



Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Origanum majorana harvested from Al-Soda, Saudi Arabia promotes mitotic arrest and apoptosis in colon cancer cells



Essam H. Ibrahim^{a,b,c,*}, Mohammad Y. Alshahrani^d, Hamed A. Ghramh^{a,b,e}, Hani Alotheid^f, Mona Kilany^{b,g}, Kareem Morsy^{a,h}, Attalla F. El-kott^{a,i}, Ramadan Taha^{a,j}, Haitham I. El-Mekkawy^a, Gamal A. EL-Shaboury^a, Ahmed A. El-Mansi^{a,k}, Mohammed E.A. Mohammed^{b,e,l}, Mahmoud A. Sayed^{b,m,n}, Ibrahim S. Yahia^{b,m,o}

^a Biology Department, Faculty of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia

^b Research Center for Advanced Materials Science (RCAMS), King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia

^c Blood Products Quality Control and Research Department, National Organization for Research and Control of Biologicals, Cairo, Egypt

^d Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Khalid University, P.O. Box 61413, Abha 9088, Saudi Arabia

^e Unit of Bee Research and Honey Production, Faculty of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia

^f Department of Basic Sciences, Faculty of Applied Medical Sciences, Al-Baha University, Al-Baha, Saudi Arabia

^g Department of Microbiology, National Organization for Drug Control and Research (NODCAR), Cairo, Egypt

^h Department of Zoology, Faculty of Science, Cairo University, 12613 Cairo, Egypt

ⁱ Department of Zoology, Faculty of Science, Damanhour University, Damanhour, Egypt

^j Department of Clinical Pathology, Faculty of Veterinary Medicine, Suez Canal University, Egypt

^k Zoology Dept., Faculty of Science, Mansoura University, Mansoura, Egypt

^l School of Allied Health Science, Faculty of Health and Life Sciences, De Montfort University, The Gateway, Leicester LE1 9BH, United Kingdom

^m Physics Department, Faculty of Science, King Khalid University, P. O. Box 9004, Abha 61413, Abha, Saudi Arabia

ⁿ Physics Department, Faculty of Science, Al-Azhar University, P. O. 71452, Assiut, Egypt

^o Nanoscience Laboratory for Environmental and Bio-medical Applications (NLEBA), Semiconductor Lab.1, Metallurgical Lab.1, Department of Physics, Faculty of Education, Ain Shams University, Roxy, 11757 Cairo, Egypt

ARTICLE INFO

Article history:

Received 20 October 2021

Revised 10 January 2022

Accepted 26 January 2022

Available online 2 February 2022

Keywords:

Origanum majorana

Anticancer

p53

Cell cycle

ROS

ABSTRACT

Colorectal cancer is reported as the third major incentive of cancer doom. The present work is meant to examine the anticancer potential of *Origanum majorana* leaf acetone extract (OMAE) to fight HT-29 human colon cancer cells. Biomolecules in OMAE were examined using Fourier Transform Infrared (FT-IR) spectroscopy. Reactive oxygen species (ROS) in OMAE was determined using an immunosorbent assay. The cytotoxic effect of OMAE was tested by MTT assay. p53 gene expression level of OMAE-treated cells was measured using quantitative real-time PCR (qPCR). Apoptotic and cell cycle arrest effects of OMAE on HT-29 were analyzed by flow cytometry. Results revealed the presence of many functional groups and considerable amount of ROS in the extract. The extract could raise p53 expression level five folds over control. OMAE arrested the HT-29 at G2/M phase. OMAE has an apoptotic effect rather than necrotic effects. Our discoveries give solid proof that *O. majorana* acetone extract has a capacity to stop colon cancer activity, at least, through the enlistment of cell division arrest as well as apoptosis. These findings can suggest the use of OMAE as a natural therapeutic candidate against the colon cancer.

© 2022 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Corresponding author.

E-mail address: essamebrahim@hotmail.com (E.H. Ibrahim).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

<https://doi.org/10.1016/j.jksus.2022.101878>

1018-3647/© 2022 The Author(s). Published by Elsevier B.V. on behalf of King Saud University.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Approximately of 1.9 million cases are reported to have cancer, of them about one-third is accounted for colorectal cancer in both women and men (WHO, 2018, n.d.). Throughout history, plants have been used as a medicine. Now, it is a magnificent source for creating new medications, of them, anticancer drugs. Identification and advancement of unprecedented effective materials derived from plants to be used as cancer therapy have gained significant recognition and made scientists give more experimental work in

cancer research. A large number of anticancer drugs are derived from plant or artificially-modified plant derivatives and phytochemicals (Amin et al., 2009; Cragg and Pezzuto, 2016; Iqbal et al., 2017). Plant-derived anticancer medications were shown to be more efficient and less harmful compared to synthetic ones. In the last few years, some plant-derived anticancer drugs are under clinical trial evaluation such as the *Taxus brevifolia* Nutt. and *Camptotheca acuminata* (the DNA topoisomerase I inhibitor) (Desai et al., 2008; Thoppil and Bishayee, 2011; Nabih et al., 2012; Greenwell and Rahman, 2015).

The mechanisms by which phytochemicals exert their anticancer effects include interference of gene expression leading to cell proliferation inhibition, induction of apoptosis, genotoxicity inhibition, increasing the anti-inflammatory and antioxidants activity, and cellular signaling pathways modulation (Greenwell and Rahman, 2015).

It is notable that cancer is a disease situation and related to evidently directed apoptosis. Recent researches proved that some plant extracts and phytochemicals could modulate the course of carcinogenesis through several ways including apoptosis. Apoptosis was shown to be one of the effective mechanisms in chemoprevention and chemotherapy studies in human trials and in animal and *in vitro* carcinogenesis models (Kuno et al., 2012; Pan and Ho, 2008; Thoppil and Bishayee, 2011).

