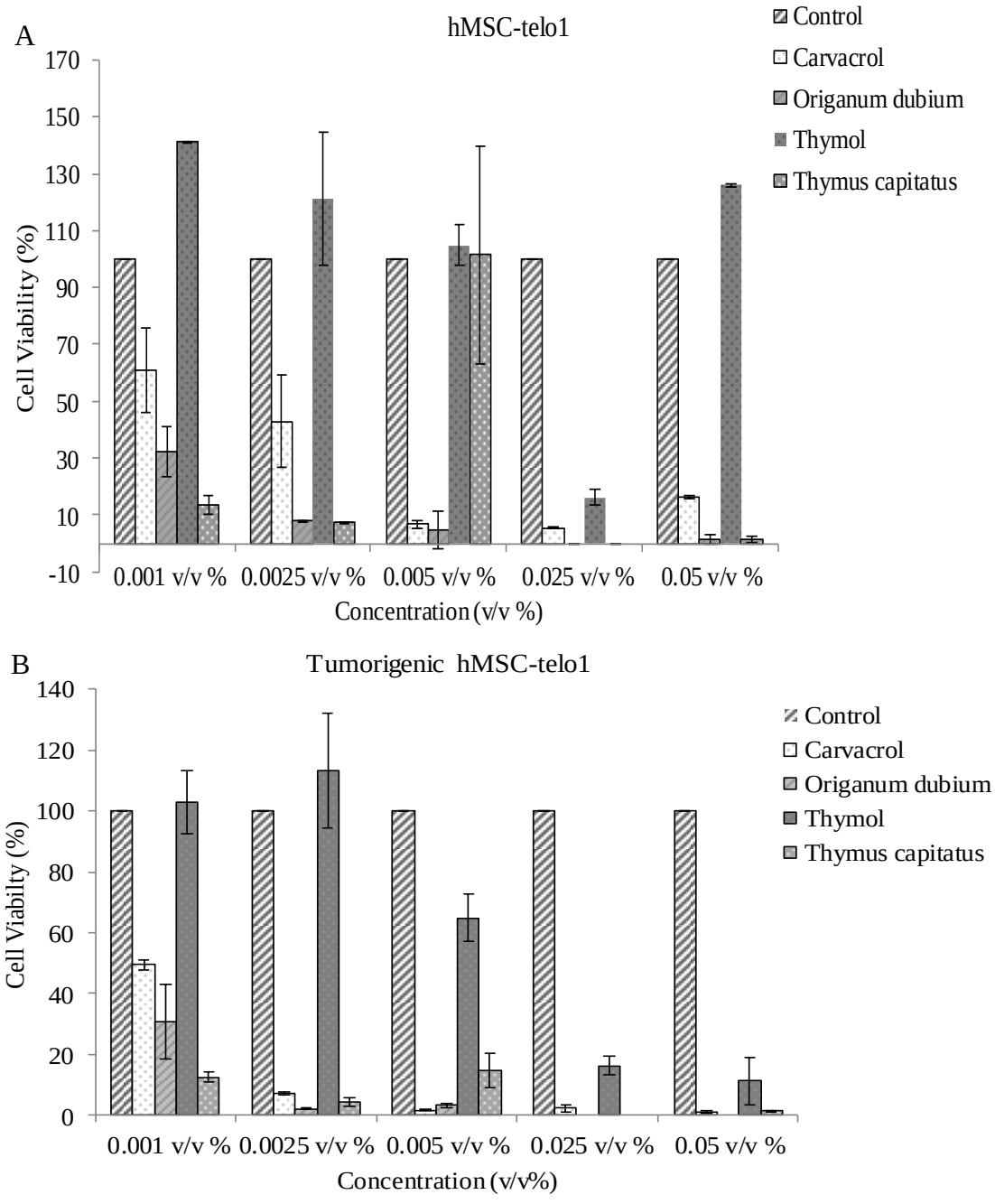
0.5 v/v % TC EO has been tested on normal and tumorigenic hMSC-telo1 cells and proliferation of both of these cell lines have been negatively impacted (D. O. Yavuz et al., 2017). Thus lower concentrations, 1×10^{-3} v/v %, 2.5×10^{-3} v/v %, 5×10^{-3} v/v %, 2.5×10^{-2} v/v %, and 5×10^{-2} v/v %, had preferred in current study.

hMSC-telo1 and tumorigenic hMSC-telo1 cells have been exposed to TC EO, OD EO, TOH and CV and MTT assay was carried out to observe the biological reaction of these cells following the application of EOs and their main components.

With 1×10^{-3} v/v %, 2.5×10^{-3} v/v %, 5×10^{-3} v/v % and 5×10^{-2} v/v % TOH treatment normal hMSC-telo1 cells had shown to preserve their viability however with 2.5×10^{-2} v/v % TOH treatment this effect had not observed. For the tumorigenic hMSC-telo1cells, TOH has been cytotoxic at higher concentrations; 5×10^{-3} v/v %, 2.5×10^{-2} v/v % and 5×10^{-2} v/v % while preserved the viability at lower concentrations (Figure 3A and 3B). 5×10^{-2} v/v % TOH augmented hMSC-telo1 cells' viability in contrast to the control cells ($p<0.001$). In regards to the tumorigenic hMSC-telo1 cells, considerable level of cell death had recorded in contrast to the control cells ($p<0.001$). Similar trend was observed for 5×10^{-3} v/v % TOH in comparison with the control cells but the difference was not significant ($p>0.05$).

Figure 3

Viability of (A) hMSC-telo1 and (B) tumorigenic hMSC-telo1cells. TC and OD EOs, TOH and CV were applied to cells at varying concentrations and kept for 24h. In order to evaluate the viable cell proportion MTT assay was done. The data are given as the mean ± SD.



