SHORT REPORT

A C G publications

Rec. Nat. Prod. X:X (202X) XX-XX

records of natural products

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

Merdiye Mavis [1*], Manal Salah Babiker Ali [2], Azmi Hanoglu [3], Yildiz Ozalp [4], Dudu Ozkum Yavuz [3], Kemal Husnu Can Baser [5] and Nedime Serakinci [*6, 7]

[1] *Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Near East University, Nicosia 99010, Turkish Republic of Northern Cyprus*
[2] *Department of Medical Genetics, Faculty of Medicine, Near East University, Nicosia 99010, Cyprus*
[3] *Department of Pharmaceutical Botany, Faculty of Pharmacy, Near East University, Nicosia 99010, Turkish Republic of Northern Cyprus*
[4] *Department of Pharmaceutical Technology, Faculty of Pharmacy, Near East University, Nicosia 99010, Turkish Republic of Northern Cyprus*
[5] *Department of Pharmacognosy, Faculty of Pharmacy, Near East University, Nicosia 99010, Turkish Republic of Northern Cyprus*
[6] *Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, European University of Lefke, Turkish Republic of Northern Cyprus*
[7] *Turkish Republic of Northern Cyprus Presidency, Selahattin Sonat Sokak, 99010, Lefkosa Turkish Republic of Northern Cyprus*

(Received October 27, 2022; Revised January 06, 2023; Accepted January 08, 2023)

Abstract: Aim of the study is to evaluate the potential use of *Thymus capitatus* (L.) Hoffm. & Link and *Origanum dubium* Boiss. essential oils and their major constituents; thymol and carvacrol in cancer therapies. For this aim hMSC-telo1 cells and their tumorigenic counterpart were exposed to varying concentrations of *Thymus capitatus* (L.) Hoffm. & Link and *Origanum dubium* Boiss. essential oils, thymol and carvacrol and cellular viability and proliferation have been evaluated by MTT assay. TUNEL assay has used for evaluation of apoptosis. Study suggested that, *Thymus capitatus* (L.) Hoffm. & Link essential oil and thymol have an enhancer effect once combined with conventional chemotherapeutic agents. 0.005 v/v % of *Thymus capitatus* (L.) Hoffm. & Link, 0.005 v/v % and 0.05 v/v % of thymol showed enchancer effect by sparing the normal hMSC-telo1 cells from the cytotoxicity of Deferasirox while these combinations were cytotoxic towards tumorigenic hMSC-telo1 cells.

Keywords: Anticancer; human mesenchymal stem cells; tumorigenic; essential oil; thymol; carvacrol.

[*] Corresponding author: E-Mail: merdiye.mavis@neu.edu.tr; (M.Maviş); nedimeserakinci@gmail.com (N. Serakinci)

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products Month-Month 202x EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.379.2210.2613
Available online: January 26, 2023

Therapeutic role of thyme and oregano essential oils in cancer therapy

1. Plant Source

Aerial parts of *Thymus capitatus* (L.) Hoffm. & Link and *Origanum dubium* Boiss. growing wild in Northern Cyprus were collected during the post- flowering phases. The former was collected from Yedidalga-35°8’36.03"N 32°48’ 11.37"E while the latter was collected from Yesilırmak- 35°10’1"N 32°44’ 01.2"E. The voucher specimens were deposited in Herbarium of the Near East University, Turkish Republic of Northern Cyprus (NEUN).

2. Previous Studies

The essential oil composition of *Thymus capitatus* (L.) Hoffm. & Link [1] and *Origanum dubium* Boiss. [2] were reported previously. The major components of *T. capitatus* were identified as thymol (62.3%) followed by *p*-cymene (10.9%), carvacrol (6.7%) and γ-terpinene (5.1%) [1].