Origanum majorana L. (*O. majorana*), a member of the Lamiaceae (mint family) is local to southern Europe, North Africa and Asia Minor. This plant is called Wazab in Saudi Arabia and has many uses with several health benefits and is utilized as an in house solution for gastrointestinal disorders, cramps, headaches, gloom, migraine, as diuretic, coughs and dizziness (Ouedrhiri et al., 2016; Vági et al., 2005). *Origanum majorana* has been reported to have a potential antimicrobial activity (Qureshi et al., 1994). Many investigations revealed that *Origanum majorana* oil (essential) and extracts (ethanolic/aqueous) have liver and kidney protective capacity against damage and genotoxicity (Leeja and Thoppil, 2007). These extracts contained many metabolites such as flavonoids, phenolic terpenoids, phenolic glycosides and sitosterol (Assaf et al., 1987; Hajlaoui et al., 2016). The *O. majorana* essential oil has a hepatoprotective, antioxidant and antimutagenic capacity (Mossa and Nawwar, 2011). *O. majorana* has been shown to exert a low anticancer activity on many hepatoma cell lines (Lin et al., 2002). Also, *O. majorana* extracts minimized the side effects of using the anticancer drug, cyclophosphamide, without affecting its cytotoxic activity (Qureshi et al., 1994).

In the current work, we examined the impact of OMAE on colon cancer cell lines regarding cytotoxicity, apoptosis, cell cycle and the level of p53 protein.

2. Materials and methods

2.1. Plant collection

In April 2021, *O. majorana* leaves were gathered from the Al Soda, Aeer, Saudi Arabia and identified. The plant was identified by an expert taxonomist at the Biology Department, King Abdul-Aziz University, Saudi Arabia.

2.2. Extract preparation

The air-dried leaves of *O. majorana* (200 g) were turned into a coarse powder utilizing an electrical processor and then soaked in acetone for 24 h with consistent mixing at room temperature. The mixture was filtered twice via filter paper (Whatman No.1). About 1.8 g were obtained after drying the filtrate. A stock solution (1%) was prepared by dissolving the obtained solid material in an

appropriate amount of acetone (180 mL) (stock solution). The stock solution was sterilized (0.45 µm syringe filter, Millipore®) and kept at –40 °C.

2.3. Functional groups analysis

Active groups present in the prepared extract were discovered utilizing Fourier Transform Infrared (FT-IR) spectroscopy according to Ibrahim et al., (Ibrahim et al., 2021b).

2.4. Estimation of reactive oxygen species

Reactive oxygen species (ROS) was estimated utilizing the same immunosorbent assay kit, method and instrument as described by Ibrahim et al., (Ibrahim et al., 2021a).

2.5. Cell line maintenance

The human colon cancer HT-29 cell line (Merk) was seeded and maintained as described previously (Ghramh et al., 2020).

2.6. Study of extract cytotoxicity against HT-29 cancer cell line

The plant extract was added to HT-29 cancer cells and percent cell viability was assessed using MTT method to calculate the IC₅₀ (concentration that produces half-maximal inhibition) as described previously (Ibrahim et al., 2021a, 2019).

2.7. Quantification of p53 gene expression

Quantification of p53 gene expression in extract-treated HT-29 cancer cells at IC₅₀ concentration was done the same way as described by Ibrahim et al., (Ibrahim et al., 2021a).

2.8. Analysis of cell cycle using flow cytometer

The cell cycle of HT-29 cancer cells was tested utilizing the Propidium Iodide Flow Cytometry Kit (Abkam) for Cell Cycle Analysis. To each well of a 6-well tissue culture plate, HT-29 cancer cells (5X10⁵) were added and incubated for 24 h before the addition of the extract at IC₅₀ concentration for 48 h. All subsequent steps were done the same way as shown by Ibrahim et al., (Ibrahim et al., 2021a). Untreated cells were used as control cultures.

2.9. Apoptotic analysis with Annexin V staining

The cell cycle of HT-29 cancer cells was tested utilizing Annexin V-FITC Apoptosis Detection Kit (abkam, Cat#: ab14085) following the procedure shown by Ibrahim et al., (Ibrahim et al., 2021a).

3. Statistical Analysis

Acquired data were expressed as means 5 results ± SEM of the number of tests. A Student's *t*-test was operated utilizing GraphPad Prism (Version 7.0) for paired or unpaired and a statistically significant *p* value of 0.05 was considered.

4. Results

4.1. Functional groups

A powerful broad peak emerged at 3341.6 cm⁻¹ attributed to stretching O–H because of phenols and alcohols (Fig. 1). Arisen strong bands (2924.1 and 2853.3 cm⁻¹) are assigned to stretching C–H bond which are identical to alkane. Emerged weak band

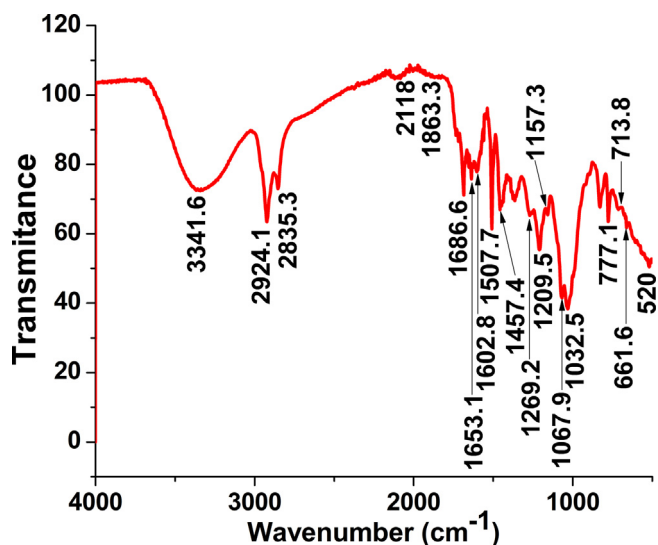


Fig. 1. FT-IR spectra of *O. majorana* acetone extract.