For all concentrations of TC EO except than 5×10^{-3} v/v % , significant cytotoxicity was observed for both normal and tumorigenic hMSC-telo1 cells. 5×10^{-3} v/v % TC EO maintained hMSC-telo1 cells' viability in contrast to control cells ($p= 0.001$), on the other hand it caused decline in the viability of tumorigenic hMSC-telo1cells ($p<0.001$).

OD EO and its major constituent CV had been cytotoxic at different levels both towards tumorigenic and normal cells at 1×10^{-3} v/v %, 2.5×10^{-3} v/v %, 5×10^{-3} v/v %, 2.5×10^{-2} v/v %, 5×10^{-2} v/v % concentrations. However amongst the tested concentrations of OD EO and CV 1×10^{-3} v/v % is the concentration that had less cytotoxic effect on non-tumorigenic and tumorigenic cells while being highly cytotoxic at higher concentrations (Figure 3A and 3B). Cytotoxicity of CV had affected the normal healthy cells in great extend compared to the control cells at all concentrations ($p= 0.030$).

OD EO and CV revealed high cytotoxicity at all concentrations used in this investigation.

No significant differences were identified between normal and tumorigenic hMSC-telo1 as 1×10^{-3} v/v %, 2.5×10^{-3} v/v %, 5×10^{-3} v/v %, 2.5×10^{-2} v/v %, 5×10^{-2} v/v % of TC EO, TOH, OD EO and CV were applied ($p>0.05$).

2. Assessment of Apoptosis of the hMSC-telo1 Cells and Their Tumorigenic Counterparts' after Applying OD EO and CV

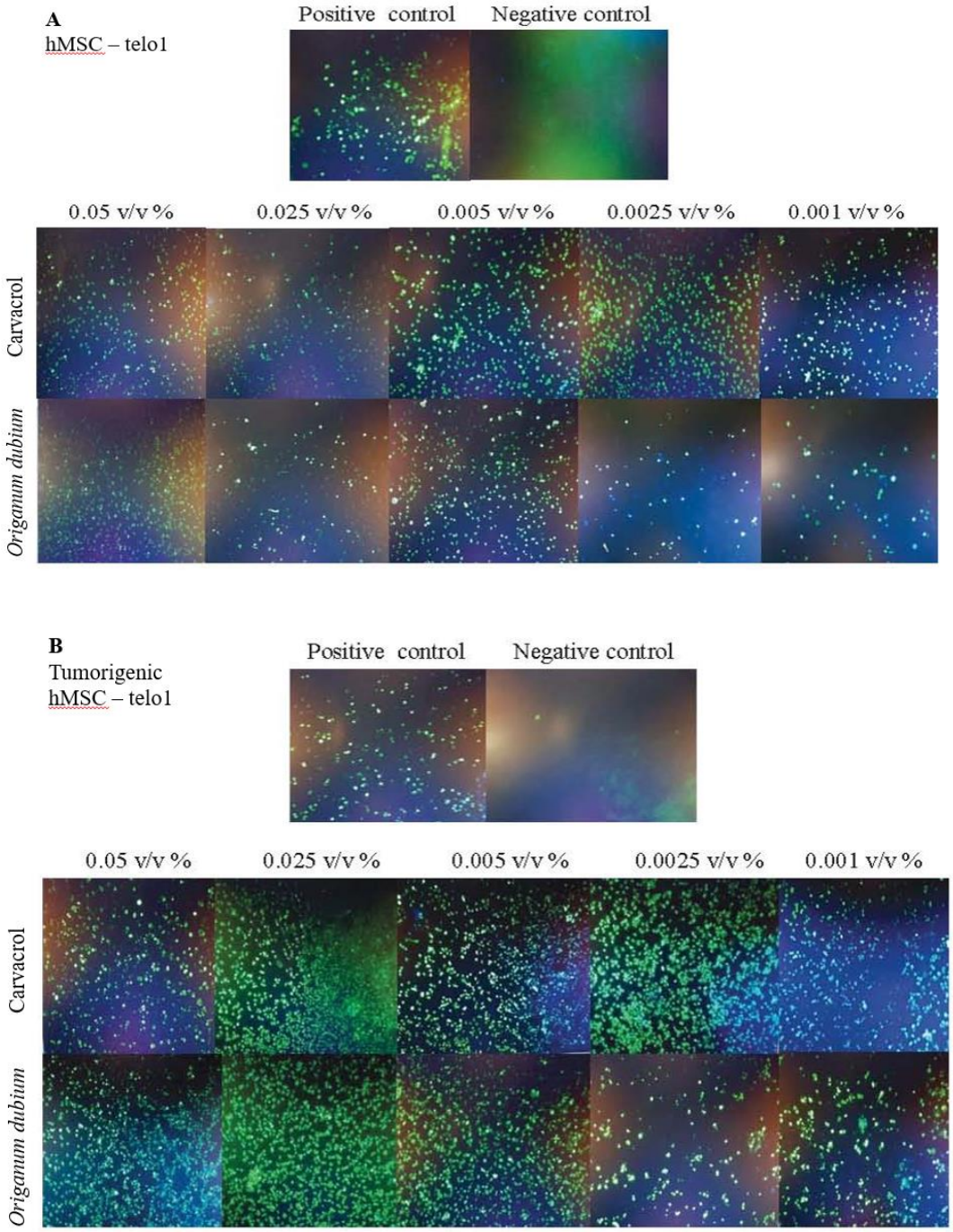
After exposing normal and tumorigenic hMSC-telo1 cells to OD EO and CV, TUNEL assay was conducted to further examine how these cells will respond to this treatment. TUNEL assay detects DNA fragmentation and the results show signals indicating cells with fragmented DNA. Fluorescent signals were observed for all concentrations and for both cell lines (Figure 4A and 4B). These findings propose that OD EO and CV can lead to cell death through DNA fragmentation. The apoptotic signals detected for tumorigenic cells after treatment with both OD EO and CV were more intense compared to normal hMSC-telo1 cells.

