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In vitro Assessment of Anticancer Properties of *Moringa peregrina* Essential Seed Oil on Different Cell Lines

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ABSTRACT

Moringa peregrina belongs to the Moringaceae family and is native to the western part of Saudi Arabia and the eastern part of Egypt, from where it has been transferred to tropical and subtropical countries. The *Moringa* trees are highly appreciated for their nutritional and medicinal properties. In the past two decades, research has focused on investigating different parts of *Moringa* as a source for anticancer preparations. However, *M. peregrina* extracts were only evaluated for their antioxidant and anti-inflammatory activities, and the literature contains few (or no) information about the cytotoxic properties reported for *M. peregrina* seed oil. Accordingly, the current work aimed to evaluate the potential anti-proliferative properties of essential oil obtained from *M. peregrina* and its effects on various cell types. Different cell lines were exposed to increasing concentrations from the seed oil (0.15 to 1000 µg/mL) for 24h, and cell toxicity was evaluated. Results revealed that all cell lines under investigation were significantly affected depending on the used oil concentration. Furthermore, cell lines showed different response to the treatment with oil according to cell type. Additionally, HepG2 and HeLa cells showed the highest response to the applied essential oil, followed by MCF-7, CACO-2 and L929, where the toxicity percentages at the maximal oil doses recorded 75.82, 75.35, 67.89, 57.50 and 53.43%, respectively. The IC_{50} value obtained for HeLa cells was 366.3 µg/mL, and increased by 1.7- and 2.0-fold for HepG2 and CACO-2 cells, respectively. The present results suggest that essential oil isolated from *M. peregrina* seeds has potential cytotoxic properties against different cancer cell lines.

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Authors' Contribution
EAE designed the study and conducted the cell culture experiments, performed statistical analysis and wrote the manuscript. MAS helped in designing the study and prepared the essential oils. HAEE and MW conceived the study. All authors drafted and approved the final manuscript.

Key words
***Moringa peregrina*, essential seed oil, cytotoxicity, medicinal plants.**

INTRODUCTION

During the last two decades, the use of microbial and plant products, has gained an increased interest in cancer therapy (El-Enshasy *et al*., 2013; Elsayed *et al*., 2014). Several compounds isolated from herbaceous medicinal plants were found to possess anticancer activities (Heo *et al*., 2014; Saleem *et al*., 2014). Extracts isolated from different parts of medicinal plants exhibited a significant inhibition of different cancer cells, *i.e.* breast, colon, lung, cervical, hepatocellular (Farooq *et al*., 2013; Kma, 2013; Olarte *et al*., 2013; Nikolić *et al*., 2014). Furthermore, medicinal plants and their extracts represented a good source for anticancer bioactive compounds currently applied in clinical trials for cancer treatment. Paclitaxel, which is isolated from *Taxus brevifolia* Nutt., is used against ovarian cancer and advanced breast cancer (Rowinsky *et al*., 1992). Podophyllotoxin, extracted from *Podophyllum emodi* is used with its derivatives (etoposide and teniposide) for the treatment of lymphomas as well as bronchial and testicular cancers (Cragg and Newman, 2005). Vincristine extracted from *Catharanthus roseus* G. Don. is mainly used with other cancer chemotherapeutics for treating different cancer types, *i.e.* leukemia, breast and lung cancer (Sultana *et al*., 2014).

Moringa peregrina belongs to the monogenic Moringaceae family containing 13 species of the genus *Moringa*. These plants are generally cultivated in tropical