(2118 cm^{-1}) might be a result of stretching $\text{C}\equiv\text{C}$ bond of alkyne. An arisen weak band (1863.3 cm^{-1}) might be because of bending $\text{C}-\text{H}$ bond of aromatic formula. Generated weak bands (1686–1653.1–1602.8–1507.7–1457.4 cm^{-1}) because of disubstituted alkenes stretching $\text{C}=\text{C}$ bond. Other peaks (strong-weak) emanated (1269.2–1209.5–1157.3 cm^{-1}) ascribed to $\text{C}-\text{O}$ stretching resultant of alkyl aryl ether. Engendered strong broad peaks (1067.9–1032.5 cm^{-1}) because of $\text{C}-\text{O}$ assigned to primary alcohols. Arisen powerful peaks (777.1–713.8 cm^{-1}) may be the results of stretching $\text{C}-\text{H}$ bond of monosubstituted benzene. Powerful peaks emanated at 661.6–520 cm^{-1} may assign to stretching $\text{C}-\text{Br}$ halo formula.

4.2. Content of ROS in the plant extract

Results of quantifying the content of ROS in the *O. majorana* leaf acetone extract using ELISA revealed that the extract contains 164.2 $\mu\text{g}/\text{mL}$ of ROS.

4.3. Extract cytotoxicity against HT-29 cancer cell line

Origanum majorana leaf extract showed a significant ($p > 0.0001$) inhibitory effects on HT-29 cell growth at the concentration 1000 $\mu\text{g}/\text{mL}$ (11% versus 100% growth rate of untreated cells, Fig. 2) up to 62.5 $\mu\text{g}/\text{mL}$. These inhibitory effects continued nonsignificantly down to the concentration of 31.25 $\mu\text{g}/\text{mL}$. The concentrations 0, 0.49, 0.98, 1.95, 3.91, 7.81 and 15.63 $\mu\text{g}/\text{mL}$ did not show any cell growth inhibition effect on the cultured cells. IC_{50} was shown to be at the concentration of 90 $\mu\text{g}/\text{mL}$.

4.4. Quantification of p53 gene expression

We used real-time RT-PCR to quantitatively analyze the expression of p53 after treatment of HT-29 cells with IC_{50} dose of *O. majorana* acetone extract *in vitro*. Results demonstrated that p53 mRNA expression increased 5.44 folds over control cells (Fig. 3).

4.5. Cell cycle and DNA content

To investigate if the inhibitory effect of the extract on the cell line was due to cell cycle arrest induction, HT-29 cells were treated with *O. majorana* acetone extract at a concentration equal to IC_{50}

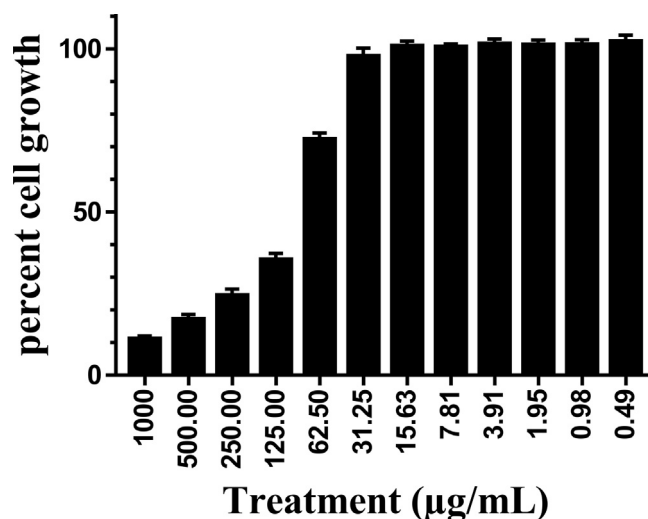


Fig. 2. Cytotoxic effects of *O. majorana* acetone extract on human colon cancer HT-29 cell line.

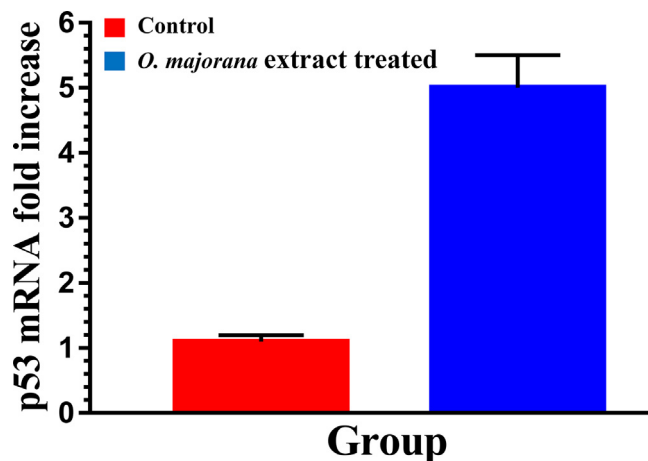


Fig. 3. Apoptotic effects of *O. majorana* acetone extract on human colon cancer HT-29 cell line.

for 48 h. Flow cytometry was used to test cell cycle progression. After the 48 h *O. majorana* acetone extract treatment, cell number in the G2/M population significantly ($p > 0.001$) increased from 18.36% (control untreated) to 32.8%. As the G2/M phase cell population increased, the G1 phase cell population decreased from 51.88% (control untreated) to 38.06% (extract treated) (Figs. 4 and 5).