Thus cytotoxicity of OD EO and CV that was shown by MTT assay, was also confirmed by TUNEL assay showing that they led cell death through apoptosis at all tested concentrations.

Overall, OD EO and CV had cytotoxic actions on both cell lines so their application at the concentrations used in the study cannot be recommended.

Figure 4

Examining apoptotic cell death. OD EO and CV applied on (A) hMSC-telo1 and (B) Tumorigenic hMSC-telo1cells at varying concentrations. TUNEL assay was performed to examine the apoptotic cell death due to the cytotoxic actions of OD EO and CV. DNase I recombinant was applied as a positive control.



3. Assessment of hMSC-telo1 Cells' and Their Tumorigenic Counterparts' Viability after Applying TC EO, TOH, TC EO/TOH and DFX Combinations

As TC EO and TOH had showed promising activity by acting in a cytotoxic fashion to tumorigenic cells and also by maintaining the viability of non-tumorigenic healthy cells, they were tested together with DFX. 5×10^{-3} v/v % TC EO and 5×10^{-2} v/v % and 5×10^{-3} v/v % TOH were preferred to be used in combination treatment as these concentrations did not lead to death of healthy cells while led to tumorigenic cell death.

Normal and tumorigenic hMSC-telo1 cells were treated with TC EO, TOH, DFX and also DFX was used together either with TC EO or TOH. Then MTT assay was done to examine the cell viability after these treatments.

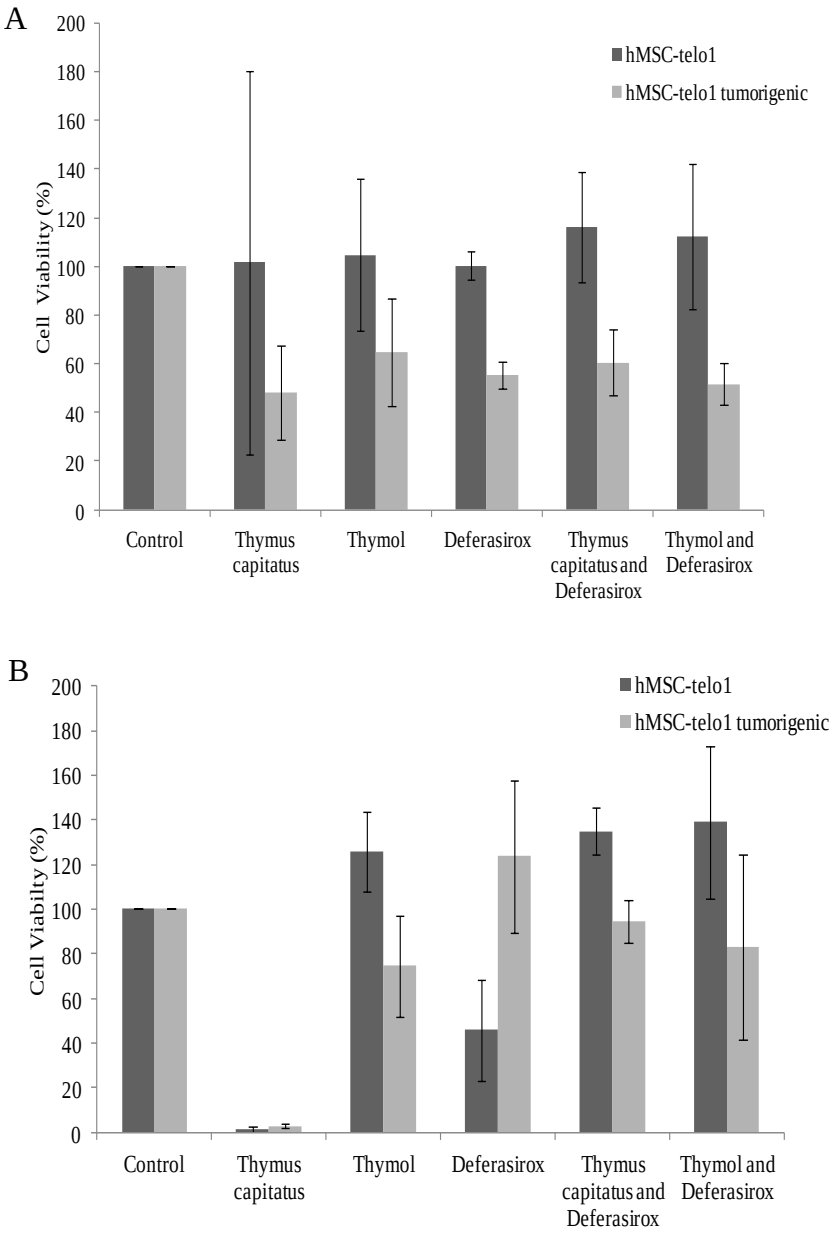
5×10^{-3} v/v % DFX, TC EO and TOH treatment lessen the amount of viable tumorigenic cells in contrast to the control cells whereas the proportion of the viable normal cells did not get affected in contrast to the control cells, though not significantly ($p>0.05$) (Figure 5A). When TC EO and TOH applied together with DFX, hMSC-telo1 cell viability enhanced in comparison with the cells exposed to only DFX. TC EO and TOH also led to tumorigenic hMSC-telo1cell death compared to control (Figure 5A) indicating that applying TC EO and TOH together with DFX can be favoured to protect the healthy cells from the actions of chemotherapeutic drugs. No significant differences were identified amongst the hMSC-telo1 cells and its tumorigenic counterpart when treated with 5×10^{-3} v/v % of TC EO, TOH, DFX or their combinations ($p>0.05$).

