Journal of Herbs, Spices & Medicinal Plants

ISSN: 1049-6475 (Print) 1540-3580 (Online) Journal homepage: www.tandfonline.com/journals/whsm20

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To cite this article: Sara S. Aldisi, Morana Jaganjac, Ali H. Eid & Ipek Goktepe (2019) Evaluation of Apoptotic, Antiproliferative, and Antimigratory Activity of *Origanum syriacum* against Metastatic Colon Cancer Cells, Journal of Herbs, Spices & Medicinal Plants, 25:3, 202-217, DOI: 10.1080/10496475.2019.1587674

To link to this article: https://doi.org/10.1080/10496475.2019.1587674

Published online: 08 Mar 2019.

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JOURNAL OF HERBS, SPICES & MEDICINAL PLANTS
2019, VOL. 25, NO. 3, 202–217
https://doi.org/10.1080/10496475.2019.1587674

Evaluation of Apoptotic, Antiproliferative, and Antimigratory Activity of *Origanum syriacum* against Metastatic Colon Cancer Cells

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ABSTRACT
The effect of an ethanolic extract of fresh leaves of *Origanum syriacum* (OSEE) on the proliferative, apoptotic, and migratory capacities of LoVo and SW620 colon cancer cells was investigated. OSEE showed a concentration-dependent apoptotic and antiproliferative effect on LoVo and SW620 cells at 500 µg mL^{-1} or higher after 48 h of exposure. Additionally, OSEE inhibited the migration of both LoVo and SW620 cells by decreasing (50–75%) wound-healing capacity at 500 and 1,000 µg mL^{-1}. The results suggest that OSEE may have potential anticarcinogenic activity in metastatic colon cancer cells through the dual effect of inhibiting cell proliferation and migration and induction of apoptosis.

ARTICLE HISTORY
Received 6 June 2018

KEYWORDS
Lovo and SW620 cells; ethanol extract; herbal medicine; Mediterranean

Introduction

Cancer is among the leading causes of morbidity and mortality worldwide. Around 13% of annual death is attributed to cancer globally (1), and this number is expected to increase by 70% in 2030 (2). Colorectal cancer is the third most common cancer worldwide, causing the fourth highest mortality globally (2).

While there are no specific causes of cancer, several risk factors are implicated in its onset. Among these risk factors, obesity, diet, low physical activity, smoking, and alcohol consumption are the top five major factors suspected of causing around 30% of cancer-related deaths (2). Despite having over 100 chemotherapy drugs, treatment failure is not uncommon, mainly due to the development of chemoresistence (3). Although improvement in cancer therapy is showing promising results in early stages, treating metastatic cancer still remains a mystery (4). There is a growing interest in seeking complementary and alternative approaches to develop novel and personalized cancer treatment derived from natural products (3).

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More than 80% of drugs were developed from natural compounds (5), highlighting the importance of continuing the search on identification of novel bioactive compounds from natural sources, such as herbs and spices which have GRASS status. Several chemotherapeutic drugs used against colon cancer, such as Taxol, Paclitaxel, and Vinblastine were derived from plants (5). More than one third of European cancer patients use some sort of alternative medicine, with herbal medicine being the most common (6). Individuals who were already using herbal medicine increased their use by three times after being diagnosed with cancer (6). This is both important and interesting as nutrition and diet are emphasized in minimizing cancer risk and prevention (7). Several recent studies established a correlation between the increased risk of breast and colon cancer development and the consumption of red meat, processed meat, and dairy (8–10) while foods rich in antioxidants have been effective in cancer prevention (10). The Mediterranean diet has been associated with decreased risk of cancer death, especially with the lower risk of colon cancer (11). This diet is characterized by high intake of olive oil, fish, fruits, herbs, and vegetables, with low meat and dairy products consumption (12). One of the components of the Mediterranean diet is *Origanum syriacum*, which has not been fully studied for its potential health benefits.

Origanum syriacum (Lamiaceae), or Syrian oregano (Za'atar) native to the Mediterranean region, is characterized by its aromatic volatile components and used for culinary purposes (13,14). Essential oil of Syrian oregano contains a variety of terpenoids, monoterpenes (15), and several compounds including carvacrol and thymol that were reported for anticancer (13,16–20), anti-inflammatory (21), cholesterol lowering (22), and antioxidant properties (16,23). In addition, *O. syriacum* volatile oil inhibited the proliferation of human breast adenocarcinoma cells *in vitro* (13). Pharmacological studies of ethanol extract derived from the aerial part of the plant showed strong anti-proliferative activities against human leukemia (THP-1) cells at a concentration of 2.126 mg mL^{-1} (24). Thymol exhibited its effect on bladder cancer cells by arresting G2/M phase of the cell cycle and inhibition of JNK and p38-dependent pathways (25). Carvacrol was also reported to induce mitochondrial-mediated apoptosis and ERK-MEK inhibition in human choriocarcinoma and human cervical cancer cells, respectively (21). This study was conducted to determine the effects of an ethanolic extracts of *O. syriacum* on the *in vitro* proliferative and migratory capacities of LoVo and SW620 metastatic colon cancer cells.

Materials and Methods

Extraction of Origanum syriacum

Fresh leaves of *O. syriacum* were purchased from Lebanon in June 2011 and brought to Qatar University. The species was identified at the Lebanese

International University, Beirut, Lebanon and the dried plant and its leaves were donated to the herbarium located at Qatar University. The mature leaves were rinsed to remove any dust or dirt and air-dried in the dark at room temperature. Dried leaves were ground into powder, 15 g of which was then suspended in 1 L 70% ethanol (99.8% purity, VWR International, Radnor, PN), and the mixture was kept in the dark for 72 h at 4°C with continuous shaking. Later, the mixture was filtered through a glass-sintered funnel and the filtrate was evaporated to dryness using a Rota-vapor (Heidloph, Schwabach, Germany) at 60°C and at 170 mbar. The extraction yield was calculated to be 13%. The crude ethanolic extract of *O. syriacum* (OSEE) was frozen at −20°C until further use. A stock of 400 mg mL^{-1} of OSEE was prepared by dissolving the powder in 100% Dimethyl sulfoxide (DMSO, 99.9% purity, Sigma-Aldrich, St. Louis, MO) and was stored at −20° C until further use. For each experiment, OSEE was diluted in media used to culture cancer cells and sterilized using 0.2 μm filter.