To date, there are very few reports on cytotoxic activity of the North Cyprus *T. capitatus* EO. Previously it was shown that *T. capitatus* has proapoptotic and anticancer activity in Colo-320, CD133+ Colo-320, and CD133– Colo-320 primary colon adenocarcinoma cell lines [3]. However, to the best of our knowledge there is not any study that has investigated the anticancer potential of Northern Cyprus specific *T. capitatus* EO on previously established and well characterized expanded life span human mesenchymal stem cells (hMSC-telo1) and their tumorigenic counterpart (tumorigenic hMSC-telo1) where hMSCs can give rise to variety of differentiated tissue and many tissues of mesenchymal origin give rise to cancer.

Previous study by our group has shown that the concentration of 0.5 v/v % of *T. capitatus* EO has an anti-proliferative effect on hMSC-telo1 and tumorigenic hMSC-telo1 cells [4]. In this current study lower concentrations (0.001 v/v %, 0.0025 v/v %, 0.005 v/v %, 0.025 v/v % and 0.05 v/v %) of *T. capitatus* EO have been tested.

The anticancer effects of major constituent of *T. capitatus*, thymol, have been observed during *in vivo* and *in vitro* studies [5]. From different experimental model studies, thymol has been reported to exert anticancer activities through different mechanisms including inducing depolarizing mitochondrial membrane potential, and activating the pro-apoptotic caspase proteins [6, 7, 8].

The major components of *O. dubium* (Lamiaceae) were determined as carvacrol (88.3%), *p*-cymene (3.8%) and γ- terpinene (2.7%) [9, 2]. There are studies showing the anticancer, antiproliferative and apoptotic potential of EOs derived from *Origanum* species and their constituents on different cell types such as leukemic cell, platelets and breast adenocarcinoma cells [10, 11].

There is no study on the potential cytotoxic activity of *T. capitatus* and *O. dubium* EOs, thymol and carvacrol on hMSC-telo1 cells and their tumorigenic counterparts.

3. Present Study

Potential cytotoxic effect of *T. capitatus* and *O. dubium* essential oils and their major components, thymol and carvacrol on hMSC-telo1 and tumorigenic hMSC-telo1 has been assessed with MTT assay. Thymol preserved the viability of normal healthy hMSC-telo1 cells at 0.001 v/v %, 0.0025 v/v %, 0.005 v/v % and 0.05 v/v % concentrations but not at 0.025 v/v % (Figure 1A and 1B). 0.005 v/v % and 0.05 v/v % concentrations of thymol enchanced the viability of normal hMSC-telo1 cells compared to control cells whilst led to a excessive tumorigenic hMSC-telo1 cell death compared to control cells.