5×10^{-2} v/v % TC EO treatment was cytotoxic towards hMSC-telo1 cells and its tumorigenic counterpart in contrast to the control cells ($p=0.001$, $p=0,013$), respectively (Figure 5B). 5×10^{-2} v/v % DFX led to decline in the amount of viable non-tumorigenic cells in contrast to control cells ($p=0.036$) however tumorigenic cell viability had augmented compared to the control cells, though not significantly ($p>0.05$) (Figure 5B). In contrast to TC EO, 5×10^{-2} v/v % TOH increased the proportion of the viable hMSC-telo1 cells while decreased the proportion for the tumorigenic ones in contrast with the control cells, but the difference was not significant ($p>0.05$) (Figure 5B). Applying TC EO or TOH together with DFX gave rise to similar results (Figure 5B). No significant

differences were identified among the hMSC-telo1 cells and its tumorigenic counterpart when treated with 5×10^{-2} v/v % of TC EO, TOH, DFX or their combinations ($p>0.05$).

Figure 5

Viability of normal and tumorigenic hMSC-telo1 cells when exposed to TC and OD EOs, their primary constituents and DFX. (A) 5×10^{-3} v/v % and (B) 5×10^{-2} v/v % TC, TOH, DFX and combination of TC/TOH and DFX were applied and to evaluate the viable cell proportion MTT assay was done. The data are given as the mean ± SD.



Discussion

Cancer is one of the key causes of mortality globally. Although widespread research is going on in the area to elucidate anticancer treatment strategies, there is still seek for novel treatment options which lead less side effects and are more effective. Reasons that prevent the current strategies not to be effective as needed can be listed as drug resistance, molecular heterogeneity and cytotoxicity of treatments on healthy normal cells. Natural products and their biologically active components can be regarded as possible candidates for anticancer therapies for their antiproliferative and cell death triggering activities (Bouhtit et al., 2021).

In this study possible cytotoxic action of TC and OD EO and their main constituents; TOH and CV on hMSC-telo1 and tumorigenic hMSC-telo1 have examined by conducting MTT assay. The novelty of our study is showing the cytotoxicity and anticancer activity of North Cyprus TC EO, OD EO, TOH and CV against tumorigenic hMSCs and also their action on normal hMSCs for the first time.

Mesenchymal stem cells (MSCs) are progenitor cells with a high self-renewal capacity and they can be isolated from several tissues. They also have various biological and immunological properties. MSCs can be used in various therapeutic applications like cell-based therapy however efficacy and safety of their use should be considered (Bouhtit et al., 2019). MSCs are simple and easy to isolate and they have great expansion potential and this makes them potential candidates for cell-based therapies (Bouhtit et al., 2019). MSC-based therapy has not been successful both in animal studies and clinical trials as there is no proper activation signal for MSCs, and due to inaccurate timing and MSC administration site (Krampera, 2011). In order to augment their therapeutic potential, MSCs are suggested to be combined with drugs (Bouhtit et al., 2019). Today combining MSCs with either a cell –product or a drug might be suggested as a potential therapeutic strategy. Combining anti-cancer drugs with MSCs can provide greater efficacy and lessen the toxicity compared to the standard treatments (Hendijani & Javanmard, 2015). As MSCs have immunological plasticity they may intensify the cytotoxicity of the drugs. In addition to that they are promoters and enhancers of the regenerative medicine and due to their regenerative and healing abilities, MSCs can shield the normal cells and injured/damaged tissues from the cytotoxic effects of the

cancer therapeutics (Bouhtit et al., 2019). They can replace the death cells and in addition to that as they have immunomodulatory functions, they provide therapeutic action. Using synthetic and semi-synthetic entities can cause side effects and they can be costly (Bouhtit et al., 2019). Thus, more safer, and effective approaches such as using natural products and their bioactive molecules should be adopted in MSC- based therapies. In this study, our results could suggest that combining EOs or their bioactive constituents with MSCs can provide a safe and effective cell- based therapy approach.

There are few studies conducted on North Cyprus TC, especially on the anticancer activity of it (D. O. Yavuz et al., 2017). There were also studies based on its cytotoxic and antimicrobial properties (Llana-Ruiz-Cabello et al., 2014) (Güvenir et al., 2020). There is a study showing that North Cyprus TC has proapoptotic and anticancer actions on human colon adenocarcinoma cell lines Colo-320, CD133+ Colo-320 (cancer stem cells), and CD133– Colo-320, where it was further triggered apoptosis of Colo-320 cells compared to CD133+ Colo-320 and CD133– Colo-320 cells (D. Ö. Yavuz et al., 2021).

A latest research deduced the cytotoxic activity of TC leaves' and stem' extracts on HepG2 hepatocellular carcinoma, A-549 pulmonary epithelial, HCT-116 colon cancer and MCF-7 breast adenocarcinoma cell lines (Younes et al., 2022). Doxorubicin was selected as a standard treatment and to determine the amount of cells that were viable and cell that were undergone apoptosis, MTT assay and apoptosis assay were done. Study showed that leaves' extract was cytotoxic towards the A-549 and HepG2 cancer cell lines and stem's extract was cytotoxic towards A-549 cells (Younes et al., 2022). Apoptosis assay was conducted for A-549 cells and it was reported that leaves' extract led into S-phase arrest and apoptosis by activating caspase-3, p53 and Bax and down regulating Bcl-2 (Younes et al., 2022).

Several investigations are stating the anticancer activities of other members of the genus *Thymus* (Lamiaceae family).

T. vulgaris has shown to halt the development of the UMSCC1 tumor cells where genes related to interferon signaling, N-glycan biosynthesis and extracellular signal-regulated kinase 5 (ERK5) signaling are being affected by the cytotoxicity of *T. vulgaris* (Sertel et al., 2011).

THP-1 leukemia cell line and peripheral blood mononuclear cells (PBMCs) have also treated with *Thymus vulgaris* L. extract and the viability of THP-1 cells was negatively affected. *Thymus vulgaris* L. extract was selectively cytotoxic towards THP-1 cells (Ayesh et al., 2014) so can be taken as a possible anticancer agent due to its selectivity.