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countries in Asia and Africa, and they range in size from small herbs to heavy trees (Singh *et al.*, 2013). This family also contains the most widely known species *M. oleifera* Lam., known as miracle tree, horseradish tree, drumstick tree (Awodele *et al.*, 2012). The plants present in this family are found to be rich in flavonoids, sterols, tocopherols, vitamin C and carotenes (Selvakumar and Natarajan, 2008; Yammuenart *et al.*, 2008). Moreover, *Moringa* species are rich in essential oils, antioxidants and sulfur amino acids (Tsaknis *et al.*, 1999; Ferreira *et al*., 2008). Although *M. oleifera* has been traditionally used as a source of nutrients, however, recent studies showed that different parts of the plant possess important biological properties. The leaves exhibited antioxidant and anticancer activities (Sreelatha *et al.*, 2011; Awodele *et al*., 2012), the park and leaf extracts showed antimicrobial activities (Yusuf and Abdul Hamid, 2013), the pods have been traditionally consumed as a stimulating cardiotonic against fainting in Thailand (Mokkhasmit *et al*., 1971), the seed extract was used to reduce lipid peroxidation (Faizi *et al*., 1998), and the root was found to have anti-fertility and anti-inflammatory activities (Padmarao *et al*., 1996; Muangnoi *et al.*, 2012). Recently, *M. peregrina* seed extracts have been shown to exhibit antioxidant and anti-inflammatory properties (Koheil *et al*., 2011).

The seed essential oil contents of the two common *Moringa* species, *i.e. M. oleifera* and *M. peregrina*, are considered to be high, ranging from 33-41 and 49.8%, respectively (Tsaknis, 1998; Rashid *et al*., 2008). The seed oil obtained from *Moringa* has been used mainly in household as well as soap and perfume applications (Ghazali and Mohammed, 2011). *Moringa* seed oil has been evaluated as a potential feedstock for the production of biodiesel (Rashid *et al*., 2008). Additionally, seed and seed oil obtained from *M. peregrina* has been evaluated for their application as a source for biofuel and biodiesel-diesel blends (Salaheldeen *et al.*, 2014, 2015). Moreover, Siddhuraju and Becker (2003) and Chuang *et al.* (2007) reported different biological activities against microbial microorganisms as well as antioxidant activities. Recently, Elsayed *et al.* (2015) evaluated the cytotoxic activities of oils from *M. oleifera* seeds on different cancer cell lines. Their results revealed that the oil significantly decreased cell viability depending on the concentration applied. Additionally, from their obtained results, HeLa cells showed the maximal decrease in cell viability, where oil treatment resulted in about 76.1% inhibition of cell viability with an IC_{50} value of 422.8 µg/mL. Due to the scarcity of anticancer studies on *Moringa* essential seed oils, the current work aimed to explore the potential anti-cancer activities of *M. peregrina* seed oils on different cell lines.

MATERIALS AND METHODS

Materials and cell lines

Dulbecco's Modified Eagle's (DMEM) cultivation medium (AppliChem, Darmstatt, Germany) was supplemented with 4.5 g/L glucose. All other reagents, chemicals and materials were of cell culture grade.

The investigation was carried out using different cell lines, which were obtained from Sigma-Aldrich Chemical Company, USA. The cell lines included: breast cancer (MCF-7); liver cancer (HepG2); colon cancer (CACO-2); cervical cancer (HeLa) and mouse fibroblasts (L929).

Preparation of seed oil

The oil under study was of commercial grade purified from *M. peregrina* seeds, and was obtained from AsiaNet Trading Est., Riyadh, Kingdom of Saudi Arabia. The extraction of the oil proceeded, where the seeds were pressed at cold temperatures to avoid any possible change in the oil properties. The oil was mixed with DMSO to prepare a stock solution (1 mg/mL). Then, DMEM medium was used to serially dilute the stock oil solution, in order to prepare the working oil solutions, which were in turn sterile-filtered. According to previous literature working with seed oils (Elsayed *et al.*, 2015), a series of working solutions ranging from 0.0 to 1000 ug/mL.

Cultivation conditions

Cells were cultivated in DMEM, containing 10% serum, 100x, 1% streptomycin/penicillin/amphotericin B solution, and were then periodically sub-cultured and transferred in a humid-CO_2 incubator (ShelLab, USA) at 5% CO_2, 37°C. Upon performing the cytotoxicity assay, cells were detached from T-flasks, then centrifuged and washed twice with sterile PBS buffer solution. Viable cell concentration was determined using Trypan Blue exclusion (Walford *et al.*, 1964), where cells having viability higher than 95% are chosen for performing the assays. Different cell types were exposed to the working solutions of *M. peregrina* seed oil ranging from 15 to 1000 µg/mL. Following cell exposure, the cytotoxic activities of the tested seed oils were screened using 3-(4,5-dimethylthiazol-2-yl),5-biphenyl tetrazolium bromide (MTT). Blanks were exposed only to the solvent in a concentration ≤ 0.5%). Following cell treatments, we investigated the plates using an inverted contrast microscope (Nikon Eclipse T500, Japan, 10 X) in to order to detect possible morphological changes.