4.6. Apoptotic effects of *O. majorana* acetone extract

The apoptotic effect of *O. majorana* acetone extract was evaluated with Annexin V staining (Figs. 6 and 7). The extract made the cells go through apoptosis, where apoptotic cell number significantly ($p < 0.05$) increased in treated cells (11.74%) over the untreated cells (0.73%). This increase in the percent of the apoptotic cells (treated) was nearly equal at the early (5.92%) and late (5.82%) stages. The decrease in percent cell growth is referred to apoptosis rather than necrosis as necrotic cells were shown to be at a normal level in treated cells (1.46%) which is nearly similar (1.19%) to untreated cells.

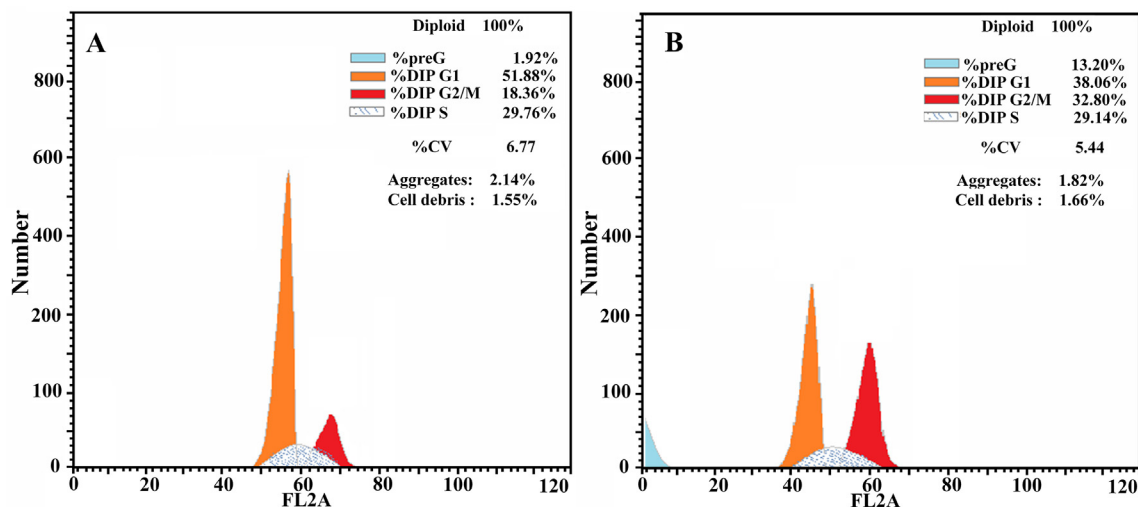


Fig. 4. Analysis of cell cycle progression. Human colon cancer HT-29 cells were untreated (A) or treated with *O. majorana* acetone extract. The distribution and percentage of cells in preG-phase, G1, S and G2/M phase of the cell cycle are indicated.

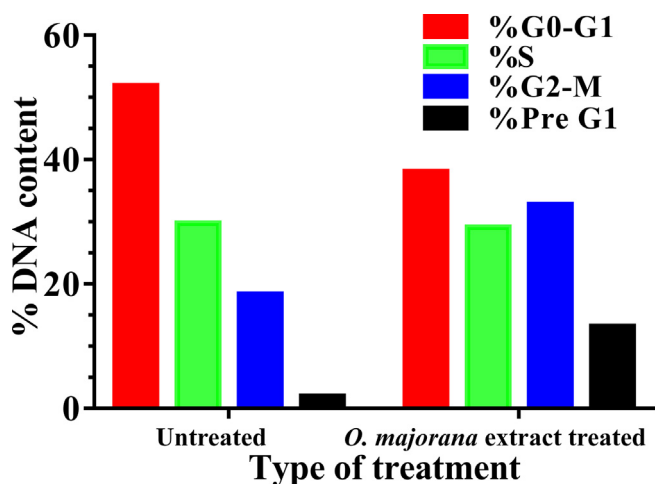


Fig. 5. % DNA content during cell cycle progression. Human colon cancer HT-29 cells were untreated or treated with *O. majorana* acetone extract. The distribution of % DNA content at early and late apoptosis as well as necrosis of the cells are indicated.

5. Discussion

To explore the anticancer capabilities of OMAE on HT-29 human colon cancer cells, we first tested the impact of different dilutions of OMAE on the proliferation of this cell line. Our outcomes indicated that the treatment of the HT-29 cells with OMAE lowered the viability of the HT-29 cells in a dose-dependent manner. The IC_{50} was around 90 μ M at 48 h.

FT-IR results in our study revealed the presence of phenolic groups in the OMAE. A previous study showed that *O. majorana* extract contains both phenolic and flavonoid components (M  abed et al., 2018). Among the phenolic compounds are the flavonoids, which have anticancer potentials by modulating the signal transduction pathways within cancer cells to inhibit cell division, metastasis and angiogenesis. Flavonoids can promote apoptosis (Ren et al., 2003).

Our results demonstrated the presence of ROS in the OMAE. In our work, OMAE was directly added to the HT-29 cells. Some workers used extracts of different sources and revealed that these extracts could trigger apoptosis in tested cells possibly through

enhanced Bax expression, and Bcl-2 protein inhibition. The increase in Bax/Bcl-2 ratio and the activation of both Caspase-3 and ROS are tangible fingers for such pathways (Murad et al., 2016; Sun et al., 2012). ROS is known to be involved in the stimulation of several cellular signaling pathways as well as transcription factors (e.g. phosphoinositide 3-kinase), mitogen-activated protein kinases, nuclear factor- κ B and p53), which can stimulate cell fate (survival or death) (Bae et al., 2011; Covarrubias et al., 2008; Kaminsky and Zhivotovsky, 2014; Zhang et al., 2016). ROS increase is usually observed in the apoptosis progress (Mat  s and S  nchez-Jim  nez, 2000; Simon et al., 2000), and its generation or upregulation is considered as a sign of apoptosis induction. Many of the anticancer drugs are natural compounds (e.g. curcumin, garlic, quercetin, cisplatin, etc.) they induce the apoptosis in cancer cells through rising the amount of ROS (Berndtsson et al., 2007; Dirsch et al., 1998; Mat  s and S  nchez-Jim  nez, 2000; Woo et al., 2003).