Cell Cultures

Two human colorectal adenocarcinoma cell lines derived from metastatic site, *LoVo* and *SW620*, were obtained from CLS Cell Lines Service GmbH (Eppelheim, Germany) and cultured in DMEM/F12 (Thermo Fisher Scientific, Waltham, MA) medium supplemented with L-Glutamine (Sigma-Aldrich, St. Louis, MO), penicillin/streptomycin (Sigma-Aldrich, St. Louis, MO), and 10% fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO). Cells were maintained at 37°C in a humidified incubator with an atmosphere of 5% CO_2.

Cell Proliferation Assay

The antiproliferative activity of OSEE was tested against *LoVo* and *SW620* cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (26). Briefly, cells were seeded at 1×10^4 cells/well density in a 96 well-microtiter plate, and then incubated at 37°C with 5% CO_2. Different concentrations of OSEE extract (0, 250, 500, 750, and 1,000 μg mL^{-1}) prepared by diluting the OSEE stock (400 mg mL^{-1}) solution in DMEM (the highest concentration contained 0.25% DMSO, which was used as a negative control) were added to the wells containing attached *SW620* and *Lovo* cells. Cyclophosphamide (CP, 98% purity, Sigma-Aldrich, St. Louis, MO), a known chemotherapeutic drug (9,26) at 500 μg mL^{-1} was used as a positive control.

After incubating cells for 24, 48, and 72 h at 37°C, the media was removed and cells were incubated with 40 μL of 0.5 mg mL^{-1} Methylthiazolyldiphenyl-tetrazolium bromide (MTT, 97.5% purity, Sigma-Aldrich, St. Louis, MO).

Then, 150 μL of 100% DMSO was added into each well and the plates were shaken for 5 min before reading the absorbance at 570nm using TECAN M200 Pro (Tecan Group Ltd., Austria) microplate reader to determine the antiproliferative effect of OSEE against *Lovo* and *SW620* cells. Each experiment was repeated three times with five technical replicates.

Apoptosis Assay

In order to determine the ability of OSEE to induce apoptosis in *LoVo* and *SW620* colon cancer cells, FITC Annexin V PI assay (BD Biosciences, Franklin Lakes, NJ) was performed according to manufacturer's instructions. Briefly, *LoVo* and *SW620* cells were treated with various concentrations of OSEE as described above in 24-well plates at density of 6×10^5 cells/well. For gating, cells were treated overnight with 5 μM camptothecin (BD Biosciences, Franklin Lakes, NJ, United States). After 48 h, cells were trypsinized and centrifuged, washed twice with phosphate buffer solution (PBS), resuspended in binding buffer, and incubated with Annexin V conjugate and propidium iodide (PI, BD Biosciences, Franklin Lakes, NJ, United States) for 15 min at room temperature. The apoptotic effect of OSEE in *LoVo* and *SW620* cells was analyzed using a BD FACS Aria III flow cytometer (BD Biosciences, Franklin Lakes, New Jersey, United States) at 494/518 nm excitation/emission mode. Cell populations were determined through gating, and percentages of cells at each population was generated by BD FACS Diva software (BD Biosciences, Franklin Lakes, New Jersey, United States).

Migration (Scratch-Wound) Assay

Wound healing assay was performed to investigate the effect of OSEE on migration of *LoVo* and *SW620* cells. The confluent monolayers of cells growing in 24-well plates were scratched using a yellow tip (20–200 μL tip) as described by El Hasassna et al. (27). Following the removal of non-adherent cells by washing with PBS, cells were treated with various concentrations of OSEE or negative control (0.25% DMSO) for 48 h at 37°C. Photomicrographs were taken using Olympus IX53 inverted microscope (OLYMPUS Co., Tokyo, Japan) at 0 and 24 h. The width of the scratch, indicative of cell migration, was measured using Olympus cell Sens software (OLYMPUS Co, Tokyo, Japan).

Data Analysis

All results were presented as the mean ± standard error of the mean (SEM) of three independent experiments conducted in five replicates (n = 15). Data were analyzed using GraphPad Prism version 7.0 (GraphPad software, Inc.

San Diego, CA) by applying one-way ANOVA to determine the effect of different concentrations of OSEE on select cells ($P \leq 0.05$). The Tukey post ANOVA test was used to compare the results of cell migration and apoptosis ($P \leq 0.05$). The inhibitory concentrations (IC_{50}) of OSEE against the metastatic cancer cells were calculated by using GraphPad Prism version 7.0.

Results

Cell Proliferation Assay

The antiproliferative activity of OSEE was tested against *LoVo* and *SW620* cells at concentrations of 0, 250, 500, 750, and 1,000 μg mL^{-1}, with 0.25% DMSO serving as a negative control and 500 μg mL^{-1} CP as a positive control. OSEE exhibited inhibitory effect at a concentration of or higher than 500 μg mL^{-1} after 48 h in both cancer cells. The percent inhibitory effect of OSEE at 500, 750, and 1,000 μg mL^{-1} against *LoVo* cells after 72 h reached rates of 52.05 ± 3.32%, 59.91 ± 7.63%, and 67.25 ± 6.12, respectively (Fig. 1). The IC_{50} values for *LoVo* cells were determined to be 901.2 and 457.5 μg mL^{-1} at 48 and 72 h, respectively, supporting the time-dependent effect of OSEE (Fig. 1). OSEE's effect against *LoVo* cells at 500 μg mL^{-1} was comparable to that of the positive control (CP) at 500 μg mL^{-1} after 24 and 48 h exposure times, while higher concentrations and longer exposure time produced more potent effect than that of CP (Fig. 1). These results demonstrated that OSEE reduced the cell proliferation in a dose- and time-dependent manner compared to a standard chemotherapy drug (CP) tested at 500 μg mL^{-1}.