Dried *T. vulgaris* has been given to rat mammary carcinoma and a syngeneic 4T1 mouse model in their diet and shown to led decrease in the tumor mass for both models (Kubatka et al., 2019).

Cervical adenocarcinoma cells, HeLa and MCF-7 were exposed to essential oil derived from *T. vulgaris* and it has been cytotoxic for both of the cell lines (A. N. Khalaf & Abed, 2021).

Thymus vulgaris L. EO with TOH as a major constituent has also tested on MCF -7, lung carcinoma H460 and acute lymphoblastic leukemia MOLT-4 cells and provided inhibition of all tested cells proportional to the dose (Niksic et al., 2021).

Another *in vitro* study has conducted with *Thymus broussonettii* EO from Morocco with CV as major constituent and it has been tested on human ovarian adenocarcinoma cell line OV1/P and on its chemoresistant complements (Ait M'Barek et al., 2007). *T. broussonettii* EO was cytotoxic for all cell lines (Ait M'Barek et al., 2007). *T. broussonettii* EO has been injected intra- tumor and it led to a decrease in the formation of solid tumor mass in mice (Ait M'Barek et al., 2007).

In regards to OD growing wild in Cyprus there are few studies. With OD there is a recent study reporting the use of OD EO for sanitation (Xylia et al., 2022). An investigation revealed that OD growing wild in Cyprus has antimicrobial activity (Karioti et al., 2006) and the other one has investigated antioxidant and anti-inflammatory measures of OD (Karioti et al., 2015).

In vitro and *in vivo* investigations carried out for *Origanum* species to examine proliferation and cancer avoiding actions.

For the *O. vulgare* essential oil there have been several studies showing the antibacterial, antifungal, antiparasitic, antioxidant, anti-inflammatory, anticancer, antihyperglycemic, anti-Alzheimer properties and also showing that it has positive effects on skin disorders (Lombrea et al., 2020).

Proliferation preventative and cytotoxic action of *O. vulgare* EO have been reported on MCF-7 and HT-29 colon adenocarcinoma cell lines (Elansary et al., 2018) (Begnini et al., 2014). These actions were also shown on HeLa cells (Elansary et al., 2018). *O. vulgare* EO had also same actions on AGS stomach cancer cells (Balusamy et al., 2018), T24 bladder cancer and mouse 4T1 cancer cells (A. R. Khan et al., 2019). *O. vulgare ssp. hirtum* EO was tested on HepG2 and HEK293 immortalized embryonic kidney cells to assess its cytotoxicity and EO decreased the number of viable HepG2 cells while not showing great toxicity towards healthy HEK293 cells (Elshafie et al., 2017). Main constituents of the EO were CV, TOH, limonene and citrol and they were also assessed for their cytotoxicity on the tested cell lines and shown that CV and TOH selectively targeted tumor cells whilst did not have any cytotoxicity towards healthy renal cells, revealing the possibility that *O. vulgare ssp. hirtum* EO can selectively target the cancer cells (Elshafie et al., 2017).

However, it was stated that *O. vulgare* did not show proliferation preventative actions towards MCF7, adenocarcinoma cell line (Al-Kalaldeh et al., 2010).

Origanum syriacum extract led to a reduction on the viability of THP-1 and PBMCs however it targeted normal cells more than the leukemic cells so it is not regarded as potential selective agent in cancer treatments (Ayesh et al., 2014).

Human lymphoblastic leukemia cell line Jurkat treated with another Lamiaceae family member *Origanum majorana* extract and the study showed that the extract had halt the proliferation proportional to dose. It was also shown that extract triggers apoptosis through increasing p53 level and decreasing Bcl-2 level (Abdel-Massih et al., 2010). *O. majorana* EO was applied on metastatic MDA-MB-231 breast cancer cell line and it was reported that the level of matrix metalloproteinases (MMPs) which play a part in invasion and metastasis were declined (Al Dhaheri et al., 2013). Al Dhaheri (2013) also presented that when chick embryo tumor growth assay had conducted with *O. majorana,* tumor growth and metastasis halted *in vivo* (Al Dhaheri et al., 2013). Another investigation testing *O. majorana* EO on HepG2, JTC- 26 cervical cancer cells and A549 lung cancer cells also demonstrated growth inbition for these cell lines (L. Zhou et al., 2021).

One of the latest investigations has suggested that one of the Lamiaceae family member *Origanum onites* L. EO has potential anticancer effect as it has been cytotoxic to different cancer cells lines (Gündogan & Nath, 2021). CV was shown to be the key constituent of the EO whilst it also contained TOH (Gündogan & Nath, 2021). It had antiproliferative action on A375 melanoma cells, MCF-7, HepG2, and HT-29 cells proportional to dose (Spyridopoulou et al., 2019).

TOH and CV are two of the key constituents of oregano and thyme EOs. EOs that are used in this investigation are derived from TC and OD grown wild in North Cyprus and their major constituents are TOH and CV, respectively.

Numerous investigations have shown the anticancer actions of TOH, CV and also the EOs containing these compounds and which is primarily because of the existence of TOH, CV or both of them.

A recent study tested TOH on U87 human malignant glioblastoma cells and it was revealed that TOH can trigger apoposis, elevate the ROS levels and also elevate the levels of proapoptotic factors Bax and p53 (Qoorchi Moheb Seraj et al., 2022). Its actions on apoptosis were also identified when bladder cancer cells were exposed to TOH where apoptotic intrinsic pathway was activated by executioner caspase-3, initiator caspase-9, release of cytochrome c, and decline in the levels of Bcl-2 (Li et al., 2017). Zeng et al. (2020) revealed that TOH has antitumor action both *in vitro* and *in vivo* (Zeng et al., 2020). Apoptosis and cell cycle arrest has been observed in colorectal cancer cells *in vitro* and *in vivo* treatment with TOH provided a considerable reduction in tumor volume as triggered apoptosis by activating BAX/Bcl-2 cascade (Zeng et al., 2020). Both *in vitro* and *in vivo* it also prevented colorectal cancer cell epithelial–mesenchymal transition (EMT), invasion, and metastasis by preventing the induction of Wnt/β-catenin cascade (Zeng et al., 2020). TOH had similar actions on the acute promyelotic cancer cell line HL-60 as led to reduction in Bcl2 and elevation in Bax levels and induction of caspase -9, -8 and -3 (Deb et al., 2011).

