



Potential role of *Moringa oleifera* on different cell lines and experimental animal model

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Abstract: Many natural products have anti-tumor effects against various forms of cancer, with various mechanisms of action including cell growth suppression, cell differentiation modulation and apoptosis induction. *Moringa Oleifera* (L) is a commonly used as phytotherapy that have an biomedical activities against many illness including cancer. In this study; *Moringa Oleifera* (L) leaves aqua's extract were evaluated for its cytotoxic potentiality against three tumor cell lines; (HepG2, Caco2 and Mcf7). Cytotoxic effects of M.O L-WX by MTT assay revealed remarkable dose dependent anti-cancer activity on Caco2 and Mcf7 while lower anticancer activity on HepG2. The IC50 value were 4 mg /mL and 9 mg /mL for CaCo2 and McF7 cancer cell lines respectively while it was 19 mg /mL for Hepg2 cells. The apoptotic effect result showed significant increase in expression of apoptotic molecules FASL of Caco2 and Mcf7 cell lines (p-value = 0.001 and 0.005 respectively when compared to the control of each cell line and also the significant increase in the activity of the caspase 8 on Caco2 and Mcf7 (p-value 0.029 & 0.007 respectively), caspase 9 (p-value 0.01 and 0.0001 respectively), and caspase 3 activity (p-value= 0.008and 0.004 respectively) when compared to its control. Analysis of the cell cycle showed substantial arrest in phases G0/G1 & G2/M phases. Also, the results showed significant increase in total oxygen capacity dependant on dose treated Finally, *Moringa Oleifera* (L) extract is a promising agent against colon and breast cancer and these results need to further evaluation to be confirmed.

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Key words: Cytotoxicity, Apoptosis, cell cycle, *Moringa oleifera*

1. Introduction:

In developing countries, medicinal plants are essential components of the indigenous medicine system that have persisted. Many of the pharmacological concepts commonly used as anticancer agents were first isolated from plants. However, due to their complex structures, many important anticancer agents are still isolated from plants so they cannot be chemically synthesized on a commercial scale. *Moringa* (*Moringa oleifera* Lam) is a form of local Indian medicinal herb that has become common in both the tropical and subtropical countries. *Moringa oleifera* is one of the Brassica order's vegetables, and belongs to the *Moringaceae* family. The *Moringaceae* is a family with a single genus with 13 recognized species (Khawaja et al., 2010). Traditionally, the *Moringa* is also well recognized and used for its health benefits in addition to being a daily used vegetable among the citizens of these areas. Thanks to its incredible healing ability for various ailments and even some chronic diseases, it has earned its name as 'the miracle tree' among commoners. Due

to its diverse uses, multiple investigations were conducted out to extract bioactive compounds from different parts of the plant (Guevara et al., 1999). Because of its affordable cost, herbal plants in medicine or known as phytomedicine are still trustworthy and widely used as one of the alternatives in medicinal field (Abalaka et al., 2009). *Moringa oleifera* is a multifunctional herbal plant that is used worldwide as human food and an alternative to medical uses. *Moringa oleifera* contains essential amino acids, carotenoids in leaves, and nutraceutical components that support the concept of using this plant as a dietary supplement or ingredient in food preparation. Some nutritional assessments were conducted in the leaves and stems. A significant factor that accounts for *Moringa oleifera*'s medicinal uses is its wide variety of essential antioxidants, antibiotics and nutrients including vitamins and minerals. *Moringa* National Institutes of Health have been recognized *Moringa Oleifera* tree was brought into the

possession of man by the will of God (the Botanical at 2007). It has been praised internationally for its ability to cure more than 300 diseases. [9] All parts of the *M. oleifera* tree, including the leaves, seeds, bark, and roots, were tested for anticancer activities. The leaves are abundant in polyphenols and polyflavonoids, which are antioxidants and possible anticancer compounds [10]. The toxic side effect of chemotherapy and radiotherapy can be reduced or minimized by medicinal plants by improving their cancer killing action, or supporting the immune systems of patients [4, 5]. The goal of this study is try to initiate cancer cell death by bioactive medicinal *M. Oleifera*.