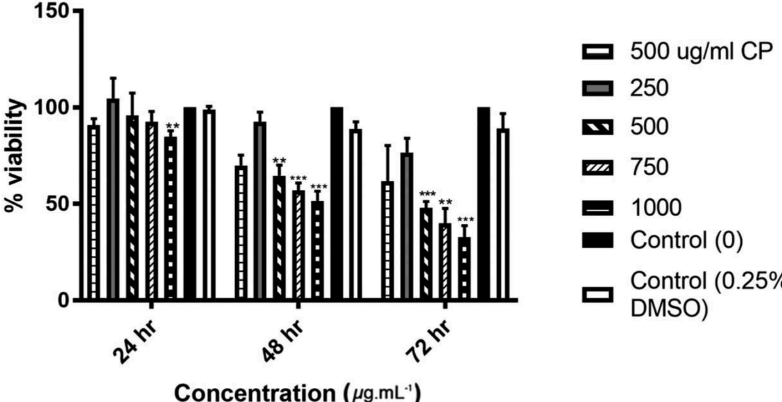

Figure 1. Effect of ethanol extracts of *Origanum syriacum* leaves on proliferation of LoVo cells after 24, 48, and 72 h of treatment. DMEM/F:12 and 0.25% DMSO were used as negative controls, and cyclophosphamide (CP) was used as a positive control. Values are mean (±SEM) for 3 trials. Asterisks (*) indicates difference compared to negative control (0) at: **(p < 0.01), *** (p < 0.001).

After 72 h of incubation, the OSEE at concentrations of 750 and 1,000 μg mL^{-1} inhibited the proliferation of *SW620* cells by 60.45 ± 8.99% and 66.07 ± 10.23% when compared to the results of negative control (0.25% DMSO), respectively (Fig. 2). The inhibitory capacity of OSEE against *SW620* cells was calculated at IC_{50} levels of 1,168 and 480.3 μg mL^{-1} after 48 and 72 h, respectively. Additionally, the antiproliferative activity of OSEE was more pronounced on *SW620* cells (44.18 ± 8.40%) at 1,000 μg mL^{-1}, compared to negative control after only 48 h of incubation. The positive control (CP) had lower antiproliferative effect on SW620 cells, having comparable activity to negative control (Fig. 2).

Apoptosis Assay

The flow cytometry studies showed that OSEE induced apoptosis in *LoVo* cells rather than necrosis (Fig. 3). As the concentration of OSEE doubled, the percentage of early apoptotic *LoVo* cells increased from 6.45% to 8.27% after 48 h (Fig. 3). At both concentrations of (500 and 1,000 μg mL^{-1}) OSEE, cells undergoing late apoptosis or necrosis was observed. As the concentration increased, number of late apoptotic/necrotic *LoVo* cells increased dramatically from 8.15% to 41.5% of the total population. Similarly, OSEE caused a concentration-dependent increase in the percentage of early apoptotic cells and late apoptotic/necrotic cells of *SW620* from 1.5% to 3.93% and 6.78% to 41.5%,

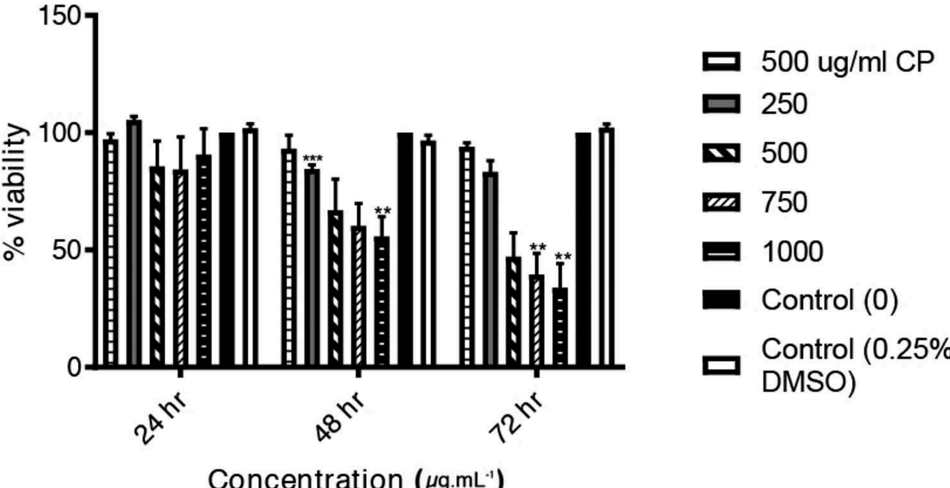

Figure 2. Effect of ethanol extract of *Origanum syriacum* leaves on proliferation SW620 cells after 24, 48, and 72 h of treatment. DMEM/F:12 and 0.25% DMSO were used as negative controls, and cyclophosphamide (CP) was used as a positive control. Values are mean (±SEM) for 3 trials. Asterisks (*) indicates difference compared to negative control (0) at: *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$).

Figure 3. Effect of ethanol extract of *Origanum syriacum* leaves on the rate of apoptosis in LoVo cells after 48 h of incubation. Percentages of cells in early apoptosis at each concentration were determined. 0.25% DMSO was used as a negative control. (a) Populations of cells after staining: Q1: PI-positive, Q2: FITC+PI-positive, Q3: unstained, Q4: FITC-positive. (b) Percentage of cells in FITC+ (early apoptotic) and FITC/PI+ (late apoptotic/necrotic) populations. Values are mean (±SEM) of 2 trials, Asterisks (***) indicates difference at $p < 0.001$.

respectively (Fig. 4). These results demonstrate that ethanolic extract of *O. syriacum* was able to induce apoptosis in both cancer cell lines by modifying cell cycle.