A recent studies showed that TOH can trigger disruption of mitochondrial functioning and cancer cells death through apoptosis (Islam et al., 2019). It also has an proliferation halting activity on cancerous cells (Islam et al., 2019). However it has

cytoprotective, antiapoptotic, anti-inflammatory and antigenotoxic activity on normal cells (Islam et al., 2019).

CV showed potential anticancer action both *in vitro* and *in vivo* investigations. It has applied on A549 cell line (Koparal & Zeytinoglu, 2003) and led to the apoptosis while another studies showed that inhibition of AXL expression and elevated malondialdehyde (MDA) and hydroxy-2′-deoxyguanosine (8-OHdG) level is responsible for the action of CV (Ozkan & Erdogan, 2012) (Jung et al., 2018).

When CV was applied on Caco-2 cancer cells the cell viability had reduced (Llana-Ruiz-Cabello et al., 2014). It also led to apoptosis of HepG2 hepatocarcinoma cells through mitochondrial - and caspase – mediated pathways (Melušová, Jantová, et al., 2014). CV can also trigger apoptosis through G1 and S phase arrest (Melušová, Slamenova, et al., 2014). CV has also halted proliferation of HCT116 and HT-29 cells (Pakdemirli et al., 2020). Another study on two colon cancer cell lines HCT116 and LoVo showed that CV inhibited their proliferation and migration. CV also induced apoptosis in these cell lines through mitochondrial apoptotic pathway and the MAPK and phosphoinositide 3-kinase/ protein kinase B (PI3K/Akt) signaling pathways where there is reduction in Bcl-2 levels and enhancement in Bax and c-Jun levels (Fan et al., 2015).

CV is applied to chemosensitive MCF-7 cells and apoptosis is triggered as Bcl-2 levels decreased, Bax and caspase -3, 6, and 9 levels increased (Al-fatlawi, Rahisuddin & Ahmad, 2014). CV has also tested on HeLa and SiHa and it had been cytotoxic towards these cell lines and apoptotic cell death had reported (Enkhtaivan et al., 2017).

The mentioned studies together with several other studies are demonstrating the anticancer activity of CV on MDA-MB-231, HepG2, DU145 (human prostate cancer) cells (F. Khan et al., 2023).

In vitro investigations also presented the anticancer activity of CV. An investigation revealed that decline in the number of tumors has observed when CV was administered to DMBA-triggered breast cancer in female rats (Rojas-Armas et al., 2020). There are also studies on hepatocellular carcinoma in Wistar rats. Reduction in the tumor growth with the administration of CV was observed in one of the studies (Subramaniyan et al., 2014). It was shown that CV led to apoptosis in hepatocellular

carcinoma and also also have chemopreventative, antiproliferative and antiangiogenic activity on hepatocellular carcinoma (Jayakumar et al., 2012) (Hanaa H. Ahmed et al., 2013).

The capability of TOH and CV to trigger apoptosis might be a used as a potent strategy to develop potential novel drugs against cancer.

In our study it was observed that at only 5×10^{-3} v/v % TC EO did not lead to a decline in the proportion of viable normal hMSC-telo1 cells, while had cytotoxic effect towards tumorigenic hMSC-telo1cells. It was highly cytotoxic towards both cell lines at other tested higher concentrations. Its major constituent TOH augmented the proportion of the normal cells and induced an considerable level of tumorigenic hMSC-telo1 cell death at 5×10^{-3} v/v % and 5×10^{-2} v/v % concentrations. Thus, our results shows that TC EO and TOH specifically targeted tumor cells while not being cytotoxic towards healthy cells and this is highly crucial and important in the development of new anticancer approaches as standard treatment options mostly lack this ability. Jaafari *et al*. in accordance with our results demonstrated that 0.5% (v/v) of TOH did not have cytotoxicity towards healthy human peripheral blood mononuclear cells, and led to the proliferation of these cells (Jaafari et al., 2007). Preserving the viability of normal cells and leading to tumorigenic cell death make the TC EO and TOH potent candidates for deducing novel anticancer treatment approaches.

Present findings are to a certain degree in line with the preceding investigation which revealed that TOH and CV had enhancing impacts on healthy MSCs for their viability, proliferation and preserving from cytotoxicity when compared to *Ptychotis verticillata* essential oil with high TOH and CV content (Bouhtit et al., 2019). Thus, the cytotoxic effect observed might be due to the presence of TOH or CV or the presence of multiple components together in EOs.

Thus, it could be suggested that 5×10^{-3} v/v % TC EO and 5×10^{-2} v/v % and 5×10^{-3} v/v % TOH can perform for augmenting the chemotherapeutic drugs whereas stimulating proliferation in healthy hMSC –telo1 cells. DFX was preferred as a standard chemotherapeutic agent in this study and considered as a positive control which is critical when comparing the outcome of novel candidate for cancer treatment (Sampaio et al., 2021). DFX is an iron chelator and an anti-cancer agent, and it has been taken as

chemotherapeutic agent as it has promising *in vitro* and *in vivo* anticancer actions towards multiple cancerous cell types. On leukemia and hepatoma cell lines, DFX shown to have *in vitro* antitumor activity (Lescoat et al., 2007) (Ohyashiki et al., 2009). Later on, its' *in vitro* antitumor action has also been recorded on solid human tumor xenografts (Lui et al., 2013). *In vitro* and *in vivo* studies showed that it can halt the proliferative capacity of pancreatic cancer cells (Harima et al., 2016) and also prevent metastasis of metastatic pancreatic cancers where pancreatic cancers generally form metastatic lesions (Amano et al., 2020). Recently it was revealed that DFX is a good candidate to be used for cervical cancer treatment as it prevented cervical cancer cell proliferation (N. Zhou et al., 2022).