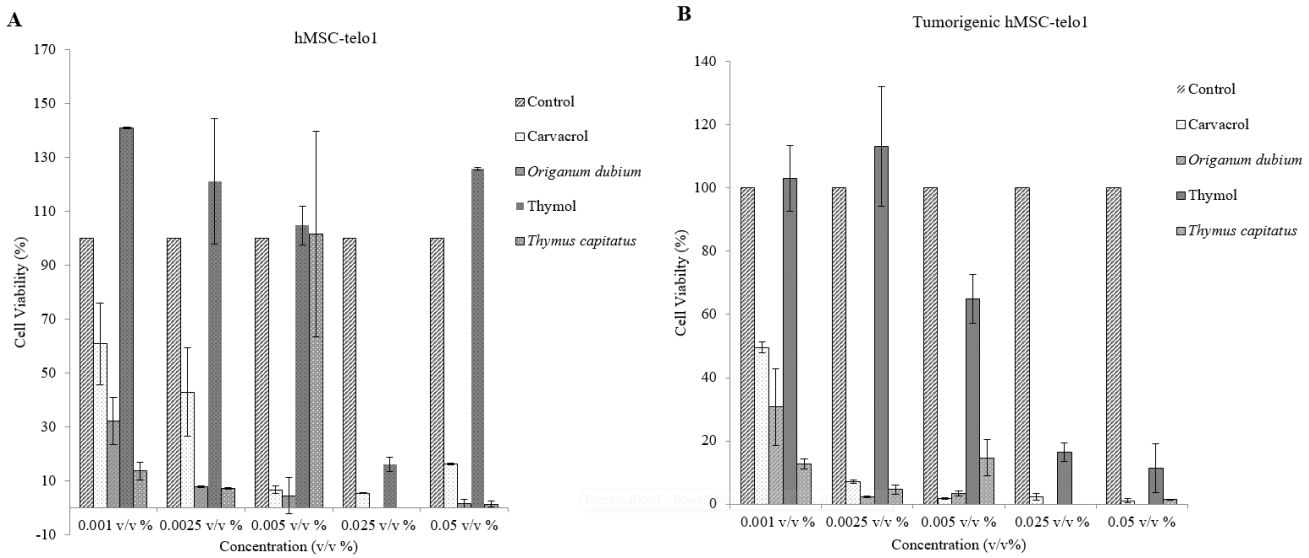

Figure 1. Viability of hMSC-telo1 cells and tumorigenic hMSC-telo1 cells. (**A**) hMSC-telo1 cells and (**B**) tumorigenic hMSC-telo1 cells were treated with different concentrations of *Origanum dubium* and *Thymus capitatus* EOs, thymol and carvacrol and incubated 24 h. The cell viability was assessed with MTT assay. The data are presented as the mean ± SD.

T. capitatus EO was also highly cytotoxic both for hMSC-telo1 cells and their tumorigenic counterpart except at 0.005 v/v % concentration where it preserved the viability of hMSC-telo1 cells compared to control cells while led to reduction in the viability of tumorigenic ones.

O. dubium EO and carvacrol, have showed varying levels of cytotoxicity at all tested concentrations both towards tumorigenic and normal hMSC-telo1 cells (Figure 1A and 1B).

To further investigate the cellular response of hMSC-telo1 and tumorigenic hMSC – telo1 cells that are subjected to *O. dubium* EO and carvacrol and to further assess the cytotoxicity of *O. dubium* EO and carvacrol towards these cells lines TUNEL assay has been performed. DNA fragmantation has been detected for all tested concentrations of *O. dubium* EO and carvacrol both for hMSC-telo1 and tumorigenic hMSC – telo1 cells with TUNEL assay (Figure 2A and 2B).

These results suggest that *O. dubium* EO and carvacrol have been cytotoxic towards both the normal hMSC-telo1 cells and their tumorigenic counterpart and their use cannot be suggested at all tested concentrations.

As 0.005 v/v % *T. capitatus* EO and 0.05 v/v % and 0.005 v/v % thymol have showed promising cytotoxic activities towards tumorigenic cells whilst preserving the normal cells, they were assessed together with Deferasirox, an anti-cancer agent, for their potential complementary activity on a chemotherapeutic agent.

MTT assay was performed to assess the biological response of hMSC-telo1 and tumorigenic hMSC-telo1 cells after incubation with 0.005 v/v % and 0.05 v/v % *T. capitatus* EO, thymol, Deferasirox, Deferasirox in combination with *T. capitatus* EO and Deferasirox in combination with thymol for 24 h.

Deferasirox, *T. capitatus* EO and thymol at 0.005 v/v % concentration have negatively affected the viability of tumorigenic hMSC-telo1 cells whilst preserved the viability of non-tumorigenic hMSC-telo1 cells compared to the control cells (Figure 3A). The viability of hMSC-telo1 cells has augmented compared once *T. capitatus* EO and thymol has used in combination with Deferasirox compared to cells treated only with Deferasirox. *T. capitatus* EO and thymol have also reduced the viability of tumorigenic hMSC-telo1 cells compared to control cells (Figure 3A) suggesting that using *T. capitatus* EO and thymol in combination with Deferasirox could be preferred to spare the viability of hMSC-telo1 cells.