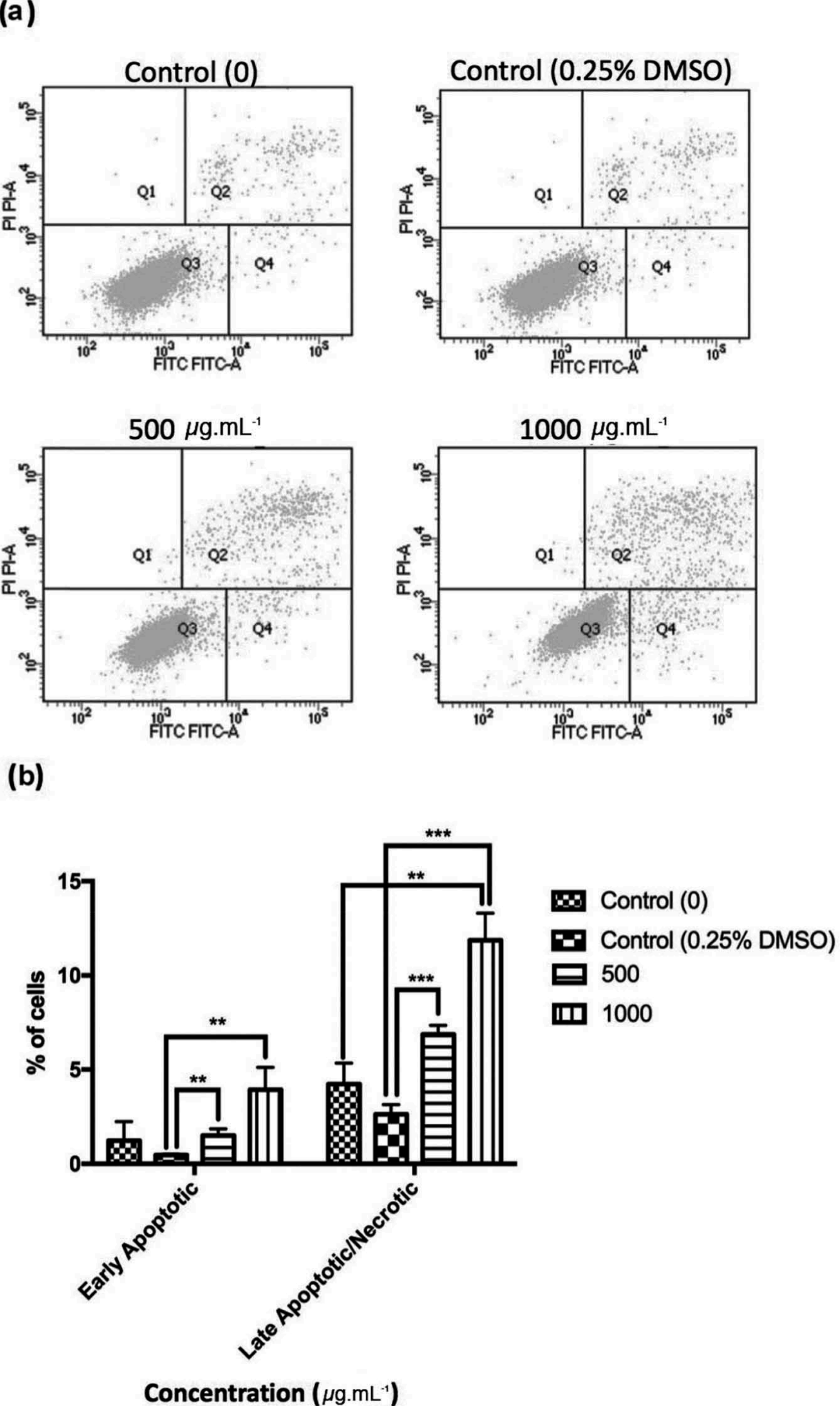

Figure 4. Effect of ethanol extract of *Origanum syriacum* leaves on the rate of apoptosis in SW620 cells after 48 h of incubation. Percentages of cells in early apoptosis and late apoptosis/necrosis were determined. 0.25% DMSO was used as negative control. (a) Populations of cells after staining: Q1: PI-positive, Q2: FITC+PI-positive, Q3: unstained, Q4: FITC-positive. (b) Percentage of cells in FITC+ (early apoptotic) and FITC/PI+ (late apoptotic/necrotic) populations. Values are mean (±SEM) of 3 trials. Asterisks (*) indicates difference compared to control (0) at: **($p < 0.01$), ***($p < 0.001$).

Migration (Scratch-Wound) Assay

At both OSEE concentrations (500 and 1,000 µg mL^{-1}) used in this assay, the migration of *LoVo* and *SW620* cells was inhibited after 24 h of exposure (Figs. 5 and 6). The migration of *LoVo* cells decreased compared to control by 47.6 ± 7.13% and 68.1 ± 15.31% at 500 and 1,000 µg mL^{-1}, respectively (Fig. 5a). Whereas, the anti-migratory effect of OSEE against *SW620* cells at 1,000 µg mL^{-1} was reduced to rates of 18.39 ± 1.81% (Fig. 6a). These results indicate that OSEE potently inhibits the migratory capacity of both *LoVo* and *SW620* cells compared to that of negative control (0.25% DMSO).

Discussion

O. syriacum has been consumed widely as a spice mix (Zaa'tar) and tea beverage in the Mediterranean region (13). Its health promoting properties have been identified as anticarcinogenic (13,16–20), anti-inflammatory (21), anti-cholesterol (22), and antioxidative (16,23). Cancer onset and progression is a multi-step process that involves uncontrollable cell division and metastasis. The process is achieved by inhibiting cell-cycle control and apoptosis, overexpression of certain pathways, and downregulation of cell adhesion molecules (26,28). Targeting these processes using extracts derived from herbs and plants or components of those has already been investigated in previous studies (13,15,29).

A key player in cancer onset is cells' ability to divide uncontrollably. Cancer cells inhibit cell cycle control by suppressing regulatory genes, such as *Rb and TP53* (30). Cancer cells overexpress proteins involved in proliferation pathways, such as ERK1/2 or JNK (25). Not only do cancer cells increase their proliferative activity, they also limit apoptosis by overexpressing anti-apoptotic proteins, such as Bcl-2 (30). Interestingly, several proteins are involved in regulating both proliferation and apoptosis. For example, p53 is involved in apoptosis and proliferation and its dysfunction is recognized as a marker of many diseases including cancer (31). Targeting both mechanisms in cancer cells is an important therapeutic approach. It is well known that chemotherapy drugs target genes/pathways to inhibit cancer.