Cytotoxicity assay

Cell viability percentage was determined by MTT as described by Mosmann (1983), with some

modifications (Elsayed *et al.,* 2015). Briefly, cells were detached from the cultivation plate surface by trypsinization, and were then washed and resuspended in PBS. Following, 96 well culture plates were inoculated with the suspended cells to reach a final concentration of 10^4 cells/100 µl/well. The cultured plates were then incubated at 5% CO_2 and 37°C for 24 h. Accordingly, the exhausted medium was pipetted out and replenished with new medium and working solutions prepared from the seed oil, and the cells were cultivated further. After another 24 h, MTT was added, and the plates were incubated for 3-4 h at 5% CO_2 and 37°C. The supernatants were then discarded and replaced with DMSO. The optical density of the precipitated crystals, which directly reflects the percentage of viability, was measured at 550 nm.

Statistical analysis

SPSS 9.0 was used for the analysis of the obtained data. Data were represented as means ± SD of triplicate experiments. ANOVA analysis was used to compare variable treatments, where $p \leq 0.05$ was used t denote statistical significance.

RESULTS

Cytotoxicity assay

Different concentrations (15-1000 µg/mL) of essential oil extracted from *M. peregrina* kernels were evaluated for their cytotoxic effect against various cell lines. After 24 h of treatment with the oil concentration, viable cell concentration was determined. Figure 1A-E represents our present findings, which obviously indicate that increased seed oil concentration has a negative impact on cell viability for all evaluated cell lines. Moreover, the cytotoxic effect was highly significant effect ($p<0.001$), and increased mainly with increasing the oil concentration. The highest cytotoxic effect was obtained at 1 mg/mL of the tested seed oil in all evaluated cell lines. The viability decreased from 100% to 24.65±0.27%, 24.18±4.52%, 42.51±4.07%, 46.57±3.45% and 32.11±0.64% for HeLa, HepG2, MCF-7, CACO-2 and L929 cells, respectively. Furthermore, HeLa, HepG2, CACO-2 and L929 cells were not significantly changed upon exposure to 62.5 or 125 µg/mL of the seed oil. On the other hand, MCF-7 cell line was not significantly changed when treated with oil concentrations between 0.0 and 62.5 µg/mL.

Considering cytotoxicity data, the results (Fig. 1F) represent the comparison of different cell types exposed to the maximal oil concentration in relation to cell toxicity. HeLa and HepG2 cells were largely affected then L929, MCF-7 and CACO-2, and the obtained cell toxicity values reached 75.35, 75.82, 67.89, 57.50 and 53.43%, respectively.

Table I lists the concentrations of the seed oil that result in an inhibition of half of the variable viable cell types. The results showed that the IC_{50} value obtained for HeLa cells was 366.3 µg/mL, while the values recorded for HepG2 and CACO-2 cells were higher by 1.7- and 2.0-fold, respectively. On the other hand, the IC_{50} values for MCF-7 and L929 cells were higher than 750 µg/mL.

Table I.- The half maximal inhibitory concentration (IC_{50}) determined for various cell types exposed to seed oil from *M. peregrina.*

Cell line	IC_{50} (µg/mL)
HeLa	366.3
HepG2	604.3
MCF-7	850.9
CACO-2	721.7
L929	935.8

Morphological alternations

The results of the influence of variable concentrations of the oil obtained from *M. peregrina* kernels on the morphology of different cells are shown (Fig. 2). It can be clearly observed that seed oil concentrations higher than 125 µg/mL significantly affected the characteristics of the growth morphology of all evaluated cell lines. Moreover, this effect was proportional to the applied concentration. Microscopic examination showed that cells are reduced in size and lost their adherence capacity. Additionally, the maximal seed oil concentration resulted in cell rounding-up and floating in relation to the morphology of the control untreated cells.