2. Materials and Methods:

1. Preparation of *M. Oleifera* aqueous extract

Moringa Oleifera Leaves were received from Moringa Arava Ltd, Sinai Egypt. In our laboratory the crude aqueous extract was prepared by mixing 1 g of dried powdered M.O leaves with 10 mL of for 5 minutes. Then the mixture was filtered into a sterile tube twice through a 2 µm pore sterile filter paper. For each set of tests, this aqueous extract stock solution (100 mg / mL) was freshly prepared and stored at 4°C for up to 5 days [17].

2. Cell lines and Cell culture:

Human carcinoma cell lines used in this study (HepG2 (liver cancer cell line), Caco2 (colon cancer cell line) and MCF7 (breast cancer cell line) were grown as monolayer culture I RPMI-1640 medium supplemented with 10% penicillin FBS 100 units / ml and streptomycin 2.00 mg / ml. It incubated the cell lines at 37 c in 5% CO₂ 95% air in humidity atmosphere in. Tumor cells were regularly sub cultured to maintain it in that phase of exponential growth. Working under the equipped laminar flow stringently attained sterile conditions.

3. MTT assay:

Cytotoxicity was measured using the MTT cell viability assay according to [Ref]. MTT (3-[4,5-dimethyl thiazole-2-yl]-2,5 diphenyltetrazolium bromide) assay is based on the yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is decreased by metabolically active cells, to generate reduction equivalents such as NADH and NADPH, in part by the action of dehydrogenase enzymes. The resulting purple formazan intracellular can be solubilized and quantified by spectrophotometric means as defined and updated by Mosmann (1983) [26].

4. FAS ligand detection

Fas antigen (CD95) is a membrane receptor which plays an important role in apoptosis induction. FAS ligand expression was detected using by using wkea supplies human factor –related apoptosis ligand

(FASL) ELISA Kit (Code No.WH-174). The FAS Ligand-specific monoclonal antibody has been coated on the microtiter strips wells. Samples (what type of sample, is it the tumor cells treated with the M.O extract or what, the control specimens are piped into these wells including standards of known FAS Ligand concentrations. The standards or samples and the biotinylated monoclonal antibody specific to FAS Ligand are incubated simultaneously during the first incubation. The enzyme Streptavidin-HRP, which binds the biotinylated antibody, is added after washing, incubated (15min at 37 °c) and washed. To induce a colored reaction product, the substrate solution is added which acts on the bound enzyme. Reaction was stopped after incubation. This colored product intensity is proportional to the FAS Ligand concentration present in the samples.

5. Caspase 9, 8 and caspase 3 activity:

Tumor cell line treated with M.O water extract for 24 hour, were assayed for Caspase-8, caspase – 9 and caspase-3 activity using the Caspase- Colorimetric Assay Kit (BIOVESION Catalog #K119-25), according to the manufacturer's protocol. Luminescence was measured after incubate at 37°C for 1-2 hour after applying substrate in a Modulus microplate luminometer at 400-or 405-nm in a microtiter plate reader.

6. DNA Fragmentation Assay

Cells were seeded in 6 wells plate with *Moringa Olifera* leaves extract for 48 hour. After treatment, the spent medium was aspirated out and the monolayer was collected by centrifugation 10 min at 4000 rpm. Mix 200 µl sample with 400 µl lysis solution, and incubate for 5 min at 65°C. A 600 µl chloroform was immediately added and emulsified by inversion (3-5 times) and then centrifuged for 2 min at 10,000 rpm, then 800 µl of freshly prepared precipitation solution was added mixed gently at room temperature for 1-2 min with several inversions, and centrifuged for 2 min at 10,000 rpm (~9400 x g). The supernatant was completely removed (avoiding drying) and then the pellet of DNA was dissolved by gentle vortexing in 100 µl of NaCl solution, then a 300 µl of cold ethanol was added, the DNA precipitated (10 min at -20 °C) and spin down (10,000 rpm (~9400 x g), 3-4 min). Then cold ethanol was removed and we dissolve DNA by gentle vortexing in 100 µl sterile deionized water. After RNase treatment, the DNA fragments were resolved in a 2% agarose gel and visualized under ultraviolet light following staining with ethidium bromide.

7. Cell cycle analysis

Tumor cells treated by the IC₅₀ dose of MOL-WX for 24 h were subjected to cytell TM cell imaging system after staining with Cytell™ cell cycle kit (GE Healthcare Life Science). The cell cycle distribution

was determined by DNA content to quantify the different phases of cell cycle. Results were represented as percentage of cells in each phase in relation to total number of cells count.