The results of the MTT assays showed that treatment with the ethanol extract derived from *O. syriacum* caused a dose- and time-dependent reduction in the growth and proliferation of *LoVo* and *SW620* cancer cells. OSEE exhibited potent inhibitory effect at 500 µg mL^{-1} or higher after 48 h of exposure in both cancer cells. When the antiproliferative activity of the crude ethanol extract of *O. syriacum* was compared to a known drug (CP), the extract showed a more pronounced antiproliferative effect on *LoVo* cells than that of CP which is a commonly used drug to treat various cancers (1,23,26). The antiproliferative effect of CP was not strongly observed against *SW-620*

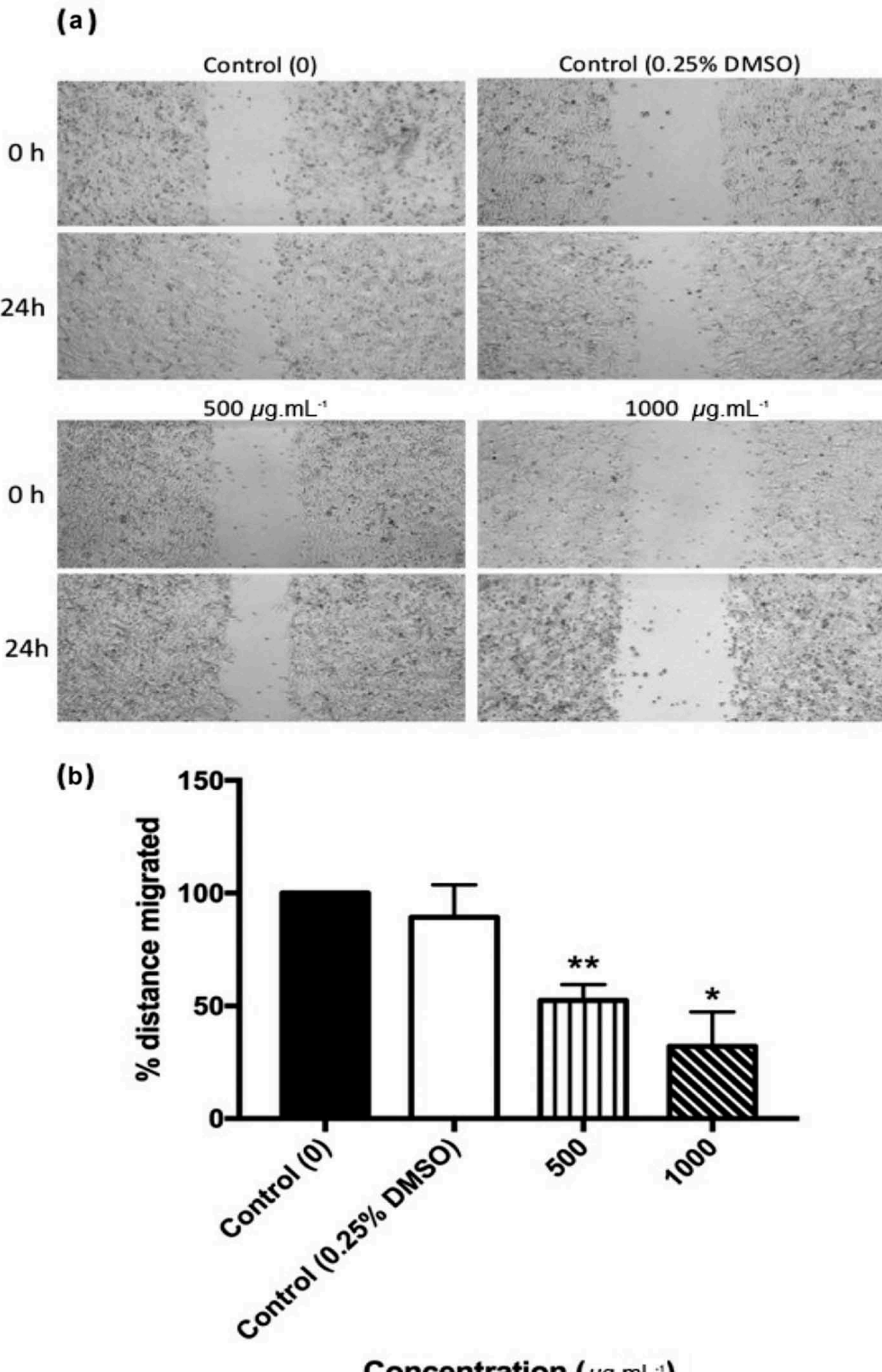

Figure 5. The anti-migratory effect of ethanol extract of *Origanum syriacum* leaves against *LoVo* cells after 24 h of incubation. (a) Wound healing in LoVo cells was significantly decreased as OSEE concentration increased, magnification 40×, (b) Quantification of anti-migration effect of OSEE. Distance migrated was calculated relative to control (0). DMEM/F:12 and 0.25% DMSO were used as negative controls. Values are means (±SEM) of 3 trials. Asterisks (*) indicate differences compared to control (0) at: *($p < 0.05$), **($p < 0.01$).