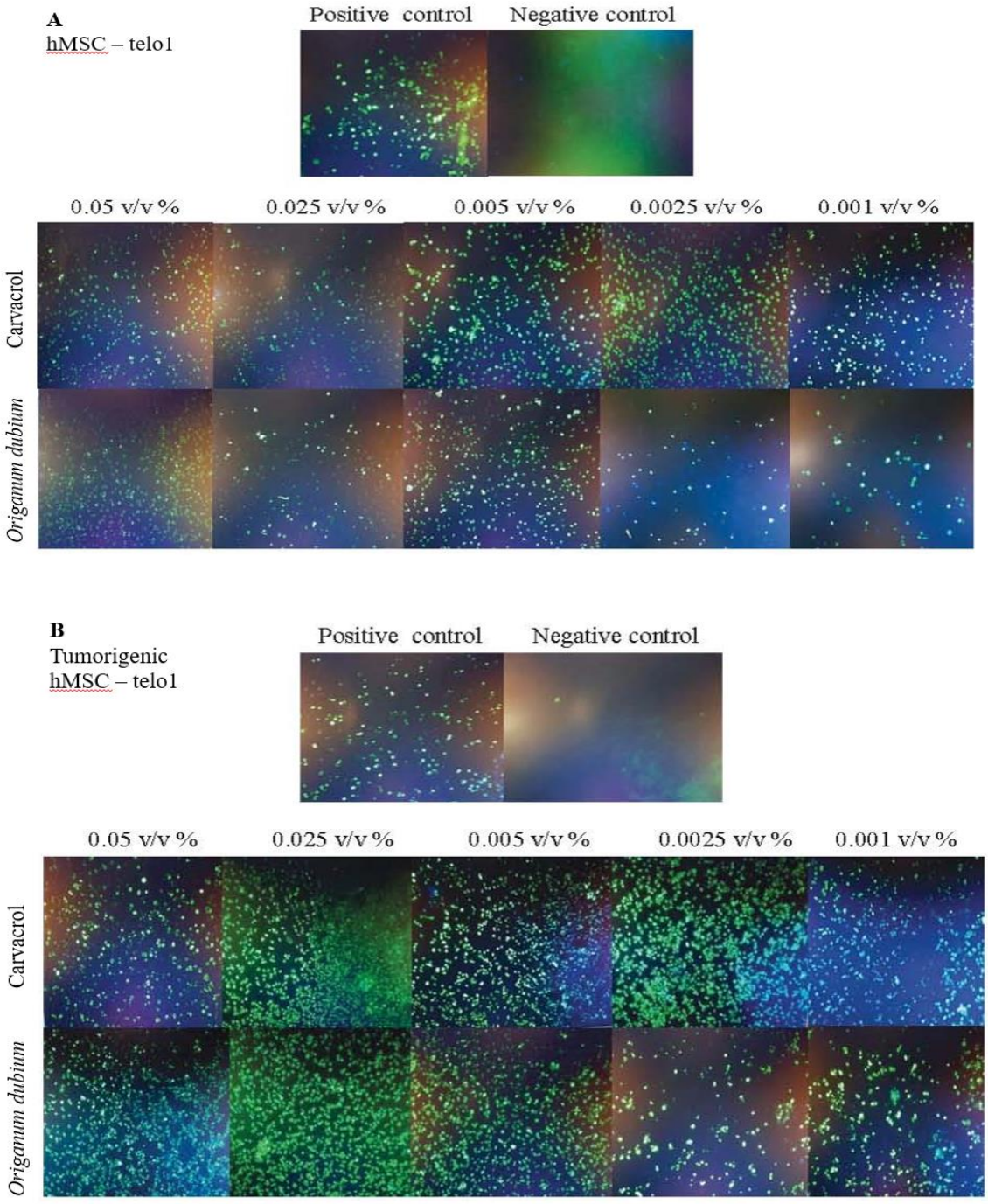

Figure 2. Detection of apoptosis. (**A**) hMSC-telo1 and (**B**) tumorigenic hMSC-telo1 cells were exposed to different concentrations of *Origanum dubium Boiss*. EO and carvacrol for 24 h and then TUNEL assay was conducted to detect if cells undergo apoptosis due to the cytotoxic effect of *O. dubium* EO and carvacrol. As a positive control DNase I recombinant has been used.