Mitotic arrest as well as apoptosis was noticed in HT-29 cells because of OMAE treatment. The capability of any anticancer agent to modulate cell cycle sequence is able to give clear data about its mechanism(s) of action to be cytotoxic. For this point of view, we test the impacts of OMAE on cell cycle apportionment utilizing flow cytometry. HT-29 cells were treated with the IC_{50} of OMAE for 48 h and its cell cycle was analyzed. This IC_{50} caused G2/M arrest on HT-29 cells. Actually, the population of G2/M has risen proving that OMAE could arrest HT-29 cells in the G2/M phase. This finding is in agreement with other workers and uses many different cell lines (Al Dhaheri et al., 2013b; Benhalilou et al., 2019).

Apoptosis has a valuable function in the demolition of the mutated and abnormal cells from the living organism as a protective mechanism to prevent the progression of cancer (Elmore, 2007; Igney and Krammer, 2002). The extract exerted apoptotic effects rather than cytotoxic effects on HT-29 cells as indicated in our results. The concentration of p53 increased more than five folds over the control. This finding is in agreement with other workers (Abdel-Massih et al., 2010). During the cell cycle, many checkpoints are being passed. These events are very important as control mechanisms. These include the G2/M checkpoint, which blocks the cycle to go through mitosis if the DNA is damaged (Taylor and Stark, 2001). The p53 protein regulates the G2/M transmission by controlling some regulatory protein complexes present in the cell (Bunz et al., 1998; Hermeking et al., 1997) or inducing the apoptosis process (Shaw et al., 1992; Yonish-Rouach et al.,

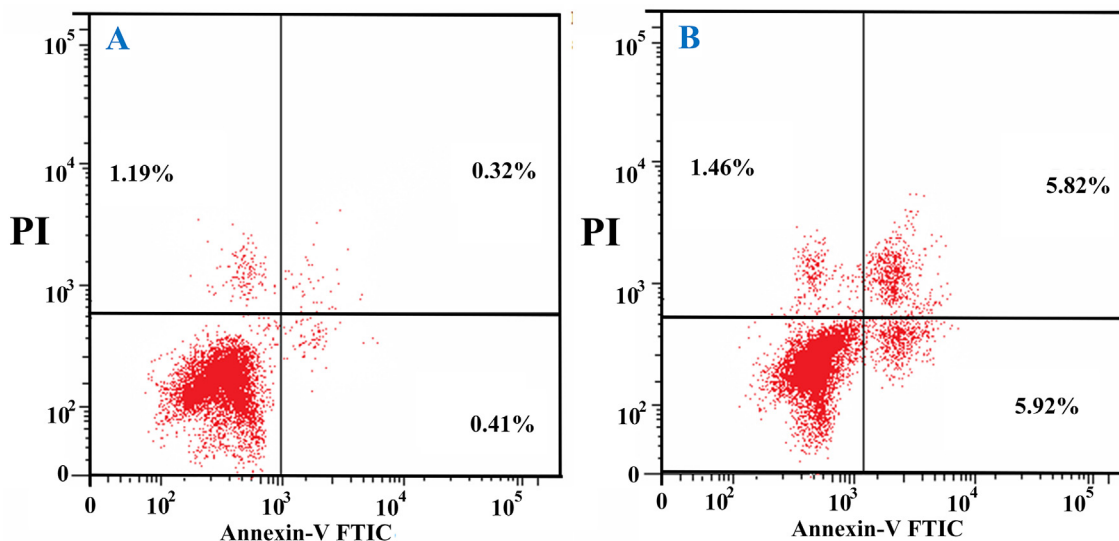


Fig. 6. Human colon cancer HT-29 cells stained with anti-Annexin V and propidium iodide showing the percentage of apoptotic cells. A: Untreated cells; B: *O. majorana* acetone extract treated cells.

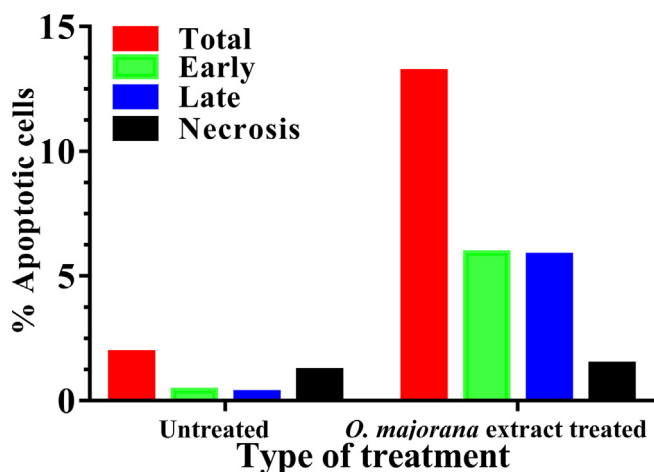


Fig. 7. % Human colon cancer HT-29 apoptotic cells after treated with *O. majorana* acetone extract.

1991). Normal p53 protein is essential in the p53-dependent pathway to control cell cycle arrest and apoptosis (Zhang et al., 2010).