Statistical Analysis:

Data statistical analysis was performed using one-way variance analysis (ANOVA) followed by homogenous subsets (Duncan) at confidence level of 5% (0.05) using the Statistical Package for the Social Science (SPSS) version 17. Multiple range tests by Duncan were used to compare treatment means.

3. Results and Discussion

One of the most versatile plants used in the traditional Indian medicine *Moringa Olifera*. Evolution of water extract from the leaves of *M. Olifera* against different cell line were studied. The results showed that *M. olifera* leaves has bioactive cytotoxic effect on Caco2, MCF7 cells with IC₅₀ 4% and 9% respectively (10g/100ml distilled water) while HepG2 is 19% (10g/100ml distilled water) as shown in Table 1 and Fig.1. These results agree with Khalafalla et al. (2010) ^{as} they deduced that water extract of Moringa leaf has cytotoxic effect on HepG2, Caco-2, and MCF-7 cells and showing chemoprevention activity [16]. Nair and Varalakshmi (2011) supported our finding as they reported that aqueous extract of *M.O* leaves caused a dose-dependent decrease in on cervical cancer cells viability and showing a significant cytotoxic activity. [17]. Our results seem to be coincident with (Jung et al, 2014) who reported that *M. oleifera* leaf extracts causes marked cell death (82% and 86% and 81%) on acute myeloid leukemia (AML), lymphoblastic leukemia (ALL) and HepG2 respectively and he also reported that hot water extract exerted a strong inhibition on HepG-2 cells growth [18]. Also Nazia et al (2015) supported our finding as *M. leaves extract caused cytotoxic effect against MCF-7 cell growth* [14]. Our result revealed that a dose dependent

increase FASL expression (Figure 2), and also significant increase in activities of all caspase enzymes (casp 8, casp 9 and casp 3) examined in this study (Figure 3) and significant DNA ladder formation, suggesting apoptotic cell death. Complete DNA degradation of Caco2 cells DNA at 8% concentration of MOL-WX, while slight degradation at 2% and 4%, comparable to control untreated cells. However a complete DNA degradation of MCF7 cells occurs at concentration 18%, and a slight degradation at concentration 4.5% and 9% as shown in (photo 1). All previous recorded parameters were detect the program of cell death and showed the apoptotic activity of MOL-WX and this result is agree with previous reported by Madi, et al. (19) which showed that the cytotoxic effect of aqueous leaf extract of Moringa mainly through apoptotic cell death, and DNA fragmenting on the HeLa cells [19]. Cell cycle analysis as shown in the (table 2 and figure 4), the result revealed different cell cycle phases arrest when compared with control untreated cells. Concerning MCF7 cells, there is sub G 1 phase arrest (19.4%) in treated cells compared to control (8.1%), also the G0\G1 phase arrest showed significant increase (59.1%) compared to in control (38.4%). However, in Caco cells the sub G1 phase arrest percent was (19.6%) when compared with control (5.1%) and G0\G1 phase arrest percent was (49.7%) when compared with control (22.4%) while S phase and G2.

M phase revealed no reliable result. This finding was agreement with Al-Asmari et al (2015), who reported that the enhancement of cell population in G0/G1 phase. (20 & 21). From previous reported finding, we conclude that MOL-WX induce apoptosis through both the intrinsic and extrinsic pathway as assessed by different parameters and also effects on cell cycle by arrest in different phases. So, it can be a promising role as inducing cytotoxic effect against cancer cells, however further studies are needed to reach the optimum result for uses.

Table 1. The IC₅₀ value of the different studied cell line

Cancer cell line	IC ₅₀ value (mg/ml)
HepG2	19
MCF7	9
CACO2	4

Table 2: Cell cycle analysis: table showed the present of cells on each cell cycle phase for the treated MCF7 cells and Caco2 cells compared to their control untreated cells

	Treated MCF7 cell	Control MCF7 cells	Treated o cells	Control Caco cells
Sub G1	19.4	8.1	19.6	5.1
C0\G1	59.1	38.4	49.7	22.4
S phase	2.2	12.8	5.5	23.1
G2\M	0.3	4.0	0.2	5.5