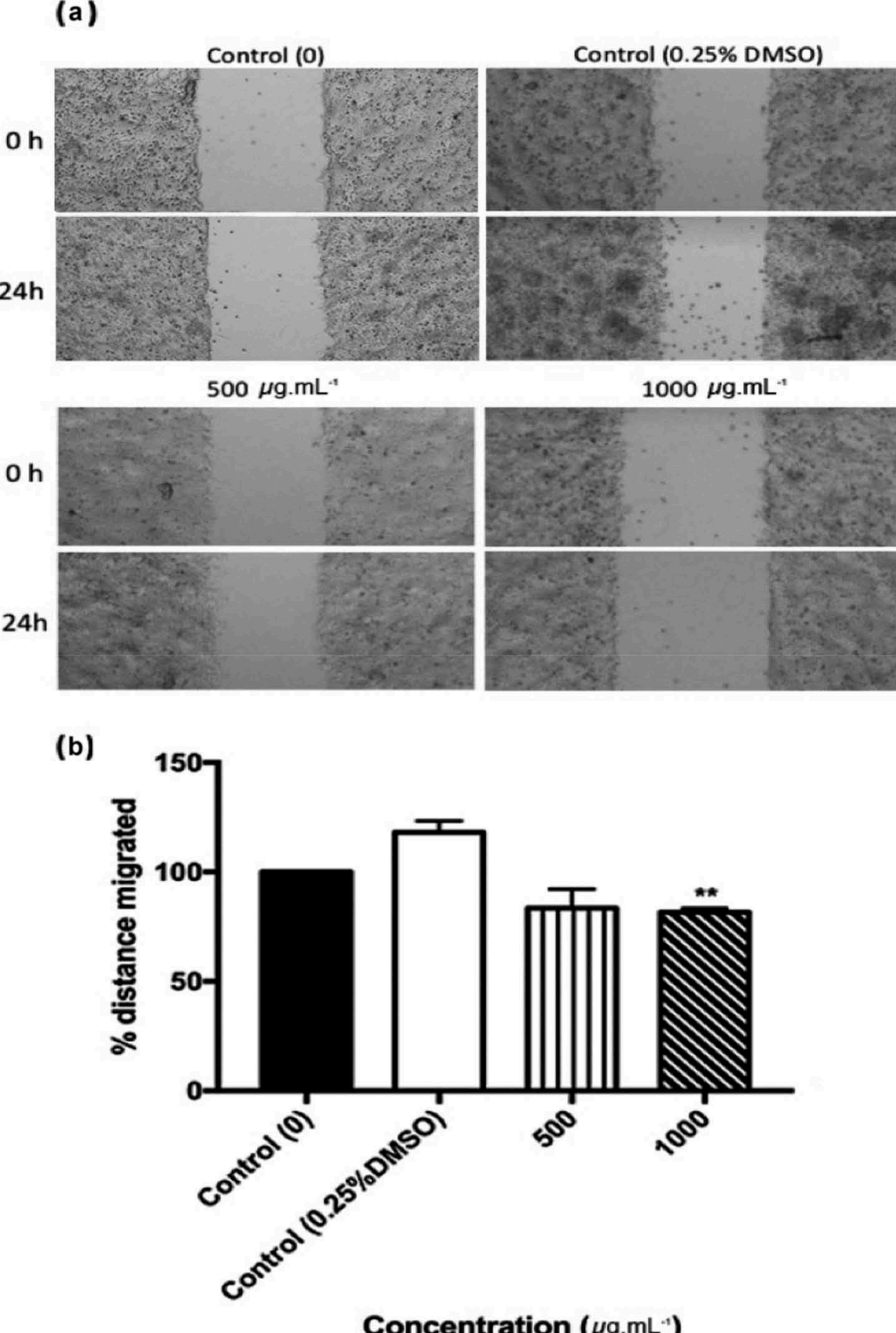

Figure 6. The anti-migratory effect of ethanol extract of *Origanum syriacum* leaves against *SW620* cells after 24 h of incubation. (a) Wound healing in SW620 cells was significantly decreased at high OSEE concentration, magnification 40×, and (b) Quantification of anti-migration effect of OSEE. Distance migrated was calculated relative to control (0). DMEM/F:12 and 0.25% DMSO were used as negative controls. Values are means (±SEM) of 2 trials. Asterisks (**) indicate significant differences compared to control (0) at $p < 0.01$.

cells (Fig. 2), which may because they are highly aggressive cells (32). The overall inhibition of colon cancer cells' proliferation after OSEE treatment might probably be attributed to "shutting down" of different cancer survival pathways including the ERK/MAPK and PI3K/Akt pathways. The hypothesis on the health-promoting potential of *O. syriacum* has been confirmed in the past. For example, carvacrol, a phenolic compound isolated from *O. syriacum*, inhibited the proliferation of prostate cancer cells through downregulating ERK1/2 expression and mediating the PI3K/Akt pathway (19). The same effect was also shown against HCT-116 and *LoVo* colon cancer cells, where carvacrol decreased expression of pAkt (17). Another phenolic compound commonly found in *O. syriacum*, thymol, inhibited the proliferation of bladder and colon cancer cells *in vitro* (16,18). The major mechanistic activity of thymol's effect was determined as the inhibition of JNK and p38 as the main mediators (16,18). The results of this study suggest that the anti-proliferative effect of ethanolic extract derived from fresh leaves of *O. syriacum* against *LoVo* and *SW620* colon cancer cells may be mainly due to growth inhibitory effects.

Apoptosis is an important mechanism that plays a key role in the suppression of tumor cells and elimination of unusual cells (33). When cells go through apoptosis, they exhibit certain morphological changes, such as membrane blebbing, cell shrinkage, formation of apoptotic bodies, chromatin cleavage, and nuclear condensation (34). In the current study, flow cytometry studies showed that OSEE induced apoptosis in *LoVo* cells rather than necrosis. After 48 h of incubation, number of early apoptotic *LoVo* and *SW620* cells increased with the increasing OSEE concentration. However, late apoptotic or necrotic cells also represented a higher percentage of the population compared to early apoptotic cells at both OSEE concentrations of 500 and 1,000 μg mL^{-1}. Notably, a much more dramatic increase was observed in late apoptotic/necrotic cells in both *LoVo* and *SW620* cells' population. These results may indicate OSEE's apoptosis inducing potential in both *LoVo* and *SW620* cells, or that OSEE produced its toxicity *via* necrosis or non-apoptosis related pathway. It has been reported that thymol induced apoptosis in different cancer cells. For example, thymol activated caspases-3/9 and decreased expression of anti-apoptotic protein, Bcl-2, in bladder cancer cells (18). Its caspase-3 modulating effect was also observed in HCT-116 colon cancer cells (16). Additionally, thymol inhibited cytochrome C in both bladder and colon cancer cells (16,18). This may provide a clue on possible pathways by which OSEE exhibited its pro-apoptotic effect on *SW620* and *LoVo* cells. Overall, these results suggest that OSEE may have potential anti-tumor activity.