Within last ten years EOs are proposed to be applied together with standard chemotherapeutic agents to lessen the side effects arising due to chemotherapeutic agents or they might provide new anticancer entities (Gautam et al., 2014). Some EO constituents also increased the cytotoxicity of the chemotherapeutic agents on several cell lines, this led to reduction in the dose of chemotherapeutics while offering the same outcome (Blowman et al., 2018).

Utilization of TC EO and TOH together with DFX augmented the proportion of the viable hMSC-telo1 cells and the deleterious actions of DFX on healthy cells have been critically eradicated.

OD EO and CV led to death of both normal and tumorigenic hMSC-telo1 cells at all tested concentrations. In order to assess the reaction of the normal and tumorigenic hMSC-telo1 cells towards OD EO and CV more, TUNEL assay was conducted and was shown that OD EO and CV induced apoptosis. Cytotoxicity of OD EO and CV was at the considerable level both for the healthy hMSC-telo1 cells and their tumorigenic counterpart so this limits their practice at concentrations chosen in the current study. Testing lower concentrations of OD EO and CV on healthy hMSC-telo1 cells and tumorigenic hMSC-telo1 cells might be useful to assess their anticancer potential as they showed cytotoxicity towards tumorigenic cells and as there are several studies mentioned above showing their possible cancer preventative actions towards different cancer cell types. The outcomes from TUNEL assay was also in line with the preceding

investigations as they revealed that the cell death due to the exposure of cells to OD EO and CV was due to the induction of apoptosis.

A systematic literature review also suggested that both CV and TOH have anticancer and antiproliferative capacity and *in vitro* carvacrol seems to be more cytotoxic towards some cell lines compared to TOH (Sampaio et al., 2021). Cytotoxicity of six monoterpenes (carvacrol, thymol, carveol, carvone, eugenol and isopulegol) have been compared as they were tested on five tumor cell lines (K-562, P-815, CEM, MCF-7 and MCF-7 gem) (Jaafari et al., 2012). As a result of that study it was shown that carvacrol is the most cytotoxix amongst all (Jaafari et al., 2012).

Investigations revealed that CV can have a negative impact on some normal cell lines while it might not have any cytotoxic effect on other normal cells (Sampaio et al., 2021). CV had cytotoxic impact on human fibroblast cells (WS-1) at higher concentration, while led to proliferation of the cells at lower concentrations (Günes-Bayir et al., 2018). For CV there has also been phase I clinical trial that healthy subjects have administered with CV (1 or 2 mg/kg daily) and there has been no side effects observed (Ghorani et al., 2021). Accordingly the link between the concentration and effect should be studied further to define concentration that does not lead to negative effect on normal cells.

If we compare the cytotoxicity of TC EO and TOH or OD EO and CV both on normal hMSC-telo1 and tumorigenic hMSC-telo1cells, it would be observed that EOs are more cytotoxic compared to their major constituents. This could be due to the synergistic action of the constituents of the EOs, especially due to the action of TOH or CV together with other components. Some studies revealed that it might be possible that the action of the major constituents is regulated by minor constituents (Bakkali et al., 2008) (Özkan & Erdoğan, 2011). Components of the EOs are responsible for important aspects such as cell penetration or cellular distribution which are important for the transport of the EO in to the target cells, thus assessing the EO first might provide more information for its biological activities (Bakkali et al., 2008).

Overall, our results suggest that TC and TOH have promising anticancer effect and can be combined to be used as enhancers of conventional chemotherapy drugs to increase their therapeutic potential. Additionally using TC and TOH in combination with

chemotherapeutic agents might provide a potent strategy to preserve or enhance the viability of normal cells and protect them form the side effects of the chemotherapeutic agents, while inducing tumorigenic cell death. At lower concentrations TC EO and TOH did not lead to reduction in the proportion of viable normal, healthy hMSC-telo1 cells, providing us a desirable property to be used in therapeutic perspective.

A systematic review conducted assess the antitumor activity of CV and TOH has suggested that more *in vivo* studies with more potent and effective methodology is needed to determine a standard and safe dose, assess the toxicity and side effects and elucidate accurate mechanisms of action (Sampaio et al., 2021). In addition to these for TC EO, OD EO, TOH and CV studies are reporting their anticancer potential however there is need for clinical trials investigating the therapeutic effectiveness of these. For cancer treatment effective targeted drug delivery is required and studies should focus on identifying cellular processes underlying the antitumor actions of EOs and/or their major constituents and novel molecular targets for EOs and/or their major elements. Pharmacokinetic status, safety, as well as toxicity of EOs should also be assessed before clinical use. These will increase the efficacy and selectivity of EOs and their constituents. Constituent of EOs with anticancer activity can also be modified synthetically to improve their activity and selectivity.

CHAPTER VI

Conclusion

In light of our and others findings it could be concluded that TC EO and its major constituent TOH are promising agents to be used in cancer therapeutic approaches as they have potential to reduce the proportion of viable tumorigenic hMSC cells and to enhance the proportion of viable normal healthy hMSC cells. Moreover, they have acted as enhancers for the therapeutic potential of conventional chemotherapy drugs. For TC EO 5×10^{-3} v/v% and for TOH both 5×10^{-3} v/v % and 5×10^{-2} v/v % are suggested as preferred concentrations. In addition, 5×10^{-3} v/v % of TC and TOH used in combination with DFX provided promising outcome compared to 5×10^{-2} v/v % in regard to their potential to reduce the viability of tumorigenic cells.