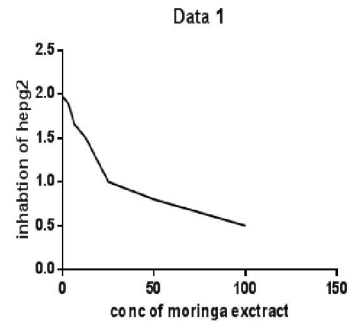


Figure 1. Cytotoxic effect of M.O water extract on the three studied tumor cell line (CACO2, MCF7 and HepG2)

Figure 2. FAS L expression on Caco2 cell line (A) and McF7 cell line after incubation with the extracts for 24 hours

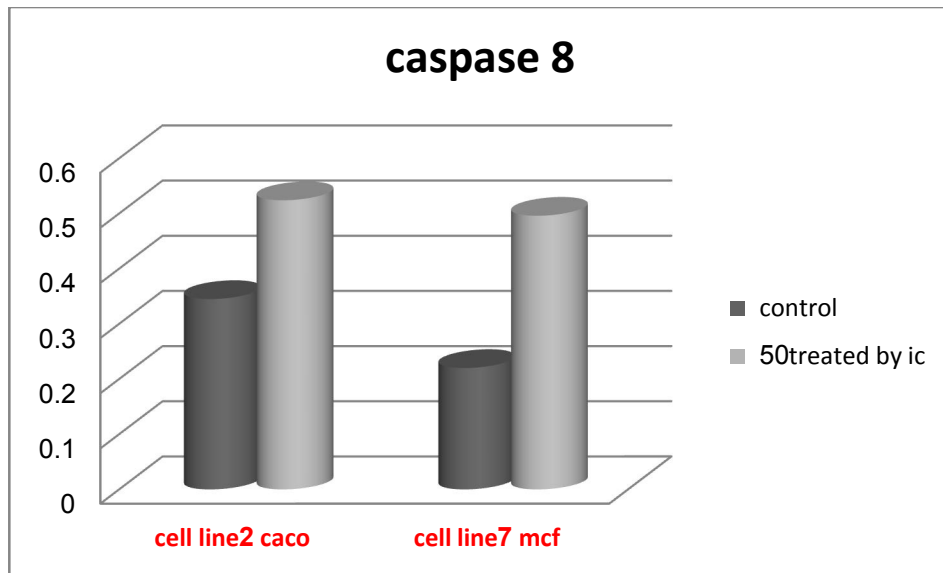


Figure 3a. Shows the effect of *Moringa olifera* extract on the induction of caspase 8 on caco2 and Mcf7 cancer cell lines

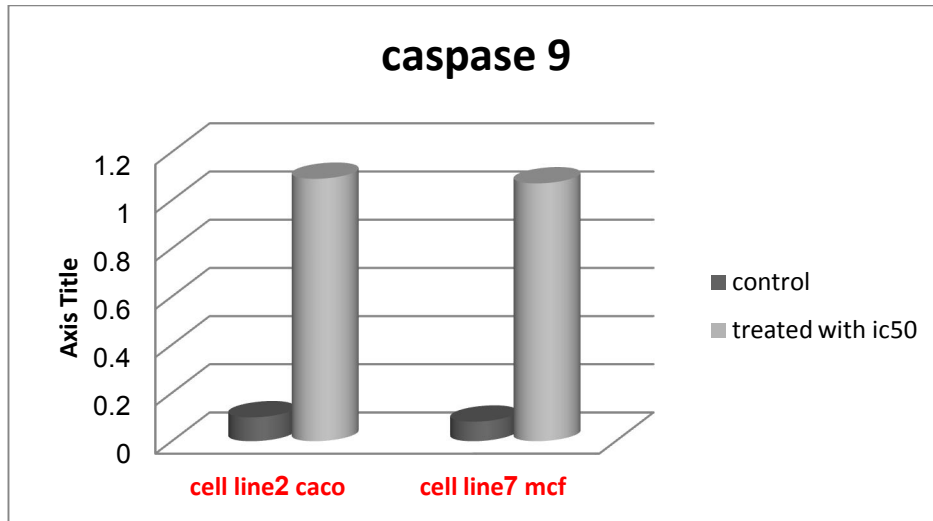


Figure 3b. Shows the effect of *Moringa olifera* extract on the induction of caspase 9 on Caco2 and MCF7 cancer cell lines