Both hMSC-telo1 and tumorigenic hMSC-telo1 cells after incubation with 0.05 v/v % *T. capitatus* EO were non-viable when compared to control cells (Figure 1B). Deferasirox at concentration of 0.05 v/v % had reduced the viability of hMSC-telo1 compared to control cells however there was not any reduction but enhancement in the viability of tumorigenic hMSC-telo1 cells compared to control cells

(Figure 3B). Unlike *T. capitatus* EO that led to a significant hMSC-telo1 cell death, its major constituent, thymol at 0.05 v/v % concentration has enhanced the viability of hMSC-telo1 cells and led to the reduction in the viability of tumorigenic hMSC-telo1 cells compared to the control cells (Figure 3B). Similar trend was observed among the hMSC-telo1 cells and tumorigenic hMSC-telo1 cells when *T. capitatus* EO or thymol was used in combination with Deferasirox (Figure 3B).

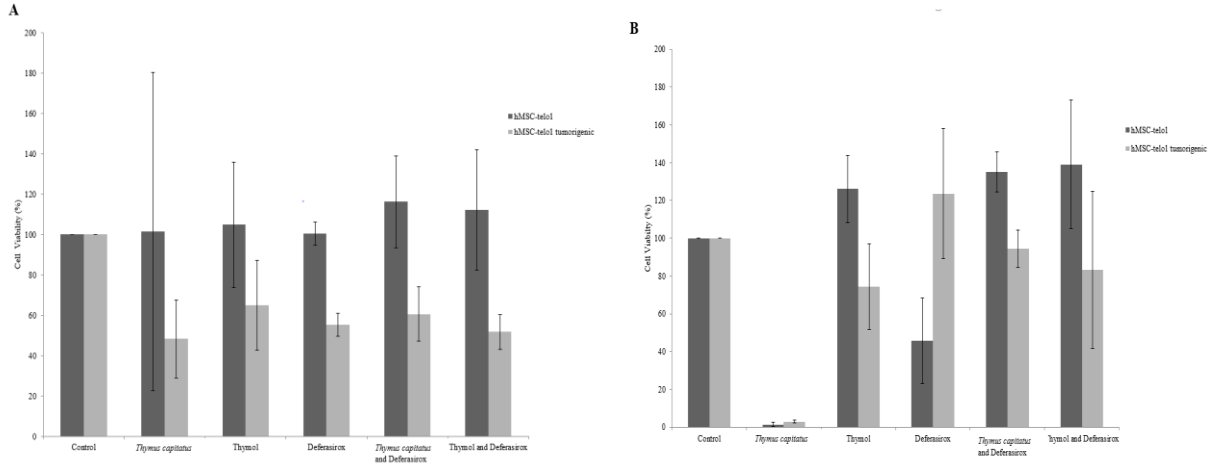

Figure 3. Comparison of response of hMSC-telo1 and tumorigenic hMSC-telo1 cells to EOs, their major components and Deferasirox. (**A**) Concentration of 0.005 v/v % and (**B**) concentration of 0.05 v/v % has been used for *Thymus capitatus*, thymol, Deferasirox and combination of *Thymus capitatus*/thymol with Deferasirox. The viability was measured with MTT assay, and the data are presented as the mean ± SD.

Thus, in this study *in vitro* observations suggest that 0.005 v/v % *T. capitatus* EO and 0.05 v/v % and 0.005 v/v % thymol can act as enhancers for chemotherapeutic agents/drugs while promoting proliferation in normal cells with proliferative capacity. When *T. capitatus* EO and thymol have been applied in combination with Deferasirox, the viability of normal, non-tumorigenic hMSC-telo1 cells have been enhanced and the negative effect of Deferasirox on non-tumorigenic hMSC-telo1 cells have been greatly eliminated by the use of *T. capitatus* EO and thymol together with Deferasirox.

They might potentially be used in combination with chemotherapeutic agents to spare the normal, healthy cells from the side effects of the chemotherapeutic agents and also to preserve and/or augment their viability. Current results are somewhat in line with the previous study which showed that thymol and carvacrol had positive effects on healthy mesenchymal stem cells in respect to their viability, proliferation and also protection from cytotoxicity [12].