Our results and previous studies suggest that combining TC EO and thymol with conventional cancer therapies might provide an effective novel approach that can enhance the therapeutic action of current cancer therapies. They can also be considered as adjuvants to standard cancer treatment options. Additionally combining TC EO or thymol with hMSCs may offer a cell-based unique approach to augment the therapeutic efficacy in cancer treatment.

Overall this and other studies demonstrated that EOs and their constituents are potent bioactive agents for pharmaceutical and therapeutic application and their use in clinical settings and applications should be considered.

Recommendations and Future Perspectives

In order to strengthen our findings and conclusions driven from the study following steps can be taken.

- In this study all experiments were done in triplicates yet further repeats had to be done.
- TUNEL assay can be conducted for the cells exposed to TC EO and TOH.
- OD and CV should be tested at lower concentrations.

- Apoptotic activities of EOs and major constituents used in the study can be considered by immunocytochemistry using antibodies towards apoptotic factors.
- The biochemical and cellular processes forming the core of the actions of tested EO and their major constituents can be investigated.
- *In vivo* investigations can be carried out to assess the anticancer capability of TC EO, OD EO, TOH and CV.
- For the efficient delivery of the tested agents to tumorigenic cells nano-encapsulation of TC EO, OD EO, TOH and CV can be performed and nano-encapsulated EOs and their constituents can be tested on normal and tumorigenic hMSC-telo1 cells. The findings can be compared with the findings of the current study.
- Apoptotic activity of EOs and major constituents can also be additionally investigated through gene and protein expression analyses.

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6.1. Master's Theses

6.2. PhD Theses

7. Publications

7.1. Articles Published in International Peer-Reviewed Journals (SCI,SSCI, AHCI, ESCI, Scopus)

1. Mavis M, Ali M, Hanoglu A, Ozalp Y, Yavuz DO, Baser KHC, Serakinci N (2023) Evaluation of Therapeutic role of Thymus capitatus (L.) Hoffm. & Link, Origanum dubium Boiss. Essential Oils and Their Major Constituents as Enhancers in Cancer Therapy. Records of Natural Products 17(4): 715-720
2. Oztenekecioglu B, Mavis M, Osum M, Kalkan R (2021) Genetic and Epigenetic Alterations in Autism Spectrum Disorder.Global Medical Genetics 15;8(4):144-148.
3. Kulaberoglu Y, Lin K, Holder M, Gai ZC, Gomez M, Shifa BA, Mavis M, Hoa L, Sharif AAD, Lujan C, Smith ESJ , Bjedov I, Tapon N , Wu G , Hergovich A (2017) Stable MOB1 interaction with Hippo/MST is not essential for development and tissue growth control. Nature Communications 8
4. Yavuz DO, Mavis M, Ates G, Hanoglu A, Hanoglu DY, Baser KHC, Serakinci N (2017) Identification of potential therapeutic role of thymus capitatus essential oil using cellular imaging. Procedia Computer Science 120: 961-966
5. Hoa L, Kulaberoglu Y, Gundogdu R, Cook D, Mavis M, Gomez M, Gomez V, Hergovich A (2016)The characterisation of LATS2 kinase regulation in Hippo-YAP signalling. Cellular Signalling 28 (5):488-497

7.2. Articles Published in Other International Peer-Reviewed Journals

7.3. Papers Presented at International Scientific Confererences and Published in Conference Proceedings

1. Mavis M, Ates G, Hanoglu A, Hanoglu DY, Yavuz DO, Serakinci, N (2016) Investigating the activity of Thymus capitatus essential oil on cancer by using human telomerase reverse transcriptase immortalized mesenchymal stem cells. FEBS Journal 283: 59

7.4. National/international Books or Book Chapters

1. Serakinci N, Cagsin H, Mavis M (2018) Use of U-STELA for Accurate Measurement of Extremely Short Telomeres. Methods in Molecular Biology. Humana Press

7.5. Articles Published in National Peer-Reviewed Journals

8. Art and Design Activities

9. Projects

10. Administrative Responsibilities

11. Memberships in Scientific and Professional Organizations

12. Awards

1. EU Scholarship Programme for the Turkish Cypriot Community (2014 -2015) Scholarship for MSc Studies of Turkish Cypriots in Europe

13. Undergraduate and Graduate Courses Taught in the Last Two Years

Academic Year	Semester	Course Name	Weekly Hours		Number of Students
			Theoretical	Practical	
2021 - 2022	**Fall**	TMG402 Kanser Genetiği	3	0	8
	Fall	MBG402 Cancer Genetics	3	0	16
	Fall	TMG205 Biyoteknoloji Teknikleri	2	2	9
	Fall	MBG205 Biotechnology Techniques	2	2	21
	Spring	TMG105 Genetiğin Temel Prensipleri	2	2	13
	Spring	MBG105 Basic Principles of Genetics	2	2	23
	Spring	TMG305 Laboratuvar Güvenliği ve Teknikleri	2	2	8
	Spring	MBG305 Laboratory Safety and Techniques	2	2	17
	Spring	TMG207 İnsan Genetiği ve Genomik	2	2	9
2022 - 2023	**Fall**	TMG402 Kanser Genetiği	3	0	8

	Fall	MBG402 Cancer Genetics	3	0	12
	Fall	TMG205 Biyoteknoloji Teknikleri	2	2	10
	Fall	MBG205 Biotechnology Techniques	2	2	22

Thesis

ORIGINALITY REPORT

15%	13%	12%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	acgpubs.org Internet Source	2%
2	www.mdpi.com Internet Source	1%
3	pdfs.semanticscholar.org Internet Source	1%
4	docs.neu.edu.tr Internet Source	1%
5	www.researchgate.net Internet Source	<1%
6	ebin.pub Internet Source	<1%
7	www.science.gov Internet Source	<1%
8	"Nutraceuticals and Cancer Signaling", Springer Science and Business Media LLC, 2021 Publication	<1%
	www.scialert.net	