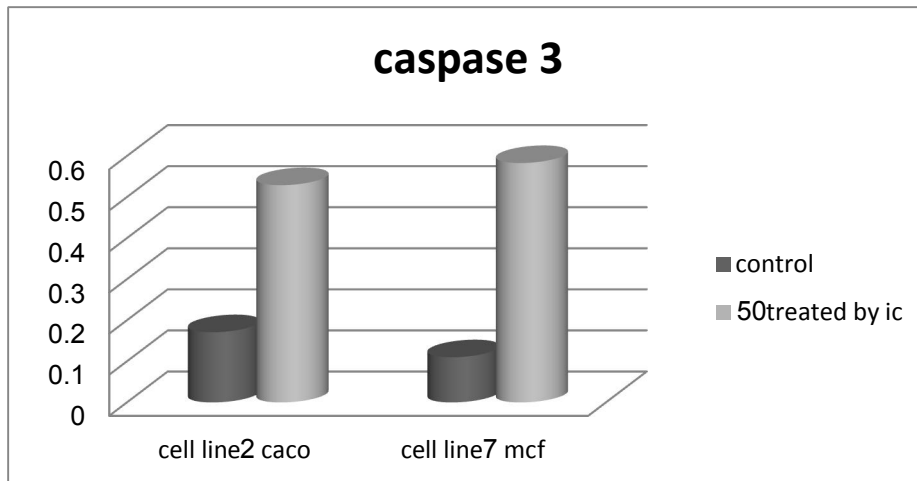


Figure 3c. Shows the effect of *Moringa olifera* extract on the induction of caspase 3 on Caco2 and MCF7 cancer cell lines