DISCUSSION

Currently, the pharmaceutical industry is depending more and more on natural resources (medicinal plants, microbes, etc.) for obtaining their major active compounds for the preparation of potential anti-cancer drugs (Elsayed *et al*., 2013; Vasanath *et al*., 2015). *M. oleifera*, is a fast growing tree resident mainly in tropical countries, and is greatly valued and used in food and health purposes (Waterman *et al*., 2014). Historically, *M. oleifera* leaves have been used to treat several acute and chronic diseases, and have been shown to exhibit many bioactive properties; reduction of blood glucose and lipid levels, inhibition of microbial and parasitic growth and reduction of inflammation, oxidative capacities and cancer development (Fahey, 2005; Awodele *et al.,* 2012;

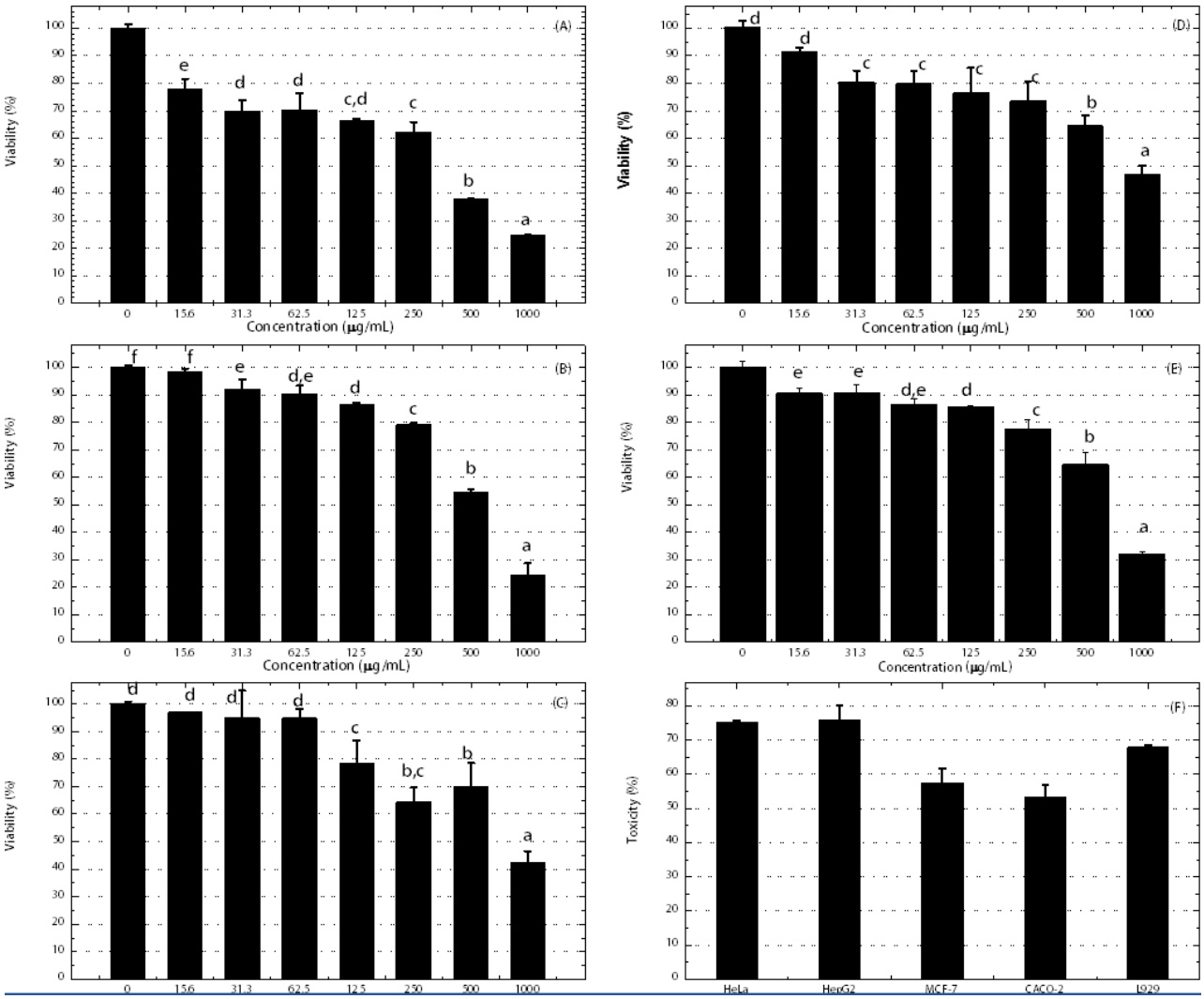

Fig. 1. Effect of increasing concentrations of oil extracted from *M. peregrina* seeds on cell viability. A, HeLa; B, HepG2; C, MCF-7; D, CACO-2; E, L929; F, Toxicity. Data are expressed as mean value ± SD. Different letters for the specific cell line represents significant difference at $p<0.05$ using one-way ANOVA.

Mbikay, 2012). Despite the fact that *M. peregrina* has been reported to show antioxidant and anti-inflammatory activities, however, few (or no) reports discuss the biological properties of its seed oil. Accordingly, the current investigation was undertaken to examine the anti-proliferative properties of *M. peregrina* seed oil against different cell lines.

The obtained results clearly indicated that the essential seed oil of *M. peregrina* has a significant effect ($p<0.05$) on cell viability of all tested cell lines, and that the reduction in cell viability is proportional to the concentration applied. Our results are in good agreement with those previously reported by Ignea *et al.* (2013) who found that grape seed extracts exhibited cytotoxic activities in PC3 and HepG2 2.2.15 cell lines. Furthermore, they also found that higher seed extract concentrations as well as longer treatment periods resulted in an increased cytotoxicity, especially in PC3 cells. When different cell lines were treated with the essential oil extracted from *M. peregrina* seeds, our results revealed that different cells vary in their response when subjected to different concentrations of the essential oil. HepG2 and HeLa cells were the most affected, followed by MCF-7, CACO-2 and L929 cells. This can be generally attributed to the fact that different cell types react differently to the administered doses