An important factor in cancer progression is metastasis. Cancer cells disintegrate from surrounding tissues to enter the blood stream and extravagate elsewhere in the body, forming a secondary tumor (30). Cancer cells

downregulate cell-adhesion molecules such as E-cadherin (22), and overexpress matrix proteases such as MMP-9 (30). As expected, not only did OSEE showed anti-proliferative and pro-apoptotic activity, but it also demonstrated a strong anti-migratory effect on *LoVo* and *SW-620* cells. This effect may be the result of several pathways, one of which might be the ERK/MAPK pathway, a key pathway involved in both cell proliferation and migration, which has been reported to be the mediator of anti-proliferative and anti-invasive activity of previously studied herbs (25,31,35). For instance, an ERK-dependent pathway was found to be the main route of cell proliferation in mouse embryonic fibroblasts, even after p53 knockdown (35). Carvacrol exhibited antimigratory effect against prostate and colon cancer cells through activation of ERK/MAPK pathway (17,20). It also exerted an effect in downregulating the expression of MMP-2 in prostate cancer cells, as well as MMP-2 and MMP-9 in both HCT-116 and *LoVo* colon cancer cells (17,20). The results of this study confirmed that *O. syriacum* possess antiproliferative, pre-apoptotic, and antimigratory effects against metastatic cancer cells. However, a few limitations of this study include the inability in identifying the bioactive chemicals and determining the molecular pathways which need be examined in greater detail.

Acknowledgments

The authors would like to thank the Toxicology Division staff at the Anti-Doping Lab Qatar for their constant support during all experimental analyses.

Conflict of Interest

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

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References

1. IARC, Cancer Fact Sheet. 2012. Retrieved from http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx.
2. WHO. 2015. Fact sheet N°297. Retrieved from http://www.who.int/mediacentre/factsheets/fs297/en/

3. Wang, Z., X. Liu, R. Ho, C. Lam, and M. Chow. 2016. Precision or personalized medicine for cancer ccemotherapy: is there a role for herbal medicine. *Mol.* 21 (7):889. doi:10.3390/molecules21070889
4. Potočnjak, I., I. Gobin, and R. Domitrović. 2018. Carvacrol induces cytotoxicity in human cervical cancer cells but causes cisplatin resistance: involvement of MEK-ERK activation. *Phytother. Res.* 23(6):1090–1097. doi:10.1002/ptr.6048
5. Molassiotis, A., P. Fernadez-Ortega, D. Pud, G. Ozden, J. A. Scott, V. Panteli, A. Margulies, et al. 2005. Use of complementary and alternative medicine in cancer patients: a European survey. *Ann. Oncol.* 16:655–663. doi:10.1093/annonc/mdi110
6. Medina-Echeverz, J., J. Fioravanti, M. Zabala, N. Ardaiz, J. Prieto, and P. Berraondo. 2007. Successful colon cancer eradication after chemoimmunotherapy is associated with profound phenotypic change of intratumoral myeloid cells. *J. Immunol.* 186:807–815. doi:10.4049/jimmunol.1001483
7. Carruba, G., L. Cocciadiferro, A. Di Cristina, O. M. Granata,C, Dolcemascolo, C. Dolcemascolo, I. Campisi, M. Zarcone, M. Cinquegrani, and A. Traina. 2016. Nutrition, aging and cancer: lessons from dietary intervention studies. *Immun. Ageing.* 13:13. doi:10.1186/s12979-016-0069-9
8. Anderson, N.J., N.D.M. Darwis, D.F. Mackay, C.A. Celis-Morales, D.M. Lyall, N. Sattar, J.M.R. Gill, and J.P. Pell. 2018. Red and processed meat consumption and breast cancer: UK biobank cohort syudy and meta analysis. *Eur. J. Cancer.* 90:73–82. doi:10.1016/j.ejca.2017.11.022
9. Gigic, B., H. Boeing, R. Toth, J. Bohm, N. Habermann, D. Scherer, P. Schrotz-King, et al. 2018. Associations between dietary patterns and longitudinal quality of life chnages in colorectal cancer patients: the colocare study. *Nutr. Cancer.* 70:51–60. doi:10.1080/01635581.2018.1397707
10. Kotecha, R., A. Takami, and J. L. Espinoza. 2016. Dietary phytochemicals and cancer chemoprevention: a review of the clinical evidence. *Oncotarget.* 7:52517–52529. doi:10.18632/oncotarget.v7i32
11. Castelló, A., E. Boldo, P. Amiano, G. Castaño-Vinyals, N. Aragonés, I. Gómez-Acebo, R. Peiró, et al. 2018. Mediterranean dietary pattern is associated with low risk of aggressive prostate cancer: MCC-Spain study. *J. Urol.* 199:430–437. doi:10.1016/j.juro.2017.08.087
12. Surh, Y. 2003. Cancer chemoprevention with dietary phytochemicals. *Nat. Rev. Cancer.* 3(10):768–780. doi:10.1038/nrc1189
13. Al-Kalaldeh, J. Z., 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.* 30:271–278. doi:10.1016/j.nutres.2010.04.001
14. Lim, W., J. Ham, F. W. Bazer, and G. Song. 2018. Carvacrol induces mitochondria-mediated apoptosis via disruption of calcium homeostasis in human choriocarcinoma cells. *J. Cell. Physiol.* doi:10.1002/jcp.27054
15. Bhalla, Y., V. Gupta, and V. Jaitak. 2013. Anticancer activity of essential oils: a review. *J. Sci. Food Agric.* 93:3643–3653. doi:10.1002/jsfa.6267
16. Chauhan, A. K., A. Bahuguna, S. Paul, and S. C. Kang. 2017. Thymol elicits HCT-116 colorectal carcinoma cell death through induction of oxidative stress. *Anticancer Agents. Med. Chem.* 17:4. http://www.eurekaselect.com/151118/article.
17. Fan, K, X. Li, Y. Cao, H. Qi, L. Li, Q. Zhang, and H. Sun. 2015. Carvacrol inhibits proliferation and induces apoptosis in human colon cancer cells. *Anticancer Drugs.* 26:813–823. doi:10.1097/CAD.0000000000000263