It is settled that interference in contact among cancer cells in point of cancer progression is an essential stage required for the acquisition of invasive discriminatory and the decrease in that contact is always accompanied by a reduced expression of E-cadherin (Berx et al., 1995). E-cadherin expression can reduce cancer cells progression and invasion (Kowalski et al., 2003). Previous study (Al Dhaheri et al., 2013a) showed that *O. majorana* could induce overexpression of E-cadherin and homotypic cell–cell aggregation but inhibited cell migration in cancer cells. We presuppose that *O. majorana* in our study exerted an anti-migratory effect on HT-29 cells in the cell culture, to some extent, via the expression of E-cadherin gene reactivation. As a result, E-cadherin overexpression is the most possible mechanism that *O. majorana* can perform its anti-cancer effect on the HT-29 cells.

6. Conclusion

O. majorana acetone extract is a good anticancer as it could arrest tested cancer cell line at G2/M phase, induce cancer cell apoptosis and upregulate p53 expression.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors would like to acknowledge the support of the Research Center for Advanced Materials Science (RCAMS), King Khalid University, Abha Saudi Arabia for funding this work through research program RCAMS/KKU/G001/21.

Author's contributions

The authors contributed equally in creating this manuscript.

References

- Abdel-Massih, R.M., Fares, R., Bazzi, S., El-Chami, N., Baydoun, E., 2010. The apoptotic and anti-proliferative activity of Origanum majorana extracts on human leukemic cell line. *Leuk. Res.* 34 (8), 1052–1056. <https://doi.org/10.1016/j.leukres.2009.09.018>.
- Al Dhaheri, Y., Attoub, S., Arafat, K., AbuQamar, S., Viallet, J., Saleh, A., Al Agha, H., Eid, A., Iratni, R., Wanjin, H., 2013a. Anti-metastatic and anti-tumor growth effects of origanum majorana on highly metastatic human breast cancer cells: inhibition of NFκB signaling and reduction of nitric oxide production. *PLoS ONE* 8 (7), e68808. <https://doi.org/10.1371/journal.pone.0068808>.
- Al Dhaheri, Y., Eid, A., AbuQamar, S., Attoub, S., Khasawneh, M., Aiche, G., Hisaindee, S., Iratni, R., Rameshwar, P., 2013b. Mitotic arrest and apoptosis in breast cancer cells induced by Origanum majorana extract: upregulation of TNF-α and downregulation of survivin and mutant p53. *PLoS ONE* 8 (2), e56649. <https://doi.org/10.1371/journal.pone.0056649>.
- Amin, A., Gali-Muhtasib, H., Ocker, M., Schneider-Stock, R., 2009. Overview of major classes of plant-derived anticancer drugs. *Int. J. Biomed. Sci.* 5, 1–11.
- Assaf, M., Ali, A., Makboul, M., Beck, J., Anton, R., 1987. Preliminary study of phenolic glycosides from Origanum majorana; quantitative estimation of arbutin; cytotoxic activity of hydroquinone. *Planta Med.* 53 (04), 343–345. <https://doi.org/10.1055/s-2006-962734>.
- Bae, Y.S., Oh, H., Rhee, S.G., Yoo, Y.D., 2011. Regulation of reactive oxygen species generation in cell signaling. *Mol. Cells* 32 (6), 491–509. <https://doi.org/10.1007/s10059-011-0276-3>.
- Benhalilou, N., Alsamri, H., Alneyadi, A., Athamneh, K., Alrashedi, A., Altamimi, N., Dhaheri, Y.A., Eid, A.H., Iratni, R., 2019. Origanum majorana ethanolic extract promotes colorectal cancer cell death by triggering abortive autophagy and