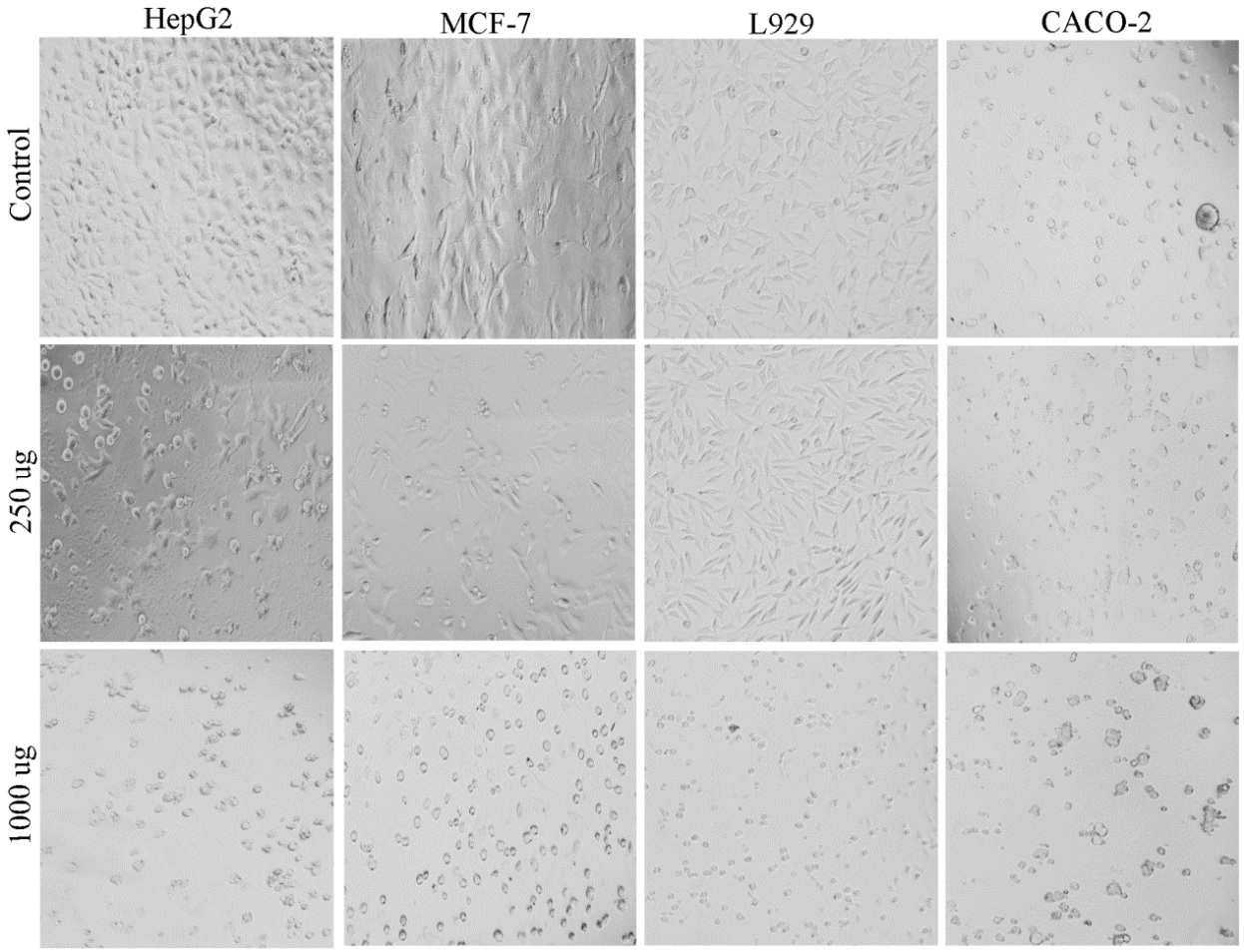

Fig. 2. Alternations in the morphological characteristics of various cells as influenced by increasing concentrations of oil extracted from *M. peregrina* seeds (10x magnification).

based on the inherent differences found in their cell membrane structure and organization (Heo *et al*., 2014). Moreover, we previously studied the anti-proliferative properties of seed oil isolated from *M. oleifera* on different cell lines, and found that the largest toxic effect was obtained on HeLa cells (Elsayed *et al*., 2015). The anti-proliferative activities of the essential oil extracted from *M. peregrina* seeds may be explained due to the presence of different fatty acids, sterols and tocopherols. Atolani *et al*. (2012) reported different cytotoxic activities for seed oils obtained from edible fruits, and they attributed this effect for the presence of omega-3 unsaturated fatty acids. The oil contents of *M. peregrina* seeds were reported to reach about 50-54.3%, where the saturated (mainly oleic and gadoleic acids) and unsaturated (mainly palmitic and stearic) fatty acids corresponded to 14.7 and 84.7%, respectively (Somali *et al*., 1984; Tsaknis, 1998). Moreover, *M. peregrina* seed oil also contains sitosterols and α-, γ- and δ-tocopherols. Scheim (2009) investigated the effect of different unsaturated fatty acids on different cancer cells, and found that they reduce tumor growth and cancer occurrence in living models. Furthermore, lipid peroxidation has been proposed as the cytotoxic mode of action of fatty acids present in the oils, where the peroxidation process produces increased levels of free radicals that alter the structure of the cell membrane of cancerous cells (Ravichandran and Johnson, 1999).

Monitoring cell morphology as affected by different concentrations of the essential seed oil revealed that cells showed abnormal characteristics of cell morphology and started to detach from plate surfaces in response to the oil concentration. These changes finally resulted in cell death. These results also are in good agreement with those previously reported (Vijayan *et al*., 2004). The authors reported morphological alternations and cell

detachment and death when they treated different cell lines with alkaloid extracts obtained from different parts of *Solanum pseudocapsicum*. Additionally, our previous results obtained with seed extracts from *M. oleifera* showed the same morphological changes (Elsayed *et al*., 2015).

In conclusion, the obtained results clearly confirm that the essential oil obtained from *M. peregrina* seeds has a significant inhibitory effect on different cell lines investigated. This effect was found to depend on the concentration used where HeLa and HepG2 cells were the most affected cell types. Finally, the current investigation can be considered as a primary investigation paving the way for future work investigating the possible mechanisms and modes of action of oil obtained from *M. peregrina* seeds on different cancer cell lines.

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Conflict of interest statement

The authors have no conflict of interest to declare

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