In the light of literature and our results, it could be suggested that combinational therapy can have a notable benefit by additionally combining complementary medicine. Thus, the combined use of EOs or their major components together with chemotherapeutic agents may be considered as a potential new approach to increase the therapeutic effect of conventional cancer therapies.

Acknowledgments

This work was partially supported by the Near East University Centre of Excellence.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID
Merdiye Mavis: 0000-0002-5493-4568
Manal Salah Babiker Ali: 0000-0003-3274-4277
Azmi Hanoglu: 0000-0002-7586-9080
Yildiz Ozalp: 0000-0001-7928-1666
Dudu Ozkum Yavuz: 0000-0001-8743-8862
Kemal Husnu Can Baser: 0000-0003-2710-0231
Nedime Serakinci: 0000-0002-1884-0839

References

[1] A. Hanoglu, D. Y. Hanoglu, B. Demirci and D. O. Yavuz (2017). 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.

[3] D. O. Yavuz, E. Becer, R. Kendirci, F. O. Kurt and H. S. Vatansever (2021). Proapoptotic and anticancer potentials of *Thymus capitatus* essential oil on colon cancer stem cells, *Cyprus J. Med. Sci*. **6**, 319-324.

[4] D. O. Yavuz, M. Mavis, G. Ates, A. Hanoglu, D. Y. Hanoglu, K. H. C. Baser and N. Serakinci (2017). Identification of potential therapeutic role of *Thymus capitatus* essential oil using cellular imaging, *Procedia Comput. Sci.* **120**, 961-966.

[5] M. T. Islam, A. B. Khalipha, R. Bagchi, M. Mondal, S. Z. Smrity, S. J. Uddin, J. A. Shilpi and R. Razina (2019). Anticancer activity of thymol: A literature-based review and docking study with emphasis on its anticancer mechanisms, *IUBMB Life*. **71**, 9-19.

[6] J. J. De La Chapa, P. K. Singha, D. R. Lee, and C. B. Gonzales (2018). Thymol inhibits oral squamous cell carcinoma growth via mitochondria-mediated apoptosis, *J. Oral Pathol. Med.* **47**, 674-682.

[7] D. D. Deb, G. Parimala, S. S. Devi and T. Chakraborty (2011). Effect of thymol on peripheral blood mononuclear cell PBMC and acute promyelotic cancer cell line HL-60, *Chem. Biol. Interact*. **193**, 97-106.

[8] M. Llana-Ruiz-Cabello, D. Gutiérrez-Praena, S. Pichardo, F. J. Moreno, J. M. Bermúdez, S. Aucejo and A. M. Cameán (2014). Cytotoxicity and morphological effects induced by carvacrol and thymol on the human cell line Caco-2, *Food Chem. Toxicol*. **64**, 281-290.

[9] K. H. C. Baser (2002). The Turkish Origanum Species, in *Oregano, the Genera Origanum and Lippia*, In: Oregano: The genera Origanum and Lippia, *ed*: Spiridon E. Kintzios, Taylor and Francis, London UK, pp. 109-117.

[10] J. Z. Al-Kalaldeh, R. Abu-Dahab and F. U. Afifi (2010). Volatile oil composition and antiproliferative activity of *Laurus nobilis, Origanum syriacum, Origanum vulgare*, and *Salvia triloba* against human breast adenocarcinoma cells, *Nutr. Res. Rev*. **30**, 271-278.

[11] I. Khan, A. Bahuguna, P. Kumar, V. K. Bajpai and S. C. Kang (2018). In vitro and in vivo antitumor potential of carvacrol nanoemulsion against human lung adenocarcinoma A549 cells via mitochondrial mediated apoptosis, *Sci. Rep.* **8**.

[12] F. Bouhtit, M. Najar, D. M. Agha, R. Melki, M. Najimi, K. Sadki, P. Lewalle, A. Hamal, L. Lagneaux and M. Merimi (2019). The biological response of mesenchymal stromal cells to thymol and carvacrol in comparison to their essential oil: An innovative new study, *Food Chem. Toxicol.* **134,** 110844.