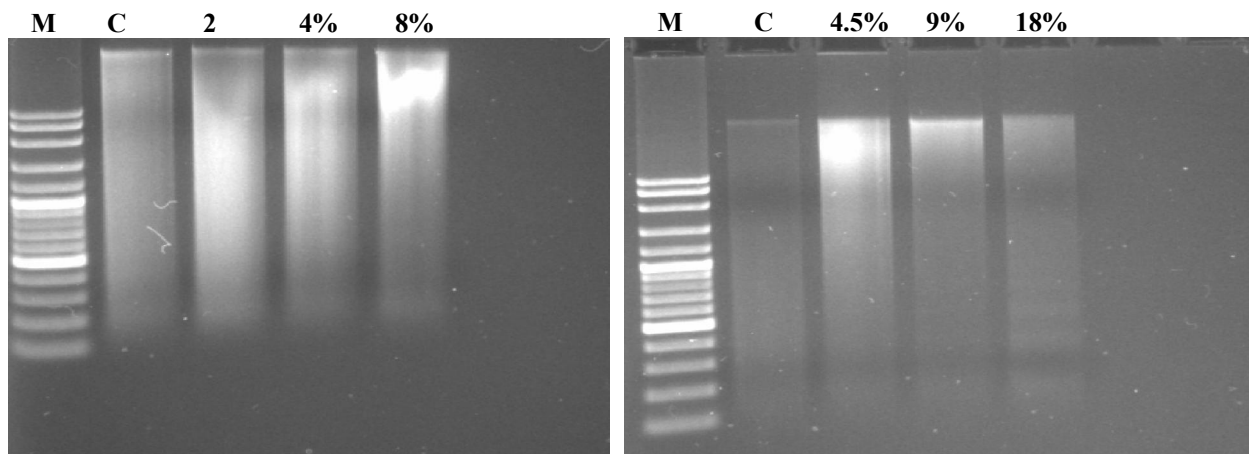
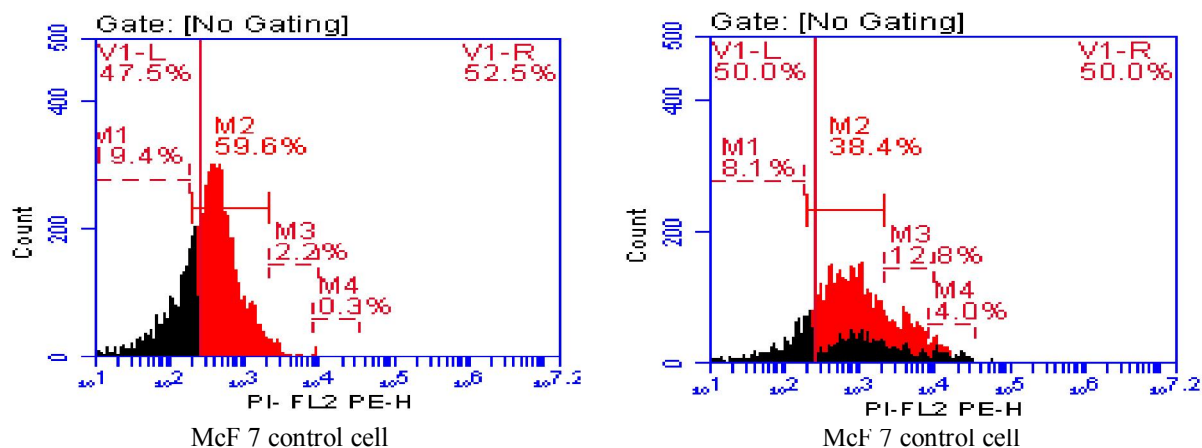
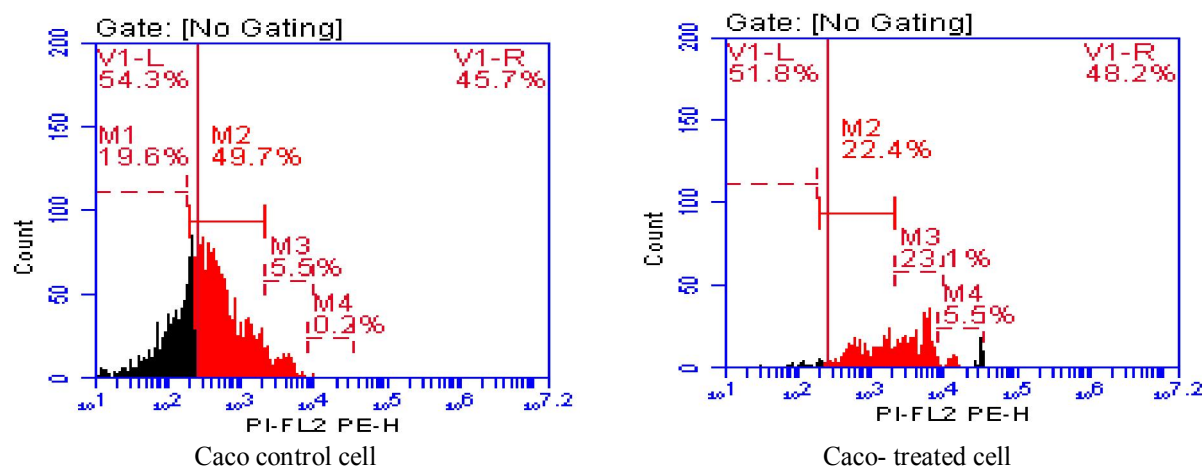


Photo 1: electrophoregram indicating DNA fragmentation on both treated and non treated Caco2(1-a) and MCF cells (1b), C = control, and M = DNA marker



Figures 5a. Flow cytometric analysis of MCF7 cell cycle for control and treated cell after 24 hr incubation with MOL-WX showing DNA phases correlated to control group (M = apoptosis sub G1, M2 =G0 /1, M3 = S phase, M 4 =G2/M)



Figures 5b. Cell cycle analysis of Caco2 cell of booth control cell (A) and treated cells after 24 hr incubation with MOL-WX showing cell population on different phase of the cell cycle correlated to control group (M = apoptosis sub G1, M2 =G0 /1, M3 = S phase, M 4 =G2/M).

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Declaration of Conflicting Interests:

The Author (s) state (s) that there is no conflict of interest.

References:

1. WHO, Cancer Fact Sheet, 2017, <http://www.who.int/mediacentre/factsheets/fs297/en>.
2. Kang Zi Khor,1 Vuanghao Lim, 1 Emmanuel J. Moses, 2 and Nozlena Abdul Samad1 The In Vitro and In Vivo Anticancer Properties of *Moringa oleifera* Evidence-Based Complementary and Alternative Medicine Volume 2018, Article ID 1071243, 14.
3. How is Chemotherapy Used to Treat Cancer? American Cancer Society. Accessed on 8/8/18, <https://www.cancerquest.org/patients/treatments/radiation-therapy> on 03/26/2019.
4. Abo KA, Adeyemi AA, Adeite DA. Ethnobotanical survey of plants used in the