- activation of the extrinsic apoptotic pathway. *Front. Oncol.* 9, 1–12. <https://doi.org/10.3389/fonc.2019.00795>.
- Berndtsson, M., Hägg, M., Panaretakis, T., Havelka, A.M., Shoshan, M.C., Linder, S., 2007. Acute apoptosis by cisplatin requires induction of reactive oxygen species but is not associated with damage to nuclear DNA. *Int. J. Cancer* 120 (1), 175–180. <https://doi.org/10.1002/ijc.22132>.
- Berx, G., Cleton-Jansen, A.M., Nollet, F., de Leeuw, W.J., van de Vijver, M., Cornelisse, C., van Roy, F., 1995. E-cadherin is a tumour/invasion suppressor gene mutated in human lobular breast cancers. *EMBO J.* 14 (24), 6107–6115. <https://doi.org/10.1002/j.1460-2075.1995.tb00301.x>.
- Bunz, F., Dutriaux, A., Lengauer, C., Waldman, T., Zhou, S., Brown, J.P., Sedivy, J.M., Kinzler, K.W., Vogelstein, B., 1998. Requirement for p53 and p21 to sustain G2 arrest after DNA damage. *Science* (80- 282 (5393), 1497–1501.
- Covarrubias, L., Hernández-García, D., Schnabel, D., Salas-Vidal, E., Castro-Obregón, S., 2008. Function of reactive oxygen species during animal development: Passive or active? *Dev. Biol.* 320 (1), 1–11. <https://doi.org/10.1016/j.ydbio.2008.04.041>.
- Cragg, G.M., Pezzuto, J.M., 2016. Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents. *Med. Principles Practice* 25 (2), 41–59. <https://doi.org/10.1159/000443404>.
- Desai, A., Qazi, G., Ganju, R., El-Tamer, M., Singh, J., Saxena, A., Bedi, Y., Taneja, S., Bhat, H., 2008. Medicinal plants and cancer chemoprevention. *Curr. Drug Metab.* 9, 581–591. <https://doi.org/10.2174/138920008785821657>.
- Dirsch, V.M., Gerbes, A.L., Vollmar, A.M., 1998. Ajoene, a compound of garlic, induces apoptosis in human promyeloleukemic cells, accompanied by generation of reactive oxygen species and activation of nuclear factor κB. *Mol. Pharmacol.* 53 (3), 402–407. <https://doi.org/10.1124/mol.53.3.402>.
- Elmore, S., 2007. Apoptosis: a review of programmed cell death. *Toxicol. Pathol.* 35 (4), 495–516. <https://doi.org/10.1080/01926230701320337>.
- Ghramh, H.A., Ibrahim, E.H., Kilany, M., 2020. Study of anticancer, antimicrobial, immunomodulatory, and silver nanoparticles production by Sidr honey from three different sources. *Food Sci. Nutr.* 8 (1), 445–455. <https://doi.org/10.1002/fsn3.1328>.
- Greenwell, M., Rahman, P.K.S.M., 2015. Medicinal plants: their use in anticancer treatment. *Int. J. Pharm. Sci. Res.* 6, 4103–4112. [https://doi.org/10.13040/IJPSR.0975-8232.6\(10\).4103-12](https://doi.org/10.13040/IJPSR.0975-8232.6(10).4103-12).
- Hajlaoui, H., Mighri, H., Aouni, M., Gharsallah, N., Kadri, A., 2016. Chemical composition and in vitro evaluation of antioxidant, antimicrobial, cytotoxicity and anti-acetylcholinesterase properties of Tunisian *Origanum majorana* L. essential oil. *Microb. Pathog.* 95, 86–94. <https://doi.org/10.1016/j.micpath.2016.03.003>.
- Hermeking, H., Lengauer, C., Polyak, K., He, T.-C., Zhang, L., Thiagalingam, S., Kinzler, K.W., Vogelstein, B., 1997. 14-3-3σ is a p53-regulated inhibitor of G2/M progression. *Mol. Cell* 1 (1), 3–11. [https://doi.org/10.1016/S1097-2765\(00\)80002-7](https://doi.org/10.1016/S1097-2765(00)80002-7).
- Ibrahim, E.H., Alshehri, A., Ghramh, H.A., Kilany, M., Morsy, K., El-Kott, A.F., Sayed, M.A., 2021a. Rosemary extract and its biogenic silver nanoparticles induce apoptosis and arrest cell cycle in HT-29 colon cancer cells. *Sci. Adv. Mater.* 13 (1), 36–49. <https://doi.org/10.1166/sam.2021.3893>.
- Ibrahim, E.H., Ghramh, H.A., Alshehri, A., Kilany, M., Khalofah, A., El-Mekkawy, H.I., Sayed, M.A., Alothaid, H., Taha, R., 2021b. *Lepidium sativum* and its biogenic silver nanoparticles activate immune cells and induce apoptosis and cell cycle arrest in HT-29 colon cancer cells. *Biomater. Tissue Eng.* 11, 195–209. <https://doi.org/10.1166/jbt.2021.2551>.
- Ibrahim, E.H., Kilany, M., Ghramh, H.A., Khan, K.A., ul, S., Islam, 2019. Cellular proliferation/cytotoxicity and antimicrobial potentials of green synthesized silver nanoparticles (AgNPs) using *Juniperus procera*. *Saudi J. Biol. Sci.* 26, 1689–1694. <https://doi.org/10.1016/j.sjbs.2018.08.014>.
- Igney, F.H., Krammer, P.H., 2002. Death and anti-death: tumour resistance to apoptosis. *Nat. Rev. Cancer* 2 (4), 277–288. <https://doi.org/10.1038/nrc776>.
- Iqbal, J., Abbasi, B.A., Mahmood, T., Kanwal, S., Ali, B., Shah, S.A., Khalil, A.T., 2017. Plant-derived anticancer agents: a green anticancer approach. *Asian Pac. J. Trop. Biomed.* 7 (12), 1129–1150. <https://doi.org/10.1016/j.apjtb.2017.10.016>.
- Kaminsky, V.O., Zhivotovsky, B., 2014. Free radicals in cross talk between autophagy and apoptosis. *Antioxidants Redox Signal.* 21 (1), 86–102. <https://doi.org/10.1089/ars.2013.5746>.
- Kowalski, P.J., Rubin, M.A., Kleer, C.G., 2003. E-cadherin expression in primary carcinomas of the breast and its distant metastases. *Breast Cancer Res.* 5, R217–R222. <https://doi.org/10.1186/bcr651>.
- Kuno, T., Tsukamoto, T., Hara, A., Tanaka, T., 2012. Cancer chemoprevention through the induction of apoptosis by natural compounds. *J. Biophys. Chem.* 03 (02), 156–173. <https://doi.org/10.4236/jbpc.2012.32018>.