18. Li, G., Y. He, J. Yao, C. Huang, X. Song, Y. Deng, S. Xie, J. Ren, M. Jin, and H. Liu. 2016. Angelicin inhibits human lung carcinoma A549 cell growth and migration through regulating JNK and ERK pathways. *Oncol. Rep*. 36:3504–3512. doi:10.3892/or.2016.5166
19. Loizzoa, M. R., F. Menichinia, F. Confortia, R. Tundisa, M. Bonesia, A. M. Saab, G. A. Statti, et al. 2009. Chemical analysis, antioxidant, antiinflammatory and anticholinesterase activities of *Origanum ehrenbergii* boiss and *Origanum syriacum* L. essential oils. *Food Chem*. 117:174–180. doi:10.1016/j.foodchem.2009.03.095
20. Lukas, B., C. Schmiderer, C. Franz, and J. Novak. 2009. Composition of essential oil compounds from different syrian populations of *Origanum syriacum* L. (Lamiaceae). *J. Agric. Food. Chem.* 57:1362–1365. doi:10.1021/jf802963h
21. Li, Y., J. M. Wen, C. J. Du, S. M. Hu, J. X. Chen, S.-G. Zhang, N. Zhang, et al. 2017. Thymol inhibits bladder cancer cell proliferation via inducing cell cycle arrest and apoptosis. *Biochem. Biophys. Res. Commun.* 491:530–536. doi:10.1016/j.bbrc.2017.04.009
22. Schwingshack, L., and G. Hoffmann. 2016. Does a mediterranean-type diet reduce cancer risk? *Curr. Nutr. Rep*. 5:9–17. doi:10.1007/s13668-015-0141-7
23. Lee, Y. S., S. Kim, S. J. Song, H. K. Hong, Y. Lee, B. Y. Oh, W. Y. Lee, and Y. B. Cho. 2016. Crosstalk between CCL7 and CCR3 promotes metastasis of colon cancer cells via ERK-JNK signaling pathways. *Oncotarget*. 7:36842–36853. doi:10.18632/oncotarget.9209
24. Ayesh, B. M., A. A. Abed, and D. M. Faris. 2014. *In vitro* inhibition of human leukemia THP-1 cells by *Origanum syriacum* L. and *Thymus vulgaris* L. extracts. *BMC Res. Notes.* 7:612. doi:10.1186/1756-0500-7-612
25. Levy, M. Y., A. Sidana, W. H. Chowdhury, S. B. Solomon, C. G. Drake, R. Rodriguez, and E. J. Fuchs. 2009. Cyclophosphamide unmasks an antimetastatic effect of local tumor cryoablation. *J. Pharmacol. Exp. Ther.* 330:596–601. doi:10.1124/jpet.109.152603
26. Al-Menhali, A. S., S. A. Jameela, A. A. Latiff, M. A. Elrayess, S. AlSayrafi, and M. Jaganjac. 2017. *Cistanche tubulosa* induces reactive oxygen species-mediated apoptosis of primary and metastatic human colon cancer cells. *J. Applied Pharm. Sci.* 7:39–45.
27. El Hasasna, H., A. Saleh, H. Al Samri, K. Athamneh, S. Attoub, K. Arafat, N. Benhalilou, et al. 2016. Rhus coriaria suppresses angiogenesis, metastasis and tumor growth of breast cancer through inhibition of STAT3, NFκB and nitric oxide pathways. *Sci Rep*. 18(6):21144. doi:10.1038/srep21144
28. Luo, Y., J. W. Wu, M. H. Lu, Z. Shi, N. Na, and J. M. Di. 2016. Carvacrol alleviates prostate cancer cell proliferation, migration, and invasion through regulation of PI3K/Akt and MAPK signaling pathways. *Oxid. Med. Cell*. doi:10.1155/2016/1469693
29. Al Dhaheri, Y., S. Attoub, K. Arafat, S. AbuQamar, J. Viallet, A. Saleh, H. Al Agha, A. Eid, R. Iratni, and H. Wanjin. 2013. Anti-metastatic and anti-tumor growth effects of *origanum majorana* on highly metastatic human breast cancer Cells: inhibition of NFkappaB signaling and reduction of nitric oxide production. *PLoS ONE*. doi:10.1371/journal.pone.0068808
30. Hanahan, D., and R. A. Weinberg. 2011. Hallmarks of cancer: the next generation. *Cell*. 144:646–674. doi:10.1016/j.cell.2011.02.013
31. Amaral, J. D., J. M. Xavier, C. J. Steer, and C. M. Rodrigues. 2010. The role of p53 in apoptosis. *Discov. Med.* 9:145–152.
32. Gupta, B. K., D. M. Maher, M. C. Ebeling, V. Sundram, M. D. Koch, D. W. Lynch, T. Bohlmeyer, et al. 2012. Increased expression and aberrant localization of mucin 13 in metastatic colon cancer. *J. Histochem. Cytochem.* 60:822–831. doi:10.1369/0022155412460678
33. Sun, Q., M. Dong, Z. Wang, C. Wang, D. Sheng, Z. Li, D. Huang, and C. Yuan. 2016. Selenium-enriched polysaccharides from *Pyracantha fortuneana* (Se-PFPs) inhibit the

growth and invasive potential of ovarian cancer cells through inhibiting beta-catenin signaling. *Oncotarget*. 7:28369–28383. doi:10.18632/oncotarget.8619
34. Doonan, F., and T. G. Cotter. 2008. Morphological assessment of apoptosis. *Methods*. 44:200–204. doi:10.1016/j.ymeth.2007.11.006
35. Drosten, M., E. Y. Sum, C. G. Lechuga, L. Simón-Carrasco, and L. C. Jacob. 2014. Loss of p53 induces cell proliferation via Ras-independent activation of the Raf/Mek/Erk signaling pathway. *Pnas*. 111:15155–15160. doi:10.1073/pnas.1417549111