- treatment of infertility and sexually transmitted diseases in southwest Nigeria. *Afr J Med Med Sci*, 29, 2000, 325–327.
5. Neto C. Antibacterial activity of some Peruvian medicinal plants from the Calle Jon de Huaylas. *J Ethnopharmacol*, 79, 2002, 133–138.
 6. O. Awodele, I. A. Oreagba, S. Odoma, J. A. Teixeira Da Silva, and V. O. Osunkalu, “Toxicological evaluation of the aqueous leaf extract of *Moringa oleifera* Lam. (*Moringaceae*),” *Journal of Ethnopharmacology*, vol. 139, no. 2, pp. 330–336, 2012.
 7. I. T. Johnson, “Phytochemicals and cancer,” *Proceedings of the Nutrition Society*, vol. 66, no. 2, pp. 207–215, 2007.
 8. P. Siddhuraju and K. Becker, “Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves,” *Journal of Agricultural and Food Chemistry*, vol. 51, no. 8, pp. 2144–2155, 2003.
 9. NIH Record. NIH Celebrates Earth Day 2008. Vol LX. No.6. March 21, 2008. http://nihrecord.od.nih.gov/newsletters/2008/03_21_2008/story4.htm.
 10. S. Nair and K. Varalakshmi, “Anticancer, cytotoxic potential of *Moringa oleifera* extracts on HeLa cell line,” *Journal of Natural Pharmaceuticals*, vol. 2, no. 3, pp. 138–138, 2011.
 11. Evidence-Based Complementary and Alternative Medicine Volume 2018, Article ID 1071243, 14 pages <https://doi.org/10.1155/2018/1071243>.
 12. C. Suphachai, “Antioxidant and anticancer activities of *Moringa oleifera* leaves,” *Journal of Medicinal Plants Research*, vol. 8, no. 7, pp. 318–325, 2014.
 13. I. L. Jung, J. H. Lee, and S. C. Kang, “A potential oral anticancer drug candidate, *Moringa oleifera* leaf extract, induces the apoptosis of human hepatocellular carcinoma cells,” *Oncology Letters*, vol. 10, no. 3, pp. 1597–1604, 2015.
 14. Nazia Hossain, M. E. S. Mirghani, Raha Bt. Raus, Optimization of *Moringa Oleifera* Leaf Extraction and Investigation of Anti Breast Cancer Activity with the Leaf Extract, (2015), ISSN 2409-3629.
 15. Mosmann, T. (Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 65, 55-63, 1983.
 16. M. M. Khalafalla, E. Abdellatif, H. M. Dafalla et al., “Active principle from *Moringa oleifera* Lam leaves effective against two leukemias and a hepatocarcinoma,” *African Journal of Biotechnology*, vol. 9, no. 49, pp. 8467–8471, 2010.
 17. Nair and K. Varalakshmi, “Anticancer, cytotoxic potential of *Moringa oleifera* extracts on HeLa cell line,” *Journal of Natural Pharmaceuticals*, vol. 2, no. 3, pp. 138-138, 2011.
 18. I. L. Jung, “Soluble extract from *Moringa oleifera* leaves with a new anticancer activity,” *PLoS ONE*, vol. 9, no. 4, Article ID e95492, 2014].
 19. N. Madi, M. Dany, S. Abdoun, and J. Usta, “*Moringa oleifera*'s Nutritious Aqueous Leaf Extract Has Anticancerous Effects by Compromising Mitochondrial Viability in an ROS-Dependent Manner,” *Journal of the American College of Nutrition*, vol. 35, no. 7, pp. 604–613, 2016.
 20. Abdulrahman Khazim Al-Asmari, Sulaiman Mansour Albalawi, Tanwir Athar, Abdul Quaiyoom Khan, Hamoud Al-Shahrani, and Mozaffarul Islam, *Moringa oleifera* as an Anti-Cancer Agent against Breast and Colorectal Cancer Cell Lines, 2015 Aug 19. doi: 10.1371/journal.pone.0135814.
 21. L. Khoshtabiat, M. Mahdavi, G. Dehghan, M. Rashidi Oxidative stress-Induced apoptosis in chronic myelogenous leukemia K562 Cells by an active compound from the dithio-Carbamate family Asian Pac. J. Cancer Prev.: APJCP, 17 (2016).

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