- Leeja, L., Thoppil, J.E., 2007. Antimicrobial activity of methanol extract of *Origanum majorana* L. (Sweet marjoram). *J. Environ. Biol.* 28, 145–146.
- Lin, L.-T., Liu, L.-T., Chiang, L.-C., Lin, C.-C., 2002. In vitro anti-hepatoma activity of fifteen natural medicines from Canada. *Phyther. Res.* 16 (5), 440–444. <https://doi.org/10.1002/ptr.937>.
- Matés, J.M., Sánchez-Jiménez, F.M., 2000. Role of reactive oxygen species in apoptosis: implications for cancer therapy. *Int. J. Biochem. Cell Biol.* 32 (2), 157–170. [https://doi.org/10.1016/S1357-2725\(99\)00088-6](https://doi.org/10.1016/S1357-2725(99)00088-6).
- Méabed, E.M.H., El- Sayed, N.M., Abou-Sreeda, A.I.B., Roby, M.H.H., 2018. Chemical analysis of aqueous extracts of *Origanum majorana* and *Foeniculum vulgare* and their efficacy on *Blastocystis* spp. cysts. *Phytomedicine* 43, 158–163. <https://doi.org/10.1016/j.phymed.2018.04.017>.
- Mossa, A.T.H., Nawwar, G.A.M., 2011. Free radical scavenging and antiacetylcholinesterase activities of *Origanum majorana* L. essential oil. *Hum. Exp. Toxicol.* 30 (10), 1501–1513. <https://doi.org/10.1177/0960327110391686>.
- Murad, H., Hawat, M., Ekhtiar, A., Aljapawe, A., Abbas, A., Darwish, H., Sbenati, O., Ghannam, A., 2016. Induction of G1-phase cell cycle arrest and apoptosis pathway in MDA-MB-231 human breast cancer cells by sulfated polysaccharide extracted from *Laurencia papillosa*. *Cancer Cell Int.* 16, 39–50. <https://doi.org/10.1186/s12935-016-0315-4>.
- Nabih, A.B., Ayman, I.E., Osama, A.A., Mohammed, H.M., 2012. Potential anticancer activity of the medicinal herb, *Rhazya stricta*, against human breast cancer. *African J. Biotechnol.* 11 (37), 8960–8972.
- Ouedrhiri, W., Balouiri, M., Bouhdid, S., Moja, S., Chahdi, F.O., Taleb, M., Greche, H., 2016. Mixture design of *Origanum compactum*, *Origanum majorana* and *Thymus serpyllum* essential oils: optimization of their antibacterial effect. *Ind. Crops Prod.* 89, 1–9. <https://doi.org/10.1016/j.indcrop.2016.04.049>.
- Pan, M.-H., Ho, C.-T., 2008. Chemopreventive effects of natural dietary compounds on cancer development. *Chem. Soc. Rev.* 37 (11), 2558. <https://doi.org/10.1039/b801558a>.
- Qureshi, S., Shah, A.H., Ahmed, M.M., Rafatullah, S., Bibi, F., Al-Bekairi, A.M., 1994. Effect of alpinia galanga treatment on cytological and biochemical changes induced by cyclophosphamide in mice. *Pharm. Biol.* 32 (2), 171–177. <https://doi.org/10.3109/13880209409082989>.
- Ren, W., Qiao, Z., Wang, H., Zhu, L., Zhang, L.i., 2003. Flavonoids: Promising anticancer agents. *Med. Res. Rev.* 23 (4), 519–534. <https://doi.org/10.1002/med.10033>.
- Shaw, P., Bovey, R., Tardy, S., Sahli, R., Sordat, B., Costa, J., 1992. Induction of apoptosis by wild-type p53 in a human colon tumor-derived cell line. *Proc. Natl. Acad. Sci. U. S. A.* 89 (10), 4495–4499. <https://doi.org/10.1073/pnas.89.10.4495>.
- Simon, H.U., Haj-Yehia, A., Levi-Schaffer, F., 2000. Role of reactive oxygen species (ROS) in apoptosis induction. *Apoptosis* 5, 415–418. <https://doi.org/10.1023/A:1009616228304>.
- Sun, S.-H., Huang, H.-C., Huang, C., Lin, J.-K., 2012. Cycle arrest and apoptosis in MDA-MB-231/Her2 cells induced by curcumin. *Eur. J. Pharmacol.* 690 (1–3), 22–30. <https://doi.org/10.1016/j.ejphar.2012.05.036>.
- Taylor, W.R., Stark, G.R., 2001. Regulation of the G2/M transition by p53. *Oncogene* 20 (15), 1803–1815. <https://doi.org/10.1038/sj.onc.1204252>.
- Thoppil, R.J., Bishayee, A., 2011. Terpenoids as potential chemopreventive and therapeutic agents in liver cancer. *WJH* 3 (9), 228. <https://doi.org/10.4254/wjh.v3.i9.228>.
- Vági, E., Rapavi, E., Hadolin, M., Vászárhelyiné Perédi, K., Balázs, A., Blázovics, A., Simándi, B., 2005. Phenolic and triterpenoid antioxidants from *Origanum majorana* L. herb and extracts obtained with different solvents. *J. Agric. Food Chem.* 53 (1), 17–21. <https://doi.org/10.1021/jf048777p>.
- WHO 2018, n.d. WHO Cancer Fact sheet (2018). Available online at: www.who.int/en/news-room/fact-sheets/detail/cancer.
- Woo, J.-H., Kim, Y.-H., Choi, Y.-J., Kim, D.-G., Lee, K.-S., Bae, J.H., Min, D.S., Chang, J.-S., Jeong, Y.-J., Lee, Y.H., Park, J.-W., Kwon, T.K., 2003. Molecular mechanisms of curcumin-induced cytotoxicity: Regulation of apoptosis through generation of reactive oxygen species, down-regulation of Bcl-XL and IAP, the release of cytochrome c and inhibition of Akt. *Carcinogenesis* 24 (7), 1199–1208. <https://doi.org/10.1093/carcin/bgg082>.
- Yonish-Rouach, E., Resnftzky, D., Lotem, J., Sachs, L., Kimchi, A., Oren, M., 1991. Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. *Nature* 352 (6333), 345–347. <https://doi.org/10.1038/352345a0>.
- Zhang, J., Wang, X., Vikash, V., Ye, Q., Wu, D., Liu, Y., Dong, W., 2016. ROS and ROS-mediated cellular signaling. *Oxid. Med. Cell. Longev.* 2016, 1–18. <https://doi.org/10.1155/2016/4350965>.
- Zhang, N., Kong, X., Yan, S., Yuan, C., Yang, Q., 2010. Huaier aqueous extract inhibits proliferation of breast cancer cells by inducing apoptosis. *Cancer Sci.* 101, 2375–2383. <https://doi.org/10.1111/j.1349-7006.2010.01680.x>.